中文版

福建农林大学（Fujian Agriculture and Forestry University）

Today is 8 20, 2017, **-2** days left until the conference begins

Conference navigation

Welcome & Invitation

Program

Invited Speakers

Committees

Registration

Abstracts Submission

Deadlines

Accommodation

VISA

Sponsors

About Xiamen

Tours

Contact Us

Welcome to the 5th ICBPI !

On behalf of the Organizing Committee, we sincerely invite colleagues to attend the 5th International Conference on Biotic Plant Interactions (5th ICBPI) in Xiamen, China on August 17-21, 2017. The theme of this five-day conference is Biotic-Plant Interactions and Sustainable Control of Pests on Crops. As a continuing effort after the 1st conference (Brisbane, Australia in 2008), the 2nd conference (Kunming, China in 2011), the 3rd conference (Yanglin, China in 2013), and the 4th conference (Nanjing, China in 2015), this conference will be organized by the State Key Laboratory of Ecological Pest Control of Fujian and Taiwan Crops, Fujian Agriculture and Forestry University and Institute of Plant Physiology and Ecology at Chinese Academy of Science, Chinese Society for Plant Biology and Chinese Society for Plant Pathology. It will bring together 800-1000 scientists all over the world and cover nine sessions: plant-fungus interactions, plant-oomycete interactions, plant-bacteria interactions, plant-virus interactions, plant-insect interactions, parasitic plants/nematodes, plant symbiosis, epigenetics in biotic plant interactions, and molecular design in crop resistance.

We are looking forward to welcoming you in Xiamen, China.

Sincerely Yours!

Zonghua Wang, Zuhua He, Regine Kahmann

Chairs of the Organizing Committee

Copyright 2016 The 5nd International Conference on Biotic Plant Interactions All rights reserved

Academic Committee

CHAIRS:

Lianhui Xie, Fujian Agriculture and Forestry University

Rongxiang Fang, Chinese Academy of Sciences

Le Kang, Chinese Academy of Sciences

Kongming Wu, Chinese Academy of Agricultural Sciences

Youyong Zhu, Yunnan Agricultural University

Regine Kahmann, Max Planck Institute for Terrestrial Microbiology, Germany

Guo-Liang Wang, Ohio State University, USA

Zuhua He, Chinese Academy of Sciences

Zonghua Wang, Fujian Agriculture and Forestry University, Minjiang University

Organizing Committee

CHAIRS:

Zonghua Wang, Fujian Agriculture and Forestry University, Minjiang University

Zuhua He, Chinese Academy of Sciences

Regine Kahmann， Max Planck Institute for Terrestrial Microbiology, Germany

MEMBERS:

Martin Dickman, Texas A&M University

Daniel Ebbole, Texas A&M University

Zhensheng Kang, Northwest Agriculture and Forestry University

John McDowell, Virginia Polytechnic Institute and State University

Youliang Peng, China Agricultural University

Qianhua Shen, Chinese Academy of Sciences

Dingzhong Tang, Fujian Agriculture and Forestry University

Weihua Tang, Chinese Academy of Sciences

Brett Tyler, Oregon State University

Chengshu Wang, Chinese Academy of Sciences

Guo-Liang Wang, Ohio State University, USA

Taiyun Wei, Fujian Agriculture and Forestry University

Jianmin Zhou, Chinese Academy of Sciences

Xueping Zhou, Chinese Academy of Agricultural Sciences

Local Organizers

State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops

Fujian University Key Laboratory for Plant-Microbe Interaction

Fujian Agriculture and Forestry University, FAFU

Minjiang University

Fujian Society for Plant Pathology

Fuzhou Zhongxinhe Conference Service Company

Sponsored by

Scientific Program of the 5th ICBPI

Time	Aug 17
8:00-23:00	Registration - The lobby of Xinglinwan Hotel

<table>
<tr><th>Time</th><th>Aug 18</th><th>Aug 19</th><th>Aug 20</th><th>Aug 21</th></tr>
<tr><td>8:00-12:10</td><td>Plenary Session I/II</td><td>Plenary Session III/IV</td><td>Plenary Session V/VI</td><td>Plenary Session VIII/IX</td></tr>
<tr><td rowspan="5">14:00-18:00</td><td rowspan="2">Concurrent Session I. Plant-bacteria interactions</td><td rowspan="2">Concurrent Session III. Plant-insect/nematode/plant parasite interactions</td><td>Plenary Session VII*</td><td rowspan="6"></td></tr>
<tr><td>Concurrent Session V. Plant resistance and crop molecular design
(d)</td></tr>
<tr><td>Concurrent Session II. Plant-fungus /oomycete interactions
(a)</td><td>Concurrent Session II. Plant-fungus /oomycete interactions
(b)</td><td>Concurrent Session II. Plant-fungus /oomycete interactions
(c)</td></tr>
<tr><td>Concurrent Session IV. Plant-virus interactions
(a)</td><td>Concurrent Session IV. Plant-microbe interactions
(b)</td><td>Young Scholar Session I</td></tr>
<tr><td>Concurrent Session V. Plant resistance and crop molecular design
(a) and (b)</td><td>Concurrent Session V. Plant resistance and crop molecular design
(c)</td><td>Young Scholar Session II</td></tr>
<tr><td>18:20-21:00</td><td>Welcome Banquet</td><td>Beer and Poster</td><td>Dinner then Culture Activity</td></tr>
</table>

***Tips:** Plenary Session VII will begin at 13:30 on Aug 20.

Opening Session and Keynote Speech, **Aug 18, Room: Main Hall (3rd floor of the Gymnasium)**			
08:00-08:10	**Opening Speech: Dr. Le Kang, Academician of CAS, Co-Chair**		Zonghua Wang
08:10-08:40	Lianhui Xie Jiasui Zhan	Prospective of the ecological pest management	

Plenary session I **Aug 18, Room: Main Hall (3rd floor of the Gymnasium)**			
08:40-09:10	Xinnian Dong	Translation in plant immune responses	Zuhua He
09:10-09:40	Youliang Peng	PacC-dependent adaptation to alkalinized host cells by *Magnaporthe oryzae* is required for rice blast disease	
09:40-10:10	**Coffee break and group photo**		

Plenary session II			
10:10-10:40	Regine Kahmann	Secreted core effectors in smut fungi: an amazing treasure box	Chengshu Wang & Daniel Ebbole
10:40-11:10	Saskia Hogenhout	Genetic components that mediate plant-insect interactions and transmission of plant pathogens	
11:10-11:40	Jin-Rong Xu	Ascosporogenesis and ascospore discharge in the wheat scab fungus *Fusarium graminearum*	
11:40-12:10	Taiyun Wei	The horizontal and vertical transmission mechanisms for rice viruses by insect vectors	
12:10-12:20	Xiaofeng Cui	Molecular Plant: from China for the world	
12:20-14:00	**Lunch**		

Concurrent session I. Plant-bacteria interactions **Aug 18, Room: 205 (2nd floor of Building 9)**			
14:00-14:20	Alberto Macho	Deciphering the molecular interface between plants and the bacterial pathogen *Ralstonia solanacearum*	Hua Lu & Wenming Wang
14:20-14:40	Gitta Laurel Coaker	Direct visualization of bacterial effector delivery during plant infection	
14:40-15:00	Hua Lu	Lux arrhythmo mediates crosstalk between the circadian clock and defense in *Arabidopsis*	
15:00-15:20	María Elena Alvarez	Chromatin alterations triggered by *Pseudomonas* infection and their effects on *Arabidopsis* defenses	
15:20-15:40	Wei Qian	Function of bacterial receptor histidine kinases in sensing host plant	
15:40-16:00	**Break**		
16:00-16:20	Jane Parker	Genetic architecture of temperature-controlled defense homeostasis in *Arabidopsis*	Haitao Cui

16:20-16:40	Zhengqing Fu	Disruption of salicylic acid signaling by a bacterial type III effector	& Gitta Laurel Coaker
16:40-17:00	Stefan Olsson	Biotic fungal-bacterial interactions with a focus on the recruitment of hyphosphere bacteria	
17:00-17:20	Yongping Duan	The effectomics of the intracellular bacterium, "Candidatus Liberibacter asiaticus" reveals a key to control citrus HLB	
17:20-17:40	Dong Wang	Swords to ploughshares: modulation of host defense molecules to promote symbiotic bacteria	
17:40-18:00	Xinhua Ding	Identification of the effectors which inhibit rice immunity and the mechanism of rice resisting against *Xanthomonas oryzae* pv. *oryzicola*	

Concurrent session II (a). Plant-fungus/oomycete interact **Aug 18, Room: Small Hall (3rd floor of the Gymnasium)**			
14:00-14:20	Richard A. Wilson	Metabolic control of effector secretion by *Magnaporthe oryzae*	Suomeng Dong & Richard A. Wilson
14:20-14:40	Yi Zhen Deng	Gcn5 regulates pathogenicity-related protein acetylation in *Magnaporthe oryzae*	
14:40-15:00	Wenbo Ma	*Phytophthora* effectors manipulate plant small RNAs to promote infection	
15:00-15:20	Qianhua Shen	Phosphorylation and transcription regulations in barley-powdery mildew interactions	
15:20-15:40	Lijun Ma	Dissecting wilt diseases using *Fusarium oxysporum–Arabidopsis* pathosystem	
15:40-16:00	**Break**		
16:00-16:20	Junfeng Liu	Structrual basis of the loss of the avirulence function of AvrPib from the rice blast fungus	Bo Zhou & Lijun Ma
16:20-16:40	Yong Hwan Lee	Phenotype plasticity of gain and loss of mutations in the rice blast fungus	
16:40-17:00	Zhonghua Ma	A specific transcript factor SREBP-1 regulates multiple stress responses and virulence in *Fusarium graminearum*	
17:00-17:20	Baoshan Chen	Genetic diversity of and development of efficient transformation system for the sugarcane smut fungus in Sothern China	
17:20-17:40	Bo Zhou	Breeding durable resistance against rice blast in rice	
17:40-18:00	Shihua Wang	The regulating mechanism of epigenetic modification on aflatoxin biosynthesis in *Aspergillus flavus*	

Concurrent session IV (a). Plant-virus interactions Aug 18, Room: 208 (2nd floor of Building 9)			
14:00-14:20	Yau-Heiu Hsu	Autophagy involved in assiting the Bamboo mosaic virus accumulation in *Nicotiana benthamiana*	Jeanmarie Verchot & Jian Ye
14:20-14:40	Dawei Li	The Barley stripe mosaic virus γb protein promotes chloroplast-targeted replication by enhancing unwinding of RNA duplexes	
14:40-15:00	Zhenghe Li	Insights into plant rhabdovirus virion morphogenesis and local movement	
15:00-15:20	Yule Liu	Autophagy functions as an antiviral mechanism against geminiviruses in plants	
15:20-15:40	Xiaorong Tao	Molecular dissection of Sw-5b-mediated broad-spectrum resistance to tospoviruses	
15:40-16:00	**Break**		
16:00-16:20	Jeanmarie Verchot	The ER stress machinery plays a central role in regulating virus infection	Na-Sheng Lin & Aiming Wang
16:20-16:40	Aiming Wang	Molecular characterization and manipulation of host factors for the control of plant potyviruses	
16:40-17:00	Kristiina Mäkinen	Potato virus A translation and replication are co-regulated by viral coat protein, two host chaperons and a protein kinase	
17:00-17:20	Jian Ye	Geminivirus as plant-dependent mutualist and biological weapon of the sweetpotato whitefly *Bemisia tabaci* MEAM1/B	
17:20-17:40	Lili Zhang	Microbiota in plant-insect vector-virus interaction	
17:40-18:00	Na-Sheng Lin	The helper virus-independent systemic movement of a viral satellite RNA in plant	

Concurrent session V (a). Plant resistance and crop molecular design Aug 18, Room: 312 (3rd floor of Building 9)			
14:00-14:20	Mark James Banfield	Engineering a plant immune receptor to extend pathogen effector recognition	Lirong Zeng & Fumiaki Katagiri
14:20-14:40	Wende Liu	Proteomic insights into lysine-acetylation mediated innate immunity in rice (*Oryza sativa*)	
14:40-15:00	Georg Felix	Specificity and diversity of plant pattern recognition receptors	
15:00-15:20	Baomin Feng	Protein Poly-ADP-ribosylation in *Arabidopsis* innate immunity	
15:20-15:40	Lirong Zeng	Role of the ubiquitin-conjugating enzymes in plant innate immunity	

15:40-16:00	**Break**		
16:00-16:20	Ping He	Transcriptional and posttranscriptional regulation of pattern-triggered immunity in plants	Mark James Banfield & Ping He
16:20-16:40	Fumiaki Katagiri	Dynamics, mechanisms, and evolution of a highly resilient plant immune signaling network	
16:40-17:00	Jian Hua	Impact of cellular status on the expression of a plant immune receptor gene in *Arabidopsis*	
17:00-17:20	Bostjan Kobe	Towards understanding TIR-domain interactions during NLR immunity receptor signalling	
17:20-17:40	Chuanyou Li	Transcriptional regulation of jasmonate signaling	

Concurrent session V (b). Plant resistance and crop molecular design **Aug 18, Room: 201 (2nd floor of Building 9)**			
14:00-14:20	Elizabeth P. B. Fontes	Inverse modulation of antibacterial and antiviral immunity by plasma membrane-associated immune complexes	Thomas Kroj & Kabin Xie
14:20-14:40	Jianfeng Li	Heterotrimeric G proteins are involved in novel immune signaling pathways in *Arabidopsis*	
14:40-15:00	Wei-Lin Wan	Differential signaling networks triggered by LRR-RK and LRR-RP-type receptors	
15:00-15:20	Junqi Song	The molecular basis of plant immunity and its interplay with DNA damage response	
15:20-15:40	Savithramma Dinesh-Kumar	Inter-organellar communication and autophagy during innate immunity	
15:40-16:00	**Break**		
16:00-16:20	Thomas Kroj	Structural and mechanistic bases of effector recognition by paired NLR immune receptors and decoy domains	Junqi Song & Elizabeth P. B. Fontes
16:20-16:40	Kee Hoon Sohn	Specificity of RIN4 function in activation or suppression of NLRs is conferred by sequence diversity in C-terminal region	
16:40-17:00	Kabin Xie	A CRISPR/Cas9 toolbox based on the endogenous tRNA processing for multiplex genome engineering	
17:00-17:20	Xilan Yu	Genes and traits responsive to growth and survival of *Pseudomonas syringae* in association with plant leaves	

Plenary session III **Aug 19, Room: Main Hall (3rd floor of the Gymnasium)**			
08:00-08:30	Yuanchao Wang	Defense and counter-defense during *Phytophthora* infection	Weihua Tang & Qianhua Shen
08:30-09:00	Bart P.H.J.Thomma	Adaptive genome evolution in the vascular wilt pathogen *Verticillium*	
09:00-09:30	Guo-Liang Wang	Dissection of the SPL11-mediated cell death and immunity pathway in rice	
09:30-10:00	Sophien Kamoun	NLR network mediates immunity to diverse plant pathogens.	
10:00-10:10	**Break**		
Plenary session IV			
10:10-10:40	Paul Schulze-Lefert	Rhizobial root microbiota membership predisposed convergent evolution of nitrogen-fixing symbiosis with legumes	Jane Parker & Baoshan Chen
10:40-11:10	Zuhua He	Genetic and epigenetic control of broad-spectrum blast resistance in rice	
11:10-11:40	Huishan Guo	Learning from the interaction between cotton and *Verticillium dahliae*	
11:40-12:10	Blake Meyers	The evolutionary complexity of micro-RNAs that target NB-LRR disease resistance genes	
12:10-12:25	Nikon NIS engineer	Advanced application of microscopic image	
12:25-14:00	**Lunch**		

Concurrent session II (b). Plant-fungus oomycete interact **Aug 19, Room: Small Hall (3rd floor of the Gymnasium)**			
14:00-14:20	Kim Elizabeth Hammond-kosack	Exploring the compatible *Fusarium*-wheat interaction using a multi-'omics' approach.	Weixing Shan & Chang Hyun Khang
14:20-14:40	Daolong Dou	Molecular interactions of *Phytophthora capsici* and its host plants	
14:40-15:00	Weihua Tang	In planta fungal profiling provides insights into *Fusarium graminearum* infection of wheat and maize	
15:00-15:20	Ryohei Terauchi	Molecular coevolution of *Magnaporthe oryzae* pathogen and rice	
15:20-15:40	Sebastian Schornack	Comparative studies of pathogenic and mutualistic root microbe interactions	
15:40-16:00	**Break**		
16:00-16:20	Fucheng Lin	Metabolomics and proteomics in *Magnaporthe oryzae*	Kim Elizabeth Hammond-kosack &
16:20-16:40	Weixing Shan	Genetic dissection of plant susceptibility to *Phytophthora* pathogens	

16:40-17:00	Suomeng Dong	Oomycete plant pathogen reprograms host pre-mRNA splicing to subvert immunity	Daolong Dou
17:00-17:20	Huiquan Liu	Rapidly evolving effector superfamilies promote host adaptation and speciation in the basal ascomycete genus *Taphrina*	
17:20-17:40	Chang Hyun Khang	Disruption of the interfacial membrane leads to *Magnaporthe oryzae* lifestyle switch and effector re-location during rice blast disease	

Concurrent session IV (b). Plant-Microbe interactions **Aug 19, Room: 208 (2nd floor of Building 9)**			
14:00-14:20	SekMan Wong	Cross protection for TMV infection in tobacco plants	Renier van der Hoorn & Xiaoming Zhang
14:20-14:40	Jianguo Wu	Small RNA-Based antiviral immunity and viral pathopoiesis in rice	
14:40-15:00	Kai Xu	Tombusvirus hijacks phospholipid PE for efficient viral replication	
15:00-15:20	Li Yang	Regulation of the growth-defense balance in plants: lessons learned from virulence effectors	
15:20-15:40	Eunyoung Chae	Activation of a plant NLR complex through heteromeric association with an autoimmune risk variant of another NLR	
15:40-16:00	**Break**		
16:00-16:20	Yongli Qiao	The molecular mechanism of suppressing host induced gene silencing by *Phytophothora* effector	SekMan Wong & Eunyoung Chae
16:20-16:40	Xiaoming Zhang	The role of a novel AGO-binding protein in plant immunity	
16:40-17:00	Fang Xie	Characterization of genes mediate rhizobial infection in *Lotus japonicus*	
17:00-17:20	Renier van der Hoorn	Evolution of the Avr2/Rcr3/Cf2 perception system	
17:20-17:40	Yansong Miao	Actin assembly regulation in plant innate immunity	
17:40-18:00	Yanping Tian	Helper component proteinase of Potato virus Y is an avirulence determinant eliciting HR in potato carrying Ny gene	

Concurrent session III. Plant-insect/nematode/plant parasite interactions **Aug 19, Room: 201 (2nd floor of Building 9)**			
14:00-14:20	Markus Albert	Detection of the plant parasite *Cuscuta reflexa* by tomato host plants	Sibao Wang &

14:20-14:40	Chengshu Wang	Genetics and metabolic responses of fungus-insect interactions	Jiang Zhang
14:40-15:00	Jinling Liao	A novel Meloidogyne effector, MgGPP, undergoes post-translational modification in host plants	
15:00-15:20	Justice Norvienyeku	Methylmalonate-semialdehyde dehydrogenase mediated metabolite homeostasis essentially regulate conidiation, polarized germination and pathogenesis in *Magnaporthe oryza*e	
15:20-15:40	Shusheng Liu	Plant-mediated interspecific competition between whiteflies	
15:40-16:00	**Break**		
16:00-16:20	Andrew Farmer Bent	Molecular mechanisms of soybean resistance to soybean cyst nematode	Markus Albert & Jinling Liao
16:20-16:40	Sibao Wang	The entomopathogenic fungus recruits gut microbiota to kill mosquitoes	
16:40-17:00	Tina Kyndt	Ascorbate oxidation level determines the hormone balance during the interaction between parasitic root-knot nematodes and rice	
17:00-17:20	Jiang Zhang	Engineering plastid genome for pest control by expression of insecticidal RNA in plastids	
17:20-17:40	Ying-Bo Mao	Elucidation of the dynamic interactions between plants and herbivorous insects	

Concurrent session V (c). Plant resistance and crop molecular design **Aug 19, Room: 312 (3rd floor of Building 9)**			
14:00-14:20	Thomas Anton Eulgem	Epigenetics-related work on EDM2/EDM3 and their roles in plant immunity	Yiji Xia & Tina Romeis
14:20-14:40	Jinlong Qiu	Stomatal opening confers immunity to bacterial leaf blight in rice	
14:40-15:00	Silke Robatzek	How endocytosis regulates immunity	
15:00-15:20	Mo Wang	The major leaf ferredoxin Fd2 regulates plant innate immunity in Arabidopsis	
15:20-15:40	Jens Stougaard	Signals and receptors involved in endosymbiosis	
15:40-16:00	**Break**		
16:00-16:20	Yiji Xia	Uncovering the molecular mechanism of AtNUDT6- and AtNUDT7-mediated immunity in plants	Thomas Anton Eulgem & Mo Wang
16:20-16:40	Tina Romeis	CDPK signaling in the activation of local and distal defense responses	
16:40-17:00	Wei Li	The rice phosphate transporter protein OsPT8 regulates disease resistance and plant growth.	

17:00-17:20	Tokuji Tsuchiya	Defense regulation by the chromatin-associated protein EDM2 in *Arabidopsis thaliana*	
17:20-17:40	Shunyuan Xiao	Membrane biogenesis and protein targeting in haustorium-invaded plant cells	

Plenary session V **Aug 20, Room: Main Hall (3rd floor of the Gymnasium)**			
08:00-08:30	Shengyang He	Bacterial pathogenesis in the phyllosphere: basic principles, climate influence and microbiota	Yau-Heiu Hsu & Wenbo Ma
08:30-09:00	Ertao Wang	Food for mutualistic mycorrhizal and parasitic fungi	
09:00-09:30	Vitaly Citovsky	Plasmodesmal localization signal of the Tobacco mosaic virus cell-to-cell movement protein	
09:30-10:00	Libo Shan	From leucine-rich repeat to malectin-like domain: differential functions of receptor-like kinases in plant immunity and growth	
10:00-10:10	**Break**		
Plenary session VI			
10:10-10:40	Jonathan Jones	Activation of defence by the RPS4/RRS1 paired NLR protein complex	Jian Hua & Andrew Farmer Bent
10:40-11:10	Hailing Jin	Cross-Kingdom RNA trafficking and environmental RNAi-Powerful innovative strategies for crop protection	
11:10-11:40	Gongyou Chen	iTAL effectors overcome bacterial blight resistance in rice	
11:40-12:10	Thorsten Nürnberger	A pore-forming toxin as microbial virulence factor and trigger of defense	
12:10-13:30	**Lunch**		

Plenary session VII **Aug 20, Room: Main Hall (3rd floor of the Gymnasium)**			
13:30-14:00	Ralph Dean	Genome enabled studies to further knowledge of rice blast infection and disease management	Yong Hwan Lee & Dawei Li
14:00-14:30	Xuewei Chen	A natural rice allele of a transcription factor confers broad-spectrum resistance	
14:30-15:00	Daoxin Xie	Virus manipulates plant hormone signaling to attract insect vectors	
15:00-15:30	Xueping Zhou	The pathogenicity factor of a geminivirus C4 induces symptoms by changing nuclear-localization of NbSKη	

15:30-16:00	Naweed Naqvi	Autophagy at the *Magnaporthe*-Rice interface	
16:00-16:20	**Break**		

Concurrent session II (c). Plant-fungus oomycete interact **Aug 20, Room: Small Hall (3rd floor of the Gymnasium)**			
16:20-16:40	Guodong Lu	*Magnaporthe* chitinase interacts with a jacalin-related lectin and promotes host colonization	Zhonghua Ma & Md. Rashidul Islam
16:40-17:00	Jiang Lu	Genome sequence of plasmopara viticola reveals effector reptorier and pathogenicity mechanisms	
17:00-17:20	Md. Rashidul Islam	Molecular based identification and formulation of cyanogenic Pseudomonas spp. controlling *Phytophthora infestans*	
17:20-17:40	Daniel Ebbole	Evolution of an effector gene family from the rice blast fungus	

Concurrent session V (d). Plant resistance and crop molecular design **Aug 20, Room: 312 (3rd floor of Building 9)**			
16:20-16:40	Yuelin Zhang	Regulation of plant immunity by multiple MAP kinase cascades	Eloise Foo & Yan Liang
16:40-17:00	Eloise Foo	Determining the site of action of strigolactones during nodulation	
17:00-17:20	Haitao Cui	A two-pronged mechanism for boosting SA defense in TNL immunity	
17:20-17:40	Pingtao Ding	Comprehensive capture-seq (Coca-seq) unravels gene regulation mechanism in plant immune signalling	
17:40-18:00	Yan Liang	Intersection between MAMP-triggered innate immunity and symbiosis	

Young Scholar **Aug 20, Room: 201 (2nd floor of Building 9)**			
16:20-16:40	Qiong Zhang	Identification and characterization of Arabidopsis genes that contribute to powdery mildew resistance via an EDS1- and SA-independent signaling pathway	Wenhui Zheng& Jun Zhao
16:40-17:00	Kevin L. Cox Jr.	Cotton is SWEET	
17:00-17:20	Sanjie Jiang	Virus infection of plants alters pollinator preference: A payback for susceptible hosts?	
17:20-17:40	M. Imran Hamid	Ecological mechanisms of microbiome associated with soybean cyst nematode suppressive soils	

17:40-18:00	Xia Yan	Functional analysis of novel rice blast fungus effectors illuminates the mechanism of plant-fungus interaction	

Young Scholar **Aug 20, Room: 208 (2nd floor of Building 9)**			
16:20-16:40	Laura Medina-Puche	A defence pathway linking plasma membrane to chloroplasts is co-opted by a virus to suppress salicylic acid signaling	Baomin Feng & Wende Liu
16:40-17:00	Muxing Liu	A novel *Magnaporthe oryzae* effector targets the Light-harvesting chlorophyll a/b-binding protein (Lhcb5) to suppress immunity in rice	
17:00-17:20	Lei Wang	Two related receptor kinases SYR1 and SYR2 of tomato act as high and low affinity receptors for the plant peptide hormone systemin	
17:20-17:40	Wenwu Ye	Transcriptional programming of *Phytophthora sojae* for organ-specific infection	
17:40-18:00	Amey Redkar	Understanding the CCG effector toolbox of white rust oomycete pathogen *Albugo candida*	

Plenary session VIII **Aug 21, Room: Main Hall (3rd floor of the Gymnasium)**			
08:00-08:30	Zhensheng Kang	The role of barberry in epidemics and virulence variation of wheat stripe rust	Yule Liu & Ryohei Terauchi
08:30-09:00	Brett Tyler	Dissecting the role of the *Phytophthora sojae* effector repertoire using CRISPR	
09:00-09:30	Dingzhong Tang	The role of a truncated NLR in plant immunity	
09:30-10:00	Jianqiang Wu	The parasitic plant dodder transfers biotic and abiotic stress-induced systemic signals among host plants	
10:00-10:10	**Break**		
Plenary session IX			
10:10-10:40	Jianmin Zhou	How does a plant receptor activate heterotrimeric G proteins?	Regine Kahmann & Fucheng Lin
10:40-11:10	Daohong Jiang	*Sclerotinia sclerotiorum*, can it be a friend?	
11:10-11:40	Guangcun He	Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation	
11:40-12:10	Roger Innes	Extracellular vesicles: An underappreciated component of the plant immune system	
12:10-12:20	Zhensheng Kang	Welcoming to the 6th ICBPI	
12:20-14:00	**Lunch**		

Plant diseases: principles of epidemics and ecological management-New perspectives, new concepts and new strategies

Dunchun He, Zujian Wu, Linping Wang, Jiasui Zhan and Lianhui Xie*
Institute of plant Virology, Fujian Agriculture and Forestry University, Fuzhou, 350002, Fujian Key Laboratory of Plant Virology, Fuzhou, 350002

1. In natural ecosystems, plant-pathogen interactions occur against a backdrop of ecological and environmental heterogeneity in which pathogen's impact is tempered by patchiness in the suitability of environments for disease development as well as by variable and small host populations. Agricultural intensification, monoculture, overuse of fertilizers and pesticides disrupt the coevolutionary dynamics typically found between plants and pathogens in unmanaged ecosystems and provide favourable environments for pathogens to rapidly reproducce and develop high infectivity and aggressiveness, particularly in rice diseases caused by viruses.

New perspectives—shifting the principles of plant disease occurrences and epidemics from single dimension of disease triangle in traditional plant pathology to multiple dimensions of double disease triangles in a globe perspective

1.1 Single dimension of disease triangle: the occurrences and epidemics of plant diseases require adequate primary inoculum of virulent pathotypes, susceptible host plants and conducible environments (Fig.1)

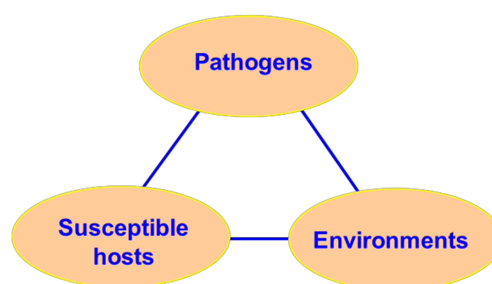

Fig.1 The disease epidemic triangle

1.2 Double disease triangles propose that the occurrences and epidemics of plant diseases are triggered by 1) a disbalanced plant life system resulted from malfunctioning metabolism of plants during their interactions with biotic and abiotic environments (Figure 2); and 2)

* Correspondence, E-mail: xielh@fafu.edu.cn

Funding project: the Fujian Technology Plan Project, China (2012N4001)

disbalanced agricultural ecosystems caused by inappropriate human intervention (Figure 3)

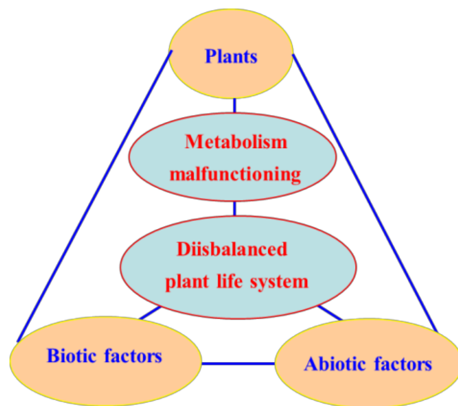

Fig. 2 Principle of disease occurrences

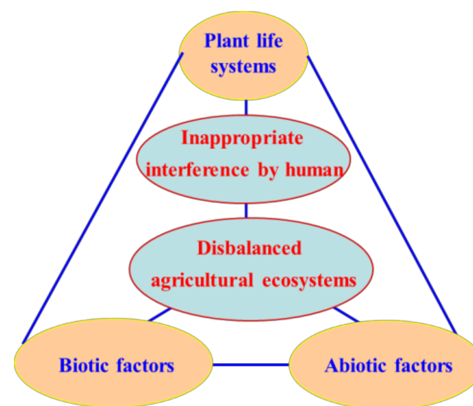

Fig. 3 Principle of disease epidemics

2. New concept: shifts from to single goal of increasing productivity to multifaceted goals including high quality and added value of production, high efficiency of using natural resources and host resistance and increased economic, social, ecological sustainability and industrialization; shifts from Integrative Pest Management by targeting to eliminate or mitigate pathogens to Ecological Pest Management by targeting to increase the health of plant populations. EPM focuses to create biotic and abiotic agricultural ecosystems conducible to the growth and development of plant population while suboptimum to the survival, reproduction and transmission of plant pathogens by scientific understanding the interaction among host plants, pathogens, vectors and environments and the impact of human activities on the interactions. It is an integration of micro and macro-ecology and micro- and macro-plant protection.

3. New strategies – the best approach to manage plant diseases is to create healthy ecosystems: macro-ecology, macro-plant protection and green production。

3.1 Overall requirement: through the concept change and scientific applications of management approaches to achieve high, good and industrializing production, high natural resources and resistance efficiency and sustainability.

3.2 Principles of management approaches: guided by the principle of plant disease epidemics, appropriate understanding the relationship of productivity and disease extents and key biotic and abiotic environments such as host genetics, density and resistance, vectors, macro- and micro climatic conditions, soil conditions, solar radiation etc to formulate management approaches for particular plant-pathogen interactions. Good plant materials, healthy seedlings

and root systems are three key components for ecological management of plant diseases.

3.3 Examples: field experiment of rice production in Youxi (50 mu or ~3.5 hectares) and Fuqing (400 mu or 28 hectares) for consecutive three years; demonstration of the technology in Chanting (1000 mu or ~67 hectares) for two years; technology radiation 50000 mu (~3500 hectares) in Changting, Jianyang and Youxi.

Translation in plant immune responses

Guoyong Xu[a], Meng Yuan[b], George Greene[a], Heejin Yoo[a], Chaoren Ai[b], Lijing Liu[a], Edward Zhuang[a], Sargis Karatyan[a], Jorge Marques[a], Jonathan Motley[a], Karolina Mukhtar[a], Wei Wang[a], Shiping Wang[b], and Xinnian Dong[a]

[a]*Howard Hughes Medical Institute-Gordon and Betty Moore Foundation, Department of Biology, Duke University, Durham, North Carolina 27708, USA*

[b]*National Key Laboratory of Crop Genetic Improvement, National Centre of Plant Gene Research (Wuhan), Huazhong Agricultural University, 430070 Wuhan*

xdong@duke.edu

A major consequence of pathogen infection is perturbation of host metabolism, including protein synthesis. However, little is known about how host cells may respond to such perturbations and selectively synthesize defense-proteins to mount immune responses. My lab showed that TBF1, a transcription factor controlling the growth-to-defense transition in plants, is tightly regulated at both transcriptional and translational levels. The TBF1 mRNA contains two upstream open reading frames (uORFs) besides the main ORF. Translation of TBF1 is normally inhibited by these uORFs, which presumably cause dissociation of the ribosome from the mRNA before it reaches the downstream TBF1 ORF. Upon induction of both pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), the inhibitory effects of uORFs are rapidly and transiently alleviated, leading to TBF1 protein translation [1]. To elucidate the regulatory mechanisms, we performed global translatome profiling, using the recently developed ribosome footprinting technology, and identified several trans-acting regulators, a highly conserved RNA sequence ("R-motif"), and many new uORFs [2]. Moreover, we used the pathogen-responsive TBF1 cassette to drive the production of defense proteins and provided the proof of concept, in *Arabidopsis* and in rice, that adding translational control to defense protein production is an effective new strategy for minimizing fitness costs associated with broad-spectrum disease resistance and reducing the selective pressure for resistant pathogens [3].

Acknowledgement: This study was initiated by CSREES of USDA 2007-35301-18022 and supported by grants from NIH 5R01 GM069594-11 and HHMI-GBMF (GBMF3032) to X.D., NSFC (31371926) to M.Y., and the NKRDPC (2016YFD0100903) to S.W.

References:

[1] K. Mukhtar, W. Wang, Y. Tada, N. Oka, C. Tucker, J. Fonseca, X. Dong (2012). Current Biology, 22, 103.
[2] G. Xu, G.H. Greene, H. Yoo, L. Liu, J. Marques, J. Motley, X. Dong. (2017) Nature, 545, 487.
[3] G. Xu, M. Yuan, C. Ai, L. Liu, E. Zhuang, S. Karapetyan, S. Wang, Dong. (2017) Nature, 545, 491.

PacC mediates lifestyle transition of *Magnaporthe oryzae* during plant infection

Xiao-Lin Chen[1], Dan He[1], Changfa Yin[1], Jun Yang[1], Jing Sun[1], Dawei Wang[1], Minfeng Xue[1], Zhigang Li[1], Zhao Peng[1], Deng Chen[1], Wensheng Zhao[1], Jin-Rong Xu[2], Nicholas J. Talbot[3], and You-Liang Peng[1]

[1]*State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of the Monitoring and Green Managements of Crop Pests, China Agricultural University, Beijing 100193*

[2]*Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907, USA*

[3]*School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4QD, UK*

Email:pengyl@cau.edu.cn

PacC transcription factor is a central component of the PacC pH signaling pathway and is required for virulence of plant fungal pathogens. Here, we report how PacC mediates lifestyle transition of the hemibiotrophic rice blast fungus *Magnaporthe oryzae* during infection. We identified nine alkaline pH-sensitive mutants of *M. oryzae* showing similarly impaired virulence. Strikingly, they were all arrested at pseudohyphae stage and disrupted in the PacC pathway, including PacC. Interestingly, host cells become alkalinized during the biotrophic growth but acidified during the necrotrophic growth. Coincidently, PacC localizes respectively to the nucleus and to the cytoplasm during the two distinct stages. Furthermore, PacC exists as both a truncated transcriptional activator and a full-length repressor that directly modulate differential expression of ~2000 fungal genes in pseudohyphae. Many enhanced genes are involved in acquisition of nutrients and energy supply while most repressed genes have the highest expression during other developments including conidiation. Establishment of the rice blast disease therefore requires different subcellular locations of PacC to reprogram gene expression for the biotrophic and necrotrophic growths.

Secreted core effectors in smut fungi: an amazing treasure box

Regine Kahmann

Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The fungus *Ustilago maydis* causes smut disease in maize. *U. maydis* is a biotrophic pathogen requiring living plant tissue for colonization. For a successful infection, plant defense responses need to be downregulated and host physiology needs to be manipulated to benefit the pathogen. To accomplish this, *U. maydis* secretes a cocktail of about 300 effector proteins. The majority of these proteins lack a functional annotation and their function remains to be uncovered. Based on a comparative analysis of six smut genomes we have identified a set of core effectors that are present in all six species. A systematic deletion of the most highly expressed effector genes in this class resulted in the discovery of mutants with strong virulence phenotypes. One of these mutants lacks the repetitive effector Rsp3. I will present evidence that Rsp3 inhibits the activity of an antifungal maize protein. Interestingly, four individual, unrelated effectors from this group are essential for virulence: individual *stp1, stp2, stp3 or stp4 mutants* (stop after penetration) deletion strains are able to form appressoria that penetrate, but hyphal growth is arrested in epidermal host tissue due to massive plant defense responses and plant cell death. A similar phenotype was already observed for mutants lacking the essential effector *pep1* (Döhlemann *et al*.,2009). Co-IP with individually tagged effectors followed by mass-spectroscopic analysis revealed that Stp1, Stp3, Stp4 and Pep1 form a complex. I will discuss our current efforts to localize the complex and to functionally characterize its components. I will address possible scenarios for the function of the complex in plant gene regulation, effector stabilization or shielding of Avr proteins.

Genetic components that mediate plant-insect interactions and transmission of plant pathogens

Saskia A. Hogenhout

Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

saskia.hogenhout@jic.ac.uk

Sap-feeding insect pests, such as aphids, psyllids, leafhoppers and planthoppers, are major agricultural pests worldwide. The green peach aphid (GPA) *Myzus persicae* alone transmits over 100 different plant viruses, and psyllids, leafhoppers and planthoppers are vectors of both viruses and bacterial plant pathogens. Mechanisms of how these insects establish feeding sites on plants and transmit plant pathogens are still largely unknown. We recently identified genetic components involved in the plant calcium burst that occurs during GPA feeding. Moreover, we found an effector protein in GPA saliva that suppresses plant calcium and ROS bursts and that is conserved in other sap-feeding insects. Our data demonstrate that establishing a feeding site involves a dynamic battle between the sap-feeding insect and plant that also promotes pathogen transmission.

A-to-I RNA editing during sexual reproduction in filamentous ascomycetes

Huiquan Liu [a], Cong Jiang [a], Jin-Rong Xu [b]
[a] *College of Plant Protection, Northwest A&F University, Yangling, Shaanxi*
[b] *Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN47907, USA*
Email: jinrong@purdue.edu

Fungi, like plants, do not have ADAR orthologs and are believed to lack A-to-I RNA editing, which is the prevalent mRNA editing in animals. However, genome-wide A-to-I editing occurs specifically during sexual reproduction in the wheat scab fungus *Fusarium graminearum*. Unlike those in animals, majority of A-to-I editing sites in *F. graminearum* occur in coding regions and over two-thirds of them result in amino acid changes, including editing of 69 pseudogenes with the UAG stop codon in their ORFs. Furthermore, *F. graminearum* differs from animals in the sequence-preference and structure-selectivity of editing sites. Genome-wide A-to-I editing also specifically occurs during sexual reproduction in *Neurospora crassa, N. tetrasperma,* and *F. verticillioides*. Some of the editing sites are conserved in these fungi and they may be functionally related to their stage-specific functions during ascus or ascospore development. Unlike in humans, RNA editing in fungi preferentially targets As in hairpin loops, which is similar to the anticodon loop of tRNA targeted by ADATs, and nonsynonymous editing events in fungi are generally beneficial and favored by positive selection. RNA editing occurred before ascus development but became prevalent during ascosporogenesis. Overall, our results indicate that A-to-I editing in fungi occurs specifically during sexual reproduction with ADAR-independent mechanisms, is generally adaptive, and may be functionally related to other stage specific genetic phenomena.

References:

[1] H. Q. Liu, QF. Wang, Y. He, L. F. Chen, C. F. Hao, C. Jiang, Y. Li, Y. F., Dai, Z. Kang, J. R. Xu. (2016) Genome Research. 26: 499-509.
[2] H. Q. Liu, Y. Li, DP Chen, ZM Qi, QH Wang, Q., JH Wang, C. Jiang, J.R. Xu. (2017). Proc. Natl. Acad. Sci. USA.

The horizontal and vertical transmission mechanisms for rice viruses by insect vectors

Taiyun Wei
Fujian Province Key Laboratory of Plant Virology, Institute of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou E-mail: weitaiyun@fafu.edu.cn

Abstract: Most persistent propagative viruses, including plant reoviruses, plant rhabdoviruses, tospoviruses, tenuiviruses, and marafiviruses, are transmitted by insect vector through two pathways: horizontal and vertical transmission [1]. For horizontal transmission of rice reoviruses, they enter the gut epithelium from the gut lumen and release into the hemolymph or other tissues of insect vector, finally invade the salivary glands, from where they are introduced back into the plant host during insect feeding. Salivary gland thus represents the key barrier for viral horizontal transmission. Recently, we found the persistent propagative *Rice gall dwarf virus* (RGDV) had evolved to exploit virus-induced filaments to perform an exocytosis-like process that enabled viral passage through the apical plasmalemma into salivary cavities, where saliva is stored. The virus-associated filaments constructed by the nonstructural protein Pns11 of RGDV could specifically attached to the surface of cavity plasmalemma through a direct interaction of Pns11 and cytoplasmic actin, the main component of cavity plasmalemma. Such attachment further induced an exocytosis-like process for viral release into the cavity, involving membrane curvature, the formation of invaginations, and the creation of vesicular compartments [2]. For vertical transmission, viruses are transmitted through the female or male to offspring insect, which has been considered as a possible mechanism for the persistence of plant viruses during periods unfavorable for horizontal transmission. In female insect, viral pathogens must pass through the follicular cells into the ovary oocytes. We found that the oocyte-entered pathways of female-specific protein vitellogenin or bacterial symbiont *Sulcia* are exploited by *Rice stripe virus* or *Rice dwarf virus* to overcome the transovarial transmission barriers in respective insect vectors [3,4]. Furthermore, RGDV exploits virus-containing tubules composed of viral nonstructural protein Pns11 to pass through the follicular cells barrier into oocyte of leafhopper vectors [5]. Interestingly, we also found RGDV infected the sperm heads of male leafhoppers. It's the first time to show that the male insect is involved in vertical transmission of plant viruses.

Reference：

[1] Hogenhout SA, Ammar ED, Whitfield AE, Redinbaugh MG (2008). Annual Review of Phytopathology, 46:327-359.
[2] Mao, Liao Z, Li J, Liu Y, Wu W, Chen H, Chen Q, Jia D, Wei T. (2017). Journal of Virology, 91: e00265-17.
[3] Huo Y, Liu W, Zhang F, Chen X, Li L, Liu Q, Zhou Y, Wei T Wei T, Fang R, Wang X. (2014). PLoS Pathogens. 10:e1003949.
[4] Jia D, Mao Q, Chen Y, Liu Y, Chen Q, Wu W, Zhang X, Chen H, Li Y, Wei T. Nature Microbiology, 2017, 2:17025.
[5] Liao Z, Mao Q, Li J, Lu C, Wu W, Chen H, Chen Q, Jia D, Wei T. Frontiers in Microbiology, 2017, 8:475.

Deciphering the molecular interface between plants and the bacterial pathogen *Ralstonia solanacearum*

Alberto P. Macho

Shanghai Center for Plant Stress Biology, CAS Center for Excellence in Molecular Plant Sciences; Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 201602.

Most bacterial plant pathogens employ a type-III secretion system to inject type-III effector (T3E) proteins directly inside plant cells. The complex infection process of *Ralstonia solanacearum* is supported by a large number of T3Es, although the function of most of them is still unknown. In order to understand the plant infection by *R. solanacearum*, we have performed screens aimed at identifying T3Es that target diverse plant functions, including immunity, responses to other environmental cues, and development. Following this strategy, we have found that *R. solanacearum* employs different T3Es to manipulate the immune responses to bacterial elicitors and hormone signalling. One of these T3Es, RipAY, is a strong suppressor of immune responses. Transient expression of RipAY in *Nicotiana benthamiana* leaves severely compromises the plant response to bacterial elicitors and to exogenous treatment with salicylic acid. Biochemical analysis shows that RipAY associates with several different cytosolic thioredoxins in plant cells. Additionally, RipAY has a domain with predicted gamma-glutamyl cyclotransferase activity, usually involved in the degradation of glutathione, a major determinant of cellular redox state. Consistent with this, we found that RipAY degrades glutathione in plant cells, and this biochemical activity is required for the suppression of immunity. Our results suggest that *R. solanacearum* employs RipAY to manipulate the redox regulation of host cells, displaying a novel virulence strategy that has a severe impact on immune responses.

Direct visualization of bacterial effector delivery during plant infection

Elizabeth Henry [a], Tania Y Toruño [a], Alain Jauneau [b], Laurent Deslandes [c], Gitta Coaker [a]
[a] *Department of Plant Pathology, University of California, Davis, One Schields Avenue, Davis, California USA*
[b] *Federal Institute of Research, University of Toulouse, 12 TRI-Genotoul, Castanet-Tolosan, France*
[c] *Laboratory of Plant-Microbe Interactions, University of Toulouse, INRA, 24 Chemin de Borde Rouge, Castanet-Tolosan, France*
glcoaker@ucdavis.edu

To facilitate infection and communicate with their hosts, pathogens secrete effector proteins that promote colonization. Most previous investigations of effector function and localization have relied on overexpression in plants. We have developed a GFP strand system that enables direct visualization of Type III effector delivery into diverse cell types. GFP is a beta barrel protein that can be divided into 11 strands. Infection of Arabidopsis expressing GFP1-10 with bacteria delivering GFP11-tagged effectors enabled direct effector detection during the course of infection by confocal microscopy. The spatial and temporal delivery of GFP11-tagged effectors during infection with the foliar pathogen *Pseudomonas syringae* and the vascular pathogen *Ralstonia solanacearum* will be presented. Collectively, these results highlight the utility of the GFP strand system to investigate effector biology in intact organisms during the course of infection.

Acknowledgement: This research was supported by an NIH grant (RO1GM092772) awarded to GC. LD was supported by an ANR grant (RADAR, ANR-15-CE20-0016-01).

References:

[1] E. Henry, T.Y. Toruño, A. Jauneau, L. Deslandes, G.L. Coaker (2017), The Plant Cell, doi: 10.1105/tpc.17.00027.

LUX ARRHYTHMO mediates crosstalk between the circadian clock and defense in Arabidopsis

Hua Lu, Department of Biological Sciences, University of Maryland Baltimore County

Abstract

The circadian clock is a timekeeper that integrates external and internal temporal cues to regulate plant growth, development, and responses to environmental stimuli. Recent studies have established a role of the circadian clock in regulating plant innate immunity. However, the molecular mechanisms underlying this role of the circadian clock have not been well understood. LUX ARRHYTHMO is an important core clock component involved in multiple transcription-translation feedback loops (TTFLs) to ensure clock accuracy. We found that the *lux-1* mutant was compromised to both basal and resistance-gene mediated defense against *Pseudomonas syringae*. *lux-1* had reduced SA accumulation upon *P. syringae* infection and suppressed high SA and constitutive defense phenotypes in the lesion mimic mutant *acd6-1* background. RNAseq analysis revealed both defense- and development-related genes are regulated by *LUX*. ChIP experiments further showed LUX binding to several TTFL gene promoters previously unidentified, expanding the TTFL network that requires LUX function. In addition, we found a direct binding of LUX to promoters of genes critical for defense signaling. Together, these data indicate that the TTFL gene LUX plays a pivotal role in the crosstalk between the circadian clock and plant innate immunity.

Chromatin alterations triggered by *Pseudomonas* infection and their effects on *Arabidopsis* defenses

Alvarez María E., Cambiagno Damián A.
CIQUIBIC-CONICET, Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Ciudad Universitaria, Haya de la Torre y Medina Allende, Córdoba 5000, Argentina.
malena@fcq.unc.edu.ar

Several evidences indicate that plant immune cascades are subject to epigenetic control. Arabidopsis tissues infected with *Pseudomonas syringae* pv *tomato* (*Pst*) alter their DNA methylation profile and this seems to modulate the activation of defense responses. In fact, some genes encoding plant immune receptors belonging to the class of nucleotide-binding leucine-rich repeat (NLR) proteins, are affected by the epigenetic control of transposon elements (TEs) located in their proximity. Interestingly, peri/centromeric heterochromatin holding most of the repetitive sequences and TEs of the genome, also modifies its epigenetic state and condensation in response to *Pst*. At early stages of infection, these regions reduce the 5-methyl cytosine (5-mC) content and lose compaction. As peri/centromeric TEs are kept silenced by 5-mC and/or dimethylation of lysine 9 of histone 3 (H3K9me2) marks, we analyze whether the *Pst* treatment activates these elements. Moreover, we tested if de-regulation of TEs affects the expression of defense genes. For this purpose, we analyzed healthy and infected tissues of wild type and mutants plants that lose H3K9me2 marks at peri/centromeric heterochromatin. Our results suggest that deregulation of TE influences the expression of disease resistance genes. Models that could explain this type of effect will be presented.

Histidine kinases of *Xanthomonas campestris*: How a phytopathogenic bacterium know its world.

Wei Qian, qianw@im.ac.cn

Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101

Two-component signal transduction system is the predominant sense-and-response mechanism of prokaryotes. The system usually contains a membrane-bound histidine kinase and a cytosolic response regulator. After detecting the environmental stimuli, the histidine kinase autophosphorylates and transfers the phosphoryl group onto the cognate response regulator, and it is the response regulator elicits adaptive responses. Although the two-component signal transduction system was identified for about three decades, how the histidine kinase sense environmental cues remains largely unknown. In the recent years, we have revealed that *Xanthomonas campestris* pv. *campestris*, a gram-negative bacterial pathogen of cruciferous plants, employs two-component signal transduction systems to directly sense iron depletion and quorum-sensing signal and regulate virulence [1-5].

Very recently, we identified a membrane-bound receptor histidine kinase of the phytopathogenic bacterium *Xanthomonas campestris*, PcrK, to be a novel bacterial receptor that specifically detects the plant hormone, cytokinin. Cytokinin physically binds to the extracytoplasmic region of PcrK to decrease its autokinase activity. Through a four-step phosphorelay, cytokinin stimulation decreased the phosphorylation level of PcrR, cognate response regulator of PcrK, to activate the phosphodiesterase activity of PcrR in degrading the second messenger 3′,5′-cyclic diguanylic acid (c-di-GMP). Perception of cytokinin by the PcrK-PcrR remarkably improves bacterial tolerance to oxidative stress by regulating the transcription of 56 genes, including the virulence-associated TonB-dependent receptor gene *ctrA*. Our results reveal an evolutionarily conserved, inter-kingdom signaling mechanism by which phytopathogenic bacteria intercept a plant hormone signal to promote their adaptation to oxidative stress.

References

[1] Cai et al. 2017. Fatty acid DSF binds and allosterically activates histidine kinase RpfC of

phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* to regulate quorum-sensing and virulence. *PLoS Pathogens*. 13:e1006304.

[2] Peng et al. 2017. An essential regulatory system originating from polygenic transcriptional rewiring of PhoP-PhoQ of *Xanthomonas campestris*. *Genetics*. doi.org/10.1534/genetics.117.200204.

[3] Wang et al. 2016. Two-component signaling system VgrRS directly senses extracytoplasmic and intracellular iron to control bacterial adaptation under iron depleted stress. *PLoS Pathogens*. 12(12):e1006133.

[4] Zheng et al. 2016. Systematic mutational analysis of histidine kinase genes in nosocomial pathogen *Stenotrophomonas maltophilia* identifies BfmAK system controlling biofilm development. *Applied and Environmental Microbiology*. 82:2444-2456.

[5] Wang et al. 2014. A three-component signaling system finetunes expression kinetics of HPPK responsible for folate synthesis by positive feedback loop during stress response of *Xanthomonas campestris*. *Environmental Microbiology*, 16:2126-2144.

Genetic variation in temperature-modulated Arabidopsis defense homeostasis

Friederike Bruessow, Jaqueline Bautor, Gesa Hoffmann & Jane E. Parker

Max-Planck Institute for Plant Breeding Research, Dept. Plant-Microbe Interactions, Cologne Germany

We are interested in understanding how plants activate and fine-tune disease resistance pathways, using *Arabidopsis* to build pathogen-induced defense pathways and networks. As part of our study, we are exploring the role of temperature, within the *A. thaliana* normal range, on defense and growth. We identified extensive within-species genetic variation in accumulation of the important resistance signaling hormone salicylic acid (SA) in response to two ambient temperatures (20°C and 16°C). Differential accessions were then selected and are being characterized for physiological, growth, metabolic, gene expression and resistance phenotypes. Our results suggest a degree of genetic plasticity in temperature-modulated SA effects on plant growth and a benefit of maintaining high SA levels on basal immunity to bacteria (*Pst* DC3000). We performed association mapping to investigate the genetic architecture of SA x temperature trait variation. From this, one well supported GWAS peak is located on the top arm of chromosome 4 and candidate gene functions, expression and effects of mutation/over-expression in relation to temperature, growth and disease resistance are being analysed. I will describe progress in dissection of this complex trait and the influence of temperature on plant physiology and immunity.

Disruption of salicylic acid signaling by a type III effector

Huan Chen[1,2], Jian Chen[1,2], Min Li[2], Ming Chang[1,2], Kaimei Xu[2], Zhenhua Shang[2], Yi Zhao[1,2], Ian Palmer[2], Yuqiang Zhang[2,3], Jon McGill[2], James R. Alfano[4], Marc T. Nishimura[5], Fengquan Liu[1,*], and Zheng Qing Fu[2]

[1]*Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, 210014*
[2]*Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA*
[3]*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, Jiangsu, 210095*
[4]*Center for Plant Science Innovation and the Department of Plant Pathology, University of Nebraska, Lincoln, NE 68588, USA*
[5]*Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80526, USA*
E-mail of presenting author: zfu@mailbox.sc.edu

SUMMARY

Gram negative plant bacterial pathogens rely on type III effectors that are injected into plant cells by the type III secretion system to suppress plant defense in order to cause diseases [1]. Although it is well known that the plant hormone salicylic acid (SA) plays a pivotal role in plant defense, the master regulator of SA signaling NPR1 has not been reported to be a target of any plant pathogen effector. SA induced upon pathogen infection facilitates the reduction of plant cytosolic NPR1 oligomers into monomers, which enter the nucleus and function as transcriptional coactivators of plant defense genes [2]. Here we show that SA promotes the interaction between the *Pseudomonas syringae* type III effector HopAB2 and NPR1. HopAB2 mediates the degradation of NPR1 through the host's 26S proteasome, dependent on HopAB2's E3 ligase activity in the presence of SA. Intriguingly, we found that NPR1 plays an important role in MAMP-triggered immunity (MTI). Thus, this work uncovers a unique strategy, in which HopAB2 targets SA-activated NPR1 and represses NPR1-dependent SA signaling outputs, thereby subverting plant innate immunity.

References:

[1] Galan, J.E. and Collmer, A. (1999). Type III secretion machines: bacterial devices for protein delivery into host cells. Science *284*, 1322-8.

[2] Mou, Z., Fan, W. and Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell *113*, 935-44.

Biotic Fungal-Bacterial Interactions

Stefan Olsson
Fujian Agriculture and Forestry University

The interaction between fungi and bacteria is probably the most common bacterial-eukaryote interaction on cellular levels on earth. Like any eukaryote in contact with bacteria the fungus has to mobilize beneficial bacteria and at the same time resist potential pathogens. To make things even worse for the fungus the whole fungal network is for mycelial fungi in cell-to cell contact with bacteria in an environment like soil. With the starting point in my own work and in two recently published reviews by me and co-authors from the fungal-bacterial interaction scientific community I will sketch some possible developments for bacterial-fungal research. These developments include both uses of recently developed methods or developing new methods but most importantly of looking into new directions with inspiration from physiological processes described and studied in other systems that are most likely involved in fungal bacterial interactions.

The effectomics of the intracellular bacterium, "*Candidatus* Liberibacter asiaticus" reveals a key to control citrus HLB

Marco Pitino and Yongping Duan

U.S. Horticultural Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Fort Pierce, FL, USA

Yongping.duan@ars.usda.gov

'*Candidatus* Liberibacter asiaticus' (Las) is the most prevalent species of Liberibacter associated with huanglongbing (HLB), a devastating disease of citrus worldwide. As an obligate plant pathogen and insect symbiont with a significantly reduced genome, Las infects all citrus varieties, causing systemic disease. To reveal the molecular interactions between Las and plant hosts, we identified 16 putative Las effectors via bioinformatics, and transiently expressed them in *Nicotiana benthamiana*. Diverse subcellular localization, including different aggregation patterns of the effector candidates, was revealed by UV- microscopy. Intriguingly, one of the 16 candidates, Las5315mp (mature protein), was localized to the chloroplast and induced cell death at 3 days post inoculation in *N. benthamiana,* which was accompanied by H_2O_2 accumulation and pronounced callose deposition in plant cells. However, LasΔ5315 induced a massive accumulation of starch in the infiltration zone, mimicking the key physiological symptom of HLB: starch accumulation. Meanwhile, we identified several host proteins that interact with Las5315mp using a yeast two-hybrid system. One of them encodes a putative intracellular receptor, belonging to NBS-LRR family and. When we co-expressed the *NBS-LRR* and the *Las5315mp* in citrus leaves, it induced hypersensitive response-like cell death. In contrast, by silencing the *NBS-LRR* gene and the ubiquitin ligase-associated protein *SGT1* gene of *N. benthamiana,* respectively, using virus-induced gene silencing (VIGS), the HR cell death phenotype was eliminated in the presence of Las5315mp. Furthermore, the VIGS-*N. benthamiana* became Las bacterial host, indicating the NBS-LRR receptor is important for HLB resistance. We are currently studying the functions of this R gene in citrus via stable transformation.

Swords to ploughshares: modulation of host defense molecules to promote symbiotic bacteria

Dong Wang
Assistant Professor
University of Massachusetts

Among major plant species, legumes have the remarkable ability to form a symbiosis with nitrogen-fixing rhizobia. Rhizobia are closely related to several pathogens of plants as well as animals, and deploy mechanisms shared with pathogenic bacteria to sustain their chronic infection in host cells. A major challenge in understanding the nitrogen-fixing symbiosis is how legume hosts support extremely high doses of rhizobia while maintaining effective innate immunity against pathogens.

One unusual feature of the legume-rhizobia symbiosis is the intracellular lifestyle of the bacteria, which exist as nitrogen-fixing organelles in host cytoplasm. This association provides the physiological conditions needed for productive nitrogen fixation, such as a microoxic environment and efficient nutrient exchange. Using the model legume *Medicago truncatula*, we show here that this arrangement additionally allows physical separation between the plant cell's need to accommodate beneficial microbes and the surveillance system against extracellular pathogens. After rhizobia transition into the intracellular environment, the host cell selectively eliminates receptors for microbe-associated molecular patterns (PAMPs) from the membrane surrounding the internalized rhizobia, using a novel mechanism to cleave and inactivate PAMP receptors at this membrane. This mechanism is specifically targeted to the intracellular compartment, which leaves the PAMP-sensing complex at the cell surface intact. Therefore, internalizing rhizobia allows the plant host to mark these bacteria as "benign" without comprising its ability to detect potential threats from other microbes.

Identification of the effectors which inhibit rice immunity and the mechanism of rice resisting against *Xanthomonas oryzae* pv *oryziola*

Haifeng Liu [abc], Zhaohui Chu [ac], Xinhua Ding [ab]

[a] *State Key Laboratory of Crop Biology, Shandong Provincial Key Laboratory of Agricultural Microbiology, Shandong Agricultural University, Taian, Shandong*

[b] *College of Plant Protection, Shandong Agricultural University, Tai an, Shandong*

[C] *College of Agronomy, Shandong Agricultural University, Tai an, Shandong*

xhding@sdau.edu.cn

Abstract

Xanthomonas oryzae pv *oryziola* (Xoc), caused bacterial leaf streak in rice, secrets the type three effector AvrRxo1 to suppress immunity in plants, but the mechanism is still poorly understood. Here, we show that AvrRxo1 acts as an ATP-dependent proteinase to degrade OsPDX1 and decreases the biosynthesis of Vitamin B6. When there is resistance gene *Rxo1*, Rxo1 protein interacts with AvrRxo1 and promotes AvrRxo1 protein entering the nucleus. Nuclear translocation of AvrRxo1 will up-regulate the expression of *OsPDX1.1* and *OsPDX1.3* and results in promoting Vitamin B6 synthesis. Vitamin B6 not only is an enzymatic cofactor in living organisms, but also plays a pivotal role in plants to cope with biotic and abiotic stresses. These results suggest that turnover of the synthesis of Vitamin B6 by manipulating AvrRxo1 plays a critical role in coevolution system between Xoc and rice.

Metabolic regulation of effector secretion by *Magnaporthe oryzae*

Guangchao Sun, Richard A. Wilson

Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska

*rwilson10@unl.edu

Abstract

Fungal pathogens suppress plant immunity by deploying cytoplasmic and apoplastic effectors into and between host cells, respectively, resulting in colonization and devastating crop losses. Regulators of effector secretion are prime targets for the management of plant diseases, but they are unknown. TOR is a conserved signaling pathway in eukaryotes that controls cell growth and development in response to nutrient sensing. In the devastating rice blast fungus *Magnaporthe oryzae*, we have previously shown that inactive TOR signaling during spore germination on coverslips or rice leaf surfaces is required for limiting mitosis, inducing autophagy and developing a functional appressorium. Conversely, we have shown how activate TOR signaling is required after penetration into rice cells to promote mitosis and initiate *M. oryzae* biotrophic growth. Whether TOR regulates effector deployment during biotrophy was not known. Here, using forward and reverse genetics, pharmacological treatments and confocal microscopy, we show how constitutive *M. oryzae* TOR activation during biotrophy attenuates fungal growth between rice cells, abolishes cytoplasmic effector secretion, and causes the inappropriate release of apoplastic effectors into host cytoplasm. Activating autophagy in TOR-active mutants, after biotrophic growth was established, remediated effector delivery and promoted cell-to-cell growth. Inactivating autophagy in wild type (after biotrophy was established) recapitulated effector dysregulation and the loss of cell-to-cell movement. We conclude that dynamic TOR status changes during biotrophy regulate effector secretion in *M. oryzae* via autophagy. This work unexpectedly links fungal metabolism, effector secretion and fungal growth in - and between - rice cells and improves our understanding of the plant-fungus metabolic interface.

Gcn5 regulates pathogenicity-related protein acetylation in *Magnaporthe oryzae*

Yi Zhen Deng*, Meiling Liang, Shulin Zhang, Chaoxiang Lin, Lian-Hui Zhang

Intergrative Microbiology Research Center, South China Agricultural University, Guangzhou, 510642
dengyz@scau.edu.cn

Abstract

Magnaporthe oryzae is the ascomycete fungus that causes the rice-blast disease. Autophagy was shown to be induced specifically upon exposure to light and essential for *M. oryzae* asexual spore formation (conidiation)[1,2]. On the other hand, autophagy is also critical for programmed cell death during conidial development and appressorial maturation[3]. A histone acetyltransferase encoding gene *GCN5* was recently identified as a key regulator in phototropic induction of autophagy and conidiation, while serving a cellular function other than autophagy induction for *M.oryzae* infection[4,5]. Therefore in this study we set out to identify more Gcn5 substrate(s) by comparative acetylome analysis between the wild-type (WT) and *GCN5*-overexpression (OX) mutant. Our results showed that the pathways of stress response, cell toxicity and death were differentially regulated by Gcn5 via acetylation on histone or non-histone proteins. Furthermore, we discovered a ferroptosis-like cell death[6] that could be uncoupled from autophagy, under regulation of Gcn5 during conidial development. Our study demonstrates that Gcn5 acts as an important (central) signal transducer in response to environmental stimuli, and modulates oxidative stress and/or ferroptosis-like cell death, via epigenetic modification on histone proteins and/or through direct modification on non-histone proteins.

References:

1. Deng, Y.Z., Ramos-Pamplona, M., and Naqvi, N.I (2009). Autophagy, 5, 33.
2. Deng Y Z, Naqvi N I (2010). Autophagy, 6(4), 455.
3. Veneault-Fourrey C, Barooah M, Egan M (2006). SCIENCE, 312(5773), 580.
4. Deng YZ, Qu Z, Naqvi NI (2015). PLoS Pathog, 11(6), e1004972.
5. Shulin Zhang, Meiling Liang, Naweed Naqvi, Chaoxiang Lin, Wanqiang Qian, Lian-Hui Zhang, Yi Zhen Deng (2017). Autophagy, accepted.
6. Lakhter AJ, Hamilton J, Dagher PC, Mukkamala S, Hato T, Dong XC, Mayo LD, Harris RA, Shekhar A, Ivan M, Brustovetsky N, Naidu SR (2014). Oncotarget;5(24), 12694.

Phytophthora effectors manipulate plant small RNAs to promote infection

Yingnan Hou[1], Yi Zhai[1], Jinqiu He[2], Wenwu Ye[3], Liping Zeng[1], Duseok Choi[1], Jinbiao Ma[2], Wenbo Ma[2]

1 Department of Plant Pathology and Microbiology, University of California Riverside, USA

2 Department of Biochemistry, Fudan University, Shanghai

3 Department of Plant Pathology, Nanjing Agricultural University

Constantly challenged by potential pathogens in the environment, plants have evolved a myriad of defense mechanisms. A growing body of evidence suggests that small RNAs are integral components of plant immune system. This is strongly supported by the identification of pathogens effectors that can suppress small RNA silencing in plant hosts. Here, we show that the *Phytophthora* suppressor of RNA silencing 2 (PSR2) specifically affects the accumulation of phased small interfering RNA (or phasiRNA) and demonstrate that the phasiRNA pathway is important for the defense of *Arabidopsis thaliana* against *Phytophthora capsici*. In particular, phasiRNAs generated from the transcripts of pentatricopeptide-repeat proteins (PPRs)-encoding genes contribute to plant resistance by silencing targeted genes in *P. capsici*. These results support a model with phasiRNAs as the executors of a host-induced gene silencing during *Phytophthora* infection. Furthermore, structural analysis of PSR2 revealed a five -helices scaffold that represents a conserved structural unit present as tandem repeats in >200 effectors of five full-sequenced *Phytophthora* species. PSR2 contains seven tandem repeats and the first two units mediate its interaction with Double Stranded RNA-binding Protein 4 (DRB4) in *Arabidopsis*. Interaction with DRB4 confers phasiRNA suppression and the virulence function of PSR2.

Phosphorylation and Transcription Regulations in Barley-Powdery Mildew Interactions

Ling Zhang, Pengya Xue, Xinyun Han, Chunlei Zhang, Qian-Hua Shen
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing
qhshen@genetics.ac.cn

Although many transcription factors (TFs) have been identified to be involved in defense transcription reprogramming and defense responses, the mechanisms that regulate the activity of these TFs are largely unknown. We have shown that three WRKYs, WRKY1/2/3, and a MYB6 are engaged in transcriptional regulation of barley defense responses against powdery mildew fungus. We report here that distinct protein kinases target different types of TFs for phosphorylation and regulation of their activity or stability. We show that barley MAPK4a can specifically interact with and phosphorylate WRKY1, but not WRKY2. Moreover, MAPK4a can interact with and phosphorylate MYB6 that was shown to antagonize WRKY1. Phosphorylation of either WRKY1 or MYB6 results in changes of its DNA-binding and transcriptional activity. We further demonstrate that WRKY3 is specifically phosphorylated by SnRK1, the SNF1-related kinases that act as energy sensor. Interestingly, WRKY3 phosphorylation leads to degradation of the TF and likely derepression of barley disease resistance to *B. graminis*. Our data reveal distinct phosphorylation and regulation mechanisms for TFs that are involved in barley and *B. graminis* fungus interactions.

***Fusarium* pathogenomics: understanding fungal pathogenicity through genomics.**

Li-Jun Ma
Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst MA, USA. E-mail: lijun@biochem.umass.edu

The root-infecting fungal pathogen *Fusarium oxysporum* is responsible for vascular wilt in over 100 different plant species. These pathogens produce thick-walled resting structures that remain viable for long periods, making disease control particularly challenging. Our study takes a systems biology approach to dissect the molecular mechanisms underlying fungal pathogenesis and host defense using the *F. oxysporum*-Arabidopsis pathosystem. Comparative genomics and comparative meta-transcriptomics were employed to study both compatible (inoculation of a *F. oxysporum* strain results in diseased plants) and incompatible (*F. oxysporum* inoculation has no negative effect on plant health) interactions by inoculating the same plant host (Col-0) with different *F. oxysporum* isolates. The study focuses on the "*primary determinative phase*", including fungal penetration and colonization from the cortex to xylem. Comparative study enables the identification of genes and pathways that contribute to the *co-evolutionary arms race* between wilt pathogens and their hosts. Distinct sets of genes from two different *F. oxysporum* strains contribute to the different disease phenotypes. Interestingly, plant genes involved in pathogen-associated molecular patterns triggered immunity (PTI) were induced in both compatible and incompatible interactions, while there are more distinct expression profiles for genes involved in effector-triggered immunity (ETI) in two different interactions.

This research was financially supported by National Science Foundation CAREER award (1652641), Burroughs Wellcome Fund Investigator award (1014893), the National Institute of Food and Agriculture National Research Initiative Hatch Grants Program grant (MAS00441), competitive Grants (2008-35604-18800 and MASR-2009-04374) and a seed grant from Massachusetts Green-energy High Performance Computing Center.

Structural basis of the loss of the avirulence function of AvrPib from the rice blast fungus

Xin Zhang[1], Dan He[1,2], Ian A. Taylor[3], Wen-Sheng Zhao[1,2], Jun Yang[1,2], Junfeng Liu[1], You-Liang Peng[1,2]

[1]Ministry of Agriculture Key Laboratory of Pest Monitoring and Green Management, College of Plant Protection, and [2]State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing 100193. [3] Macromolecular Structure Laboratory, The Francis Crick Institute, Mill Hill Laboratory, The Ridgeway, Mill Hill, London, NW7 1AA, UK..

Abstract:

Fungal effectors are weapons for pathogens to fight with plants. Most of these effectors share rare sequence homology to function-known proteins, and the mechanism of the evolution and varieties between them are largely unknown.

To better understand the recognition effectors by its immune receptor, we have determined crystal structure of AvrPib, an effector from *Magnaporthe oryzae*. Despite lack on the sequence similarity, the crystal structure of AvrPib shows high structural homology to that of MAX effectors, suggesting AvrPib is also a MAX effector. In nature isolates, residues V39 and V58, which locate in the hydrophobic core of the structure, cause loss function of AvrPib by single-point mutation (V to A). Solubility of the mutants with the V39A or V58A mutation is much lower than the wild type in *E. coli* or tobacco. CD data also show that these mutants lost partial or full of secondary structure elements of the wild-type one. These data reveal that one of the mechanisms of AvrPib to avoid the recognition by the immune receptor should be unstablized the normal fold induced by the mutated residues in its hydrophobic core.

Phenotype plasticity of gain and loss of mutations in the rice blast fungus

Yong-Hwan Lee

Department of Agricultural Biotechnology and Center for Fungal Genetic Resources, Seoul National University, Seoul 08826, Korea (email: yonglee@snu.ac.kr)

Genetic mutations via both gain-of-function (GOF) and loss-of-function (LOF) approach have been applied to study gene function. In this study, we showed for the first time the large scale functional analysis of both type of mutants at same gene using *Magnaporthe oryzae*. Phenotypic analysis of 190 genes revealed that screening of both type of mutant confers new or additional phenotypes depends on dose of expression, resulting 141 genes (74.2%) necessary for pathogenicity-related phenotypes. Among the 141 genes, the screen identified known 9 genes, implicated in the pathogenicity-related processes of the rest of the genes. In addition, four GOF mutants resulted in increased disease severity, while corresponding LOF mutants caused decreased disease severity, suggesting appearance of phenotypes correlated with the quantity of their transcripts. These studies demonstrate that analysis of GOF mutant combined with LOF mutants at same gene determine and accelerate more precise function of gene.

A novel transcript factor SREBP-1 governs virulence and multiple stress responses in *Fusarium graminearum*

Zunyong Liu, Yanni Yin, and Zhonghua Ma
Institute of Biotechnology, Zhejiang University, Hangzhou 310058.
E-mail of presenting author: zhma@zju.edu.cn

Sterol regulatory element binding proteins (SREBPs) including UPC2, SrbA are a class of basic helix-loop-helix transcription factors that regulate azole resistance in yeasts and *Aspergillus* spp. The UPC2 and SrbA orthologs, however, are not associated with azole resistance in *Fusarium graminearum*, the causal agent of Fusarium head blight of small grain crops. After tested the sensitivity of more than 1000 *F. graminearum* gene deletion mutants, we identified a novel SREBP transcription factor (named SREBP-1 hereafter), which regulated sensitivity of *F. graminearum* to azole fungicides. The full length SRBP-1 cDNA was unable to restore the defects of the yeast UPC2 mutant and *vice versa*. Combining yeast one hybrid, ChIP-qPCR, and ChIP-seq assays, we identified a cis-element of SREBP-1, and found that SREBP-1 bound the promoters of more than 110 genes including 19 genes in the ergosterol biosynthesis pathway in *F. graminearum*. Consistently, ΔFgSREBP-1 produced less ergosterol and showed increased sensitivity to anime fungicides and the HMG-CoA reductase inhibitors, lovastatin and fluvastatin. In addition, ΔSREBP-1 also exhibited increased sensitivity to DNA damaging agents. Unexpectedly, ΔSREBP-1 displayed reduced resistance to phytolexin, and subsequently decreased virulence on wheat head and corn silk. Interestingly, SREBP-1 interacted with itself to form a homodimer. Additionally, we found that SREBP-1 was subject to several post-translation modifications including phosphorylation and sumoylation that are important for its biological functions. Taken together, these results indicated that the novel transcription factor SREBP-1 regulates multiple stress responses and virulence in *F. graminearum*.

Genetic diversity and development of efficient transformation system for the sugarcane smut fungus in Sothern China

Shan Lu [a,b], Xiaorui Shen [b], Zhiqiang Wang [a], Baoshan Chen [a,b]
[a] *State Key Laboratory of Conservation and Utilization of Subtropical Agro-Bioresources, Nanning*
[b] *College of Life Science and Technology, Guangxi University, Nanning*
chenyaoj@gxu.edu.cn

The genome sequences of 14 isolates of the sugarcane smut pathogen, basidiomycete fungus *Sporisorium scitamineum,* collected from sugarcanes in provinces of Southern China were determined. While the genome sequence of isolate JG36 was resolved at chromosome level to be 19.32 Mb, 22 chromosomes with 6469 putative genes, the rest of the isolates were at 100X or more coverage with 200-300 scaffolds. Genetic analysis with 12 genes involved in diploid hyphal develoment reveals that the 14 isolates fall into 3 clades with clear lineage. Despite of the availability of genome sequence information, lack of efficient gene manipulation system hindered the gene functional study. Here, we report the successful adaption of T-DNA-based CRISPR/Cas9 systems for site-precise gene knock-out and knock-in efficient in the *S. scitamineum*. Efficiency of gene disruption ranged between 12.8%-39.1% for six tester genes, and in-cis complementation efficiency greater than 74.5%. Of particular advantage of this system in both targeted gene knock-out and gene complementation can be achieved by simply modifying the vector and the desired transformants can be identified using drug resistance coupled with PCR. Thus facilitate the manipulation of fungi genes for functional analysis and could potentially be utilized for other basidiomycete fungi. Large scale gene functioning studies are underway.

Acknowledgement: This study was supported in part by the National Key Basic Research Program and Guangxi Collaborative Innovation Initiative for Sugarcane and Sugar Industries.

Breeding for durable resistance against rice blast to reduce rice yield loss and fungicide dependency

Bo Zhou
The Division of Genetics and Biotechnology, International Rice Research Institute, Philippines

Rice blast caused by the fungal pathogen Magnaporthe oryzae imposes constant threats to the stable rice production worldwide. The utilization of host R genes has been widely accepted as the most effective and economical strategy for tackling this devastating disease. Nevertheless, due to the quick adaptation assumed to be mediated by the mutation of avirulence genes in the pathogen, newly deployed R genes usually erode shortly in the field. In farmer's pratice, intensive application of fungicides is widely adopted, causing additional costs and significant damage to the environment. In this presentation, I will introduce an integrated strategy by combining several factors for breeding durable resistance to rice blast. Firstly, the non-race specific resistance gene/QTLs displaying partial but durable resistance should be incorporated for a basal shield against rice blast. Secondly, race specific resistance genes should be deployed economically by understanding their working model and chromosomal locations. Lastly, pathogen surveillance tools should be in place for the strategic selection of effective resistance genes in different regions and timely rotation of different combinations. The ongoing research activities on rice blast resistance at IRRI will be also updated in the presentation.

The regulating mechanism of epigenetic modification on aflatoxin biosynthesis in *Aspergillus flavus*

Zhenhong Zhuang, Silin Ren, Huahui Lan, Jun Yuan, Shihua Wang*

Key Laboratory of Pathogenic Fungi and Mycotoxins of Fujian Province, and School of Life Sciences,

Fujian Agriculture and Forestry University, Fuzhou, 350002

**Corresponding authors. E-mail: wshyyl@sina.com.*

A. flavus is a kind of conmon pathogenic fungus of crops. It could produce toxic and carcinogenic secondary metabolites aflatoxins, contaminating crops and agricultural products. Protein phosphorylation is an important and reversible posttranslational modification and regulates many life actions. By high-throughput LC-MS/MS, 544 phosphorylated peptides and 283 phosphorylated proteins were identified, containing 598 high confidence phosphorylated sites. One phosphorylated protein Ste11 was selected for functional study. *ste11* deletion strain (Δ*ste11*) and *ste11* complementation strain (Δ*ste11::ste11*) were constructed. It was found that the colony growth, conidia production, sclerotial formation and host infection were suppressed in Δ*ste11* mutant. Especially, aflatoxins biosynthesis was suppressed seriously after *ste11* was deleted. The point mutants, S187A and S187D for Ste11 were also constructed. The results demonstrated that Ste11 played significant roles in morphogenesis and aflatoxins biosynthesis and the phosphorylation of 187th S in Ste11 was closely related to the function of Ste11.

Our further results also demonstrated that succinylation, Sumoylation and acetylation were also involved the aflatoxins biosynthesis and pathogenicity, and that the succinylated site (370th K) in succinylated protein AflE played vital roles in the function of AflE. Based on these analyses and results, the post-translational modifications have been proved to regulate the morphogenesis and aflatoxins biosynthesis in *A. flavus*.

Keywords: *Aspergillus flavus*, aflatoxins, epigenetic modification

Autophagy is involved in assisting the accumulation of *Bamboo mosaic virus* in *Nicotiana benthamiana*

Ying-Ping Huang, Ying-Wen Huang, Yung-Jen Hsiao, Siou-Cen Li, Ching-Hsiu Tsai and Yau-Heiu Hsu

Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan 402

yhhsu@nchu.edu.tw

Autophagy plays a vital role on the cellular homeostasis to maintain good nutritional status and as part of the innate immunity system in responding the pathogen invasion. In this study, we investigated whether the autophagy is involved in the infection cycle of *Bamboo mosaic virus* (BaMV), a single-stranded positive-sense RNA virus. Initially, we have observed that BaMV infection would induce autophagy but not trigger the cell death on *Nicotiana benthamiana*. Surprisingly, the autophagy induced by BaMV could support rather than diminish the accumulation of BaMV in *N. benthamiana* leaves revealed by the loss- and gain-of function assays. Furthermore, the class III PI3K inhibitor 3-methyladenine which blocks the formation of autophagosome negatively regulates the accumulation of BaMV. These results suggest that autophagosome formation is critical in the accumulation of BaMV. The pull-down experiment with the antibody against NbATG8, the autophagosome marker gene, revealed that both plus- and minus-strand BaMV RNAs were associated with NbATG8. Besides, the results of confocal microscopy indicated that the induced autophagosome might be derived from chloroplasts and containing both the BaMV viral RNA and its RdRp. Overall of these results suggest that BaMV infection targeting to chloroplast for replication might induce autophagy to selectively engulf the portion of viral RNA-containing chloroplast, the chlorophagy. The virus-induced autophagic body derived from the virus-containing chloroplasts would either provide an alternative site for viral RNA replication or a shelter away from the silencing.

The *Barley stripe mosaic virus* γb protein promotes chloroplast-targeted replication by enhancing unwinding of RNA duplexes

Kun Zhang[+a], Xuejiao Jin[+a], Meng Yang[a], Zhihao Jiang[a], Pengfei Wang[b], Xiuling Cao[a], Ning Yue[a], Byung-Ho Kang[b], Yongliang Zhang*[a], Dawei Li[*a]

[a] *State Key Laboratory of Agro-Biotechnology a, College of Biological Sciences, China Agricultural University, Beijing 100193*
[b] *School of Life Sciences, Center for Cell and Developmental Biology, The Chinese University of Hong Kong, Hong Kong*

RNA viruses encode various RNA binding proteins that function in many steps of viral infection. These proteins function as RNA helicases, methyltransferases, RNA-dependent RNA polymerases, RNA silencing suppressors, RNA chaperones, movement proteins, and so on. Although many of the proteins bind the viral RNA genome during different stages of infection, our knowledge about the coordination of their functions is limited. In this study, we describe a novel role for the *Barley stripe mosaic virus* (BSMV) γb as an enhancer of αa RNA helicase activity, and we show that the γb protein is recruited by the αa viral replication protein to chloroplast membrane sites of BSMV replication. Electron tomography (ET) of the chloroplast during BSMV infection was performed, revealing an overall structure of BSMV replication sites characterized by the clustering of outer membrane-invaginated vesicles in inner membrane-derived packets, and the diverse morphology of cytoplasmic invaginations with membrane-invaginated vesicles at the periphery and openings of varied sizes. Molecular analyses reveal the localization of αa replicase protein and validate its crucial role in rearranging the chloroplast membranes. Mutagenesis or deletion of γb from BSMV resulted in reduced positive strand (+) RNAα accumulation, but γb mutations abolishing viral suppressor of RNA silencing (VSR) activity did not completely eliminate genomic RNA replication. In addition, *cis*- or *trans*-expression of the *Tomato bushy stunt virus* p19 VSR protein failed to complement the γb replication functions, indicating that the direct involvement of γb in BSMV RNA replication is independent of VSR functions. These data support a model whereby two BSMV-encoded RNA-binding proteins act coordinately to regulate viral genome replication and provide new insights into strategies whereby double-stranded viral RNA unwinding is regulated, as well as formation of viral replication complexes.

References:

1. Kun Zhang[+], Yongliang Zhang[+], Meng Yang, Songyu Liu, Zhenggang Li, Xianbing Wang, Chenggui Han, Jialin Yu, Dawei Li[*]. 2017. The *Barley stripe mosaic virus* γb protein promotes chloroplast-targeted replication by enhancing unwinding of RNA duplexes. ***PLoS Pathogens*** 13(4): e1006319.
2. Xuejiao Jin, Zhihao Jiang, Kun Zhang, Pengfei Wang, Xiuling Cao, Ning Yue, Xueting Wang, Xuan Zhang, Yunqin Li, Dawei Li, Byung-Ho Kang, Yongliang Zhang*, 2017 Three-dimensional analysis of chloroplast structures associated with virus infection, ***Plant Physiology*** (Accepted).

Insights into plant rhabdovirus virion morphogenesis and local movement

Kai Sun[a], Shasha Qian[a], Xin Zhou[a], Zhenghe Li[a]

[a] *State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou*

lizh@zju.edu.cn

Plant rhabdoviruses and many other plant negative-stranded RNA viruses have unique enveloped virion structures amongst plant viruses. The minimal infectious unit of rhabdoviruses are ribonucleoprotein complexes (RNP) consisting of viral genome encapsidated by the nucleoprotein (N), phosphoprotein (P) and RNA polymerase (L). During morphogenesis, the RNP assemble and bud into intracellular membranes to acquire host-derived lipids and the viral transmembrane glycoprotein (G protein). However, mechanisms underlying the envelopment processes, as well as the viral movement entities are poorly understood for plant rhabdoviruses. Sonchus yellow net virus (SYNV) is a plant nucleorhabdovirus that replicates in the nuclei of infected cells and buds from the inner nuclear membrane into perinuclear spaces. To investigate SYNV budding and movement, we used a newly established reverse genetic system to generate recombinant SYNV G or matrix protein (M) mutants. Both mutants were defective in virus budding and compromised in local and systemic movement. Additional biochemical and genetic evidence suggests that SYNV budding is directed by concerted actions of the M protein and the G protein C-terminal tail. The fact that M and G double mutation did not abolish infectivity suggest that SYNV may initiate systemic infection independent of virion formation. We further provided evidence that the P protein contains a nuclear exporting signal that may mediate viral RNP egress from infected nuclear, which were then assisted by sc4 movement protein during local and systemic trafficking. These data suggest that the SYNV RNPs are the minimal movement entities, and the G and M proteins-driven virion envelopment process facilitates movement.

Acknowledgement: This work is supported by the China National Science Foundation (No. 31671996 and 31470255).

Role of Autophagy in plant-virus interactions

Yakupjan Haxim, Asigul Ismayil, Qi Jia, Yan Wang, Xiyin Zheng, Yule Liu*

School of Life Sciences, Tsinghua University, Beijing 100084

E-mail: yuleliu@mail.tsinghua.edu.cn

Autophagy is an evolutionarily conserved process that recycles damaged or unwanted cellular components. We first found that autophagy is linked to plant immunity. Subsequently, cytoplastic glyceraldehyde-3-phosphate dehydrogenases (GAPC) were found to negatively autophagy and plant immunity. Recently, we found that the plant autophagic machinery targets the virulence factor βC1 of Cotton leaf curl Multan virus (CLCuMuV) for degradation through its interaction with the key autophagy protein ATG8. A V32A mutation in βC1 abolished its interaction with NbATG8f, and virus carrying βC1V32A showed increased symptoms and viral DNA accumulation in plants. Furthermore, silencing of autophagy-related genes ATG5 and ATG7 reduced plant resistance to the DNA viruses CLCuMuV, Tomato yellow leaf curl virus, and Tomato yellow leaf curl China virus, whereas activating autophagy by silencing GAPC genes enhanced plant resistance to viral infection. Thus, autophagy represents a novel anti-pathogenic mechanism that plays an important role in antiviral immunity in plants.

References:

[1] Y. Haxim, A. Ismayil, Q. Jia, Y. Wang, X. Zheng, T. Chen, L. Qian, N. Liu, Y. Wang, S. Han, J. Cheng, Y. Qi, Y. Hong, Y. Liu (2017). Autophagy functions as an antiviral mechanism against geminiviruses in plants. ***Elife***, 6, e23897

[2] G. Xu, S. Wang, S. Han, K. Xie, Y. Wang, J. Li, Y. Liu (2017). Plant Bax Inhibitor-1 interacts with ATG6 to regulate autophagy and programmed cell death. ***Autophagy***, 13(7):1161-1175

[3] S. Han, Y. Wang, X. Zheng, Q. Jia, J. Zhao, F. Bai, Y. Hong, Y. Liu (2015). Cytoplastic Glyceraldehyde-3-Phosphate Dehydrogenases Interact with ATG3 to Negatively Regulate Autophagy and Immunity in *Nicotiana benthamiana*. ***Plant Cell***, 27(4):1316-31

Sw-5b NLR Confers Broad-spectrum Resistance to Tospoviruses through Recognition of a Conserved 21-amino-acid Viral Effector Epitope

Min Zhu [1,†], Lei Jiang [1,†], Baohui Bai [1], Wenyang Zhao [1], Xiaojiao Chen [1], Jia Li [1], Yong Liu [2], Zhengqiang Chen [1], Boting Wang [1], Chunli Wang [1], Qian Wu [1], Qianhua Shen [3], Savithramma P. Dinesh-Kumar [4] and Xiaorong Tao [1,*]

[1] Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095;[2] Institute of Plant Protection, Hunan Academy of Agricultural Sciences, Changsha 410125; [3] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing; [4] Department of Plant Biology and The Genome Center, College of Biological Sciences, University of California, Davis, California, United States of America.
[†]These authors contributed equally to this work.
Email: taoxiaorong@njau.edu.cn

Plant uses both cell surface-resident pattern-recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat (NLR) receptors to detect various pathogens. Plant PRRs typically recognize conserved pathogen-associated molecular patterns (PAMPs) to provide a broad-spectrum pathogen resistance. In contrast, plant NLRs generally detect pathogen strain-specific effectors and confer race-specific resistance. Here, we demonstrate that tomato Sw-5b NLR confers broad-spectrum resistance against American type tospoviruses by recognizing a conserved 21-amino-acid peptide region within viral movement protein NSm (NSm^{21}). Sw-5b NB-ARC-LRR domains directly associate with NSm^{21} *in vitro* and *in planta*. Domain swap, site-directed mutagenesis and structure modeling analyses identified four polymorphic sites in the Sw-5b LRR domain that are critical for the recognition of NSm^{21}. Furthermore, recognition of NSm^{21} by Sw-5b likely disturbs the residues adjacent to R927 in the LRR domain to weaken the intramolecular interaction between LRR and NB-ARC domains, thus translating recognition of NSm^{21} into activation of Sw-5b. Natural variation analysis of Sw-5b homologs from wild tomato species of South America revealed that the four polymorphic sites in the Sw-5b LRR domain evolved in a step-wise manner and are all necessary to confer resistance to tospovirus infection. Results described here provide a new example of a plant NLR mediating broad-spectrum resistance through recognition of a small conserved PAMP-like region within the pathogen effector.

The IRE1/bZIP60 pathway and Bax inhibitor 1 suppress systemic potyvirus and potexvirus accumulation in plants

Omar Arias Gaguancela [a], Lizbeth Pena Zunigaa, Ida Elisabeth Johansenb, Aiming Wangc, Yasuyuki Yamajid, and Jeanmarie Verchot[e]

[a] *Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater OK 74078, USA*

[b] *Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark*

[c] *Southern Crop Protection and Food Research Centre, AAFC, 1391 Sandford Street, London, Ontario N5V 4T3, Canada*

[d] *Laboratory of Plant Pathology, Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*

[e] *Texas A&M Agrilife Research and Extension Center at Dallas, 17360 Coit Road, Dallas, Texas 75252 USA*

Email presenting author: jm.verchot@ag.tamu.edu

RNA viruses infecting animals and plants depend upon the biosynthetic and transport properties of the ER-Golgi network for success in replication and completion of their infection cycles. Viruses will stimulate lipid synthesis to expand the ER surface area, and remodel portions of the ER into protected microenvironments for replication. ER stress sensors spanning the lipid bilayer recognize changes in the ER and respond to environmental stressors that cause the ER to malfunction. The inositol requiring enzyme (IRE1) is an endoplasmic reticulum (ER) stress sensor, that when activated, splices the bZIP60 mRNA producing a truncated transcription factor that upregulates genes involved in the unfolded protein response (UPR) [1]. Bax inhibitor 1 (BI-1) is another ER stress sensor that regulates cell death in response to environmental assaults [2]. The potyvirus 6K2 and potexvirus TGB3 proteins are known to reside in the ER, serving respectively as anchors for the viral replicase and movement protein complex. This study used GFP tagged *Turnip mosaic virus* (TuMV), *Plantago asiatica mosaic virus* (PlAMV), *Potato virus Y* (PVY) and *Potato virus X* (PVX) to determine that the IRE1/bZIP60 pathway and BI-1 machinery are induced early in virus infection in *Arabidopsis thaliana*, *Nicotiana benthamiana*, and *Solanum tuberosum*. Homozygous *ire1a-2*, *ire1b-4*, and *ire1a-2*/*ire1b-4* mutant Arabidopsis plants were inoculated with these potyviruses and potexviruses and this led to greater virus systemic accumulation. Silencing *BI-1* expression also resulted in systemic necrosis. These data suggest that ER stress

activated pathways led by IRE1 and BI-1 respond to invading potyvirus and potexviruses to restricts virus infection and enable physiological changes enabling plants to tolerate virus assault [3].

Acknowledgement: Special thank you to Professor Shigetou Namba for valuable conversations. This research was funded by NIFA Plant-Associated Microbes and Plant-Microbe Interactions Award Number: 2014-67014-21715.

References:

1. Chen Y, Brandizzi F. IRE1: ER stress sensor and cell fate executor. Trends Cell Biol. 2013;23(11):547-55. doi: 10.1016/j.tcb.2013.06.005. PubMed PMID: 23880584; PubMed Central PMCID: PMCPMC3818365.
2. Ishikawa T, Uchimiya H, Kawai-Yamada M. The role of plant Bax inhibitor-1 in suppressing H2O2-induced cell death. Methods Enzymol. 2013;527:239-56. doi: 10.1016/B978-0-12-405882-8.00013-1. PubMed PMID: 23830635.
3. Gaguancela OA, Zuniga LP, Arias AV, Halterman D, Flores FJ, Johansen IE, et al. The IRE1/bZIP60 Pathway and Bax Inhibitor 1 Suppress Systemic Accumulation of Potyviruses and Potexviruses in Arabidopsis and Nicotiana benthamiana Plants. Mol Plant Microbe Interact. 2016;29(10):750-66. doi: 10.1094/MPMI-07-16-0147-R. PubMed PMID: 27578623.

The potyviral RNA-dependent RNA polymerase is a multifunctional protein and plays contrasting diverse roles in viral infection

Fangfang Li, Yinzi Li, Cheng Xiaofei, Ruyi Xiong, Changwei Zhang and Aiming Wang.

London Research and Development Centre, Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON N5V 4T3, Canada.

Potyviruses represent the largest group of known plant RNA viruses. Like other positive-sense single-stranded RNA viruses, potyviruses have a small and compact genome that encodes a large polyproteins with a molecular mass of about 350 kDa, and a relatively small polyprotein resulting from a transcriptional slippage-led frameshift in the P3 coding region. The two polyproteins are processed into a number of intermediate precursors and 11 mature proteins. Since nuclear inclusion protein b (NIb), the only potyviral RNA-dependent RNA polymerase, is absolutely required for viral genome replication, we studied NIb in the past ten years using *Turnip mosaic virus* (TuMV) as a model virus. We found that NIb is a multifunctional protein and plays contrasting roles in viral infection. In addition to its essential role as an RNA polymerase, NIb can interact with a number of host proteins and recruit them to promote viral infection. NIb can also be sumoylated in the nucleus. Sumoylaiton of NIb inhibits NPR1 sumoylation, leading to downregulation of the NPR1-mediated resistant pathway and suppression of the host immune response. Interestingly, the NIb protein of several other potyviruses, such as *Potato virus Y* (PVY), *Pepper mottle virus* (PepMoV), and *Pepper severe mosaic virus* (PepSMV), can function as an avirulence (Avr) protein that triggers the hypersensitive response (HR) in plants carrying corresponding *R* genes. Taken together, potyviral NIb catalyzes biosynthesis of the replicative intermediates as a generator of the RNA silencing inducer, recruit host factors to assist in viral replication as a hijacker, inhibit NPR1-mediated resistance as a virus-encoded innate immunity suppressor (VIIS), and interacts with R proteins to trigger immunity response as an effector triggered immunity (ETI) elicitor. Thus, a single viral protein can play contrasting roles in viral infection, reflecting the complex and specific virus-plant interactions as a result of the co-evolutionary arms race.

Potato virus A translation and replication are regulated by viral coat protein, two host chaperons and a protein kinase

Kristiina Mäkinen, Andres Lõhmus, Jane Besong-Ndika, Anders Hafren and Konstantin I. Ivanov
Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland
Kristiina.makinen@helsinki.fi

In addition to genome encapsidation, many non-structural functions have been attributed to viral coat proteins (CPs) [1]. Potyviruses form a large family of economically important positive-stranded plant RNA viruses. In the current study we sought to understand the molecular mechanisms underlying the CP-mediated regulation of translation and replication of potato virus A (PVA; a potyvirus) and the interplay between CP, host proteins and viral RNA in these processes. Our results suggest that PVA CP inhibits viral RNA translation through CP translated from viral RNA *in cis* [2]. The action of two host chaperons, heat shock protein 70 and CP-interacting protein (CPIP, a HSP40 family member), was found to relieve the translation inhibition likely by detaching CP from viral RNA and targeting it to degradation via ubiquitin-proteasome pathway [3]. High CP concentration was found to overpower the function of CPIP and stop PVA gene expression. The RNA-binding property of PVA CP and its capacity to inhibit translation were both found to be regulated by host casein kinase 2 [4,5]. We found that both the functional chaperon system and CP phosphorylation are essential for PVA replication. We propose that the transient translational block formed by the non-phosphorylated CP allows sufficient time for replication complex formation and contributes to the switch of PVA RNA as a template for translation to one for replication. We further propose that later in the infection, when CP inhibits viral RNA translation through co-translational interactions between excess CP accumulated *in trans* and CP translated from viral RNA *in cis,* viral RNA could be sequestered from translation and specifically selected for encapsidation. PVA protein HCpro was found to stabilize CP possiblyby helping the progress of encapsidation.

References:

[1] K. Ivanov and K. Mäkinen (2012). Current Opinion in Virology,2, 712.
[2] J. Besong-Ndika, K.I. Ivanov, A.Hafrèn, T. Michon, and K. Mäkinen (2015). J. Virol. 89, 4237.
[3] A. Hafren, D. Hoffius, G.Rönnholm, U. Sonnewald, and K. Mäkinen (2010).Plant Cell 22, 523.
[4] K. Ivanov, P. Puustinen, R. Gabrenaite, H.Vihinen, L. Rönnstrand, L. Valmu, N. Kalkkinen, and K. Mäkinen, (2003). Plant Cell 15, 2124.
[5] A. Lõhmus, A. Hafren, and K. Mäkinen, (2017). J. Virol., 91, e01316-16.

Geminivirus as plant-dependent mutualist and biological weapon of the sweetpotato whitefly *Bemisia tabaci* MEAM1/B

Pingzhi Zhao[1], Xiangmei Yao[1], Yanwei Sun[1], Jian Ye[1]
[1]*State Key laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101.*
**Email of presenting author, Email: jianye@im.ac.cn*. Tel: 8610-64807363

Over the past decades of years, changes in the predominance of insect-transmitted viruses in agricultural ecosystems have occurred due to virus invasion. One of the most notable trends has been the emergence of an insect vector-sweet potato *Bemisia tabaci* as driving forces in the emergence of plant viruses in agrosystems worldwide. Vector-borne pathogens such as geminivirus promote the performance of whitefly in ways that influence the frequency and nature of interactions between hosts and vectors as defined as Indirect Mutualism. Previous work has reported a phytohormone Jasmonic acid (JA)-mediated terpene biosynthesis is suppressed in begomovirus-infected plants, leading to reduced resistance to whiteflies which transmit these viruses. We identified the proteins encoded by the monopartite or bipartite begomoviruses as the key genetic factor that suppresses JA-mediated terpene biosynthesis. Recently, we found that begomoviruses have evolved sophisticated strategies to counter multiple-layer resistances against whitefly. In this talk we will focus on a spillover effect conferred by the infection of begomovirus *Tomato yellow leaf curl* (*China*) *virus*, in which with enhanced performance of its vectors *Bemisia tabaci* MEAM1/B but delayed the development of non-vector insects, eg. Cotton ballworm (*Helicoverpa armigera)* and two-spotted spider mite (*Tetranychus urticae*). The results show that virus impacts biological invasion by inhibiting vector's competitor. Our results gives another explanation for the global successful spread of a supervector insect and its carried virus.

Microbiota in plant-insect vector-virus interactions

Liying Chen [a], Qiong Li [a] and Lili Zhang [a]

[a] *Institute of Microbiology, Chinese Academy of Sciences, No 3 Yard 1 West Beichen, Beijing*

Plant leaves are the major sites for insect feeding and virus inoculation; however, the extremely high sequence identities among bacterial 16S ribosomal DNA, plant mitochondrial 18S and chloroplast 16S rDNAs have strongly hampered the analysis of bacterial communities within leaves. In this work, we established an efficient methodology for specific amplification of prokaryotic genes without interference of host organelles. Based on this sequencing platform, we clarified the bacterial communities harbored by leaves of rice seedlings. Moreover, this methodology makes it possible to identify symbiotic bacteria from rice seed in which the host organelles largely outnumber bacterial cells. Small black planthoppers (SBPHs) living in each rice-grown environment were analyzed bacterial communities, which revealed that the SBPHs harbored both intracellular and extracellular bacteria, most of the extracellular bacteria also hosted by the corresponding rice leaves, indicating obtained through feeding. However, *Delftia*, identified as the dominant leaf-borne bacterium, was not acquired by the insects. When rice-field SBPHs were changed to greenhouse plants that harbored fewer bacterial species, most of the acquired extracellular bacteria were unable to retain, suggesting loose relationship. Two kinds of bacteria, including *Wolbachia* and *Acinetobacter*, were confirmed tight relationship with SBPH, while *Acinetobacter* also existed within the rice leaf. Taken together, these results indicated that the SBPH bacterial communities are closely related to the feeding environments within rice leaf; however, the bacterial symbiosis is host dependent. Future work would investigate the function of the symbiotic bacteria, including *Wolbachia*, *Acinetobacter* and *Delftia*, on the transmission of vector-borne viruses.

The helper virus-independent systemic movement of a viral satellite RNA in plant

Na-Sheng Lin
Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Satellite RNAs (satRNAs), parasites of viruses, depend on their helper viruses (HVs) for replication, encapsidation, and efficient spread. However, it remains largely unknown how satRNAs interact with viruses and the cellular machinery to undergo trafficking. We used Bamboo mosaic potexvirus satRNA (satBaMV) as a working material, because satBaMV-encoded P20 protein is required for satBaMV systemic trafficking in *Nicotiana benthamiana*. By grafting, when satBaMV expressed in transgenic plants in the absence of HV, the transgene derived satBaMV, uncoupled from HV replication, was able to move autonomously across a graft union. Co-immunoprecipitation analysis revealed that a nucleolar protein fibrillarin was associated with the P20 protein complex. Silencing fibrillarin suppressed satBaMV phloem-based movement following grafting or coinoculation with HV. Confocal microscopy revealed that the P20 protein co-localized with fibrillarin in the nucleoli and formed punctate structures associated with plasmodesmata. The mobile satBaMV RNA appears to exist as ribonucleoprotein (RNP) complex composed of P20 and fibrillarin, suggesting the autonomous movement of satBaMV via the fibrillarin-satBaMV-P20 RNP complex in phloem-mediated systemic trafficking.

Engineering a plant intracellular immune receptor for extended pathogen effector recognition

Mark J Banfield [a], Marina Franceschetti [a], Juan-Carlos De la Concepcion [a]
[a] *Dept. Of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, UK*
mark.banfield@jic.ac.uk

Plant NLR immune receptors (nucleotide-binding and leucine-rich repeat receptors) survey the intracellular environment for signatures of non-self, typically the presence and/or activity of translocated pathogen effector proteins. Plant NLRs typically contain three domains, N-terminal CC or TIR, NB-ARC, and C-terminal LRR, but the prevalence of non-canonical domains in these receptors is being realised. Many of these integrated domains (NLR-IDs) may have their evolutionary origin as virulence-associated effector targets that were recombined into NLRs to act as traps or baits. Little is known about how these NLR-IDs recognise effectors and enable activation of immune signalling. The biochemical and structural basis of recognition of the rice blast pathogen (*Magnaporthe oryzae*) effector protein AVR-Pik with the rice NLR Pikp, *via* the direct binding of the effector to a heavy metal associated (HMA) domain contained with the NLR, has been described [1]. We have now extended these studies to derive the molecular basis of differential recognition of variant AVR-Pik alleles from the pathogen with Pik NLRs from rice. We reveal a correlation between effector binding affinity to the Pik-HMA *in vitro*, and *in planta* immunity-related readouts. Protein crystal structures of various AVR-Pik/Pik-HMA complexes, including examples that promote immunity-related readouts and those that do not, reveal protein/protein interfaces that likely underpin disease resistance mediated by Pik NLRs. Recently, we have used the information from this unpublished work to engineer Pik-HMAs with bespoke recognition capabilities.

References:

References should be indicated as consecutive numbers placed in square brackets, e.g. [1] and [2,3], etc., and referenced as in the example below.

[1] A. Maqbool, H. Saitoh, *et al*. (2015). eLife, e08709.

Proteomic insights into lysine-acetylation mediated innate immunity in rice (*Oryza sativa*)

Yehui Xiong[a], Kai Zhang[a], Guo-Liang Wang[a,b], Wende Liu[a*]

[a] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193*

[b] *Department of Plant Pathology, Ohio State University, Columbus, OH, 43210, USA*

Email: wendeliu@126.com

Lysine acetylation is a dynamic and reversible post-translational modification that plays an important role in the gene transcription regulation. Previously, we combined a highly sensitive immune-affinity purification method (used pan anti-acetyl-lysine antibody conjugated agarose for immunoaffinity acetylated peptide enrichment) with high-resolution LC–MS/MS, and generated a high quality proteome-scale data for lysine-acetylation (Kac) sites and Kac proteins in the model monocot plant rice (*Oryza sativa*) [1]. A total of 1337 Kac sites in 716 Kac proteins with diverse biological functions and subcellular localizations were identified in rice seedlings. In addition, protein interaction network analysis revealed that a variety of signaling pathways are modulated by protein acetylation. KEGG pathway category enrichment analysis indicated that glyoxylate and dicarboxylate metabolism, carbon metabolism, and photosynthesis pathways are significantly enriched. To further understanding of how Kac functions in biotic stress responses in rice, we performed the label-free quantitative proteomics analysis of acetylome of rice in response to fungal and bacterial pathogen-associated molecular pattern (PAMPs) chitin and flg22 treatment. Further in-depth functionally studies of chitin and flg22 induced rice Kac proteins will provide insight into the function of acetylation in regulation of plant immunity.

References:

[1] Xiong Y., Peng X., Cheng Z., Liu W., and Wang GL. (2016) A comprehensive catalog of the lysine-acetylation targets in rice (*Oryza sativa*) based on proteomic analyses. J Proteomics, 138:20-9

Specificity and diversity of plant pattern recognition receptors

Markus Albert, Philippe Chatelain, Ursula Fürst, Bettina Kaiser, Lei Wang, Yi Zeng, Judith Fliegmann and Georg Felix

Zentrum für Molekulare Biologie der Pflanzen ZMBP, Universität Tübingen, Germany;

Plant genomes encode multi-membered families of structurally related receptor kinases (RKs) that regulate developmental processes in response to endogenous ligands or act as immunoreceptors detecting a variety of microbe- or pathogen associated molecular patterns (M-/PAMPs). In our work we study how specificities of immunoreceptors for particular MAMPs arise and evolve. As model receptors we use the two LRR (leucine rich repeat)-receptor kinases FLS2 and EFR of Arabidopsis that detect the two bacterial proteins flagellin and EF-Tu, respectively. Functional perception of the flg22 epitope of flagellin is found in a broad range of plant species belonging to various families of seed plants. Several phytopathogenic bacteria, like *Agrobacterium tumefaciens* for example, have evolved modified flg22-epitopes to evade recognition by ordinary FLS2 receptors. In a screen for plants that, in turn, have evolved a detection system for $flg22^{Atum}$, we found that some Vitis species carry a modified FLS2, termed $FLS2^{XL}$ for extended ligand recognition, that detects the modified flg22 epitope with the same efficiency as authentic flg22. Heterologous expression of $FLS2^{XL}$ in Arabidopsis or tobacco leads to clearly increased resistance against *A. tumefaciens* (Fürst et al., unpublished).

In contrast to FLS2, occurrence of a functional EFR seems restricted to species belonging to the order of *Brassicales*. However, structurally closely related receptor kinases are present in most if not all angiosperms but, so far, only two other members of this receptor family have been matched with their cognate ligands, RaxX in the case of XA21 from rice[1] and csp22 from cold shock protein in the case of CORE from tomato[2], respectively. Thus, EFR, XA21, and CORE all act as pattern recognition receptors (PRRs) but they have specificities for structurally unrelated bacterial PAMPs. We observed that even the RLK with highest homology to EFR, a RLK from Arabidopsis that we termed Eli for EFR-like receptor, was completely blind to the elf18 peptide. In an approach to study how specificity of EFR might

have evolved, we replaced specific LRR-domains of EFR with the corresponding domains from Eli and assayed these chimeric receptors for ligand binding and functionality in activation of defense responses. The results show that the majority of the 23 LRRs in EFR are relevant for detecting the elf18 ligand, indicating that several changes affecting the surface of the protein are required to change Eli into a functional EFR. Most interestingly, while lacking responsiveness to authentic elf18 some of the hybrid receptors exhibited high specificity for elf-derived variants inactive on EFR, thus forming novel, synthetic, ligand-receptor pairs (Albert et al., unpublished).

1 Pruitt, R. N. *et al*. The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. *Sci Adv* **1**, e1500245, doi:10.1126/sciadv.1500245 (2015).

2 Wang, L. *et al*. The pattern-recognition receptor CORE of Solanaceae detects bacterial cold-shock protein. *Nat Plants* **2**, 16185, doi:10.1038/nplants.2016.185 (2016).

Protein poly(ADP-ribosyl)ation in *Arabidopsis* innate immunity

Baomin Feng[1], Libo Shan[2] and Ping He[1]

[1]*Department of Biochemistry and Biophysics, and Institute for Plant Genomics & Biotechnology, Texas A&M University, College Station, TX 77843*

[2]*Department of Plant Pathology and Microbiology, and Institute for Plant Genomics & Biotechnology, Texas A&M University, College Station, TX 77843*

Protein poly(ADP-riobsyl)ation, or PARylation, is the covalent attachment of ADP-ribose polymer from NAD^+ to a target protein and has been implicated in plant innate immunity. From a genetic screening, we identified *Arabidopsis* poly(ADP-ribose) glycohydrolase 1 (*parg1*) mutant which showed enhanced immune gene expression. In *Arabidopsis*, PARG1 removes the protein PARylation catalyzed by poly(ADP-ribose) polymerases (PARPs). Consistently, the *parp* mutants show globally compromised immune gene induction and susceptibility to bacterial pathogens. To elucidate the mechanism of PARylation in plant immunity, we used *Arabidopsis* protein microarray coupled with *in vitro* PARylation assay to globally identify the PARP2 targets. 56% of identified targets are predicted to localize in nuclei, consistent with the nuclei localization of PARP2. One candidate target, DAWDLE (DDL, a FHA domain protein), positively regulates the defense against bacterial pathogens. PARP2 interacts with and PARylates DDL, and flg22, the N-terminal 22aa peptide of flagellin as a MAMP, could enhance the interaction and DDL PARylation. Mass Spectrometry and mutagenesis analyses revealed multiple PARylation sites on DDL. Genetic complementation assay suggests that DDL PARylation is critical for its roles in immunity, but not for its function in development or microRNA biogenesis. Our study identified the first set of plant PARylated proteins, and established that PARylation of DDL is a novel regulatory mechanism of innate immunity.

Currently, we are characterizing the PARG1 interactors identified from yeast two hybrid screening. Preliminary results suggest that one CCCH Zinc finger protein, SZF1, interacts with PARG1 and plays a positive role in plant defense. SZF1 might execute its immune function through targeting specific 3'UTRs and suppress translation. We are investigating potential PARylation of SZF1 and its effects on SZF1's function. This work implies that PARylation might regulate *Arabidopsis* immunity at multiple levels.

Role of the ubiquitin-conjugating enzymes in plant innate immunity

Bangjun Zhou[a, b], Ravi V. Mural[b], Xuanyang Chen[b, 1], Matt E. Oates[c], Richard A. Connor[b], Gregory B. Martin[d], Julian Gough[c] and Lirong Zeng[a, b]

a) Center for Plant Science Innovation and Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583. b) Biology Department, University of Arkansas, Little Rock, AR 72204. c) Department of Computer Science, University of Bristol, Bristol, BS8 1UB, UK. d) The Boyce Thompson Institute for Plant Research and Department of Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853. 1) Current address: Department of Agronomy, Fujian Agriculture and Forestry University, Fuzhou, 35002

Ubiquitination has emerged in recent years as a key regulatory mechanism underlying plant innate immunity against various pathogens. Of the three classes of enzymes involved in ubiquitination, ubiquitin-conjugating enzymes (E2) have been often incorrectly considered to play merely an auxiliary role in the ubiquitination process and few E2 enzymes have been investigated in plants. Our recent study revealed the tomato UBC13 type E2s, Fni3 and SlUBC13-2 and their cofactor, Suv positively regulate plant immunity. To further characterize the role of E2 in plant innate immunity, we identified and cloned forty tomato genes encoding E2 proteins. Thioester assays indicated the majority of the genes encode enzymatically active E2. Phylogenetic analysis classified the forty tomato E2 into thirteen groups, of which only members of group III were found to act with AvrPtoB, a *Pseudomonas syringae* pv. *tomato* (*Pst*) effector that uses its ubiquitin ligase (E3) activity to suppress host immunity. Knocking-down the expression of group III E2 genes in *Nicotiana benthamiana* diminished AvrPtoB-promoted degradation of the Fen kinase and AvrPtoB suppression of host immunity-associated PCD. Importantly, silencing group III E2 genes also resulted in reduced pattern-triggered immunity (PTI). By contrast, PCD induced by several effector-triggered immunity (ETI) elicitors was not affected on group-III-silenced plants. Functional characterization suggested redundancy among group III members for their role in the suppression of plant immunity by AvrPtoB and in PTI. These results suggest AvrPtoB has evolved a strategy for suppressing host immunity that is difficult for the plant to thwart. We also uncovered the involvement of non-group III E2 members in plant immunity through functioning at the endoplasmic reticulum (ER) site. Our work builds a foundation and provides critical inroads into understanding the regulation of plant immunity by an often-overlooked group enzymes in ubiquitination.

Transcriptional and posttranscriptional regulation of pattern-triggered immunity in plants

Ping He
Department of Biochemistry & Biophysics
Institute for Plant Genomics & Biotechnology
Texas A&M University
College Station, TX 77843

Lack of specialized immune cells, plants have evolved a multilayered innate immune system to fend off pathogen threats. To understand how plants establish effective immunity yet without running amok, our lab has designed various sensitive and high throughput genetic screens in the reference plant *Arabidopsis*. A screen to understand defense activation with *Arabidopsis* expressing a luciferase reporter gene under the control of an immune responsive gene identified a series of *Arabidopsis* Genes Governing Immune gene Expression (*AGGIE*). *AGGIE1* encodes RNA polymerase II C-terminal domain (CTD) phosphatase-like 3 (CPL3) that regulates immune gene expression by modulating the phosphorylation dynamics of eukaryotic RNA polymerase II transcription machinery in plant pattern-triggered immunity. *AGGIE2* encodes poly(ADP-ribose) glycohydrolase 1 (PARG1) that regulates immune gene expression by modulating protein poly(ADP-ribosyl)ation post-translational modification. Another *AGGIE* gene encodes a protein localizing to RNA Processing bodies (P-bodies), the non-membranous cytoplasmic ribonucleoprotein foci related to 5'-to-3' mRNA decay, a critical posttranscriptional control of eukaryotic gene expression. P-bodies are dynamically modulated during plant immune responses and regulate plant pattern-triggered immunity. We further reveal that P-body-mediated mRNA decay ensures a rapid posttranscriptional down-regulation of certain immune-related genes that may otherwise negatively impact plant immunity.

Dynamics, mechanisms, and evolution of a highly resilient plant immune signaling network

Noriyuki Hatsugai, Qi Zheng, Xiaotong Liu, and Fumiaki Katagiri
[a] *Department of Plant and Microbial Biology, Microbial and Plant Genomics Institute, University of Minnesota, St Paul, MN 55108, USA.*
katagiri@umn.edu

Microbial pathogens can evolve much faster than plants and compromise the plant immune signaling network. Thus, the immune signaling network needs to be highly resilient against perturbations to its internal components, so that its underlying mechanisms are effectively concealed from pathogen evolution. To overcome the identifiability problem associated with a highly resilient network, we reduced the network to a network of four signaling sectors, the jasmonate (JA), ethylene (ET), PAD4, and salicylate (SA) sectors, in the model plant Arabidopsis and simultaneously impaired the four sectors by quadruple mutations (*quad*). Pattern-triggered immunity (PTI) triggered by the bacterial molecular pattern flg22 and effector-triggered immunity (ETI) triggered by the bacterial effector AvrRpt2 were largely abolished in *quad* [1]. Then the network functions and the signal flows among the four sectors were analyzed in comprehensive combinatorial states of the sectors. This analysis enabled a conceptual reconstitution of the network at the sector scale, which allowed simpler interpretations of mechanistic relationships among the sectors underlying network resilience [1,2]. Using network reconstitution, we also demonstrated that transcriptome response during flg22-PTI is highly resilient against loss of some of the sectors [3]. Furthermore, studying the network with highly impaired resilience in *quad*, we discovered another signaling sector that mediates strong inhibition of ETI signaling by PTI signaling (ETI-Mediating PTI-Inhibited Sector, EMPIS). We speculate that the role of this inhibition is to limit ETI when PTI is not compromised: such a mechanism would limit a negative impact of unnecessary immunity on plant fitness. I will also discuss how such a resilient network may have evolved.

Acknowledgement: This research was supported by grants from National Science Foundation to FK, IOS-1121425 and MCB-1518058.

References:

[1] K. Tsuda et al. (2009) PLOS Genet 5, e1000772
[1] Y. Kim et al. (2014) Cell Host Microbe 15, 84
[1] R. A. Hillmer et al. (2017) PLOS Genet 13, e1006639

Impact of cellular status on the expression of a plant immune receptor gene in Arabidopsis

Jian Hua
Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14953, USA

Expression and activity of intracellular immune receptors are tightly regulated in plants for effective defense responses as well as balance between growth and immunity. The disruption of an evolutionarily conserved calcium-binding protein BON1 results in upregulation of an immune receptor gene *SNC1* and consequently a constitutive defense response in Arabidopsis. We are using this *bon1* autoimmune mutant to understand the cellular signal and signaling that impact the expression of the immune receptor gene *SNC1*. We provide evidence that BON1 functions together with autoinhibited calcium ATPases 10 and 8 (ACA10 and ACA8) to regulate calcium signals in Arabidopsis. The steady level of calcium concentration is increased in both *aca10* and *bon1* mutants, and cytosolic calcium oscillation imposed by external calcium treatment was altered in *aca10* and *bon1* mutants in guard cells. In addition, calcium and pathogen induced stomatal closure was compromised in the *aca10* and *bon1* mutants. Furthermore, we identified a mutant form of calcium dependent kinase as a suppressor of the *bon1* mutant. Taken together, these data indicate that calcium signal and calcium signaling are critical for the expression of immune receptor gene *SNC1* and plant immunity.

Towards understanding TIR-domain interactions during NLR immunity receptor signalling

Bostjan Kobe[a], Thomas Ve[a,b], Shane Horsefield[a], Hayden Burdett[a], Simon J. Williams[a,c], Michael J. Landsberg[a], Edward Egelman[d], Parimala R. Vajjhala[a], Kathryn J. Stacey[a], Peter N. Dodds[e]

[a]*University of Queensland, Brisbane, Australia,* [b]*Institute for Glycomics, Griffith University, Southport, Australia,* [c]*Australian National University, Canberra, Australia.* [d]*University of Virginia, Charlottesville, USA,* [e]*CSIRO Agriculture, Canberra, Australia*

b.kobe@uq.edu.au

NLRs (nucleotide binding, leucine-rich repeat receptors) are used by the plant immune system to detect effector proteins secreted into the plant cell by potential pathogens, as part of effector-triggered immunity. To signal downstream, NLRs contain either a TIR (Toll/interleukin-1 receptor) domain or a CC (coiled-coil) domain at their N-termini. TIR domains are found in animals, plants and bacteria, e.g. in TLRs (Toll-like receptors) and TLR adaptors in animals. While it is known that signaling depends on self-association and homotypic association of TIR domains, crystal structures have revealed few common association modes [1,2]. We have targeted TIR domains from mammals, plants and bacteria (determining crystal structures for human TLR adaptor proteins MAL [3] and SARM (unpublished), the bacterial protein TcpB from *Brucella melitensis* [4] and the plant immune proteins L6 from flax [5], RPS4, RRS1 [6], SNC1 and RPP1 from Arabidopsis [7] and RPV1 from grape [8]). These crystal structures have started to reveal common trends in association modes, in particular for bacterial and plant TIR domains. Furthermore, for the TLR adaptors MAL and MyD88, we have been able to reconstitute large assemblies and determine the structure of the filamentous assembly of MAL by cryo-electron microscopy [9]. As an unexpected twist, we (unpublished) and other [10] have shown that the TIR domain of the TLR adaptor SARM possesses self-association-dependent NAD+ cleavage activity. Jointly, these studies suggest a general mechanism of function of TIR domains, which involves "signaling by cooperative assembly formation (SCAF)" with prion-like features that is consistent with signaling in other innate immunity pathways.

References:

[1] T. Ve et al (2015) Apoptosis 20, 250
[2] Nimma et al (2017) Curr Opin Struct Biol 43, 122
[3] Valkov et al (2011) Proc Natl Acad Sci USA 108, 14879
[4] Alaidarous et al (2014) J Biol Chem 289, 654
[5] Bernoux et al (2011) Cell Host Microbe 9, 200
[6] Williams et al (2014) Science 344, 299
[7] Zhang et al (2017) Proc Natl Acad Sci USA
[8] Williams et al (2016) Frontiers Plant Sci 7, 1850
[9] Ve et al (2017) Nat Struct Mol Biol
[10] Essuman et al (2017) Neuron 93, 1334

Mediator links the jasmonate receptor to transcriptionally active chromatin

Lin Li, Chunpeng An, Qingzhe Zhai, and Chuanyou Li

State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101.

Jasmonoyl-isoleucine (JA-Ile), the active form of the plant hormone jasmonate (JA), is sensed by the F-box protein CORONATINE INSENSITIVE 1 (COI1), a component of a functional Skp–Cullin–F-box (SCF) E3 ubiquitin ligase complex. Sensing of JA-Ile by COI1 rapidly triggers genome-wide transcriptional changes that are largely regulated by the basic helix-loop-helix transcription factor MYC2. However, it remains unclear how the JA-Ile receptor protein COI1 relays hormone-specific regulatory signals to the RNA polymerase II general transcriptional machinery. Here, we report that the plant transcriptional co-activator complex Mediator directly links COI1 to the core promoter of MYC2 target genes. MED25, a subunit of the Mediator complex, physically interacts with COI1 on the core promoters of MYC2 targets and facilitates COI1-dependent degradation of Jasmonate–ZIM domain (JAZ) transcriptional repressors. MED25 and COI1 influence each other's enrichment on the core promoters of MYC2 target genes. Furthermore, MED25 physically and functionally interacts with HISTONE ACETYLTRANSFERASE1 (HAC1), which plays an important role in JA signaling by selectively regulating H3K9 acetylation of MYC2 target promoters. Moreover, the enrichment and function of HAC1 on the core promoter of MYC2 targets depend on COI1 and MED25. Therefore, the MED25 interface of Mediator links COI1 with HAC1-dependent H3K9 acetylation to activate MYC2-regulated transcription of JA-responsive genes. This study exemplifies how a single Mediator subunit integrates the actions of both genetic and epigenetic regulators into a concerted transcriptional program. This mechanism likely operates in other plant hormones whose receptors are localized in the nucleus.

Inverse modulation of antiviral and antibacterial immunity by immune RLK complexes

Elizabeth P B Fontes et al.

Department of Biochemistry and Molecular Biology/ National Institute of Science and Technology in Plant-Pest Interactions- Universidade Federal de Viçosa- Viçosa, MG, Brazil.

Plants deploy different immune receptors to recognize pathogens and defend themselves against pathogen attacks. Previous studies have shown that the activation of PAMP-triggered immunity (PTI) is fine-tuned by several levels of negative controls to prevent autoimmunity. In the current study, we demonstrated that the antiviral immune receptor NUCLEAR SHUTTLE PROTEIN-INTERACTING KINASE 1 (NIK1) acts as an additional negative regulator of antibacterial immunity. Plants with *nik1* null alleles exhibited dwarf morphology, enhanced disease resistance to the bacteria *Pseudomonas syringae* pv. *tomato* and *P. syringae* pv. *Maculicola* and enhanced PAMP immune response, a phenotype reversed by NIK1 complementation. Furthermore, NIK1 interacted constitutively with BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1) and FLAGELLIN SENSING 2 (FLS2) and these protein complexes were disrupted by flagellin, which shift the equilibrium towards FLS2/BAK1complex formation. NIK1 overexpression negatively impacted the formation of an active immune complex, whereas loss of NIK1 function enhanced FLS2/BAK1 complex formation. We will present additional data showing how the phosphorylation status of these immune receptors affect the flagellin-dependent release of NIK1 from BAK1 or FLS2, and the dynamics of immune complex formation.

Heterotrimeric G proteins are involved in novel immune signaling pathways in Arabidopsis

Jian-Feng Li

State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510006

Heterotrimeric G proteins composed of Gα, Gβ and Gγ subunits are evolutionarily conserved signaling modules involved in diverse biological processes in plants and animals. The role and action of G proteins, particularly Gα, remain largely enigmatic in plant innate immunity. Here we show that Arabidopsis Gα (GPA1) and Gβ (AGB1) in conjunction with Receptor for Activated C Kinase 1 (RACK1) mediate a new immune signaling pathway activated by bacteria-secreted proteases, where RACK1 serves as an essential scaffolding protein to determine the specific activation of MPK3/6 but not MPK4. In addition, the *gpa1* mutant also exhibits multiple immunodeficiencies in response to the bacterial flagellin epitope flg22. We uncovered that GPA1 plays a pivotal role in an immune pathway involving the flg22 receptor FLS2, co-receptor BAK1, Regulator of G Signaling 1 (RGS1), and AGB1, in which flg22 elicits GPA1/AGB1 dissociation from the FLS2/BAK1/RGS1 receptor complex. Consequently, flg22-induced degradation was detected for FLS2, BAK1 and RGS1 but not for GPA1 or AGB1. We further found that GPA1 constitutively interacts with the NADPH oxidase RbohD as a downstream effector to potentiate flg22-induced ROS burst independent of the central cytoplasmic kinase BIK1. Taken together, our work sheds multiple novel insights into the functions and regulatory mechanisms of heterotrimeric G proteins in *Arabidopsis* innate immunity.

Differential signaling networks triggered by LRR-RK and LRR-RP-type receptors

Wei-Lin Wan [a], Maricris Zaidem [b], Joachim Kilian [a], Jian-Min Zhou [c], Detlef Weigel [b], Andrea A. Gust [a], Thorsten Nürnberger [a]

[a] *Center for Plant Molecular Biology and Plant Biochemistry, University of Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany*

[b] *Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tuebingen, Germany*

[c] *State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, 100101 Beijing*

wei-lin.wan@zmbp.uni-tuebingen.de

Plant cell surface receptors sense microbial pathogens by recognizing microbial structures called pathogen or microbe-associated molecular patterns (PAMPs/MAMPs). There are two major types of plant pattern recognition receptors: (1) plant receptor-like proteins and receptor-like kinases carrying extracellular leucine-rich repeat domains (LRR-RP and LRR-RK) and (2) plant receptor-like proteins and receptor-like kinases carrying extracellular lysin motifs (LysM-RP and LysM-RK). Although many studies focus individually on the signal pathways triggered by different receptor types during the establishment of immunity to microbial infection, the exact overlap and the differences between these pathways remain largely unknown. In this study, *Arabidopsis thaliana* responses to flg22 and nlp20, via their corresponding receptor types, FLS2 (LRR-RK) and RLP23 (LRR-RP), were compared. Systematic analyses of various plant immune responses revealed that nlp20 triggers only slow and weak early responses such as ROS accumulation and MAPK activation. However, compared to flg22, nlp20 is capable of inducing higher levels of the phytohormones ethylene and salicylic acid. In contrast, flg22 triggers early responses (ROS, MAPKs) faster and stronger, and also causes more extensive transcriptome reprograming. Through a mutational screen focusing on genes with known roles in plant immunity, we found that BIK1 may play different roles in signaling after flg22 and nlp20 treatments. The results present a more complete picture of MAMP-triggered immunity and may lead to the discovery of key components participating in plant immunity.

The interplay between DNA damage and plant immune responses

Junqi Song[1], Brian Keppler[2], Robert Wise[3] and Andrew F. Bent[2]

[1]Texas A&M AgriLife Research Center at Dallas, Dallas, TX 75252; [2]Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI 53706; [3]Department of Biology, University of Wisconsin - Oshkosh, Oshkosh, WI 54901

DNA damage repair and immune responses are two fundamental processes that have been characterized extensively, but the links between them remain largely unknown. We previously identified multiple genes that play a dual role in homologous recombination and transcriptional regulation of plant-defense genes. Moreover, we discovered that multiple microbial bacterial, fungal and oomycete plant pathogens with diverse life styles induce double-strand-breaks to host plant DNA. These suggested an interplay between DNA damage and plant immune responses. Here we report that poly(ADP-ribosyl)ation plays an important role in plant immune systems in response to infection. Poly(ADP-ribosyl)ation is a post-translational modification and contributes to multiple molecular and cellular processes with a prominent role in DNA damage repair. Human PARP1, the founding and most characterized member of the PARP family, accounts for more than 90% of overall molecular and cellular PARP activity in response to DNA damage while PARP2 supplies a minor portion of this PARP activity. We found that Arabidopsis PARP2 rather than PARP1 plays the predominant role in poly(ADP-ribosyl)ation and organismal resilience in response to either chemically-induced DNA damage or pathogen infections. Hence, core aspects of plant poly(ADP-ribosyl)ation are mediated by substantially different enzymes than in animals, indicating the likelihood of substantial differences in regulation. Collectively, our findings suggest that the two ancient surveillance mechanisms, DNA damage and plant immune responses, are intricately interconnected.

Inter-organellar communication and autophagy during innate immunity

Savithramma P Dinesh-Kumar[1], Jongchan Woo[1], Ugrappa Nagalakshmi[1], Neeraj Lal[1], Eunsook Park[1], Alexander Nedo[2], and Jeffrey Caplan[2,3]

[1]*Department of Plant Biology and The Genome Center, College of Biological Sciences, One Shields Avenue, UC Davis, CA 95616*

[2]*Department of Biological Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711*

[3]*Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711*

Macroautophagy, hereafter referred to as autophagy, is a dynamic process that is conserved across eukaryotes and entails the engulfment of cellular components or cargoes in double membrane vesicles called autophagosomes. Autophagosomes are then targeted to the vacuole/lysosome for degradation or recycling. It has been well established that recycling of long-lived cellular proteins and organelles by autophagy is an important adaptive response to nutrient deprivation. However, recent studies have revealed that autophagy participates in other diverse biological processes including innate immunity and programmed cell death (PCD). We will discuss emerging perspectives on autophagy, cell death, and innate immunity. In addition, we will discuss strategies to identify small-molecule regulators of autophagy for disease control.

Emerging evidence suggests that chloroplasts play an important function during innate immunity and they also have a central role in the production of immune signals. Our recent findings demonstrated that chloroplasts dynamically change their morphology by sending out stroma-filled tubular projections known as stromules during immune responses (*Caplan et al., Developmental Cell 34:45-57*). Interestingly, stromules form complex associations with the nuclei and subsequent clustering of chloroplasts around nuclei during immune response. We will discuss these findings and our recent results on the role of cytoskeleton on stromule formation and chloroplast association with nuclei during plant innate immunity.

Structural and mechanistic bases of effector recognition by paired NLR immune receptors and decoy domains

Thomas Kroj
INRA UMR BGP, Campus International de Baillarguet, TA A-54/K, 34398, Montpellier, France
Keywords: plant immunity, immune receptors, NLR, effectors, cell death, rice blast, *Magnaporthe oryzae*

Nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) are important receptors in plant immunity and allow specific recognition of pathogen effectors. Based on our work on the detection of the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by the rice NLR RGA5, we recently developed the hypothesis that some NLRs recognize effectors by integrated decoy domains. Comparative genomic analysis showed that NLRs carrying integrated decoy domains are frequent and widespread. We identified them in 31 land plants, from mosses to angiosperms, and they represent, on average, 7% of the NLRs. This 'integrated decoy model' was further supported by work in other experimental systems and has been widely accepted.

By detailed structure-function analysis we further deciphered the molecular details of the binding of AVR-Pia and AVR1-CO39 to the integrated decoy domain of RGA5, a heavy metal-associated domain most related to the yeast copper chaperon ATX1 (RATX1 domain). This demonstrated that the direct RGA5-RATX1/effector binding is strictly required for effector recognition but only of moderate affinity and acts in concert with the association of the effectors to additional sites in RGA5. This combination of integrated decoy domains with additional independent effector-NLR interactions seems to confer robust effector recognition that is resilient to effector mutations. We will present first results on how knowledge on the molecular details of effector recognition by integrated decoy domains can be exploited for the modification of the recognition spectrum of NLRs.

Specificity of RIN4 function in activation or suppression of NLR is conferred by sequence diversity in C-terminal region

Maxim Prokchorchik[1,3] and Kee Hoon Sohn[1,2,3]

[1]Department of Life Sciences and [2]School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology, Pohang, South Korea, [3]Bioprotection Research Centre, Institute of Agriculture and Environment, Massey University, Palmerston north, New Zealand.

Plant innate immunity relies on two layers of pathogen detection. Cell surface-localized pattern recognition receptors detect pathogen-associated molecular patterns (PAMPs) of invading microorganisms and activate PAMP-triggered immunity (PTI). Successful pathogens must circumvent PTI to colonize plants, and many bacterial pathogens use type III secretion (T3S) to deliver effectors that suppress PTI into plant cells. Effectors can be detected directly or indirectly by plant disease resistance (R) proteins, which then activate effector-triggered immunity (ETI) generally together with a hypersensitive response (HR) of the infected tissue. Plant pathogenic bacteria, *Pseudomonas syringae* and *Erwinia amylovora*, translocate a type III secretion-dependent effector protein, AvrRpt2, in host plant cells. AvrRpt2 is a cysteine protease that cleaves its host target protein RIN4. AvrRpt2-directed cleavage of RIN4 is recognized by CC-NB-LRR type immune receptors RPS2 and MR5 in Arabidopsis and Malus, respectively. Interestingly, RPS2 and MR5, although both recognize AvrRpt2, do not share significant sequence homology. We found that RPS2 but not MR5 shows auto-activity as shown by a rapid programmed cell death when transiently expressed in *Nicotiana benthamiana* leaf cells. Thus, we hypothesized that the mechanisms by which RPS2 and MR5 are activated by AvrRpt2-directly cleavage of RIN4 differ from each other. Further mechanistic details of AvrRpt2-triggered activation of MR5 will be presented.

A CRISPR/Cas9 Toolbox Based On the Endogenous tRNA Processing for Multiplex Genome Engineering

Kabin Xie[a], Dan Ding[a], Hao Liu[a], Ling-ling Chen[a] and Yinong Yang[b]

[a] *National Key Laboraroty of Crop Genetic Improvement, Huazhong Agricultural University, No. 1 Shizishan street, Wuhan 430070*

[b] *Department of Plant pathology and Environmental Microbiology, Pennsylvania State University, University Park, State College, Pennsylvania 16802, USA*

E-mail of presenting author: kabinxie@mail.hzau.edu.cn

The cluster regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated system 9 (Cas9) becomes a revolutionary tool for genome editing in life sciences. We previously demonstrated that the RNA-guided genome editing using Cas9 and single guide RNA (gRNA) was efficient in rice [1]. In genome editing practice, the off-target effect which may cause unwanted mutations is one of the major concerns for CRISPR/Cas9. Thus, selecting highly specific targeting sequence is essential for CRISPR/Cas9 technology. To this end, we established CRISPR-PLANT (http://www.genome.arizona.edu/crispr) and CRISPR-P 2.0 (http://cbi.hzau.edu.cn/cgi-bin/CRISPR2) to provide web tools for plant gRNA design [2, 3]. Recently, we established a polycistronic-tRNA-gRNA (PTG) method for multiplex genome editing using CRISPR/Cas9 [4]. This method hijacks the endogenous tRNA processing system to produce many gRNAs from one artificial *PTG* genes consisting of tRNA-gRNA cassettes, and then the mature gRNAs direct Cas9 to edit multiple genomic targets. Our studies showed that the PTG method was robust and efficient to simultaneously edit up to 8 and 6 targets in rice and human cell lines, respectively. Because the tRNA processing is conserved in different organisms, this method could be broadly used to construct sophisticated tools for genome engineering. Taking together, we established an integrated platform of plant CRISPR/Cas9 technologies which provide useful tools for crop genome engineering.

References:

[1] K. Xie and Y. Yang (2013). Mol Plant, 6, 1975.
[2] K. Xie, J. Zhang, and Y. Yang (2014). Mol Plant, 7, 923.
[3] H. Liu, et al. (2017). Mol Plant, 10, 530.
[4] K. Xie, B. Minkenberg, and Y. Yang (2015). Proc Natl Acad Sci USA, 112, 3570.

Genes and traits responsive to growth and survival of *Pseudomonas syringae* in association with plant leaves

Xilan Yu[a], Gwyn Beattie[b], Steven Lindow[c]
[a] *Hubei Collaborative Innovation Center for Green Transformation of Bio-resources, College of Life Sciences, Hubei University, Wuhan, Hubei*
[b] *Departments of Plant Pathology and Microbiology, Iowa State University, Ames, IA, USA*
[c] *Department of Plant and Microbial Biology,University of California, Berkeley, CA, USA*
E-mail of presenting author: yxl13728@outlook.com

Many bacterial foliar pathogens including the agriculturally important plant pathogen *Pseudomonas syringae* have complex interactions with their host plants. These interactions include multiplication and survival on leaf surfaces, modification of the environment to favor growth, invasion of plant leaves, multiplication in the intercellular spaces, and evasion of plant defense responses, often with the subsequent induction of disease lesions. As one of the most extensively studied *P. syringae* strains, *P. syringae* pv. syringae B728a exhibits a pronounced epiphytic stage followed by an apoplastic stage; that is, it establishes populations on leaf surfaces and then in the leaf interior. *P. syringae* likely encounters distinct environmental conditions in these distinct stages of their interactions with plants. We performed global transcriptome profiling of B728a to understand the environmental conditions that B728a experiences and the cellular traits that it uses to grow and survive during these stages of its lifecycle[1]. One important finding is that water limitation is a major stress influencing *P. syringae* growth and survival both on and in plant leaves. *P. syringae* can adapt to water stress during leaf colonization, in part, by accumulating compatible solutes[2]. The disaccharide trehalose is a particularly potent compatible solute that contributes to water stress tolerance in *P. syringae*. We characterized trehalose production in B728a and also in DC3000, a *P. syringae* strain that is less tolerant to water stress than B728a and a poor epiphytic colonist. We specifically investigated the relative contribution of distinct trehalose biosynthetic pathways to trehalose production and water stress tolerance in these two strains. As *P. syringae* has been shown to manipulate water level in plant leaves for virulence[3]，our work could shed light on compatible solutes involvement in bacterial pathogencity as well as osmotolerance during plant-pathogen interactions.

[1] Xilan Yu, et al. (2013). *Proc Natl Acad Sci U S A*, 110, E425.
[2] Brian Freeman, et al. (2010). *Environmental Microbiology*, 12, 1468.
[3] Xiufang Xin, et al. (2016). *Nature*, 539, 524.

Defense and Counter-Defense in the Apoplastic Battlefield during Plant-Pathogen Interaction

Yuanchao Wang

Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095

In nature, plants are under constant challenge by a wide variety of microbes. During the long run of evolution, plants have developed innate immunity to resist infection by the majority of microbes. For example, plants recognize microbial attack via perception of conserved microbial molecules by cell surface immune receptors and thereby operating effective defense. In the arms race with plants for survival, pathogenic microbes evolved sophisticated strategies to modulate plant immunity to favor successful infection. Recently, we identified the glycoside hydrolase XEG1 from the soybean root rot pathogen *Phytophthora sojae*. XEG1, on the one hand functions as a virulence factor essential for *Phytophthora* infection, and on the other hand acts as a conserved PAMP that can be recognized by multiple plant species. In this talk, I will present data to explain how XEG1 regulates *Phytophthora* virulence and plant immunity.

Adaptive genome evolution in the vascular wilt pathogen *Verticillium*

Bart P.H.J.Thomma

Fungi cause severe crop losses and threaten food security worldwide. The soil-borne fungal pathogen Verticillium dahliae causes vascular wilt disease on hundreds of plant species, and disease control is challenging because resistance in plants is rare. Moreover, V. dahliae has a flexible genome allowing it to escape host immunity and maintain aggressiveness. Through comparative population genomics we try to unravel mechanisms to establish genomic diversity that is essential for adaptive genome co-evolution during the continued arms race with host plants. These analyses have revealed lineage-specific regions within V. dahliae genomes that are important for virulence. Interestingly, these regions are enriched for in planta-expressed effector genes encoding secreted proteins that enable host colonization. Some of these effectors enable host specificity.

The Receptor-like Kinase SDS2 Modulated by the E3 Ligase SPL11 Controls Cell Death and Immunity in Rice

Jiangbo Fan[1,2], Pengfei Bai[2], Yehui Xiong[1,3], Yuese Ning[1], Chongyang Zhang[1], Ruyi Wang[1], Xiangzong Meng[4], Jinggeng Zhou[4], Mo Wang[2], Chan Ho Park[2], Maria Bellizzi[2], Wende Liu[1], Jong-Seong Jeon[5], Libo Shan[4], Guo-Liang Wang[1,2]

[1]*State Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193*
[2]*Department of Plant Pathology, The Ohio State University, Columbus, OH 43210, USA*
[3]*School of Life Science, Tsinghua university, Beijing, 100084*
[4]*Department of Plant Pathology and Microbiology, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843, USA*
[5]*Graduate School of Biotechnology, Kyung Hee University, Yongin 17104, Korea.*

Abstract

Programmed cell death (PCD) plays a critical role in plant immunity. The E3 ubiquitin ligase SPL11 is a negative regulator of PCD and immunity in rice. SPL11 interacts with the Rho GTPase-activating protein (RhoGAP) SPL11-INTERACTING PROTEIN 6 (SPIN6), and thereby negatively regulates the Rho GTPase OsRac1, a central regulator of rice PTI and ETI signaling. However, how SPL11-mediated PCD and immunity is regulated remains unclear. In this study, we show that SPL11 cell-death suppressor 2 (SDS2), an S-domain receptor-like kinase, positively regulates PCD and immunity in rice. The *sds2* mutant shows reduced immune responses and enhanced susceptibility to the blast fungus *Magnaporthe oryzae*. Conversely, over-expression of *SDS2* in rice leads to constitutive PCD accompanied by elevated immune responses and enhanced resistance to *M. oryzae*. SDS2 interacts with and phosphorylates SPL11, which in turn ubiquitinates SDS2 for degradation. In addition, SDS2 interacts with receptor-like cytoplasmic kinases, OsRLCK118/176, that phosphorylate the NADPH oxidase OsRbohB to positively regulate immunity. Thus, the plasma membrane-resident protein complex consisting of SDS2, SPL11, and OsRLCK118/176 fine tunes PCD and immunity in rice.

NLR network mediates immunity to diverse plant pathogens

Chih-Hang Wu [a], Ahmed Abd-El-Haliem [b], Tolga O. Bozkurt[a,c], Khaoula Belhaj[a], Ryohei Terauchi[d,e], Jack H. Vossen[b] and Sophien Kamoun[a]

[a] *The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.*

[b] *Plant Breeding, Wageningen University and Research, Wageningen, The Netherlands.*

[c] *Department of Life Sciences, Imperial College London, London, United Kingdom.*

[d] *Division of Genomics and Breeding, Iwate Biotechnology Research Center, Iwate, Japan.*

[e] *Laboratory of Crop Evolution, Graduate School of Agriculture, Kyoto University, Kyoto, Japan.*

sophien.kamoun@tsl.ac.uk

Both plants and animals rely on nucleotide-binding domain and leucine-rich repeat-containing (NLR) proteins to respond to invading pathogens and activate immune responses. An emerging concept of NLR function is that “sensor” NLR proteins are paired with “helper” NLRs to mediate immune signaling. However, our fundamental knowledge of sensor/helper NLRs in plants remains limited. In this study, we discovered a complex NLR immune network in which helper NLRs in the NRC (NLR-required for cell death) family are functionally redundant but display distinct specificities toward different sensor NLRs that confer immunity to oomycetes, bacteria, viruses, nematodes, and insects. The helper NLR NRC4 is required for the function of several sensor NLRs including Rpi-blb2, Mi-1.2 and R1, whereas NRC2 and NRC3 are required for the function of the sensor NLR Prf. Interestingly, NRC2, NRC3 and NRC4 redundantly contribute to the immunity mediated by other sensor NLRs including Rx, Bs2, R8 and Sw5. NRC family and NRC-dependent NLRs are phylogenetically related clustering into a well-supported superclade. Using extensive phylogenetic analysis, we discovered that the NRC-superclade has probably emerged over 100 million years ago from an NLR pair that diversified to constitute up to one half of the NLRs of asterids. These results reveal a complex genetic network of NLRs by linking evolutionary history to immune signaling. We propose that this NLR network increases robustness of immune signaling to counteract rapidly evolving plant pathogens.

Reference:

[1] C.H. Wu, A. Abd-El-Haliem, T.O. Bozkurt, K. Belhaj, R. Terauchi, J.H. Vossen, S. Kamoun (2017). PNAS, doi: 10.1073/pnas.1702041114.

Rhizobial root microbiota membership predisposed convergent evolution of nitrogen-fixing symbiosis with legumes

Paul Schulze-Lefert

Abstract

Rhizobia are a paraphyletic group of soil-borne bacteria defined by their ability to induce nodule organogenesis in legume roots and fix atmospheric nitrogen for plant growth. In non-leguminous plants, species within the Rhizobiales order define a core lineage of the plant microbiota, suggesting alternative forms of interactions with plant hosts. We compared more than 1,300 whole-genome sequences of Rhizobiales isolates, including microbiota members from non-legumes, and show that the set of genes required for nodulation and nitrogen fixation in legume symbiosis was acquired multiple independent times within each Rhizobiales sublineage. The majority of root-associated rhizobia colonize and promote root growth in the crucifer Arabidopsis without nitrogen fixation, indicating these are rhizobial traits of an ancestral root association. Thus, the capacity for nodulation and nitrogen fixation in legumes was likely acquired from a predisposed root association in multiple subsequent events, constituting an example of convergent evolution.

Epigenetic regulation and signaling of durable and broad-spectrum blast resistance in rice

Yiwen Deng, Keran Zhai, Dongyong Yang, Zuhua He

National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200032
Corresponding author: zhhe@sibs.ac.cn

Breeding for broad-spectrum and durable disease resistance has been the ideal target of crop breeding. Although many resistance (R) genes have been identified, very few R genes have been adopted for broad-spectrum and durable disease resistance in long-term crop breeding programs due to either resistance breakdown or defense cost. We identified that the rice *Pigm* locus contains an nucleotide-binding leucine-rich repeat (NLR) receptor cluster and confers durable and broad-spectrum resistance to fungal blast (*Magnaporthe oryzae*) without yield penalty. Within the NLR cluster, PigmR confers broad-spectrum resistance; while another NLR PigmS interacts with and competitively attenuates the homodimerization of PigmR to suppress the PigmR-mediated resistance. The expression of *PigmS* and thus the Pigm-mediated resistance are subjected to tight epigenetic regulation by RNA-dependent DNA methylation. Our study therefore reveals a novel mechanism of balancing broad-spectrum resistance and yield through epigenetic regulation of a pair of antagonistic NLRs. To further dissect the PigmR-mediated broad-spectrum resistance, we conducted screening for downstream PigmR-interacting proteins (PIPs), and found one of PIPs, PIP1, which functions in PigmR-mediated resistance. PIP1 shows nuclear localization in a PigmR-dependent manner. Interestingly, the PigmR-induced nuclear accumulation of PIP1 could be inhibited by PigmS. PIP1 possesses DNA binding and transcriptional activation capacity, indicating a novel class of transcription factors. In support of its function in defense, we found PIP1 directly binds to the promoter of *OsWAK14* and activates *OsWAK14* expression. We therefore propose that the translocation of PIP1 into nucleus for activating gene expression is critical to the PigmR-mediated blast resistance.

Learning from the interaction between cotton and *Verticillium dahliae*

Hui-Shan Guo

State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 10010 (guohs@im.ac.cn)

Verticillium dahliae Kleb. is a soil-borne fungal pathogen that causes wilt disease in a wide range of crops (e.g. cotton, sunflower, potato and tomato) all over the world and causes huge annual loss in agriculture.

To investigate the infection mechanism, we generated T-DNA insertional mutants from a virulent isolate of *V. dahliae* (Gao et al., 2010) and GFP-labeled *V. dahliae* (Zhao et al., 2014), we provided for the first time the molecular features for accurate identification of hyphopodium, an infectious structure in *V. dahliae*, finding that hyphopodium-specific VdPls1 and VdNoxB, a tetraspanin and a catalytic subunit of membrane-bound NADPH oxidases, for ROS production is required for tip-high Ca^{2+} elevation and penetration peg formation during plant infection by *V. dahliae* (Zhao et al., 2016). We further observed that VdNoxB regulates the cytoskeletal organization of the sepin ring, requiring VdSep5, partitioning the hyphopodium and invasive hypha and form the fungus-host penetration interface for delivery of secretory proteins during root infection by *V. dahliae*, suggesting that hyphopodium is the apparatus that not only breaches host cells but also provides a unique interface for the secretion of fungal effectors (Zhou et al., 2017). A *Verticillium*-specific secretory protein, namely VdSCP7, is secreted by *V. dahliae* and accumulates in the plant nucleus to function as an effector and alter plant immunity (Zhang et al., 2017).

In studying plant immunity against *Verticillium* pathogens, we found for the first time that plants (e.g. cotton, tomato and Arabidopsis) naturally export endogenous microRNAs (e.g. miR166 and miR159) into *V. dahliae* to specifically target cross-kingdom virulence genes silencing in the fungi and confer disease resistance (Zhang et al., 2016a). More importantly, we successfully applied this cross-kingdom RNA silencing (RNAi), an approach called host-induced gene silencing (HIGS), to protect cotton plants against *V. dahliae*.

RNAi-expressing cotton plants gained significant resistance to vascular wilt disease in the laboratory condition and in the field (Zhang et al., 2016b).

Gao, F., Zhou, B.J., Li, G.Y., Jia, P.S., Li, H., Zhao, Y.L., Zhao, P., Xia, G.X., and Guo, H.S*. 2010. A glutamic acid-rich protein identified in *Verticillium dahliae* from an insertional mutagenesis affects microsclerotial formation and pathogenicity. PLoS One 5:e15319.

Zhao, P., Zhao, Y.-L., Jin, Y., Zhang, T., and Guo, H.-S*. 2014. Colonization process of Arabidopsis thaliana roots by a green fluorescent protein-tagged isolate of *Verticillium dahliae*. Protein & Cell 5:94-98.

Zhao, Y.-L., Zhou, T.-T., and Guo, H.-S*. 2016. Hyphopodium-Specific VdNoxB/VdPls1-Dependent ROS-Ca2+ Signaling Is Required for Plant Infection by *Verticillium dahliae*. PLOS Pathogens 12:e1005793.

Zhou, T.-T., Zhao, Y.-L., and Guo, H.-S*. 2017. Secretory proteins are delivered to the septin-organized penetration interface during root infection by *Verticillium dahliae*. PLOS Pathogens 13:e1006275.

Zhang, L., Ni, H., Du, X., Wang, S., Ma, X.-W., Nürnberger, T., Guo, H.-S*., and Hua, C*. 2017. The *Verticillium*-specific protein VdSCP7 localizes to the plant nucleus and modulates immunity to fungal infections. New Phytologist 215:368-381.

Zhang, T., Zhao, Y.L., Zhao, J.H., Wang, S., Jin, Y., Chen, Z.Q., Fang, Y.Y., Hua, C.L., Ding, S.W., and Guo, H.S*. 2016a. Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. Nature Plants 2:16153.

Zhang, T., Jin, Y., Zhao, J.-H., Gao, F., Zhou, B.-J., Fang, Y.-Y., and Guo, H.-S*. 2016b. Host-Induced Gene Silencing of the Target Gene in Fungal Cells Confers Effective Resistance to the Cotton Wilt Disease Pathogen *Verticillium dahliae*. Molecular Plant 9:939-942.

The evolutionary complexity of microRNAs that target NB-LRR disease resistance genes

Blake Meyers

Donald Danforth Plant Science Center, St. Louis, Missouri, & University of Missouri – Columbia, Division of Plant Sciences, Columbia, Missouri, USA

Plants implement several mechanisms to control the transcript level of *NBS-LRR* (nucleotide binding site leucine-rich repeat) defense genes, since high expression of plant *NBS-LRR* defense genes is often lethal to plant cells perhaps associated with fitness costs. As negative transcriptional regulators, diverse miRNAs target *NBS-LRRs* in eudicots and gymnosperms. We are using genome-wide approaches across many sequenced land plants genomes. We found a close association between the diversity of *NBS-LRRs* and miRNAs. In the genomes which have diverse miRNAs targeting *NBS-LRRs*, the miRNAs typically target highly duplicated *NBS-LRRs*. In comparison, the heterogeneity of *NBS-LRRs* was relative higher in Poaceae and Brassicaceae genomes which have few miRNA-targeted *NBS-LRRs*. We observed that duplicated *NBS-LRRs* from different gene families gave birth to new miRNAs periodically. Most of these newly-emerged miRNAs target the same conserved, encoding protein motif of NBS-LRRs, consistent with a model of convergent evolution for these miRNAs. By assessing the interactions between miRNAs and *NBS-LRRs*, we found nucleotide diversity in the wobble position of the codons in the target site drives the diversification of miRNAs. These data support a co-evolutionary model of plant *NBS-LRRs* and miRNAs to explain how plants balance the benefits and costs of *NBS-LRR* defense genes.

Exploring the compatible Fusarium-wheat interaction using a multi-'omics' approach

Neil A. Brown[a], Ana Machado[a], Catherine Walker[a] [a] Wing Sham Lee[a], Martin Urban[a], Robert King[b], Kostya Kanyuka[a] and Kim Hammond-Kosack[a]

[a] *Biointeractions and Crop Protection Department, Rothamsted Research, Harpenden AL5 2JQ, UK.*

[b]*Computational and Analytical Sciences Department, Rothamsted Research, Harpenden AL5 2JQ, UK*

E-mail: kim.hammond-kosack@rothamsted.ac.uk

Fusarium graminearum (*Fg*) is a major fungal pathogen of wheat crops globally, causing Fusarium head scab/ ear blight disease. This floral disease results in reduced grain yield and lower grain quality due to contamination with various harmful trichothecene mycotoxins. During compatible interactions, *Fg* colonization advances by hyphae growing within the extracellular spaces of the wheat rachis tissue without causing macroscopic symptoms. Later, hyphae penetrate the wheat cells coincident with the induction of host cell death [1]. Here we report on the characterization of the early *Fg* transcriptome during symptomless wheat floral infection [2] and the identification of various small secreted *Fg* effectors, which when transiently overexpressed using the Barley Stripe Mosaic Virus vector system (BSMV-VOX) [3], lead to enhanced *Fg* fungal infection.

Acknowledgement: This research is funded by the BBSRC.

References:

[1] Brown, N.A et al., (2010) Fungal Biology 114, 555.
[2] Brown, N.A et al., (2017) Mol. Plant Pathology, on line early, doi: 10.1111/mpp.12564
[3] Lee, W-S et al., (2012) Plant Physiology 160, 582.

Molecular interactions of *Phytophthora capsici* and its host plants

Qi Li, Ji Wang, Qimeng Zhang, Fen Zou, Yanyu Chen, Meixiang Zhang,
Danyu Shen, Daolong Dou*

Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095,

**Correspondents:* ddou@njau.edu.cn

Phytophthora capsici, an oomycete pathogen, causes many devastating diseases on a broad range of plant species, including model plant *Arabidopsis thaliana* and hundreds of vegetable crops such as pepper and cucumber. *P. capsici* produces several hundreds of predicted RxLR effector proteins to regulate host immunity during interactions. However, the mechanisms of host manipulation via these RxLR effectors are still largely unknown.

We identified several *P. capsici* RxLR effectors that may induce cell death on *Nicotiana benthamiana* and are required for full virulence of the pathogen. Among them, RxLR242 may activate hypersensitive response (HR)-liking phenotypes in several plants and exhibits high polymorphism abundance in different strains. Interestingly, gene silencing of *RxLR242* leads to successful infection on *N. tabacum* plant, which is a non-host plant of *P. capsici*. The results suggest the gene may play an important role to determine the host range. RxLR207 can enhance plant disease resistance by accelerating cell death. Using yeast two-hybrid system, we found that it interacts with several RNA-recognition motif (RRM) containing proteins of *Arabidopsis*. The corresponding *RRM* mutant lines also show altered disease resistant levels, suggesting that these genes have noteworthy roles in plant immunity. Transcriptome analysis indicates a set of *Arabidopsis* genes are regulated by *RXLR207,* ~60% of which are identical to the *RRM* genes-dependent genes. The results indicate that RXLR207 regulate cell death and plant immunity probably through interaction with these RRM proteins.

At the meantime, we tested interactions of the critical components (e.g. EDS1 and NPR1) of plant immunity pathway with RxLR effectors. The results showed that EDS1 and NPR1 are targeted by RxLR103 and RxLR48, respectively. Both effectors can suppress plant defense on *N. benthamiana* based on agroinfiltration-mediated transient expression. The corresponding gene-silenced transformants showed significantly reduced virulence on *N. benthamiana* and *Arabidopsis*, indicating that both of them are required for full virulence. ROS burst and

callose deposits were more abundant at the sites inoculated with *RxLR48*-silenced transformant, compared with the wild type. But the molecular mechanisms of RxLR48 targeting NPR1 is still under exploration. On the other hand, RxLR103 interacts with EDS1 through its lipase-like domain, followed by interfering with formation of EDS1/PAD4 complex.

Understanding of *Fusarium graminearum*-Host molecular interactions by cellular tracking and gene profiling

Yan Guo, Wanqiu Wang, Yanzhang Wang, Weihua Tang
Center for Excellence in Molecular Plant Science, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, 200032
whtang@sibs.ac.cn

Fusarium graminearum can cause severe crop diseases such as Fusarium head blight on wheat and Gibberella stalk rot on maize, and contaminate grains with mycotoxins. Cultivars highly resistant to *F. graminearum* are still not available, therefore it is needed to comprehensive understand *F. graminearum* pathogenicity to explore new approaches for resistance design. The host infection process of *F. graminearum* is complex and temporal-spatial specific. We tracked *F. graminearum* infection of wheat coleoptile and maize stalk at cellular level, and obtained the specific fungal infection transcriptomes [1,2]. We further elucidated the infection strategy transition of *F. graminearum*, and discovered that *F. graminearum* remodels membrane lipids to adapt apoplast phosphate starvation during maize stalk infection.

Acknowledgement: This work was supported by the National Key Research and Development Program of China (2016YFD0100600), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB11020500).

References:

[1] X-W. Zhang, L-J. Jia, Y. Zhang, G. Jiang, X. Li, D. Zhang, W-H. Tang* (2012) In planta stage-specific fungal gene profiling elucidates the molecular strategies of Fusarium graminearum growing inside wheat coleoptiles. Plant Cell 24: 5159

[2] Y. Zhang, J. He, L-J. Jia, T-L. Yuan, D. Zhang, Y. Guo, Y. Wang, W-H. Tang* (2016) Cellular Tracking and Gene Profiling of Fusarium graminearum during Maize Stalk Rot Disease Development Elucidates its Strategies in Confronting Phosphorus Limitation in the Host Apoplast. PLoS Pathogens 12: e1005485

Molecular coevolution of *Magnaporthe oryzae* pathogen and rice

Ryohei Terauchi
Iwate Biotechnology Research Center, Kitakami, Iwate, Japan
Kyoto University, Muko, Kyoto, Japan

Rice blast caused by *Magnaporthe oryzae* is the most devastating disease of rice worldwide. To understand the interactions between *M. oryzae* and rice, we have been studying three sets of pathogen avirulence effector gene (*AVR*) and host *R*-gene: *M. oryzae AVR-Pia*/ rice *Pia*, *AVR-Pii*/*Pii* and *AVR-Pik*/*Pik*. In all three cases, paired *NLR* genes function together to recognize an AVR; *Pia* consists of *RGA4* and *RGA5*, *Pii* consists of *Pii-1* and *Pii-2*, and *Pik* consists of *Pik-1* and *Pik-2*. RGA5 and Pik-1 harbors HMA integrated domain (ID) whereas Pii-2 contains NOI ID. In this paper, I will overview the three molecular interactions and focus on the possible molecular co-evolution of *AVR-Pik*/*Pik*.

Structures and processes impacting on the microbial colonisation of plants

Sebastian Schornack
University of Cambridge, Sainsbury Laboratory (SLCU), sebastian.schornack@slcu.cam.ac.uk

The study of plant-biotic interactions has unravelled important immunity mechanisms which restrict microbial invasion. Conceptually, successful pathogens and symbiotic plant colonising microbes suppress immunity. They also exploit or benefit from additional host mechanisms for their entry and establishment. Remarkable examples for broad host microbes are *Phytophthora palmivora* oomycetes which can infect hundreds of host species and diverse organs and tissues. Arbuscular Mycorrhiza fungi establish symbiotic interactions with the majority of all land plants and species of Rhizobacteria can form a nitrogen fixing nodule symbiosis in most legumes.

By utilising these microbes and their interactions with legumes, tobacco, barley and Arabidopsis we aim to discover, understand and modulate general plant mechanisms for microbial colonisation.

Time-resolved dual transcriptomics of *P. palmivora* root colonisation has helped us to identify infection relevant induced microbial effector proteins as well as early induced plant genes involved in microbial sensing and signalling. Furthermore, we identified and characterised a conserved eukaryotic protein which impacts on microbial entry by altering biochemical and physical properties of the plant cell wall. These findings add to our understanding of common and specific plant colonisation mechanisms and may provide alternative strategies for quantitative plant disease resistance.

Metabolomics and proteomics in *Magnaporthe oryzae*

Shuang Liang, Huan-Bin Shi, Xue-Ming Zhu, Lin Li, Xiao-Hong Liu, Fu-Cheng Lin*
State Key Laboratory for Rice Biology, Biotechnology Institute, Zhejiang University, Hangzhou
**Corresponding author: fuchenglin@zju.edu.cn*

Magnaporthe oryzae, the causal agent of rice blast, has been chosen as a model to study the interaction between fungi and plants. Common to many other plant pathogenic fungi, *M. oryzae* elaborates a signature penetration structure, the appressorium, to infect its host. Autophagy is a conserved and complex process in organisms and plays vital roles in plant pathogenic fungi, impacting growth, morphology, development, and pathogenicity. In our research, the metabolomics and proteomics had been expoled in *M. oryzae*. We detected the detailed differential chemical compounds between autophagy-deficienct mutants and the wild-type strain Guy11. At present, GC-MS, LC-QTOF and nano-LC-TripleTOF were used to analyze the significantly different metabolites and proteins between the wild-type strain Guy11 and the autophagy-deficient mutant Δ*Moatg1*. As a result, 34 metabolites related to autophagy were detected in the mycelium, including sugar, amino acids and glycerophospholipid, and so on.

Genetic and molecular dissection of susceptibility of *Arabidopsis thaliana* to the oomycete pathogen *Phytophthora parasitica*

Weixing Shan, Guangjing Fan, Tingting Li, Fengyan Deng, Qiaona Pan, Yang Yang, Xiaoyu Qiang, Yu Du

State Key Laboratory of Crop Stress Biology for Arid Areas and College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100

wxshan@nwafu.edu.cn

The outcome of interactions between plants and pathogens can be typically described as incompatible and compatible, both are the result of complex interplay between many factors of both partners. Compared with the knowledge of incompatible interaction in which pathogen avirulence genes and corresponding plant disease resistant genes are involved, little is known on the compatible interaction. To identify genes involved in plant susceptibility to infection by oomycete pathogens, we employed the *Arabidopsis thaliana-Phytophthora parasitica* as a model oomycete pathosystem and screened for *A. thaliana* T-DNA insertional mutants. This led to the identification of a set of Arabidopsis mutants resistant to *P. parasitica* infection. We also used conserved *Phytophthora* RXLR effectors to identify their host targets that mediate plant susceptibility. Characterization of *Arabidopsis* mutants and effector targets led to the identification of several different genes that were further confirmed host susceptible factors using multiple approaches to be negatively regulating plant disease resistance to *P. parasitica*. We present in this report genetic and molecular evidence for the involvement of diverse genes in plant immunity. Characterization of the identified *Arabidopsis* mutants will facilitate improved understanding of genetic network that mediates plant susceptibility to oomycete pathogens.

Acknowledgement: This work was supported by National Natural Science Foundation of China (#31125022 and #31561143007) and the Programme of Introducing Talents of Innovative Discipline to Universities (project 111) from the State of Administration for of Foreign Experts Affairs.

An oomycete plant pathogen reprograms host pre-mRNA splicing to subvert immunity

Jie Huang[1], Lianfeng Gu[2], Ying Zhang[1], Tingxiu Yan[1], Guanghui Kong[1], Liang Kong[1], Baodian Guo[1,3], Min Qiu[1], Yang Wang[1], Maofeng Jing[1], Weiman Xing[3], Wenwu Ye[1,6], Zhe Wu[4], Zhengguang Zhang[1,6], Xiaobo Zheng[1,6], Mark Gijzen[5], Yuanchao Wang[1,6], Suomeng Dong*[,1,6]

1. Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095.

2. Basic Forestry and Proteomics Center, Haixia Institute of Science and Technology (HIST), Fujian Agriculture and Forestry University, Fuzhou 350002.

3. Shanghai Center for Plant Stress Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201602.

4. Department of Biology, South University of Science and Technology of China, Shenzhen, Guangdong 518055.

5. Agriculture and Agri-Food Canada, London, Ontario, N5V 4T3, Canada.

6. Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), 210095.

** corresponding author: Suomeng Dong (smdong@njau.edu.cn)*

The process of RNA splicing influences many physiological processes including plant immunity. However, how plant parasites manipulate host RNA splicing process remains unknown. Here we demonstrate that PsAvr3c, an avirulence effector from oomycete plant pathogen *Phytophthora sojae*, physically binds to and stabilizes soybean (*Glycine max*) serine/lysine/arginine rich proteins GmSKRPs *in vivo*. The SKRPs are novel proteins that associating with spliceosome components, and are plant negative regulators for *Phytophthora* infection. Analysis by RNA-seq data indicates that differential splicing of 400 soybean genes, including defense related genes, are altered in GmSKRP1 and PsAvr3c over-expressing lines compared to control plants. Representative splicing events mediated by GmSKRP1 and PsAvr3c are tested by infection assays or by transient expression in soybean plants. Our results show that a plant pathogen effector can reprogram host RNA splicing to promote disease, and we propose that pathogens evolved such strategies to defeat host immune systems.

Novel superfamilies of effector proteins with modular structures drive host adaptation and speciation in the basal ascomycete genus *Taphrina*

Huiquan Liu[a], Yimei Zhang[a], Qiang Zhang[a], Manli Sun[a], Shijie Zhang[a], Qinhu Wang[a], Zhongshou Wu[a], Jin-Rong Xu[b]

[a] *State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100.*

[b] *Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. USA.*

liuhuiquan@nwsuaf.edu.cn

The basal ascomycetes in genus *Taphrina* have strict host-specificity and coevolution with their host plants, making them appealing models for studying genomic basis of speciation, a fundamental issue in biology. We therefore compared genome features of 11 strains from 8 species that have distinct host ranges. All *Taphrina* species have small genomes of 12.0-15.7 Mb with variable repeat contents of 0.86%-8.26%, and high gene density. Their chromosome number and size vary both between and within species. They possess a fast-speed subgenome that serves as a cradle for host adaptation and infection. At least two superfamilies of candidate secreted effector proteins (CSEPs) with modular structures unique to *Taphrina* were identified and found to have undergone extensive expansion and contraction in different species. CSEP genes are commonly organized in gene clusters that form distinct AT-rich isochore-like regions in *Taphrina* genomes, which may be related to epigenetic regulation of gene expression. Furthermore, most CSEPs are up-regulated during plant infection and they evolve faster than other secreted proteins. In addition to displaying signatures of positive selection, functional characterization of selected CSEP genes confirmed their roles in suppression of plant defense responses. Overall, our results showed that rapidly evolving CSEP superfamilies in the fast-speed genome have play important roles in adaptation and speciation of *Taphrina* pathogens, possibly by affecting various aspects of host-pathogen interactions.

Acknowledgement: This work was supported by the Foundation for the Author of National Excellent Doctoral Dissertation of PR China (grant no. 201471).

Disruption of the interfacial membrane leads to *Magnaporthe oryzae* effector re-location and lifestyle switch during rice blast disease

Chang-Hyun Khang, and Kiersun Jones, Jie Zhu, Cory Jenkinson, Dong Won Kim
Deptartment of Plant Biology, University of Georgia, Athens, GA 30602, USA
ckhang@uga.edu

To cause the devastating rice blast disease, the hemibiotrophic fungus *Magnaporthe oryzae* produces invasive hyphae that are enclosed in the plant-derived interfacial membrane, known as the extra-invasive hyphal membrane (EIHM), in living rice cells. Little is known about when the EIHM is disrupted and how the disruption contributes to blast disease. Here we show that EIHM disruption depends on the hyphal growth stage in first-invaded cells of susceptible rice. Our approach utilized GFP secreted from invasive hyphae as an EIHM integrity reporter. Secreted GFP (sec-GFP) accumulated in the EIHM compartment but appeared in the host cytoplasm when the EIHM integrity was compromised. Live-cell imaging coupled with sec-GFP and various fluorescent reporters revealed that the loss of EIHM integrity leads to shrinkage and eventual collapse of the host vacuole, and subsequently, the invaded cell died in a contained manner due to the presumed closure of plasmodesmata. We report that the EIHM disruption and the host cell death are landmarks that define three distinct infection phases (early biotrophic, late biotrophic, and transient necrotrophic phases) within the first-invaded cell before biotrophy is reestablished in subsequently invaded cells. *M. oryzae* effectors exhibited infection phase-specific localizations, including entry of the apoplastic effector Bas4 into the host cytoplasm through the disrupted EIHM during the late biotrophic phase. Understanding how the infection phase-specific cellular dynamics is regulated and linked to host susceptibility will offer potential targets that we can exploit to control blast disease.

Acknowledgement: This work was supported by the Agriculture and Food Research Initiative competitive grants program, Award number 2014-67013-21717 from the USDA National Institute of Food and Agriculture.

Cross protection between TMV and HLSV, TMV and its mutant with an internal poly(A) tract

Sek-Man Wong [a, b, c]

[a] *Department of Biological Sciences, National University of Singapore, Singapore 119543,*

[b] *Temasek Life Sciences Laboratory, Singapore 117604,*

[c] *National University of Singapore Suzhou Research Institute, Suzhou Industrial Park, Jiangsu.*

Email: dbswsm@nus.edu.sg

Cross protection is the phenomenon through which a mild strain virus suppresses symptoms induced by a closely related severe strain virus in infected plants. *Hibiscus latent Singapore virus* (HLSV) and *Tobacco mosaic virus* (TMV) are species within the same genus of tobamovirus. HLSV protects *Nicotiana benthamiana* against TMV-U1 strain, inducing mild symptoms instead of severe systemic necrosis caused by TMV infection. Both real time RT-PCR and western blot shown that HLSV accumulation increased in cross protected plants. Microarray was conducted to monitor global host genes during cross protection. A total of 1,938 genes were changed in response to HLSV infection and 1,826 genes were changed in response to cross protection by HLSV. By a 20-day time course, host genes *NbVPE1a, NbACO*, *NbSAR8.2m*, *NbWIPK, NbWRKY8*, *NbTOM1* and *NbHsp101*, corresponded to TMV accumulation. Other host genes, *NbARP1*, *NbCaM3*, *NbCP2* and *NbPI*, were up-regulated only by HLSV. Results also indicated that *NbTOM1* is competed by HLSV and TMV during mixed infection. *NbCP2* was up-regulated by HLSV and facilitated HLSV in viral accumulation. With the overexpression of *NbCP2*, both HLSV and TMV accumulation increased in single virus infection. The overexpression of NbCP2 can only facilitate HLSV accumulation in HLSV+TMV 100:1 mixed infection. At the same time, the silencing of *NbCP2* can be overcome by HLSV infection. *NbCP2* can be up-regulated by HLSV infection in cross protected plants. Taken the serial amount of TMV inocula in large scale of cross protection and HLSV/TMV accumulation shift in cross protected plants, these results indicated that the up-regulation of *NbCP2* facilitated HLSV accumulation in cross protected plants and the reduced accumulation of TMV delayed cell death. The expressions of host defense response genes were up-regulated after TMV infection, as compared to TMV-43A-infected plants. Host defense response to TMV-43A infection was lower than that to TMV. The absence of UPD might contribute to the reduced host response to TMV-43A.

Acknowledgement: We thank Ministry of Education, Republic of Singapore for financial support through Tier 1 research grant R-154-000-A34-114.

References:

[1] S. Guo, S-M. Wong (2017). Virus Research, in press. http://dx.doi.org/10.1016/j.virusres.2017.01.004.

[2] Y. Wen, GX-Y. Lim, S-M. Wong (2013). **PLoS ONE,** 8: e73725. doi:10.1371/journal.pone.0073725.

Small RNA-Based antiviral immunity in rice

Jianguo Wu[1,] and Yi Li [2]

[1]*State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Province Key Laboratory of Plant Virology, Institute of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou, 350002.*

[2]*State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing 100871.*

E-mail: wujianguo81@126.com; liyi@pku.edu.cn

As well established from many previous studies, small RNA-mediated RNA silencing is a widely conserved mechanism of host defense against many pathogens in plants and other organisms. In higher plants, where more than 90% of viruses have a highly replicating RNA genome, the RNA-based nature, high efficiency and trans-acting property of RNA silencing make it a potent defense mechanism against virus infection. MicroRNAs (miRNAs) have emerged as a critical role in plant defense and viral offense systems. However, a role for host miRNAs in defense against viral infection in plants has thus far remained elusive.

Rice and other cereals such as maize, barley, wheat, all monocots, are among the most important food crops worldwide. Viral pathogens are a major and constant threat to the productivity of these and other crops throughout the world. A single crop is often susceptible to infection by multiple viruses. Thus far, there are no effective strategies to empower any of these cereal crops with resistance against infection by multiple viruses. In this manuscript, we report our discovery that monocot-unique ARGONAUTE 18 (AGO18) is specifically induced by viral infection and up-regulates AGO1 and AO (L-ascorbate oxidase) in antiviral response. Over-expression of AGO18 in transgenic rice confers strong and broad-spectrum resistance to evolutionarily diverse viruses. Mechanistically, we found that AGO18 competes with AGO1 to bind miR168 and miR528 an established negative regulator of AGO1 and ROS (Reactive oxygen species) homeostasis. Our results support a novel model that AGO18 sequestration of miR168 and miR528 leads to elevated levels of AGO1 and ROS to embark on a robust defense response against viral infection. Therefore, understanding the mechanisms of antiviral defense in rice will help to control viral epidemics and guarantee food security.

Tombusvirus hijacks phospholipid PE for efficient viral replication.

Kai Xu [ab], Peter Nagy [b]
[a] *College of Life Sciences, Nanjing Normal University, Nanjing*
[b] *Department of Plant Pathology, University of Kentucky, Lexington, USA*
Corresponding: xukai@njnu.edu.cn

Positive strand RNA viruses utilize host sub-cellular membranes to build viral replication compartments (VRCs). Role of different lipid components in viral replication was less understood. Tomato bushy stunt virus (TBSV), in the *Tombusvirus* genus, is a plant positive strand RNA virus with ~4.7k nt long RNA genome. TBSV initiate viral replication on host peroxisomal membranes. By using a liposome based *in vitro* viral replicase assemble assay, phosphatidylethanolamine (PE) was identified as the major lipids for TBSV replication. PE was shown to be enriched at the VRCs by hijacking PE-rich Rab5-endosomes through the actin network. Inhibition of PE biosynthesis or disrupting Rab5 function both inhibited replication of TBSV or another tombusvirus Carnation italian ringspot virus (CIRV). Increasing cellular PE content by disrupting PE methyltransferase *CHO2* greatly increased TBSV or CIRV replication. Biochemical assay showed that TBSV replication protein p33 could bind to PE membranes, and more importantly TBSV RNA genome could associate to viral replication proteins that associated to PE membranes. By using Rab5 GTPase labeled endosomal membrane marker, TBSV induced donut-like replication compartments could be visualized under the laser-scanning confocal microscopy for the first time, providing valuable information on the biogenesis and morphology of VRCs caused by viral invasion.

Regulation of the growth-defense balance in plants: lessons learned from virulence effectors

Li Yang[abc], Paulo José Pereira Lima Teixeira[bc], Surojit Biswas[bc], Omri M. Finkel[bc], Yijian He[bc], Isai Salas-Gonzalez[bc], Marie E. English[bc], Petra Epple[bc], Piotr Mieczkowski[b], Jeffery L. Dangl[bc]

[a]*Department of Plant Pathology, University of Georgia*

[b]*Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*

[c]*Howard Hughes Medical Institute*

bioyangli@gmail.com

Activated defense is usually at the expense of fast growth, which is known as the ‘growth-defense tradeoff’. Plants strategically regulate the output of the phytohormone signaling network to balance these two vital processes. Effector proteins derived from pathogens can physically interact with host cellular components to attenuate defense response or redirect resource allocation. TCP transcription factors, which regulate multiple developmental processes, were recently discovered as convergent targets of effectors from three different pathogens: *Golovinomyces orontii*, *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis*. I will discuss how virulence effectors fine tune the growth-defense crosstalk by precisely manipulating a subset of the Jasmonate (JA) regulon, to avoid pleiotropic host responses.

Activation of a plant NLR complex through heteromeric association with an autoimmune risk variant of another NLR[1]

Diep T. N. Tran[a], Eui-Hwan Chung[b,c], Rebecca Schwab[a], Jeffery L. Dangl[b,c], Detlef Weigel[a], Eunyoung Chae[a]

[a]*Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany*

[b]*Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA*

[c]*Howard Hughes Medical Institute, University of North Carolina, Chapel Hill, NC 27599, USA*

eunyoung.chae@tuebingen.mpg.de

When independently evolved immune receptor variants meet in hybrid plants, they can activate immune signaling even in the absence of non-self recognition. Such autoimmune risk alleles have recurrently evolved at a complex NLR locus in *A. thaliana*, *DM2*. One of these risk alleles activates signaling in the presence of a particular variant encoded at another NLR locus, *DM1*. We show that the risk variants of DM1 and DM2 NLRs signal through the same pathway that other plant NLRs deploy upon non-self recognition. This involves heteromeric association of DM1 and DM2, with the P-loops of both proteins contributing to signaling. In the absence of the DM2 risk variant, DM1 forms inactive homo-oligomers. Activation of the DM1 complex relies on conformation of the heteromerizing NLR. Autoimmunity triggered by cooperative action of this NLR pair thus suggests that activity of a signaling complex depends on the sum of activation potential of partner NLRs.

References:

[1] Tran DTN, Chung EH, Habring-Müller A, Demar M, Schwab R, Dangl JL, Weigel D, Chae E. (2017) *Current Biology* 2017 Apr;27(8):1148-1160.

The molecular mechanism of suppressing host induced gene silencing by *Phytophothora effector*

Yongli Qiao

Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081

Phytophthora contains important plant pathogens that cause devastating diseases of crops. *Phytophthora* pathogens deliver an array of effectors to alter host physiology and defense responses. Here, we show that two effectors (Avh18 and Avh146) from the oomycete plant pathogen *Phytophthora sojae* suppress RNA silencing in plants by inhibiting the biogenesis of small RNAs. Ectopic expression of *Phytophthora* suppressors of RNA silencing 1 and 2 (PSR1 and PSR2) enhances plant susceptibility to both a virus and *Phytophthora*, and PSR1 can bind to an evolutionarily conserved RNA helicase domain protein in plants. This protein, designated PSR1-Interacting Protein 1 (PINP1), regulates the accumulation of both microRNAs and endogenous small interfering RNAs in Arabidopsis, and silencing of PINP1 leads to developmental defects and hypersusceptibility to *Phytophthora* infection. These phenotypes are reminiscent of transgenic plants expressing PSR1, supporting PINP1 as a direct virulence target of PSR1. A similar function of PINP1 homologous genes in development and immunity was also observed in *Nicotiana benthamiana*. Taken together, these results indicate PINP1 as a previously unidentified component of RNA silencing that regulates distinct classes of small RNAs in plants.

Plant resistance function mediated by AGO2 and AGO2-binding protein

Xiaoming Zhang[a]
[a] *State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing*
zhangxm@ioz.ac.cn

Small RNAs (sRNAs) are 20 to 30 nucleotide (nt)-long noncoding RNA molecules that regulate gene expression in eukaryotes through a process generally termed RNA silencing. RNA silencing machinery plays critical roles in plant resistance to viruses and other pathogens. To determine the function of RNA silencing in plant resistance to bacteria, we inoculated the *Arabidopsis* with *Pst* DC3000 and detected the accumulation of small RNAs (sRNAs) at genome level by deep sequencing. We found that the accumulations of the miRNA, nat-siRNA, NBS-LRR siRNA and other sRNAs are specifically regulated by pathogen infection. To understand the mechanism of plant immunity mediated by RNA silencing, we studied the function of AGOs in plant immunity and uncovered that miR393b* loaded into AGO2 can increase plant immunity by modulating the secretion of immunity proteins. Our further studies showed that AGO2 prefers sRNA duplexes without central mismatches while AGO1 can load sRNA duplexes with central mismatches. AGO proteins need associating with other proteins to perform regulation functions. Although the functions of AGOs have been practically uncovered, the components interact with AGOs are largely unknown. We then studied the proteins interact with AGO2 and found one of the candidates, AGOBP1, can physically interact with AGO2. This protein can change the loading efficiency of sRNAs into AGO2 which finally alters plant resistance function to pathogens.

References:

[1]Huang J., Yang M, Lu L.,* Zhang X* (2016). Frontiers in Microbiology. 7.
[2]Huang, J., Yang, M., Zhang, X* (2016). J Integrative Plant Biology. 58(4):312-27

Characterization of genes mediate rhizobial infection in *Lotus japonicus*

Xiangxiao Kong[1], Xiaolin Li[1], Liping Qiu[1], J. Allan Downie[2], and Fang Xie[1]

[1] *National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

[2] *John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom*

The rhizobial infection in legume is a multifaceted developmental process that is driven by the bacteria but is ultimately under the control of the host. For most legumes, the bacteria invade to the host plants from the tunnel-like infection thread to the cortex nodule primordia. To understand the molecular mechanisms of infection thread formation we performed a forward genetics screening for the mutants abolished the infection thread formation in *L. japonicus*. We reported that Nod factor induced cell wall degradation and actin cytoskeleton rearrangement genes expression and mediate infection thread initiation and formation. Here we identified a putative receptor-like kinase RINRK, which specific required for rhizobia infection but not nodule organogenesis. *RINRK* was induced by Nod factors and specific expressed in rhizobia infected root hairs and in young uninfected nodules. Moreover, RINRK interact with Nod factor receptors NFR1 and NFR5 *in vivo* and *in vitro*. Consistent with role in rhizobia infection we found that *RINRK* is required for earlier nodulin genes induction. We propose that RINRK is a novel component of the Nod factor receptor complex, specifically mediating processes necessary for rhizobia infection.

Pathogen recogniton by the Rcr3/Cf-2 perception system of tomato

Jiorgos Kourelis[a], Shivani Malik[a], Judith K. Paulus[a], Anja Hörger[a], Sophien Kamoun[b] and Renier A. L. van der Hoorn[a]

[a]*Plant Chemetics Laboratory, University of Oxford, South Park Road, Oxford, UK;* [b]*The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich, UK;* renier.vanderhoorn@plants.ox.ac.uk

The *Cf-2* resistance gene of tomato encodes a receptor-like protein and confers recognition of Avr2, a small cysteine-rich protein secreted by the fungal tomato pathogen *Cladosporium fulvum* [1]. Perception of Avr2 by Cf-2 triggers the hypersensitive response (HR) and requires Rcr3, a secreted papain-like cysteine protease of tomato [2]. Avr2 inhibits Rcr3, indicating that Rcr3 is a virulence target of Avr2, and that Cf-2 acts by guarding Rcr3 [3]. However, Avr2 also inhibits Pip1, a close paralog of Rcr3 [4], and the depletion of *Pip1* in the absence of *Cf-2* caused more increased susceptibility than *Rcr3* depletion, suggesting that Pip1 is the operative target of Avr2 and Rcr3 acts as a decoy [5]. Rcr3 is also inhibited by the cystatin-like EpiC1 and EpiC2B of the oomycete pathogen *Phytophthora infestans* [6], and by the chagasin-like Cip1 of the bacterial pathogen *Pseudomonas syringae* [7], but the Rcr3-EpiC/Cip1 interactions do not trigger HR with Cf-2. These 'stealthy effectors' might evade recognition because the interaction with Rcr3 is relatively weak. By contrast, EpiCs have higher inhibitory activity to C14, a more distantly related protease [8], whilst Cip1 inhibits more strongly C14 and Pip1, a paralog of Rcr3 [7]. Our current studies show that: i) co-expression of Avr2/Rcr3/Cf-2 induces HR in *N. benthamiana*; ii) Rcr3 orthologs are conserved in Solanaceous species and can trigger HR with Avr2 and Cf-2; and iii) functional differences between Rcr3 and Pip1. Our studies highlight a new mechanism of Rcr3 inhibitor perception by Cf-2 that can be exploited to broaden resistance specificity by Cf-2 to other pathogens and to other plants.

References:

[1] Dixon et al., *Cell* 1998; [2] Kruger et al. *Science* 2001; [3] Rooney et al., *Science* 2003; [4] Shabab et al., *Plant Cell* 2008; [5] Ilyas et al., *Curr. Biol*. 2014; [6] Song et al., *PNAS* 2009; [7] Shindo et al., *PLoS Pathog*. 2016; [8] Kaschani et al., *Plant Physiol*. 2011.

Actin assembly regulation in plant innate immunity

He Sun[1], Zhu Qiao[1], Khi Pin Chua[1], Alma Tursic[1], Xu Liu[4],Yong-Gui Gao[1,3], Xingliang Hou[4], Yuguang Mu[1], Yansong Miao[1,2*]

[1]*School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore*
[2]*School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637551, Singapore*
[3]*Institute of Molecular and Cell Biology, A*STAR, Singapore 138673, Singapore*
[4]*South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650*
yansongm@ntu.edu.sg

Regulating the turnover and rearrangement of actin cytoskeleton networks plays important roles in plant cells during plant development and plant defense responses. Rapid polymerization and depolymerization highly drive the dynamic reorganization of the actin cytoskeleton. Actin filament mediated vesicular trafficking delivers numerous signal transductions. An extensive set of actin-binding proteins (ABPs) function in a competitive and cooperative manner for actin assembly in plants[1, 2]. Recent studies have shed light on formin-profilin-actin complex's functionin plant actin polymerization, and their involvement in signal transduction and plant innate immune responses. Arabidopsis thaliana contains multiple isoforms of profilin, formin, and actin, whose partnership-specificity are important in regulating actin assembly at different physiological or pathological states. To understand the underlying mechanisms by which the actin nucleation complexes,formin-profilin-actin, are dynamically modulated to coordinate the plant immune response and the "growth-defense trade-off" phenomenon, we used in vitro biochemistry and in vivo cell biology approaches to study the formin-profilin-actinfunction in plants and during plant defense responses. Our works discovered novel regulatory roles in plant actin polymerization and actin-mediated plant growth during defense responses.

References:

1. Henty-Ridilla, J.L., et al., *The plant actin cytoskeleton responds to signals from microbe-associated molecular patterns*. PLoS Pathog, 2013. **9**(4): p. e1003290.
2. Day, B., et al., *The pathogen-actin connection: a platform for defense signaling in plants*. Annu Rev Phytopathol, 2011. **49**: p. 483-506.

Helper component proteinase of *Potato virus Y* is an avirulence determinant eliciting HR in potato carrying *Ny* gene

Yanping Tian[a,b], Shuwen Wang [b], Lu Wang [b], Yameng, Luan[b], Jari P.T. Valkonen[a]

[a] *Department of Agriculture Sciences, University of Helsinki, Latokartanonkaari 7, Helsinki, Finland.*

[b] *College of Plant Protection, Shandong Agricultural University, NO.61 Daizong Street, Tai'an City, Shandong Province*

Yanping.tian @sdau.edu.cn

Potato virus Y (PVY), the type member of genus *Potyvirus*, is one of the most common and destructive viruses infecting potato and other crops of *Solanaceae*. Different PVY strains have been identified based on the recognition between resistance genes and different PVY isolates. Identifying the avirulence genes recognized by the resistance genes is crucial understanding the interaction mechanisms between viruses and resistance genes. Study has revealed that PVY^C HCpro is the avirulence gene corresponding to *Nc* gene. Here, our results showed that HCpro of PVY^O could elicit the hypersensitive responses (HR) on the potato carrying Ny_{tbr} gene. Furthermore, the central domain of PVY^O HCpro is essential for induction of HR in presence of Ny_{tbr}, which is adjacent but not overlapped with the essential region for inducing necrosis responses in presence of *Nc* gene. High correlations between conserved motifs structure in predicted models and their ability to elicit HR in potato cultivars carrying Ny_{tbr} gene was found. Results from further constructed mutants confirmed the structure-function relationship between PVY^O and Ny_{tbr} gene. In together, our resluts indicated that three dimentional structure of HCpro is essencial for its function.

Detection of the plant parasite *Cuscuta reflexa* by tomato host plants

Markus Albert
Center for Plant Molecular Biology, University of Tuebingen, Tuebingen, Germany
markus.albert@zmbp.uni-tuebingen.de

Plant species of the genus *Cuscuta* are holoparasites which infect both dicotyledonous and monocotyledonous host plants. One notable exception is tomato, which is specifically resistant to *Cuscuta reflexa*. We discovered that tomato responds to a small, secondarily modified, proteinaceous factor occurring exclusively in various *Cuscuta* spp. with plant immune responses typically activated after the recognition of microbe-associated molecular patterns (MAMPs). We identified the tomato cell surface receptor-like protein CUSCUTA RECEPTOR 1 (CuRe1) as a critical component of the perception system for the parasite-associated molecular pattern. CuRe1 is sufficient to confer responsiveness to this Cuscuta factor and increased resistance to parasitic *C. reflexa* when heterologously expressed in otherwise susceptible host plants, such as *Nicotiana benthamiana*. In an ongoing project, we aim to decipher the structure and sequence of the yet unidentified parasitic molecular pattern that triggers defense via CuRe1. In general, our findings underline that plants recognize parasitic plants as non-self, similar to the perception of microbial or pest pathogens, and provide potential for engineering resistance to parasitic plants in crops.

Genetic and metabolic interactions between fungi and insects

Chengshu Wang
Shanghai Institute of Plant physiology and Ecology, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032
Email: cswang@sibs.ac.cn

In nature, there are about one thousand known fungal species capable of killing insects, most of which are ascomycetes. The species like *Metarhizium* spp. and *Beauveria bassiana* have been developed as environmentally friendly biocontrol agents against different insect pests and the genetically tractable systems to study the mechanisms of fungus-insect interactions. Phylogenetic analysis revealed that fungal entomopathogenicity is polyphyletic, so similar expansions of insect cuticle degrading proteases and chitinases reflect a convergent evolution during the arms race of fungus-insect interactions. Genome survey of different *Metarhizium* species with varied host range indicated that the specialist species with a narrow host range diverged first and then the transitional species followed by the generalists in association with the co-evolution of insect hosts [1]. To initiate pathogenic infections, insect fungi like *M. robertsii* evolved with divergent adhesins to mediate spore adherence to insect and plant surfaces, a mammalian perilipin-like protein to regulate the appressorium turgor pressure for insect cuticle penetration, a collagen-like protein and LysM effectors to camouflage cell wall components for evading host immunity. In addition, *in vivo* metabolomics analysis revealed the dynamics of small molecules were produced by both the fungi and insect hosts during fungal invasion of hosts. In particular, the small molecules such as the cyclodepsipeptide destruxins produced by *M. robertsii* [2] and dibenzoquinone oosporein by *B. bassiana* [3] could also be deployed by the fungi to inhibit host innate immune responses to facilitate fungal infection [4].

References:

[1]. Hu X, Xiao G, Zheng P, Shang Y, Su Y, Zhang X, et al. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. Proc Natl Acad Sci U S A. 2014; 111:16796-801.
[2]. Wang B, Kang Q, Lu Y, Bai L, Wang C. Unveiling the biosynthetic puzzle of destruxins in *Metarhizium* species. Proc Natl Acad Sci U S A. 2012; 109:1287-92.
[3]. Feng P, Shang Y, Cen K, Wang C. Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc Natl Acad Sci USA. 2015; 112:11365-70.
[4]. Wang CS, Wang SB. Insect pathogenic fungi: genomics, molecular interactions, and genetic improvements. Annu Rev Entomol. 2017; 62:73-90.

A novel meloidogyne effector, MgGPP, undergoes post-translational modification in host plants

Jinling Liao[1,2,3]
1 Laboratory of Plant Nematology, South China Agricultural University, Guangzhou,
2 Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou,
3 Guangdong Eco-Engineering Polytechnic, Guangzhou
jlliao@scau.edu.cn

Plant pathogen effectors can recruit the host post-translational machinery to mediate their post-translational modification (PTM) and regulate their activity to facilitate parasitism, but few studies have focused on this phenomenon in the field of plant-parasitic nematodes. In this study, we show that the plant-parasitic nematode *Meloidogyne graminicola* has evolved a novel effector, MgGPP, that is exclusively expressed within the nematode subventral esophageal gland cells and up-regulated in the early parasitic stage of *M. graminicola*. The effector MgGPP plays a role in nematode parasitism. Transgenic rice lines expressing MgGPP become significantly more susceptible to *M. graminicola* infection than wild-type control plants, and conversely, in planta, the silencing of MgGPP through RNAi technology substantially increases the resistance of rice to *M. graminicola*. Significantly, we show that MgGPP is secreted into host plants and targeted to the ER, where the N-glycosylation and C-terminal proteolysis of MgGPP occur. C-terminal proteolysis promotes MgGPP to leave the ER, after which it is transported to the nucleus. In addition, N-glycosylation of MgGPP is required for suppressing the host response. The research data provide an intriguing example of in planta glycosylation in concert with proteolysis of a pathogen effector, which depict a novel mechanism by which parasitic nematodes could subjugate plant immunity and promote parasitism and may present a promising target for developing new strategies against nematode infections.

Methylmalonate-Semialdehyde dehydrogenase mediated metabolite homeostasis essentially regulate conidiation, polarized germination and pathogenesis in *Magnaporthe oryzae*

Justice Norvienyeku[1,2#], Zhenhui Zhong[1,2#], Lili Lin[1,2#], Zonghua Wang[1,2,3*]

1. State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, 350002.

2. Fujian-Taiwan Joint Center for Ecological Control of Crop Pests and College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, 350002.

 # These authors contributed to this work equally.

3. College of Ocean Science Minjiang University, Fuzhou, 350108.

** To whom correspondence should be addressed. Email: wangzh@fafu.edu.cn.*

Plants generate multitude of aldehydes under abiotic and biotic stress conditions. Ample demonstrations have shown that rice-derived aldehydes enhance the resistance of rice against the rice-blast fungus *Magnaporthe oryzae*. However, how the fungal pathogen nullifies the inhibitory effects of host aldehydes to establish compatible interaction remains unknown. Here we identified and evaluated the *in vivo* transcriptional activities of *M. oryzae* aldehyde dehydrogenase (ALDH) genes.

Transcriptional analysis of *M. oryzae* ALDH genes revealed that the acetylating enzyme Methylmalonate-Semialdehyde Dehydrogenase (MoMsdh/MoMmsdh) elevated activities during host invasion and colonization of the fungus. We further examined the pathophysiological importance of *MoMSDH* by deploying integrated functional genetics, and biochemical approaches. *MoMSDH* deletion mutant Δ*Momsdh* exhibited germination defect, hyper-branching of germ tube and failed to form appressoria on hydrophobic and hydrophilic surface. The *MoMSDH* disruption caused accumulation of small branch-chain amino acids, pyridoxine and AMP/cAMP in the Δ*Momsdh* mutant and altered Spitzenkörper organisation in the conidia.

We concluded that *MoMSDH* contribute significantly to the pathogenesis of *M. oryzae* by regulating the mobilization of Spitzenkörper during germ tube morphogenesis, appressoria formation by acting as metabolic switch regulating small branch-chain amino acids, inositol, pyridoxine and AMP/cAMP homeostasis.

Plant-mediated interspecific competition between whiteflies

Shu-Sheng Liu
Email: shshliu@zju.edu.cn
Institute of Insect Sciences, Zhejiang University, Hangzhou 310058

Whiteflies of the *Bemisia tabaci* complex and other members in the family Aleyrodidae include some important crop pests worldwide. Many of the species often occur sympatrically and show niche overlap, where they have complex interspecific interactions including competition. The process of interspecific competition is dynamic and should be investigated in view of the intrinsic differences between species in the context of the environments where they interact. Host plant is a major environmental factor mediating interspecific relationships that may lead to competitive displacement of some species by the others, such as what would occur in biological invasions. In this presentation, first, I will show that competition trajectory and eventual outcome between competitors may vary depending on the host plant on which they live. Such host plant-mediated interspecific competition has been extensively observed under controlled conditions and further supported by circumstantial evidence from the field. I then try to explore the possible mechanisms of plant effects including effects on whitefly behaviour, survival and reproduction. In addition, I will try to touch on the mechanisms of apparent competition mediated by various factors including natural enemies, whitefly-transmitted viruses and human-mediated disturbance such as insecticide application and vegetation management. Finally, I will try to suggest some strategies to use multidisciplinary approaches, including ecological, behavioural, genomic, metabolic and proteomic approaches, to investigate the mechanisms underlying whitefly interspecific interactions including competition.

Resistance to cyst nematodes via altered α-SNAP housekeeping proteins

Andrew Bent, Adam Bayless, Ryan Zapotocny, John Smith, Derrick Grunwald
Department of Plant Pathology, University of Wisconsin - Madisoin, Madison, WI 53706 USA
afbent@wisc.edu

Alpha-SNAP and NSF are highly conserved housekeeping proteins of plants and animals that facilitate cellular vesicular trafficking by mediating SNARE bundle disassembly and re-use. We have discovered that plant polyploidy has allowed a unique mechanism of disease resistance to emerge, in which variant forms of alpha-SNAP exhibit incompletely dominant-negative activity that disrupts vesicle trafficking. This toxicity apparently disrupts the plant syncytium (the biotrophic interface that feeds cyst nematode pathogens), and a fine balance is required to achieve toxicity to pathogens while not imposing yield drag on the plant. Soybean cyst nematode is consistently the most damaging disease of U.S. soybeans, one of the world's main food crops. The soybean *Rhg1* (resistance to *Heterodera glycines*) locus is widely used by plant breeders as the best available disease resistance locus to reduce the damage caused by SCN. We discovered about five years ago that the relevant genes at *Rhg1* do not encode proteins normally associated with disease resistance. Instead, resistance is mediated by copy number variation of three genes at the *Rhg1* locus, including two types of alleles encoding proteins similar to alpha-SNAP. We more recently discovered that the *Rhg1* resistance-associated alpha-SNAPs are defective proteins that bind less well to NSF, disrupt SNARE/NSF complexes, and disrupt vesicle trafficking. The proteins are cytotoxic when overexpressed. SCN-resistant soybeans carry genes at other loci that encode wild-type alpha-SNAPs, which compensate for the presence of *Rhg1*. The *Rhg1*-encoded defective alpha-SNAP variants are highly expressed at the nematode feeding site. Apparently, soybeans use this toxic alpha-SNAP to disrupt the function of soybean syncytium cells at the nematode feeding site, and thereby disrupt nematode growth and reproduction. We continue to discover and will report on additional mechanistic components of this novel and economically very important plant disease resistance trait, and have also gained evidence that *Rhg1*-mediated resistance can function in other plant species against other cyst nematode species.

Insect pathogenic fungus recruits the gut bacteria to promote mosquito killing

Ge Wei [a,b,#], Yiling Lai [a,#], Guandong Wang [a,b,#], Sibao Wang [a,*]

[a] *Key Laboratory of Insect Developmental and Evolutionary Biology, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, 300 Feng Lin Road, Shanghai*

[b] *University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing*

E-mail: sbwang@sibs.ac.cn

Mosquitoes transmit a wide range of pathogens that cause diseases such as malaria, dengue, yellow fever, and Zika, which have a devastating impact on human health [1]. Efforts to control the mosquito-borne diseases are hampered by increased resistance of vectors to chemical insecticides. The entomopathogenic fungus *Beauveria bassiana* provides an attractive alternative to chemical insecticides, and is equally effective against insecticide-resistant and insecticide-susceptible mosquitoes [2]. The insect gut microbiota plays crucial roles in modulating host immune homeostasis and the interactions between the host and intestinal pathogens [3]. Unlike viruses, bacteria and parasites, which need to be ingested to cause disease, entomopathogenic fungi infect insects through the cuticle and proliferate in the hemolymph [4]. However, possible interactions between the gut microbiota and entomopathogenic fungi are unknown.

Here we show that *B. bassiana* interacts with the gut microbiota to accelerate mosquito death. After topical fungal infection, mosquitoes with gut microbiota die significantly faster than mosquitoes without microbiota. Furthermore, fungal infection causes dysbiosis of mosquito gut microbiota with a significant increase in the gut bacterial load and a significant decrease in the bacterial diversity. In particular, the opportunistic pathogenic bacterium *Serratia marcescens* overgrows in the midgut and translocates to the hemocoel, which promotes fungal killing of mosquitoes. We further reveal that fungal infection down-regulates antimicrobial peptides and dual oxidase (Duox) expression in the midgut. Duox down-regulation in the midgut is mediated by secretion of the toxin oosporein from *B. bassiana*. Our findings reveal the important contribution of the gut microbiota in *B. bassiana*-killing activity, providing new insights into the mechanisms of fungal pathogenesis in insects, and yielding new strategies for biological control of mosquitoes.

Acknowledgement: This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant XDB11010500) and One Hundred Talents Program of the Chinese Academy of Sciences (Grant 2013OHTP01).

References:

[1] J.M. Medlock, et al. (2012). Vector Borne Zoonotic Dis, 12, 435.
[2] B.G.J. Knols, T. Bukhari, M. Farenhorst (2010). Future Microbiol, 5, 339.
[3] P. Engel, N.A. Moran (2013). FEMS Microbiol Rev, 37, 699.
[4] C. Wang, S. Wang (2017). Annu Rev Entomol, 62, 73.

Ascorbate oxidation level determines the hormone balance during the interaction between parasitic root-knot nematodes and rice

Tina Kyndt[a], Richard Singh[a,] Adelahu Mekonene[a], Ruben E.M. Verbeek[a], Ashley Haeck[a], Kristof Demeestere[a], Raimund Tenhaken[c], Shahid Siddique[b], Michael Frei[b a]

[a] *Faculty of Bioscience Engineering, UGent, Coupure links 653, 9000 Ghent, Belgium*

[b] *Rheinische Friedrich-Wilhelms-University of Bonn, INRES - Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany*

[c] *University of Salzburg, Kapitelgasse 4, 65020 Salzburg, Austria*

Ascorbate, vitamin C (AsA), is the major antioxidant buffer in the plant apoplast and becomes oxidized to dehydroascorbate during the oxidative burst phenomenon, caused by environmental challenges or pathogen attack. mRNA-seq studies on root knot nematode (*M. graminicola)*-induced galls and giant cells in rice roots, revealed that genes involved in ascorbate biosynthesis, oxidation/reduction and transport are differentially expressed, in comparison with uninfected rice root cells. This research was set-up to investigate the role of AsA in the interaction between plants and these sedentary nematodes. First, HPLC-UV measurements of AsA showed a specific accumulation of AsA in galls at 3 and 7 days after infection, while the remainder of the infected root system contained similar AsA levels as uninfected roots. Infection experiments on rice plants 24h after external application with 20 mM AsA and/or 20 U/ml Ascorbate oxidase, showed that oxidized AsA, but not reduced AsA, triggers a strong defense response against root knot nematodes. These data were validated by infection experiments on AsA biosynthesis mutants and AsA peroxidase mutants, all showing increased susceptibility. Hormone measurements (UHPLC-HRMS) on these mutants revealed disturbances in their hormonal profile, mainly in their root jasmonate and auxin levels, which could explain their enhanced susceptibility. Collectively, our data point to a role for oxidized ascorbate in hormone biosynthesis and plant defense in the course of the rice-root-knot nematode interaction.

Acknowledgement: The Special Research Fund of Ghent University is acknowledged for their financial support in the BOF13/GOA/030 project. The authors also acknowledge the financial support (AUGE/11/016) from the Hercules Foundation of the Flemish Government for the UHPLC-Q-ExactiveTM mass spectrometry equipment available at the EnVOC research group and used for hormone analysis. We also want to thank Lies Harinck and Lien De Smet for the technical support.

Engineering Plastid Genome for Pest Control by Expression of Insecticidal RNA in Plastids

Jiang Zhang [a]

[a] *College of Life Sciences, Hubei University, Wuhan, 430062*
Tel: (86) 15827188121 E-mail: zhangjiang@hubu.edu.cn

Plastid (chloroplast) genetic engineering has a great promise in plant biotechnology. It has several attractive advantages such as high-level of transgene expression owing to the polyploidy of the plastid genetic system, transgene containment via maternal inheritance and absence of gene silencing and pleiotropic effects. It has been demonstrated that double-stranded RNAs (dsRNAs) targeted against essential genes can trigger a lethal RNAi response in insect pests. Previously, we found chloroplasts can be capable of stably accumulating long dsRNAs, in which case dsRNA expression from the plastid genome provide better protection against Colorado potato beetle (CPB), a notorious agricultural pest, than dsRNA expression from the nuclear genome (Zhang et al., Science, 2015). Encouraged by this primary success, we aimed to employ this strategy to target another important insect pest, cotton bollworm (CBW). However, feeding transplastomic plants expressing dsRNA against essential genes of CBW did not cause the gene silencing and suppress the growth of CBW larvae. While no effective RNAi responses were observed in CBW, we found that a secreted RNA nuclease from midgut cell of CBW larvae can quickly degrade the ingested dsRNA, which might impede the effect of RNAi. From this findings, we proposed an approach how to escape the activity of this RNA nuclease and control the insects that showed no or less sensitivity to RNAi. In the end, I will summarize an updated working model of plastid-mediated crop protection and the potential application of this technology (Zhang et al., Trends in Biotechnol.).

Key Words: Crop protection, Plastid genetic engineering, RNAi, RNA nuclease

References:

[1] **Jiang Zhang**, Sher Afzal Khan, Claudia Hasse, Stephanie Ruf, David G. Heckel, Ralph Bock. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. **Science**, 2015, 347(6225): 991-994.
[2] **Jiang Zhang**, Sher Afzal Khan, David G. Heckel, Ralph Bock. Next generation insect-resistant plants: RNAi-mediated crop protection. **Trends in Biotechnology**. doi:10.1016/j.tibtech.2017.04.009

Elucidation of the dynamic interactions between plants and herbivorous insects

Chen Chun-Yu, Chen Fang-Yan, Song Wei-Meng, Wang Dan-Dan, Chen Xiao-Ya, Mao Ying-Bo

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai

Email: ybmao@sibs.ac.cn

The plant-insect co-evolution has been a driving force of present-day biodiversity on the earth. We are interested in the molecular mechanism of plant-insect competitions. According to their different feeding habits most insects can be divided into chewing and sucking groups. We found that the plant responses to chewing and sucking insects are quite different. The plant hormone Jasmonate (JA) plays an important role in defense against chewing insects. In Arabidopsis the JA signaling is more active in young plants, and the miR156-targeted SPLs, a master regulator of plant phase transition, is responsible for the deterioration of JA response at maturation stage. However, defense metabolites accumulate constitutively, replenishing the arsenal along with plant growth and development. On the other side, insects have a rich array of P450s, which play a fundamental role in resistance or tolerance to phytoalexins and agrochemicals, as evidenced by our plant-mediated RNAi assay. Besides, insect oral secretions may contain components that function as effectors to interfere with host defense. Data obtained from the investigation will enrich our knowledge on the mechanisms of plant-insect interaction and contribute to development of new strategies for pest control.

References:

[1] Y.B. Mao, Y.Q. Liu, D.Y. Chen, F.Y. Chen, X. Fang, G.J. Hong, L.J. Wang, J.W. Wang, X.Y. Chen (2017). Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance. Nature Communications, 8, 13925.

[2] X.Y. Tao, X.Y. Xue, Y.P. Huang, X.Y. Chen, Y.B. Mao (2012) Gossypol-enhanced P450 gene pool contributes to cotton bollworm tolerance to a pyrethroid insecticide. Molecular Ecology, 21:4371.

A Transposon-associated mechanism of NLR immune receptor expression control in *Arabidopsis thaliana*

Thomas Eulgem [a], Tokuji Tsuchiya [b], Jianqiang Wang [a] and Yan Lai [a, c]

[a] *Department of Botany & Plant Sciences, Institute for Integrative Genome Biology, Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA*

[b] *Department of Applied Biological Sciences, College of Bioresource Science, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*

[c] *College of Life Sciences, Fujian Agriculture and Forestry University, No.15 Shangxiadian Road, Cangshan District, Fuzhou, Fujian, 350002*

thomas.eulgem@ucr.edu

Transposable elements (TEs) play critical roles in genome evolution by creating genetic and epigenetic variation. We showed that *COPIA-R7*, a TE inserted into the *Arabidopsis thaliana* NLR-type immune receptor gene *RPP7*, recruited the histone mark H3K9me2 to this locus. H3K9me2 is a suppressive epigenetic signal that transcriptionally silences TEs. At *COPIA-R7* this mark affects the choice between two alternative *RPP7* polyadenylation sites and, thereby, influences the critical balance between RPP7-coding and non RPP7-coding transcripts. As NLR genes are often organized in clusters containing TEs, recruitment of TE-associated regulatory mechanisms to NLR expression control is likely common. H3K9me2 levels at *COPIA-R7* and *RPP7* are controlled by EDM2, a nuclear localized PHD-finger protein. Besides EDM2, we identified additional components contributing to epigenetically-controlled alternative polyadenylation. These and further examples for TE-mediated NLR expression control will be described at the meeting.

Acknowledgement:

Our work on EDM2 has been supported by the following grants from the National Science Foundation of the United States: IOS-0724838, IOS-1052556 and MCB-1330905.

Reference:

[1] T, Tsuchiya, T, Eulgem (2013). Proc Natl Acad Sci USA, 110, E3535

Stomatal opening confers immunity to bacterial leaf blight in rice

Dandan Zhang, Caijuan Tian, Jin-Long Qiu

State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101 E-mail: qiujl@im.ac.cn

Environmental conditions affect the outcome of host-pathogen interactions, but the interplay between biotic and abiotic factors is still poorly understood. Stomata are microscopic pores on the leaf surface surrounded by two specialized guard cells. They regulate gas exchange and water transpiration in response to environmental cues, and also function in innate immunity by limiting pathogen entry by active closing. This so-called stomatal defense can be triggered by microbial-associated molecular patterns (MAMPs). In turn, bacterial pathogens have evolved virulence factors that suppress stomatal closure and so permit entry and colonization of host tissue. However, the possibility that plants possess a counter-defense against the deleterious effect of effector-induced stomatal reopening has not been fully examined. Here we report evidence that, paradoxically, stomatal opening confers post-invasive immunity against bacterial pathogens in rice. We found that the resistance of rice ABA deficient plants to bacterial blight pathogen was at least partially due to increased stomatal opening. In addition, artificial opening of stomata conferred enhanced resistance to that pathogen. Moreover, a rice mutant with constitutively open stomata exhibited strong resistance to bacterial leaf blight. Our work reveals a novel role of stomata in plant immunity that provides new insight into the interactions between plant, pathogen and environment.

How endocytosis regulates immunity

Sara Ben Khaled, Michaela Kopischke, Gildas Bourdais, Lydia Rickett, Mary Tetlow and Silke Robatzek

The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

Our main research focus has been how cells defend themselves against infection of their extracellular space by microbial pathogens. Conserved microbial patterns activate cell surface receptors that are essential for host immunity, and induce their internalization. Over the years we have characterized immune receptor-mediated endocytosis, a process conserved across different receptor family members. Yet, the significance of receptor-mediated endocytosis in the regulation of immune signalling remained controversial. We recently have revealed that endocytosis of cell surface immune receptors is a mechanism for sustaining cellular responsiveness to microbial patterns, a process involved in microbe-induced stomatal closure, to confer long-term anti-bacterial immunity.

This work is supported by the Gatsby Charitable Foundation and a grant by the European Research Council (ERC).

The major leaf ferredoxin Fd2 regulates plant innate immunity in Arabidopsis

Mo Wang[a,b], Lu Rui[b], Haojie Yan[c], Hua Shi[b], Wanying Zhao[a], Jinshan Ella Lin[a], Kai Zhang[a], Joshua J. Blakeslee[a], David Mackey[a], Dingzhong Tang[b], Zhongmin Wei[d] and Guo-Liang Wang[a]

a. Department of Plant Pathology, Ohio State University, Columbus, Ohio, United States of America.

b. Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou 350002

c. State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing

d. Plant Health Care, Inc., Seattle, Washington, United States of America

E-mail of presenting author: wangmo108@163.com

Increasing numbers of studies have indicated that the chloroplast plays an important role in plant immunity. Various defense signaling molecules, including reactive oxygen species (ROS), salicylic acid (SA) and jasmonic acid (JA), are synthesized in the chloroplast [1]. Ferredoxins, the main distributors for transferring electrons to various acceptor systems in the chloroplast, contribute to redox regulation and antioxidant defense in plants [2]. However, their function in plant immunity is still unknown. In this study, we show that expression of the major leaf ferredoxin gene Fd2 is suppressed by Pseudomonas syringae pv tomato (Pst) DC3000 infection, and knocking out of Fd2 (Fd2-KO) [3] in Arabidopsis leads to more susceptibility to hemibiotrophic and biotrophic pathogens. Upon Pst DC3000 infection, Fd2-KO mutant accumulates higher levels of jasmonic acid and displays compromised salicylic acid-related immune responses. In addition, Fd2-KO also shows defects in pathogen-associated molecular patterns-triggered immunity. However, Fd2-KO shows enhanced R-protein mediated resistance to Pst DC3000/AvrRpt2 infection, suggesting that Fd2 plays a negative role in effector-triggered immunity. Furthermore, Fd2 interacts with FIBRILLIN4 (FIB4), a harpin-binding protein localized in chloroplasts [4]. Interestingly, Fd2, but not FIB4, localizes to stromules extended from chloroplasts. Taken together, our results demonstrate that Fd2 is an important component involved in plant immunity.

Acknowledgement: **We thank** R. Scheibe and H-B. Wang for providing Nossen and *Fd2-KO* seeds. This work is supported by a grant from Plant Health Care Inc.

References:

[1] M. S. Padmanabhan and S. P. Dinesh-Kumar (2010). Molecular plant-microbe interactions, 23, 1368.

[2]G. Hanke and P. Mulo (2013). Plant, cell & environment, 36, 1071.

[3]I. Voss, M. Koelmann, J. Wojtera, S. Holtgrefe, C. Kitzmann, J. E. Backhausen and R. Scheibe (2008). Physiologia plantarum, 133, 584.

[4]D. K. Singh, S. N. Maximova, P. J. Jensen, B. L. Lehman, H. K. Ngugi and T. W. McNellis (2010). Plant physiology, 154, 1281.

Signals and receptors involved in endosymbiosis

Jens Stougaard
Centre for Carbohydrate Recognition and Signalling, Department of Molecular Biology and Genetics, Aarhus University, Denmark

Legumes form endosymbioses with rhizobia and arbuscular mycorrhizal fungi, host endophytes, support a rhizosphere community and like other plants they are attacked by pathogens. One of the features enabling legumes to distinguish between these very different microbes appears to be a large family of LysM receptor kinases monitoring microbial signals. LysM receptor kinases have been shown to play a crucial role for perception of rhizobial Nod factors while others have not been studied. The function of some of these receptors in perception of signal molecules including lipochito-oligosaccharides, exopolysaccharides and chitin derived signal molecules and in plant-microbe interaction will be presented together with the genetic and biochemical methods used for functional studies. Biochemical approaches for detailed characterization of ligand – LysM receptor interactions will be presented and a model for legume recognition of rhizobial bacteria and pathogens will be discussed.

Unraveling the molecular mechanism of the NUDT6/NUDT7-mediated immune response in Arabidopsis

Hailei Zhang, Shoudong Zhang, Shuying Pang, Kaien Li, Yiping Wang, Yiji Xia
Department of Biology, Hong Kong Baptist University

Nudix proteins are a diverse subfamily of pyrophosphatases found in all classes of organisms. They have a wide range of substrates but most of them hydrolyze nucleoside diphosphate derivatives. They are involved in DNA repairing, removing toxic nucleotide derivatives, controlling the levels of metabolic intermediates, and RNA decapping. The Arabidopsis NUDT6 and NUDT7 have been found to act as negative regulators of plant immunity. The nudt6 nudt7 mutant displays severe growth retardation due to autoimmunity. The autoimmunity phenotype of nudt6 nudt7 is suppressed completely by eds1 and partially by npr1, eds5, and snc1-11. Interestingly, a high concentration of ammonium in growth medium can also suppress autoimmunity caused by nudt6 nudt7 and several other constitutive disease resistance mutations. The nudt6 nudt7 phenotype is epigentically unstable. In vitro enzymatic assay revealed that preferred substrates of NUDT6 and NUDT7 are nucleotide derivatives containing an ADP moiety, including NADH, NAD, and ADP-ribose (ADPR). Our Recent study has revealed that NUDT6 and NUDT7 function in RNA processing.

CDPK signaling in the activation of local and distal defense responses

Tiziana Guerra [a,b], Guido Durian [a,c], Katharina Hake [a], Heike Seybold [a,d], Na Liu [e], Benjamin Conrad [a], Silke Schilling [a], Dingzhong Tang [e], Tina Romeis [a]

[a]*Department of Plant Biochemistry, Dahlem Centre of Plant Sciences, FU Berlin, Berlin, Germany*
[b]*Leibniz Institute of Vegetable and Ornamental Crops, 14979 Großbeeren, Germany*
[c]*Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland*
[d]*Environmental Genomics, Botanical Institute, Christian-Albrechts University of Kiel, Kiel, Germany*
[e]*Fujian Agriculture and Forestry University, Fuzhou, Fujian*
tina.romeis@fu-berlin.de

Plant recognition of pathogens via receptor-mediated perception processes either in the context of PAMP-triggered immunity (PTI) or effector-triggered immunity (ETI) is fundamental to the activation of local and systemic defence responses and is prerequisite to plant resistance. Changes in the intracellular calcium concentration has been recognized as an early key feature in both scenarios. *Arabidopsis thaliana* calcium-dependent protein kinase CPK5 has been characterized by us as a crucial component which undergoes pathogen signal-derived calcium-sensing leading to enzyme activation [1]. Thereby, CPK5 plays a dual role in innate immune signalling: CPK5 mediates rapid defense signal propagation within 45 min of PAMP stimulation, and CPK5 is responsible for enhanced salicylic acid-dependent resistance to bacterial pathogens [2, 3]. Whereas NADPH-oxidase RBOHD-catalyzed ROS is required for rapid signal transmission to distal plant sites, the enzyme is dispensable for enhanced resistance in CPK5-overexpressing plants [2]. We investigated whether and how CPK5 is required for transcriptional reprogramming at distal plant sites. Furthermore, CPK5 function was assessed for transcription factor regulation during the onset and maintenance of systemic acquired resistance at distal sites of the plant (2 days after local stimulation). Remarkably, CPK5 also contributes to effector-triggered immune complex signalling in an *exo70b1* background, which requires the atypical NLR-receptor TN2 [4]. These data suggest different calcium/CPK-dependent signalling circuits in local and distal plant tissue during PTI and ETI.

To independently screen for *in vivo* phosphorylation substrates of CPKs in immune signalling we applied conditional *in vivo* phosphoproteomics. A transcription factor has been identified, which became transiently phosphorylated by the kinase within an intrinsic disordered region.

Phosphorylation, confirmed *in vivo* and *in vitro*, is necessary to trigger enhanced target gene expression, and the role of the CPK/TF link in PTI and ETI will be discussed.

References:
[1] H. Seybold et al. (2014). New Phytol. 204. 782-90.
[2] U. Dubiella, H. Seybold et al. (2013). Proc Natl Acad Sci U S A. 21. 8744-9.
[3] T. Romeis and M. Herde (2014). Curr. Opin. Plant Biol. 20. 1-10.
[4] N. Liu et al. (2017). Plant Cell. 29. 746-759.

The Rice Phosphate Transporter Protein OsPT8 Regulates Disease Resistance and Plant Growth

Zheng Dong, Li Li, Lihua Li, Liangying Dai, Wei Li*
Hunan Provincial Key Laboratory of Crop Germplasm Innovation and Utilization and College of Bio-safety Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128

**E-mail: liwei350551@163.com*

The absorption of nutrients and disease resistance are two indispensable physiological processes in plant. Whereas, it is still largely unknown whether there is cross talk between their molecular signaling pathways. BWMK1, a rice mitogen-activated protein kinase, is a regulator of disease resistance. In this study, we identified OsPT8, a member of phosphate transporters (PTs)Pht1 familyin the rice (*Oryza sativa*), as an interactor of BWMK1 and characterized the functions of OsPT8. Under low phosphate (Pi) conditions, *OsPT8* gene is induced and the *OsPT8* overexpression plants show shorter root length and higher plant height than the control plants. However, the differences in development between *OsPT8-OX* and the control plants are reduced upon the increase of Pi concentration. Interestingly, we found overexpression of *OsPT8* suppresses rice disease resistance against pathogens *Magnaporthe oryzae* and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Accordingly, the transcription level of the related resistance genes, such as *OsPR10* and *OsNPR1*, are inhibited in *OsPT8-OX* plants after inoculation of these pathogens. Moreover, *OsPT8* gene is induced, whereas, the *BWMK1* gene is suppressed after the pathogens inoculation. These results demonstrate that OsPT8 is involved in transduction of Pi signaling for development and negatively regulates disease resistance in rice.

Defense regulation by the chromatin-associated protein EDM2 in *Arabidopsis thaliana*

Tokuji Tsuchiya [a], Thomas Eulgem [b]

[a] *Department of Applied Biological Sciences, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*

[b] *Center for Plant Cell Biology, Institute for Integrative Genome Biology, Department of Botany & Plant Sciences, University of California, Riverside, CA 92521, USA*

tsuchiya.tokuji@nihon-u.ac.jp

Of central importance for pathogen resistance of plants are NLR (Nucleotide-binding domain and Leucine-rich Repeat) receptor proteins. Previous studies have reported that NLR protein and transcript levels are under tight control to allow maximal pathogen protection while avoiding spurious defense activation and detrimental autoimmunity. The nuclear localized Arabidopsis chromatin-associated protein EDM2 elevates transcript levels of the *NLR*-gene *RPP7*. Both EDM2 and RPP7 are required for race-specific immunity of Arabidopsis against the pathogenic oomycete *Hyaloperonospora arabidopsidis*. EDM2 has typical features of epigenetic regulators, such as nuclear localization signals and a module of 2 ½ atypical PHD-finger units. PHD-finger domain has been reported to bind to histones. Consistent with this, we found the PHD-finger units of EDM2 to specifically bind to *in vitro* histone H3 peptides with certain post-translational histone modifications (PHMs). Thus our data indicates EDM2 to decode epigenetic information to *NLR* transcriptional control. In addition, we identified EMSY-like chromatin remodelers and ubiquitin E3 ligases as EDM2-intracting proteins. At 5th ICBPI, we present new data on analyses in EDM2 and its interactors, linking effects of EDM2 on chromatin regulation to *RPP7* expression and thus resistance to *H. arabidopsidis*. EDM2 serves as a paradigm for the transcriptional regulation of *NLR*-genes in general, providing insights on the involvement of chromatin-associated processes in the tight control of *NLR*-gene function.

Acknowledgement:
Our work has been supported by Nihon University Competitive Grant for Research.

References:

[1] T.Tsuchiya, T,Eulgem (2011). Mol Plant Microbe Interact, 24, 1573
[2] T.Tsuchiya, T,Eulgem (2013). Proc Natl Acad Sci USA, 110, E3535
[3] T.Tsuchiya, T,Eulgem (2013). Sci Rep, 3, 1701
[4] T.Tsuchiya, T,Eulgem (2014). Plant Signal Behav, 9, e29202

Membrane biogenesis and protein targeting in haustorium-invaded plant cells

Xianfeng Ma[1,2], Qiong Zhang[1], Ying Wu[1], Harley King[1], Marcela Rojas-Pierce[3], Xingyao Xiong, Shunyuan Xiao[1]

[1]*Institute of Biosciences and Biotechnology Research & Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20850, USA*
[2]*Hunan Provincial Key Laboratory for Germplasm Innovation and Utilization of Crop, Hunan Agricultural University, Changsha 410128*
[3]*Department of Plant and Microbial Biology, North Carolina State University*
Raleigh, NC 27695, USA

Many fungal and oomycete pathogens use similar feeding structures called haustoria to secrete effectors to suppress host immunity and steal nutrients from plant cells. The extrahaustorial membrane (EHM) represents the host-pathogen interface and a critical battleground. The broad-spectrum resistance protein RPW8.2 from Arabidopsis is specifically targeted to the EHM where it activates haustorium-focused defenses. The origin and biogenesis of the EHM is not known. By using RPW8.2 and other RPW8 family members as tools, we demonstrated that the EHM is most likely synthesized *de novo*, which is consistent with our observation that RPW8.2 is targeted via the Trans Golgi Network (TGN) to the EHM during EHM biogenesis. To understand how RPW8.2 is precisely targeted to the EHM, we have recently conducted a large-scale genetic screen and found that loss of VTI11, a Qb SNARE at the TGN required for vesicle trafficking from the TGN to the vacuole, results in mis-targeting of RPW8.2 to the plasma membrane and loss of resistance to powdery mildew. Interestingly, *vti11* mutant plants in the absence of *RPW8.2* exhibits enhanced disease resistance to adapted powdery mildew, suggesting that a TGN-localized SNARE complex containing VTI111 is essential for correct sorting of RPW8.2 and perhaps other membrane proteins involved in plant immunity at the TGN.

"Disease-Climate-Microbiome" interactions in the phyllosphere

Sheng Yang He

Howard Hughes Medical Institute, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Email: hes@msu.edu

A major roadblock to global food sufficiency is persistent loss of staple crops to pathogen infections. Greater efforts are needed to accelerate the buildup of a comprehensive knowledge base that explains how plant diseases occur; how plants defend against microbial pathogens; and how dynamic climate conditions impact plants, pathogens, and their interactions. In 1960, RB Stevens formulated the famous "Disease Triangle" concept [1], proposing that plant disease outbreaks require not only a susceptible plant and a virulent pathogen, but also conducive environmental conditions. For practical reasons, however, most contemporary investigations into plant-pathogen interactions have not devoted enough effort to understanding why climatic conditions, such as humidity and temperature, have a profound effect on pathogen virulence and host susceptibility at the molecular level. Moreover, these studies often ignore the potentially pervasive effect a plant's endogenous microbiome may have on basic plant health and host-pathogen interactions. I will give an example of interplays between disease, humidity and microbiota during *Pseudomonas syringae* infection of *Arabidopsis thaliana* leaves. Our results suggest that a better understanding of plant-pathogen interactions should increasingly consider the multi-dimensional nature of "disease-environment-microbiome" interactions that are more reflective of what occur in nature.

1. R. B. Stevens (1960) Plant Pathology, an Advanced Treatise. Vol. 3, Academic Press, New York

Food for mutualistic mycorrhizal and parasitic fungi

Yina Jiang[1], Wanxiao Wang[1], Qiujin Xie[1], Na Liu[2,3], Lixia Liu[1], Dapeng Wang[1], Xiaowei Zhang[1], Chen Yang[1], Dingzhong Tang[2,3], Ertao Wang[1]*

[1] *National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

[2] *Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Fujian Agriculture and Forestry University, Fuzhou 350002*

[3] *State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101*

* *For correspondence E-mail: etwang@sibs.ac.cn*

Abstract

Arbuscular mycorrhiza (AM) formation is a widespread symbiotic interaction between 80-90% of land plants and soil fungi. The plant benefits from enhanced inorganic nutrient supply mediated by the fungal hyphae network in the soil. In return, the fungi draw organic nutrients from the plant. Organic nutrients are thought to be supplied primarily in the form of sugars. However, within the fungus, most carbon is stored in lipids that are transported throughout the mycelium. Here we show that the AM fungus *Rhizophagus irregularis* is a fatty acid auxotroph and fatty acids synthesized in the host plant are transferred to the fungus during AM symbiosis. We find that the transfer is depedent on the RAM2 and peri-arbuscular membrane-localized ABC transporter-mediated plant lipid export pathway. We further show that fatty acids synthesized in plants also can be transferred to the pathogenic fungus *Golovinomyces cichoracerum*. Plants defective in fatty acid biosynthesis are impaired in AM symbiosis and show defects in colonization by the pathogenic *G. cichoracerum*. Given the abundance of lipids in *R. irregularis*, we suggest that the AM fungus reprograms its host plant to secure fatty acids as a major carbon source, and that a pathogenic fungus similarly recruits the fatty acid biosynthesis program to facilitate host invasion.

Plasmodesmal localization signal of TMV MP

Cheng Yuan[1], Sondra G. Lazarowitz[2] and Vitaly Citovsky[1]

[1] *Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794-5215, USA;* [2] *Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853*

The modern era of virology began with the discovery of Tobacco mosaic virus (TMV). Since then, TMV has served as an experimental and conceptual model for studies of viruses and dissection of virus-host interactions. Specifically, the TMV cell-to-cell movement protein (MP) has emerged as the paradigm for dissecting the molecular details of cell-to-cell transport through plant intercellular connections, the plasmodesmata. However, one of the most fundamental and key functional features of TMV MP, its putative plasmodesmal localization signal (PLS), has not been identified. To address this limitation, we have identified the plasmodesmal localization signal (PLS) in the *Tobacco mosaic virus* (TMV) cell-to-cell movement protein (MP). TMV MP PLS encompasses amino acid residues between positions 1 to 50, with residues Val-4 and Phe-14 potentially representing critical sites for PLS function that most likely affect protein conformation or protein interactions. We then show that PLS interacts with the Arabidopsis synaptotagmin A (SYTA), a mediator of contacts between the ER and the plasma membrane known to be involved in cell-to-cell transport of TMV MP. The PLS sequence is both necessary and sufficient for interaction with SYTA, and the plasmodesmal targeting activity of PLS is substantially reduced in a SYTA knockdown plant line. Thus, SYTA may represent the host factor that recognizes PLS and stabilizes its association with the cell membrane at plasmodesmata.

From leucine-rich repeat to malectin-like domain: differential functions of receptor-like kinases in plant immunity and growth

Libo Shan
Department of Plant Pathology and Microbiology, and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843

Plants have evolved a largely expanded collection of cell surface-resident receptor-like kinases (RLKs) to sense the external or internal signals, and relay the signaling cascades to various downstream outputs that are central to plant growth, development, immunity and stress adaptation. The largest group of RLKs are LRR-RLKs with an extracellular leucine-rich repeat (LRR) domain. Several LRR-RLKs, including brassinosteroid hormone receptor BRI1 and bacterial flagellin receptor FLS2, heterodimerize with BAK1, also known as SERK3, and other SERKs, upon the cognate ligand perception. We recently show that BAK1/SERKs also heterodimerize with ERECTA family LRR-RLKs controlling stomatal patterning upon perception of EPF peptides, and HAESA family LRR-RLKs controlling floral organ abscission upon perception of IDA peptides. Thus, BAK1/SERKs are shared coreceptors in diverse signaling receptorsomes and modulate distinct cellular responses in plant immunity, growth and cell differentiation. Apparently, the different functions of SERKs in diverse signaling pathways appear to be uncoupled, and different SERKs possess unequal contribution to different physiological responses. The differential functions of RLKs in plant immunity and growth are also supported by our recent genetic screen in which we identified ANXUR1 (ANX1), a malectin-like domain-containing RLK as an important negative regulator in plant immunity triggered by two tiers of immune receptors: pattern recognition receptors (PRRs) sensing microbial signatures and intracellular nucleotide-binding domain leucine-rich repeat (NLR) proteins recognizing pathogen effectors. ANX1 complexes with both PRR FLS2/BAK1 complex and NLR proteins and modulates immune receptor functions. Interestingly, the mutation that affects ANX1 function in plant immunity does not disrupt its function in controlling pollen tube growth during fertilization, suggesting an uncoupled function of ANX1 during plant immunity and sexual reproduction.

Mechanisms of RPS4/RRS1 NLR immune complex derepression

Jonathan D G Jones[a], Zane Duxbury[a], Yan Ma[a], Sung Un Huh[a], Hailong Guo[a], Pingtao Ding[a]
[a] *Sainsbury Lab, Norwich Research Park, Colney Lane, Norwich, UK*
jonathan.jones@tsl.ac.uk

Diverse microbes cause plant disease, and plants have evolved a robust innate immune system that recognizes pathogen molecules and then activates defense. Immunity involves both cell surface receptors and also intracellular Nucleotide-binding, Leucine-rich Repeat (NLR) immune receptors, often encoded by Resistance (R) genes.

Some resistances require two co-functioning NLR proteins. The adjacent, divergently transcribed, Arabidopsis RPS4 and RRS1 genes, encoding TIR-NLR proteins. Both are required for resistance to bacteria that deliver AvrRps4 or PopP2 effectors, and for resistance to certain Colletotrichum strains. RRS1 carries a C- terminal WRKY domain targeted by AvrRps4 and PopP2, suggesting these effectors target WRKY domains. We investigate how the RPS4/ RRS1 complex activates defense upon effector recognition.

Prior to activation, the complex is maintained in an inactive state. Our data suggest that molecular interactions between the RRS1 WRKY domain and the RRS1 Domain 4 (which lies between the LRR and the WRKY domain) are key to this negative regulation of the complex. We propose that disruption of these interactions by the action of effectors is key to derepression of the complex and to subsequent activation of defence.

Williams et al.. Science (2014) vol. 344 (6181) pp. 299-303
Sohn et al. *(2014)* PLOS Genetics doi: 10.1371/journal.pgen.1004655
Saucet et al (2015) Nat. Comms 6 pp. 6338
Huh et al (2017) Plos Path 13(5) 1006376
Sarris et al (2015) Cell 161 pp. 1089-1100

Bidirectional cross-Kingdom RNAi and exosome-mediated small RNA trafficking between Arabidopsis and the fungal pathogen *Botrytis cinerea*

Qiang Cai, Baoye He, and Hailing Jin*
Department of Plant Pathology and Microbiology, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, CA 92521.

Small RNAs (sRNAs) are a class of short non-coding RNAs that mediate gene silencing in a sequence-specific manner. We have demonstrated that some sRNAs from eukaryotic pathogens, such as *Botrytis cinerea*, the fungal pathogen that causes grey mold disease on more than 1000 plant species, could be translocated into host plant cells and suppress host immunity genes for successful infection. Recently we have found that transgenic plants expressing hairpin RNAs that targeting *Botrytis* Dicer1 and Dicer2 genes could effectively block the generation of fungal sRNA effectors and suppress grey mold disease. These findings demonstrate an important role of bidirectional Cross-Kingdom RNAi in host – pathogen interactions. However, the mechanism of the sRNA trafficking between plant hosts and eukaryotic pathogens are largely unknown.

We observed a drastic increase of multivesicular bodies (MVBs) and extracellular vesicles (EVs) at the infection sites, and the mutate of ARA6, a MVB marker, displayed enhanced susceptibility to wild type strain but not the dcl1dcl2 strain of *B. cinerea*. These results suggest that MVB-associated EV trafficking is likely to play a role in sRNA trafficking from plant to *B. cinerea*. We thus profiled sRNA populations from *B. cinerea* cells and EVs isolated from the infected Arabidopsis leaves, and identified a panel of plant endogenous sRNAs present in both fractions, which target fungal genes involved in pathogenicity. Furthermore, we discovered two Arabidopsis exosome markers that were co-localized with MVBs inside the plant cells, and displayed an increased secretion into apoplast spaces at the infection sites after *B. cinerea* inoculation. The identified plant endogenous mobile sRNAs were detected in the exosomes. These data confirmed that plants deliver sRNAs into fungal cells through exosomes and silence fungal genes involved in pathogen virulence.

Xanthomonas oryzae iTALEs Overcome *Xa1*-Mediated Resistance in Rice

Zhiyuan Ji[a,b]*, Chonghui Ji[b]*, Bo Liu[b], Lifang Zou[a], Gongyou Chen[a]† and Bing Yang[b]†

[a] *School of Agriculture and Biology/State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai 200240.*

[b] *Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA.*

*† gyouchen@sjtu.edu.cn(G.C.) & byang@iastate.edu (B.Y.). * These authors contributed equally.*

Pathogenic microbes and their host plants have followed a 'zigzag' course co-evolving new virulence strategies in pathogens and counteracting resistance mechanisms in hosts. TALEs (transcription activator-like effectors) play an important role in the pathogenesis of *X. oryzae*pv. *oryzae* (Xoo) and *X. oryzae* pv. *oryzicola* (Xoc), the causal agents of bacterial blight and leaf streak, respectively, in rice. TALEs target host genes of susceptibility (*S* gene) or resistance (*R* gene) in a sequence specific manner. However, C-terminally truncated TALEs, here referred to as iTALEs (interfering TAL effectors), are prevalent in the majority of Xoo and Xoc strains, and their roles in pathogenesis are largely unknown. Here we first used PXO99^A, a representative of *X. o*.pv. *oryzae* that is virulent in a large number of rice varieties and contains nine gene clusters totaling nineteen individual TALE genes, to generate a series of PXO99^A mutants, including a TALE-free strain by sequentially deleting individual TALE gene clusters. Surprisingly, deletion of the cluster 3 (*Tal3a*/*Tal3b*, two previously regarded pseudogenes) enabled the mutant, ΔTal3, incompatibles to IRBB1 but compatible to some rice lines including IR24. *Tal3a* and *Tal3b* are expressed and encode identical N-termini, distinct central repetitive and C-terminal domains, and the nuclear localization motifs but lacking the transcriptional activation domain. Correspondingly, the introduction of *Tal3a*or *Tal3b* to ΔTal3 enabled ΔTal3 to cause disease in IRBB1 comparable to the parent strain PXO99^A. IRBB1 and IR24 are near isogenic rice lines for *Xa1*, which was identified as a nucleotide-binding site leucine-rich (NLR) repeat type *R* gene from Kougyoku and IRBB1. Detailed structure analysis of Tal3a indicates that the unique N- and C-terminal regions are essential for iTALEs to suppress the *Xa1*-mediated disease resistance. The widespread iTALE genes among the majority of both Xoo and Xoc pathogens counteract the broad recognition of TALEs by *Xa1* and effectively restrict the spectrum of the otherwise broad resistance

triggered by TALEs in *Xa1*-containing rice. Mutagenesis and functional complementation with several iTALE genes in Xoo and Xoc indicate that iTALE genes are evolutionarily conserved and functionally equivalent to contribute pathogen virulence by suppressing *Xa1* resistance to bacterial leaf blight and leaf streak diseases.

Acknowledgements

This research was supported by the National Science Foundation of China research grant 31230059 (G.C.), the State Key Basic Research and Development Project of China grant 2012CB114003 (G.C.), the Special Fund for Agro-scientific Research in the Public Interest of China 201303015 (G.C.) and the US National Science Foundation research grants 2012-1238189 and IOS-1258103 (B.Y.).

A pore-forming toxin as microbial virulence factor and effector in immune defense

Thorsten Nuernberger
University of Tuebingen, Center for Plant Molecular Biology, Tuebingen, Germany

Members of the superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) are found in bacteria, fungi and oomycetes. A subset of these proteins causes leaf necrosis on dicot, but not on monocot plants. NLP cytotoxicity is required crucial for microbial virulence and a necrotrophic lifestyle of the producing microbe. X-ray crystallography-based analyses of two microbial NLPs revealed substantial fold conservation of these proteins with sequence-unrelated cytolytic toxins produced by marine organisms (actinoporins). Actinoporins bind to animal host sphingomyelin prior to membrane pore formation and cytolysis. While plants do not produce sphingomyelins, we show that the target site for NLP toxins is a dicot-specific glycosylated sphingolipid. Binding induces a conformational switch within NLP cytolysins thereby mediating membrane attachment and pore formation. These findings explain the unusual host selectivity of NLP toxins.

Many NLP proteins harbor a twenty amino acid stretch (nlp20) that mediates host immune activation in *Brassicaceae* including *Arabidopsis*. This peptide is recognized by a ternary immune receptor complex comprising ligand-binding leucine-rich receptor protein RLP23 and two co-receptors, SOBIR1 and BAK1. Stimulus-dependent complex formation will be discussed along with potential use of this and related receptors in engineering crops with heightened immunity to microbial infection.

Genome enabled studies to further knowledge of rice blast infection and disease management

Ralph A. Dean
Center for Integrated Fungal Research, Department of Entomology and Plant Pathology, North Carolina State University, USA.

The filamentous fungus *Magnaporthe oryzae* is the causal agent of rice blast disease and the most destructive pathogen of rice worldwide. Following conidia germination on the leaf surface, a specialized infection structure, the appressorium forms. Infection is described as being hemibiotrophic, which is characterized by a biotrophic phase during the early stages of infection followed by a necrotrophic phase defined by host cell death and lesion formation. We are undertaking a global approach to dissect the infection process and I will present the integration of data from transcriptomic, proteomic and post translational proteomics approaches to model appressorium formation. I will also present recent findings functionally charactering candidate effectors. Candidates were screened for host cell death suppressive abilities by transiently expressing them within the leaves of *Nicotiana benthamiana* and challenging them with known plant cell death inducing genes. I will describe the properties of those found to act as suppressors. Finally, I will present work on how we are using knowledge of genes involved in pathogenesis to manage rice blast disease through Host-Induced Gene Silencing (HIGS). Results on the effectiveness of HIGS as well as work investigating how small RNAs are taken up by the fungus from the host will be discussed.

A natural allele of a transcription factor in rice confers broad-spectrum blast resistance

Weitao Li[1,6], Ziwei Zhu[1,6], Mawsheng Chern[2,3,6], Junjie Yin[1,6], Chao Yang[1,6], Li Ran[1,6], Mengping Cheng[4], Min He[1], Kang Wang[1], Jing Wang[1], Xiaogang Zhou[1], Xiaobo Zhu[1], Zhixiong Chen[1], Jichun Wang[1], Wen Zhao[1,5], Bingtian Ma[1], Peng Qin[1], Weilan Chen[1], Yuping Wang[1], Jiali Liu[1], Wenming Wang[1], Xianjun Wu[1], Ping Li[1], Jirui Wang[4], Lihuang Zhu[5], Shigui Li[1], Xuewei Chen[1,*]

[1]*State Key Laboratory of Hybrid Rice, Key Laboratory of Major Crop Diseases & Collaborative Innovation Center for Hybrid Rice in Yangtze River Basin, Rice Research Institute, Sichuan Agricultural University at Wenjiang, Chengdu, Sichuan 611130*
[2]*Department of Plant Pathology, University of California, Davis, California, USA*
[3]*Joint Bioenergy Institute, Emeryville, California, USA*
[4]*Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Chengdu, Sichuan 611130*
[5]*State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101*
[6]*These authors contributed equally*
**Correspondence: xwchen88@163.com*

Abstract

Rice is a staple diet of nearly half the world's population and rice blast is often a destructive disease that results in significant crop loss. Non-race-specific resistance has been more effective in controlling crop diseases than race-specific resistance because of its broad spectrum and durability. Through a genome-wide association study, we here report the identification of a natural allele of a C_2H_2-type transcription factor in rice that confers non-race-specific resistance to blast. A survey of 3,000 sequenced rice genomes reveals that this allele exists in 10% of rice, suggesting that this favorable trait has been selected through breeding. This allele causes a single nucleotide change in the promoter of the *bsr-d1* gene, which results in reduced expression of the gene through the binding of the repressive MYB transcription factor, and consequently, an inhibition of H_2O_2 degradation and enhanced disease resistance. Our discovery highlights this novel allele as a strategy for breeding durable resistance in rice.

Key Words: Genome-wide association study (GWAS), Single nucleotide polymorphism (SNP), Hydrogen peroxide (H_2O_2), C2H2, MYB, Transcription factor, Broad-spectrum, Resistance, Blast disease, Rice

Molecular basis of virus-induced attraction of insect vectors for efficient viral transmission

Dewei Wu[a], Tiancong Qi[a], Wan-Xiang Li[b], Le Kang[c], Shou-wei Ding[b] and Daoxin Xie[a]

[a] *School of Life Sciences, Tsinghua University, Beijing*

[b] *Department of Plant Pathology and Microbiology, University of California, Riverside, CA, USA*

[c] *State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing*

E-mail of presenting author: daoxinlab@mail.tsinghua.edu.cn

To survive in stressful environment, plants have evolved sophisticated mechanisms to defend against various biotic and abiotic stresses. Such plant defense systems depend largely on inducible expression of various defense-related genes, which are primarily manipulated by plant hormones. Plant hormones, which are characterized by the property of serving as chemical messengers, play essential roles in regulating various developmental processes and diverse defense responses. Our research interests are focused on elucidating perception mechanisms of plant hormones jasmonate (JA) and strigolactone (SL). We have uncovered receptors for jasmonate (JA) and strigolactone (SL): COI1[1] serves as a JA receptor[2] that reversibly senses the active JA molecules to regulate diverse plant developmental processes and various plant defense responses[3-11]; D14 acts as a non-canonical receptor[12-13] for SL, which generates and irreversibly binds the active SL molecule (CLIM) to trigger SL-regulated plant responses.

In contrast to the well-described phenomenon that plants employ hormonal signals to regulate plant defense against pathogens and insect pests, we recently found that pathogen virus has evolved a novel mechanism to hijack plant hormone pathway and modify plant volatile compounds to attract more insect vectors for efficient viral transmission and infection[14]. We will discuss how cucumber mosaic virus employs viral effector protein 2b to hijack the host JA signaling pathway for inducing odor-dependent aphid attraction.

References:

[1] D. Xie, B.F. Feys, S. James, M. Nieto-Rostro, J.G. Turner (1998). Science, 280, 1091.
[2] J. Yan, C. Zhang, M. Gu, Z. Bai, W. Zhang, et al. (2009). Plant Cell, 21, 2220.
[3] P. Hu, W. Zhou, Z. Cheng, M. Fan, L. Wang, et al. (2013). Mol Cell, 50, 504.
[4] T. Qi, J. Wang, H. Huang, B. Liu, H. Gao, et al. (2015). Plant Cell, 27, 1634.
[5] T. Qi, H. Huang, S. Song, D. Xie (2015).Plant Cell, 27, 1620.
[6] T. Qi, H. Huang, D. Wu, J. Yan, Y. Qi, et al. (2014). Plant Cell, 26, 1118.
[7] T. Qi, S. Song, Q. Ren, D. Wu, H. Huang, et al. (2012). Plant Cell, 23, 1795.
[8] S. Song, H. Huang, H. Gao, J. Wang, D. Wu, et al. (2014). Plant Cell, 26, 263.
[9] S. Song, T. Qi, H. Huang, Q. Ren, D. Wu, et al. (2011). Plant Cell, 23, 1000.
[10] S. Song, T. Qi, M. Fan, X. Zhang, H. Gao, et al. (2013). PLoS Genetics, 9, e1003653.
[11] H. Cheng, S. Song, L. Xiao, H.M. Soo, Z. Cheng, et al. (2009). PLoS Genetics, 5, e1000440.
[12] R. Yao, Z. Ming, L. Yan, S. Li, F. Wang, et al. (2016). Nature, 536, 469.
[13] R. Yao, F. Wang, Z. Ming, X. Du, L. Chen, et al. (2017). Cell Res, 27, 838.
[14] D. Wu, T. Qi, W. Li, H. Tian, H. Gao, et al. (2017). Cell Res, 27, 402.

Tomato leaf curl Yunnan virus-encoded C4 induces cell division through impacting NbSKη-mediated phosphorylation and stability of Cyclin D 1.1 in *Nicotiana benthamiana*

Yuzhen Mei[a], Xiuling Yang[b], Xueping Zhou[a, b*]

[a] *State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, Zhejiang, 310058*

[b] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193*

The whitefly-transmitted geminiviruses induce severe developmental abnormalities in plants. Geminivirus-encoded C4 protein functions as one of viral symptom determinants that could induce abnormal cell division. However, the molecular mechanism by which C4 contributes to cell division induction remains unclear. Here we report that *Tomato leaf curl Yunnan virus* (TLCYnV) C4 interacts with a glycogen synthase kinase 3 (GSK3)/SHAGGY-like kinase, designed NbSKη, in *Nicotiana benthamiana*. Pro32, Asn34, and Thr35 of TLCYnV C4 are critical for its interaction with NbSKη and required for C4-induced typical symptoms. Interestingly, TLCYnV C4 directs NbSKη to the membrane and reduces the nuclear-accumulation level of NbSKη. The relocalization of NbSKη by C4 impacts the NbSKη-mediated phosphorylation and stability of nucleus-located Cyclin D1.1 (NbCycD1;1) and increases the accumulation level of NbCycD1;1 contributing to the cell division induction. Moreover, *NbSKη–RNAi*, *35S::NbCycD1;1* transgenic *N. benthamiana* plants have the similar phenotype as *35S::C4* transgenic *N. benthamiana* plants on callus-like tissues formation resulted from abnormal cell division induction. Thus, this study provides new insights into mechanism of how a viral protein hijacks NbSKη to induce abnormal cell division in plants.

Autophagy at the *Magnaporthe*-Rice interface

Naweed I. Naqvi[a], Yizhen Deng[b], Ziwei Qu[a]
[a] *Temasek Life Sciences Laboratory, 1 Research Link, Singapore.*
[b] *College of Agriculture, South China Agricultural University*
Email: naweed@tll.org.sg

Phototropic response and circadian rhythms are critical for growth, differentiation and environmental adaptation in fungi. However, the precise regulators of the intracellular metabolic response during such dark-light rhythms have not been fully characterized yet. The blast fungus, *Magnaporthe oryzae*, initiates conidiation in response to phototropic and nutritional cues. We found that autophagy is induced specifically upon exposure to light and is essential for asexual development in *M. oryzae*. Comparative RNAseq analyses provided insights into novel signal transduction cascades underlying such phototropic induction of conidiation and in adaptation to the host milieu. We identified a histone acetyltransferase that negatively regulates light-dependent induction of autophagy during conidiation *in planta*. Such repression of autophagy occurs via the epigenetic regulation of the Atg7 function in *M. oryzae*. Furthermore, we identified a novel circadian-regulated *Twilight* (*TWL*) function essential for phototropic response and pathogenesis in *M. oryzae*. Expression of *TWL* showed a distinct circadian rhythm, and its transcript level peaked at subjective twilight. Mutually exclusive phosphorylation acetylation cycles govern the intracellular dynamics and regulation of the Twl protein. Acetylated GFP-Twl remained cytosolic in the dark, whereas the light-induced phosphorylated (via Snf1 kinase) version translocated to the nucleus. Twilight function was required for regulating the mRNA levels of several important transcription/repair factors such as Tfb5, which translocates into the nucleus during the phototropic response. Lastly, we provide mechanistic insights into the role of selective autophagy (mitophagy) in nutrient and redox homeostasis in the adaptation of the blast pathogen to the host milieu; and propose a regulatory mechanism that underlies the induction of autophagy by important environmental clues such as light and nutrients in the rice blast pathosystem.

***Magnaporthe* chitinase interacts with a jacalin-related lectin and promotes host colonization**

Yijuan Han[1,2], He Li[2], Lihua Liu[2], Linlin Song[1,2], Xiangzi Zheng[1,2], Chengzeng Lin[1,2], Jie Zhou[1,2], Shihua Wang[1,2], Daniel Ebbole[3], Zonghua Wang[1,2], Guo-dong Lu[1,2]

1 State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, Fujian

2 Key Laboratory of Biopesticide and Chemistry Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou

3 Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, United States of America

Abstract

●The genome of the rice blast fungus *Magnaporthe oryzae* encodes fifteen GH_18 family chitinases. In this study we characterized the function of an extracellular chitinase, MoChi1,and its interaction with host protein OsMBL1, a jacalin-related lectin in rice *Oryza sativa*).

●Deletion of *MoChi1* gene resulted in reduced aerial hyphal formation and altered the sensitivity to cell wall stress. *MoChi1* deletion mutants also displayed reduced induction of the defense-related genes expression and pathogenicity in rice.

in rice.

●MoChi1 was confirmed to interact with OsMBL1 through yeast two hybrid assay, co-immunoprecipitation and BiFC assays. Transcription of *OsMBL1* was induced by PAMPs and *M.oryzae* infection. Elevated expression levels of *OsMBL1* led to activation of defense-related genes and enhanced rice resistance to the blast disease. OsMBL1 could bind to chitin and competitive binding assays revealed that interaction between MoChi1 and OsMBL1 inhibits the chitin binding.

●Our study suggests that the fungal chitinase MoChi1 degrades chitin and may act to suppress chitin-elicited rice immunity through its interaction with a jacalin-related lectin OsMBL1.

Key words: Rice, Mannose-Binding Lectin-Like Protein, *Magnaporthe oryzae*, Chitinase, interaction, chitin recognition

Genome sequence of *Plasmopara viticola* reveals effector reptorier and pathogenicity mechanisms

Jiang Lu
Shanghai Jiao Tong University and Key Lab of Guangxi Crop Genetic Improvement

Plasmopara viticola，an oomycete pathogen causing downy mildew disease of grapevine，is one of the most devastating diseases in viticulture worldwide. Here we report a 101.3 Mb whole genome sequence of *P. viticola* isolate ‘JL-7-2’ obtained by a combination of Illumina and PacBio sequencing technology. The *P. viticola* genome contains 17,014 putative protein-coding genes， including a total of 1,301 putative secreted proteins, of which 100 plus putative RXLR effectors and 90 CRN effectors were identified. Expression patterns of the effector genes varied and could be categorized into different groups as the pathogen interacting with the host. High-throughput in plant localization studies revealed that the PvRxLRs accumulated in different cell compartments. Most of the PvRxLRs could fully suppress programmed cell death elicited by a range of cell death-inducing proteins，while small percentage of them could not suppress PCD but rather induced PCD in the tobacco system. The former appeared acting as broad suppressors of cell death to manipulate immunity in the host plant. Findings from this study greatly enhance our knowledge on pathogenicity and mechanism of interactions between this biotrophic pathogen and its host.

Molecular based identification and formulation of cyanogenic *Pseudomonas* spp. controlling *Phytophthora infestans*

[a]Md. Rashidul Islam and [b]Md. Nizam Uddin

[a&b]*Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

E-mail: rasha740177@yahoo.com

ABSTRACT

Late blight caused by an oomycete, *Phytopthora infestans* is one of the devastating diseases of potato and tomato worldwide. Every year the disease incurs a loss estimated around $16 billion in the developing countries and in Bangladesh the loss estimated around $ 880 million which include the yield loss and the cost for fungicides application. However, growers are interested to use bio-agents instead of chemical fungicides to control late blight to avoid hazards and production cost. Recently, bacterial bioagents producing hydrogen cyanide are drawing attention to control plant pathogens [1]. Cyanogenic *Pseudomonas* spp has been reported as potential bioagents to control late blight pathogen, *Phytophthora infestans* [2]. In the present study, a total of 200 bacterial isolates obtained from phylloplane of potato plants of different varieties were screened for the production of cyanide and only six bacterial isolates were identified as cyanogenic indicated by a color change from yellow to orange of a filter paper saturated with 1 % picric acid solution and wetted with 10 % sodium carbonate solution fixed to the lid of a petridish containing LB medium with glycine at temperature ranged from 8-30°C. Polymerase chain reaction (PCR) results with primer PsEG30F (5′-ATYGAAATCGCCAARCG -3′) and PsEG790R (5′-CGGTTGATKTCCTTGA- 3′) specific to the conserved sequences of RNA polymerase sigma factor 70 (*rpo*D) gene [3] producing a 760 bp amplicon confirmed the bacterial isolates are *Pseudomonas* spp. Sequence analyses by Blast program revealed that these *Pseumonas spp* are closely related to *Pseudomonas* spp (CCV18038.1), *Pseudomonas putida* (WP_023532290.1), *Pseudomonas fulva* (AKC04323.1) and *Pseudomonas parafulva* (WP_058639614.1). *In vitro* growth inhibition showed that all cyanide producing *Pseudomonas* spp completely inhibited the

growth of *Phytophthora infestan*. The bacterial isolates were formulated in Talcum powder and the bacterial cells are viable for up to six months. These *Pseudomonas* spp has the growth promoting activity when seeds of some crop plants are treated. The growing ability of these cyanogenic *Pseudomonas* spp at cool to warm temperature (8-30°C) suggesting its potential application in controlling late blight of potato in Bangladesh.

References:

[1] S. R. Dürr. (2014) Master's thesis submitted to Department of Forest- and Soil Sciences, Institute of Soil Research University of Natural Resources and Life Sciences, Vienna.
[2] Hunziker L, Bönisch D, Groenhagen U, Bailly A, Schulz S, Weisskopf L. (2015) Appl Environ Microbiol 81:821.
[3] M. Mulet, A. Bennasar, J. Lalucat, E. Garcı´a-Valde´ 2009. Molecular and Cellular Probes 23: 140.

Evolution of an effector gene family from the rice blast fungus.

Daniel J. Ebbole [a], Mei-lian Chen [a,b], Yijuan Han [b], Wenhui Zheng [b], Jian Chen [b], Guohua Duan [a,b], Guodong Lu [b], Zonghua Wang [b]

[a] *Department of Plant Pathology & Microbiology, Texas A&M University, College Station, Texas, 77843 USA*

[b] *Key Laboratory of Biopesticides and Chemical Biology, Ministry of Education/Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian*

d-ebbole@tamu.edu

A 21 member gene family of the rice blast fungus (*Pyricularia oryzae*) is expressed primarily during infection and colonization of rice. At least one of the gene family members is delivered into rice cells. This gene is able to suppress programmed cell death induced by expression of BAX1 in *Nicotiana benthamiana*. Little allelic variation leading to amino acid substitutions were found in analysis of rice isolate genome sequences, however, presence/absence polymorphism and other null alleles were noted for most members of the gene family. Some gene family member alleles are unique and diagnostic of rice-adapted isolates. In populations of *P. oryzae* infecting other hosts, amino acid substitutions relative to the rice isolate sequences were common. The number of sequences of isolates from non-rice hosts is limited, however, the gene family haplotypes within host-specific populations have shown limited variation, with the exception of isolates from wheat. Several of the haplotypes in the wheat-infecting population resemble those from other grass hosts, suggesting that genetically distinct grass-infecting populations have contributed individuals to the wheat-infecting population. *P. grisea* is a pathogen of *Digitaria* grasses and the gene family has diverged from *P. oryzae* to such as an extent that orthology of the 18 *P. grisea* gene family members is only detectable for a few of the genes. We propose adaptation to a host species involves selection operating on gene family members. Gene family members occur in Colletotrichum pathogens of grasses, possibly through an ancient gene transfer event.

Regulation of plant immunity by multiple MAP kinase cascades

Yuelin Zhang
Department of Botany, University of British Columbia, Vancouver, BC, Canada

MAP kinase signaling is an integral part of plant immunity. Arabidopsis MPK4 is a component of two independent MAP kinase cascades and functions in regulating development as well as plant defense. The MEKK1-MKK1/2-MPK4 kinase cascade is important for basal resistance and is protected by the NLR protein Suppressor of *mkk1 mkk2* (SUMM) 2. The ANPs-MKK6-MPK4 cascade plays an essential role in cytokinesis. Analysis of SUMM3 revealed that SUMM2 senses the disruption of the MEKK1-MKK1/2-MPK4 kinase cascade through a substrate protein of MPK4, CRCK3. Reverse genetic analysis showed that the ANPs-MKK6-MPK4 cascade also plays important roles in regulating plant immune responses. Loss of function of MKK6 or ANP2/ANP3 results in constitutive activation of plant defense responses. The autoimmune phenotypes in *mkk6* and *anp2 anp3* mutant plants can be largely suppressed by a constitutively active *mpk4* mutant. The constitutive defense response in *anp2 anp3* is dependent on the defense regulators PAD4 and EDS1, but not SUMM2, suggesting that the ANP2/ANP3-MKK6-MPK4 cascade negatively regulates a SUMM2-independent defense response pathway. Our reverse genetic analysis also revealed that a third MAP kinase cascade consisting of MPK3/MPK6, MKK4/MKK5 and two redundant MAPKKKs functions downstream of PAMP receptors to regulate basal resistance against bacterial pathogens.

Determining the site of action of strigolactones during nodulation

Eloise Foo,[a] Erin L. McAdam[a], Cassandra Hugill[a], Sebastien Fort[b], Eric Samain[b], Sylvain Cottaz[b], Noel W. Davies[c] and James B. Reid[a]

[a]*School of Biological Sciences, University of Tasmania, Private Bag 55, Hobart-AUSTRALIA*

[b]*University. Grenoble Alpes, CERMAV, F-38000 Grenoble, Grenoble-FRANCE*

[c]*Central Science Laboratory, University of Tasmania, Private Bag 74, Hobart-AUSTRALIA*

eloise.foo@utas.edu.au

Strigolactones (SLs), a recently characterized hormone group, influence the ability of legumes to associate with nitrogen-fixing bacteria[1,2]. In this study we determine the stage at which SLs act during nodulation. We show that SLs promote infection thread formation, as a null SL-deficient mutant forms significantly less infection threads than wild type plants. We found no evidence that SLs influence physical events before or after infection thread formation, as SL-deficient plants displayed a similar ability to influence rhizobial growth, produce flavonoids and induce root hair curling in response to rhizobia or rhizobial produced Nod factors as wild type plants. Ongoing studies are examining whether this influence of SLs is on bacterial symbiont and/or the plant partner. We found no evidence to suggest that SLs influence nodule function, as SL-deficient nodules fix nitrogen at a similar rate to wild type nodules. This influence of SLs on infection thread formation was accompanied by induction of some early nodulation (*ENOD*) genes, well-known markers of the early events during nodule formation. Importantly, SL synthesis is up-regulated by core elements of the Nod factor signalling pathway and this requires the downstream transcription factor *NSP2* but not *NIN*. This, together with the fact that the expression of certain SL biosynthesis genes can be elevated in response to rhizobia/Nod factors suggests that Nod factors may induce SL biosynthesis. SLs appear to influence nodulation largely independently of ethylene action, as SL-deficient and ethylene insensitive double mutant plants display essentially additive phenotypes and we found no evidence that SLs influence ethylene synthesis or vice versa.

1. M.J. Soto, M. Fernandez-Aparicio, V. Castellanos-Morales, J.A. Garcia- Garrido, M.J. Delgado, H. Vierheilig H, *Soil Biol Biochem*. **2010** 42, 383–385
2. E. Foo, N.W. Davies NW *Planta* **2011,** 234, 1073-1081

A two-pronged mechanism for boosting SA defense in TNL receptor-triggered immunity

Haitao Cui[1,2], Jingde Qiu[1], Yue Zhou[1], Deepak Bhandari[1], Jaqueline Bautor[1] and Jane E. Parker[1]

[1]*Department of Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Carl-von-Linné Weg 10, 50829 Cologne, Germany*

[2]*Key Laboratory of Ministry of Education for Genetics, Breading and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and University, Fuzhou 350002*

In plant immunity, pathogen-activated intracellular NLR receptors rapidly mobilize resistance pathways but the downstream signaling mechanisms remain obscure. Enhanced disease susceptibility 1 (EDS1) - phytoalexin deficient 4 (PAD4) complexes control transcriptional defense reprogramming and resistance triggered by Toll-Interleukin1-Receptor (TIR)-family NLRs (TNLs). Induction of the salicylic acid (SA) hormone defense sector provides one crucial barrier against biotrophic pathogens. Arabidopsis EDS1/PAD4 complexes promote SA accumulation by inducing expression of the major SA synthesis gene, *ICS1*. We show that EDS1/PAD4 bolster SA-regulated resistance by inhibiting transcription activity of MYC2 (myelocytomatosis oncogene homolog 2), a master regulator of SA-antagonizing jasmonic acid (JA) hormone pathways. Interference with MYC2 boosts SA defenses independently of induced SA synthesis, thereby counteracting actions of a potent bacterial JA mimic, coronatine. While PAD4 mediates interaction with MYC2 *in planta,* suppression of MYC2 transcription activity requires an intact EDS1/PAD4 complex and EDS1 contribution beyond stabilizing PAD4. These data uncover an immune receptor signaling circuit that intersects with hormone pathway crosstalk to reduce bacterial pathogen growth.

Comprehensive capture-seq (Coca-seq) unravels gene regulation mechanism in plant immune signalling

Pingtao Ding [a], Bruno Pok Man Ngou [a], Ram Krishna Shrestha [a], Dan MacLean [a], Dawn Nagel [b], Jose Pruneda-Paz [b], Steve A. Kay [c], Jonathan DG Jones [a]

[a] The Sainsbury Laboratory, Norwich, United Kingdom, [b] University of California, San Diego, United States, [c] University of Southern California, Dornsife, United States

Pingtao.Ding@sainsbury-laboratory.ac.uk

Unlike the vertebrate immunity, plants lack a circulatory system. In order to achieve a certain threshold of inducible immunity, plants have evolved intracellular immune receptors to recognize pathogenic effectors deployed by infectious microbes. Here we are using versatile paired nuclei-localized immune receptors RPS4 and RRS1 from Arabidopsis as our model [1], for they together can confer resistance to multiple pathogens, including bacteria *Ralstonia solanacearum*, *Pseudomonas syringae*, *Xanthomonas campestris* and fungus *Colletotrichum higginsianum*. Similar to inflammosome signalling in mammalian innate immunity, upon pathogenic ligand recognition, RPS4/RRS1 can trigger cell death and resistance in planta. However how the downstream defence genes are switched on is largely unknown. Here we are reporting two distinct groups of master transcription factors (calmodulin-binding and MYC-like TFs), identified from a high-throughput yeast one-hybrid screen, play important but divergent roles in early defence genes activation and cell death. To investigate the molecular details of how these TFs regulate the target defence gene expressions, we generated customized clusters of 120nt RNA probes to capture/enrich the gene-of-interest sequences from ATAC-seq, RNA-seq and ChIP-seq libraries. From this comprehensive capture-seq (Coca-seq) datasets, we aim to identify key components sitting on the target gene bodies in the chromatin context and in relation to the activation of early time-point plant immune signalling.

References:

[1] PF Sarris et al (2016). Cell, 161-5: 1089-1100.

Intersection between MAMP-triggered innate immunity and symbiosis

Yan Liang
Institute of Biotechnology, Zhejiang University, Hangzhou

N-acetyl-D-glucosamine (GlcNAc)-containing microbial molecules can either act as MAMPs (Microbe-Associated Molecular Patterns) which induce plant innate immune responses or symbiotic signals which control the initiation of symbiosis. Chitooligosaccharides (COs), a β1-4 linked polymer of GlcNAc [degree of polymerization (dp)=6-8)], are fungal MAMPs, whereas lipochitooligosaccharides (LCOs), acylated COs with modification, are the key symbiotic signaling molecules, such as Nod factors for the legume-rhizobium symbiosis and Myc factors for the arbuscular mycorrhiza (AM) symbiosis. GlcNAc-containing molecules, whether they be MAMPs or symbiotic signals, are recognized by the lysin motif (LysM)-containing receptor kinases (LYK) or receptor-like proteins (LYP) in plants. In Arabidopsis, COs are recognized by LYK5-CERK1 complex, structurally similar to the model of the Nod factor recognition mediated by NFR1-NFR5 complex in legume plants. Recently, we found Arabidopsis chitin receptor LYK5 protein levels were regulated by E3 ligase Plant U-BOX (PUB) 13, which was identified through searching for the ortholog of MtPUB1, the E3 ligase interacting with Nod factor receptor[1]. The similarity of receptor model and regulatory mechanism between COs and LCOs raises the question how plants distingush these similar molecules and show apparently opposite responses. Therefore, we compared CO and LCO recognition in tomato plants, which can form symbiosis with AM fungi. Among the four members in the CERK1/NFR1 subclade, we found one LYK maintained the presumed ancestral function of CO recognition, however, its paralogous gene underwent neofunctionalization to recognize AM fungi, which might have evolved to become a Nod factor receptor in legumes. Taken together, these studies further supports our previous hypothesis that LCO recognition might have evolved from plant-fungal pathogen interaction.

References:

[1] Liao D, Cao Y, Sun X, Espinoza C, Nguyen CT, Liang Y*, Stacey G*. (2017) *New Phytologist,* DOI: 10.1111/nph.12898 (*co-corresponding author)

Identification and characterization of Arabidopsis genes that contribute to powdery mildew resistance via an EDS1- and SA-independent signaling pathway.

Qiong Zhang, Robert Berkey, Jiexiu Ouyang and Shunyuan Xiao
Institute for Bioscience and Biotechnology Research, University of Maryland College Park, Rockville, MD20850

Despite tremendous progress towards understanding the molecular mechanisms of host immunity and pathogenesis in recent years, how plants as host mount appropriate defense responses against pathogens with different levels of adaptation, and how pathogens manipulate and derive nutrients from host cells still remain poorly characterized. One of the most powerful approaches to address these questions is to identify and functionally characterize additional immune components and/or host targets required for fungal pathogenesis through genetic screens. Since conventional genetic screens have been saturated, we designed and performed a novel and sensitive mutant screening in the background of an immunocompromised mutant called *eps* (in which three major immune components are knocked out) for single point-mutations that render *eps* either "eds" (for enhanced disease susceptibility) or "edr" (for enhanced disease resistance) to well-adapted and poorly-adapted powdery mildew fungi. Strikingly, we identified over 20 "edr" mutants that display remarkable resistance to both a well-adapted and a poorly-adapted powdery mildew pathogen despite the (super) susceptibility of the parental types. We also identified mutants that show resistance to one but not the other powdery mildew species, along with mutants that display "eds" to both. Whole-genome sequencing and genetic complementation analysis revealed that several novel genes likely contribute to innate immunity through an EDS1- and salicylic acid-independent signaling pathway. Therefore, our findings will not only lead to a better understanding of host-immunity mechanisms, but also enable engineering of crop resistance against fungal pathogens using new gene-editing technologies.

Cotton is SWEET

Kevin L. Cox Jr.[1] and Libo Shan[1]

[1] *Department of Plant Pathology and Microbiology, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, Texas 77843*

Cotton is an economically important crop as it provides fiber, oil, and foodstuff. Its yield is constantly at risk being lowered due to various biotic stresses, including bacterial blight of cotton (BBC) caused by Xanthomonas citri subsp. malvacearum (Xcm). While the disease was previously controlled by resistance genes, it has surprisingly re-emerged in the U.S. within the last five years. By combining transcriptome profiling with transcription activator-like (TAL) effector-binding element (EBE) prediction, we show that GhSWEET10, encoding a functional sucrose transporter, is induced by Avrb6, a TAL effector determining Xcm pathogenicity. Activation of GhSWEET10 by designer TAL effectors (dTALEs) restores virulence of Xcm avrb6 deletion strains, whereas silencing of GhSWEET10 compromises cotton susceptibility to infections. We show via an extensive survey of GhSWEET transcriptional responsiveness to different Xcm field isolates that additional GhSWEETs may also be involved in BBC. Preliminary data with cotton cultivars commonly used in fields has suggested a correlation between SWEET transcriptional induction and disease severity. These findings advance our understanding of the disease and resistance in cotton and may facilitate the development cotton with improved resistance to BBC.

Virus infection of plants alters pollinator preference: A payback for susceptible hosts?

Sanjie Jiang[1], Simon C. Groen[1], Alex M. Murphy[1], Nik J. Cunniffe[1], Jack H. Westwood[1], Matthew P. Davey[1], Toby J.A. Bruce[2], John C. Caulfield[2], Oliver J. Furzer[1], Alison Reed[1], Sophie I. Robinson[1], Elizabeth Miller[1], Christopher N. Davis[1], John A. Pickett[2], Heather M. Whitney[3], Beverley J. Glover[1], and John P. Carr[1]

[1]*Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK.*

[2]*Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.*

[3]*University of Bristol, School of Biological Sciences, Bristol BS8 1UG, UK.*

Presenting Author Email: sj437@cam.ac.uk

Corresponding Author Email: jpc1005@hermes.cam.ac.uk

Virus infection induces changes in plant volatile emission profiles and this can make plants more attractive to insects, such as aphids, that are viral vectors. However, it is unknown if virus-induced alterations in volatile production affect plant-pollinator interactions. We found that volatiles emitted by cucumber mosaic virus (CMV)-infected tomato (*Solanum lycopersicum)* and *Arabidopsis thaliana* plants altered the foraging behaviour of bumblebees (*Bombus terrestris*). Virus-induced changes in volatiles emitted by tomato were identified by gas chromatography-coupled mass spectrometry. In tomato, CMV infection made plants emit volatiles attractive to bumblebees. Bumblebees pollinate tomato by sonicating flowers which releases pollen and enhances self-fertilization and seed production. If bumblebees were allowed to pollinate flowers from of mock-inoculated and CMV-infected tomato plants, the greatest increase in seed yield was seen for CMV-infected plants [1]. Increased pollinator preference may increase plant reproductive success in two ways: i) as female parents, by increasing the probability that ovules are fertilized; ii) as male parents, by increasing pollen export. Mathematical modeling suggested that in the wild, such increases to the number of offspring of infected susceptible plants resulting from increased pollinator preference could outweigh underlying strong selection pressures favoring pathogen resistance, allowing genes for disease susceptibility to persist in plant populations. We speculate that enhanced pollinator service for infected individuals in wild plant populations might provide mutual benefits to the virus and its susceptible hosts.

[1] Groen SC *et al*., 2017. Virus Infection of Plants Alters Pollinator Preference: A Payback for Susceptible Hosts? ***PLoS Pathogens***. 2016.12 (8): e1005790. doi: 10.1371/journal.ppat.1005790

Ecological Mechanisms of Microbiome Associated with Soybean Cyst Nematode Suppressive Soils

M. Imran Hamid[a,b], Muzammil Hussain[b], Meichun Xiang[b], Xingzhong Liu[b]

a Department of Plant Pathology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan

b State key lab of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing

Presenting author: mian_108@hotmail.com

mian_108@yahoo.com

Disease-suppressive soils are exceptional ecosystems in which crop plants suffer less from specific soil-borne pathogens by exploiting microbial consortia for protection against infections [1]. Soybean cyst nematode (SCN; *Heterodera glycines*) is the most destructive pest of soybean worldwide and its suppressive soils have been identified in some localities [2, 3]. The rhizosphere and cyst microbiomes may play vital role to protect soybean in the SCN suppressive soils. To decipher the ecological mechanisms involving in the development of specific soil suppression, we collected soil samples from the fields with different soybean monoculture years from several locations of northeast of China. Growth room pot experiments and by using the ultra-high-throughput sequencing approach, we identified the key bacterial and fungal taxa involved in SCN suppression. The long term monoculture soils showed natural suppression against SCN than the short term monoculture soils. At genus level, the *Pseudomonas*, *Purpureocillium* and *Pochonia* that have been documented to suppressing SCN were much more abundant in long term monoculture soils than that in short term monoculture soils [3, 4]. Furthermore, long term monoculture suppressive soils were taken into account for further investigation to test the disease suppressive ability by using different treatments designed as i) suppressive soil (S), ii) conducive soil (C), iii) conducive soil mixed with 10% (w/w) suppressive soil (CS), iv) suppressive soil treated at 80°C for 1 hr (S80), and v) suppressive soil treated with formalin (SF). Suppressiveness transferred by adding 10% of suppressive soil to conducive soil had a more pronounce effect on cyst microbiome variation than rhizosphere microbiome. A short heat disturbance (80 °C for 1 h) or formalin treatment

of suppressive soil reduced disease protection and resulted in the significant variation of rhizosphere and SCN cyst microbiome. Our results suggested that the plants engage a subset of functional microbial groups in the rhizosphere for initial defense upon nematode attack and later on colonize the nematode cysts to response for suppression of SCN in disease-suppressive soils.

References:

1. M. Mazzola (2004). Annual Review of Phytopathology, 42, 35.
2. X. Z. Liu, J. Q. Li, D. S. Zhang (1997). International Journal of Nematology, 7, 18.
3. S. Chen (2007). Biocontrol Science and Technology, 17, 125.
4. M. I. Hamid, M. Hussain, Y. Wu, X. Zhang, M. Xiang, X. Liu (2017). FEMS Microbiology Ecology, 93(1), fiw222.

Functional analysis of novel rice blast fungus effectors illuminates the mechanism of plant-fungus interaction

Xia Yan[1], Darren M. Soanes[1], Magdalena Martin Urdiroz[1], Lauren S. Ryder[1], Bozeng Tang[1], Michael J. Kershaw[1], Nicholas J. Talbot[1]

1 School of Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, EX4 4QG, United Kingdom

The first line of defence against fungal pathogens by plants is the detection of pathogen-associated molecular patterns by pattern recognition receptors to trigger PAMP-triggered immunity (PTI). Pathogens that overcome PTI and successfully invade host cells do so by delivering effectors that interfere with PTI, leading to effector-triggered susceptibility (ETS). We have identified a family of novel effector-encoding genes in the rice blast fungus *Magnaporthe oryzae* in this study. Transcriptome analysis was applied to identify genes that were differentially expressed during plant infection and which were temporally co-regulated during the early stages of tissue colonisation by the fungus. Putative effector genes were characterized by targeted gene deletion and protein localization using live-cell imaging. We characterized a group of *MEP (Magnaporthe effector* protein) genes, apoplastic *MEP* effectors outlined invasive hyphae while cytoplasmic *MEP* effectors accumulated at the biotrophic interfacial complex (BIC). Specifically, *MEP1* and *MEP3* were involved in suppression of plant immunity, respectively. Potential effector interacting partners in rice were identified using yeast two hybrid analysis and co-immunoprecipitation in infected plant tissue. The discovery of *MEP* effector targets in rice indicates plant defence response may involve key signaling hubs. The characterization of novel rice blast fungus effectors and their interacting partners in rice sheds new insights into understanding the plant-fungus interaction.

A defence pathway linking plasma membrane to chloroplasts is co-opted by a virus to suppress salicylic acid signaling

Laura Medina-Puche, Tabata Rosas-Diaz, Liping Wang, Tamara Jimenez-Gongora, Dan Zhang, Xue Ding, Chanhong Kim, Yuanyuan Zhang, Shanshan Lv, Renyi Liu, Rosa Lozano-Duran*

Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, Shanghai

*laura@sibs.ac.cn, lozano-duran@sibs.ac.cn**

Plant pathogens need to suppress plant defence responses in order to establish a successful infection. RNA silencing is considered the main plant anti-viral defence; in recent years, however, it has become increasingly clear that plants have additional strategies against viral invasion, which include production of defensive hormones such as salicylic acid (SA) and jasmonic acid. C4 is a small protein encoded by *Tomato yellow leaf curl virus* (TYLCV) which is essential for infectivity, but of which the molecular function remains obscure. Our results indicate that C4 is localized in two different subcellular compartments, namely plasma membrane and chloroplasts. This double localization correlates with the presence in the C4 protein of two targeting signals: an N-myristoylation motif required for plasma membrane localization, and a chloroplast transit peptide. Interestingly, we have found that these two targeting signals are present in a number of pathogen effectors, as well as in a subset of plant proteins, many of which have been ascribed a role in defence. This finding suggests that a pathway may exist in plants linking plasma membrane and chloroplasts to regulate defence, and that this putative pathway is hijacked by plant pathogens, presumably to suppress these responses. Strikingly, transcriptome analysis of Arabidopsis transgenic plants expressing C4 shows a clear repression of SA biosynthesis and responses. Treatment with pathogen-associated molecular patterns (PAMPs) to activate defence triggers a re-localization of C4 from plasma membrane to chloroplasts, and expression of C4, or a non-myristoylable version of C4 that localizes to chloroplasts exclusively, inhibits SA production in response to PAMP treatment. Activation of SA biosynthesis in response to PAMPs requires retrograde signaling from the chloroplast to the nucleus. Interestingly, C4 does not affect responses to exogenously applied SA, but suppresses up-regulation of PAMP-responsive nuclear genes that

are activated by retrograde signaling. Based on these results, our current hypothesis is that activation of plant defence leads to the re-localization of C4 from plasma membrane to chloroplasts, where it interferes with retrograde signaling, suppressing SA biosynthesis to promote the viral infection.

A novel *Magnaporthe oryzae* effector targets the Light-harvesting chlorophyll a/b-binding protein (Lhcb5) to suppress immunity in rice

Muxing Liu [a], Zhengguang Zhang [a]

Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, and Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing.

In the plant-pathogen interaction, plants depend on innate immunity systems to defense the attacking pathogens. To establish successful colonization, pathogens secreted effectors to interfere with plant innate immunity [1]. It is clear that, these effectors can either stay in the extracellular space between the plant plasma membranes and the fungal cell walls, or translocate into the plant cells [2] [3] [4] [5].

In previous study, we found that Qc-SNARE protein MoSyn8 mediates intracellular trafficking and affects the secretion of effectors [6]. In order to discover more effector molecules regulated by MoSyn8, we performed two-dimensional electrophoresis (2-DE) to investigate the protein secretion in the Δ*Mosyn8* mutant. Further study found a secreted protein MoPrs (Protein Regulated by MoSyn8) can be secreted into the host cells to suppress host immune response. The rice cDNA library screening found that MoPrs targets light-harvesting chlorophyll a/b binding protein Lhcb5 in rice. We also found Lhcb5 protein responses to the infection of *Magnaporthe oryzae*. In the rice-*M.oryzae* interaction, Lhcb5 is induced to be phosphorylated, which produced ROS and caused cell death of rice to inhibit the infection of *M.oryzae*. MoPrs can inhibit the phosphorylation of Lhcb5, suppressing the immune response regulated by Lhcb5 and promoting the infection of *M. oryzae*.

[1] Jones JD, Dangl JL, 2006. The plant immune system. *Nature* **444**, 323-9.
[2] Kankanala P, Czymmek K, Valent B, 2007. Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell* **19**, 706-24.
[3] Tanaka S, Brefort T, Neidig N, *et al*., 2014. A secreted Ustilago maydis effector promotes virulence by targeting anthocyanin biosynthesis in maize. *Elife* **3**, e01355.
[4] Giraldo MC, Dagdas YF, Gupta YK, *et al*., 2013. Two distinct secretion systems facilitate tissue invasion by the rice blast fungus Magnaporthe oryzae. *Nat Commun* **4**, 1996.
[5] Khang CH, Berruyer R, Giraldo MC, *et al*., 2010. Translocation of Magnaporthe oryzae effectors into rice cells and their subsequent cell-to-cell movement. *Plant Cell* **22**, 1388-403.
[6] Qi Z, Liu M, Dong Y, *et al*., 2016. The syntaxin protein (MoSyn8) mediates intracellular trafficking to regulate conidiogenesis and pathogenicity of rice blast fungus. *New Phytol* **209**, 1655-67.

Two related receptor kinases SYR1 and SYR2 of tomato act as high and low affinity receptors for the plant peptide hormone systemin

Lei Wang [a], Elias Einig [a], Markus Albert [a], Judith Fliegmann [a], Axel Mithoefer [b], Hubert Kalbacher [c], Georg Felix [a]

[a] *Center for Plant Molecular Biology, University of Tuebingen, Tuebingen, Germany*

[b] *Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany*

[c] *Interfaculty Institute of Biochemistry, University of Tuebingen, Tuebingen, Germany*

lei.wang@zmbp.uni-tuebingen.de; markus.albert@zmbp.uni-tuebingen.de; georg.felix@zmbp.uni-tuebingen.de

Plant peptides play important roles regulating growth, development and interaction with other organisms [1]. Discovered more than a quarter-century ago as the first plant peptide hormone, systemin was shown to be critical for systemic wound response and anti-herbivore defense in tomato [2, 3]. The receptor for this peptide hormone remained mysterious since the receptor SR160 proposed earlier [4] is a tomato homolog of the brassinosteroid receptor BRI1 and its role as systemin receptor could not be corroborated in later work [5, 6]. Starting with the observation that the wild tomato *S. pennellii*, in contrast to the cultivated tomato *S. lycopersicum*, lacks sensitivity to systemin, we mapped the trait responsible for systemin responsiveness by using a collection of introgression lines between these two species [7] and cloned two closely related leucine-rich repeat receptor like kinases (LRR-RLKs) that defined sensitivity to systemin. Heterologous expression of these receptors, named Systemin Receptor 1 (SYR1) and Systemin Receptor 2 (SYR2), confered systemin responsiveness to *Nicotiana benthamiana* and *Arabidopsis thaliana*, corroborating their role as systemin receptors. SYR1 exhibited specific, high-affinity binding for systemin whereas SYR2 acted as a low-affinity receptor. Complementing SYR1 into the introgression line lacking systemin receptors showed that presence of this receptor, although not decisive for local and systemic wound responses, was important for defense against the generalist insect herbivores *Spodoptera littoralis*.

References:

[1] P. Tavormina, et al. (2015). The Plant Cell, 27(8), 2095-2118.
[2] G. Pearce, D. Strydom, S. Johnson, C. A. Ryan (1991) Science, 253(5022), 895-899.
[3] C. A. Ryan, G. Pearce (1998). Annual review of cell and developmental biology, 14(1), 1-17.
[4] J. M. Scheer, C. A. Ryan (2002). Proceedings of the National Academy of Sciences, 99(14), 9585-9590.
[5] N. Holton, et al. (2007). The Plant Cell, 19(5), 1709-1717.
[6] R. Malinowski, et al. (2009). Plant molecular biology, 70(5), 603-616.
[7] Y. Eshed, D. Zamir (1995). Genetics, *141*(3), 1147-1162.

Transcriptional programming of *Phytophthora sojae* for organ-specific infection

[a]Wenwu Ye, [a]Yang Wang, [a]Long Lin, [a]Jiawei Wu, [a]Yachun Lin, [a]Yuanchao Wang
[a]*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095*
Email: yeww@njau.edu.cn

The different organs of a plant are significantly different in structure, metabolism, and defense response, however, the mechanisms on how plant pathogens regulate for organ-specific infection were largely unknown. Effector proteins are secreted by many plant pathogens to promote infection, e.g., the genome of the soybean root rot pathogen *Phytophthora sojae* contains hundreds of effector genes. We have found that different effector genes could be transcriptionally programmed following infection, and the mechanism facilitated the effectors target different functional branches of the plant defense response. To learn our next hypothesis that *P. sojae* transcriptionally reprogram effector genes to facilitate its organ-specific infection in soybean, we recently compared the transcriptomes of *P. sojae* during the early stage of its infection in soybean roots and leaves. We identified the differentially expressed genes and found many enriched pathogenicity-related gene families were likely associated with the response of pathogen against different plant cell structure components and defense stresses. Functions of several candidate genes were further studied based on CRISPR/CAS9-mediaed gene knock out. A bZIP transcription factor of *P. sojae* was revealed to play a specific role in the infection of soybean roots. The results revealed that transcriptional programming are important for successful infection and host environment adaptability of *Phytophthora* pathogens.

Understanding the CCG effector toolbox of white rust oomycete pathogen *Albugo candida*

Amey Redkar[1], Volkan Cevik[1,2], Kate Bailey[1], Hossein Borhan[3], Eric Holub[4] and Jonathan D.G. Jones[1]

[1]*The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH, United Kingdom.*

[2] *Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom*

[3]*Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N0X2, Canada.*

[4]*University of Warwick, School of Life Sciences, Warwick Crop Centre, Wellesbourne, CV35 9EF, United Kingdom.*

E-mail: amey.redkar@tsl.ac.uk

Abstract:

Albugo candida is an obligate biotrophic oomycete pathogen that causes white blister rust disease in Brassicaceae, and comprises many physiological races that infect distinct host species. Some *A. candida* races can also infect various Arabidopsis accessions, thus facilitating the characterization of effectors and resistance genes that are involved in this obligate biotrophic patho-system. *A. candida* induces a potent immuno-compromised state following infection of susceptible host plants, which can enable different pathogens to colonize and reproduce in the same tissue [1]. Co-habitation of different races on the same host is therefore possible, and could be an important means of generating novel races through the exchange of effector repertoires. Our analyses of multiple *A. candida* genomes reveal the presence of novel class of secreted CX_2CX_5G (abbreviated as CCG) effector family. Every physiological race has around 60-80 CCG secreted proteins which also shows presence/ absence polymorphism. Multiple CCG effectors are recognized by a resistance gene White Rust Resistance 4 (*WRR4*) from the model *Arabidopsis thaliana* (Col-0) [2]. Some of these candidate CCG proteins also confer an enhanced susceptibility to other oomycete pathogens in stable transgenic *A. thaliana* lines. Current experiments aim to functionally characterize these CCG effectors especially for their potential involvement in *A. candida* mediated immune suppression. Recent progress on these aspects will be presented.

Acknowledgement: I am thankful to The Genetics Society, UK for Junior Scientist Travel Award to attend this meeting, and an EMBO fellowship to support my work.

References:

[1] K. Belhaj, L.M. Cano, D.C. Prince, A. Kemen, K. Yoshida, Y.F. Dagdas, G.J. Etherington, H.J. Schoonbeek, H.P. van Esse, J.D.G. Jones, S. Kamoun, S. Schornack (2017). Cellular Microbiology, 19, e12628.

[2] M.H. Borhan, N. Gunn, A. Cooper, S. Gulden, M. Tor, S. Roger-Rimmer, E.B. Holub (2008). MPMI, 21(6), 757.

The role of barberry in virulence variation and epidemics of wheat stripe rust in China

Zhensheng Kang, Jie Zhao, YuanTian, Yuanyuan Zhao, Qiao Li, Gangming Zhan, Lili Huang
State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling 712100, Shanxi
**correspondence author: kangzs@nwsuaf.edu.cn*

Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a destructive disease of wheat worldwide. Virulence variation of the pathogen contributes to produce new races, resulting in breakdown of wheat resistance, and causing subsequent epidemics of the disease. Recently, sexual cycle of *Pst* was found based on identification of barberry (*Berberis*) as alternate host, making it possible to understand roles of sexual reproduction in virulence variation of *Pst* and alternate hosts in epidemics of the disease. In China, our investigations found that many various barberry species widely distributed in epidemic areas of the disease and most of them were alternate hosts for *Pst* based on artificial inoculation. More importantly, a large number of *Pst* samples were recovered in succession from naturally infected barberry species. Some were known races including current predominant races and the other were new races based on virulence tests. Barberry-derived *Pst* samples were originated from the western China in which susceptible barberry widely distributed in quantity or species and sexual reproduction of *Pst* on barberry occurred regularly. Our results indicated that the western China could be the center of origin of *Pst* and hotspot for the pathogen variation. High variation frequencies were revealed by phenotypic and genotypic analyses for sexual progeny of *Pst*, suggesting that sexual reproduction is a major approach for emergence of new races by sexual recombination.

Dissecting the role of the *Phytophthora sojae* effector repertoire using CRISPR

Brett M. Tyler[a], Felipe Arredondo[a], Anna Co[a], Linkai Cui[a,b], Madeleine Duquette[a], Yufeng Fang[a], Biao Gu[a,c], Brent Kronmiller[a], Chidambareswaren Mahadevan[a,d], Jianqiang Miao[a,e], Hua Wise[a]

[a] *CeOregon State University, Corvallis, Oregon, USA;* [b] *Henan University of Science and Technology, Luoyang;* [c] *Northwest A&F University, Yangling;* [d] *Rajiv Gandhi Centre for Biotechnology, Kerala, India;* [e] *China Agricultural University, Beijing*

Oomycete pathogens cause destructive diseases on a huge range of plants of importance to agriculture, horticulture and natural ecosystems. Many of these pathogens are highly adaptable, readily overcoming chemical and genetic control measures, and jumping to new host species. Genomic studies have implicated hundreds to thousands of genes as potentially contributing to the virulence of oomycete pathogens. The products encoded by these genes include transporter proteins, toxins, secreted effector proteins, hydrolytic enzymes, and inhibitors of plant defense enzymes. In the soybean pathogen, *Phytophthora sojae*, the genome contains nearly 400 genes that encode RxLR effectors that can enter plant cells to target specific components of the plant signaling and defense machinery. Surprisingly however, as many of these RxLR genes are completely or nearly silent. In any one *P. sojae* strain, around 75% of the RxLR genes have a transcript level less than 0.1% of the highest expressed effector (*Avr1a*). Only 5% of the RxLR genes (21 genes) account for 90% of the RxLR transcripts. However, the genes that are silent and those that are most transcribed varies extensively from strain to strain. In a comparison of four strains, 39% of the most highly transcribed genes showed over 500-fold variation. These results suggest that *Phytophthora sojae* achieves extensive pathogenic plasticity through transcriptional polymorphisms. Evolutionary modeling has suggested that the most highly transcribed genes may be essential for virulence, in spite of their high levels of transcriptional variation. We have used dsRNA-mediated transient gene silencing to assess the contributions to virulence of RxLR genes that are highly and consistently transcribed during infection. The results indicate that around one-third of these genes are individually essential for full virulence. More recently, we have been using CRISPR-mediated gene knockouts to assess the contributions of RxLR genes

to virulence. This technology is very efficient in *P. sojae* and readily yields homozygous knockouts via gene conversion. While some knockouts have not confirmed results obtained from gene silencing, one effector gene, *Avh180*, was identified as important for root infection and essential for leaf infection. In order to delve into the mechanisms of genomic plasticity in *P. sojae,* we selected for *P. sojae* variants that could infect leaves in spite of an *Avh180* deletion. RNA sequencing of these second site suppressor strains has revealed that unsilencing of diverse non-effector genes can overcome the loss of the effector gene.

The role of a truncated NLR protein in plant immunity

Na Liu[1,2], Katharina Hake[3], Wei Wang[2,4], Ting Zhao[2], Lu Rui[1], Tina Romeis[3], Dingzhong Tang[1,2*]

[1]*Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou 350002*

[2]*State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101*

[3]*Department of Plant Biochemistry, Dahlem Centre of Plant Sciences, Freie Universität Berlin, 14195 Berlin, Germany*

[4]*University of Chinese Academy of Sciences, Beijing 100049*

**Corresponding author: (email: dztang@genetics.ac.cn)*

ABSTRACT

Plant disease resistance (R) proteins play critical roles in plant immunity by directly or indirectly recognizing pathogen effectors, and leads to effector triggered immunity. Most of the R proteins are intracellular, and belongs to nucleotide binding (NB) domain and leucine-rich repeat (LRR)-containing (NLR) immune receptors. Besides those full length NLR immune receptors, there are many atypical NLRs that lack LRR domain in plant genome. Although those truncated NLR proteins are implicated in plant immunity, the function of those proteins are not well understood. To study the mechanism of plant immune response, we previously performed a mutant screen for enhanced disease resistance to powdery mildew. In this screen, we identified Arabidopsis *exo70B1-3* mutant, which shows activated defense responses upon infection and express enhanced resistance to fungal, oomycete and bacterial pathogens. EXO70B1 is one of the eight subunits of exocyst complex, which plays a critical role in exocytosis. In a screen for mutations that suppress *exo70B1* resistance, we identified multiple alleles of a truncated NLR, TIR-NBS2 (TN2), suggesting that loss-of-function of EXO70B1 leads to activation of the TN2-mediated plant immunity. TN2 is atypical NLR-like protein because it lacks the LRR domain common in typical NLR receptors. In addition, TN2 interacts with EXO70B1 in yeast and *in planta*, suggesting that pathogen effectors could evolve to target EXO70B1 to manipulate plant secretion machinery, and TN2 could monitor EXO70B1 integrity as part of an immune receptor complex. In the same suppressor screen, we found that *exo70B1*-activated autoimmune responses also require *CPK5*, a

Calcium-dependent protein kinase that functions as calcium sensor and plays important roles in plant immunity. However, the CPK5 homologs CPK4, CPK6, and CPK11, which were previously reported to function redundantly with CPK5 in effector-triggered immunity, did not contribute to *exo70B1*-associated phenotypes, indicating that CPK5 plays a unique role in plant immunity. We showed that membrane localization and kinase activity of CPK5 are critical for its function in the *exo70B1*-mediated pathway. Overexpressing *CPK5* results in TN2-dependent autoimmunity and enhanced disease resistance, reminiscent of the *exo70B1* phenotypes. Ectopic expression of *CPK5* in the *exo70B1* mutant led to constitutive CPK5 protein kinase activity, which was not detectable in *tn2* mutants. Furthermore, TN2 interacts with the CPK5 N-terminal variable and kinase domains, stabilizing CPK5 kinase activity *in vitro*. This work uncovers a direct functional link between an atypical immune receptor and a crucial component of early immune signaling. Recent progress on this project will be discussed.

References:

Liu, N., Hake, K., Wang, W., Zhao, T., Romeis, T., Tang, D. CALCIUM-DEPENDENT PROTEIN KINASE5 associates with the truncated NLR protein TIR-NBS2 to contribute to *exo70B1*-mediated immunity. ***Plant Cell.*** 2017, 29: 746–759

Zhao, T., Rui, L., Li J., Nishimura M., Vogel J., Liu, N., Liu, S., Zhao, Y., Dangl, J., Tang, D. A truncated NLR protein, TIR-NBS2, is required for activated defense responses in the *exo70B1* mutant. ***PLoS Genetics***. 2015, 11: e1004945.

The parasitic plant dodder transfers biotic and abiotic stress-induced systemic signals among host plants

Christian Hettenhausen, Jingxiong Zhang, Juan Li, Guojing Shen, Huifu Zhuang, and Jianqiang Wu
Department of Economic plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming
wujianqiang@mai.kib.ac.cn

Cuscuta spp. (dodders) are stem parasites that naturally graft to their host plants to draw water and nutrients, and multiple adjacent hosts can often be parasitized by one or more *Cuscuta* plants simultaneously, forming plant clusters. Although it is known that metabolites, proteins, and mRNAs are transferred from hosts to *Cuscuta* and *Cuscuta* even facilitates host-to-host virus movement, yet it remains elusive if *Cuscuta* bridge connections mediate ecologically meaningful communications in these plant clusters. We show that when two host plants are bridge-connected by *Cuscuta* parasites, insect herbivory on one host induced large transcriptomic changes in the attacked local leaves, undamaged systemic leaves of the attacked plant, as well as in leaves of the systemic host, indicating that *Cuscuta* connections enabled a new form of herbivory-induced inter-plant systemic signaling. Detailed analysis indicated that this type of inter-plant signaling can be found between conspecific or heterospecific hosts of different families and even occur in several consecutively *Cuscuta*-connected host plants over long distances (> 100 cm). Importantly, herbivory on one host plant elevated defensive metabolites in the other systemic *Cuscuta* bridge-connected hosts, resulting in enhanced defense against insects. Furthermore, we provide compelling evidence that *Cuscuta* bridges also transfer nutrient deficiency-induced systemic signaling among different hosts. Namely, when one of the *Cuscuta*-bridge connected hosts is stressed by nitrogen deficiency, a systemic signal travels to other hosts and reconfigures their transcriptomes. We also show that there is intensive crosstalk between hosts, which reshapes the transcriptomic responses of N-starved hosts and hosts under normal conditions. Thus, *Cuscuta* facilitates plant-to-plant signaling and influences host trophic interactions with insects and plant adaptation to nutrient stresses.

How does a plant immune receptor activate heterotrimeric G proteins

Xiangxiu Liang , Miaomiao Ma, and Jian-Min Zhou
[S]*State Key Laboratory of Genome Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, #1 West Beichen Road, Chaoyang Disrict, Beijing 100101*
jmzhou@genetics.ac.cn

Higher plants deploy a large repertoire of Pattern-Recognition Receptors (PRRs), composed of Receptor Kinases (RKs) or Receptor-Like Proteins localized on plant cell surface, to perceive molecular patterns that are released by the pathogen or the host plant during infection and activate pattern-triggered immunity. An extensively studied immune RK is FLS2, which perceives the bacterial flagellin epitope flg22 and forms an active receptor complex with its co-receptor BAK1, a receptor-like kinase. The receptor complex also includes the receptor-like cytoplasmic kinase BIK1, which acts as a rate-limiting factor that relay the signal to activate multiple downstream signaling components. The stability and activity of the receptor complex is subject to sophisticated regulation. For example, we and others have shown that BIK1 stability is regulated by the calcium-dependent protein kinase CPK28 [1] and heterotrimeric G proteins consisted of XLG2 (Gα), AGB1 (Gβ), and AGG1/2 (Gγ) [2], the latter are directly coupled to the FLS2 receptor complex. Ongoing research in my laboratory addresses dynamically control the homeostasis of BIK1 and regulation of the aforementioned heterotrimeric G proteins by the FLS2 receptor complex. We will present our new findings concerning how the ubiquitin-proteasome system interacts with heterotrimeric G proteins and CPK28 to form a regulatory module to dynamically regulate BIK1 stability and immunity. We will also discuss major differences between plant and animal heterotrimeric G proteins and how the plant heterotrimeric G proteins maybe activated upon flg22 perception.

References:

[1] J. Monaghan, S. Matschi, O. Shorinola, H. Rovenich, A. Matei, C. Segonzac, F.G. Malinovsky, J.P. Rathjen, D. MacLean, T. Romeis, T., *et al*. (2014). Cell Host Microbe 16, 605.

[2] X. Liang, P. Ding, P., K. Lian, J. Wang, J., M. Ma, L. Li, L. Li, M. Li, X. Zhang, S. Chen *et al*. (2016). eLife *5*, e13568.

Sclerotinia sclerotiorum, can it be a friend?

Hongxiang Zhang, Zheng Qu, Jiatao Xie, Jiasen Cheng, Bo Li, Tao Chen, Yanping Fu, Guoqing Li, Daohong Jiang

State Key Laboratory of Agricultural Microbiology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070
E-mail: daohongjiang@mail.hzau.edu.cn

Sclerotinia sclerotiorum is a worldwide spread notorious ascomycetous fungus, it could attack more than 400 species of plants and leads to huge economic losses eachyear on many economic important crops. In China, stem rot of rapeseed (*Brassica napus*) caused by *S. sclerotiorum* is the major disease that restricts the production efficiency of rapeseed, especially at the middle and lower reaches of the Yangtze River where the rainfall is very rich during the rapeseed flowering season. *S. sclerotiorum* forms sclerotia, dormant structure to overseasons, on killed plants at the late stage of infection. Sclerotia could germinate both carpogenically to produce ascospores and myceliogenically to produce hyphae. *S. sclerotiorum* invades plant via either ascospores or hyphae, usually, ascospore-infection is initiated on petals and senescent tissues, and the hyphae-infection starts at the stem base near ground surface. *S. sclerotiorum,* as a typical necrotrophic pathogen, produces oxalic acids and other organic acids and plant cell wall-degrading enzymes to kill plant cells and tissues before invading. Researches revealed that oxalic acid produced by *S. sclerotiorum* has other functions related to pathogenicity, and effectors or effector-like small proteins have been identified in *S. sclerotiorum* [1, 2], thus, the pathogenicity of *S. sclerotiorum* is more complicate than once we thought. Previously, we isolated and identified a hypovirulence-associated DNA virus, SsHADV-1, from a hypovirulent strain DT-8. This virus has very strong infectivity; its virus particles could directly infect hyphae of *S. sclerotiorum*. Either spraying virus particles or hyphal fragments of virus-infected strains on aerial parts of plants could protect plants against the infection of virus-free *S. sclerotiorum* [3, 4]. Here, we further find that this virus-infected *S. sclerotiorum* could live on rapeseed, and enhance the resistance against *S. sclerotiorum* and *Botrytis cinerea* and promote the growth of inoculated plants. Thus, *S. sclerotiorum* could be a friend for us when infected by mycoviruses.

Acknowledgement: This research was supported by the National Natural Science Foundation of China (Grants 31430070), and China Agriculture Research System (Grant CARS-13)

References:

[1] B.Williams, M. Kabbage, H. Kim, R. Britt, M. B. Dickman. (2011). PLoS Pathogens 7: e1002107
[2] X. Lyu, C. Shen, Y. Fu et al. (2016). PLoS Pathogens 12: e1005435
[3] X. Yu, B. Li, Y. Fu et al. (2010). Proc Natl Acad Sci U S A 107: 8387.
[4] X. Yu, B. Li, Y. Fu et al. (2013). Proc Natl Acad Sci U S A 110: 1452.

Allelic diversity in resistance gene enables rice to combat brown planthopper variation

Guangcun He (gche@whu.edu.cn)
National Key Laboratory of Hybrid Rice, College of Life Sciences
Wuhan University, Wuhan 430072

Brown planthopper (*Nilaparvata lugens*, BPH) is one of the most destructive insect pests of rice (*Oryza sativa*). This insect is believed to have undergone a host shift from *Leersia* plants to rice ~0.25 million years ago. After that, BPH evolved as a monophagous insect herbivore of the rice. During the course of its coevolution with rice, BPH has evolved in virulence and multiple biotypes have been reported to infested different rice varieties.

In rice, resistance to BPH has been determined for many accessions in the cultivated varieties and wild species. Since the resistance gene *Bph1* was first identified in cultivated rice, about 30 genes for BPH-resistance have been reported so far. These genes show different resistance spectrums and levels against biotypes of BPH. Interestingly, most of BPH-resistance genes are mapped on several chromosome regions in clusters. The cluster on the long arm of chromosome 12 (12L) is the largest one that harbors eight BPH-resistance genes, including the most widely used *Bph1* and *Bph2*. Recently, we isolated *Bph9* in 12L cluster via map-based cloning strategy. We showed that the other BPH-resistance genes in the cluster are alleles of *Bph9*. This gene locus showed wide sequence diversity in the rice germplasm. This finding showed that allelic diversity in *Bph9* enables rice to combat planthopper variation. Among the isolated BPH-resistance genes, most encode the NB-LRR proteins. *Bph3* and *Bph15* encode the lectin receptor-like kinases (LecRKs). The *bph29* and *Bph32* encode novel proteins. Exploring diversity in BPH-resistance genes is essential in modern rice improvement programs. It is expected that more details and mechanisms will be elucidated in the near future and eventually facilitate the development of BPH-resistant rice variety.

Extracellular Vesicles: An Underappreciated Component of the Plant Immune System

Brian Rutter and Roger Innes

Exosomes are extracellular vesicles (EVs) that play a central role in intercellular signaling in mammals by transporting proteins and small RNAs. Plants are also known to produce EVs, particularly in response to pathogen infection. The contents of plant EVs have not been analyzed, however, and their function is unknown. Recent work in the Innes laboratory has revealed that plant EVs are highly enriched in proteins involved in biotic and abiotic stress responses, and carry miRNAs. In addition, EV secretion is enhanced in plants infected with *Pseudomonas syringae* and in response to treatment with salicylic acid. These findings suggest that EVs represent an important component of the plant immune system. In this talk I will present our ongoing investigations into the possible functions of EVs, and our initial investigations into the genetic requirements for EV biosynthesis.

A mutant involved in EPS signals to mediate infection thread formation in *Medicago truncatula*

Yali zhu[1,2], Fang Xie[1]

[1] *National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

[2] *University of the Chinese Academy of Sciences*

E-mail: xiefang@sibs.ac.cn

In the legume–rhizobium symbiosis, bacteria invade into host roots mainly through plant-made tunnels structure, infection thread. Bacteria produced signal molecules including Nod factor and exopolysaccharides (EPS) are essential for the infection thread formation. The Nod factor signals perception and signal transduction have been widely studied during the recent decades, but for EPS signals, only EPS receptor EPR3 been found in *Louts japonicus* recently. One mutant was found in *Medicago truncatula* which may involve in EPS signal transduction pathway. This mutant have normal infection and nodulation phenotype when inoculated with wildtype rhizobia Rm1021, however, the infection events was great reduced after inoculated with *exo* mutant. This gene was induced by rhizobia and expressed in the infected root hairs, nodule primordia and the mature nodules' meristem zone. We're working on the molecular mechanism of how this protein perceive and transduce EPS signaling during nodulation process.

Receptor Histidine Kinase RavS of *Xanthomonas campestris* Is A Novel Cyclic-di-GMP Effector that Regulates the Lifestyle Transition from Virulence to Free-Living

Shou-Ting Cheng, Wei Qian

State Key Laboratory of Plant genomics, Institution of Microbiology, Chinese Academy of Sciences, Beijing 100101

Two-component signal transduction system(TCS) and cyclic di-GMP(c-di-GMP) regulation system are two major regulatory systems in bacteria cells. TCS, composed by a membrane-bound histidine kinase(HK) and a cytoplasmic response regulator(RR), is one of

the most important machinery to detect and respond to environmental and cellular signals.C-di-GMP is a ubiquitous second messenger that modulates various physiological processes of bacteria. Here we revealed that in *Xanthomonas campestris*(*Xcc*), the causative agent of black rot disease of cruciferous plants, TCS RavA-RavR and another HK RavS form a three-component signaling system. Among them, RavR is a response regulator containing an EAL domain and a degenerated GGDEF domain. Inactivation of *ravA* and *ravR* significantly decreased EPS production and bacterial virulence but increased swimming ability and flagella development, while inactivation of *ravS* resulted in decreased bacterial swimming ability and seriously inhibited flagella development. Epistasis analysis revealed that *ravS* is a downstream gene of *ravR*. We found that RavR could negatively control the phosphorylation level of RavS by accepting its high energy phosphate group. C-di-GMP specifically binds RavS to enhance the latter's phosphotransferase activity towards RavR, which acts as a phosphate sink on this occasion. In addition, RavR could also positively regulate the phosphorylation level of RavS by hydrolyzing cellular c-di-GMP. Phosphorylation level of RavS, regulated by RavR and c-di-GMP, is critical in controlling *Xcc* virulence and swimming ability. Our results revealed that bacterial HK is potential c-di-GMP effector, which gives insight into the understanding of bacterial regulation of life-style transition.

Prc Protease Cleavages Sensor Region of Receptor Histidine Kinase VgrS of *Xanthomonas campestris* to Promote Stress Tolerance by Sequestering Phosphorylation Signaling

Chaoying Deng [a], Huan Zhang [a], Li Wang [a], Yue Pan [a], Wei Qian [a]
[a] *State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beichenxi Road, Chaoyang District, Beijing*
qianw@im.ac.cn

Cellular signaling transduction system is of great importance for organisms, which could be generally classified into two groups: reversible and irreversible. Proteolysis catalyzed by proteases is a prominent irreversible signaling cascade in modulating cell physiology [1, 2], while protein phosphorylation catalyzed by kinases/phosphatase is reversible [3]. In

prokaryotes, crosstalk between two signaling pathways attracts much attention in recent years; however, how proteolysis controls protein phosphorylation by cleaving receptor kinases remains to be an opening question. The major technical challenge is that the identification of the physiological substrates of proteases is quite difficult.

Our previous studies revealed that in *Xanthomonas oryzae* pv. *oryzae*, the causative agent of rice bacterial blight disease, encodes at least six PDZ-domain containing proteases. Inactivation of one coding gene, *prc* (also named *tsp*), resulted in substantial virulence attenuation and hypersensitivities to multiple cell envelope stresses [4, 5]. We continue to identify the physiological substrates of Prc and investigate the role of Prc-catalyzed proteolysis in controlling the environmental adaptation in *Xanthomonas campestris* pv. *campestris*, which is more genetic amenable than *X*. *oryzae* pv. *oryzae*. Our results revealed that under osmolarity stress, Prc directly binds and cleaves the sensor region of VgrS, a canonical HK controlling the bacterial virulence and stress responses. Proteolysis of VgrS sensor remarkably decreased the autokinase activity of this HK, which results in reprogram of the VgrS-triggered gene expression and significantly promote the bacterial resistance to osmolarity stress. Since Prc-like proteases and HKs are signaling proteins commonly distributed in the kingdom of gram-negative bacteria, our results suggest that inactivation of HK by periplasmic protease might be a general and important crosstalk between TCS and proteolysis regulations.

References:

[1] E. Gur, D. Biran, E. Z. Ron (2011). Nature Reviews Microbiology 9, 839.
[2] R. J. Tomko, Jr., M. Hochstrasser (2013). Annual Review of Biochemistry 82, 415.
[3] T. Hunter (1995). Cell 80, 225.
[4] C. Y. Deng, A. H. Deng, et al (2014). Molecular Plant-Microbe Interactions 27, 101.
[5] W. Qian et al (2005). Genome Research 15, 757.

Chromatin Immunoprecipitation-Sequencing Analysis Revealed the Global Regulator Clp of *Xanthomonas campestris* Modulates Transcription of *vgrS*

Baozhen Ren, Huan Zhang, Wei Qian*
*State Key Laboratory of Plant Gemonics，Institute of Microbiology，Chinese Academy of Sciences，100101 Beijing　*E-mail：qianw@im.ac.cn*

Clp（CAP-like protein）is a global transcription regulator in *Xanthomonas*. The regulatory role

of Clp in controlling gene transcription has been studied by mutational and transcriptional approach. Here we employ chromatin immunoprecitipation method to refine the Clp regulon and investigate the architecture of the promoter region of Clp-regulated genes. ChIP-seq analysis identified 346 and 73 downstream genes of Clp when the bacterium grew in rich NYG and minimal XVM2 medium，respectively. These genes belong to 15 functional groups. The consensus binding motif of Clp was dissected. Among the Clp regulon, we focused on a target gene *vgrS*, which encodes a histidine kinase of the two-component signal transduction system. Epistatistical analysis demonstrated that Clp is the positive regulation of *vgrS* transcription. It directly binds to the promoter region of *vgrS*, rather than *vgrR*, to control its expression. Phenotype screening revealed that the above regulatory cascade is important to bacterial resistance to Zn stress. An oxidordeutase gene is proved to be regulated by Clp-VgrS pathway during stress tolerance. Our results make a better understanding for Clp regulation mechanism in *X. campestris* pv. *campestris*.

References:

[1] Li Wang, Yue Pan, Zhi-Hui Yuan, Huan Zhang, Bao-Yu Peng, Fang-Fang Wang, Wei Qian. PLoS Pathog. Two-Component Signaling System VgrRS Directly Senses Extracytoplasmic and Intracellular Iron to Control Bacterial Adaptation under Iron Depleted Stress. 2016 Dec; 12(12): e1006133.
[2] Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem. 2000;69:183–215.

Two-Component Signaling System VgrRS Directly Senses Extracytoplasmic and Intracellular Iron to Control Bacterial Adaptation under Iron Depleted Stress

Li Wang[a], Yue Pan, Zhi-Hui Yuan, Huan Zhang, Bao-Yu Peng, Fang-Fang Wang, Wei Qian*[a]

[a] *State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing*

E-mail: qianw@im.ac.cn

Both iron starvation and excess are detrimental to cellular life. For pathogenic bacteria, iron supply is critical since they always live in iron-limited environments produced by host immune responses. However, how bacteria sense and respond to iron is incompletely understood [1, 2]. We combined genetic, biochemical, and biophysical methods to reveal that

in the phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris*, a membrane-bound receptor histidine kinase, VgrS, senses extracytoplasmic iron limitation in the periplasm, while its cognate response regulator, VgrR, detects intracellular iron excess. Under iron-depleted conditions, dissociation of Fe^{3+} from the VgrS activates the VgrS autophosphorylation and subsequent phosphotransfer to VgrR. Chromatin immunoprecitipation and high throughput sequencing demonstrated that in reacting to iron-depleted environments, VgrR controls the expressions of hundreds of genes. Among them, the transcription of a TonB-dependent receptor gene, *tdvA*, is repressed tightly by phosphorylated VgrR. This regulation is critical to efficient iron uptake and bacterial virulence since activation of *tdvA* is detrimental to these processes. When the intracellular iron accumulates, the VgrR-Fe^{2+} interaction dissociates not only the binding between VgrR and the *tdvA* promoter, but also the interaction between VgrR and VgrS. This relieves the repression in *tdvA* transcription to impede continuous iron uptake and avoids possible toxic effects of excessive iron accumulation. Our results revealed a signaling system that directly senses both extracytoplasmic and intracellular iron to modulate bacterial iron homeostasis.

Reference:

[1] J.E. Cassat, E.P. Skaar (2013). Cell Host Microbe, 13, 509.

[2] T. Kobayashi, N. K. Nishizawa (2012). Annu Rev Plant Biol, 63, 131.

Systematic Mutational Analysis of Two-Component Signaling Systems of *Lonsdalea quercina subsp. populi* Identifies Lqpn0375-Lqpn0376 as A Virulence Regulator

Ruo-Lan Yang[a], Chao-Ying Deng[b], Ai-Ning Li[a], Wei He[a], Wei Qian[b]

[a] *School of Forestry, Beijing Forestry Univercity, Beijing*

[b] *State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing*

Populus canker, which was first found in Henan Province in 2006, was a serious bacterial disease caused by the Gram-negative bacterium *Lonsdalea quercina subsp. populi*. However,

how the pathogenic bacterium regulate virulence factors during pathogenesis remains unclear. Two-component signal transduction system (TCSTS), consisting of a membrane-spanning sensor histidine kinase (HK) and a cytoplasmic response regulator (RR), is the most canonical regulatory machinery utilized by prokaryotes to respond to diverse environmental stimuli. To date, the TCSTS of *L. quercina* N-5-1 has never been experimentally investigated. Here, we annotated 42 putative histidine kinase and response regulator genes encoded by the genome of *L. quercina* N-5-1. By insertional inactivation, we successfully constructed 32 gene mutants. Phenotypic characterization identified mutants with deficiencies in bacterial growth, swimming motility, and pathogenicity. Among them, a TCSTS (Lqpn0375- Lqpn0376) was genetically confirmed to regulates virulence of *L. quercina* N-5-1. In addition, inactivation of the two genes resulted in significant decreases of tolerance to hydrogen peroxide stress, chloramphenicol stress and swimming motility. Chromatin immunoprecipitation together with high-throughput sequencing revealed 161 genes that were regulated by the system. Electrophoresis mobility shift assay (EMSA) assay confirmed that three genes which encode a glycosyl transferase family 1(*Lqpn0434*), bacterioferritin (*Lqpn3037*) and chemotaxis protein (*Lqpn3270*), were directed bound by transcription factor Lqpn0375 *in vitro*, and qPCR confirmed that Lqpn0375 is the regulator of these genes. To our best knowledge, our work is the first effort to systematically investigate the TCSTS in pathogenic bacteria of populus, which help us to understand the bacterial pathogenesis and development antimicrobial approaches to defend the diseases.

References:

[1] W Qian, Z.J. Han, J Tao, C.Z. He (2008). Genome-scale mutagenesis and phenotypic characterization of two-component signal transduction systems in Xanthomonas campestris pv. campestris ATCC 33913. Mol Plant Microbe Interact 21:1128–1138.

[2] T Toth, T Lakatos, A Koltay (2013). *Lonsdalea quercina* subsp *populi* subsp nov., isolated from bark canker of poplar trees. International Journal of Systematic and Evolutionary Microbiology. 63, 2309-2313.

Fatty Acid DSF binds and Activates Receptor Histidine Kinase RpfC of *Xanthomonas campestris* by Releasing the Autoinhibition from Juxtamembrane Domain

Zhen Cai[a,b,#], Zhi-Hui Yuan[a,b,#], Huan Zhang[a,b], Yue Pan[a,b], Yao Wu[a], Xiu-Qi Tian[a,b], Fang-Fang Wang[a], Li Wang[a], Wei Qian[a,*]

[a]*State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing*. [b]*School of Biological Sciences, University of Chinese Academy of Sciences, Beijing*

As well as their importance to nutrition, fatty acids (FA) represent a unique group of quorum sensing chemicals that modulate the behavior of bacterial population in virulence. However, the way in which full-length, membrane-bound receptors biochemically detect FA remains unclear. Here we provide genetic, enzymological and biophysical evidences to demonstrate that in the phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris*, a known medium-chain FA diffusible signal factor (DSF) binds directly to the N-terminal, 22 amino acid-length sensor region of a receptor histidine kinase (HK), RpfC. The binding event remarkably activates RpfC autokinase activity by causing an allosteric change associated with the dimerization and histidine phosphotransfer (DHp) and catalytic ATP-binding (CA) domains. Six residues were found essential for sensing DSF, especially those located in the region adjoining to the inner membrane of cells. Disrupting direct DSF-RpfC interaction caused deficiency in bacterial virulence and biofilm development. In addition, two amino acids within the juxtamembrane domain of RpfC, Leu^{172} and Ala^{178}, are involved in the autoinhibition of the RpfC kinase activity. Replacements of them caused constitutive activation of RpfC-mediated signaling regardless of DSF stimulation. Therefore, our results provide direct evidence to demonstrate that DSF is the ligand of RpfC. It also revealed a biochemical mechanism whereby FA activates bacterial HK in an allosteric manner, which will assist in future studies on the specificity of FA-HK recognition during bacterial virulence regulation and cell-cell communication.

Tomato targets of the RipH1, 2, 3 type III effectors from *Ralstonia solanacearum*, towards the understanding of contribution to disease

Alice Morel[a], Patrick Barberis[a], Cyrus Sabbagh[a], Gaofei Jiang[a], Fabien Lonjon[a], Fabienne Vailleau[a], Stéphane Genin[a] and Nemo Peeters[a]
[a] *Laboratory of Plant-Microbe Interactions (LIPM), UMR CNRS-INRA 2594/441, 24 chemin de Borde Rouge – Auzeville, CITY: Castanet-Tolosan, France [FR]*
alice.morel@inra.fr

One of the major virulence determinants of plant pathogenic *Ralstonia* species is the type III secretion system that enables it to inject proteins (also called "Ralstonia Injected Proteins" or Rip) into the host cells. Some of these known type III effectors from the *Ralstonia solanacearum* species complex seem to be conserved, as they are present in all known sequenced strains.

Among these conserved effectors, we showed that the members of the RipH paralogous family (RipH1, RipH2, RipH3) are required for the virulence of *R. solanacearum* on several host plants (tomato, Arabidopsis, *Medicago truncatula*) with a genetic redundancy on tomato. Using yeast-two-hybrid screenings we have identified several tomato targets of these three RipH. Several of these targets are localized in the nucleus of the plant cell, including a few transcription factors. Linked with the cytoplasmic and nuclear localization of the RipH, we are investigating the role of these effectors in the manipulation of the host defenses pathways against the bacterium.

Here will be presented our current advancement on the analysis of the effect of the RipH on their tomato targets, in a global effort to understand the contribution of these T3Es to the virulence mechanisms of the bacterium.

Inhibition of apoplastic subtilases at the plant-pathogen interface

Daniela J. Sueldo[1], Nattapong Sanguankiattichai[1], Tram Ngoc Hong[1], Farnusch Kaschani[2] and Renier A. L. van der Hoorn[1]
[1]*Plant Chemetics Lab, Department of Plant Sciences, University of Oxford;* [2] *Analytics Core Facility Essen, University of Duisburg-Essen*

During plant-pathogen interactions, the plant apoplast becomes a true battlefield, with both plant and pathogen secreting hydrolytic enzymes and inhibitors into the extracellular space. Plant subtilases are abundant serine proteases that accumulate in the apoplast during infection.

Though numerous reports suggest that subtilases are involved in plant defence, it is still unclear how these extracellular proteases contribute to plant immunity and how their activity is modulated. Activity-based Protein Profiling (ABPP) is a technique that allows monitoring protein activities without the need of previous knowledge of substrates or even purification of the enzyme. Using ABPP we have identified that the activity of apoplastic subtilases is suppressed in *N. benthamiana* upon infection by *Pseudomonas syringae* pv. *tomato* (PtoDC3000) by an inhibitor that is a heat-stable protein and larger than 3kDa. To determine the identity of the inhibitor we performed immunoprecipitation assays in *N. benthamiana* of epitope-tagged subtilases followed by PtoDC3000 infection. We identified several proteins that co-purify with apoplastic subtilases, including protease inhibitors. Preliminary data assessing the biological relevance of these interactions will be presented at the meeting. Finally, to address such antagonistic protein interactions at a larger scale we have performed a large-scale ABPP experiment followed by mass spectrometry using a cocktail of probes that target different hydrolytic activities known to differentially accumulate in the apoplast during pathogen challenge. We will present data showing the power of ABPP to monitor the activity of >100 different enzymes during bacterial infection.

Time series RNA-seq reveals early root responses to ISR-inducing *Pseudomonas simiae* WCS417 and a link with ISR

Ioannis A. Stringlis*, Silvia Proietti*, Marcel C. Van Verk, Richard Hickman, Christos Zamioudis and Corné M.J. Pieterse

Plant-Microbe Interactions, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

Email: I.Stringlis@uu.nl

The ability of roots to discriminate between pathogenic and beneficial microbes is crucial for plant health. In Arabidopsis, beneficial *Pseudomonas simiae* WCS417 rhizobacteria trigger an induced systemic resistance (ISR) that is effective against a broad range of pathogens. The early responses activated in the roots upon perception of WCS417 are largely unknown. Here, we used time-series RNA-seq to compare the early root responses to living WCS417 with that of the bacterial and fungal elicitors flagellin ($flg22^{Pa}$ of *P. aeruginosa* and $flg22^{417}$ of WCS417) and chitin, respectively. Our data show that the early transcriptional root responses to $flg22^{Pa}$

and chitin differ in timing, but display a large overlap in gene identity. The majority of the upregulated genes are involved in immunity, while the downregulated genes are predominantly related to development. Interestingly, the transcriptional response of roots to flg22^{Pa} was highly similar to flg22^{417}, despite the 5 amino acids differing between the two peptides. Even though 83% of the transcriptional changes inflicted by living WCS417 overlapped with the flg22^{417} profile, 50% of the flg22^{417} profile was not affected by living WCS417, suggesting active suppression by the living bacteria. Interestingly, we observed differential effects by WCS417 and flg22^{417} on a set of auxin-responsive genes, and that the transcriptomic profile of roots in response to WCS417 has a strong auxin signature. This is in line with the plant growth-promoting activities of WCS417 on Arabidopsis. Further experiments showed that intact auxin signaling is also required for the onset of WCS417-ISR. Together, these findings suggest that WCS417 is recognized as a potential attacker, but that a large part of the flagellin-triggered response is swiftly suppressed, possibly to overcome host immunity in order to establish a mutually beneficial interaction.

Pseudomonas syringae **hijacks MYC2-family transcription factors to dampen** ***EDS1*** **induction for enhancing virulence**

Jingde Qiu[a], Haitao Cui[a,b], Dacheng Wang[a], Patrick von Bonn[a], Jane Parker[a]

[a] *Department of Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Carl von Linné Weg 10, Cologne, 50829, Germany*

[b] *Plant Immunity Center, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, 350002*

jingde@mpipz,mpg.de

Plant hormones crosstalk interactions are essential in response to biotic stresses, among which salicylic acid (SA) and jasmonic acid (JA) are well known for their mutual antagonistic role in modulating responses to different pathogens. *Pseudomonas (P.) syringae*, a hemi-biotrophic pathogen, secretes virulence factor coronatine (COR), a mimic of jasmonic acid isoleucine, to manipulate JA signaling for promoting virulence [1]. The transcription factor (TF) MYC2 with its homologs MYC3 and MYC4 regulates different aspects of JA-dependent responses, including promoting *P. syringae* virulence [2,3]. *P. syringae* pv. *tomato* (*Pst*) DC3000 utilizes COR to hijack MYC2-dependent JA signaling to suppress SA-dependent defenses, which was demonstrated by showing bacterial COR activates transcriptional activities of NAC TFs genes via MYC2 transcriptional activity, leading to

inhibition of SA accumulation through NAC TFs' direct suppression on *ICS1* while promotion on *BSMT1*, genes involved in SA biosynthesis and metabolism, respectively [4,5]. However, we do not know whether and how MYC2 overcomes SA-independent defenses to enhance *Pst* DC3000 infection. Here, we will show MYC3 and MYC4 act additively with MYC2 in suppression of SA-independent defense, and present progress in analysis of MYC2-family TFs suppression on *EDS1*-dependent defense during *Pst* DC3000 infection.

Reference

[1] Melotto, M., et al. (2006). Cell, 126(5): p. 969-80.
[2] Fernandez-Calvo, P., et al. (2011). Plant Cell, 23(2): p. 701-15.
[3] Schweizer, F., et al. (2013). Plant Cell, 25(8): p. 3117-32.
[4] Laurie-Berry, N., et al. (2006). Molecular Plant-Microbe Interactions, 19(7): p. 789-800.
[5] Zheng, X.Y., et al. (2012). Cell Host Microbe, 11(6): p. 587-96.

MYB72-dependent root exudates modulate rhizosphere signaling

Ke Yu[a*], Ioannis Stringlis[a*], Ronnie de Jonge[a], Kirstin Feussner[b], Marcel van Verk[a], Roeland Berendsen[a], Peter Bakker[a], Ivo Feussner[b], Corné Pieterse[a]

[a] *Plant–Microbe Interactions, Department of Biology, Utrecht University, Utrecht, The Netherlands*

[b] *Department of Plant Biochemistry, Georg-August-University Goettingen, Albrecht-von-Haller-Institute for Plant Sciences, Goettingen, Germany*

k.yu@uu.nl

Signaling between plant roots and beneficial microbes is fundamental for the establishment of mutualistic associations. Plants benefit from such associations as mutualists can, e.g. elicit induced systemic resistance (ISR), enhance nutrient uptake and promote plant growth. In turn, plants have a role in shaping functions and composition of the root microbiome. It has been shown that ISR-inducing microbes can hijack iron deficiency responses of their hosts. Under iron deficiency, *Arabidopsis* roots synthesize and secrete iron-mobilizing phenolics in a MYB72-dependent manner. However, relatively little is known about the microbial responses to these compounds in root exudates. In this study, we investigated the effects of MYB72-dependent root exudates on the ISR model strain *Pseudomonas simiae* WCS417r as well as on the root microbiome. We found that MYB72-dependent root exudates affect gene expression of WCS417r and influence the early assemblage of the root microbiome. By identifying key microbial responses in the rhizosphere to these microbe-induced plant exudates, we aim to better understand the signaling that takes places between plant and associated mutualist.

Specific suppression of MAP kinase activation – a novel function of the bacterial effector protein AvrRpt2

Lennart Eschen-Lippold [a], Dierk Scheel [a], Justin Lee [a]

[a] *Dept. Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle, Germany*

leschen@ipb-halle.de

Mitogen-activated protein kinase (MAPK) cascades are central regulators of diverse developmental, stress and defense signalling pathways in plants. Upon recognition of invading pathogens or treatment with pathogen-associated molecular patterns (PAMPs), at least four MAPKs are rapidly activated (MPK3, MPK4, MPK6, MPK11) that target diverse substrate proteins for phosphorylation. This post-translational modification represents a crucial mechanism to regulate protein abundance, activity and expression. To interfere with defense signalling and to modify the cellular homeostasis in their favour, the attacking pathogens evolved mechanisms to directly deliver effector molecules into the host cells that target specific signalling components. Several bacterial effectors were described to interfere with MAPK activation. As an example, HopAI1 targets MPK3, MPK4 and MPK6 to inactivate them by removing the phosphate group from the threonine in the TEY motif. We previously identified a novel function of the *Pseudomonas syringae* effector protein AvrRpt2, which specifically suppresses PAMP-induced activation of MPK4 and MPK11, but not of MPK3 and MPK6. Several defense-related responses are affected in AvrRpt2-expressing plants, correlating with enhanced susceptibility against *Pseudomonas syringae* and *Botrytis cinerea* [1]. Our work now focusses on the understanding of the underlying molecular mechanism. For this purpose, we screened several putative AvrRpt2 homologs from different plant- or soil-associated bacteria for MPK4/MPK11-suppression activity [2]. Additionally, we analyse the role of AvrRpt2's protease activity for the specific MPK4/11 suppression.

References:

[1] L. Eschen-Lippold, X. Jiang, J.M. Elmore, D.M. Mackey, L. Shan, G.L. Coaker, D. Scheel, J. Lee (2016). Plant Physiology, Vol. 171, p. 2223.

[2] L. Eschen-Lippold, D. Scheel, J. Lee (2016). Plant Signaling & Behavior, Vol. 11, No. 12, e1257456.

Shigella effector IpaB suppresses plant cell death and immunity

Sung Hee Jo[a,b], and Jeong Mee Park[a,b]*

[a] *Plant Systems Engineering Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon 34141, South Korea;* [b] *Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, Korea University of Science and Technology (UST), Daejeon 34113, South Korea; * Correspondence: jmpark@kribb.re.kr*

Shigella is a major public health burden in developing countries as a causative organism of bacterial heterogeneity. *Shigella* uses a type three secretion system (T3SS) for entry into the host, replication and signaling for endopathology. In particular, IpaB, one of the T3SS, is a multifunctional effector and is known to modulate host cell death in response to a bacterial infection step. The induction mechanism of caspase-1-dependent macrophage apoptosis by IpaB is well known at the molecular level, but the mechanism of inhibition of apoptosis by IpaB is not well known. In this study, we have attempted to investigate the virulence function of *Shigella* IpaB in plants, expecting to release new functions of IpaB because there is no caspase-mediated apoptotic pathway in plants. Overexpression of IpaB on *N. benthamiana* leaves did not induce cell death, but interestingly, co-expression of IpaB suppressed non-host pathogen-induced plant cell death responses. We also tested the ability of IpaB to control plant immunity using bacterial phytopathogen, *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 expressing IpaB. As a results, the virulence of *Pst* DC3000 expressing IpaB was 10 times higher than that of wild- type *Pst* DC3000, suggesting that IpaB could interact with plant immunity-related factors in plant cells. From these results, we found that IpaB of *Shigella* retained its function as a virulence factor in plants and could potentially reveal new host targets or functions of IpaB using plants.

[1] S.A. Killackey, M.T. Sorbara, S.E. Girardin (2016). Frontiers in Cellular and Infection Microbiology, 6, 38

[2] Shih-Chun Yang, Chi-Feng Hung, I.A. Aljuffali, Jia-You Fang (2015). Microbiological Research, 181, 43

[3] SuayibU stun, Petra Muller, Ralf Palmisano, Michael Hensel, Frederik Bornke (2012). New Phytologist, 194, 1046

Identification of the bacterial factors involved in PGPR-induced systemic resistance of plants.

Tsai, Wei-Hsiang[a] and Chen, Chao-Ying[a]

[a] *Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan*

Email: *waltwns@gmail.com*

Some of plant growth-promoting rhizobacteria (PGPR) also increase whole-plant resistance against biotic and abiotic stresses, a phenomenon of induced systemic resistance (ISR) [1]. A PGPR strain named *Bacillus cereus* C1L, isolated from the rhizosphere of *Lilium formosanum* in Taroko National Park, Hualien County, Taiwan, was proven able to reduce disease severity of lily gray mold and southern corn leaf blight by soil-drench application. The aim of this study was to investigate the mechanism of *B. cereus* C1L-induced plant systemic resistance and identify the bacterial genes involving in. To find the related genes, a transposon-insertion library of *B. cereus* C1L previously constructed was used to screen the mutants decreasing in the ISR ability on *Nicotiana benthamiana* against a fungal pathogen *Botrytis cinerea*. In the mutant, M177, the transposon was located to the gene encoding macrolide ATP-binding cassette (ABC) transporter permease. A lack of expression of the ABC transporter permease gene in M177, as shown by reverse transcription-polymerase chain reaction, would restrict the secretion of bacterial products playing role(s) in the ISR activation. The secreted products of mutant M177 and wild-type strain *B. cereus* C1L culturing in *N. benthamiana* root exudate-containing medium were then analyzed by LC-MS/MS and several distinct products were shown to be the candidates as ISR elicitors.

Reference:

[1] Ahemad, M. and Kibret, M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. of King Saud University – Science 26, 1–20.

Biological Control of Tobacco Bacterial Wilt by Injection Inoculation of Lytic phage against *Ralstonia solanacearum*

CAI Liu-ti，LU Ning, WANG Han-cheng

Guizhou Academy of Tobacco Science. Longtanba Road #29, Guanshanhu District, Guiyang 550081

cailiuti01@163.com

Tobacco bacterial wilt, caused by *Ralstonia solanacearum* （Rs）, is one of major diseases to flue-cured tobacco. It is difficult to prevent and control the bacterial wilt，not only because Rs is a complex specie, which can infects more than 200 plant species belonging to more than 50 botanical families, including important crops such as potato, tomato, pepper, tobacco and banana, but also its many virulence factors and complicated virulence regulation network system. At present, There is still cannot control it occurs effectively in fields. In order

to look for bio-control potential agent for control the pathogen, here, lytic phage ϕPB2 against Rs was applied to control tobacco bacterial wilt by the method of needle inoculation in potting experiment. Tobacco (*Nicotiana tabacum*) cv. K326 plants were grown in ϕ25-cm pots in a greenhouse. When 30-Day-old tobacco seedlings were transplanted into pots, four treatments were designed after injured Roots (T0:poured with 15ml sterile water as control; T1: poured with 20ml（10^8cfu/ml）Rs ZP strain. T2: poured with 20ml（10^8cfu/ml）Rs ZP strain, as well as poured with 20ml（10^5pfu/ml）phage ϕPB2. T3: poured with 20ml（10^8cfu/ml）Rs ZP strain, as well as inoculation 500ul（10^5pfu/ml）phage ϕPB2 with needle injection), each treatment contains six plants.

The potting experiments showed that T1 treatment began to show wilt symptoms at 15 days post-inoculation. Disease incidence in treatment T3 was lower than in T1 and T2 within 21days after the wilt symptoms appeared. Tobacco plants in treatment T3 wilt symptoms appearance was delayed 3 days than in treatment T1, and its disease index was only 37.51% and 32.44% of the T1 and T2 respectively at 21st days after wilt symptoms appeared. Those results suggested that phage ϕPB2 might be used as a potential agent in the bacterial wilt control strategies depended on their application manners.

Acknowledgment：Supported by “Construction and application study on lytic phage against *Ralstonia solanacearum* caused tobacco bacterial wilt”(Qiankehe support No. [2017]2606) .

Characterization of a novel D-phenothrin-degrading bacterial strain *Pseudomonas fulva* P31

Jingjing Yang, Yanmei Feng, Hui Zhan, Fang Yang, Shaohua Chen*

State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Guangdong Province Key Laboratory of Microbial Signals and Disease Control, Integrative Microbiology Research Centre, South China Agricultural University, Guangzhou 510642

E-mail: shchen@scau.edu.cn

D-phenothrin is one of the most popular synthetic pyrethroid insecticides (SPs) for its broad spectrum and high insecticidal activity. However, persistent use of D-phenothrin has resulted in serious environmental contamination and raised public concerns on human health. Here, a novel bacterial strain was isolated from active sludge, which was able to degrade 100% of 50 $\mu g \cdot mL^{-1}$ D-phenothrin in 72 hours. The strain was identified as *Pseudomonas fulva* on the basis of morphology, physio-biochemical properties, and 16S rRNA gene analysis as well as

Biolog Microstation system. The culture conditions for biodegradation were optimized at 29.5 °C, 7.3 of pH and 0.3 $g\cdot L^{-1}$ of an inoculum size. In addition, strain P31 showed high tolerance to D-phenothrin and strong ability to degrade D-phenothrin with a K_i of 482.1673 $\mu g\cdot mL^{-1}$ and a q_{max} of 0.0455 h^{-1}. Strain P31 was also found to be highly effective in degrading various SPs, including permethrin, cypermethrin, deltamethrin, cyfluthrin, cyhalothrin, and fenpropathrin, which similar to D-phenothrin are also widely used insecticides with environmental contamination problems with the degradation process following the first-order kinetic model. Taken together, our results suggest that *P. fulva* P31 may be an ideal microorganism for bioremediation of the SPs-contaminated environments.

Acknowledgement: The authors gratefully acknowledge the grant from the National Natural Science Foundation (No.31401763).

Beneficial Plant-*Streptomyces* interactions: case study of lettuce drop disease controlled by endophytic streptomycetes

Xiaoyulong Chen[1], Maria Bonaldi[2], Armin Erlacher[3], Andrea Kunova[2], Cristina Pizzatti[2], Marco Saracchi[2], Gabriele Berg[3] and Paolo Cortesi[1]

[1] *Laboratory of Plant Pathology and Biocontrol, Collage of Tobacco Science, Guizhou University, Guiyang*

chenxiaoyulong@sina.cn

[2] *Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via Giovanni Celoria 2, 20133, Milano, Italy*

[3] *Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria*

Yield losses caused by phytopathogens should be minimized to maintain the food quality and quantity for the demand of massively growing human population. Thus, searching for sustainable solutions to suppress phytopathogens, as well as to increase the yield is gaining high public interests. *Streptomyces*, abundant in soil, are a group of filamentous bacteria producing a variety of beneficial secondary metabolites, gifting them the potential to be developed as bio-pesticides and bio-fertilizers. We labeled two bioactive *Streptomyces* strains with EGFP marker to investigate their interactions with lettuce using confocal laser scanning microscopy (CLSM), and evaluated their biocontrol activities against *Sclerotinia sclerotiorum* on lettuce, as well as PGP activities on several economically important horticultural plants. The abundant colonization of young lettuce seedling by two *Streptomyces* strains demonstrated their

capability to interact with the host from early stages of seed germination and root development up to two weeks. Plant-strain specific PGP activity was observed; e.g., *S*. *cyaneus* ZEA17I promoted the growth of lamb lettuce but not that of tomato. When they were applied to *S*. *sclerotiorum* inoculated substrate in growth chamber, *S*. *exfoliatus* FT05W and *S*. *cyaneus* ZEA17I significantly reduced lettuce basal drop incidence by 42.3% and 53.9%, respectively, compared to the control (P < 0.05). Furthermore, under field conditions, *S*. *exfoliatus* FT05W and *S*. *cyaneus* ZEA17I reduced the disease incidence by 40% and 10% respectively. Our results indicate the greatly promising potential of *Streptomyces* for exploitations as BCAs and PGPB in agro-ecosystems.

Acknowledgements:
The authors thank Prof. Mervyn Bibb (John Innes Centre, UK), for kindly providing the plasmid pIJ8641, as well as prof. Flavia Marinelli (University of Insubria, Italy), for donor strain *E*. *coli* ET12567 for *Streptomyces* transformation.
Keywords: *Streptomyces*. Colonization. Biocontrol. Plant growth promotion

References:

[1] **Chen, X.**, Pizzatti, C., Bonaldi, M., Erlacher, Saracchi, M., A., Kunova, A., Berg, G., Cortesi, P. Biological control of lettuce basal drop and host plant colonization by rhizospheric and endophytic streptomycetes. ***Frontiers in Microbiology***. 2016：7:714.

[2] **Chen, X.**, Bonaldi, M., Erlacher, A., Kunova, A., Pizzatti, C., Saracchi, M., Berg, G., Cortesi, P. Biocontrol of two *Streptomyces* spp. strains against lettuce basal drop caused by *Sclerotinia sclerotiorum*. ***Phytopathology***. 2016：106 (6):S7

[3] Bonaldi, M., **Chen, X***, Kunova, A., Pizzatti, C., Saracchi, M., Cortesi, P. Colonization of lettuce rhizosphere and roots by tagged *Streptomyces*. ***Frontiers in Microbiology***. 2015：6:25.
Co-first author

[4] Kunova, A., Bonaldi, M., Saracchi，M., Pizzatti, C., **Chen, X.**, Cortesi P. Selection of Streptomyces against soil borne pathogens by a standardized dual culture assay and evaluation of their effects on plant growth. ***BMC Microbiology*** 16(272):1-11

Interaction of *Pseudostellaria heterophylla* with quorum sensing and Quorum quenching bacteria mediated by root exudates in a consecutive monoculture system

Liaoyuan Zhang[a,b], Zewang Guo[b], Huifang Gao[b], Jung-Kul Lee[a*], Wenxiong Lin[b*]
[a] *Department of Chemical Engineering, Konkuk University, Seoul 143-701, Republic of Korea*
[b] *Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education,College of Life Sciences, Gutian Edible Fungi Research Institute, Fujian Agriculture and Forestry University, Fuzhou, Fujian*

province, 350002

1365677053@qq.com

Abstract: Many plant-pathogenic bacteria were dependent on quorum sensing (QS) to evoke disease. In this study, the population of QS and quorum quenching (QQ) bacteria was analyzed in consecutive monoculture system of *Pseudostellaria heterophylla*. The isolated QS strains were identified as *Serratia marcescens* with *SwrIR*-type QS system and exhibited a significant increase over the years of monoculture. Only one QQ strain was isolated from new planted soil sample and identified as *Bacillus thuringiensis* which secreted lactonase to degrade QS signal molecules. Inoculation of *S. marcescens* to *P. heterophylla* root could rapidly cause wilt disease, which was alleviated by *B. thuringiensis*. Furthermore, the expression of lactonase encoded by the *aiiA* gene in *S. marcescens* resulted in reduction of its pathogenicity, implying that toxic effect of *S. marcescens* on the seedlings was QS-regulated. Meanwhile, excess lactonase in *S. marcescens* led to reduction in antibacterial substances, exoenzymes, and swarming motility, which might contribute to pathogensis on the seedlings. Root exduates and root tuber extracts of *P. heterophylla* significantly promoted the growth of *S. marcescens*, whereas a slight increase of *B. thuringiensis* was observed by both samples. These results demonstrated that QS-regulated behaviors in *S. marcescens* mediated by root exudates played an important role in replanting disease of *P. heterophylla*.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (No. 31301858 and No. 31501694), Research Fund for the Doctoral Program of Higher Education of China (No. 20133515120011), the KU Research Professor program of Konkuk University and 2015 KU Brain Pool fellowship of Konkuk University.

Genomic identification of a PGPR strain (*Pseudomonas* sp. M30-35) from *Haloxylon ammodendron* rhizosphere

Aolei He, Qi Zhao, Jinlin Zhang*

State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou,730020

Author e-mail: heal15@lzu.edu.cn; *Corresponding author e-mail: jlzhang@lzu.edu.cn

Haloxylon ammodendron, a C4 perennial succulent xero-halophytic shrub with excellent tolerance to both drought and salinity, is naturally distributed in desert region. In our previous work, *Pseudomonas* sp. M30-35 was isolated from the rhizosphere soil of *H. ammodendron* in Tengger desert, Minqin County, Gansu Province. In this work, the complete genome of M30-35 was sequenced by Pacific Biosciences (PacBio) RS Ⅱ and Single Molecule Real Time (SMRT) sequencing technology. The genome of M30-35 contains a single chromosome that is 4,926,954bp in the length with 54.3％G+C content (Figure 1). In total 6,299,610 paired-end

reads (1,558,033,092 bp) corresponding to 209 folds of genomic coverage were generated. We found several kinds of genes responsible for plant growth promotion, such as genes participating in phosphate solubilizing, IAA biosynthesis, ACC deaminase, trehalose production, and genes responsible for alleviating oxidative stress in plants, which are helpful to explore the potential interaction mechanism between plant and M30-35. Subsequently, the PGPR property of this bacterium strain was preliminarily evaluated in perennial ryegrass (*Lolium perenne* L.). The results showed that application of M30-35 improved the growth of ryegrass seedlings under 0, 150 and 300mM NaCl, especially, shoot dry weight and root volume was significantly increased by M30-35, compared to control (Luria broth medium) and *Escherichia coli* DH5α (Figure 2). This study will provide a theoretical basis for PGPR strains from desert plant rhizosphere to be used in agricultural production and new research methods for further exploring the growth promotion mechanism of PGPR strains.

Keywords: Desert plant rhizosphere; PGPR strain; Genome sequence; Gene identification

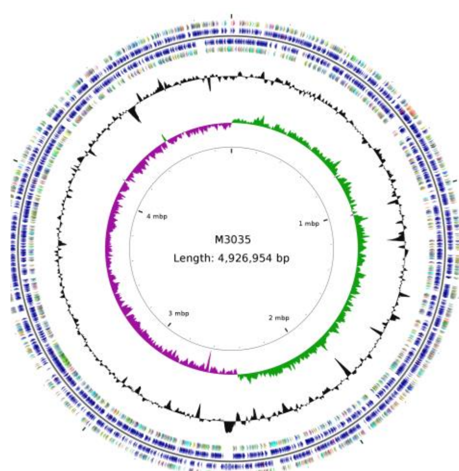

Figure 1. Circular representation of the *Pseudomonas* sp. M30-35 genome

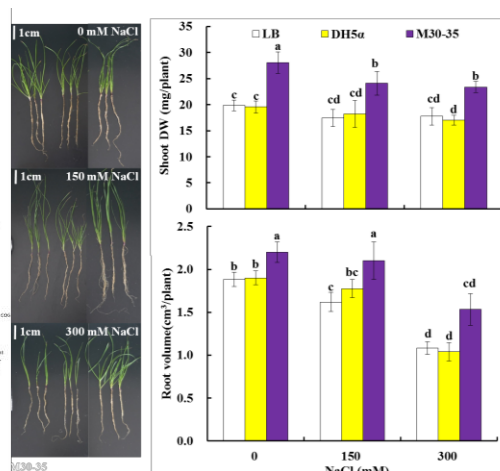

Figure 2. Effects of M30-35 on shoot dry weight and root volume of perennial ryegrass (*Lolium perenne* L.)

Mechanism of bacterial blight disease resistance in *Oryza meyeriana* Baill in Yunnan province mediated by ubiquitination

Fu Jian, Wu Jinhu, Chen yue, Wang bo, Wang lingxian, Ke xue, Yin fuyou, Cheng zaiquan

Biotechnology & Genetic Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650223, Yunnan.

E-mail of presenting author: fugein@aliyun.com

Oryza meyeriana Baill from Yunnan province is a high resistant wild rice ecotype to bacterial blight disease (BB). This feature is very prominent in *oryza*. But the mechanism of resistance is unclear. Ubiquitination is one of the most refined regulation systems of protein degradation

in plant. Its function involve in disease resistance. Until now, there is no report about the disease resistance mechanism in *O. meyeriana* mediated by ubiquitination.
In this study, the transcriptomic analysis was carried out by RNA sequencing of *O. meyeriana* leaves, inoculated with Xoo to understand the transcriptional responses and interaction between the host and pathogen. Totally, 57,313 unitranscripts were de novo assembled from 58.7 Gb clean reads and 14,143unitranscripts were identified after Xoo inoculation. The significant metabolic pathways related to the disease resistance enriched by KEGG, were revealed to plant-pathogen interaction, phytohormone signaling, ubiquitin mediated proteolysis, and phenylpropanoid biosynthesis. Further, the ubiquitination enzyme (E1, E2, E3) genes were also identified to be differentially expressed in response to Xoo infection. These genes mainly included 24 ubiquitin genes, 5 ubiquitin active enzyme (E2) gene, 8 ubiquitin conjunction enzyme (E2) gene and 94 ubiquitin ligases enzyme (E3) genes. Three subgroups of *E3*, HECT, RING-H2 and XB3, were identified. It has been reported that some genes belong to these subgroup of *E3* related to disease resistance in rice, such as xb3 regulated BB. 40 *E3* genes were chosen to analyze expression pattern after infected by Xoo in *O. meyeriana*. The result showed that 17 *E3* genes were up-regulated after inoculation as the early response (1h, 2h, 4h and 8h). A *ubr4* homologous gene and two *E3* genes were up-regulated after 48h to 72h inoculation. The structures of 4 *xb3*genes were very different to the homologous genes of rice, and their many ankyrin repeat domain implied that they can bind more proteins. So this study demonstrated that ubiquitination mediated *O. meyeriana* against Xoo infection. This could be an useful information for further investigating the molecular mechanism associated with disease resistance in *O. meyeriana*.

Acknowledgement: This study supported by Natural Science Foundation of China (31560384)

Diversity of endophytes associated with TCM plants and the endophytes effects on accumulation of secondary metabolic products in the host plants

Jihong Jiang* Bo Yuan Junjuan Wang YongSun

(The Key Laboratory of Biotechnology for Medicinal Plant of Jiangsu Province, Jiangsu Normal University Xuzhou221116)

**Correspondence author: E-mail:jhjiang@jsnu.edu.cn*

Endophytes are microorganisms that live inside the host plant tissues which have novel

metabolites exhibiting a variety of biological activities . The research on plant endophytes has been become a hot topic in the research filed of microbes, natural products and others.

As the research on plant-microbe interaction upsurge, chemical diversity bearing pharmaceutical potential reached beyond the plant kingdom and offered an expended view promising to transform glimpses of reductionist research of the past years to snapshots of an exuberant world of systems biology by microbial source which forms a huge diversity in nature and is one of the largest unexplored reservoirs forming a ware house of natural bioactive compounds on the earth. Hence this has generated more attention and interest over other source such as plant due to various drawbacks.

In comparison with rhizosphere and phyllosphere bacteria, endophytes are likely to interact more closely with their host. Endophytes can indirectly benefit plant growth by preventing the growth or activity of plant pathogens through competition for space and nutrients, production of hydrolytic enzymes, antibiosis, induction of plant defence mechanisms and through inhibition of pathogen-produced enzymes or toxins.

In recently, we have got many endophytes from traditional Chinese medical plants and study their effects on the hosts. The main research works on endophyte and are showed as following:

1. About 1000 strains were isolated from the medical plants, and was studied in detail for its taxonomic position. Comparative 16S rRNA gene sequence analysis showed that those strains belonged to more than 40 genera of actinomycetes including rare actinomycetes. Most of them showed several functions.
2. Endophytes represent a new area of research and offer wide range of benefits to host plants. endophytes displayed different plant growth promoting traits. Our results provide strong evidence that the role of habitat-adapted symbiotic ACC deaminase producing putative endophytes in the performance of medicinal plants consists not only in the improved the host growth, but also in the improvement host flavonoids and alkaloids production. We also explored the endophytes' effects on the accumulation of secondary metabolic products in the host plants.

The troponin C, a behavior regulating component from *Nephotettix cincticeps* may serve as a potential target for control of the rice pest

Hanhong Lan*, Xiaojing Hong, Ranran Huang, Xin Lin, Qinghuang Li, Kaihui Li,, Tao Zhou

School of Biological Sciences and Biotechnology, Minnan Normal University, Zhangzhou, Fujian, 363000.

Abstract

Nephotettix cincticeps, migrating insects, threatens the rice production by sucking plant host and transmitting rice dwarf virus in Southeast Asia and Southern China. Behavior regulation of insect may become a novel strategy in the comprehensive management of the rice pest. Troponin C (TnC) plays important roles in regulating insect behavior by modulating muscle activity. However, characterizations and functions of TnC from *N. cincticeps* (Nc-TnC) remain unknown. Here, we successfully identified and characterized Nc-TnC to be a member of EF-hand protein with Ca^{2+}-bingding motif. We demonstrated that Nc-TnC ubiquitously transcribed at all development stages and special tissues in adult insects, with relative higher levels at the adult stage and in the intestinal canal. Microinjection-based transient knockdown of Nc-TnC significantly decreased survival rates, amounts of honeydew and weight of *N. cincticeps*, thus adversely affecting the performance of the rice pest. Furthermore, we revealed that rice dwarf virus (RDV) infection caused death-related cytopathological changes; in which interaction of RDV Pns10 with Nc-TnC may depress accumulation of Nc-TnC and be also a pivotal reason for adverse affects on insect. Our data deepen understanding of Nc-TnC function and its interaction with RDV; it also implied Nc-TnC may serve as a potential target for *N. cincticeps* control.

Keywords: *Nephotettix cincticeps*, Troponin C, expression profiles, RNAi, rice dwarf virus, interaction

The diversity of endophytic bacteria associated with garlic and their effects on plant fungal pathogen

Li le xi, Zhou liang ying, Gao Wenyuan, Wang Junjuan, Sunyong, Jiang jihong*

(The Key Laboratory of Biotechnology for Medicinal Plant of Jiangsu Province/College of Life Science, Jiangsu Normal University, Xuzhou 221116, Jiangsu)

Abstract: The garlic endophytes are important microbes in garlic. Due to the long-term

symbiotic relationship, the metabolism of garlic endophytes is stable, and these microbes have strong inhibition effects on various plant pathogen. In this study, 205 endophytic bacteria strains were isolated from garlic bulb that sampled from 20 garlic. Using nine plant fungal pathogen (*Alternaria solani*, *Cladosporium capsici*, *Pyricularia oryzae* Cav., *Sclerotium cepivorum* Berk., *Ceratocystis fimbriata*, *Pseudoperonospora cubensis*, *Fusarium oxysporum*, *Physalospora piricola*, *Ascochyta citrullina*) as indicator, the in vitro antagonism bioassays were conducted. It is found that 93 endophytic bacteria had bacteriostatic activity, among them six strains showed strong inhibition effect on all the nine fungi. Based on 16 rRNA sequencing, the six bacteria belong to *Pseudomonas aeruginosa*, *Pseudomonas* sp. enrichment culture clone and *Enterobacter aerogenes*. Also, we found 38 strains inhibited the growth of *Sclerotium cepivorum* (white rot pathogen)，the inhibition rate is range from 16.63-64.58%. Among them, the inhibition rate of 17 strains is more than 50%. These results provide the basis for the development of microbial fungicide, and it is helpful to exploit new microbial resources for the biocontrol of plant disease.

Key words: garlic, endophytic bacteria, bacteriostatic activity

Correspondence author:jhjiang@jsnu.edu.cn

Isolation of Plant Growth Promoting Rhizobacteria from Alkalic Soil

Li Lixin[a], Lv Min[a,b], Wang Mingjing[a], Chen Tao[b]

[a] *Alkali Soil Natural Environmental Science Center, Northeast Forestry University, Harbin*

[b] *Biotechnology Research Institute, CAAS, Beijing*

E-mail: lixinli0515@163.com

Plant growth promoting rhizobacteria (PGPR) enhance plant growth and development under stress conditions. PGPR can tolerate adverse conditions, including salinity and drought, with promoting plant growth and yield. PGPR exist in the rhizosphere near the root surface and establish an association inside the root to promote plant growth. Our aim is to isolate PGPR from alkalic soil, identify the isolates through 16S rRNA sequence analysis and morphological and biochemical characteristics as well, and reveal the mechanism between PGPE and plants under alkalic stress.

References:

References should be indicated as consecutive numbers placed in square brackets, e.g. [1] and [2,3], etc., and referenced as in the example below.

[1] Sami Ullah and Asghari Bano (2015). Can. J. Microbiol. 61: 307–313

ITD6 is Required for Infection Thread Formation and mRNA Export in *Lotus japonicus*

Xiaolin Li[a], Miaoxia Liu[a], J. Allan Downie[b] and Fang Xie[a]*

[a] *National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

[b] *John Innes Centre, Norwich NR4 7UH, United Kingdom*

E-mail:xiefang@sibs.ac.cn

In eukaryotes cells, mRNA nuclear export is a dynamics and highly regulated process which enhances control over the timing and level of translation. Legume plants symbiotic association with nitrogen-fixing bacteria and leading to nitrogen-fixing nodules formation. Rhizobia invasion to legume plants generally through infection thread. Here, we identified a mutant *itd6* (infection-thread deficient 6) in *L. japonicus*, which only can entrap bacteria in the infection pocket and occasional form few abnormal infection thread, thereby it cannot form effective nodule to fix nitrogen. ITD6 encode a function unknown protein by map-based cloning, sequencing and function complementation assay. The *ITD6* is induced by rhizobia and specific expressed in infected root hairs and all cell layers of developing nodule primordia. Moreover, the nodulation specific transcription factor NIN directly binds to the *ITD6* promoter to activate its expression. A nucleoporins protein and a RNA-binding protein interact with ITD6 from a yeast-two-hybrid screening. Further function analysis shown that *itd6* mutant accumulate more poly(A) mRNAs in the nucleus, likely resulting from reduced mRNA export activity. Taken together, ITD6 was identified as a new component required for infection thread formation, and ITD6 protein contributing to the transfer of mature mRNA from the nucleus to the cytosol.

Function of the SCAR/WAVE Component During the Infection Thread Growth in Lotus japonicus

Lushuang Li [a], Liping Qiu[a] and Fang Xie*

[a] *National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai*

ysli@sibs.ac.cn

Rhizobial infection of legume root hairs requires a rearrangement of the actin cytoskeleton to enable the establishment of plant-made infection structures called infection threads.In the SCAR/WAVE (Suppressor of cAMP receptor defect/WASP family verpolin homologous protein) actin regulatory complex is conserved from animals to plants and,generally,is composed of the five subunits SCAR/WAVE,PIR121,NAP125,BRICK and ABI.Three of the five subunits have been shown to participate in root hair infection in Lotus japonicus by Mesorhizobium loti[1, 2].Lotus ABI-like proteins (ABIL) and BRICK have not been characterized functionally,so far.Here we demonstrate that abil lore Insertion mutant and microRNA knock-down of the BRICK gene leads to delay the nodule formation.However,infection threads extend into the root cortex that were normal in abil mutants.Those reminder us that these nodule defects were due to the hindering process of releasing from the infection into the cortex. Short root hairs of the mutants had mostly transverse or web-like actin filaments,while bundles of actin filaments in wild-type root hairs were predominantly longitudinal. The N-terminal SCAR-homology domain,ABIL and BRICK two of them interact with each other. In addition, we found that ABIL expression is enhanced by M. loti and that this is directly regulated by the NODULE INCEPTION (NIN) transcription factor.

References:

[1] Yokota, K., et al., *Rearrangement of Actin Cytoskeleton Mediates Invasion of Lotus japonicus Roots by Mesorhizobium loti*. Plant Cell, 2009. **21**(1): p. 267.

[2] Qiu, L., et al., *SCARN a Novel Class of SCAR Protein That Is Required for Root-Hair Infection during Legume Nodulation*. Plos Genetics, 2015. **11**(10): p. e1005623.

From Dark to light: an in vitro infection system of a soil-borne pathogen

Yao Lu [a], Xin Jin [a], Haibin Lu [a], Qin Chen [a]

[a] *Agronomy colledge, Northwest Agriculture & Forest University, No3. Taicheng Road, Yangling, Shaanxi*

luhaibin011@163.com

Ralstonia solanacearum, a devastating soil-borne pathogen, attacks wide range of crops and causes bacteria wilt, which leads to huge loss of crop production. But little is known how *Ralstonia* interacts with plant root and exerts infection strategies because visualization of root

infection is not possible. To visualize the *Ralstonia* infection, we are trying to establish an *in vitro* infection system between potato and *Ralstonia*. A serial of different concentrations of pathogen were inoculated to potato explants and investigated disease development for 8-10days. Explants infiltrated with 0.1OD showed water loss in leaves at 3dpi. Some of them fell down to ground at 6dpi, and then all of them were killed around 8dpi. As we expected, wilt symptom on explants treated with 0.01OD *Ralstonia* were delayed two days comparing with 0.1OD-treated potato explants. In a word, the established *in vitro* infection system will help us to understand the interplay at early infection stage between *Ralstonia* and potato.

Acknowledgement: The project was supported by the National Natural Science Foundation of China (No. 31601703), the Research Foundation for advanced Talents and the fundamental Research Funds for Science and Technology Innovation of the Central Universities from Northwest A&F University (No. Z111021601 & No. Z109021706).

Genetic diversity and application of endophyte from JUNCAO

Mingyue Ni

Fujian University Key Laboratory for Functional Genomics of Plant Fungal Pathogens, Fujian Agriculture and Forestry University, Fuzhou 350002

Nimingyue12138@163.com

Abstract: Plant endophytic bacteria is a huge treasure trove of resources, with significant research and development value. Plant endophytic bacteria with the host of long-term co-evolution, as an important part of the plant ecosystem. In the plant growth and development, nutrient absorption, stress stress and secondary metabolites and other physiological and biochemical has a significant role. In this study, we mainly studied the species and physicochemical properties of endophytic bacteria in different areas, and the inhibition of rice diseases and nitrogen fixation.

Acknowledgement:Prof. Guodong Lu,Wenyu Ye

References:

[1] Qiang Li,Jun Liu,Dongpo Zhou,Jing Zhu.Biotechnology Bulletin.2006,03:33~37.

[2] Zhen Yang,Jun Cao.Journal of Microbiology.2016 vol. 36:1~6.

Screening and Identification of Antagonistic Bacterium Strains against *Verticillium dahliae* from Endophytic Bacteria

Lin Zhang[a,b], Wenpeng Li[b], Xiaoyan Yin[b], Qiuhong Niu[b*]
[a] *State Key Laboratory of Cotton Biology, Henan Key Laboratory of Plant Stress Biology, School of Life Sciences, Henan University, Kaifeng 475004*
[b] *Department of Life Science and Biotechnology, Nanyang Normal University, Nanyang, 473000*
E-mail of presenting author: qiuhongniu@hotmail.com

Cotton Verticillium Wilt is a world-epidemic disease caused by *Verticillium dahliae*. It has a serious influence on the quality of cotton and cotton farmers' income. Biological control which people pay more attention now is a highly effective measure for management of cotton Verticillium wilt.

Endophytic bacteria have good prospects in biocontrol application. Existing in plants, endophytic bacteria take a favorable biocontrol position, so they can endure the plant self-defense reaction and directly act on pathogens, which can provide comprehensive and effective protection for the host plants.

Eighty bacterial strains were isolated in cotton from Kaifeng, Henan province. The antagonistic assay in plates showed that there were 13 strains which had antagonistic activity to *V. dahliae* among them. After second screening, one strain numbered QY1 showed the highest antagonistic activity. The homogeneous analysis demonstrated that the deduced nucleotide sequence of the 16S rRNA sequence reached to be 98% homology with *Kosakonia cowanii* 888-76^T (CP019447.1). The bacterium was classified to be *Kosakonia* genus.

To investigate whether the extracellular protease secreted by this strain has the effect of inhibiting *V. dahliae*, the extracellular protease of the bacteria QY1 was isolated and purified. A homogeneous extracellular protease was purified by chromatography, and the hypothesis of proteinaceous factor in the inhibition of *V. dahliae* was confirmed by the experiments of inhibiting *V. dahliae* when treated with the homogenous protease. Characterization of the purified protease revealed the molecular mass of 20 kDa and the optimum activity at pH 8, 45°C. The gene clone for the protease is in progress.

Key words: *Verticillium dahliae*; Endophytic bacteria; *Kosakonia*; antagonistic bacteria; protease

The *Ralstonia solanacearum* type III effector RipAY targets plant redox regulators to suppress immune responses

Yuying Sang[ab], Yaru Wang[abc], Yali Wei[abc], Hong Ni[a], Yimin She[a], Nemo Peeters[d], Alberto P Macho[ab*]

[a] Shanghai Center for Plant Stress Biology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 201602.
[b] CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031.
[c] University of Chinese Academy of Sciences, Beijing.
[d] LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan 31326, France
* Corresponding author

The subversion of plant cellular functions is essential for bacterial pathogens to proliferate in host plants and cause disease. Most bacterial plant pathogens employ a type III secretion system to inject type III effector (T3E) proteins inside plant cells, where they contribute to the pathogen-induced alteration of plant physiology. In this work, we found that the *Ralstonia solanacearum* T3E RipAY suppresses plant immune responses triggered by bacterial elicitors， phytohormone salicylic acid and *Pseudomonas syringae* DC3000 in *Nicotiana benthamiana*. Further biochemical analysis indicated that RipAY associates in planta with thioredoxins from *N. benthamiana* and Arabidopsis. Interestingly, RipAY displays γ-glutamyl cyclotransferase (GGCT) activity to degrade glutathione in plant cells, which is required for the reported suppression of immune responses. Given the importance of thioredoxins and glutathione as major redox regulators in eukaryotic cells, RipAY activity may constitute a novel and powerful virulence strategy employed by *R. solanacearum* to suppress immune responses and potentially alter general redox signaling in host cells.

Acknowledgement: We thank members of the Macho laboratory and Rosa Lozano-Durán for suggestions in this project. Research in the Macho laboratory is supported by the Shanghai Center for Plant Stress Biology (Chinese Academy of Sciences), National Natural Science Foundation of China (grant 31571973) and the Chinese 1000 Talents Program.

GCR2 is the Binding Protein of *N*-3oxo-hexanoyl-homoserine Lactone, a Bacterial Quorum-Sensing Signal Molecule, in Plants

Pengfei Hou [a], Qian Zhao [a,b], Qiushi Ai [a], Zhe Zhang [a], Fang Liu [a,b], Zhenhua Jia [a,b], Shuishan Song [a,b]
[a] Biology Institute, Hebei Academy of Sciences, 46th South Street of Friendship, Shijiazhuang

[b] *Hebei Engineering and Technology Center of Microbiological Control on Main Crop Disease, 46th South Street of Friendship, Shijiazhuang*
E-mail: shuishans620@163.com

Abstract: *N*-acyl-homoserine lactones (AHLs), the quorum-sensing signals used by Gram-negative bacteria[1], can be perceived by their host plants, thereby modulate gene expression and cellular responses in plant[2,3]. However, little is known about AHL signal perception and transduction. In our study, Arabidopsis protein GCR2 was proved to be the plant receptor of bacterial *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) by diversity receptor kinetics analysis including Biotin labeled-ELISA assay, Radioligand assay, Microscale Thermophoresis (MST) assay and Bio-Layer Interferometry (BLI) assay. Bioinformatics and site-directed mutagenesis analysis indicated that Lys (355) and Val (14) of GCR2 were the key amino acids in the interact region of GCR2 binding to 3OC6-HSL. Molecular genetics and qRT-PCR analysis indicated that GCR2 participated in promotion of Arabidopsis primary root elongation induced by 3OC6-HSL. Split-ubiquitin system analysis indicated that 3OC6-HSL induced the dissociation of GCR2 and Gα protein in the downstream signal transduction. The out-come of this study will enrich the biological functions of GCR2 and be great importance of further understanding AHL-mediated cross-kingdom communication between bacteria and host plant.

Acknowledgement: This work was financially supported by projects from the National Basic Research Program of China (No. 2015CB150600), the National Natural Science Foundation of China (No. 31601144) and the Natural Science Foundation of Hebei Province (No. C2015302020).

References:

[1] J.P. Pearson, K.M. Gray, L. Passador et al. (1994). Proc. Natl. Acad. Sci. USA. 91: 197.
[2] F. Liu, Z. Bian, Z. Jia et al. (2012). Mol. Plant Microbe Interact. 25: 677.
[3] C. Miao, F. Liu, Q. Zhao et al. (2012). Biochem. Biophys. Res. Commun. 427: 293.

G Protein Signaling Participate in Elevation of Intracellular Calcium Induced by *N*-acyl-homoserine Lactones, the Bacterial Quorum-Sensing Signals, in Arabidopsis

Lu Cheng [a], Qian Zhao [a,b], Fang Liu [a,b], Xiangye Cao [a], Zhenhua Jia [a,b], Shuishan Song [a,b]
[a] *Biology Institute, Hebei Academy of Sciences, 46th South Street of Friendship, Shijiazhuang*

[b] *Hebei Engineering and Technology Center of Microbiological Control on Main Crop Disease, 46th South Street of Friendship, Shijiazhuang*
E-mail: shuishans620@163.com

Abstract: *N*-acyl-homoserine lactones (AHLs) are the quorum-sensing (QS) signal molecules used to coordinate the population behaviors in gram-negative bacteria**.** Recent evidence demonstrates that plants are able to respond to bacterial AHLs, including intracellular calcium elevation[1] and G protein-mediated AHL-induced plant root elongation [2]. However, little is known about the function of G protein signaling in AHL-mediated calcium elevation within cells. In this study, electrophysiological approaches were used to detect the changes of transmembrane Ca^{2+} current in Arabidopsis treated with AHLs. The increased Ca^{2+} current induced by *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) were enhanced in *GPA1*-overexpressing plant *cGα*, but were abolished in *GPA1* functional-deficiency mutant *gpa1-4*. Pharmacological assay showed that the transient-increase in $[Ca^{2+}]$cyt levels induced by 3OC8-HSL were enhanced by G protein activator CTX treatment, while abolished by treating with G protein inhibitor PTX. In functional-deficiency mutant *gcr2-2 of GCR2*, a G protein coupled receptor, the Ca^{2+} current elevation effect induced by *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) were also abolished, but could be restored in *GCR2* complimentary transgenic lines. The concentration of cellular cAMP, a G protein-actived secondary messenger to regulate plasma membrane calcium channel, were increased by 3OC8-HSL, and this effect was enhanced by Gα overexpressing, while weakened by Gα functional-deficiency mutating. These data suggested that G protein signaling play a positive role in plant intracellular calcium elevation induced by bacterial AHLs.The out-come of this study will be great importance of further understanding AHL-mediated cross-kingdom communication between bacteria and host plant.

Acknowledgement: This work was financially supported by projects from the National Basic Research Program of China (No. 2015CB150600), the National Natural Science Foundation of China (No. 31601144) and the Natural Science Foundation of Hebei Province (No. C2015302020).

References:

[1] S. Song, Z. Jia, J. Xu et al. (2011). Biochem. Biophys. Res. Commun. 414: 355.
[2] F. Liu, Z. Bian, Z. Jia et al. (2012). Mol. Plant Microbe Interact. 25: 677.

A common regulatory mechanism of the LsrB protein from both symbiotic and pathogenic bacteria interacting with their hosts

Guirong Tang, qiong Li, Ningning Li, Li Luo*

Shanghai Key Laboratory of Bio-energy Crops, School of Life Sciences, Shanghai University, Shanghai 200444;

qingxiong51@126.com

Reactive oxygen species (ROS) are important regulatory signals that are involved in bacterium-host interactions. In *Sinorhizobium meliloti*, a LysR regulator, LsrB, senses ROS and regulates the expression of downstream genes to modulate the development of nitrogen-fixing nodules on host alfalfa roots. A LsrB homologue from *Brucella* species is required for virulence in mice. The plant pathogen *Agrobacterium tumefaciens* also contains a *lsrB* homologous gene, and it had previously been unclear as to whether the LsrB proteins from these different bacteria were capable of regulating interactions with their hosts in a conserved manner. Here, we find that the LsrB protein directly binds to the target promoter region to control gene expression in *A. tumefaciens* by forming predictive dimers. The LsrB proteins from both *A. tumefaciens* and *B. abortus* were able to replace their homologues in *S. meliloti* to work in alfalfa nodulation, as they were recognized by anti-$LsrB_{sm}$ antibodies in either *A. tumefaciens* or *S. meliloti* cells. The *lsrB* deletion mutant of *A. tumefaciens* was more sensitive to oxidants and produced less succinoglycan. Importantly, the mutant showed decreased attachment and transformation efficiency in host plants. Several genes involved in exopolysaccharide production, attachment and ROS metabolism were downregulated. The LsrB dimers and protein-DNA complexes were found in *A. tumefaciens* cells. Our findings demonstrate that the LsrB proteins from both symbiotic and pathogenic bacteria can sense oxidation signals via Cys residues, reprogramme gene expression, and modulate interactions with hosts.

Effect of secondary symbiont PABS (*Hamitonella defensa*) on the fitness and relative abundance of *Buchnera aphidicola* of wheat aphid, *Sitobion miscanthi* (Hemiptera: Aphididae)

Qian Li[1], Jia Fan[1], Jingxuan Sun[1], Julian Chen[1*]

[1] *The State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193.*

Abstract

Bacterial symbiont is a close association with the most insect species, such as the most severely agricultural pests, aphid. Insect endo-symbiont has been reported involved in host development and ecology fitness. Here, two strains were screened with the same symbionts background except the difference with or without *Hamitonella defensa* (PABS), the PABS-infected strain, named YX (collected from Yu Xi wheat field in Yun Nan Province), and the PABS-free, named DZ (collected from De Zhou wheat field in Shan Dong Province). By artificial infection, the PABS-free DZ and PABS-re-infected (named DZ-P) strains with the same identical genetic background were built in this study. Furthermore, the influence of PABS on *S. miscanthi* was uncovered with ecological fitness measurement. The results indicated that PABS increased the fitness of *S. miscanthi*, as evidenced through higher weight of adult, higher percent wingless aphid rate and more total number of offspring. However, the indices of longevity did not change significantly even exhibited a decreasing trend. The results of quantitative PCR showed that the relative abundance of primary symbiont *Buchnera aphidicola* in different development stages of DZ-P strains were significantly higher than DZ strains except in first instar. Consequently, our finding indicates that PABS may indirectly improve the fitness of *S. miscanthi* by stimulate the proliferation of *B. aphidicola*. Therefore, further investigations are needed to reveal the mechanisms of interaction among PABS, *B. aphidicola* and host aphid.

Key words: *Sitobion miscanthi*, endo-symbionts, *Hamitonella defensa* (PABS), fitness, *Buchnera aphidicola*, relative abundance

Plants from the *Solanaceae* family perceive the *Ralstonia solanacearum* csp22 peptide but not flagellin-derived peptides

Yali Wei[1,2], Carlos Caceres-Moreno[1,2], Tamara Jimenez-Gongora[1,2], Yuying Sang[1], Rosa Lozano-Duran[1] and Alberto P. Macho[1,*].

[1]*Shanghai Center for Plant Stress Biology, CAS Center for Excellence in Molecular Plant Sciences; Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 201602.*

[2]*University of Chinese Academy of Sciences, Beijing.*

* *Corresponding author*

Abstract

Plant basal resistance against bacterial pathogens involves the detection of conserved bacterial molecules, such as the components of the flagellum, which are perceived as pathogen-associated molecular patterns and activate plant immune responses. *Ralstonia solanacearum* is often considered one of the most destructive bacterial pathogens due to its lethality, unusually wide host range, persistence, and broad geographical distribution. In spite of the extensive research on plant immunity over the last years, the molecular patterns of *R. solanacearum* that are perceived by plant cells are poorly understood. A conserved peptide of bacterial flagellin, flg22, is usually employed as paradigm of molecular pattern perceived by plants, but no elicitor activity has been detected so far for *R. solanacearum* flg22, probably due to several polymorphisms in its aminoacid sequence compared to flg22 peptides from other bacteria. Recent reports have shown that other epitopes from flagellin are able to elicit immune responses in specific plant species. In light of these reports, we decided to test whether *R. solanacearum* flagellin contains other epitopes able to induce immune responses in plants from the *Solanaceae* family, which comprises the most economically relevant hosts for *R. solanacearum*. Using either purified peptides or recombinant proteins we did not find any elicitation activity of flagellin from *R. solanacearum* in several plant species from the *Solanaceae* family. Searching for additional peptides from *R. solanacearum* that may be perceived by plants, we found several protein sequences in *R. solanacearum* similar to the consensus of the elicitor peptide csp22, present within the cold-shock protein of several bacterial species. Purified *R. solanacearum* csp22 (Rs_csp22) peptide was indeed able to

trigger immune responses in *N. benthamiana* and tomato, but not in *Arabidopsis thaliana*. However, expression of the tomato CORE receptor in *A. thaliana* transgenic plants conferred responsiveness to Rs_csp22. Our results shed light on the potential mechanisms for perception of *R. solanacearum* by plants, paving the way for improving current approaches to generate sustainable sources of resistance to *R. solanacearum*.

Biodiversity of endophytes associated with *Pinellia ternate* and the PGP interaction between the endophytes and the host plant

Mengke Xu，Panpan Zhang，Dan Li, Bo Yuan, Liang Zhang Jihong Jiang*

The Key Laboratory of Biotechnology for Medicinal Plant of Jiangsu Province

Jiangsu Normal University,Xuzhou 221116, Jiangsu Province

This study aims to investigate the diversity of endophyte from the medicinal plant *Pinellia ternata* and potential plant growth promoting activities, and evaluate the effect of plant growth promoting endophytes on the growth and the accumulation of secondary metabolite of *P.ternata*. We isolated 53 endophytic bacteria from root, stem and leaf of *P.ternata*. 16S rRNA gene sequencing showed that 35 representative endophytic bacteria belonged to two phyla (*Firmicutes* and *Proteobacteria*) and seven different genera, the genera *Bacillus* was the most predominant isolates. We found that many endophytic bacteria had the potential growth promoting activities based on screening experiment. The result showed that 78% strains were equipped with potential ability of nitrogen fixation, 48% strains had the ability of phosphate solubilization, 30% strains had the ability of organic phosphorus solubilization, 96% strains could produce siderophores, 57% strains had the potential ability of ACC deaminase, 65% strains could produce indole-3-acetic acid, 87% strains could produce cellulase, 74% strains could product xylanase, 70% strains could product protease, 22% strains could product amylase.

We chose two endophytic bacteria strains (KLBMPPT001 and KLBMPPT024) to explore the effects on growth promotion. The results indicated that strains KLBMPPT001 and KLBMPPT024 significantly promoted the growth of host plant *P. ternate* and affected the the accumulation of *P. ternata* secondary metabolites. Compared with control group, these strains

increased the shoot length up to 55%-194%, root length up to 90%-307%, leaf area up to 121%-232%, fresh weight up to 6%-231%, dry weight up to 17%-165%, and the accumulation of total alkaloids increased up to 50%-140%.

In order to further confirm the colonization of endophytic bacteria in the host plant *P. Ternate*, we successfully introduced the plasmid pHT43-GFP into strain KLBMPPT024 and performed GFP track .

This research provides the foundation for the effect of endophytes on the production and quality of the host plant and solve the problems of medicinal plant *P. ternate* resources shortage and accumulation of active ingredients.

Correspondence author: E-mail:jhjiang@jsnu.edu.cn

RS01385*, a Lipopolysaccharide O-antigen Ligase, affects virulence determinant in *Ralstonia solanacearum

Jinli Yan[a b], Lianhui Zhang[a b*]

[a]*Key Laboratory for Conservation and Utilazation of Subtropical Agro-Bioresources, College of Agriculture, South China Agricultural University, Guangzhou*

[b]*Guangdong Province Key Laboratory of Microbial Signals and disease control, and Integrative Microbiology Research Centre, South China Agricultural University, Guangzhou*

E-mail of presenting author: 2417617672@qq.com

Ralstonia solanacearum , a soilborne bacterium, is the causal agent of bacterial wilt and infects over 200 plant species in 50 families. This pathogen is lethal to many solanaceous species, including eggplant. Extracellular polysaccharide is a key virulence determinant in *Ralstonia solanacearum*. Extracellular polysaccharide (EPS) and virulence on solid medium were reduced, when wide type strain is subcultured. Here, to identify genes controlled extracellular polysaccharide change in *Ralstonia solanacearum* , we generated a transposon Tn5 insertional mutagenesis library, and subculture transformants. A mutant was identified to show still produced a lot of extracellular polysaccharide. HiTAIL-PCR was used to identify flanking sequences of Tn5 insertion in mutuant. Sequence analysis revealed insertion of Tn5 into *RS01385*, encoding O-antigen ligase, which was based on data from NCBI(https://www.ncbi.nlm.nih.gov/).In order to study the function of *RS01385*, we generated the *RS01385* delta mutant. Targeted deletion of *RS01385* resulted in reduction swimming or swarmming motility, but incresed in production of EPS by EPS precipitation asssy .And the resulting mutant had a characteristic of higly hyperviscosity. In summary, *RS01385* is an important for controling EPS production in *R.solanacearum*. Studying on

Polysaccharides of the strong virulent strain EP1 will facilitate investigation of pathogenic mechanisms.

Down regulation of biofilm formation by Cd^{2+} on *Bacillus subtilis* 1JN2 affected its biocontrol efficiency against *Ralstonia* wilt on tomato

Wei Yang[a], Haixia Yan[b], Shimo Li[a], Yuming Luo[a*]

a: Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Science, Huaiyin Normal University, No 111，West Changjiang Road, Huai'an.

b: Agro-Tech Extension and Service Center, No 55，West Jiankang Road, Huai'an.

Bacillus subtilis 1JN2 was demonstrated to be an effective biocontrol agent against *Ralstonia* wilt on tomato in our previous work. While the biocontrol efficiency would be affected by heavy metal that appeared in the rhizosphere soil of the host plants due to the industrial pollutions. Here we aimed to investigate the reasons of how heavy metal affected the biocontrol efficacy of *B.subtilis* 1JN2 using Cd^{2+}. According to the results, low content Cd^{2+} that less than 10 mg/L had no effects on the biofilm formation of 1JN2 while more than 15 mg/L Cd^{2+} in the media could inhibit the biofilm formation. And 0-25 mg/L Cd^{2+} had no effects on the cell growth of *B.subtilis* 1JN2. Greenhouse experiments demonstrated that 15mg/L artificial Cd^{2+} in the tomato rhizosphere could inhibit the colonization of *B.subtilis* 1JN2 which leading to the decrease of biocontrol efficacy against *Ralstonia* wilt, only 10^3 CFU/mL of 1JN2 was detected one week post treated with 10^8 CFU/ mL but 10^6 CFU/ mL could be detected without Cd^{2+} in the soil. However the additional Cd^{2+} had no effects on the colonization of *Ralstonia solanacearum* FJ100 which was the pathogen caused wilt symptom on tomato. Biocontrol efficacy against *Ralstonia* wilt by 1JN2 was decreased 43.2% when 15mg/L artificial Cd^{2+} treated compared to the control without Cd^{2+}.

A *Ralstonia solanacearum* type-III effector targets components of NLR chaperone complex in plant cells

Gang Yu[1‡], Liu Xian[1,2‡], Yuying Sang[1], Nemo Peeters[3], Alberto P. Macho[1]*
[1]*Shanghai Center for Plant Stress Biology, CAS Center for Excellence in Molecular Plant Sciences; Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai.*
[2]*University of Chinese Academy of Sciences, Beijing.*
[3]*LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France.*
*‡ Indicates equal contribution. * For correspondence: alberto.macho@sibs.ac.cn.*

Ralstonia solanacearum, a widely distributed, Gram-negative, soil-borne phytopathogen, is the causal agent of bacterial wilt disease of more than 50 families 250 plant species, including economically important crops, such as tomato, potato, banana, ginger, pepper and tobacco. Single strains of *R. solanacearum* can inject a repertoire of more than 70 proteins into the host plant cells via type-III secretion system (T3SS) to manipulate the plant cellular functions. Here we report Rip32 (*Ralstonia* injected protein 32), a *Ralstonia* type-III effector protein, which significantly compromised plant immunity triggered by bacterial elicitors and other effectors in *Nicotiana benthamiana*. Affinity purification coupled with LC-MS/MS identified a member of NLR chaperone complex as one of Rip32 potential interacting proteins in plant cells. This association was further confirmed by independent interaction assays. Constitutive expression of Rip32 altered Arabidopsis plant development. Given its multifaceted roles in the suppression of immune responses, Rip32 is a novel uncharacterized effector and might play important roles during bacterial infection.

Isolation, expression and antimicrobial activity of antimicrobial protein of Bacillus subtilis strain XF-1

Jing Zhao[a,b], Yi-Xing Wu[a,c], Hon-Ling HO[d], Zhou-Jun Chen[c], Xing-Yu Li[a], Zi-Chao Mao[c], Yue-Qiu He[a,c*]
[a]*National Engineering Center of Application Technologies for Agricultural Diversity, Yunnan Agricultural University (YAU), Kunming 650201；* [b] *Yuxi Normol University,Yuxi 653100；* [c]*Faculty of Agronomy and Biotechnology, YAU, Kunming 650201；*[d]*Department of Biology, State University of New York, New Paltz, NY12561, USA*

ynfh2007@163.com

Background Objective: There are many reports on the use of Bacillus subtilis, either singly or in combination with other micro-organisms, as an effective bio-control agent against plant-pathogenic fungi especially those which are soil-borne (Gu ZR et al,2004). Bacillus

subtilis XF-1, isolated in 2006 from field soil in Yunnan, where crucifers were infected by Plasmodiophora brassicae, resulting in clubroot disease. Our previous unpublished study indicated that a water-soluble protein produced by XF-1 possessed significant antimcirobial activity and could be a potential candidate for the control of P. brassicae. It was heat resistant and could remain active even after being kept at 121℃ for 20 min.

Methods: After cultured at 30℃ for 48 h, crude protein of from strain XF-1 was extracted by 80% ammonium sulfate precipitation and then dialysed. The obtained protein was purified by gel chromatography and chitin-based affinity chromatography. Relying on protein sequence, the gene sequence of the protein was cloned.

Results: The purified protein showed a single band with a molecular weight of 25 kD in gel after SDS-PAGE and still kept the better heat resistance after ten minutes heated at 100℃. Relying on protein sequence, the gene sequence of the protein was obtained by comparing XF-1 genome with the alignment result, and the gene was named with PBT1 (Plasmodiophora brassicae toxicity). By cloning and expressing the gene PBT1, the results showed that, gene PBT1, which cloned from strain XF-1, encode a complete ORF (open reading frame) with the 753 bp. Compared with Bacillus subtilis 168（GenBank accession numbe: AL009126.3）, the gene has the difference with 15 base pairs and shared 98% sequence similiarity. According to the prediction to signal peptide, CD(conserve domain), Motif, prosite, signal peptide lies at the 31th and 32th amino acids; 4 type of structure sites may be exist: N-myristoylation site (7 sites),

protein kinase C phosphorylation site; casein kinase II phosphorylation site(6), N-glycosylation site. By alignment of amino acid sequences translated, the result indicated that the differences from B 168 locate in the 12th, 50th, 59th, 73th, 96th amino acids and the five amino acids of Valine, Glutamic acid, Valine, Valine and Valine are replaced with methionine, Glutamine, Alanine, threonine and Serine.

References:

Gu ZR, Wu W, Gao XH, Ma CZ. 2004. Antifungal substances of Bacillus subtilis strain G3 and their properties. Acta Phytopathol. Sinica. 34(2): 166-172.

Keywords: Bacillus subtilis XF-1；PBT1；Plasmodiophora brassicae； Antimicrobial effect

Functional screening identifies *Ralstonia solanacearum* phylotype IIB/1 clade virulent effectors interfering with potato immunity

Xueao Zheng[a], Dong Cheng, Bingsen Wang, Xiaojing Li, Guozhen Zhao, Mengshu Huang, Yanping Li, Wenhao Li, Xiaodan Tan, Huilan Chen[b]

[a] *Potato Biology and Biotechnology Key Laboratory of Ministry of Agriculture ; Huazhong Agricultural University, Wuhan, 430070.*

zhengxueao@webmail.hzau.edu.cn

Abstract:

Ralstonia solanacearum in phylotype IIB/1 clade seriously constrains potato production worldwide as there are no efficacious means to minimize its damage on potato growth and development.

The type 3 secretion system effectors of *R*. *solanacearum* enhance their colonization by interfering with potato immunity. The effectors are diversified in phylotype IIB/1 clade present more than 50 effectors in each strains. Mutants lacking one or more effector genes might not affect the pathogenicity indicated that functional redundancy exist among the effector network. Therefore, different perspective should be brought to detect virulent effectors.

In order to identify virulent effectors more efficiently, we subjected 30 phylotype IIB/1 core effectors to three screening systems: growth inhibition screens in yeast; HR (induced by INF1\R3a&Avr3a) suppression screens by agroinfiltration in *N*. *benthamiana*; potato inoculation assay with effector mutants. These systems complemented mutually to identify core virulent effectors.

Further work is focusing on biochemical function and those characterized effectors will be used to investigate the potato immunity as a molecular probe.

Alkaloids Constrain Bacterial Endophyte Assemblages in Different Organs of *Lycoris radiate*

Zhou Jia–Yu, Wang Ren (✉)

Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Xuanwu District, Nanjing

Presenting Author: zhoujiayu@cnbg.net

Corresponding Author: jswangren@aliyun.com

The distribution of bacterial endophyte varies with different organs of host plant. However, factors driving tissue–specific distribution of bacterial endophyte are largely unknown. *Lycoris radiate*, an important medicinal plant, contains abundant Amaryllidaceae alkaloids that have antineoplastic, antimicrobial, antiviral, and antimalarial abilities. Our

study shows that alkaloid types and contents vary with different organs of *L. radiate*. Thus, we propose that tissue–specific distribution of Amaryllidaceae alkaloids may affect the bacterial endophyte assemblages in different organs of *L. radiate*.

Diversity of bacterial endophyte in different organs of *L. radiate* was monitored by high–throughput sequencing. Bacterial communities in different organs were quite different. Gammaproteobacteria was dominating in flowers. The core microbiome in stems consisted of Gammaproteobacteria, Betaproteobacteria, and Alphaproteobacteria. Mollicutes was dominating in leaves. The core microbiome in bulbs mainly included Mollicutes and Betaproteobacteria. The preponderant classes in roots were Actinobacteria and Alphaproteobacteria. Alpha diversity of bacterial endophyte in stems and roots was higher than others. Beta diversity of bacterial endophyte in different organs was significantly different. Contents of seven main alkaloids (Galanthamine, Lycoramine, Lycorine, Pseudolycorine, Lycorenine, Tazettine, and Narciclasine) and five main precursors for alkaloid biosynthesis (trans–Cinnamic acid, *p*–Coumaric acid, Caffeic acid, Protocatechuic aldehyde, and L–Tyramine) in different organs of *L. radiate* were compared. Canonical Correspondence Analysis (CCA) two–dimensional ordination diagram showed Pseudolycorine, Lycorenine, Tazettine, trans–Cinnamic acid, Caffeic acid, Protocatechuic aldehyde, and L–Tyramine were important factors affecting the tissue–specific diversity of bacterial endophyte. Importantly, Variation Partitioning Analysis (VPA) showed that total alkaloid content could explain 96.92% of total factors that drive the tissue–specific diversity of bacterial endophyte in *L. radiate*. Summarily, alkaloids constrain bacterial endophyte assemblages in different organs of *L. radiate*, which may be due to the antimicrobial ability of Amaryllidaceae alkaloids. Inversely, as potential candidates of inducers that promote plant secondary metabolite accumulations, variations in endophytes assemblages may feedback and affect alkaloid accumulations in *L. radiate*.

Potential of a new strain of *Bacillus licheniformis* X-1 as a biocontrol agent of Fusarium wilt on strawberry

ZHOU Tong [a], Xu Yong-ping [a,b,c,*], LI Xiao-yu [a,b], WANG Li-li [a,b],
CHEN Yan [a], ZHANG Nan [a], WANG Jia-ning [a], Jia Cang-cang [a], QU Fang-jing [a]

[a] *Animal Biotechnology and Nutrition Lab, School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116024, Liaoning;*

[b] *Ministry of Education Center for Food Safety of Animal Origin, Dalian 116620;*

[c] *Dalian SEM Bio-Engineering Technology Co. Ltd., Dalian 116620*

E-mail: zhou1114120215@163.com

Chemical pesticides are widely used in agriculture, which endangers both environmental health and food safety. Biocontrol is a safer and sustainable strategy for safe and profitable agricultural productivity [1]. We isolated a novel bacterial strain X-1 from the rhizosphere soil of strawberry, which can suppress mycelial growth and conidial germination of strawberry Fusarium wilt pathogen (*F. oxysporum f. sp. fragariae*). The strain was identified as *Bacillus licheniformis* using morphological, biochemical, physiological, and phylogenetic 16S rDNA sequencing data. *Bacillus licheniformis* X-1 was inoculated in organic fertilizer (OF) for preparing bio-organic fertilizer (BIO). The effects of BIO in controlling strawberry Fusarium wilt and its functional mechanisms were studied in this paper. The pot experiment results showed that the applications of BIO could decrease the incidence of strawberry Fusarium wilt by 65.8% and disease index by 67.2%, and the biocontrol efficiency was about 80.5%. Furthermore, the application of BIO could significantly increase in strawberry seedling fresh weight (25.8%), seedling length (18.4%), and root length (48.5%) compared to the control. After application of BIO, the activities of polyphenol oxidase (PPO), peroxidase (POD), phenylalanine (PAL) and superoxidase (SOD) were increased. Meanwhile, the content of maleic dialdehye (MDA) was decreased, it reduced the plant cell membrane lipid peroxidation damage. These results suggest that X-1 has potential commercial application as a biofertilizer or biocontrol agent.

References:

[1] Shafi, J., Tian, H., & Ji, M. (2017). Bacillus species as versatile weapons for plant pathogens: a review. Biotechnology & Biotechnological Equipment,31(3), 446-459

Dual function of a secreted metalloprotease in the *Ustilago maydis*-maize pathosystem

Bilal Ökmen[a], Bastian Kemerich[a], Daniel Hilbig[a], Raphael Wemhöner[a], Sara Theimann[a], Jörn Aschenbroich[b], Kerstin Schipper[b], Gunther Doehlemann[a]

[a] *The Terrestrial Microbiology Laboratory, Institute of Botany, University of Cologne, Cologne, Germany*

[b] *Institute of Microbiology, Heinrich-Heine University, Düsseldorf, Germany*

bilal.oekmen@uni-koeln.de

Abstract

Chitinases are widely distributed across diverse biological systems. While fungal chitinases are responsible for cell-wall remodeling during growth and morphogenesis, plant chitinases are strongly induced upon pathogen attack and establish an important arsenal of plants against pathogens. In this study, we have shown that a metalloprotease (UmFly1) of *Ustilago maydis* is involved in modification of both endogenous fugal chitinase (Cts1) and plant chitinase (ZmChiA). UmFly1 is involved in cleavage of maize ZmChiA to inhibit its enzymatic activity. Consistent with this, deletion mutants of *umfly1* showed drastically reduced virulence compared to the wild-type strain. In addition to the virulence defect, Δ*umfly1* mutants also impaired in cell separation in the yeast stage leading to formation of aggregates, but not impaired in growth, filamentation and pathogenic differentiation. Biochemical analysis of the endogenous *U. maydis* chitinase Cts1 revealed that UmFly1 is required for C-terminal processing of Cts1, which leads to the activation of Cts1. These results indicate a dual function of UmFly1 in *U. maydis* cell separation and virulence. We hypothesize that during co-evolution with its host, endogenous function of UmFly1 adapted to cleavage of maize chitinases to avoid hydrolytic activity of host chitinases, reflecting its functional adaptation to a pathogenic life style. Complementation of Δ*umfly1* mutants with Fly1 homolog from non-pathogenic *Moesziomyces bullatus* (*MbFly1*), a close relative of *U. maydis*, also support that hypothesis. While MbFly1 fully complements cell separation phenotype of the mutant, it can only weakly complement virulence and ZmChiA cleavage activity.

Plant jasmonate ZIM domain genes: shedding light on structure and expression patterns of JAZ gene family in sugarcane

Feng Liu[a,b], Tingting Sun[a,b], Ling Wang[a,b], Weihua Su[a,b], Shiwu Gao[a,b], Yachun Su[a,b]*, Liping Xu[a,b], Youxiong Que[a,b]*

[a] *Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002*

[b] *Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou 350002*

E-mail of presenting author: 18359162091@163.com

Abstract: Sugarcane smut caused by *Sporisorium scitamineum* is one of the most severe fungal diseases in sugarcane industry. Using a molecular biological technique to mine sugarcane resistance genes can provide gene resources for further genetic engineering of sugarcane disease-resistant breeding. In this study, seven differentially expressed sugarcane jasmonate ZIM (zinc-finger inflorescence meristem) domain (*JAZ*) genes, *ScJAZ1–ScJAZ7*, were mined from the transcriptome of sugarcane after inoculation with *S. scitamineum*. Bioinformatics analysis revealed that these seven *ScJAZ* genes encoded basic proteins that contain the TIFY and CCT_2 domains. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis demonstrated that the *ScJAZ1–ScJAZ7* genes are tissue specific and differentially expressed under adverse stress. During *S. scitamineum* infection, the transcripts of *ScJAZ4* and *ScJAZ5* were both upregulated in the susceptible genotype ROC22 and the resistant genotype Yacheng05-179; *ScJAZ1*, *ScJAZ2*, *ScJAZ3*, and *ScJAZ7* were downregulated in Yacheng05-179 and upregulated in ROC22; and the expression of *ScJAZ6* did not change in ROC22, but was upregulated in Yacheng05-179. The transcripts of the seven *ScJAZ* genes were increased by the stimuli of SA and ABA, particularly MeJA. The expression of the genes *ScJAZ1–ScJAZ7* was immediately upregulated by the stressors H_2O_2, NaCl, and $CuCl_2$, whereas slightly induced after treatment with $CaCl_2$ and PEG. In addition, the expression of *ScJAZ6* as well as seven tobacco immunity-associated marker genes was upregulated, and antimicrobial activity against *Pseudomonas solanacearum* and *Fusarium solani* var. *coeruleum* was observed during the transient overexpression of *ScJAZ6* in

Nicotiana benthamiana, suggesting that the *ScJAZ*6 gene is associated with plant immunity. In conclusion, the different expression profiles of the *ScJAZ1-ScJAZ7* genes during *S. scitamineum* infection, the positive response of *ScJAZ1-ScJAZ7* to hormones and abiotic treatments, and the function analysis of the *ScJAZ6* gene revealed their involvement in the defense against biotic and abiotic stresses. The findings of the present study facilitate further research on the *ScJAZ* gene family especially its regulatory mechanism in sugarcane.

Acknowledgement: This work was funded by the National Natural Science Foundation of China (31501363 and 31101196), Research Funds for Distinguished Young Scientists in Fujian Agriculture and Forestry University (xjq201630), Natural Science Foundation of Fujian province, China (2015J06006), the earmarked fund for the Modern Agriculture Technology of China (CARS-20), and the Program for New Century Excellent Talents in Fujian Province University (JA14095).

A sugarcane pathogenesis-related protein, ScPR10, plays a positive role in defense responses under *Sporisorium scitamineum*, SrMV, SA and MeJA stresses

Qiong Peng[a,b], Yachun Su[a,b], Hui Ling[a,b], Waqar Ahmad[a,b], Shiwu Gao[a,b], Jinlong Guo[a,b], Youxiong Que[a,b], Liping Xu[a,b]*

[a] *Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002*

[b] *Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou 350002*

E-mail of presenting author: syc2009mail@163.com

Abstract: Plant fungal and viral diseases are the major concerns in sugarcane industry. Many antifungal and antivirus components, including pathogenesis-related (PR) proteins, have been identified. The pathogenesis-related protein 10 (PR10), is the dominant group in PR families, involved in the plant defense mechanism. In this study, *ScPR10* (GenBank Acc. No. KT887884), a 701 bp-length *PR10* gene with a 483 bp-length open reading frame, was

isolated from sugarcane. Its transient expression in the leaves of *Nicotiana benthamiana* indicated that, the function role of ScPR10 is likely in the nucleus, and it increased the level of H_2O_2 accumulation in leaf cells. Moreover, ScPR10 could also enhance the resistance of *N. benthamiana* leaves to infection by *Pseudomonas solanacearum* and *Fusarium solani* var. *coeruleum*. Quantitative real-time PCR analysis revealed that *ScPR10* was not constitutively expressed in sugarcane tissues due to its high expression in the buds and scant presence in root tips. In addition, the transcript of *ScPR10* could be induced by a pathogenic fungus (*Sporisorium scitamineum*) and a virus (*Sorghum mosaic virus*, SrMV) in the resistant sugarcane cultivars, while it was down-regulated in the susceptible ones. After exposure to salicylic acid (SA) and methyl jasmonate (MeJA), *ScPR10* peaked at 6 h and 12 h, respectively. These results suggest that *ScPR10* can play a positive role in sugarcane defense responses to *S. scitamineum*, SrMV, SA, and MeJA stresses.

Acknowledgement: This work was funded by the National Natural Science Foundation of China (31571732) the earmarked fund for the Modern Agriculture Technology of China (CARS-20), the research Funds for Distinguished Young Scientists in Fujian Agriculture and Forestry University (xjq201630), and the Natural Science Foundation of Fujian province, China (2015J06006).

Transcriptional Regulation of Effectors in rice blast fungus *Magnaporthe oryzae*

Bozeng Tang, Xia yan, Darren Soanes, Michael Kershaw and Nick Talbot

University of Exeter School of Biosciences, 98452, Exeter, Devon, United Kingdom of Great Britain and Northern Ireland
bt284@exeter.ac.uk

To cause rice blast disease, the filamentous fungus, *Magnaporthe oryzae*, secretes effectors to modulate metabolism of its host and suppress plant immunity. Effector genes are only expressed *in planta* once the pathogen has invaded its host, and show only low basal expression levels in other developmental stages. In *M. oryzae* a large number of effectors have been identified and some have been partially characterized by means of identifying their interacting partners in host plants. To date, however, very little is known regarding how

plant-specific expression of fungal effectors is regulated. To investigate the mechanisms which govern transcriptional regulation of effectors, we established a mutant screen to identify putative regulators of effector gene expression. We have screened for mutants showing constitutive expression of GFP-labelled effectors in conidia and during mycelial growth in order to identify potential transcriptional regulators and signaling components. We are now carrying out genome sequencing and bulked segregant analysis to identify novel effector regulators and will report on progress toward their characterization.

Aspergillus flavus Ash1 involves in fungal virulence and pathogenicity

Zhenhong Zhuang, Shihua Wang*

Key Laboratory of Pathogenic Fungi and Mycotoxins of Fujian Province, and School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, 350002

*School of Life Sciences, Fujian Normal University, Fuzhou, Fujian 350108 *Corresponding authors: Wang SH, Tel/Fax: +86 591 87984471, E-mail: wshyyl@sina.com.*

Abstract: As an important epigenetic modification, post-translational modifications of key amino acids on histone tails are revealed as “Histone code”. Ash1 (absent, small, or homeotic discs 1) functions as lysine histone methyltransferase, and this study aimed at elucidating the bio-function of *ash1* on epigenetic regulation in *Aspergillus flavus*. The study revealed that the *Δash1* strain increased conidiation when compared to WT (wild type) and Com-*ash1* (*ash1* gene complementation) strains. It was found that *ash1* gene significantly up-regulated the development of radiation growth of mycelium and sclerotia, which was confirmed by Q-PCR on sclerotia regulators, *nsdC* and *nsdD*. TLC and HPLC analysis showed that aflatoxin B1 (AFB1) productuon was dramatically down-regulated when *ash1* gene was deleted. The expression level of AFB1 bio-synthesis genes (*aflC*, *aflD*, *aflK*, and *aflQ*) and regulation genes (*aflR* and *aflS*) were further analyzed by Q-PCR, and the results showed that all these AFB1 bio-synthesis and regulation genes were down-regulated in Δ*ash1* strain compared to WT and Com-*ash1* stains. Pathogenicity analysis showed that *ash1* gene played an important role in crop infections and animal aspergillosis in *A. flavus*. On the peanut and corn surface, the AFB1 bio-synthesis and the conidia producing capacity were significantly

down-regulated when *ash1* gene was deleted, which suggested that *ash1* gene was required in the process of mycotoxin production and offspring transmission of *A. flavus* among crops. All the results in this study showed that histone lysine methylation transferase Ash1 played a critical role in morphogenesis, AFB1 bio-synthesis and pathogenicity of *A. flavus*.

Key words: Histone methyltransferase; *ash1* gene; *Aspergillus flavus;* pathogenicity; secondary metabolites

The research was supported by National 973 Program of the Ministry of Science and Technology of China (No. 2013CB127802), the grants of the National Natural Science Foundation of China (No.31600118, No.31172297, and No.31400100), The Fund for Cultivation of outstanding youth science and technology talents in Fujian Agriculture and Forestry University (xjq201410).

How do pathogen effectors exploit the host symplast

Xiaokun Liu, Catherine Mansfield, Christine Faulkner
Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, UK
Xiaokun.kun@jic.ac.uk

It is well established that pathogens invade plant cells to establish an infection. Biotrophic pathogen secrete effectors into host cells in order to manipulate host processes to the pathogen's benefit as part of their infection strategy. While much research focuses on the mechanism by which pathogens introduce effectors into host cells, there is evidence that effectors can spread from the infected cells to surrounding non-infected cells. The oomycete pathogen of Arabidopsis Hyaloperanospora arabidopsids (Hpa), invades host cells via the formation of haustoria, structures that are the presumed site of nutrient and effector exchange between host and pathogen. We observed that the SUC2 promoter is activated in cells which contain Hpa haustoria, as well as in surrounding cells. This suggests that some signal, possibly an effector, moves from the infected cells to surrounding non-infected cells to activate the SUC2 promoter. We have begun screening Hpa effectors for those that can move between cells in host tissues. In our preliminary screen of six Hpa effectors we identified two effectors that could move between cells in host tissues. We expect that many effectors can transit between host cells and tissues via plasmodesmata and this process enhances infection success. For example, the activation of the SUC2 sucrose transporter promoter around

infected cells suggest that Hpa effectors may alter the distribution of sugars around an infection site, presumably to the benefit of the pathogen. We hypothesise that this be a common element of the mechanism of infection for many pathogens. We aim to identify and characterise effectors from different pathogens that move between host cells to manipulate host processes in non-infected cells, focusing on Hpa and C. higginsianum (a fungal pathogen). The results will give an insight into how pathogens exploit the symplast and non-infected cells to promote infection, characterising a poorly considered element of plant-pathogen interactions.

Identification and characterization of the *Verticillium dahliae* effector that is responsible for cotton defoliation

Jinling Li[a], Luigi Faino[a], Grardy C.M. van den Berg[a],Tingli Liu[b], Baolong Zhang[b], Longfu Zhu[c], Bart P.H.J. Thomma[a]

[a]*Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*

[b]*Provincial Key Laboratory of Agrobiology, Jiangsu Academy of Agricultural Sciences, Nanjing 210095*

[c]*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070*

E-mail:jinling.li@wur.nl

Key words: comparative genomics, effector, defoliation, *Verticillium dahliae*

Plant pathogens from diverse taxonomic origins have been shown to secrete effector proteins into host plants to manipulate host physiology and establish infection. *Verticillium dahliae* is a soil-born fungus that causes Verticillium wilt disease in a wide range of crops, including cotton and olive. *V. dahliae* strains have previously been characterized as defoliating and non-defoliating strains based on their ability to cause defoliation on cotton, but the *V. dahliae* gene(s) that are involved in cotton defoliation remain unknown thus far. Here, we present a comparative genomics study defoliating and non-defoliating strains of *V. dahliae* that enabled us to identify a region of about 20 kb that specifically occurs in multiple defoliating strains. In this region, we were subsequently able to uncover a single highly-expressed gene that encodes a putative effector protein. Currently, we are performing experiments to confirm the role of this effector in cotton defoliation.

Delivery of Cytoplasmic and Apoplastic Effectors from *Phytophthora infestans* Haustoria by Distinct Secretion Pathways

Shumei Wang[1], Petra C Boevink[2], Ruofang Zhang[3], Stephen C Whisson[2], Paul RJ Birch[1,2]*

[1]*Division of Plant Sciences, University of Dundee (at JHI), Errol Road, Invergowrie, Dundee DD2 5DA, UK*

[2]*Cell and Molecular Sciences, James Hutton Institute, Errol Road, Invergowrie, Dundee DD2 5DA, UK.*

[3]*Potato Engineering and Technology Research Centre of Inner Mongolia University, West College Road 235, Hohhot, 010021.*

smwang@dundee.ac.uk

Potato blight, a ravaging disease caused by the oomycete *Phytophthora infestans,* is a major threat to global food security [1].*P. infestans* secretes effector proteins that are delivered inside (cytoplasmic) or can act outside (apoplastic) plant cells to neutralise host immunity [2]. Little is known about how and where effectors are secreted during infection, yet such knowledge is essential to understand and combat crop disease. We show that the cytoplasmic effector Pi04314, expressed as an mRFP-fusion protein with a signal peptide to secrete it from plant cells, does not passively re-enter the cells upon secretion. However, Pi04314-mRFP expressed in *P. infestans* is translocated from haustoria, which form intimate interactions with plant cells, to accumulate at its sites-of-action in the host nucleus and nucleolus [3]. The well-characterised apoplastic effector EPIC1 [4] was also secreted from haustoria. EPIC1 secretion was inhibited by brefeldin A (BFA), demonstrating that it is delivered by conventional Golgi-mediated secretion. In contrast, Pi04314 secretion was insensitive to BFA treatment, indicating that the cytoplasmic effector follows an alternative route for delivery to the inside of plant cells. *P. infestans* haustoria are thus sites for delivery of both apoplastic and cytoplasmic effectors during infection, following distinct secretion pathways.

[1] W.E Fry, *et al*. (2015). *Phytopathology*, **105**:966-981

[2] S. Kamoun (2006).*Ann Rev Phytopathol* **44,** 41-60

[3] P.C Boevink, *et al*. (2016).*Nat Commun*, **7**:10311

[4] J. Song *et al*. (2009). *Proc Natl Acad Sci (USA)* **106,** 1654-1659

Effects of melanin biosynthesis genes SCD1 and THR1 deficiency in *Sclerotinia sclerotiorum*

Yue Liang [a], Wei Xiong [b], Caixia Chen [a], Huiying Sun [a]
[a] *Department of Plant Pathology, Shenyang Agricultural University, Shenyang, Liaoning*
[b] *School of Life Sciences, Chongqing University, Chongqing*
liangyuet@126.com

Sclerotinia sclerotiorum is a necrotrophic plant pathogen causing significant loss on crop and vegetable worldwide. Sclerotium plays critical roles as the survival structure in life cycle and is the primary inoculum in disease cycle. Sclerotial pigmentation resulted from melanin can protect fungi from adverse environmental conditions [1]. Two hypothetical proteins (SCD1 and THR1) involved in melanin biosynthesis have been identified in sclerotial development [2]. Phylogenetic analysis of the deduced amino acid sequences of SCD1 and THR1 indicated that they are highly similar to orthologs in *Botrytis cinerea*. Transcript analysis found that expression of *SCD1* increased in sclerotia than in mycelia at each developmental stage whereas *THR1* showed up-regulation during sclerotial maturation only. Gene replacement of *SCD1* and *THR1* resulted in abnormal mycelial growth (slower radical growth, less biomass, increased pH value in liquid medium and larger branching angle of apical hypha) and sclerotial development (delayed initial formation, less in number, limited melanization and impaired capability of UV resistance). However, pathogenicity and virulence of *S. sclerotiorum* were not affected by mutation of either *SCD1* or *THR1* based on infection assays on the leaves of tomato and rapeseed. In summary, this study demonstrated that *SCD1* and *THR1* are involved in melanin biosynthesis, which effects on mycelium growth, sclerotial formation and sensitivity to UV-irradiation but not pathogenicity of the fungus.

Acknowledgement: This study was supported by the Scientific Research Foundation for the Introduced Talents of Shenyang Agricultural University (20153040) and the Natural Science Foundation Project of CQ (CSTC, 2011BB1101).

References:

[1] M.D. Bolton, B.P.H.J. Thomma, B.D. Nelson (2006) *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Molecular Plant Pathology, 7, 1–16.
[2] Y. Liang, M.H. Rahman, S.E. Strelkov, N.N.V. Kav. (2010) Developmentally induced changes in the sclerotial proteome of *Sclerotinia sclerotiorum*. Fungal Biology, 114, 619–627.

Functional study of mitogen-activated protein (MAP) kinase pathways in *Sporisorium scitamineum* mating/filamentous growth

Bin Zhang , Yixu Wang, Yizhen Deng, Lianhui Zhang
College of Agriculture, South China Agricultural University, Guangzhou, 510642
Petperpan657@163.com

Sugarcane smut caused by *Sporisorium scitamineum* leads to great losses in yield and sugar content of sugarcane in China and worldwide, and therefore becomes one of the most serious constraints to global sugarcane production. We previously identified b locus as a conserved mating-type locus in *S. scitamineum*. However, the cellular mechanism underlying b-locus-regulating mating and/or filamentous growth remains largely unknown. Our transcriptome analysis revealed that two signal transduction pathways mediated by MAP kinases, Hog1 and Cek1 respectively, were both up-regulated in the b-deletion mutant compared to the WT. In this study, we functionally verified these two pathways in *S. scitamineum* mating and/or filamentous growth, by targeted deleting *HOG1* and *CEK1* respectively, and systematically characterizing colonial morphology, cell wall integrity, response to various stress, mating and/or filamentous growth, etc.

Isolation and Identification of Endophytic Fungi from Arecanut (*Areca catectu* L.)

SONG Wei-wei, NIU Xiao-qing, TANG Qing-hua, QIN Wei-quan*

Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang 571339, songweiwei426@aliyun.com

Endophytes with many varieties are widely distributed and almost exist in all aquatic and terrestrial plants. Endophytes are rich in secondary metabolites and play an important role in biological control of plant diseases. The host plants infected by endophytes usually grow fast, have a strong resistance to adversity and diseases, and are immune to animal attack compared to uninfected plants [1].

Areca catechu L. is the main economic crop in Hainan, which is one of the most important southern herbal medicine resources. The diversity of endophytic fungi in arecanut (*Areca catechu* L.) was discussed in this study. A total of 47 endophytic fungi were isolated from the arecanut by tissue separation method. Then rDNA-ITS sequence analysis was used for these isolates' identification and the phylogenetic tree was constructed. The results showed that 47 isolates belonged to 8 genus, including *Penicillium*、*Fusarium*、*Phyllosticta*、*Curvularia*、*Nigrospora*、*Colletotrichum*、*Acremonium* and *Exserohilum*. The dominant endophytic genus in arecanut were *Penicillium* and *Fusarium*, which accounted for 31.91%

and 27.66% respectively among the identified fungi. The research provided theoretical basis for subsequent development and utilization of endophytes in arecanut.

Acknowledgement: This work was supported by the Key Project of Hainan Province (ZDYF2016058) and Central Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences (No.1630152017015).

References:

[1] B. Liu, X. F. Zheng, D. G. Sun, C. Q. Ruan, G. C. Fan, Y. P. Duan (2011). Acta Ecologica Sinica, 31(24):7325-7342

Functional analysis of the exocyst complex in *Fusarium oxysporum* f. sp. *Cubense*

Xin Zhou[a], Pusheng Guo[a], Weizhong Chen[a], Kaili Wu[a], Yingzi Yun[a], Zonghua Wang[a,b]

[a]*State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002*

[b]*Minjiang University, Fuzhou 350002*

yingziyun@fafu.edu.cn, zonghuaw@163.com

Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp *cubense* (Foc), is considered to be one of the most destructive of all plant disease and a serious threat to worldwide banana production. The disease-causing ability of Foc is mostly mediated by secreted virulence factors such as enzymes, toxins and specialized effectors which are usually delivered to extracellular space or into the host through exocytosis pathway. The exocyst complex directs the precise docking and tethering of secretory vesicles to the target plasma membrane for the final SNARE-mediated membrane fusion event. Here we identified the presence of an octameric exocyst complex in Foc composed by eight subunits: Sec3, Sec5, Sec6 Sec8, Sec10, Sec15, Exo84 and Exo70. And the exocyst complex localizes both to the tips ahead of Spitzenkörper and septa of growing vegetative hyphae in Foc. We found the polar localization relying on actin and microtubule cytoskeleton is involved in tip exocytosis, and the septa localization only requiring microtubule cytoskeleton is related to nontip directed exocytosis which, however did not show in the hypha of *F. graminearum*. In addition, the intact exocyst complex and GTPae Sec4 exocyst binding parterner are required for the subcellular localization. Among eight Foc components only two subunits: Sec5 and Exo70, encoding

genes can be successfully knocked out which were also highly conserved in *Magnaporthe oryzae* and *F. graminearum,* and *ΔFocEXO70* mutation leads to defects on vegetative growth, conidiation and virulence. Our study provides evidence in Foc that exocyst plays a regulatory role in pathogenicity, asexual production and vegetative growth and further sheds insight of the mechanism underlying pathogenicity.

Functional analysis of the flippases family in *Fusarium graminearum*

Pusheng Guo[a], Xin Zhou[a], Kaili Wu[a], Weizhong Chen[a], Yingzi Yun[a], Zonghua Wang[a,b]

[a]*State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002*

[b]*Minjiang University, Fuzhou 350002*

yingziyun@fafu.edu.cn, zonghuaw@163.com

Fusarium head blight caused by *Fusarium graminearum* poses a great challenge to grain production and food security. Extracellular enzymes and mycotoxin (such as DON) are important virulence factors for *F. graminearum*, the biosynthesis and secretion of these factors have close relationships with cellular membrane traffic process. Lipid molecules (including phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine *et al*.) distribute asymmetrically in phospholipid bilayer layer, which is important to mediate the function of membrane traffic. Flippases hydrolyze ATP to transfer lipid molecules from far cytoplasmic layer to near cytoplasmic layer to maintain lipids asymmetrically distribution. We analyzed biological function of flippases in *F. graminearum* with reverse genetic methods. There are 5 genes encoded flippases in *F. graminearum*, which were named as *FgDNFA*, *FgDNFB*, *FgDNFC1*, *FgDNFC2* and *FgDNFD*. We succeed to constructed deletion mutant for these 5 flippase genes respectively, and analyzed phenotypes for these mutants, including vegetative growth, conidiation, sexual process, DON production and pathogenicity. Based on the phenotypes data we found that FgDnfA plays an important role in regulating vegetative growth of hyphae, conidiation, formation of ascospore and virulence on wheat-heads; and FgDnfA, FgDnfB and FgDnfD also take part in synthesis of toxin DON. Meanwhile, we built double genes deletion mutants for these 5 flippase genes. Double genes deletion mutant of *FgDNFB* and *FgDNFD* displayed serious growth defects and also lost the abilities for

sporulation and virulence, which indicated functional redundance existed between FgDnfB and FgDnfD.

Allelopathy of wheat and maize straw decomposed products on *Rhizoctonia cerealis* Vander Hoeven and the GC-MS analysis

Qi Yong-zhi, Ma Lu-lu, Wang Fang-fang, Jin Jing-jing, Jia Wei, Zhen Wen-chao
College of Plant Protection, Agricultural University of Hebei, 2596#, Lekai South street, Nanshi District, Baoding 071001, Hebei Province
Email: qiyongzhi1981@163.com. Correspondence: wenchao@hebau.edu.cn

Over the past decade, Wheat Sharp Eyespot which mainly caused by *Rhizoctonia cerealis* Vander Hoeven (RC) occurred more than 1 million hectares every year in the maize and wheat straws returning to field of northern China. The allelopathy of straw decomposition products (SDP) had been reported as the major reason for the occurrence of soil-borne diseases. To investigate the allelopathy of the wheat and maize SDP on RC, culture dish experiments were conducted. The results showed that the mycelial growth and sclerotia formation of RC were both promoted by the wheat and maize SDP at concentrations of 0.03, 0.06 and 0.12 g•mL^{-1}, with the response index (*RI*) from 0.13 to 0.45, and were inhibited at concentration of 0.24 and 0.48 g•mL^{-1} of the maize SDP, significantly. But, no significant effects were found of all the concentrations of wheat and maize SDP on average weight of the sclerotia, and of wheat SDP at concentrations of 0.24 and 0.48 g•mL^{-1} on the mycelial growth and sclerotia formation. The compounds extracted from the SDPs by ethyl acetate were identified by gas chromatography-mass spectrometry. GC-MS results showed that the compounds of the maize SDP included organic acids, esters, hydrocarbons, amides and aldehydes, with the relative proportions 25.26%, 24.01%, 17.22%, 14.39% and 7.73%, respectively. P-hydroxybenzoic acid (9.21%), dibutyl phthalate (6.94%), 3-phenyl-2-Acrylic (5.06%), 4-hydroxy 3, 5-dimethoxybenzoic acid (2.26%), hexanoic acid (1.73%), 8-octadecenoic acid (1.06%), and 3-(4-hydroxy-3-methoxy-phenyl)-2-acid (1.04%) were detected in maize SDP. The compounds of wheat SDP included hydrocarbons, organic acids, esters and amides, with the proportions 37.75%, 28.34%, 14.39% and 12.33%, respectively. P-hydroxybenzoic acid (7.30%), 4-hydroxy-3, 5-dimethoxybenzoic acid (6.35%), hexanoate (2.48%), salicylic acid (1.84%), palmitoleic acid (1.56%), 2-amino-5-methoxybenzoic acid

(1.56%), and 3-(4-hydroxy-3-methoxy-phenyl)-2-acid (1.45%) were also detected in wheat SDP.

Acknowledgement: This study was funded by the "Natural Science Foundation of Hebei Province, China (C2016204211) and Science and Technology Research Project of higher education institutions of Hebei Province, China (ZD2016162)".

Jasmonic acid effects on appressoria formation in *Magnaporthe oryzae*

Yingyao Liu [a]

[a] *Temasek Life Science Laboratory, 1 Research Link National University of Singapore, Singapore*

yingyao@tll.org.sg

Jasmonic acid is a classic plant hormone that mediates several aspects of development including the immune response against bacterial and fungal pathogens. Recently, we showed that *Magnaporthe oryzae,* the causal fungal pathogen of the rice blast disease, produces Jasmonic acid. In addition, the intrinsic jasmonate is hydroxylated via the Antibiotic Biosynthesis Monooxygenase (ABM) activity in *M. oryzae* to produce 12OH-JA, which is used to suppress the defense response (1). The *ABM*-deletion mutant elicits a strong defense response in the host and shows a complete loss of ability to colonize the rice plants. [1]. Therefore, we explored the role(s) of intrinsic jasmonic acid and its derivates in the development and pathogenesis of *M. oryzae*.

A gene-deletion mutant *opr3*Δ (that lacks a key reductase in JA biosynthesis) showed strong defects in appressorium formation, similar to the phenotypes found in mutants that defective in cyclic AMP signaling (eg *pth11*Δ and *cpka*Δ) in the blast fungus [2]. By characterizing and quantifying the effect of exogenous JA and cAMP on the aforementioned strains, we propose that JA participates in the morphogenesis of infection structures, in concert with the canonical cAMP signaling in *M. oryzae*. Our preliminary data suggest the involvement of a conserved MAPK cascade (3) as the direct effector downstream of the jasmonic acid signaling in *M. oryzae*.

References:

[1] R. N. Patkar *et al*., (2015). *Nature Chemical Biology*, 11, 733-740.
[2] R. Ramanujam *et al*., (2010). *PLOS Pathogens*, 6(5), e100089.
[3] Xu, J. R., & Hamer, J. E. (1996). *Genes & Development*, 10(21), 2696-2706.

Phytophthora infestans RXLR effector interacts with S factor NRL1 to promote turnover of SWAP70

Qin He [a], Shaista Naqvi [a], Hazel McLellan [a], Petra Boevink [b], Eleanor Gilroy [b], Ingo Hein [b], Paul Birch [a, b]

[a] Division of Plant Science, University of Dundee; and [b]Cell and Molecular Sciences; both located at James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK
E-mail of presenting author: He.Qin@hutton.ac.uk

Plant pathogens deliver effectors into plant cells to manipulate host processes. Much attention has been focused on identifying their host targets. Recently, we reported that *Phytophthora infestans* RXLR effector Pi02860 targets NRL1, a host non-phototrophic hypocotyl 3/root phototropism 2 (NPH3/RPT2)-like protein, in the host cytoplasm and at the cell plasma membrane [1]. We found Host NRL1 is a susceptibility factor and its activity is required for Pi02860 to promote disease and suppress the INF1 cell death. A dimerization-dead NRL1 mutant attenuates infection and loses its ability to suppress INF1-triggered cell death. NRL1-mut reduces the ability of Pi02860 to attenuate INF1-mediated HR, emphasizes the Pi02860 needs a functional NRL1 for its effector activity. NRL1 interacts with a guanine nucleotide exchange factor (GEF), SWAP70, which localises to endosomes. Virus-induced gene silencing of SWAP70 in *N. benthamiana* resulted in enhanced *P. infestans* colonization and compromised INF1-triggered cell death. Overexpression of SWAP70 showed reduced *P. infestans* infection and accelerated INF1-triggered cell death, indicating that this host protein acts as a positive regulator of immunity. Suppression of INF1-triggered cell death by Pi02860 was significantly attenuated by co-expression with SWAP70. Interesting, Co-expression of Pi02860 and NRL1 with SWAP70 reduces the abundance of SWAP70 in MG132 sensitive manner. The NRL1 dimer mutant with Pi02860 prevent turnover of SWAP70. VIGS of NRL1 reduces 02860-mediated degradation of SWAP70 and has less impact of Pi02860 on preventing SWAP70 mediated INF1 cell death acceleration. Critically, Pi02860 enhances the interaction between NRL1 and SWAP70. We argue that Pi02860 uses host protein NRL1 to target and promote turnover of SWAP70, potentially blocking host vesicle trafficking to suppress immunity.

References:

[1] Yang L, McLellan H, Naqvi S, He Q, Boevink PC, Armstrong M, Giuliani LM, Zhang W, Tian Z, Zhan J, Gilroy EM, Birch PR. Potato NPH3/RPT2-Like Protein StNRL1, Targeted by a *Phytophthora infestans* RXLR Effector, Is a Susceptibility Factor. Plant Physiol, 2016, 171(1): 645-57

Kingdom-wide analysis of autophagy-related genes in fungi

Qinhu Wang, Huiquan Liu, Cong Jiang, and Jin-Rong Xu*

State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100

Presenting author: wangqinhu@nwafu.edu.cn

** Corresponding autohor: jinrong@purdue.edu*

Autophagy is a fundamental degradation process for cellular homeostasis and nutrient recycling. To date, 41 autophagy-related (*ATG*) genes were identified in yeast. It is known that many of the *ATG* genes are conserved in eukaryotes. Fungi is one of the most diverse organism have many genomes were sequenced, however, whether there are lineage-specific *ATG* genes loss and duplication remains unclear. Here, by systematic analysis the *ATG* genes in the kingdom of Fungi, several lineage-specific *ATG* genes loss and duplication events were uncovered. We found that among the 41 *ATG* genes, 20 are highly conserved in all the fungi, 7 are conserved in Ascomycota, and 14 are Saccharomycetes specific. Even in Saccharomycetes, the number of *ATG* genes is highly variable. Two lineages of typical intracellular obligate pathogens, Microsporidia and Pneumocystis, have lost almost all of the *ATG* genes. However, *ATG1* and *ATG15* are kept in most of Microsporidia. Except the 7 *ATG* genes duplication in early-diverging fungi Mucoromycotina, we found that autophagy marker gene *ATG8* is duplicated in dermatophytes, and the two *ATG8s* are differently expressed. Besides, many lineage-specific duplications were observed in the *ATG15* and *ATG22*, which are required for the breakdown of autophagic bodies. Evaluation of conservation of complexes involved in autophagy revealed that most of them contain both conserved and non-conserved element. Moreover, we found that 24 of the 25 *ATG* genes in *Fusarium graminearum* are targeted by A-to-I RNA editing. Our findings have shed lights on the conservation, evolution, and regulation of the *ATG* genes in fungi.

Linking the indigenous mycobiota with different varieties of wheat and rice grains

Xin Zhou[a, b], Wen Chen[c], Lei Cai[a]

[a] *State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No.1 Beichen West Road, Chaoyang District, Beijing 100101.*

[b]*University of Chinese Academy of Sciences, No.19(A) Yuquan Road, Shijingshan District, Beijing 100101.*

[c]*Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, 960 Carling Ave., Ottawa, ON, K2W 1C7, Canada.*

Email: Zhouxin5518@163.com

Microbiome associated with grain and rhizosphere of crops, is a key determinant to the health and productivity of crops. In this study, grain and rhizosphere samples from resistant varieties of wheat (*Triticum aestivum* L., resistant to Fusarium Head Blight) and rice (*Oryza sativa* L.,

resistant to rice blast) were collected from six provinces in China during the harvest seasons 2015/2016. The mycobiota was recovered by sequencing the amplicons of internal transcribed spacer (ITS), using Illumina MiSeq sequencing technology. The preliminary results showed that the most abundant genera in rice grains were *Alternaria, Cladosporium, Fusarium, Kondoa, Ophiosphaerella, Sakaguchia, Solicoccozyma, Sporobolomyc*es and *Ustilaginoidea* and the most abundant genera in wheat grains were *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Mycosphaerella*, *Penicillium*, *Tilletia* and *Wallemia*. The core mycobiota of rice grains were *Nigrospora*, *Occultifur*, *Sakaguchia* and *Ustilaginodidea* while the core mycobiota of wheat grains were *Cystofilobasidium*, *Rhizopus* and *Sclerostagonospora*. The distinct microbiota profile associated with wheat or rice reflects host-mediated selection of microbiomes, which is also shaped by geography such as some important rice pathogens, including *Cercospora*, *Curvularia*, *Pyricularia* and *Ustilaginoidea* were significantly more frequently recognized in grain samples collected in Central and Southern regions of China than samples collected in Northeast China. The occurrence frequency of these pathogenic fungi in different areas of China might be informative in making strategy to guide local farmers to control the crop diseases and improve yield and quality of rice and wheat grains.

References:

[1] Chen, W.., et al., 2016. Agriculture, Ecosystems & Environment. 226, 43-55.
[2] Coleman - Derr, D., et al., 2015. New Phytologist, 209(2), 798-811.
[3] Edwards, J., et al., 2015. Proceedings of the National Academy of Sciences, 112(8), E911-920.
[4] Links, M. G., et al., 2014. New Phytologist, 202(2), 542-553

Application of *Lilium* defense-related protein LsGRP1 in the disease control of gray mold on lily

Shih, Yu-Ting.[a] and Chen, Chao-Ying[ab]
[a]*Master Program for Plant Medicine, National Taiwan University, Taipei, Taiwan*
[b] *Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan*
E-mail: r04645006@ntu.edu.tw

Lily is a bulbous flower crop with highly economic importance. Beside of soil-borne diseases and pests, gray mold disease, caused by *Botrytis elliptica* (Berk.) Cooke, affects lily production enormously. How to effectively control gray mold disease is a main concern of lily farmers. Defense-related LsGRP1 (*Lilium* ‘Star Gazer’ glycine-rich protein 1) is a protein with increased expression in lily which exhibits salicylic acid-induced resistance against gray mold disease. According to previous report, LsGRP1 may enhance plat immunity *via* its

antimicrobial activity [1]. In this study, the potential of LsGRP1 to induce plant defense against gray mold pathogen was investigated, and the application methods were optimized. At first, LsGRP1 recombinant protein produced by *Escherichia coli* was infiltrated into the middle leaves of lily to evaluate its effect on the induction of disease resistance in un-infiltrated upper and lower leaves. The optimal dose, application site, and the time points of application were determined by comparing the efficacy of disease suppression. In order to lower the cost, the total lysate of the LsGRP1 recombinant protein without purification from sonicated *E. coli* cells was applied directly to lily. On the other hand, the examination of the protection effect of crude LsGRP1 recombinant protein on the gray mold disease of strawberry and tomato were undergoing. Expectedly, a novel disease control agent could be developed for gray mold disease in different plants to maintain crop production and environmental sustainability.

References:

[1] Lin, C.H., Chang, M. W., and Chen, C.Y. (2014). Phytopathology 104:340-346.

Distinct regions of *Phytophthora sojae* effector Avh240 determine its dimerization and localization in plasma membrane

Baodian Guo [a, b], Haonan Wang [a], Bo Yang [a], Maofeng Jing [a], Haiyang Li [a], Qinli Hu [b], Fangfang Wang [b], Yan Wang [a], Wenwu Ye [a], Suomeng Dong [a], Weiman Xing [b*], Yuanchao Wang [a*]

[a] *Department of Plant pathology, Nanjing Agriculture University, Nanjing, 210095*

[b] *Shanghai Center for Plant Stress Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 201602*

E-mail of presenting author: 2016202012@njau.edu.cn

Phytophthora pathogens secrete a large number of effectors into plants to subvert plant immunity to promote infection. Previously we identified an RXLR effector Avh240 which is highly induced during *Phytophthora sojae* infection and can also inhibit the cell death triggered by PAMPs and effectors in plants. To understand the molecular details of its virulence function, we solved the crystal structure of Avh240 and confirmed that it forms a dimer *in vitro* and *in vivo*. From the dimerization interface, we identified two amino acids essential for dimer-formation of Avh240. Interestingly, the mutant of Avh240, which does not self-associate, can no longer promote *Phytophthora* infection. We generated several deletion

mutants based on Avh240 secondary structure and demonstrated that the two α-helices determined its plasma membrane localization. Furthermore, the mutant of Avh240, which lacks of these two α-helices, lost the ability to promote *Phytophthora* colonization. Overall, our study reveals that both the structural basis of an RXLR effector dimerization and the localization in plasma membrane required for its virulence function.

Phytophthora sojae effector Avr1b can be delivered into soybean cells by heterologous PI3P-binding proteins during infection.

Qunqing Wang[a,b], Felipe Arredodo[b], Eli Perez[b], Brett Tyler[b]

[a] *Department of Plant Pathology, Shandong Agricultural University, Taian 271018*

[b] *Botany and Plant Pathology, OREGON STATE UNIVERSITY, Corvallis, OR, USA*

wangqunqing@163.com

Oomycete and fungal pathogens secrete effector proteins that can enter plant cells to modify the physiology of their hosts[1]. A major class of effectors produced by oomycetes contains RXLR motifs that mediate entry of these effectors into plant cells[2]. Previous research showed that RXLR effectors can enter host cells in the absence of any pathogen[3]. Furthermore, these effectors can bind to specific lipids including phosphatidylinositol -3-phosphate (PI3P)[4]. PI3P-binding requires the RXLR motif, plus in some cases, C-terminal regions of the protein. Previously we showed that PI3P binding is required for the effectors to enter into host cells when the purified proteins are introduced into root or leaf tissue[5]. Here we show that the RXLR motif of Avr1b is sufficient for cell entry in vivo, independent of the C-terminal PI3P binding residues. In order to validate that PI3P-binding mediates host cell entry *in planta*, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RXLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. The Avr1b and various derivative mutant proteins can be specifically detected in culture supernatants after de-glycosylation, indicated that Avr1b is post-translationally modified.

References:

[1] Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. Cell, 124(4), 803-814.

[2] Jiang, R. H., Tripathy, S., Govers, F., & Tyler, B. M. (2008). RXLR effector reservoir in two

Phytophthora species is dominated by a single rapidly evolving superfamily with more than 700 members. Proceedings of the National Academy of Sciences, 105(12), 4874-4879.

[3] Dou, D., Kale, S. D., Wang, X., Jiang, R. H., Bruce, N. A., Arredondo, F. D., ... & Tyler, B. M. (2008). RXLR-mediated entry of *Phytophthora sojae* effector Avr1b into soybean cells does not require pathogen-encoded machinery. The Plant Cell, 20(7), 1930-1947.

[4] Kale, S. D., Gu, B., Capelluto, D. G., Dou, D., Feldman, E., Rumore, A., ... & Lawrence, C. B. (2010). External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. Cell, 142(2), 284-295.

[5] Tyler, B. M., Kale, S. D., Wang, Q., Tao, K., Clark, H. R., Drews, K., ... & Fudal, I. (2013). Microbe-independent entry of oomycete RxLR effectors and fungal RxLR-like effectors into plant and animal cells is specific and reproducible. Molecular Plant-Microbe Interactions, 26(6), 611-616.

Pectin acetylesterase is required for the virulence of oomycete plant pathogen *Peronophythora litchii*

Guanghui Kong, Lang Wan, Wensheng Yang, Wen Li, Liqun Jiang, Junjian Situ, Minhui Li, Pinggen Xi, Zide Jiang*

Department of Plant Pathology/Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642

Pectin is a major component of the primary cell wall of higher plants. Some galacturonyl residues in the backbone of pectinaceous polysaccharides are often *O*-acetylated, and the resulting acetylesters change dynamically. This processes involve both enzymatic acetylation and deacetylation. Our study found pectin acetylesterases (PAEs), which can remove of acetyl-substituents, are widespread in the plant pathogenic oomycete and showed sequence and transcriptional polymorphism. We also characterized the functions of *PlPAE4* and *PlPAE5* from *P. lithii* and found that *PlPAE5* knockout mutants inoculated into lychee were less invasive than the wild-type *P. litchii* strain SHS3, demonstrating PAE5 functions in the *P. litchii* infection. Ectopic expression of PAE5 on *Nicotiana benthamiana* could promote the infection of *Phytophthora capsici*. This study firstly reported that PAE protein is involved in the infection of oomycete plant pathogen.

Polymerization of SKRP proteins is required for negative regulation of plant immunity against *Phytophthora*

Ling Chen [a],¶, Jie Huang [a],¶, Xinyu Lu [a], Yuanchao Wang [a], Suomeng Dong [a]*

[a] *Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095*

*¶ authors contribute equally in this project. * corresponding author: smdong@njau.edu.cn*

E-mail of presenting author: 2016202001@njau.edu.cn

Alternative splicing (AS) of pre-mRNA plays critical roles in regulating plant processing. Previously, we demonstrated that *Glycine max* serine/lysine/arginine rich proteins (GmSKRPs) are associated with plant RNA splicesome complex and function as negative regulators of plant immunity. However, little is known about the biochemical mechanism of SKRPs in plant immunity. Here we show that tomato SKRP (SlSKRP) can form polymer in vivo and in vitro. Interestingly, a short C-terminal domain which resembles monkeytail domain that is required for Rna14p heteropolymerization in yeast, is widely conserved among plant SKRP proteins. Mutagenesis of the monkeytail domain compromised the polymerization of SlSRKP in vivo and in vitro. Previously, it is shown that SlSKRP is associated with splicesome components. To examine whether polymerization of SlSRKP is required for splicesome association, bimolecular fluorescence complementation (BiFC) assay was used. BiFC data reveal that SlSRKP monkeytail mutant (SlSKRP^{MK}) can still interact with spliceosome components such as U1, however, SlSKRP^{MK} speckle colocalization with SlU2AF35 and SlU2AF65 is significantly reduced. Furthermore, transient expression of SlSKRP^{MK} no longer enhanced plant susceptibility to *Phytophthora* pathogens, suggesting that polymerization of SKRP proteins and their association with some of the spliceosome components are important for SKRP biological functions. Now, we are using CRISPR/Cas9 genome editing tool to knockout SlSKRP in tomato to investigate the genetic role of SlSKRP in plant immunity and the underlying mechanism.

The immunity triggered by a small cysteine rich effector *SCR2* from *Phytophthora infestans* requires proteolytic cleavage by host protease

Shuaishuai Wang [a], Rongkang Xing [a] Liliana Chao [b], Chihhang Wu [b], Wenwu Ye [a], Zhengguang Zhang [a], Yuanchao Wang [a], Xiaobo Zheng [a], Vivianne Vleeshouwers [c], Renier van der Hoorn [d], Sophien Kamoun [b], Suomeng Dong [a],*

[a] *College of Plant Protection, Nanjing Agricultural University, Nanjing,*

[b] *The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kindom*

[c] *Wageningen UR Plant Breeding, Wageningen University and Research Centre, Droevendaalsesteeg 1, Wageningen 6708 PB, The Netherlands*

[d] *Department of Plant Sciences, University of Oxford, South Parks Road,Oxford OX1 3RB, United Kingdom*

** corresponding author: smdong@njau.edu.cn*

E-mail of presenting author: 2015202022@njau.edu.cn

Oomycete, a group of filamentous plant pathogens, is responsible for many notorious crop diseases such as potato late blight that threaten the global food security. It has been known that apoplastic players such as small cysteine rich (SCR) effector genes are highly induced during oomycete infection, however, the molecular mode of actions of SCR effectors remain largely unknown. A preliminary bioinformatics analysis combined with high-throughput *in planta* screening assay resulted in the identification of a *Phytophthora infestans* cysteine rich effector *SCR2*, which could trigger significant reactive oxygen burst, defence related gene induction and hypersensitive response in *Nicotiana benthamiana* and potato. Unlike many other *SCR* effector genes, *SCR2* orthologs are conserved in genomes of a variety of oomycete including species from *Phytophthora*, *Hyaloperonospora*, *Pythium* and *Plasmopara*. Furthermore, chemical inhibition assay suggested that serine proteases are involved in SCR2 perception by plants. Additionally, it is found that SCR2 protein could be proteolytic cleaved by serine protease P69B, and this action can be compromised by adding P69B protease inhibitors such as EPI1 from *P. infestans*. Interestingly, transient overexpression EPI inhibitors significantly impair SCR2 triggered immune response in *N. benthamiana*, suggesting plant P69B protease activity is involved in SCR2 triggered immunity. In summary, we identified a novel SCR effector with immune induction activity, and studying the underlying mechanism covers that manipulation of proteolytic cleavage of apoplastic effectors maybe an important layer of plant-oomycete interaction.

A potato chloroplast kinase is required for *Rpivnt1* mediated immunity against late blight pathogen

Chuyun Gao [a],¶, Huawei Xu [a],¶, Jie Huang [a], Qinli Hu [b], Tingxiu Yan [a], Chih-hang Wu [c], Joe Win [c], Yuanchao Wang [a], Xiaobo Zheng [a], Vivianne Vleeshouwers [d], Weiman Xing [b], Sophien Kamoun [c], Suomeng Dong [a],*

[a] *College of Plant Protection, Nanjing Agricultural University, Nanjing, 210095*

[b] *Shanghai Center for Plant Stress Biology, CAS, 3888 Chenhua Road, Shanghai, 201602*

[c] *The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kingdom*

[d] *Wageningen UR Plant Breeding, Wageningen University and Research Centre, Droevendaalsesteeg 1, Wageningen 6708 PB, Netherlands*

*¶ authors contribute equally in this project. * corresponding author: smdong@njau.edu.cn*

E-mail of presenting author: 2014802185@njau.edu.cn

Avirulence (AVR) effectors, recognized by host immune receptors, are a group of key players during plant and microbe interaction. However, little is known about how plant nucleotide binding leucine-rich repeat (NB-LRR) receptors recognize effectors of filamentous plant pathogens, such as late blight pathogen *Phytophthora infestans*. It is known previously that *P. infestans* effector Avrvnt1 can be recognized by cognate receptor protein Rpivnt1 from *Solanum venturii*. Here we identified an Avrvnt1 ortholog Avrvnt1^{Pipo} that cannot be recognized by Rpivnt1, which provide an opportunity to study Avrvnt1-Rpivnt1 interaction mechanism. Firstly, we mapped a single residue mutation in Avrvnt1^{Pipo} that determined the hypersensitive response (HR) phenotype of Avrvnt1/Rpivnt1 by using Gain-Of-Function assay. Secondly, we performed a yeast two-hybrid screening and verified a putative Avrvnt1 binding protein chloroplast kinase GLYK from *Solanum tuberosum*. Thirdly, transient silencing of *NbGLYK* in *N. benthamiana* specifically compromised Avrvnt1 triggered HR and the defective HR phenotype can be complimented by overexpression of synthetic *GLYK* constructs. All the other HR responses mediated by Avr and NB-LRR pairs are not affected in our result, suggesting GLYK may serve as a guardee protein in plant perception of Avrvnt1 effector. Further genetic and biochemical roles of *GLYK* in plant immunity require further investigations.

Phytophthora sojae **essential effector Avh238 suppresses ethylene biosynthesis to promote infection in soybean**

Bo Yang, Maofeng Jing, Baodian Guo, Yuyin Wang, Haonan Wang, Wenwu Ye, Yan Wang, Suomeng Dong, Yuanchao Wang

Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095
E-mail: 2014202004@njau.edu.cn

Phytophthora pathogens secrete an arsenal of effectors to manipulate host innate immunity. However, the molecular basis underlying the effector functions remains largely enigmatic. Here we found that *Phytophthora sojae* uses an essential effector Avh238 to target a key enzyme EBRE (ethylene biosynthesis related enzyme) in the ethylene (ET) biosynthesis process in soybean. By destabilizing EBRE, Avh238 suppresses ET biosynthesis and promotes *Phytophthora* infection. Silencing of EBRE or inhibition of ET signaling

significantly increases the susceptibility of soybean to *P. sojae* infection, supporting a role for EBRE and ET in plant immunity. Moreover, the wild-type *P. sojae* but not the Avh238 knockout mutant inhibits ET induction and promotes *P. sojae* infection in soybean. This work highlights the ET biosynthesis pathway as an anti-*P. sojae* defense mechanism and a direct effector target.

Deletion of a new gene attenuates virulence of Fusarium oxysporum f. sp. cubense race 4

Minhui Li, Yuncong Yuan, Xiaolin Xie, Zide Jiang*

Department of Plant Pathology, South China Agricultural University, Wushan Road 483, Guangzhou,

liminhui@scau.edu.cn

Fusarium oxysporum f. sp. *cubense* (FOC), the casual agent of banana fusarium wilt, is one of the most destructive pathogens threatening the banana production worldwide. However, the molecular mechanisms underlying the virulence and pathogenicity of this fungal pathogen are still poorly understood. Based on the result of pathogenicity test, a T-DNA insertional mutant named W2987 was selected for its significantly reduced virulence. And sequence analysis shows that a new gene encoding a hypothetical protein is located at the downstream of T-DNA insertion. In order to know gene function, knockout of the specific gene were conducted through split marker homologous recombination in FOC race 4 isolate XJZ2. The gene knockout mutants showed no significant differences with the wild type (WT) strain XJZ2 on the morphology and growth rate of the colony. Compared with WT, sporulation of the gene knockout mutants was reduced. And pathogenicity tests showed that gene knockout mutants couldn't penetrate the cellophane paper and produce macerated symptom when inoculating on the fruit of tomato. Compared with WT, the gene knockout mutants showed the reduced virulence to the host banana plantlets. The results show the new gene encodes a specific protein that attenuates virulence of FOC.

Cas9-mediated gene replacement in *Peronophythora litchii*

Junjian Situ, Liqun Jiang, Lang Wan, Guanghui Kong, Pinggen Xi, Minhui Li, Zide Jiang*

Department of Plant Pathology / Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642

**Corresponding author : zdjiang@scau.edu.cn*

Peronophythora litchii causes downy blight of litchi fruits as well as tender leaves and panicles rot of litchi plant. *P. litchii* is an oomycete that shares a range of morphological features with true fungi. Although the basic biological characteristics of *P. litchii* have been described, functional genomics studies have been hampered by the lack of efficient method for genome engineering. Recently, the CRISPR/Cas9 system have been established successfully in *Phytopthora sojae*, which provide a novel strategy for testing the functions of specific genes in oomycete microorganisms.

Genomics analyses have been identified 245 candidate RxLR effectors in *P. litchii*. Here, we have implemented this CRISPR/Cas9 system in *P. litchii*, using the candidate RxLR effector gene as the target. To accomplish this, PEG-mediated protoplast transformations were conducted by using plasmids pYF2-PsNLS-hSpCas9 and pYF2.3G-Ribo-sgRNA as well as the donor template. Through PCR analysis of the transformants, we found evidence for gene replacement events in four of the effector genes. The results were confirmed by subsequent Sanger sequencing. Of four RxLR effectors, deletion of one impaired the virulence of *P. litchii*. Therefore, our results validated that this powerful tool can be applied in the litchi downy blight pathogen. Future studies aim to the mechanism how the effectors contribute to *P. litchi* virulence.

References:

[1] Y. Fang, B. M. Tyler (2016). Efficient disruption and replacement of an effector gene in the oomycete *Phytophthora sojae* using CRISPR/Cas9. *Mol. Plant Pathol.* 17, 127–139.

[2] W. Ye, Y. Wang, D, Shen, D. Li, T. Pu, Z. Jiang, Z. Zhang, X. Zheng, B. M. Tyler, Y. Wang (2016). Sequencing of the litchi downy blight pathogen reveals it is a *Phytophthora* species with downy mildew-like characteristics. *Mol. Plant–Microbe Interact,* 7(29), 573-583.

Gpa3, a Guanine nucleotide-binding protein regulates sexual mating by mediating cAMP signal in *Sporisorium scitamineum*

Enping Cai[a], Rongrong Wu[a], Shuquan Sun[b], Jinpei Wang[a], Dan Mei[a], Yizhen Deng[b], Zide Jiang[b*], Changqing Chang[a*]

[a] *Guangdong Innovative and Entepreneurial Research Team of Sociomicrobiology Basic Science and Frontier Technology, South China Agricultural University, Wushan Road 483, Guangzhou*; [b] *Department of Plant Pathology/Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Wushan Road 483, Guangzhou*

changcq@scau.edu.cn

Sugarcane smut caused by *Sporisorium scitamineum* is one of the most devastating diseases in sugarcane-growing areas. The fungus is dimorphic, which produces haploid sporidia by budding, while during infection, the compatible sporidia from two mating types conduct sexual mating to form virulent dikaryotic hyphae. In contrast to the wealth information of the maize pathogen *Ustilago maydis*, the sexual mating molecular process of *S. scitamineum* is largely unknown. This study investigated a sexual mating defective mutant *9M22* identified from T-DNA insertional mutant library of *S. scitamineum*. Hi-tail PCR analysis showed that the T-DNA insertion located at promotor region in *GPA3*. The deletion mutants of *GPA3*, obtained by homologous recombination knockout approach, also displayed a mating-defective phenotype. Expression of pheromone precursor gene (*MFA1*), pheromone receptor gene (*PRA1*) and cAMP synthetic gene (*UAC1*, encoding an adenylyl cyclase) were significantly attenuated in *gpa3*-deletion mutants. The supplement of cAMP for *gpa3*-deletion mutants could entirely recover the mating ability. The results demonstrated that *Gpa3* coupling pheromone receptor acts as a molecular switch on sexual mating ability by controlling the cAMP-PKA signaling pathway in *S. scitamineum*.

Farnesyltransferase beta subunit protein Ram1 is essential for pheromone production and sexual mating in *Sporisorium scitamineum*

Shuquan Sun[a], Lingyu Li[b], Xiaomeng Zhang[a], Yizhen Deng[a], Changqing Chang[b*], Zide Jiang [a*]

[a] Department of Plant Pathology/Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Wushan Road 483, Guangzhou; [b] Guangdong Innovative and Entepreneurial Research Team of Sociomicrobiology Basic Science and Frontier Technology, South China Agricultural University, Wushan Road 483, Guangzhou

zdjiang@scau.edu.cn

Sugarcane smut caused by *Sporisorium scitamineum* results in serious losses in sugar yield and quality in sugarcane-growing areas globally. Sexual mating among this dimorphic fungus is a major determinant of morphological transitions linked to virulence while the molecular mechanism is still poorly studied. Fungal pheromone a-factor was well characterized in budding yeast *Saccharomyces cerevisiae*. The mature a-factor terminating in a C-terminal CAAX motif (where C is cysteine, A is usually aliphatic, and X is any residue) is modified with prenylation, proteolysis, and carboxymethylation for secretion. The farnesyltransferase (FTase) beta subunit protein specifically recognizes the cysteine residue prior to prenylation by alpha subunit. Here we report *ram1* deletion mutants of the FTase beta subunit encoding gene defective in sexual mating in *S. scitamineum*. Several *ram1* deletion mutants from two compatible wild-type mating-types were obtained by PEG-mediated protoplast transformation method. The sexual mating ability of Δ*ram1* mutants with their compatible wild-type sporidia were significantly attenuated comparing to wild types, and that of Δ*ram1* mutants from two compatible wild-type backgrounds were completely lost. Comparing to the wild type, expression of precursor synthesis genes (*MFA*) and G-protein (*GPA3*) were down-regulated in Δ*ram1* mutants. Additionally, the supplement of synthetic pheromone for Δ*ram1* mutants could enhance their sexual mating partly. Based on these results, we concluded that Ram1 functions as upstream regulator of *S. scitamineum* pheromone production and is involved in virulence.

MoHst4p, a histone deacetylase, is required for vegetative growth ,conidiogenesis and full pathogenicity in *Magnaporthe oryzae*

Shulin Zhang[a b], Meiling Liang[a b], Xue Cao[a b], Yizhen Deng[a b], Lianhui Zhang[a b]

[a]Key Laboratory for Conservation and Utilazation of Subtropical Agro-Bioresources, College of Agriculture, South China Agricultural University, Guangzhou, 510642

[b]Guangdong Province Key Laboratory of Microbial Signals and disease control, and Integrative

Microbiology Research Centre, South China Agricultural University, Guangzhou, 510642.

E-mail of presenting author: zhangsl80h@163.com

Histone modification is key epigenetic regulation important in many cellular events, including gene expression, DNA replication and repair, chromatin compaction and cell-cycle control. Previous studies showed *ScHST3* and *ScHST4* were required for deacetylation of histone H3 in *Saccharomyces cerevisiae*. Here, we identified an *HST4* ortholog, *MoHST4*, in the rice-blast fungus *Magnaporthe oryzae*. Targeted deletion of *MoHST4* resulted in slow radial growth and significant reduction in conidia. The *Mohst* mutant could form appresorium on inductive artificial surface and host leaf surface, but the ability of penetration into host is relatively decreased, therefore the mutant displayed reduced pathogenicity on barely leaves. Taken together, *MoHST4* is crucial for vegetative growth, conidiogenesis and full virulence on host plants, but not for appressorium formation.

***Falciphora oryzae*, a beneficial fungus, stimulates the lateral root growth in Arabidopsis**

Xun Sun and Yan Liang

Institute of biotechnology, Zhejiang University, Hangzhou

sunxun@zju.edu.cn

Falciphora oryzae (previously named *Harpophora oryzae*), a dark septate endophyte originally isolated from wild rice (*Oryza granulata*) in China, has been reported that it is able to enhance plant growth and immunity in cultivated rice (*Oryza sativa*). In this study, we investigate whether *F. oryzae* can colonize *Arabidopsis thaliana*, and promote its growth and development. Similar to the colonization pattern in rice, *F. oryzae* mainly colonizes on the differentiation zone of Arabidopsis root, and stays in the epidermal and cortical cells without penetration to the stele. The most dramatic change after *F. oryzae* inoculation in Arabidopsis is the root architecture: increased lateral root growth but reduced primary root length, resembling the effect of auxin, a major plant growth hormone. *DR5*, the auxin responsive element, expression is highly upregulated in the lateral root primordia after *F. oryzae* inoculation, no matter whether GUS (β-glucuronidase) and GFP (Green fluorescence protein)

reporters were used. Consistently, the auxin polar transport mutant, *aux1-22*, and auxin signaling mutant, *axr1-1*, both show reduced responses to *F. oryzae* inoculation. These results suggest that *F. oryzae* might secret the growth-promoting factors which active the auxin signaling pathway directly or indirectly, leading to the increase of lateral root growth. Interestingly, we found *F. oryzae* secreted the growth-promoting factors before physical contact with Arabidopsis root. In addition, signals from plant roots are required for the production of the growth-promoting factors in fungi, because the supernatant from free living *F. oryzae* does not enhance the lateral root growth. Taken together, our results suggest that a signal communication occurs between *F. oryzae* and Arabidopsis roots, and the signal from *F. oryzae* promotes Arabidopsis lateral root growth and initiates the fungal colonization.

Roles of the superoxide dismutase family of *Fusarium graminearum* in oxidative stress response and virulence

Yan-Zhang Wang[a], Yan Guo[a,b], Ni Zuo[a,b], Dong Zhang[a], Wei-Hua Tang[a]*

[a] *National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032*

[b] *University of the Chinese Academy of Sciences.(Email: whtang@sibs.ac.cn)*

Fusarium graminearum is a devastating phytopathogen that cause head blight, ear blight and stalk rot in wheat and maize, as well as other cereals. The interaction of *F. graminearum* and host plants is accompanied by accumulation of reactive oxygen species (ROS), such as $O_2^{\bullet -}$ and H_2O_2 at infection site. Superoxide dismutase (SOD) converts $O_2^{\bullet -}$ into O_2 and H_2O_2, thereby scavenging the toxic effects of $O_2^{\bullet -}$.Our previous studies demonstrated that the cytosolic Cu-Zn SOD1 is responsible for the detoxification intracellular $O_2^{\bullet -}$ in *F. graminearum*. In this work, we investigated roles of other four SODs in *F. graminearum*. We constructed mutants of these *SOD* genes by the split-marker recombination approach. Only mutants of *SOD2*, *SOD3*, and *SOD5* were obtained, and no mutants of *SOD4* could obtain despite several attempts, suggesting that *SOD4* may be essential. The assays for the sensitivity

of *SOD* mutants to various stresses showed that △*SOD5* mutants were more sensitive to cell wall-perturbing agents Congo red and membrane stress agent SDS, implying defects in cell wall and membrane integrity; △*SOD2* mutants showed increased sensitivity to an intracellular superoxide radical-generating agent menadione, similar to △*SOD1* mutants, suggesting a role of SOD2 in removing intracellular superoxide radicals. △*SOD5* mutants displayed reduced pathogenicity in wheat coleoptiles, and △*SOD2* mutants showed a weak pathogenicity in wheat spiketlets. Currently, in-depth dissection of these SODs in scavenging ROS to establish colonization and infection is underway.

Acknowledgement: This work was supported by the National Key Research and Development Program of China (2016YFD0100600), the National GMO project (2016ZX08009-003) and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB11020500).

References:

[1]S.H. Yao, Y. Guo, Y.Z. Wang, D. Zhang, L. Xu, W.H. Tang(2016). Fungal Gent Biol. 91:32-42

The rice false smut pathogen *Villosiclava virens* secretes a class v chitinase to suppress plant immunity

Guo-Bang Li, Ning Du, Zhi-You Gong, Yan Li, Fu Huang, Jing Fan, Wen-Ming Wang

Rice Research Institute, Sichuan Agricultural University, Chengdu, 611130

2472630931@qq.com

Rice false smut, caused by a special ascomycete fungal pathogen *Villosiclava virens* (*Vv*) (anamorph: *Ustilaginoidea virens* (Cooke) Takahashi), is becoming one of the most serious diseases in rice production[1,2]. Pathogen effectors play pivotal roles during pathogenesis. However, the functions of most *Vv* effectors are unknown. Previously, transcriptome analysis showed that *Uv_5918*, encoding a class v chitinase, displayed high expression during infection of the rice flower. Uv_5918 was validated to contain a functional signal peptide, suggesting that Uv_5918 is a secreted protein. Subcellular localization assay identified that Uv_5918 contained a functional nuclear export signal (NES) and a nuclear localization signal (NLS). Heterologous expression of *Uv_5918* can compromise pathogen-associated molecular patterns (PAMPs)-induced immunity in rice (*Oryza sativa*) and *Arabidopsis thaliana*, such as inhibition of reactive oxygen species burst, reduced accumulation of callose and weakened

expression of defense-related genes. Taken together, our data indicate that *Uv_5918* may play a role in pathogenesis by suppression of host defense.

Acknowledgement：This work was supported by the National Natural Science Foundation of China (grants 31501598 to J. Fan).

References:

[1] E. Tanaka, T. Ashizawa, R. Sonoda, et al (2008).*Mycotaxon* 106, 491–501.
[2] M.C. Cooke (1878).*Grevillea* 7, 13–15.

Integrated transcriptome and metabolome analysis of *Oryza sative* L. upon infection with *Rhizoctonia solani*, the causal agent of rice sheath blight

Wenlei Cao[a], Bin Xu[a], Limin Yuan[b], Xijun Chen[a], Xuebiao Pan[a], Shimin Zuo[a,*]

[a], *Jiangsu Key Laboratory of Crop Genetics and Physiology/**Co-Innovation Center for M**odern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009*
[b], *Testing center of Yangzhou University*

Presenting author: wlcao@yzu.edu.cn

Sheath blight (SB), caused by necrotrophic fungus *Rhizoctonia solani* Kühn (*R.solani*), gives rise to severely grain losses in rice. In this study, an integrated approach of transcriptomics and metabolomics was employed to try to uncover potential mechanism and to mine the candidate genes/pathways regulating rice SB resistance. YSBR1 is a novel germplasm with stable and high resistance to SB, which has been identified by using greenhouse and field trials. Histological analysis by SEM showed that the growth of *R. solani* mycelium was apparently suppressed in YSBR1 but not in Lemont (susceptible) after 60 HAI. *R. solani* infection was able to trigger substantial metabolic reprogramming in rice, particularly in primary metabolism and energy metabolism. Phenylalanine and tyrosine, which have been confirmed to involve in plant-pathogen interaction via the biosynthesis of secondary metabolites, were specially increased in YSBR1 infected. Total of 332/62 (Lemont/YSBR1) and 2606/748 (Lemont /YSBR1) DEGs at 10 and 20 HAI were identified, respectively. A total of 247 unique DEGs were identified specifically in YSBR1, which were annotated to involve

in several pathways, like amino acid metabolism, secondary metabolic, energy metabolism, photosynthesis, and sugar metabolism pathways. Through combining genetic analysis and omics evidence, 6 candidate genes localized within the known SBR QTL region (Lemont/YSBR1), encoding cellulose synthase-like family A, transcription factors TFⅡS, elongin A, EF hand family protein, calmodulin-related calcium sensor protein, ERF transcription factor, and thaumatin, have been identified.

Plant recognition of a novel *Phytophthora* PAMP XEG1

Yan Wang[a], Yuanpeng Xu[a], Yujing Sun[a], Jiaming Qi[a], Huibin Wang[a], Yuanyuan Shao[a], Bowen Wan[a], Wenwu Ye[a], Suomeng Dong[a], Yuanchao Wang[a]

[a]*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095*

Email: yan.wang@njau.edu.cn

Phytophthora species are notorious plant pathogens that cause great damage on crops. Soybean root rot caused by *Phytophthora sojae* is destructive to soybean and often leads to an annual loss up to tens of billions dollars. Recently, we identified XEG1, a novel pathogen-associated molecular pattern (PAMP) in *P. sojae*. XEG1 belongs to the glycoside hydrolase family 12 and is widely distributed across microbial taxa. XEG1, on the one hand, is essential for *Phytophthora* virulence, and on the other hand can be recognized by plants and triggers cell death in various plant species. In this study, we employed *Nicotiana benthamiana* as a model plant and characterized the function of membrane-localized receptors in XEG1 recognition. In this way, we identified the recognition receptor that responses to XEG1. In addition, we found a novel co-receptor that participates in defense-signaling mediated by XEG1 and several other PAMPs. Collectively, this study provides novel insights on understanding plant innate immunity against *Phytophthora* pathogens and will facilitate the development of durable disease resistance resources.

References:

Z. Ma, T. Song, L. Zhu, W. Ye, Y. Wang, Y. Shao, S. Dong, Z. Zhang, D. Dou, X. Zheng, B.M. Tyler and Y. Wang. A *Phytophthora sojae* glycoside hydrolase 12 protein is a major virulence factor during soybean infection and is recognized as a PAMP. Plant Cell 27(7):2057-2072.

Phosphate status-dependent control of plant immunity to root-infecting fungi

Tae-Hong Lee [a], Kei Hiruma [a, b], Kentaro Okada [a], Midori Tanaka [a], Paul Schulze-Lefert [c], Yusuke Saijo [a, b]

[a] *Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan*

[b] *Japan Science and Technology Agency (JST), Precursory Research for Embryonic Science and Technology (PRESTO), Kawaguchi, Japan*

[c] *Max Planck Institute for Plant Breeding Research, Cologne, Germany*

E-mail of presenting author: saijo@bs.naist.jp

When key nutrients are scare, plants often recruit and rely on root-associated beneficial microbes for nutrient acquisition, but need to retain effective resistance against pathogens. We pursue the hypothesis that plants modulate defense responses to microbial challenge, according to the availability of key nutrients, such as inorganic phosphate (Pi), and the behavior of their encountered microbes. A transcriptome analysis indicates that defense responses to damage-associated molecular pattern (DAMP) via PEPRs, but not to the fungal microbe-associated molecular pattern (MAMP) chitin, are enhanced in Pi-starved roots of *Arabidopsis thaliana*. This seems to reinforce tryptophan-derived secondary metabolism, involving the production of the antimicrobials camalexin and indole glucosinolates that contribute to anti-fungal resistance, consistent with PEPR-mediated resistance against *Colletotrichum higginsianum* [1]. We address the biological significance and molecular basis of PEPR signaling sensitization under low Pi conditions, by employing a beneficial endophyte, *C. tofieldiae*, and its pathogenic relative *C. incanum* [2]. Our results point to the existence of separate requirements for the PEPR pathway and Pi starvation response (PSR) components between the two opposite fungal interactions.

References:

[1] K. Yamada et al. (2016). EMBO J, 35, 46-61.

[2] K. Hiruma et al. (2016). Cell 165, 464-474.

Activities and metabolic effects of salicylhydroxamic acid against *Alternaria alternata* examined using the Biolog Phenotypic MicroArray

Hancheng Wang [a], Zhifan Zhang [b], Yanfei Huang [c], Changqing Zhang [c]
[a] *Guizhou Academy of Tobacco Science, Guiyang 550081*
[b] *Zhengan County Tobacco Company, Zunyi Tobacco Company, Zunyi Zhengan 563400*
[c] *College of Agriculture, Yangtze University, Jingzhou 434025*
E-mail:xiaobaiyang126@hotmail.com

Abstract: Salicylhydroxamic acid (SHAM) is a chemical that has same roles as flavonoids in plant, which could enhance the efficacy of strobilurins by inhibiting alternative respiration. This study was conducted to investigate the *in vitro* activity of SHAM to *A*. *alternata* by "hyphal growth rate" and "spore germination" methods, and also the phenotypic fingerprint of the pathogen under pressure of SHAM using BIOLOG phenotype MicroArray (PM) [1]. Result showed that SHAM had poor effect on mycelial growth and conidial germination of the pathogen, with both EC_{50} values high than 100 mg/L. Using PM plates 1 to 10, 950 different growth conditions were analyzed. When compared with the treatment of no chemicals, *A*. *alternata* was still able to metabolize 45 tested carbon sources under the pressure of 100 mg/L SHAM. Metabolism of the carbons, such as m-Inositol, D-Galactose which belongs to the galactose, was inhibited. Utilization of nitrogen sources rate decreased 3.46% when compared with no chemicals. Amino acid and polypeptides significantly inhibited included L-cysteine, L-proline, N-phthaloyl-L-glutamic acid, ethanolamine, β-phenylethylamine, tyramine, formamide, D,L-lactamide, uric acid, parabanic acid trp-Val, tyr-Ile, and tyr-val. The pathways of these metabolic substrates essentially are tricarboxylic acid cycle. Utilization of phosphorus sources and sulfur sources significantly inhibited included dithiophosphate, d-Mannose-1-Phosphate, o-phospho-D-tyrosine, 2-deoxy-D-glucose 6-phosphate, O-phospho-L-tyrosine, L-cysteine, and S-methyl-L-cysteine. Metabolism of 2-deoxy-D-glucose 6-phosphate, and O-phospho-L-tyrosine happens in glycolysis, and the others happened in tricarboxylic acid cycle. Under the pressure of 100 mg/L SHAM, metabolism of *A*. *alternata* were not affected in biosynthetic pathway (PM5), osmolytes (PM9), and various pH (PM10) conditions.

Acknowledgement: This work was supported by National Natural Science Foundation of China (31501679, 31360448) and Guizhou Tobacco Company (201305, 201714).

References:

[1] Wang, H. *et al*. Metabolic activities of five botryticides against *Botrytis cinerea* examined using the Biolog FF MicroPlate. *Sci*. *Rep*. **6**, 31025; doi: 10.1038/srep31025 (2016).

Genome comparison of three *Magnaporthe oryzae* field isolates reveals genome variations and isolate-specific genome content

Wenjuan Wang, Shen Chen, Jing Su, Jianyuan Yang, Congying Wang, Aiqing Feng, Liexian Zeng, Xiaoyuan Zhu*

Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640;

E-mail of presenting author: juanlook@163.com

Rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases to stable rice production worldwide. The most effective and economic approach for controlling rice blast is to deploy the broad spectrum resistant genes and its derived resistance cultivars. The rice cultivar Mabayinzhan from Shaoguan, Guangdong in China, have displayed good field resistance for several years. However, the resistance of cultivar Mabayinzhan has been broken in recent years, which may due to the mutation of cognate avirulence genes in fugal field population. To understand the pattern of the mutation of *Magnaporthe oryzae* field isolates under natural selection forces, we carried out the whole genome resequencing approach to analyze the genomes of three field isolates 2012M-6, 2013M-1 and 2013M-2. By comparing the *de novo* genome assemblies of the three isolates against the reference strain 70-15, we identified several extensive polymorphisms including unique genes, SNPs， small Indels and large Indels, as well as the unmapped sequence. The three isolates 2012M-6, 2013M-1 and 2013M-2 are highly similar in the genome sequence, while the isolate 2012M-6 was found carrying *AvrPib* gene through the analysis of secreted proteins. By genome comparison analysis, we provided results that the three field isolates from South China had very small genome variation, while maybe contain isolate-specific genome content against 70-15 and other resequencing strains in China.

Keywords: *Magnaporthe oryzae,* field isolates, genome resequencing, genome comparison.

The *FgSRP1* SR-protein gene is important for plant infection and mRNA processing in *Fusarium graminearum*

Yimei Zhang[1], Xuli Gao[1], Manli Sun[1], Huiquan Liu[1], and Jin-Rong Xu[1,2*]

[1] *NWAFU - Purdue Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling,*

Shaanxi 712100.

[2] *Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.*

E-mail: xinongyimei@163.com

The versatile functions of SR (serine/arginine-rich) proteins in pre-mRNA splicing and processing are modulated by reversible phosphorylation [1,2]. Previous studies showed that FgPrp4, the only protein kinase among spliceosome components, is important for intron splicing and the FgSrp1 SR protein is phosphorylated at five conserved sites in *Fusarium graminearum* [3]. In this study, we showed that the *Fgsrp1* deletion mutant rarely produced conidia and caused only limited symptoms on wheat heads and corn silks. Deletion of *FgSRP1* also reduced ascospore ejection and deoxynivalenol (DON) production. Interestingly, *FgSRP1* had two transcript isoforms due to alternative splicing and both of them were required for its normal functions in growth and DON biosynthesis. FgSrp1 localized to the nucleus and interacted with FgPrp4 in vivo. Deletion of all four conserved phosphorylation sites but not individual ones affected the *FgSRP1* function, suggesting their overlapping functions. RNA-seq analysis showed that the expression of over thousands of genes and splicing efficiency in over 300 introns were reduced. Taken together, *FgSRP1* is important for conidiation, and pathogenesis and alternative splicing is important for its normal functions. The FgSrp1 SR protein is likely important for mRNA processing or splicing of various genes in different developmental and infection processes.

References:

[1] Cho, S. *et al* (2011). Interaction between the RNA binding domains of Ser-Arg splicing factor 1 and U1-70K snRNP protein determines early spliceosome assembly. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 8233-8238, doi:10.1073/pnas.1017700108.

[2] Zhou, Z. & Fu, X. D (2013). Regulation of Splicing by SR proteins and SR Protein-Specific Kinases. *Chromosoma* **122**, 191-207, doi:10.1007/s00412-013-0407-z.

[3] Gao, X. *et al* (2016). FgPrp4 Kinase Is Important for Spliceosome B-Complex Activation and

Splicing Efficiency in *Fusarium graminearum*. *PLoS genetics* **12**, e1005973, doi:10.1371/journal.pgen.1005973.

The *PKR1* regulatory subunit of PKA is involved in regulating growth, sexual and asexual development, and pathogenesis in *Fusarium graminearum*

Chaoqun Li[a], Yonghui Zhang[b], Huan Wang[a], Ju Zhang[a], Manli Sun[a], Jin-Rong Xu[b], and Chenfang Wang[a*]

[a] *State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi 712100.*

[b] *Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.*

E-mail: wangchenfang@nwafu.edu.cn

Fusarium graminearum is a causal agent of the wheat scab disease and a producer of deoxynivalenol (DON) mycotoxins. Treatment with exogenous cAMP or deletion of *PDE2* cAMP phosphodiesterase increased its DON production. To better understand the role of the cAMP-PKA pathway in *F. graminearum*, in this study we functionally characterized the *PKR1* gene encoding the regulatory subunit of PKA, the only known intracellular target of cAMP in fungi. Mutants deleted of *PKR1* were viable but had severe defects in growth, conidiation, and plant infection. The *pkr1* deletion mutant produced compact colonies with shorter aerial hyphae that were increased in the number of nuclei in hyphal compartments. Mutant conidia were morphologically abnormal and appeared to undergo rapid autophagy-related cell death. The *pkr1* mutant was blocked in perithecium development but increased in DON production. It had a disease index less than 1 and failed to spread from the inoculated kernels to neighboring spikelets. The *pkr1* mutant was unstable and spontaneous suppressors with faster growth rate were often produced on older cultures. A total of 67 suppressor strains that grew faster than the original mutant were isolated. Three of them had similar growth rate and colony morphology with the wild type but were still defective in conidiation. Sequencing analysis with 18 candidate PKA-related genes in three representative suppressor strains identified mutations only in the *CPK1* catalytic subunit gene. Further characterization showed that 10 of the rest 64 suppressor strains also had mutations in

CPK1. Overall, these results showed that *PKR1* is important for regulating hyphal growth, reproduction, pathogenesis, and DON production, and mutations in *CPK1* were partially suppressive to deletion of *PKR1* in *F. graminearum*.

Functional characterization of frequency genes in *Magnaporthe oryzae* and *Fusarium graminearum*

Qiang Zhang [a], Xuli Gao [a], Qinhu Wang [a], Cong Jiang [a,*] and Jin-Rong Xu [a,*]

[a] *State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest Agricultural and Forestry University, Yangling, Shaanxi 712100.*

E-mail of presenting author: cjiang@ nwafu.edu.cn

Circadian rhythm organizes inner physiology with respect to the external world, providing life with the ability to anticipate and thereby better prepare for major fluctuations in its environment. To determine the importance of the circadian rhythm in the rice blast fungus *Magnaporthe oryzae*, here we functionally characterized the *MoFRQ* gene, an ortholog of *Neurospora crassa frq* which is the central component of the oscillator. Light not only stimulated the expression of *MoFRQ*, but also promoted MoFrq accumulation into the nucleus. The *Mofrq* deletion mutant is defective in aerial hyphal growth under constant light. The conidiation of the mutant was significantly reduced in comparison with the wild-type Guy11. MoFrq and Htf1 form a heterodimer to regulate the conidia formation from the conidiophores. The *Mofrq* mutant rarely caused typical lesions on rice or barley leaves, indicated a significant reduction in virulence. Defects of the *Mofrq* mutant in appressorium formation and penetration were detected as well. The mobilization and degradation of glycogen from conidia to appressoria was regulated *MoFRQ*, which might be responsible for the defect of *Mofrq* mutant in penetration. The PKA activity in the *Mofrq* mutant was also reduced. Therefore, *Mofrq* mutant was defective in vegetative growth, conidiation, appressoria penetration and signaling activation. On the other hand, we also deleted the ortholog of *MoFRQ* in *Fusarium graminearum*, but found *FgFRQ* was dispensable for both vegetative and invasive growth. We then expressed *FgFRQ* in the *Mofrq* mutant, but failed to complement the defects of *Mofrq* mutant in growth and plant infection. Although *N. crassa* was not a pathogenic fungus,

NcFRQ gene fully complemented the function of its ortholog in *M. oryzae*. Interstingly, *NcFRQ* and *MoFRQ* but not *FgFRQ* has a strong bais for non-optimized codons, suggested this codon usage was important for the function of *FRQ* genes.

Acknowledgement: We thank Drs. Huiquan Liu and Chenfang Wang for fruitful discussions. This work was supported by Zhongying Young Scholars of Northwest A&F University.

Functional analyses of putative G protein-coupled receptor genes in *Fusarium graminearum*

Shulin Cao [a], Cong Jiang [a], Huiquan Liu[a], Chunlan Wu[a], Tao Yin[a], Jin-Rong Xu[a,b]

[a] *State Key Laboratory of Crop Stress Biology for Arid Areas, Purdue-NWAFU Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100.*

[b] *Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. USA*

xycjl100@yeah.net

G protein-coupled receptors (GPCRs) are seven transmembrane α-helices proteins that detect extracellular signals outside the cell and activate intercellular signaling pathways. GPCRs and their downstream signaling are important for various physiological processes and contribute to many human diseases. Nevertheless, few GPCRs have been identified in fungal genomes and most of them had not been functionally characterized. In this study, totally 85 GPCRs were predicted in *F. graminearum* based on the genome information.. We generated genes deletion mutants of all those GPCRs and assayed their defects in growth, conidiation, sexual reproduction and virulence. Three GPCRs mutants were defective in wheat infection and DON production but all the deletion mutants were normal in vegetative growth and conidiation. Interestingly, two GPCR genes (*FgPRE2* and *FgCGR1*) were important for sexual development. The *Fgpre2* mutant still produced perithecia but the size was smaller, whereas the *Fgcgr1* mutant failed to form mature perithecia. Both of them were normal in hyphal fusion and male fertility. However, *FgCGR1* gene was not necessary for the perithecial initials formation but required for late stage of perithecia. Expressing dominant active *gzgpa1*^{G42R} in the *FgCGR1* deletion mutant restored its defect in perithecia development. We further showed FgCgr1 interact with GzGpal and the third intracellular loop of FgCgr1 was

responsible for the interaction. Among all GPCRs deletion mutants, only three of them attenuates the virulence on wheat and two had defects in perithecia development. These results suggest most of the GPCRs genes have overlapping function in development and infection of *F. graminearum*.

Acknowledgement: This work was supported by Chinese Universities Scientific Fund (Z109021618)

Functional characterization of FgPrp6 in *Fusarium graminearum*

Chaohui Li [a], Xuli Gao [a], Qiaojun Jin [a] and Jin-Rong Xu [a,b*]

[a] *College of Plant Protection, Northwest A&F University, Yangling, Shaanxi*

[b] *Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States*

chaohuili@yeah.net

The pre-mRNA processing factors Prp6 is an essential component of the U4/U6-U5 tri-snRNP. Prp4 is the only protein kinase in spliceosome. Prp6 was phosphorylated by Prp4 but its function and regulation mechanism during the process of splicing are not well-characterized. In *Fusarium graminearum*, the *Fgprp4* deletion mutant was found to have severe growth defects and produced spontaneous suppressors and suppressor mutations were identified in *FgPRP6* in our previous research [1]. In this study, we further collected 300 subcultures of spontaneous sectors. Thirteen mutations on 11 amino acid sites of FgPrp6 were identified in 25 suppressor strains by sequencing analysis. The FgPrp6 protein has an N-terminal PRP6_N domain and 19 tetratricopeptide repeats (TPRs). In humans, five hPrp4-phosphorylation sites have been identified in the linker region of hPrp6 between the PRP6_N domain and TPR repeats [2]. Sequence alignment showed that two of them, T252 and T261, are conserved in FgPrp6 and its orthologs from other filamentous fungi. Whereas 3 mutation sites A229, R230, L234 are in the linker region, 7 mutation sites R282, R284, L287, T293, E308, R318, E343 are in the first TPR repeat and not far away from the conserved Prp4-phosphorylation sites. On those 10 sites twelve suppressor mutations may have similar effects on FgPrp6 functions as phosphorylation by FgPrp4 in *F. graminearum*. To verify putative Prp4-phosphorylation sites T252 and T261, the *FgPRP6*^{T252A}-3xFlag and *FgPRP6*^{T261A}-3xFlag alleles were co-transformed with the *FgPRP6* knock out construct into wild type strain PH-1. All resulting *Fgprp6/FgPRP6*^{T252A}-3xFlag and

Fgprp6/*FgPRP6*^{T261A}-3xFlag transformants were normal in growth and conidiation. It is possible that FgPrp6 is phosphorylated at multiple residues and the phosphorylation at individual sites may be functional redundancy. Further verification of phosphorylation sites and functional analysis of the suppressor mutations are still ongoing.

References:

[1] X. Gao, Q. Jin, et al. (2016). PLoS Genet, 12.
[2] M. Schneider, H.H. Hsiao, et al. (2010). Nat Struct Mol Biol, 17.

Phytophthora infestans RxLR effector PITG_22798 modulates potato immune responses through interaction with Knox and KAUA proteins

Zhou Jing, Ren Yajuan, Tian Zhendong

Potato Biology and Biotechnology Key Laboratory of Ministry of Agriculture;Key Laboratory of Potato Biology and Biotechnology, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, 430070

tianzhd@mail.hzau.edu.cn

Phytophthora infestans secretes number of RxLR effector proteins into host cells to modulate plant immune responses and promote colonization. *P. infestans* RxLR effector PITG_22798 was up-regulated during early infection of potato. Transient expression of PITG_22798 promotes *P. infestans* colonization on *N. benthamiana* leaves. High level expression of PITG_22798 could induce cell death, which depends on SGT1-mediated signaling and could be suppressed by the *P. infestans* effector, AVR3b [1]. PITG_22798 locates in the host nucleus and interacts with potato Knox and KAUA protein. Protein interaction leads to PITG_22798 re-localization with KAUA protein. Both of interacts proteins are negative regulators of potato late blight resistance, indicating *P. infestans* effector PITG_22798 modulates potato immune responses through multiple ways. PITG_22798 promotes *P. infestans* colonization might through enhancing Knox and KAUA negative regulation functions.

References:

1. H. Wang, Y. Ren, J. Zhou, J. Du, J. Hou, R. Jiang, H. Wang, Z. Tian*, C. Xie*(2017) Int. J. Mol. Sci. 18, 409-413.

CRISPR-mediated mutagenesis confirms that RXLR effector gene *Avr1b* is essential for avirulence on soybean plants expressing resistance gene *Rps1b*

Biao Gu [a, b], Yufeng Fang [b], Kai Tao [b], Hua Zhao [c], Weixing Shan [c], Brett M. Tyler [b]

[a] *College of Plant Protection, Northwest A&F University, 3 Taicheng Road, Yangling*

[b] *Center for Genome Research and Biocomputing and Department of Botany and Plant Pathology, Oregon State University, Corvallis, USA*

[c] *College of Agronomy,, Northwest A&F University, 3 Taicheng Road, Yangling*

bgu@nwafu.edu.cn

The diploid *Phytophthora* pathogens are less well-documented in molecular genetics studies because of frequent deletion of exogenous genes and dramatic variations of gene silencing efficiency. By taking advantage of the adapted CRISPR/Cas9 genome editing system [1], we generated the *Phytophthora sojae* RXLR effector gene *Avr1b* Knock-out (KO) and Knock-in (KI) mutants to confirm its avirulence activities on soybean plants with the corresponding resistant gene *Rps1b*.

Three *Avr1b*-targeted sgRNAs were individually inserted into pYF515 vector for expression of both sgRNA and *Cas9* gene. Each pYF515-sgRNA plasmid was combined with a homology direct repair (HDR) donor plasmid carrying mCherry in place of Avr1b. The combinations were introduced into protoplasts via PEG-mediated transformation, in order to replace *Avr1b* in *P. sojae* strain P7063. On average, 30-70 independent colonies were regenerated from 1-3 x 10^6 treated protoplasts. The different sgRNAs produced different efficiencies of homologous recombination. SgRNA 114R produced the highest editing efficiency, with a frequency of homozygous replacement events of 70%.

The specific virulence of three transformants carrying mCherry in place of Avr1b was evaluated by soybean hypocotyl and detached leaf inoculation assays. The gain of virulence of all selected Avr1b KO transformants on Rps1b plants indicates Avr1b was required for the avirulence phenotype. However, one control transformant that retained Avr1b and the P7063 wild type strain induced hypersensitive responses on Rps1b-expressing soybeans.

In order to complement the Avr1b KO, a sgRNA-resistant version of *Avr1b* was created and re-introduced into Avr1b KO transformant 114-7. The gain of avirulence in 114-7 confirmed that the sgRNA-resistant Avr1b could still be recognized by Rps1b. In summary, we have used CRISPR-mediated knock-out and knock-in strategies to confirm that Avr1b of *P. sojae* is essential for avirulence on soybean plants expressing resistant gene *Rps1b*.

Acknowledgement: This work was supported by NSFC grant (#31301644) and CSC fellowship (#201506305046) to B.G. and USDA NIFA grant #2011-68004-30104 to B.M.T. .

References:

[1] Y. Fang, L. Cui, B. Gu, F. Arredondo, B.M. Tyler. 2017. Curr. Protoc. Microbiol. 44:21A.1.1

The histological and transcriptomic analysis of incompatible interaction between wild potato *Solanum pinnatisectum* and *Phytophthora infestans*

Hua Zhao[a], Xiaoli Cao[a], Qin Chen[a], Biao Gu[b]

[a] *College of Agronomy, Northwest A&F University, No.3 Taicheng Road, Yangling,* [b] *College of Plant Protection, Northwest A&F University, No.3 Taicheng Road, Yangling*

zhaohua362@nwsuaf.edu.cn

The stacking of disease resistance components from diverse wild potato species into cultivated potato is illustrated to be a promising and durable approach to control potato late blight cauesd by *Phytophthora infestans*[1]. A Mexican wild potato species *Solanum pinnatisectum* exhibited considerable resistance against multiple *P. infestans* isolates with different SSR genotypes that enable us to further dissect its genetic resistence mechanism.

Histological characterization showed that more than 80% of the zoospores could germinate within 3 hours post inoculation (hpi) on both *S. pinnatisectum* and the susceptible control *S. cardophyllum*. However, there were limited and disorganized hyphal branches formed on the *S. pinnatisectum* epidermal cells by 9 hpi. At this time point, on the contrary, a confluent hyphal network was distinctively observed around the inoculation site and the hyphae extensively penetrated into mesophyll cells of susceptible cultivar. The necrosis was visible on infected mesophyll cells of *S. pinnatisectum* at 9 hpi and after 12 hpi the cell death was dominant that indicates hypersensitive response (HR) was induced upon *P. infestans* infection. By 3 days post inoculation, typical lesions with numerous sporangia developed on *S. cardophyllum*, while macroscopic necrosis appeared on leaf epidermis of *S. pinnatisectum* that results in remarkable delay of subsequent lesion development.

Gene expression and comparative transcriptomic analysis identified dramatic up-regulation of plant immunity-related genes corresponding to biotic stresses. Transient expression of known *P. infestans* avirulence genes demonstrated six out of ten *Avr* genes were able to trigger cell death in *S. pinnatisectum*, while only three *Avr* genes were recognized by *S. cardophyllum*. Transcriptomic data mining discovered 24 up-regulated LRR genes at 9 and 12 hpi, which may be responsible for the active defence response-based cell necrosis in *S. pinnatisectum*.

Acknowledgement: We are grateful to Prof. Weixing Shan for providing of *Phytophthora infestans* isolates. The project was supported by Natural Science Basic Research Plan in Shaanxi Province of China (# 2015JQ309).

References:

[1] V. G. A. A. Vleeshouwers, S. Raffaele, J. H. Vossen et al., (2012). Annu. Rev. Phytopathol. 49, 507–531.

The Mechanism of FgSnu114 in Regulation of Spliceosome Activation in *Fusarium graminearum*

Chaoni Song[1], Xiaoping Li[1], Jin-Rong Xu[1,2], Qiaojun Jin[2].
[1] *Purdue-NWAFU Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100.*
[2] *Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.*
E-mail: 2528303202@qq.com

Pre-mRNA splicing takes place in the spliceosome. Snu114, an important component of the U5 snRNP, regulates Brr2's activity of unwinding U4/U6 during splicesome activation. Here, we show that four spontaneous mutations in different domains of Snu114 suppressed the defects caused by *FgPRP4* deletion in *Fusarium graminearum*. All the suppressor strains are normal in growth and sexual reproduction, but are still impaired in plant infection. Interestingly, in sequence alignment the K695Δ mutation in FgSnu114 is the same site of human Snu114 that interacts with Brr2 [1], indicating this mutation may affect the interaction between FgSnu114 and FgBrr2. We further verified the suppressor mutations by introducing FgSnu114^{D222N} into the *Fgprp4* mutant. Our study will contribute in understanding the mechanism of Snu114 in regulating Brr2's function.

Keywords: Pre-mRNA splicing, spontaneous mutations, interaction

References:

[1] Agafonov, D. E., Kastner, B., Dybkov, O., Hofele, R. V., Liu, W. T., & Urlaub, H., et al. (2016). Molecular architecture of the human u4/u6.u5 tri-snrnp. *Science, 351*(6280), 1416.

Mutations in FgSad1 suppress *Fgprp4* and alter its iteraction with Brr2, Prp8, Snu114, and Snu66 in *Fusarium graminearum*

Xiaoping Li, Chaoni Song, Jin-Rong Xu, Qiaojun Jin.
State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A &F University, Yangling, Shaanxi
E-mail :jqiaojun@nwsuaf.edu.cn

Sad1 is required for the assembly of the U4/U6 di-snRNP and maintaining the integrality of U4/U6.U5 tri-snRNP in budding yeast and human, respectively. However, detailed knowledge about its regulation mechanism during the process of splicing is rare. In a previous study, suppressor mutations of the *Fgprp4* mutant were identified in FgPrp6, FgPrp31, FgPrp8,

and FgBrr2 in the plant pathogenic fungus *Fusarium graminearum*. In this study, we further identified 7 mutations in FgSad1 also suppressed *Fgprp4*.in 14 suppressor strains. All the 14 suppressors were still defective in sexual reproduction and plant infection. Compared to the Sad1 in *Saccharomyces cerevisia* that does not contain Prp4 kinase, FgSad1 has an extra 50aa N-terminal region rich in serine and arginine. Interestingly, truncation of the 50 aa resulted in severe growth defects and instability, but did not affect the nuclear localization of FgSad1, indicating that the 50 aa RS rich region is important for FgSad1's function but disposable for its localization. We further assayed the effect of suppressor mutations on the interaction between Sad1 and other spliceosome proteins. Both in yeast two hybrid and co-IP assays, P258S and S269P mutations enhanced the interactions between FgSad1 and FgSnu66，FgBrr2, FgPrp8, or FgSnu114， while D76N and L512P decreased the interaction between FgSad1 and FgSnu114. Our results suggests that FgSad1 helps to maintain the integrality of tri-snRNP via mediating the interaction between itself and FgBrr2、FgSnu114、FgSnu66 and FgPrp8.

Uncover the role of autophagy in neutralized phosphorous acid-induced resistance against *Phytophthora parasitica* in tomato

Huang, Y. S., Lin, C. P., and Liou, R. F.
Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan
r03633018@ntu.edu.tw

Neutralized phosphorous acid (NPA) is widely used for the management of plant diseases caused by oomycete pathogens including *Phytophthora parasitica*. While it has been proposed that phosphonate, the effective component of NPA, may function through inhibiting pathogen growth and enhancing plant resistance, the detailed mechanism remains to be elucidated. To identify genes which might play key roles in NPA-induced resistance, we performed microarray analysis and found many genes which are differentially expressed in NPA-pretreated tomato plants in response to infection by *P. parasitica*. Of special interest, some of these genes are involved in the biosynthesis of secondary metabolites and lipids. As well, autophagy-related genes including *Sl*ATG3, *Sl*ATG6, and *Sl*ATG18b (a homolog of AtATG18b) were significantly upregulated. Analysis by qRT-PCR indicated that these genes

are indeed upregulated in response to infection by *P. parasitica*. To uncover the role of autophagy in the interaction between the pathogen and host, we downregulated the expression of autophagy-related genes by *Tobacco rattle virus*-mediated gene silencing and then infected the silenced plants with *P. parasitica*. The results indicated that silencing of *Sl*ATG3, *Sl*ATG6, *Sl*ATG18b did not alter tomato susceptibility towards the pathogen regardless of NPA treatment. However, downregulation of *Sl*ATG8-4, a potato ATG8CL homolog encoding a key protein of autophagosome, enhanced disease symptom in the NPA-treated plants. Examination by confocal microscopy of the subcellular distribution of *Sl*ATG8-2-GFP demonstrated that infection by *P. parasitica* induced the formation of autophagosomes in tobacco epidermal cells. These results suggest the involvement of autophagy in plant response to infection by *P. parasitica* and an important role of *Sl*ATG8-4 in NPA-induced resistance.

Acknowledgement: *P. parasitica*, NPA-induced resistance, autophagy, transcriptome analysis

References:

[1] C. M. Rojas, M. Senthil-Kumar, V. Tzin, and K. S. Mysore. 2014. Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Plant Sci., vol. 5.

[2] Y. F. Dagdas et al. 2016. An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. Elife, vol. 5, p. e10856.

[3] Y. Cao, and D. J. Klionsky. 2007. Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. Cell research 17:839–849.

[4] S. Han, Y. Wang, X. Zheng, Q. Jia, J. Zhao, F. Bai, Y. Hong, and Y. Liu. 2015. Cytoplastic Glyceraldehyde-3-Phosphate Dehydrogenases Interact with ATG3 to Negatively Regulate Autophagy and Immunity in *Nicotiana benthamiana*. The Plant Cell 27:1316–1331.

[5] A. P. Hayward, J. Tsao, and S. P. Dinesh-Kumar. 2009. Autophagy and plant innate immunity: Defense through degradation. Seminars in Cell & Developmental Biology 20:1041–1047.

[6] D. Hofius, D. Munch, S. Bressendorff, J. Mundy, and M. Petersen. 2011. Role of autophagy in disease resistance and hypersensitive response-associated cell death. Cell Death & Differentiation 18:1257–1262.

[7] S. I. Kwon, H. J. Cho, S. R. Kim, and O. K. Park. 2013. The Rab GTPase RabG3b Positively Regulates Autophagy and Immunity-Associated Hypersensitive Cell Death in *Arabidopsis*. PLANT PHYSIOLOGY 161:1722–1736.

[8] Z. Lai, F. Wang, Z. Zhang, B. Fan, and Z. Chen. 2011. A critical role of autophagy in plant resistance to necrotrophic fungal pathogens: Autophagy in plant disease resistance. The Plant Journal 66:953–968.

[9] H. D. Lenz, E. Haller, E. Melzer, K. Kober, K. Wurster, M. Stahl, D. C. Bassham, R. D. Vierstra, J. E. Parker, J. Bautor, A. Molina, V. cudero, T. Shindo, R. A. L. van der Hoorn, A. A. Gust, and T. Nürnberger. 2011. Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens: Autophagy controls plant basal immunity. The Plant Journal 66:818–830.

[10] B. Levine, and G. Kroemer. 2008. Autophagy in the Pathogenesis of Disease. Cell 132:27–42.

[11] Y. Liu, M. Schiff, K. Czymmek, Z. Tallóczy, B. Levine, and S. P. Dinesh-Kumar. 2005. Autophagy Regulates Programmed Cell Death during the Plant Innate Immune Response. Cell 121:567–577.
[12] M. Seay, S. Patel, and S. P. Dinesh-Kumar. 2006. Autophagy and plant innate immunity. Cellular Microbiology 8:899–906.

Exploration on *Sclerotinia sclerotiorum* virus diverity and their potential application for fungal disease mangement

Songsong Wu[1, 2], Jiasen Cheng [1, 2], Yanping Fu[2], Tao Chen[2], Daohong Jiang[1, 2] , Jiatao Xie[1, 2*]

1 State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei Province

2 The Provincial Key Lab of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei Province

E-mail of presenting author: jiataoxie@mail.hzau.edu.cn

Rapeseed (*Brassica napus*) stem rot caused by *Sclerotinia sclerotiorum* is the most important disease on rapeseed in China. Mycoviruses are viruses that infect fungi and replicate in fungal cells, and they are found in nature commonly. Hypovirulence-associated mycoviruses of fungal plant pathogens have attracted much attention because of their potential as biological control agents against plant fungal diseases. Hypovirulent strains of *S. sclerotiorum* are increasingly recognized to harbor great diverse mycoviruses. They possess diverse genomes of mostly ssRNA, dsRNA and rarely circular ssDNA. Those newly founding mycoviruses associated with hypovirulence contribute to exploit new potential virocontrol agents for rapeseed rot disease. However, the spread of RNA mycoviruses is limited among vegetatively incompatible individuals, and this limitation is regarded as one of the critical factors responsible for reducing the efficacy of hypovirulence-associated RNA mycoviruses in controlling fungal diseases. The hypovirulence-associated Sclerotinia sclerotiorum mycoreovirus 4 (SsMYRV4), was found to function as a potent inhibitor of G proten signaling pathway, ROS production and vegetative incompatibility-mediated PCD. Furthermore, SsMYRV4-infected strain could easily accept other viruses through hyphal contact and these viruses could be efficiently transmitted from SsMYRV4-infected strain to other vegetatively incompatible individuals. Thus, we concluded that SsMYRV4 is capable of suppressing host vegetatively incompatible reaction and facilitating heterologous viruses transmission among host individuals. These findings may enhance our understanding of virus ecology, and provide a potential strategy to utilize hypovirulence-associated RNA mycoviruses to control fungal diseases.

A Secretory Protein of *Sclerotinia Sclerotiorum* That Interacts with the Chloroplast Calcium Sensor CAS to Suppress Host Defense

Liguang Tang[1, 2], Guogen Yang[1, 2], Jiatao Xie[1, 2], Yanping Fu[2], Tao Chen[2], Daohong Jiang[1, 2], Jiasen Cheng[1, 2*]

1 State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei Province

2 The Provincial Key Lab of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei Province

E-mail of presenting author： tangliguanghzau@163.com

Abstract: A large of secreted proteins diverse plant pathogens have been identified and played critical roles in manipulating plant immune response. A candidate effector protein from *S. sclerotiorum*, named SSITL, was previously reported to be essential in suppression of plant immunity at the early stage of infection. However, the molecular mechanism that SSITL suppresses plant immunity against *S. sclerotiorum* infection has not been clarified. Here, yeast-2-hybrid assay results showed that SSITL interacts with Arabidopsis calcium sensing receptor (CAS), which is a chloroplast-localized protein and is responsible for activating of immune signaling in plants. The interaction between SSITL and CAS was confirmed *in planta* by co-immunopricipitation (co-IP). Meanwhile, SSITL can translocate into plant cells and move cell to cell in plants. Arabidopsis T-DNA insertion mutant *cas-1* and *SSITL*-expressing Arabidopsis showed increased susceptibility to *S. sclerotiorum* wild type strain Ep-1PNA367 and *SSITL* silenced transformant A10 compared to the wild type Col-0. Moreover, *CAS*-overexpressing plant showed increased resistance to Ep-1PNA367 and A10, indicating that the resistance to *S. sclerotiorum* was mediated by CAS in Arabidopsis. N-terminal truncated SSITL and C-terminal truncated SSITL cannot interact with CAS, and expression of N-terminal or C-terminal truncated SSITL in Arabidopsis also do not affect the plant resistance to Ep-1PNA367. Furthermore, SA biosynthesis related genes that act downstream of CAS signaling pathway were significantly suppressed by SSITL, suggesting that CAS mediated immune response was interfered by the interaction between SSITL and CAS. In summary, SSITL is a potential effector which may manipulate plant immune response through suppressing of CAS regulated SA biosynthesis pathway to promote

infection of *S. sclerotiorum*.

Interactions of beneficial root fungi with *Arabidopsis thaliana*

Kei Hiruma [a,c], Paul Schulze-Lefert[b], and Yusuke Saijo [a,c]

[a] *Department of Biological Sciences, Nara Institute of Science and Technology, 630-0192, Nara, Japan*

[b] *Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany*

[c] *JST PRESTO, JAPAN*

E-mail: hiruma@bs.naist.jp

In nature plants often rely on root-associated beneficial fungi for uptake of nutrients from soils. *Colletotrichum tofieldiae* (*Ct*), an ascomycete fungal endophyte, that was isolated form natural habitats of Arabidopsis in Spain, colonize Arabidopsis roots and promote plant growth under low phosphate conditions via transferring phosphorus to plants [1]. In contrast to *Ct C.incanum*, the close related species of *Ct*, inhibit plant growth in low phosphate condition, suggesting that *Ct*-mediated plant growth promotion is determined by relatively subtle differences observed in these species. *Ct* strains have been isolated not only from Arabidopsis grown in Spain but also from many other plant species grown in large geographic area across Eurasia, including monocots. These suggest a broad host range and geographic distribution for this fungal species. However it is not currently clear how these *Ct* strains with more than 99 % genome similarity each other interacts with Arabidopsis in low phosphate conditions. In this study we have used several *Ct* strains, which have been isolated from Europe and Japan, to test whether these *Ct* strains have ability to establish mutualistic interactions with Arabidopsis in low phosphate conditions. We will present our progress in these research lines.

References:

[1] Hiruma et al. Cell. 165, 464-474.

Functional characterization of U4/U6·U5 tri-snRNP specific protein FgSnu66 in *Fusarium graminearum*

Manli Sun[a], Cong Jiang[a], Jin-Rong Xu[a, b*]

[a]*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi,*

[b]Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America
sml666@nwafu.edu.cn

Pre-mRNA splicing is catalyzed by a ribonucleoprotein (RNP) complex called spliceosome. *PRP4* is the only kinase associated with spliceosome. Unlike that in other eukaryotic organisms, *FgPRP4* kinase gene was not a lethal gene in *Fusarium graminearum*. The *Fgprp4* deletion mutant had severe defects in growth and produced spontaneous suppressors to recover the growth rate [1]. In this study, a nine amino acid tandem duplication mutation was identified in FgSnu66, an essential component of U4/U6·U5 tri-snRNP [2]. The suppressor strain S37 had this nine amino acid tandem duplication mutation was normal in growth rate and sexual reproduction. We thus assayed its intron splicing by RNA-seq. Although the intron splicing efficiency of S37 failed to reach the level of the wild-type PH-1, it was much higher than that in the *Fgprp4* mutant. Among 321 suppressors of *Fgprp4* mutant, only 20 were FgSnu66 associated suppressor mutations. R477H and R477C partially recovered the phenotypes but did not influence the nucleus localization of FgSnu66. Because most suppressor mutations of FgSnu66 was localized in the C-terminal region, we therefore deleted this region in *Fgprp4* mutant and found it was suppressive to *Fgprp4*. Interestingly, the C-terminal truncation of FgSnu66 had a stronger interaction with FgPrp4, FgPrp8, FgSnu114 and FgSad1 than the full length protein in Y2H assays. Overall, our results indicated both suppressor mutations and C-terminal truncation in FgSnu66 recovered the defects of *Fgprp4*, and the C-terminal of FgSnu66 might play a negative role in interacting with other key components of spliceosome.

References:

[1] X.L. Gao, Q.J. Jin, C. Jiang, Y. Li, C.H. Li, H.Q. Liu, Z.S. Kang, J.R. Xu (2016). PLoS Genetics. 12. 1.
[2] G. Alexander, N. Gitte, B. Josette, M. Matthias, L. Reinhard, F. Patrizia (1999). The EMBO Journal. 18. 4535.

Subcellular localization of the *Phytophthora infestans* RXLR effector AVR1 and its corresponding resistance protein R1

Yu Du[1,2], Jeroen Berg[1], Francine Govers[1]&Klaas Bouwmeester[1]
[1]Laboratory of Phytopathology, Wageningen University and Research, 6708PB Wageningen, The Netherlands
[2]College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100

Phytophthora infestans causes late blight disease on potato and tomato. It secretes hundreds of effectors that modulate host cellular processes to promote colonization. Well known are the RXLR effectors that are translocated into host cells and often play dual roles. On the one hand they manipulate the host cell machinery to facilitate pathogen colonization; on the other hand,when recognized bya corresponding host resistantprotein,they trigger effector-triggered immunity (ETI). The mechanisms underlying ETI are poorly understood. This study focused on the RXLR effector AVR1 and its corresponding resistance protein R1, and addressed the question in which subcellular compartment effector perception and plant defence activation takes place. We investigated the subcellular localization of both R1 and AVR1. Fusing nuclear localization/export signals and mutatedversions of these signals to R1 and AVR1 allowed us to target R1 and AVR1 to different subcellular compartments. In this way we determined the subcellular localizations that are required to elicit R1-mediated immunity and AVR1-suppressed host defence responses.

Acknowledgement: This work was supported by the Programme of Introducing Talents of Innovative Discipline to Universities (project 111) from the State of Administration for of Foreign Experts Affairs, P. R. China.

The meiosis-specific activator *FgAMA1* is important for ascospore formation in *Fusarium graminearum*

Chaofeng Hao [a], Jie Liang [a], Huiquan Liu [a], Jin-Rong Xu [b]

[a]*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi;*

[b]*Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA.*

Email address: chaofenghao2015@163.com

Wheat head blight (WHB) caused by *Fusarium graminearum* is one of the most destructive diseases on wheat, barley, and maize, causing great yield losses worldwide. Because ascospores are the primary inoculum for plant infection, sexual reproduction is a critical step in disease cycle. In *Saccharomyces cerevisiae*, *AMA1* is a meiosis-specific regulator of anaphase promoting complex (APC/C) [1]. It is important for yeast meiosis and post-meiosis cytokinesis. Interestingly, the *AMA1* ortholog *FgAMA1* in *F. graminearum* is a pseudogene. The coding region of *FgAMA1* contains a premature stop codon TAG that is changed to TGG during sexual reproduction by

A-to-I RNA editing [2]. In this study, we characterized the functions of *FgAMA1* and its UAG to UGG editing event. The *Fgama1* deletion mutant was normal in growth, conidiation, and plant infection on wheat head but defective in ascospore morphology, which is consistent with the specific expression of *FgAMA1* at later stages of sexual development. Although the number of mutant ascospores in one asci was generally eight but all of the ascospores were round or oval shaped, and no septum were in them. The mutant ascospore contained two nuclei while for wild type there were four components in one ascospore and each component contained one nucleus. In addition, some mutant ascospores were budding inside the asci. Furthermore, we showed that expression of the wide type or edited allele of *FgAMA1* but not un-editable allele rescued the *Fgama1* defects, and the three-prime untranslated region (3'-UTR) was essential for the function of *FgAMA1*. Overall, our results indicate that *FgAMA1* is dispensable for meiosis but important for ascosporogenesis in *F. graminearum*.

Reference

[1] Cooper, K. F.*et al*. Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosome in yeast. (2000). *PNAS* **97(26)**, 14548–14553.

[2] Liu, H. *et al*. Genome-wide A-to-I RNA editing in fungi independent of ADAR enzymes. (2016). *Genome Research* **26**, 499-509.

Lysine acetylation functions in DON production in *Fusarium graminearum*

Jiaxing Qin[a], Cong Jiang[b], Shanyue Zhou*[a]

[a]*College of Agronomy and Plant Protection, The Key Lab of Integrated Crop Pests Management of Shandong Province, Qingdao Agricultural University, Qingdao, Shandong 266109*

[b]*State Key Laboratory of Crop Stress Biology for Arid Aeras, Northwest A&F University, Yangling, Shaanxi 712100*.

Fusarium graminearum is a disastous fungal pathogen which causes Fusarium head blight (FHB) disease on wheat, barley and other small grain cereals. In addtion to severe yield loss and quality damage, the pathogen produces trichothecene mycotoxin deoxynivalenol (DON) in the course of plant infection which poses a threat to the health of human and animals.

Bioinformatics analysis revealed that a gene *FgHAT*1 is pridicted to ecode a GCN5

acetyltransferase in *Fusarium graminearum* and there is one conserved bromo-domain in the C-terminal of the amino-acid sequence. To investigate the functions of the gene in the pathogen, the gene was deleted by split-PCR. Assayed with competitive ELISA, no DON was produced by the gene deletion mutant. As a conserved protein modification, lysine acetylation has been well demonstrated to play important roles in cellular metabolic pathways. So we deduce that DON production might be regulated by lysine acetylation in *Fusarium graminearum*. To confirm our deduction, we performed a global acetylome comparation between the gene deletion mutant and the wild type strain PH-1. The results showed that there are 93 acetylated proteins differentially expressed, among which 50 are up-regulated and 43 are down-regulated in the gene deletion mutant. And futher, GO analysis showed that most of the differential acetylated proteins were involved in metabolic process and cellular process. In KEGG pathway analysis, most of the proteins were mapped into the carbon metabolism, glycolysis /gluconeogenesis, the tricarboxylic (TCA) cycle and pyruvate metabolism.

The results of the acetylome analysis are consistent with our deduction which indicated that lysine acetylation plays crucial role in DON production in *Fusarium graminearum*.

*Corresponding author: zhoushanyao@126.com.

Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi

Yina Jiang[1], Wanxiao Wang[1], Qiujin Xie[1], Na Liu[2,3], Lixia Liu[1], Dapeng Wang[1], Xiaowei Zhang[1], Chen Yang[1], Xiaoya Chen[1], Dingzhong Tang[2,3], Ertao Wang[1*]

[1] National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032,

[2] Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Fujian Agriculture and Forestry University, Fuzhou 350002,

[3] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101,

** For correspondence author*

Presenting author (underlined) E-mail: yinajiang@alyun.com

Arbuscular mycorrhiza (AM) formation is a widespread symbiotic interaction between 80-90% of land plants and soil fungi. The plant benefits from enhanced inorganic nutrient supply

mediated by the fungal hyphae network in the soil. In return, the fungi draw organic nutrients from the plant. Organic nutrients are thought to be supplied primarily in the form of sugars. However, within the fungus, most carbon is stored in lipids that are transported throughout the mycelium. Here we show that the AM fungus Rhizophagus irregularis is a fatty acid auxotroph and fatty acids synthesized in the host plant are transferred to the fungus during AM symbiosis. We find that the transfer is dependent on the RAM2 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 2) and peri-arbuscular membrane-localized ATP binding cassette transporter-mediated plant lipid export pathway. We further show that fatty acids synthesized in plants also can be transferred to the pathogenic fungus *Golovinomyces cichoracerum*. Plants defective in fatty acid biosynthesis are impaired in AM symbiosis and show defects in colonization by the pathogenic *G. cichoracerum*. Given the abundance of lipids in *R. irregularis*, we suggest that the AM fungus reprograms its host plant to secure fatty acids as a carbon source, and that a pathogenic fungus similarly recruits the fatty acid biosynthesis program to facilitate host invasion.

References:

[1] YN Jiang, WX Wang, QJ Xie, N Liu, LX Liu, DP Wang, XW Zhang, C Yang, XY Chen, DZ Tang, ET Wang. (2017) Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science*.DOI: 10.1126/ (*Science* 08 Jun 2017: eaam9970).

Function of the Ss-SFH1 transcription factor in *Sclerotinia sclerotiorum*

Ling Liu, Qiaochu Wang, Hongyu Pan

College of Plant Sciences, Jilin University, Changchun, 130062

E-mail of presenting author: panhongyu@jlu.edu.cn

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic plant pathogen with a worldwide distribution. Here, a gene named *Ss-Sfh1* was cloned in *S. sclerotiorum*, which encodes a snf5-box-containing protein[1]. The predicted Ss-SFH1 protein also has a GATA Zn-finger domain in a C-terminal region. To investigate the role of Ss-SFH1 in *S. sclerotiorum*, the partial sequence of Ss-SFH1 was cloned and RNA interference (RNAi)-based gene silencing method was employed to alter the expression of Ss-SFH1. RNA silenced mutants Ss-SFH1

RNA levels reduced exhibited slow hyphal growth. Appressoria and oxalic acid accumulation were reduced in *Ss-Sfh1* RNAi mutants. Disease assays demonstrated that pathogenicity in RNAi-silenced strains was significantly compromised with the development of a smaller infection lesion on soybean leaves. In addition, *Ss-Sfh1*-silenced transformants were more tolerant to oxidative stress compared with the wild-type strain[2]. Furthermore, the expression levels of putative probably involved in ROS production-related genes were significantly different in silenced strains[3]. All the results suggest that Ss-SFH1 is involved in hyphal growth, virulence and the tolerance to oxidative stress in *S. sclerotiorum*.

Acknowledgement: This work was financially supported by the National Natural Science Foundation of China (31271991, 31471730).

References:

[1] Ghosh R. and Campos, MK (2015). Mol Biol Cell.26(9):1764-81.

[2] Shi L and Gong L.(2015)Fungal Genet Biol. 81:201-11

[3] Moriwaki, A and Kubo, E. (2006).FEMS Microbiol. Lett. 257, 253–261.

Gene differential expression analysis of the interaction between *Sclerotinia sclerotiorum* and *Glycine max*

Qiaochu Wang, Hailong Cheng, Ling Liu, Jinliang Liu, Yanhua Zhang, Xianghui Zhang, Hongyu Pan

College of Plant Sciences, Jilin University, Changchun,

E-mail of presenting author: panhongyu@jlu.edu.cn

Sclerotinia sclerotiorum(Lib.) de Bary is a filamentous pathogenic fungus. It has broad host range and causes substantial losses of important crops. In this study, a transcriptional factor Ss-Nsd1 gene from *S. sclerotiorum* was cloned, then *Ss-Nsd1* gene knock-out mutant was obtained. Infection cushion was not found in *Ss-Nsd1* knock-out mutant, meanwhile the mutant lost the ability to invade the host plant. However the secretion of toxins such as oxalic acid was not affected.

To illustrate the forming pathway of infection cushion and find functional genes in primary infection, RNA-seq technique was used to search differentially expressed genes. Fourteen soybean vavieties were screened, and susceptible variety Soybean No.1 and the resistant variety Jennon 28 were selected as experimental hosts for this study. In initial

formation period the infection cushion was found in 24h after the infection. The strains of wild type UF-70 and the mutant ΔSs-Nsd1 infected two soybean vavieties in 24h and 48h respectively, the RNA-sequencing was proceeded.

The results of transcriptional analysis showed that there were 516 differentially expressed genes in the initial infection stage of *S. sclerotiorum*, in which 293 genes related to molecular function. Among 293 genes, there are 23 transmembrane transporters, 28 zinc ion bindings, 26 dehydrogenases, 3 ligases, 29 transferases, 2 peroxidase, 14 transcription factors and so on; Among 516 differentially expressed genes, there are 278 biological process related genes, 245 metabolic related genes, 106 cellular components related genes including 42 membrane composition related genes. On the basis of these results, virulent genes of *S. sclerotiorum* were deeply excavated from the hydrolytic enzymes, detoxification, secondary metabolites biosynthesis, oxalic acid production, and generation of reactive oxygen species.

Acknowledgement: This work was financially supported by the National Natural Science Foundation of China (31471730).

References:

[1] J.T Alkooranee, T.R Aledan, Xiang J et al(2015). American Journal of Plant Sciences,6,1662.
[2] D Liberti, JA Rollins, K.F Dobinson(2013). Molecular Plant-Microbe Interactions,26,768.

Screening and identification proteins that interact with transcription factor SsMCM1 in *Sclerotinia sclerotiorum*

Tingtao Xu, Xiaoli Liu, Ling Liu, Qiaochu Wang, Jinliang Liu, Xianghui Zhang, Hongyu Pan, Yanhua Zhang

College of Plant Sciences, Jilin University, Changchun 130062,

E-mail of presenting author: yh_zhang@jlu.edu.cn

The *Sclerotinia sclerotiorum* (Lib.) de Bary is one of agriculture's most devastating necrotrophic fungal plant pathogens. It infects 408 different plant species from 278 genera encompassing 75 plant families and causes unrestricted lesion development and tissue maceration of its hosts[1].

One family of transcription factors that are well conserved in eukaryotic organisms is MADS-box proteins. Its founding members are MCM1 (yeast), Agamous (plant), Deficiens (Drosophila) and serum response factor (SRF, human) [2]. The putative *SsMCM1* gene was cloned in our lab, and it was highly similar to the orthologues *S. cerevisiae* Mcm1, including a conserved DNA-binding domain. SsMCM1 function was investigated using RNA interference.

Our results suggest that the MADS-box transcription factor SsMCM1 is involved growth and virulence in *S.sclerotiorum*[3].

Considering its importance, SsMCM1 may also interact and form heterodimers with other protein in *S. sclerotiorum* to regulate growth and virulence. Yeast two-hybrid and Bimolecular Fluorescent Complementary have been applied to identify proteins that physically associate with protein SsMCM1 in *S.sclerotiorum*. 12 putative SsMCM1 interacting proteins were identified. SsIP37 contains the Pmp3 family domain; SsIP43 contains the PGM3 family domain; SsIP44 contains the Mpv17-PMP22 domain; SsIP86 contains the Catalase domain; SsIP98 contains the Cyclin domain; SsIP106 contains the RGL11 domain. The other interacting proteins function are unknown. SsCdc28 (SSIG_02296) was cloned which highly homologous to yeast Cdc28. Yeast two-hybrid assays indicating that Cdc28 physically interacts with SsIP86. This study clarified the interaction between SsMCM1-SsIP86-SsCdc28. It will be important to reveal the regulatory network and regulation mechanism of SsMCM1 transcription factor in *S. sclerotiorum*.

Acknowledgement: This work was supported in part by the National Natural Science Foundation of China (No.31101394, 31471730).

References:

[1] Dickman, M.B, Fluhr, R (2013). Annu. Rev. Phytopathol, 51, 1-25.

[2] Sebastian Soyk, Zachary H (2017). Cell, 169, 1–14.

[3] Qu X, Yu B (2014). International Journal of Molecular Sciences, 15, 8049-8062.

Host adaptation of the fungal effector Pit2: can we call it decoy?

Johana Misas-Villamil[1]*, André N. Mueller [2]*, Ute Meyer[1], Helen Dauben[1], Fatih Demir [3], Pitter Huesgen[3], Gunther Doehlemann[1,2]

[1] *Botanical Institute and Cluster of Excellence on Plant Sciences, University of Cologne, Cologne, Germany.*

[2] *Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.*

[3] *Central Institute for Engineering, Electronics and Analytics, Forschungszentrum Jülich, Jülich, Germany.*

* *Equal contribution, for correspondence: jmisas@uni-koeln.de*

Ustilago maydis is a biotrophic fungus responsible of the corn smut disease in maize. To manipulate its host *U. maydis* secretes a set of effectors into the extracellular interface aiming to downregulate immune responses and so achieving a successful colonization. One of those effectors is Pit2, a secreted cysteine protease inhibitor essential for maintenance of biotrophy

[1]. Pit2 contains a 14 amino acids protease inhibitor domain (PID14) that itself can inhibit cysteine proteases and is essential for fungal virulence [2]. The closest related barley pathogen *U. hordei* contains a Pit2 ortholog with only low overall sequence similarity to UmPit2 but a conserved PID14 motif. *In vitro* experiments showed that UhPID14 has the potential to inhibit maize cysteine protease activity although its efficiency is reduced compared to UmPID14. Remarkably, *U. maydis* Pit2 deletion mutants complemented with UhPit2 cannot rescue the tumor formation phenotype. This phenotype could be explained through a host specificity function of Pit2. In maize, UmPit2 is cleaved in small peptides by apoplastic cysteine proteases, probably releasing the inhibitory portion from the PID14 motif. After cleavage, the PID14 inhibitory domain might be in close proximity to the cysteine protease achieving faster and enhanced inhibition. In this manner Pit2 performs as a trap, first acting as a substrate and then as an inhibitor to efficiently block immune responses. In contrast, UhPit2 is stable in the maize apoplast which might explain the lower suppression of cysteine protease activity *in vivo*. These experiments indicate that Pit2 effector performance is driven by at least two evolutionary steps during host adaptation: 1) Pit2 evolves the capacity to inhibit apoplastic cysteine proteases and 2) Pit2 exploits host proteases to improve their inhibition.

References:

[1] Doehlemann G, Reissmann S, Aßmann D, Fleckenstein M, Kahmann R (2011).Two linked genes encoding a secreted effector and a membrane protein are essential for *Ustilago maydis*-induced tumour formation. MolMicrobiol 81: 751–766.

[2] Mueller AN, Ziemann S, Treitschke S, Aßmann D, Doehlemann G (2013) Compatibility in the *Ustilago maydis*–maize interaction requires inhibition of host cysteine proteases by the fungal effector Pit2. PLoS Pathogens 9: e1003177.

The *Fusarium oxysporum* Avr2-Six5 effector pair alters exclusion selectivity of plasmodesmata.

Lingxue Cao, Ben J.C. Cornelissen and Frank L. W. Takken

Molecular Plant Pathology, Swammerdam Institute for Life Science, University of Amsterdam, Amsterdam, 1098 XH, Netherlands,

f.l.w.takken@uva.nl

Pathogens use effector proteins to manipulate their hosts. During infection of tomato the fungus *Fusarium oxysporum* secretes the effectors Avr2 and Six5. Whereas Avr2 suffices to trigger *I-2*-mediated cell death in heterologous systems, both effectors are required for

I-2-mediated disease resistance in tomato. How Six5 participates in triggering resistance is unknown. Using BiFC assays we found that Avr2 and Six5 interact at plasmodesmata. Single-cell transformation revealed that a 2xRFP marker protein and GFP-tagged Avr2 translocate only to neighbouring cells in the presence of Six5. Six5 alone does not alter plasmodesmal transductivity as 2xRFP was only translocated in the presence of both effectors. In *SIX5*-expressing transgenic plants the distribution of virally expressed Avr2-GFP, and subsequent onset of *I-2*-mediated cell death, differed from that of wild type tomato. Together these data imply that Six5 functions as a fungal movement protein; by aiding translocation of Avr2 it contributes to virulence in susceptible plants but induces resistance in the presence of *I-2*.

A basidiomyceteous fungi-specific *PsCaMKL1* encoding a CaMK-like protein kinase is required for the virulence and oxidative tolerance of *Puccinia striiformis* f. sp. *tritici*

Min Jiao, Dan Yu, Chenglong Tan, Jia Guo, Dingyun Lan, Ershang Han, Tuo Qi, Ralf Thomas Voegele, Zhensheng Kang, Jun Guo*

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi,

E-mail: 413498711@qq.com; guojunwgq@nwafu.edu.cn

Calcium/calmodulin-dependent kinases (CaMKs) are Ser/Thr protein kinases (PKs) that respond to changes in cytosolic free Ca^{2+} and play diverse roles in eukaryotes. In fungi, CAMKs are generally classified into four families CAMK1, CAMKL, RAD53 and CAMK-Unique. Among these, CAMKL is the largest family. In some fungal plant pathogens few CaMKLs have been shown to be responsible for pathogenesis, but little is known about their roles in rust fungi. In this study, we functionally characterized a novel PK gene, *PsCaMKL1*, from *Puccinia striiformis* f. sp. *tritici* (*Pst*). PsCaMKL1 belongs to a group of PKs that are evolutionarily specific to basidiomyceteous fungi. It shows a highly conserved intra-species polymorphism and cytoplasmic localization in wheat protoplasts. *PsCaMKL1* transcripts are highly induced at early infection stages, whereas are down-regulated in barely germinated urediospores. Overexpression of *PsCaMKL1* in fission yeast increased resistance

to environmental stresses. Knock down of *PsCaMKL1* using host-induced gene silencing (HIGS) reduced the virulence of *Pst* accompanied by enhanced reactive oxygen species (ROS) accumulation and a hypersensitive response. These results suggest that *PsCaMKL1* is a novel pathogenicity factor that exerts it virulence function by regulating ROS production in wheat.

Acknowledgements: This study was financially supported by the National Basic Research Program of China (No. 2013CB127700), the National Natural Science Foundation of China (No. 31371889 and 31171795), and the 111 Project from the Ministry of Education of China (No. B07049).

Host-induced gene silencing of an important pathogenicity factor *PsCPK1* in *Puccinia striiformis* f. sp. *tritici* enhances resistance of wheat to stripe rust

Tuo Qi, Xiaoguo Zhu, Chenlong Tan, Peng Liu, Jia Guo, Zhensheng Kang, Jun Guo*

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi

E-mail: 617669891@qq.com; guojunwgq@nwafu.edu.cn

Rust fungi are devastating plant pathogens and cause a large economic impact on wheat production worldwide. To overcome this rapidly loss of varieties resistance, we generated stable transgenic wheat plants expressing short interfering RNAs (siRNAs) targeting potentially vital genes of *Puccinia striiformis* f. sp. *tritici* (*Pst*). Protein kinase A (PKA) has been proved to play important roles in regulating the virulence of phytopathogenic fungi. *PsCPK1*, a PKA catalytic subunit gene from *Pst*, is highly induced at the early infection stage of *Pst*. The instantaneous silencing of *PsCPK1* by *barley stripe mosaic virus* (BSMV)-mediated host-induced gene silencing (HIGS) results in a significant reduction in the length of infection hyphae and disease phenotype. These results indicate that *PsCPK1* is an important pathogenicity factor by regulating *Pst* growth and development. Two transgenic lines expressing the RNA interference (RNAi) construct in a normally

susceptible wheat cultivar displayed high levels of stable and consistent resistance to *Pst* throughout the T_3 to T_4 generations. The presence of the interfering RNAs in transgenic wheat plants was confirmed by northern blotting, and these RNAs were found to efficiently down-regulate *PsCPK1* expression in wheat. The present study addresses important aspects for the development of fungal-derived resistance through the expression of silencing constructs in host plants as a powerful strategy to control cereal rust diseases.

Acknowledgement: This work was supported by National Basic Research Program of China (No. 2013CB127700) and National Natural Science Foundation of China (No. 31371889；NO. 31171795).

A nucleus-localized effector from *Phytophthora* hijacks a host histone acetyltransferase to enhance plant susceptibility

Haiyang Li [a], Haonan Wang [a], Maofeng Jing [a], Jinyi Zhu [a], Baodian Guo [a], Yang Wang [a], Yachun Lin [a], Han Chen [a], Zhenchuan Ma [a], Yan Wang [a], Wenwu Ye [a], Suomeng Dong [a], Yuanchao Wang [a]

[a] *Department of Plant Pathology, Nanjing Agriculture University, Nanjing, 210095*

2014202015@njau.edu.cn

Abstract: Filamentous fungi and oomycetes pathogens secrete various of intracellular effectors to manipulate host immunity during infection. Identification of plant targets of these effectors will help to uncover the mechanisms on how effectors suppress PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) in plants. Previously we have determined that PsAvh52, a RxLR effector secreted from soybean root rot pathogen *Phytophthora sojae*, can suppress the cell death induced by both the effectors and PAMPs in *Nicotiana benthamiana*. Here, we demonstrated that PsAvh52 interacts with a soybean histone acetyltransferase (GmHAT1), a key factor manipulating epigenetic modifications. GmHAT1 localizes in plant cell cytoplasm, but it is re-localized from the cytoplasm to nucleus when co-expressed with PsAvh52. The nucleus-localized GmHAT1 promotes the expression of plant susceptible genes and enhances susceptibility to plant disease by

increasing the level of histone acetylation. Taken together, these results indicate that PsAvh52 manipulates epigenetic modifications to enhance *Phytophthora sojae* colonization in soybean.

Key words: *Phytophthora sojae*, effector, plant susceptibility, epigenetic modifications

A *Phytophthora* effector manipulates host histone acetylation and reprograms defense gene expression to promote infection

Liang Kong[1], Xufang Qiu[1], Jiangang Kang[1], Yang Wang[1], Han Chen[1], Jie Huang[1], Min Qiu[1], Yao Zhao[1,2], Guanghui Kong[1], Zhenchuan Ma[1], Yan Wang[1,3], Wenwu Ye[1,3], Suomeng Dong[1,3,]*, Wenbo Ma[2] and Yuanchao Wang[1,3,4,]*

[1]*Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095,*

[2]*Department of Plant Pathology and Microbiology, University of California, Riverside, California, CA 92521, USA*

Abstract: Immune response during pathogens infection requires extensive transcription reprogramming. A fundamental mechanism of transcriptional regulation is histone acetylation. However, how pathogens interfere with this process to promote disease remains largely unknown. Here, we demonstrate that the cytoplasmic effector PsAvh23 produced by the soybean pathogen *Phytophthora sojae* acts as a modulator of histone acetyltransferase (HAT) in plants. PsAvh23 binds to the ADA2 subunit of the HAT complex SAGA and disrupts its assembly by interfering the association of ADA2 with the catalytic subunit GCN5. As such, PsAvh23 suppresses H3K9 acetylation mediated by the ADA2/GCN5 module and increases plant susceptibility. Expression of *PsAvh23* or silencing of *GmADA2*/*GmGCN5* resulted in mis-regulation of defense-related genes, likely due to decreased H3K9 acetylation levels at the corresponding loci. This study highlights an effective counter-defense mechanism by which a pathogen effector suppresses the activation of defense genes by interfering with the function of the HAT complex during infection.

Population structure of *Fusarium* species from cereal crops in China

Jianhua Wang[a,b], Jingbo Zhang[b], Mbacke Ndoye[b], Heping Li[b], Yucai Liao[b].

[a] *Institute for Agri-Food Standards and Testing Technology, Shanghai Academy of Agricultural Sciences, 1000 Jinqi Road, Shanghai 201403,*

[b] *Molecular Biotechnology Laboratory of Triticeae Crops and College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070*

jianhuawang163@163.com

Fusarium species can infect wheat, maize and other cereal grain crops, is an economically important plant pathogenic fungus in China and throughout the world. More importantly, *Fusarium* mycotoxins accumulated in infested grains pose a serious health threat to humans and animals. Diseased maize ears and wheat spikes were collected from fields during the growing season in China. Fungal colonies displaying morphological characteristics of *Fusarium* spp. were purified by the single-spore technique and characterized at the species level by morphological observations and *translation elongation factor 1-α* (*TEF*) gene sequencing. In total, 1651 *Fusarium* strains were isolated and these strains were identified to 20 *Fusarium* species. The results indicated that the *Fusarium graminearum* clade were predominant on wheat, while *F. verticillioides* was the predominant one on maize in China. In this study, we first isolated *F. temperatum*, *F. kyushuense*, *F. andiyazi*, *F. anthophilum*, *F. lacertarum*, *F. sacchari*, *F. meridionale*, *F. commune* and *F. boothii* from maize, while *F. sacchari* was found to be new recorded *Fusarium* specie from wheat in China. The population structure analysis of the pathogen is very important for the plant-pathogen interaction research, pathogen and mycotoxins control. The report contributes to an improved understanding of the composition of *Fusarium* species on wheat and maize in China, which will be useful for exploring appropriate disease management strategies in the field.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (31401598), National Basic Research Program of China (Grant 2013CB127801), Shanghai Agriculture Commission Basic Research Program (2014NO.7-3-7).

Isolation and Identification of Agriculture Beneficial Microorganisms with Multifunctional Roles in Contaminated Farmland

Wen Tang[a], Pin Su[a], Yong Liu[a], Deyong Zhang[a]

[a]*Hunan Academy of Agricultural Sciences, Hunan Plant Protection Institute, Changsha ,*
wiwitina@163.com

Abstract: In current agriculture, many farmlands are contaminated with industrial e-ffluents, pesticide residues, and chemical fertilizers[1,2]. As a result, current biological control agents are facing multiple challenges coming from pest management and soil quality improvement[3]. Microorganisms with properties of disease management, growth promotion, and environmental amendments are urgently desired in many contaminated areas. Therefore, we carried out the isolation and identification of agriculture benefici-al microorganisms with multifunctional roles which meet requirements of farming in c-ontaminated areas. In combination with the traditional methods of the microorganism isolation, part of rhizospheric soil samples were obtained from health plant in disease occurred fields. Particularly, others samples were obtained from microbial diversity lan-ds including the forest of National Nature Reserve. Isolated bacterial strains were emp-loyed individually with the *Magnaporthe oryzae* and *Athelia rolfsii* under the antagoni-stic experiment to identify strains significantly inhibiting pathogens. An effective strain was screened by dual experiment. Currently, we isolated 110 bacterial strains from th-e rhizospheric soil, sixes strains have antagonistic with *Magnaporthe oryzae*, which inhibited mycelial growth as to dead, and one strain has antagonistic with *Athelia rolfsii*, which inhibited mycelial growth as the same.

Keywords: Biological control; Rhizospheric microorganism; Rice Blast and Southern Blight; Contaminated farmlands.

References:

[1] Jiemin.Cheng, Ying Zhang, Yan Wang. Potential Environmental Problems Resulted from Contaminated Farmland and Solution for Land Consolidation in China(2016).Transactions of the Chinese Society of Agricultural Engineering.

[2] Leo Horrigan, Robert S Lawrence, Polly Walker. How Sustainable Agriculture Can Address the Environmental and Human Health Harms of Industrial Agriculture(2002).Environmental Health Perspectives,110(5):445-456.

[3] S.Tingay, A.Mitchell, J.Kochman.(2002). Prospects for Disease Biocontrol(2002). The Australian Cottongrower,23(1).

A conserved host target of oomycete Avr3a family effectors negatively regulates plant resistance to *Phytophthora*

Tingting Li [a], Qinhu Wang [a], Guangjin Fan [a], Ruirui Feng [a], Weiwei Li [a], Meixiang Zhang[a], Yu Du [a], Brett M. Tyler [b], Weixing Shan [a,*]

[a] *State Key Laboratory of Crop Stress Biology for Arid Areas and College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100,*

[b] *Center for Genome Research and Biocomputing and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.*

Presenting author: tingting.li@nwafu.edu.cn

** Corresponding author: wxshan@nwafu.edu.cn*

Many crops are destroyed by the destructive oomycetes [1]. It is known that these pathogens secrete hundreds of RXLR effectors to manipulate host immunity [2]. However, whether some conserved host proteins are targeted by the pathogen effectors remains unclear. In this study, by independent bimolecular fluorescence complementation, yeast two-hybrid, and co-immunoprecipitation experiments, we showed that the plant RIP1 (RXLR-effector Interacting Protein 1) was conservatively targeted by *Phytophthora* Avr3a family effectors. Phylogenetic analysis revealed that the RIP1 sub-family proteins were fast evolving in plants. Besides, both qPCR and GUS reporter assays showed that *RIP1* was heavily induced during the *Arabidopsis*-*Phytophthora* interaction. To investigate the role of *RIP1* in plant defense to *Phytophthora* attacks, we overexpressed *AtRIP1* in *Arabidopsis thaliana* and silenced *NbRIP1* in *Nicotiana benthamiana*. And subsequently inoculation analysis showed that *RIP1* negatively regulated disease resistance of host plants to *P. parasitica*, *P. capsici*, and *P. infestans*. Cell death assays showed that *RIP1* could suppress *INF1*-triggered cell death (ICD), and was necessary for the ICD - suppression activity of Avr3a^{KI}. Western blot results showed that Avr3a could increase accumulation of RIP1 protein. In conclusion, our results show that a negative regulator of host resistance, RIP1, is conservatively targeted by Avr3a family effectors, possibly through stabilizing its accumulation to suppress plant basal defense.

References

[1] W.E. Fry, P.R.J. Birch, H.S. Judelson, et al. (2015). Phytopathology 105, 966.

[2] R.G. Anderson, D. Deb, K. Fedkenheuer, et al. (2015). Mol Plant Microbe In 28, 1063.

MoLeu1-mediated leucine synthesis pathway is required for asexual development and pathogenicity of *Magnaporthe oryzae*

Haolang Jiang[a], Wei Tang[a], Qiaojia Zheng[a], Guiyuan Zhao[a], Jiao Liu[a], Rufeng Wang[a], Zonghua Wang[a,b]

[a] *State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002.*

[b]*Minjiang University, Fuzhou 350002*

Rice blast, caused by the filamentous phytopathogen *Magnaporthe oryzae*, is one of the most destructive diseased of rice worldwide. Recent studies have revealed that leucine is not only severed as fundamental substrates for proteins synthesis but also important for initiating signal transduction pathways that modulate translation initiation. Isopropylmalate isomerase, encoded by *LEU1*, catalyzes the conversion of α-isopropylmalate to β-isopropylmalate which is an essential stage for leucine biosynthesis in budding yeast. In this study, we functionally characterized the Leu1 homologous protein MoLeu1 in *Magnaporthe oryzae* by generating its knock-out mutants. Compared to the mycelial stage, *MoLEU1* exhibited much higher transcription levels in the conidial and infectious stages. Deletion of *MoLEU1* led to obvious defects on hyphal growth, sporulation and pathogenicity on rice. Moreover, the Δ*Moleu1* mutant was unable to sustain hyphae growth on MM medium. Exogenous leucine fully restored vegetative growth and partially restored conidiation, appressorium formation and pathogenicity. We found that MoLeu1 was localized to the cytoplasm at different stages of fungal development. Taken together, our results showed that MoLeu1 is required for vegetative growth, conidiation, appressorium formation, pathogenicity and leucine biosynthesis.

The distribution of *Rhizoctonia cerealis* Vander Hoeven in different growth stages of Wheat

Jin Jing-jing, Li Hai-yan, Ma Lu-lu, Jia Wei, Qi Yong-zhi, Zhen Wen-chao

College of Plant Protection, Agricultural University of Hebei, 2596#, Lekai South street, Nanshi District, Baoding 071001, Hebei Province

Abstract: In order to definite the distribution of *Rhizoctonia cerealis* (RC) in different growth

stages of wheat, the biomass of RC in wheat tissues which collected from Baoding and Xinji experiment stations were determined by the QPCR technology. The results showed that the distribution of RC in wheat tissues was similar between Baoding and Xinji. From the whole growth stage of wheat, the biomass of RC in whole plant was the lowest at tillering stage. Then, it increased obviously. Compared to that before overwintering, the biomass of RC at returning green stage increased by 202.7%.The RC was 3909 nanogram per gram dry tissue at jointing stage，which was 13 times higher than that at tillering stage. Plant tissues obviously accelerated the aging of wheat tissues at heading stage and the expansion of RC on wheat tissues was inhibited, with 3% decline. In addition, the biomass of RC in different wheat tissues at each growth stage was also analyzed. During tillering stage and before overwintering stage, the biomass of RC was distributed mainly in the first leaf sheath of the main stem and the radio was about 33% of the total biomass of RC in whole wheat tissues. RC mainly infected subcrown internodes at returning green stage for the lack of aboveground wheat tissues. The second leaf sheaths of the main stem and tillers were mainly infected at the erecting stage. Although the spring-grown leaves and there were internodes had grown at jointing stage, it still has 44% of the total biomass of RC distributed in the leaf sheaths of leaves growing before winter. When it came to heading stage, 21% of the total RC in wheat tissues transferred to the leaf sheath of the third spring-grown leave of the main stem, the leaf sheaths of the second spring-grown leave of the tillers, and the first internodes of the main stem and tillers.

Key words: Rhizoctonia cerealis Vander Hoeven, growth stages of Wheat, QPCR technology

Acknowledgement: This study was funded by the "Natural Science Foundation of Hebei Province, China (C2016204211) and Science，Technology Research Project of higher education institutions of Hebei Province, China (ZD2016162)，and the Key Technologies R&D Program of China during the 12th Five-Year Plan period (2013BAD07B05)"

Email:jingjing1809@yeah.com
Correspondence: qiyongzhi1981@163.com, wenchao@hebau.edu.cn

Characterization of VdASP F2 secretory factor from *Verticillium dahliae* by a fast and easy gene knockout system

Chengjian Xie[a,b], Xingyong Yang[a, b]*

[a]*School of Life Sciences, Chongqing Normal University, Chongqing 401331*

[b]*The Chongqing Key Laboratory of Molecular Biology of Plant Environmental Adaptations, Chongqing Normal University, Chongqing 401331*

** corresponding author*

Tel: +86-13452957948; E-mail: yangxy94@swu.edu.cn

Abstract:

The vascular wilt fungus *Verticillium dahliae* produces persistent resting structures known as microsclerotia, which enable long-term survival of this plant pathogen in soil. The completed genome sequence of *V. dahliae* has facilitated large-scale investigations of individual gene functions using gene-disruption strategies based on *Agrobacterium tumefaciens*-mediated transformation (ATMT). However, the construction of gene-deletion vectors and screening of deletion mutants have remained challenging in *V. dahliae*. In this study, we developed a fast and easy gene knockout system for *V. dahliae* using ligation-independent cloning and fluorescent screening. We identified secretory factor VdASP F2 in a T-DNA insertion library of *V. dahliae* and deleted the *VdASP F2* gene using the developed knockout system. Phenotypic analysis suggests that *VdASP F2* is not necessary for *V. dahliae* growth on potato dextrose agar under various stress conditions. However, on semisynthetic medium or under limited nutrient conditions at lower temperatures, the *VdASP F2* deletion mutant exhibited vigorous mycelium growth, less branching, and a significant delay in melanized microsclerotial formation. Further assessment revealed that *VdASP F2* was required for the expression of *VDH1* and *VMK1*, two genes involved in microsclerotial formation. Cotton inoculated with the *VdASP F2* deletion mutant wilted, demonstrating that *VdASP F2* is not associated with pathogenicity under normal conditions. However, after inducing microsclerotial formation and incubation at low temperatures, cotton infected with the *VdASP F2* deletion mutant did not exhibit wilt symptoms. In conclusion, our results show that *VdASP F2* plays an important role in the response of *V. dahliae* to adverse environmental conditions

and is involved in a transition to a dormant form for prolonged survival.

Sorting nexin (MoVps17) is required for fungal development and plant infection by regulating endosome dynamics in the rice blast fungus

Huawei Zheng[a], Zhongkun Guo[a], Yang Xi[a], Mingyue Yuan[a], Yahong Lin[a], Congxian Wu[a], Yakubu Saddeeq Abubakar[a], Xianying Dou[a], Guangpu Li[b], Zonghua Wang[a,c*], Wenhui Zheng[a*], Jie Zhou[a*]

[a] *State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou*

[b]*Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, USA*

[c]*College of Ocean Science, Minjiang University, Fuzhou, 350108*

jiezhou@fafu.edu.cn, wenhuiz@126.com, wangzh@fafu.edu.cn

Vps17 is a sorting nexin (SNX) and a component of the retromer which is involved in protein trafficking. To date, the role of such intracellular trafficking events in development and pathogenicity of the rice blast fungus (*Magnaporthe oryzae*) remains unclear. We investigate the functional relationship between the SNX and the cargo-selective complex (CSC) of the fungal retromer by genetic analysis, live cell imaging and immunological assay. Our data show that the MoVps17 null mutation causes defective in growth, development, and pathogenicity in *M. oryzae*. MoVps17 is localized to endosomes, which depends on phosphatidylinositol 3-kinase (PI3K) activity and PI3K activity plays an important role during fungal development and infection. The PX and BAR domains are both essential for the endosomal localization and function of MoVps17. We further show that MoVps17 and MoVps5 can interact with each other in yeast two-hybrid assays, but not directly interact with CSC components. Live cell imaging suggests that MoVps17 can regulate fusion and budding of early endosomes and endocytosis. Taken together, our results suggest that retromer SNX MoVps17 show specificity function with CSC and demonstrate that MoVps17 plays an important role in regulation of endosome dynamics in phytopathogens, which is required for fungal development and plant infection.

Exploring the functional relationship between MoYpt7 and MoVps35

Congxian Wu[a], Jie Zhou[a]
[a] *State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou*

jiezhou@fafu.edu.cn

Rab GTPases and retromer complex are important transport-related proteins in eukaryotic cells. It hasbeen shown that Rab7 (Ypt7) interacts with receptor-selection subunit of retromer complex Vps26/Vps29/Vps35in *Saccharomyces cerevisiae* and mammalian cells which indicate the retromer trimer subcomplex may be a downstream effector of Rab7. *Magnaporthe oryzae* is an important pathogen of graminaceous crops. Previous studies have shown that MoYpt7 and MoVps35, a core member of retromer complexare pathogenicity-related genes of *M. oryzae*, and the phenotype of these two genes knockout mutants are similar. In order to explore the relationship between this two proteins, we first conducted Co-IP experiments, which shown that MoVps35 interacts with MoYpt7. Co-expression of mCherry-MoYpt7 and MoVps35-GFP in the wild-type strain Guy11 revealed that mCherry-MoYpt7 co-localizes with MoVps35-GFP. In addition, the localization of GFP-MoYpt7 was not significantly changed in *ΔMovps35*, but the localization of MoVps35-GFP in MoYpt7 knockout mutant was changed compared to its localization in Guy11.Overexpression of MoYpt7 in *ΔMovps35* did not restore the phenotype of *ΔMovps35* mutant. Similarly, overexpression of MoVps35 in *ΔMoypt7* did not restore the phenotype of *ΔMoypt7*. Co-IP assay showed that both MoYpt7-CA (GTP-boundstates) and MoYpt7-DN (GDP-boundstates) interact with MoVps35. Taken together, these results indicated that MoYpt7 interacts with MoVps35 in rice blast fungus, but MoVps35 might not act as the downstream effector of MoYpt7. Their interaction mechanism remains to be further investigate.

Epigenetic control of transposon mobilization in *Magnaporthe oryzae*

Mingyue Shi Huakun Zheng Xiaofeng Chen Zonghua Wang
Fujian University Key Laboratory for Functional Genomics of Plant Fungal Pathogens, Fujian Agricul ture and Forestry University, Fuzhou 350002

Abstract *Magnaporthe oryzae*, the causal agent of rice blast and wheat blast disease, is a

serious threat to global food security, while the complicated population and rapid mutation of avirulence (AVR) genes of the fungus tremendously restricts the persistantly effective diseases management. Further study on molecular mechanism underlying the rapid mutation is of great scientific and practical importance. A large amount of the transposable elements (TEs) in the genome are closely involved in the rapid mutation of the fungal AVR genes, however, the regulatory mechanism for the TE mobilization is not clear yet. Recently, DNA methylation has been proved to play a role in regulating the transcriptional activity rather than the mobilization of TEs in *M. oryzae,* indicating that the epigenetic regulation of TE mobilization is likely more complex than expected. Therefore, in this study, we will apply functional genomics and epigenetic approaches to further explore the regulatory mechanism for TE mobilization in *M. oryzae*, through analyzing the interrelation among DNA methylation, histone methylation, and small RNA-mediated DNA methylation, and their potential reciprocal action on the regulation of TE mobilization. Our results will provide new theoretical basis for understanding the rapid mutation mechanism of AVR genes in the rice blast fungus. Evaluation of the DNA methylation at the sixth position of the purine adenosine by using the modification specific antibody α-6mA. The mycelia grown in CM broth for 3 days and 5 days respectively were collected for DNA preparation, and then subjected to dot blot using the α-6mA antibody. We found that both the genome of Guy11 and FJ81278 carry the 6mA modification.

References:

1 Tamaru H, Selker E U. A histone H3 methyltransferase controls DNA methylation in Neurospora crassa.[J]. Nature, 2001, 414(6861):277-83.

2 Wicker T, Sabot F, Hua-Van A, et al. A unified classification system for eukaryotic transposable elements[J]. Nature Reviews Genetics, 2007, 10(4):276.

3 Lanciano S, Carpentier M C, Llauro C, et al. Sequencing the extrachromosomal circular mobilome reveals retrotransposon activity in plants.[J]. Plos Genetics, 2017, 13(2):e1006630.

4 Saze H, Kakutani T. Differentiation of epigenetic modifications between transposons and genes[J]. Current Opinion in Plant Biology, 2011, 14(1):81-87.

Cytoplasmic Dynein Light Intermediate Chain2 (*MoD2LIC*) Dependent Endocytosis Regulates Radial Growth, Conidio-genesis and pathogenicity in *Magnaporthe oryzae*

Lili Lin[1,2#], Justice Norvienyeku[1,2#], Waheed Abdul[2], Sami Rukaiya Aliku[1], Frankine Jagero Otieno[2], Xiaomin Chen[1], Zonghua Wang[1,2,3*]

1. State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, 350002

2. Fujian-Taiwan Joint Center for Ecological Control of Crop Pests and College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, 350002

 # These authors contributed to this work equally.
3. College of Ocean Science Minjiang University, Fuzhou, 350108
** To whom correspondence should be addressed. Email: wangzh@fafu.edu.cn.*

Magnaporthe oryzae is a filamentous fungus that causes rice blast and has now been used as a model organism to study the interaction between pathogenic fungi – host plant interaction. Research findings available showed that the retrograde transport system is indispensable in the parasitic life of most pathogenic fungi including the cosmopolitant destructive ascomycete fungus *M. oryzae*. Cytoplasmic dynein motor proteins facilitate the progression of the retrograde transport system by conveying cellular cargoes and protein towards cell centre. In this study, we used targeted gene deletion and overexpression techniques evaluate the cellular and pathogenicity role of Cytoplasmic Dynein Light Intermediate Chain2 (*MoD2LIC)* in the rice blast fungus. Our results showed that the growth of *MoD2LIC* deletion and over-expression mutants were significantly inhibited compared to the Guy11 wild type strain. We also observed that the *MoD2LIC* deletion mutant failed to produce conidia whilst the conidiation abilities of the *MoD2LIC* over-expression mutants were drastically reduced. We additionally showed that both *MoD2LIC* and *MoD2LIC-OE* strains lost their pathogenicity completely. These results indicate that *MoD2LIC has* an important effect on the growth and pathogenicity of *Magnaporthe oryzae* and further suggest that the activities of *MoD2LIC* is tightly regulated during morphological, reproductive and infectious development of the rice blast fungus.

Phytophthora utilizes effector to hijack plant BAG7 and bZIP28 in the ER for successful infection

Maofeng Jing[a,b], Yurong Li[c], Bo Yang[a,b], Haonan Wang[a,b], Haiyang Li[a,b], Bodian Guo[a,b], Daolong Dou[a,b], Yuanchao Wang[a,b] and Martin B. Dickman[c]

[a] *Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095,* [b] *Key Laboratory of Integrated Management of Crop Diseases and Pests, Nanjing 210095,* [c] *Department of Plant Pathology and Microbiology, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843, USA*

E-mail of presenting author: jingmf@njau.edu.cn

In order to promote colonization, *Phytophthora* pathogens secrete an array of specific effector proteins to manipulate host innate immunity. *Phytophthora* uses an essential effector Avh262 to stabilize Binding immunoglobulin Proteins (BiPs) in the ER, which act as negative regulators of plant resistance to *Phytophthora* and ER stress-triggered cell death, resulting in attenuated plant defense responses [1]. However, little is known about how *Phytophthora* hijacks the host BiPs to regulate the ER machinery for successful infection. In this work, we show that Avh262 interacts with both AtBAG7, an ER-localized cochaperone that helps maintain for the maintenance of the unfolded protein response, and bZIP28, an ER membrane-tethered transcription factor *in planta* [2,3]. The translocation of AtBAG7 and bZIP28 from the ER to the nucleus is required for activating the downstream pathways. Here we show that AtBAG7 negatively regulates plant resistance to *Phytophthora*, in a positive bZIP28, also plays a positive role. We also found that the crosstalk between PTI and ER stress pathway, which PAMPs trigger the translocation of AtBAG7 and bZIP28 from the ER to the nucleus for upregulating the expression of ER stress-associated defense genes. However, this processing can be prevented by the *Phytophthora* effector Avh262-mediated accumulation of BiPs.

References:

[1] Jing et al. (2016). Nature Communications. 7:11685.

[2] Li et al. (2016). New Phytologist. doi: 10.1111/nph.14388

[3] Williams et al. (2010). PNAS. vol. 107, 6089

A novel class of conserved effectors with ribonuclease domains is involved in virulence of phytopathogenic *Colletotrichum* fungi on plants

Naoyoshi Kumakura[a], Suthitar Singkaravanit-Ogawa [b], Pamela Gan [a], Ayako Tsushima [a, c], Mari Narusaka[d], Yoshihiro Narusaka[d], Yoshitaka Takano[b], Ken Shirasu [a, c]

[a] *Center for Sustainable Resource Science, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Japan*

[b] *Graduate School of Agriculture, Kyoto University, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto, Japan*

[c] *School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan*

[d] *Research Institute for Biological Sciences Okayama, 7549-1 Yoshikawa, Kibityuo-cho, Kaga-gun, Okayama, Japan*

naoyoshi.kumakura@riken.jp

Members of the genus *Colletotrichum* infect many commercially important crops. Previously, we sequenced and annotated the genomes of several *Colletotrichum* species. *Colletotrichum* fungi secrete small effector proteins, which are important for successful plant invasion. Bioinformatically, we identified 252 common genes expressed during infection encoding small-secreted proteins conserved in four *Colletotrichum* species. From a screen of these effectors, we identified a family of effectors encoding secreted ribonucleases (SRNs).To characterize these further, we established knock out mutants of SRN genes in *C. orbiculare* and found that they are involved in virulence of the pathogen. In *C. orbiculare*, SRN has four homologs, SRN1-4. Only quadruple mutants of SRNs, but not triple mutants, showed reduced virulence on plants, indicating that all SRNs have a redundant function. All four SRNs have a ribonuclease domain. In *N. benthamiana*, transiently expressed SRN1, 2, and 4 induced cell death. A ribonuclease catalytic residue mutated SRN2 did not induce cell death, suggesting that ribonuclease activity is required for cell death. SRN homologs are widely conserved in fungi and they are dramatically expanded in *Blumeria graminis*, a phytopathogen that causes powdery mildew on grasses [1]. Our findingstogether with those from previous studies imply that SRNsare a novel class of effectors whose function in virulence may be conserved in many fungal phytopathogens.

References:

[1] C. Pedersen, *et al*. (2012). BMC Genomics, 12, 694

MoHrd1-mediated endoplasmic reticulum-associated degradation pathway (ERAD) is required for appressorium development and pathogenicity of *Magnaporthe oryzae*

Wei Tang[1], Haolang Jiang[1], Qiaojia Zheng[1], Xuehang Chen[1], Zonghua Wang[1*]

[1]: State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002

E-mail of presenting author: tangw@fafu.edu.cn

Most of membrane and secretory proteins are folded, assembled and modified in the endoplasmic reticulum (ER). Cells had evolved the ER quality control (ERQC) machineries to maintain the dynamic process, including ERAD and unfolded protein responses (UPR) pathways. Once protein folding is disturbed, the toxic accumulation of misfolded or unassembled proteins actived UPR to enhance the protein folding capacity of ER. Meanwhile, proteins that cannot be refolded are subjected to ERAD and modified by ubiquitination, and degraded by the 26S proteasome. Our previously studies characterized the role of bZIP transcrption factor MoHac1 of UPR pathway in *M. oryzae*. Here, we further explore the functions of an E3 ubiquitin ligase MoHrd1-mediated ERAD pathway, and found that both MoHrd1 and MoDer1 localizate to ER, disruption of *MoHRD1*, but not *MoDER1* (another protein of ERAD), caused reduced pathogenicity, while double deletion of *MoHRD1* and *MoDER1* resulted in significant defects in hyphae growth and sporulation, and lost pathogenicity, suggesting that MoHrd1 synergizes MoDer1 is required for full pathogenicity. Additonally, the Δ*Mohrd1/Moder1* double mutant is defective in appressorium formation, most of appressorium exhibit anomocytic, and appressorium penetration is blocked. Furthermore, MoHrd1 is involved in the degradation of ERAD substrate and ER stress response, MoHrd1 and MoDer1 also mediate crosstalk between the ERAD and UPR pathway in *M. oryzae*. Collectively, these results showed that MoHrd1-mediated ERAD pathway regulates infection-related development in *M. oryzae*, and ERAD-associated molecular mechanism on pathogenisis requires further investigations.

Taxonomy of *Colletotrichum* from Different Economic Crops

Yangyan Zhu[a,b], Hongyan Li[a,b], Jiawei Huang[a,b], Hongli Hu[a, b, *]

[a]*College of Plant Protection,, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002*

[b]*State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002*

* *Email of corresponding author: huhongli7905@gmail.com*

Anthracnose is a common disease on many crops, which is caused by *Colletotrichum* species. But the taxonomy of *Colletotrichum* is very complex and now divided into 9 species complex and some ungrouped species[1] .During our research on fungal diversity from Fujian, Yunnan, Sichuan economic crops, 175 strains were obtained from 49 plants after isolation and purification. 85 strains were identified as *Colletotrichum* species among the 112 identified strains using morphology and multi-loci (ITS, β-tubulin，TEF, ACT, etc) phylogenetic analyses. They were identified to 5 *Colletotrichum* species complex. Among them, 5 *Colletotrichum* species were found on tea, i.e., *C. camelliae*, *C. fructicola*, *C. karstii*, *C. siamense* and *C. ti*, and 3 *Colletotrichum* species were identified on mango, i.e., *C. fioriniae, C. gloeosporioides* and *C. siamense*. More *Colletotrichum* species remained to be identified.

References

[1] P.F. Cannon, U. Damm, P.R. Johnston, and B.S. Weir (2012). Studies in Mycology, 73: 181–213.

The 5-oxoprolinase is required for conidiation, sexual reproduction, virulence and deoxynivalenol production of *Fusarium graminearum*

Piao Yang[1] #, Yunyun Chen[1] #, Huiming Wu[2], Wenqin Fang[1], Qifu Liang[1], Yangling Zheng[2], Stefan Olsson[1, 3], Dongmei Zhang[1], Jie Zhou[2], Zonghua Wang[1, 4] *, Wenhui Zheng[1] *

Piao Yang, E-mail: 1160204025@fafu.edu.cn (PY)

In eukaryotic organisms, the 5-oxoprolinase is one of the six key enzymes in the γ-glutamyl cycle that is involved in the biosynthetic pathway of glutathione (GSH, an antioxidative tripeptide counteracting the oxidative stress). To date, little is known about the biological functions of the 5-oxoprolinase in filamentous phytopathogenic fungi. In this study, we investigated the 5-oxoprolinase in *Fusarium graminearum* for the first time. In *F. graminearum,* two paralogous genes (*FgOXP1* and *FgOXP2*) were identified to encode the

5-oxoprolinase while only one homologous gene encoding the 5-oxoprolinase existing in other filamentous phytopathogenic fungi as well as in *Saccharomyces cerevisiae*. Deletion of *FgOXP1* or *FgOXP2* in *F. graminearum* led to significant defects in its virulence on wheat. This is likely caused by an observed decreased deoxynivalenol (DON, a mycotoxin) production in the gene deletion mutant strains as DON is one of the best characterized virulence factors of *F. graminearum*. The *FgOXP2* deletion mutant strains were also defective in conidiation and sexual reproduction while the *FgOXP1* deletion mutant strains were normal in those phenotypes. Double deletion of *FgOXP1* and *FgOXP2* led to more severe defects in conidiation, DON production and virulence on plants, suggesting that *FgOXP1* and *FgOXP2* function redundantly. Although transformation of *MoOXP1* into *ΔFgoxp1* was able to complement *ΔFgoxp1*, transformation of *MoOXP1* into *ΔFgoxp2* failed to restore its defects in sexual development, DON production and pathogenicity. Taken together, these results suggest that *FgOXP1* and *FgOXP2* are likely to have been functionally diversified and play significant roles in fungal development and full virulence in *F. graminearum*.

Increasing crop productivity and resilience in smallholder farms: an experimental test

Li-Na Yang[1], Zhe-Chao Pan[2], Wen Zhu[1], E-Jiao Wu[1], Dun-Chun He[1], Ruey-Shyang Chen[3], Qi-Jun Sui[2], Jiasui Zhan[1] (Email: jiasui.zhan@fafu.edu.cn)

[1]Fujian Key Laboratory of Plant Virology, Institute of Plant Virology, Fujian Agricultural and Forestry University, Fuzhou, Fujian 350002; [2]Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences, No. 2238, Beijing Road, Kunming, Yunnan, 650200; [3]Department of Biochemical Science &Technology, National Chiayi University, Taiwan

Smallholder farming systems play a crucial role in agricultural and ecological sustainability. Despite the developed world’s emphasis on highly mechanized agriculture, a substantial part of world crop production occurs under smallholder conditions in which social, economic or simply physical reasons (e.g. terracing) limit mechanization. While limiting opportunities for labor efficiencies, the lack of mechanization actually provides the opportunity to promote ecologically and evolutionarily based

agronomic practices that maximize productivity and yield sustainability. Previous studies have variously demonstrated the positive impact of mixed planting strategies on host productivity as a consequence of reduced disease impact. Here we present data from a complex series of trials involving within-species diversification of potato – the world's third largest food crop which is grown extensively in China (22% of world production) under smallholder production conditions, – showing that the benefits of mixed planting strategies extend beyond significant increases in yield and production resilience, and reduction in the amount and fluctuation of late blight disease to increased soil microbial diversity, improved soil nutrition status and reduced evolutionary rates and aggressiveness of the associated *Phytophthora infestans* pathogen. Together this combination of increased yield productivity and stability, reduced pathogen evolution and improved soil health provide the economic and ecological basis for sustainable agriculture in smallholder systems.

Taxonomy of *Pestalotiopsis* and Closely Related Genera from Fujian Economic Crops

Tian Fang[a,b], Chengquan Luo [a,b], Hongli Hu[a, b, *]

[a] *College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002*

[b] *State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002*

** Email of corresponding author: huhongli7905@gmail.com*

Species of *Pestalotiopsis* and morphologically similar genera occur commonly as plant pathogens, or represent a fungal group known to produce a wide range of chemically novel, diverse metabolites. *Pestalotiopsis* was a taxonomically confused genus and now is divided into 3 genera, i.e., *Pseudopestalotiopsis*, *Neopestalotiopsis* and *Pestalotiopsis*, mainly based on the morphology of three middle cells[1,2]. During our research on fungal diversity from Fujian economic crops, 65 samples from 32 plant species in Fujian province were collected and isolated, resulting in 36 *Pestalotiopsis*-like strains from the obtained 160 strains. They were further identified to genus or species level based on colony morphology, conidia characteristics and multi-loci (ITS, β-tubulin，TEF, etc) phylogenetic analyses. Based on these data, 5 strains were identified to belong to *Neopestalotiopsis*, 24 strains to *Pestalotiopsis*, and 7 strains to *Pseudopestalotiopsis*. Among them, 15 strains are from mango and 5 strains from tea. Species identification is still going on.

References:

[1] S.S.N. Maharachchikumbura , K.D. Hyde, J.Z. Groenewald, et al. (2014). Studies in Mycology, *Pestalotiopsis* revisited, 79:121-186.

[2] Y. Song (2015). Taxonomy of the genus *Pestalotiopsis* and evaluation of its DNA barcoding. Guizhou University.

Evidence for local adaptation and pleiotropic effects associated with melanization in a plant pathogenic fungus

Wen Zhu[a], Jiasui Zhan[a], Bruce A. McDonald[c*]

[a] *Fujian Key Laboratory of Plant Virology, Institute of Plant Virology, Fujian Agricultural and Forestry University, Fuzhou, Fujian 350002;*

[b] *Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland*

Corresponding author: Bruce A. McDonald email: bruce.mcdonald@usys.ethz.ch

Abstract:

We combined a common garden experimental design with digital image analysis to determine how melanization responds to thermal and fungicide stress in 126 strains of *Rhynchosporium commune* sampled from nine global field populations. We found that temperature and fungicide stress significantly affected the degree of melanization. The nine field populations showed similar thermal reaction norms in response to different temperatures, but they showed quite different reaction norms in response to fungicide stress. Significant correlations were found between the degree of melanization and the local environment, including mean annual temperature, latitude, and relative humidity, suggesting that melanization is a locally adaptive trait. We also found that melanization is highly correlated with virulence and fungicide resistance, and that there is a trade-off between growth rate and melanization, suggesting that melanization has pleiotropic effects in *Rhynchosporium commune*.

Keywords: melanization, thermal reaction norm, fungicide resistance, local adaptation, *Rhynchosporium commune*

Perturbation and rerouting of host selective autophagy by the Irish famine pathogen *P. infestans*

Pooja PANDEY[1], Alex LEARY[1], Yasin TUMTAS[1], Nattapong SANGUANKIATTICHAI[1] , Radoslaw KOWALCZYK[1], Maria E. SEGRETIN[1,2], Yasin F. DAGDAS[3], Sophien Kamoun[3], Tolga O. BOZKURT[1]

[1]*Imperial College London, Department of Life Sciences, London, UK.*

[2]*INGEBI-CONICET, Obligado 2490, C.A.B.A., C1428ADN, Argentina*

[3]*The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK.*

E-mail of contact: o.bozkurt@imperial.ac.uk

A selective form of autophagy organized by autophagy cargo receptors contributes to immunity in plants. Autophagy cargos are enclosed in autophagosomes, double-membrane vesicles that are coated by conserved ubiquitin-like ATG8 protein either to be degraded or relocated. In plants ATG8 is expanded into multiple isoforms, possibly to mediate selective autophagy. However, we know little about how selective autophagy is regulated and contributes to immunity. Recently, we discovered that the *Phythophthora infestans* effector PexRD54 subverts host defences mediated by plant autophagy cargo receptor Joka2. PexRD54 outcompetes Joka2 for binding the solanaceous ATG8 isoform ATG8CL. To better understand the molecular mechanisms of selective autophagy in plants, we exploited PexRD54, which stimulates autophagosome formation through ATG8CL binding. We discovered that PexRD54 stimulates autophagosome formation by coupling host vesicle transport regulators to ATG8CL-coated autophagosomes. Furthermore, effector-labelled autophagosomes are delivered toward the pathogen interface, possibly to allocate cellular resources. Strikingly, we also identified two diverse plant RabGAPs with validated ATG8 binding motifs that compete with the effector and Joka2 for ATG8CL binding. Finally, our proteomics screen for Joka2 interactors in pathogen infected tissue revealed defense proteins and related signaling components. Our results implicate effector-mediated employment of host components in autophagosome biogenesis and show that effectors can serve as tools to study molecular mechanisms of selective autophagy.

The analysis of root-knot nematode resistance in rice

Zobaida Lahari[a], Stanley Dimkpa[b], Roshi Shrestha[b], Adam H Price[b] & Godelieve Gheysen[a]

[a]*Dept. of Molecular Biotechnology, Ghent University, Coupure Links 653, B-9000, Ghent, Belgium*

[b] *Institute of Biological & Environmental Sciences, University of Aberdeen, Aberdeen, AB243UU, UK*

godelieve.gheysen@ugent.be

The root-knot nematode *Meloidogyne graminicola* is the most important root-parasitic nematode of Asian rice (*Oryza sativa*) and causes significant yield losses on rice production. So far no strong and reliable resistance has been reported in Asian rice accessions against *M. graminicola*. In a screening conducted by Dr. Adam Price (University of Aberdeen) of 331 rice accessions against *M. graminicola* it had been shown that the two *O. sativa* accessions LD 24 and Khao Pahk Maw appeared to be resistant to *M. graminicola* [1]. Subsequently I have confirmed and analysed in detail the resistance level of these two rice accessions against *M. graminicola*. It was found that at 9 h after nematode inoculation, the number of nematodes attracted to the root tips of LD 24 and Khao Pahk Maw was not significantly different in comparison with the susceptible controls. At 2 days after inoculation (dai) only few nematodes were found inside the roots of LD 24 and Khao Pahk Maw while >50 were found in the susceptible controls. Eventually at 17 dai the number of galls and also the number of nematodes were significantly higher in the susceptible controls than LD 24 and Khao Pahk Maw. At 17dai LD 24 and Khao Pahk Maw had also very few females (5 in Khao Pahk Maw and <1 in LD 24, in comparison with >100 in the susceptible controls). So these two Asian rice accessions appear ideal donors for breeding root-knot nematode resistance. The data from screening the segregating F2 population (developed from a cross between rice accessions Vialone and LD24, highly susceptible and resistant to *M. graminicola* respectively) for resistance against *M. graminicola* indicates LD24 is resistant due to the presence of a major resistance gene.

Reference:

[1] Stanley O N Dimkpa, Zobaida Lahari, Roshi Shrestha, Alex Douglas, Godelieve Gheysen and Adam H Price (2016). Journal of Experimental Botany. Vol. 67, No. 4 p. 1191.

Ecological Mechanisms of Microbiome Associated with Soybean Cyst Nematode Suppressive Soils

M. Imran Hamid[a,b], Muzammil Hussain[b], Meichun Xiang[b], Xingzhong Liu[b]

a Department of Plant Pathology, University College of Agriculture,

University of Sargodha, Sargodha, Pakistan

b State key lab of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing

Presenting author: mian_108@hotmail.com

mian_108@yahoo.com

Disease-suppressive soils are exceptional ecosystems in which crop plants suffer less from specific soil-borne pathogens by exploiting microbial consortia for protection against infections [1]. Soybean cyst nematode (SCN; *Heterodera glycines*) is the most destructive pest of soybean worldwide and its suppressive soils have been identified in some localities [2, 3]. The rhizosphere and cyst microbiomes may play vital role to protect soybean in the SCN suppressive soils. To decipher the ecological mechanisms involving in the development of specific soil suppression, we collected soil samples from the fields with different soybean monoculture years from several locations of northeast of China. Growth room pot experiments and by using the ultra-high-throughput sequencing approach, we identified the key bacterial and fungal taxa involved in SCN suppression. The long term monoculture soils showed natural suppression against SCN than the short term monoculture soils. At genus level, the *Pseudomonas*, *Purpureocillium* and *Pochonia* that have been documented to suppressing SCN were much more abundant in long term monoculture soils than that in short term monoculture soils [3, 4]. Furthermore, long term monoculture suppressive soils were taken into account for further investigation to test the disease suppressive ability by using different treatments designed as i) suppressive soil (S), ii) conducive soil (C), iii) conducive soil mixed with 10% (w/w) suppressive soil (CS), iv) suppressive soil treated at 80°C for 1 hr (S80), and v) suppressive soil treated with formalin (SF). Suppressiveness transferred by adding 10% of suppressive soil to conducive soil had a more pronounce effect on cyst microbiome variation than rhizosphere microbiome. A short heat disturbance (80 °C for 1 h) or formalin treatment of suppressive soil reduced disease protection and resulted in the significant variation of

rhizosphere and SCN cyst microbiome. Our results suggested that the plants engage a subset of functional microbial groups in the rhizosphere for initial defense upon nematode attack and later on colonize the nematode cysts to response for suppression of SCN in disease-suppressive soils.

References:

1. M. Mazzola (2004). Annual Review of Phytopathology, 42, 35.
2. X. Z. Liu, J. Q. Li, D. S. Zhang (1997). International Journal of Nematology, 7, 18.
3. S. Chen (2007). Biocontrol Science and Technology, 17, 125.
4. M. I. Hamid, M. Hussain, Y. Wu, X. Zhang, M. Xiang, X. Liu (2017). FEMS Microbiology Ecology, 93(1), fiw222.

Identification of new Cuscuta factors

Max Körner, Volker Hegenauer and Markus Albert

Center for Plant Molecular Biology, University of Tuebingen, Auf der Morgenstelle 32, 72076 Tuebingen, Germany

max.koerner@zmbp.uni-tuebingen.de

Parasitic plants are a constraint on agriculture worldwide. Plants of the genus *Cuscuta* spp. are obligate holoparasites with a broad host spectrum for nearly all dicotyledonous plants. As leaf- and rootless plants, *Cuscuta* spp. wind around stems of host plants and penetrate host tissue with haustoria. They directly connect to the vasculature and withdraw water, nutrients and carbohydrates. Thus, the haustorium development and the establishment of a connection to the host represent essential steps in the parasite's life cycle.

Little is known concerning the development of such host-parasite connections on molecular level. In this project we want to gain knowledge about specific molecular signals of *Cuscuta* spp. that get sensed by host plants and manipulate them towards susceptibility or resistance, respectively. On the host plant side, we are interested in identifying receptors that recognize parasitic molecules and further induce cellular signaling programs related to susceptibility or development. To identify novel *Cuscuta* factors that reprogram the host cellular signaling, we cloned the promoters of host genes that are upregulated at *Cuscuta* infection sites and fused them to the luciferase reporter gene. In a promotor::luciferase based bio-assay, we now screen

different haustorial *Cuscuta*-extract preparations for bioactive Cuscuta factors.

The HPL branch of the oxylipin pathway increases susceptibility of tobacco to the whitefly *Bemisia tabaci*

Wen-Hao Deng, Ping Li, Chao Liu, Xiao-Wei Wang, Shu-Sheng Liu, Yin-Quan Liu*

Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and , Institute of Insect Sciences, Zhejiang University, Hangzhou 310058;

E-mail address: dengwenhao0608@163.com

Abstract: Green leaf volatiles (GLVs) are products of the hydroperoxide lyase (HPL) branch of the oxylipin pathway and they can influence the performance of herbivores. However, the roles of GLVs in defense against the whitefly *Bemisia tabaci* are not well studied. Here we report that the tobacco HPL pathway affects the survival and fecundity of whitefly. Using genetically modified method, we constructed transgenic tobacco plants overexpressing *HPL* gene. Biological assays showed that the survival rates and fecundity of the whitefly on transgenic plants were significantly higher than that on wild type plants. Moreover, whiteflies were obviously attracted by transgenic tobacco plants. On the contrary, Virus-induced gene silencing (VIGS) assays showed silencing of *HPL* gene and *ADH* gene were adverse to the performance of whiteflies. When tobacco plants were daubed with (*Z*)-3-Hexenol and (*E*)-2-hexenal in lanolin, chemicals of GLVs, the survival rates and fecundity of the whitefly were increased. Taken together, our results suggest the HPL pathway may negatively modulate tobacco resistance to whitefly.

Acknowledgement: This work was supported by National Basic Research Program of China (2013CB127604) and the Special Fund for Agro-scientific Research in the Public Interest of China (201203036).

References:

[1] A. Kessler, I.T. Baldwin (2001), *Science*, 291, 2141-2144.

[2] I. Feussner, C. Wasternack (2002), *Annu. Rev. Plant Biol.*, 53, 275-297.

[3] K. Kishimoto, K. Matsui (2008), *Phytochemistry*, 69, 2127-2132.

[4] K. Shiojiri, K. Kishimoto (2006), *Proc. Natl Acad. Sci. USA,* 103, 16672-16676.

Subterranean infestation by *Holotrichia parallela* larvae is associated with changes in the peanut (*Arachis hypogaea* L.) rhizosphere microbiome

Lili Geng[a], Gaoxiang Shao[a], Meiling Wang[a], Xiaoxiao Sun[a], Jie Zhang[a]

[a] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing*

llgeng@ippcaas.cn

Hypothesizing that infestation by subterranean insect larva may influence the composition of rhizosphere microbial communities, a model composed of *Holotrichia parallela* larva, peanut (*Arachis hypogaea* L.) and rhizosphere microorganisms was established to investigate the belowground tritrophic interaction. Deep sequencing of V3 and V4 hypervariable regions of 16S rRNA gene was used to characterize the rhizosphere bacteria of infested and uninfested peanuts. A total of 2,673,656 reads were generated and an average of 2558 OTUs were obtained for each sample. The core rhizosphere bacteria consisted of 639 OTUs, which were found in 95% of peanut rhizosphere samples. Proteobacteria was the most abundant bacterial phylum in the core rhizosphere of peanuts, with a relative abundance of 59.4%. Comparisons of rhizosphere bacterial community structure of peanuts with those infested by *H. parallela* larva revealed that the relative abundance of *Proteobacteria* and *Bacteroidetes* increased, while that of *Actinobacteria* decreased in the rhizosphere with infestation. A significant shift in bacterial communities was observed within 24 h after infestation by principal component analysis and non-metric multi-dimensional scaling analysis. For the 332 genera identified in 24 h treatment, infestation of white grubs led to the significant changes of abundance of 67 genera. For the 365 genera in 48 h treatment, infestation led to the changes of 50 genera. The enrichment of *Pseudoxanthomonas* was driven mainly by *Pseudoxanthomonas Mexicana* at species level and the relative abundance of genus *Acidovorax* significantly decreased in both treatment groups. Our results indicate that rhizosphere microbial community changed and specific bacteria species were increased during the early infestation stage of the white grub. This study will be useful for elucidating the function of rhizosphere microbiome in the interaction between subterranean pests and crops.

A transgenic strategy for controlling plant bugs (*Adelphocoris suturalis*) through expression of double-stranded RNA homologous to fatty acyl-coenzyme A reductase in cotton

Jing Luo[1,2]*, Sijia Liang[1]*, Jianying Li[1], Zhongping Xu[1], Lun Li[1], Bangqin Zhu[2], Zhe Li[2], Chaoliang Lei[2],Keith Lindsey[3], Lizhen Chen[1,2], Shuangxia Jin[1] and Xianlong Zhang[1]

[1]*National Key Laboratory of Crop Genetic Improvement,, Huazhong Agricultural University, Wuhan 430070*

[2]*Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070;*

[3]*Department of Biosciences, Durham University, Durham, DH1 3LE, UK;*

[*] *Corresponding author: jsx@mail.hzau.edu.cn and lzchen@mail.hzau.edu.cn*

Plant bugs (Miridae species), which are sap-sucking insects, have emerged as major pests of cotton in China. Most Miridae species are not sensitive to commercial *Bacillus thuringiensis* (Bt) cotton, resulting in significant economic losses and an increased application of insecticide, which eventually may compromise the future of Bt cotton. We demonstrate that *FATTY ACYL-COA REDUCTASE* (*AsFAR*) plays an essential role in the reproduction of the bug *Adelphocoris suturalis*. Down-regulation of *AsFAR* expression by injection of double-stranded RNA suppresses ovarian development and female fertility, resulting in females producing few viable offspring. To determine the viability of an RNA interference approach to limit *FAR* expression and reproductive ability in *A. suturalis*, a dsRNA targeting the *AsFAR* gene (*dsAsFAR*) of *A. suturalis* was expressed in transgenic cotton plants. *AsFAR* transcription levels were significantly downregulated in *A. suturalis* feeding on the transgenic plants. In contained field trials, the transgenic cotton lines significantly suppressed the development of *A. suturalis* populations and were resistant to damage caused by plant bug infestation. These results suggest a new strategy for the management of plant bug pests of cotton.

Keywords: double-stranded RNA, fattyacyl-coenzyme A reductase (FAR), pest control, plant bugs (Adelphocoris suturalis), RNA interference, transgenic cotton.

Comprehensive analysis of cotton miRNA response to whitefly infestation offers new insights into plant-herbivore interactions

Jianying Li[1], Shuangxia Jin[1*]

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070

** Corresponding author: jsx@mail.hzau.edu.cn*

Abstract: Although the regulatory functions of miRNAs and their targets have been characterized in model plants, their underlying role in the cotton response to herbivore infestation has not been determined. To address this, we performed small RNA and degradome sequencing between resistant and susceptible cotton cultivars following infestation with the generalist herbivore whitefly. In total, 260 miRNA families and 241 targets were identified. Quantitative-PCR analysis revealed that several miRNAs and their corresponding targets exhibited dynamic spatio-temporal expression patterns. Moreover, 17 miRNA precursors were generated from 29 long intergenic non-coding RNA (lincRNA) transcripts. Genome-wide discovery also led to the identification of 85 phased small interfering RNA (phasiRNA) loci. Among these, nine *PHAS* genes were triggered by six miRNAs, including leucine-rich repeat (LRR) disease resistance protein, auxin response factor (ARF), and MYB transcription factors. Through modeling and the experimental data, we explored and expanded the miR390-tasiARF cascade during the cotton response to whitefly. Virus-induced gene silencing (VIGS) of *ARF8* in HR cotton plants altered auxin accumulation, which was phenotypically manifested in the HR cultivar as increased tolerance to whitefly infestation. These results provided comprehensive analyses of lincRNAs, miRNAs, phasiRNAs and their corresponding targets and highlight the essential roles of miRNA in the cotton response to herbivore infestation.

Keywords: cotton, whitefly, insect resistance, lincRNA, miRNA, phasiRNA

α-Farnesene and Ocimene Induce Metabolite Changes by Volatile Signaling in Neighboring Tea (*Camellia Sinensis*) Plants

Lanting Zeng [a, b], Yinyin Liao [a], Ziyin Yang [a, b, *]

[a] *South China Agricultural Plant Molecular Analysis and Genetic Improvement, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650*

[b] *University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049*

[*] *Corresponding author. Ziyin Yang, Tel: +86-20-38072989, Email address: zyyang@scbg.ac.cn.*

Email address of lanting Zeng: zenglanting@scbg.ac.cn

Herbivore-induced plant volatiles (HIPVs) act as direct defenses against herbivores and as indirect defenses by attracting herbivore enemies. However, the involvement of HIPVs in within-plant or plant-to-plant signaling is not fully understood. Furthermore, in contrast to model plants, HIPV signaling roles in crops have hardly been reported. Here, we investigated HIPVs emitted from tea (*Camellia sinensis*) plants, an important crop used for beverages, and their involvement in tea plant-to-plant signaling. To ensure uniform exposure to HIPVs, jasmonic acid (JA) was used to simulate herbivore attacks. Metabonomics techniques based on ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry and gas chromatography-mass spectrometry were employed to determine metabolite changes in healthy tea plants exposed to JA-stimulated volatiles. JA-stimulated volatiles mainly enhanced the amounts of 1-*O*-galloyl-6-*O*-luteoyl-α-D-glucose, assamicain C, 2,3,4,5-tetrahydroxy-6-oxohexyl gallate, quercetagitrin, 2-(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-*4H*-chromen-8-yl)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-*2H*-pyran-3-yl, 3,4-dimethoxybenzoate, 1,3,4,5,6,7-hexahydroxyheptan-2-one, and methyl gallate in neighboring undamaged tea leaves. Furthermore, -farnesene and β-ocimene, which were produced after JA treatments, were identified as two of main JA-stimulated volatiles altering metabolite profiles of the neighboring undamaged tea leaves. The two JA-stimulated volatiles were indirectly involved in tea plant-to-plant communications. This research advances our understanding of the ecological functions of HIPVs and can be used to develop crop biological control agents against pest insects in the future.

Acknowledgement: optional This work was supported by grants from the Guangdong Innovation Team of Modern Agricultural Industry Technology System (2016LM1143), the National Natural Science Foundation of China (31670690), and the Guangdong Natural Science Foundation for Distinguished Young Scholar (2016A030306039)

TYLCCNV pathogenicity factor βC1 interacts with NtSKP1 to improve the peformance of whiteflies on tobacco plants

Zou Chi[a], Shu Yan-Ni[a], Liu Shu-Sheng[a], Wang Xiao-Wei[a]

[a]*Institute of Insect Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou*

zouchichi@126.com

Abstract Plant-mediated interactions between insects and viruses transmitted by insects are important determinants of the population dynamics of both types of organisms in the field. Our previous work shows that a worldwide invasive whitefly can establish mutualism with the begomovirus *Tomato yellow leaf curl China virus* (TYLCCNV) via crop plants and the viral pathogenicity factor βC1 determines the mutualism between whiteflies and TYLCCNV. In the present study, we used the βC1 protein of TYLCCNV as a bait to screen tobacco cDNA library through yeast two hybrid system and found that the tobacco protein SKP1 (S-phase kinase associated protein 1) interacts with βC1. Then we confirmed this interaction in *vitro* and in *vivo* through pull-down and BiFC, respectively. Besides, silencing the gene *NtSKP1* enhanced the survival and the fecundity of the whitefly on tobacco plants. These results indicate that βC1 play an important role in mediating vector-virus mutualistic relationships through the interaction with the NtSKP1 in tobacco.

Key word: TYLCCNV, βC1, NtSKP1, whitefly, mutualism

A defence pathway linking plasma membrane to chloroplasts is co-opted by a virus to suppress salicylic acid signaling

Laura Medina-Puche, Tabata Rosas-Diaz, Liping Wang, Tamara Jimenez-Gongora, Dan Zhang, Xue Ding, Chanhong Kim, Yuanyuan Zhang, Shanshan Lv, Renyi Liu, Rosa Lozano-Duran*

Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, Shanghai

*laura@sibs.ac.cn, lozano-duran@sibs.ac.cn**

Plant pathogens need to suppress plant defence responses in order to establish a successful infection. RNA silencing is considered the main plant anti-viral defence; in recent years, however, it has become increasingly clear that plants have additional strategies against viral invasion, which include production of defensive hormones such as salicylic acid (SA) and jasmonic acid. C4 is a

small protein encoded by *Tomato yellow leaf curl virus* (TYLCV) which is essential for infectivity, but of which the molecular function remains obscure. Our results indicate that C4 is localized in two different subcellular compartments, namely plasma membrane and chloroplasts. This double localization correlates with the presence in the C4 protein of two targeting signals: an N-myristoylation motif required for plasma membrane localization, and a chloroplast transit peptide. Interestingly, we have found that these two targeting signals are present in a number of pathogen effectors, as well as in a subset of plant proteins, many of which have been ascribed a role in defence. This finding suggests that a pathway may exist in plants linking plasma membrane and chloroplasts to regulate defence, and that this putative pathway is hijacked by plant pathogens, presumably to suppress these responses. Strikingly, transcriptome analysis of Arabidopsis transgenic plants expressing C4 shows a clear repression of SA biosynthesis and responses. Treatment with pathogen-associated molecular patterns (PAMPs) to activate defence triggers a re-localization of C4 from plasma membrane to chloroplasts, and expression of C4, or a non-myristoylable version of C4 that localizes to chloroplasts exclusively, inhibits SA production in response to PAMP treatment. Activation of SA biosynthesis in response to PAMPs requires retrograde signaling from the chloroplast to the nucleus. Interestingly, C4 does not affect responses to exogenously applied SA, but suppresses up-regulation of PAMP-responsive nuclear genes that are activated by retrograde signaling. Based on these results, our current hypothesis is that activation of plant defence leads to the re-localization of C4 from plasma membrane to chloroplasts, where it interferes with retrograde signaling, suppressing SA biosynthesis to promote the viral infection.

Investigating the function of the V2 protein from *Tomato yellow leaf curl virus* in the endoplasmic reticulum

Liping Wang, Tamara Jiménez-Góngora, Dan Zhang, Xue Ding, Pengfei Fan, Rosa Lozano-Durán

Shanghai Center for Plant Stress Biology, University of Chinese Academy of Sciences. No. 3888 Chenhua Road, Shanghai.

wlp@sibs.ac.cn

Tomato yellow leaf curl virus (TYLCV), a member of the family *Geminiviridae*, is a

single-stranded DNA virus posing a serious threat to tomato production worldwide. The V2 protein encoded by TYLCV functions as an RNA-silencing suppressor and is essential for the virus systemic infection, although the molecular mechanisms underlying those activities remain poorly understood. Using subcellular co-localization studies, we have determined that V2 localizes in the endoplasmic reticulum(ER) of plant cells. Interestingly, the ER has been shown to play an important role in host-virus interactions, and some animal viruses manipulate the unfolded protein response (UPR)pathway to promote the infection. It has been recently shown that plant viruses could also activate the UPR pathway, although the mechanisms by which they do so, as well as the relevance for the viral infection, are not fully understood. Given that V2 accumulates in the ER, we are exploring whether V2 interferes with the function of this subcellular compartment, including the ER stress response, UPR, and ER-associated degradation (ERAD). Our results will shed light on the potential role of the ER in the geminivirus infection.

In planta expression of a viral protein modifies plant development

Pengfei Fan*, Dan Zhang*, Tabata Rosas-Diaz, Laura Medina-Puche, Rosa Lozano-Duran

Shanghai Center for Plant Stress Biology, Shanghai Institutes of Biological Sciences, University of Chinese Academy of Sciences, Shanghai 200031

*These authors contributed equally to this work

zhangdan@sibs.ac.cn

Tomato yellow leaf curl virus (TYLCV) (genus *Begomovirus*, family *Geminiviridae*) causes the most devastating viral disease of tomato, giving rise to severe economic losses. TYLCV is transmitted by the whitefly *Bemisia tabaci*, and, in addition to tomato, can infect other cultivated crops (including bean, pepper, tobacco, and potato) as well as weeds. TYLCV has a single 2,787 nucleotides covalently closed genomic circular ssDNA, and encodes two large open-frames (ORF) on the viral strand (CP and V2), and four on the complementary strand (C1-C4). C4 has been described as a symptom determinant and essential for infectivity tomato, but its molecular function is unclear. In this work, we show that C4 is required for

symptom development in *Nicotiana benthamiana*, where it localizes at the plasma membrane (PM) and in chroloplasts. Transgenic expression of C4 in Arabidopsis causes dramatic developmental alterations. In the aerial part of the plant, expression of C4 causes phenotypes including dwarfism, smaller leaves, late flowering, fewer seeds and siliques, twisted stems, crinkled siliques, and stem enations. Additionally, C4-expressing plants display fewer lateral roots. Our results show that the developmental phenotypes caused by C4 require its PM localization and depend on the tissue where this viral gene is expressed. Our results can shed light not only on the virulence function of C4, but also on the regulation of specific plant developmental processes.

A phloem-restricted virus interacts with BREVIS RADIX domain-containing proteins and manipulates phloem development

Tabata Rosas-Diaz, Pengfei Fan, Laura Medina-Puche, Dan Zhang and Rosa Lozano-Duran
Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, No. 3888 Chenhua Road, Shanghai.
tabatarosas@sibs.ac.cn

The C4 protein from the virus *Tomato yellow leaf curl virus* (TYLCV) is essential for the infection and localizes to plasma membrane and plasmodesmata (PD) in a myristoylation-dependent manner, although its exact molecular function is still unclear. Arabidopsis plants expressing C4, but not a non- myristoylable version of this protein ($C4_{G2A}$), show strong developmental alterations. Through a yeast two-hybrid screen we have found that C4 interacts with BREVIS RADIX (BRX) domain-containing proteins, including BRX and BRX-like 2, and subsequently confirmed these interactions *in vivo* and *in vitro*. Through bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer/fluorescence-lifetime imaging microscopy (FLIM-FRET), the C4-BRX and C4-BRXL2 interactions can be detected at the plasma membrane. In Arabidopsis, *BRX* has been described as a transcriptional co-regulator involved in protophloem development. Interestingly, C4-expressing plants (but not $C4_{G2A}$-expressing plants) show a phloem over-proliferation phenotype. We will present results suggesting that a phloem-limited virus, TYLCV, might target BRX-containing proteins to manipulate phloem development as a virulence strategy.

The interaction between *Turnip mosaic virus* encoded proteins and AtSWEET1 protein in *Arabidopsis thaliana*

Ying Sun, Yan Wang, Xianghui Zhang, Yanhua Zhang, Hongyu Pan, Jin-liang Liu
Department of Plant Protection, College of Plant Sciences, Jilin University, Changchun 130062
E-mail of presenting author: jlliu@jlu.edu.cn

Turnip mosaic virus (TuMV) is an important species of the genus *Potyvirus* and has an exceptionally broad host range in terms of plant genera and families of any potyviruses. It occurs worldwide and causes great losses to agricultural production. The symptom formation of a plant viral disease results from molecular interactions between the virus and its host plant. SWEET (sugars will eventually be exported transporters) protein family is a new class of sugar transporters that play an important role in the interaction of host-pathogens[1]. It has been reported that plants infected with fungi or bacteria will induce partial expression of the SWEET gene involved in pathogen-host interaction[2,3]. However, the SWEET protein family has not been found to be involved in the interaction between virus and host.

In order to study whether *Arabidopsis thaliana* 17 *AtSWEET* genes were induced by virus infection. The expression of *AtSWEET* genes were detected to infect *Arabidopsis thaliana* by infectious cDNA clone of TuMV. The result showed that most of the *AtSWEET* genes were induced expression. It suggested that the *AtSWEET* gene family is involved in the interaction of TuMV and *Arabidopsis thaliana*.

A yeast two hybrid library of *Arabidopsis thaliana* cDNAs was screened using the P3 protein as bait. AtSWEET1 in *Arabidopsis thaliana* was identified to interact with TuMV P3 protein. It in deeply confirmed that AtSWEET1 interacted with TuMV-encoded P3, HC-Pro, VPg and NIa-Pro protein by yeast two-hybrid assay and Bimolecular fluorescence complementation assay (BiFC). It suggested that SWEET protein family play an important role in the interaction between the virus and the host. The role of AtSWEET1 in TuMV infection was under investigation.

Acknowledgments: This work was supported by the Natural Science Foundation of China (31201485).

References:

[1] K.L. Cox, F.H. Meng, K.E. Wilkins, et al (2017). Nature communications, Doi:10.1038/ncomms15588.
[2] L.Q. Chen (2014). New Phytologist, 201, 1150-1155.
[3] P.N. Dodds, E.S. Lagudah (2014). Starving the enemy. Science, 354, 1377-1378.

A viral effector suppresses cell-to-cell spread of silencing by targeting a plasmodesmal protein

Tabata Rosas-Diaz[1], Dan Zhang[1], Liping Wang[1], Xue Ding[1], Tamara Jimenez-Gongora[1], Zhengyan Feng[1], Pengfei Fan[1], Xinyan Zhao[1], Tan Li[1], Xiaokun Liu[2], Eduardo R Bejarano[3], Jian-Kang Zhu[1], Christine Faulkner[2], Weiman Xing[1], Rosa Lozano-Duran[1]

[1]*Shanghai Center for Plant Stress Biology, Shanghai*. [2]*John Innes Centre, Norwich, United Kingdom*. [3]*University of Malaga, Malaga, Spain*.

C4 from the virus *Tomato yellow leaf curl virus* (TYLCV) localizes to plasma membrane and plasmodesmata (PD) in a myristoylation-dependent manner and is essential for the infection, although its exact molecular function remains elusive. Interestingly, we found that C4 can suppress the silencing spread in transgenic plants expressing a hairpin against the *Sulfur* gene from a SUC2 promoter (which results in bleaching around leaf veins (SUC-SUL plants)), and this effect requires plasma membrane/PD localization. Through a yeast two-hybrid screen we identified a plant receptor-like kinase (RLK) as an interacting partner of C4, and subsequently confirmed this interaction *in vivo* and *in vitro*. Through bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer/fluorescence-lifetime imaging microscopy (FLIM-FRET), the C4-RLK interaction can be detected at the plasma membrane and in PD. Notably, cell-to-cell spread of silencing in the SUC-SUL background is promoted by overexpression of the RLK, and inhibited when this protein and its closest homologue, which also interacts with C4, are absent. The interaction of the RLK with C4 maps to its kinase domain, and our results suggest that it interferes with the function of the plant protein at two different levels: i) by affecting its kinase activity; and ii) by interfering with binding of other interacting partners. Taken together, our data show that a plant RLK plays a role in cell-to-cell spread of silencing, and that this function is inhibited by C4 from TYLCV.

High expression of foot-and-mouth disease virus (FMDV) VP1 by fusion with viral silencing repressor in *N. benthamiana*.

Hyo Ju Choi[1,2], Jeong Mee Park[1,2*]

[1]*Plant Systems Engineering Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), Deajeon 34141, South Korea*

[2]*Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, Korea University of Science and Technology (UST), Daejeon 34113, South Korea;*Corespondence : jmpark@kribb.re.kr*

Foot-and-Mouth Disease Virus (FMDV) is a highly contagious and an economically important issue in the livestock industry. There is currently no effective treatment for FMDV. Recently, there have been several reports on the development of plant-derived FMD vaccines, but the low expression levels of antigenic proteins have been pointed out as limitations of plant-derived vaccines. In this study, we attempted to produce a high level of FMDV structural protein VP1 with high solubility using the potato virus X (PVX)-based expression system in *Nicotiana benthamiana*. Upon transient expression, VP1 protein expression was higher in PVX-based vector than in plant binary vector. As a way to increase the amount of VP1 protein, we co-expressed PVX:VP1 with various viral RNA silencing repressors, such as TCV CP (P38), TBSV P19, TuMV HC-Pro. Among them, the effect of increasing VP1 expression by P38 was greatest. Furthermore, in order for p38 protein to continuously increase VP1 expression during PVX infection, VP1 fused with p38 was expressed in PVX-vector. As a result, expression of VP1 was stably detected in both infected and systemic leaves up to 14 days. Overall, we improved the VP1 protein expression and stability using a plant virus-based expression system. These results may be helpful in the development of plant-derived animal vaccines.

References:

[1] K.L.Hefferon (2011). Virology, 433, 1-6
[2] J.Zisahewski , M.Sack, R. Fischer (2015) Biotechnology Journal, 107-116

Studying the perinuclear localization of chloroplasts induced by the Rep protein from *Tomato yellow leaf curl virus*

Ding Xue, Liping Wang, Rosa Lozano-Duran

Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, No. 3888 Chenhua Road, Shanghai

dingxue@sibs.ac.cn，lozano-duran@sibs.ac.cn

The family *Geminiviridae*, comprising viruses with small circular single-stranded DNA genomes, is one of the largest and most important families of plant viruses. Geminiviruses

pose a major threat to sustainable agriculture and food security globally. Investigating the molecular mechanisms underlying pathogenicity and spread of geminiviruses is a crucial step towards the design of effective strategies for agriculture protection. We are using *Tomato yellow leaf curl virus* (TYLCV), the main virus affecting tomato production, as a model to investigate the interaction between the virus genes and the host plant. TYLCV is mainly transmitted by an insect vector, the whitefly *Bemisia tabaci*, and only has one single-stranded circular DNA genome encoding six proteins from six open reading frames (ORF): two in the virion sense orientation, CP and V2, and four in the complementary orientation, C1 (also known as replication-associated protein or Rep), C2 ,C3 and C4. The Rep/C1 protein is the only viral protein essential for virus replication. We have observed that transient expression of Rep/C1 in *Nicotiana benthamiana* can induce the perinuclear localization of chloroplasts. Expression of TYLCV can also induce this effect, which seems to require production of reactive oxygen species. Treatment with the defensive hormones salicylic acid also results in perinuclear localization of chloroplasts. We hypothesize that this phenotype is caused by activation of the plant defence responses upon perception of Rep/C1 or its activity.

Uncovering the global plant gene expression and the role of the viral proteins during citrus leprosis virus C infection

Gabriella D. Arena [a,b], Pedro Luis Ramos-González [c], Luana Rogério [a], Clare Casteel [d], Bryce W. Falk [d], Juliana Freitas-Astúa [c,e] and Marcos A. Machado [a]

[a] *Laboratório de Biotecnologia, Centro APTA Citros Sylvio Moreira/IAC, Cordeirópolis/SP, Brazil*

[b] *Instituto de Biologia, Universidade Estadual de Campinas, Campinas/SP, Brazil*

[c] *Laboratorio de Bioquímica Fitopatológica, Insituto Biológico, São Paulo/SP, Brazil*

[d] *Department of Plant Pathology, University of California, Davis/CA, USA*

[e] *Embrapa Mandioca e Fruticultura, Cruz das Almas/BA, Brazil*

gdiasarena@ucdavis.edu

Citrus leprosis virus C (CiLV-C) is the prevalent virus causing a disease that affects the citrus industry in Latin America. Differently from other plant viruses, CiLV-C is unable to accomplish the systemic movement in any of its known hosts, being restricted to the feeding sites of its mite vector. A previous study suggests that this atypical locally-restricted infection is a consequence of a hypersensitive-like response (HR). To better understand the molecular mechanisms behind the plant-virus interaction, we used RNA-seq to assess the global

response of Arabidopsis along the course of the CiLV-C infection. At the earliest stage (6 h after infestation with viruliferous mites), the plant response to CiLV-C infection is undetectable. Plant transcriptome is progressively reprogrammed and high number of genes is differentially expressed at the pre-symptomatic stage (6 days after infestation). Gene set enrichment analysis revealed the modulation of plant immune pathways. Genes involved in the salicylic acid (SA) pathway and hypersensitive response (HR) were over-represented, and mainly up-regulated. To clarify the role of the CiLV-C proteins in triggering such responses, we expressed them individually in *Nicotiana benthamiana*. Agrobacterium-mediated transient expression of none of the CiLV-C proteins produces a visible altered phenotype but that using the p61 ORF. Expression of this protein consistently leads to a burst of reactive oxygen species, increased expression of SA- and HR-related genes and cell death. Mimicry of responses typically observed during CiLV-C-plant interaction put forward elements, indicating p61 as the putative viral effector to be neutralized by plant defenses.

Transcriptome analysis of *Phalaenopsis* orchid with synergistic infection of *Cymbidium mosaic virus* and *Odontoglossum ringspot virus*

Hsuan Pai[a], Kuan-Chieh Tseng[b], Yi-Fan Chiang-Hsieh[b], Chia-Hung Chien[b], Han-Qin Zheng[b], Shu-Chuan Lee[a], Wen-Chi Chang[b] and Na-Sheng Lin[a]

[a] *Institute of Plant and Microbial Biology, Academia Sinica, NanKang, Taipei, 115, Taiwan*

[b] *College of Biosciences and Biotechnology, Institute of Tropical Plant Sciences, National Cheng Kung University, Tainan, 70101, Taiwan*

pai@gate.sinica.edu.tw

Synergistic viral interaction is commonly hallmarked with reinforced virus colonization and hindered host growth in mixed infected plants [1]. Here we characterized viral synergism between the two major orchid viruses, *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV), in *Phalaenopsis amabilis*, with signs of chlorotic ringspots, enhanced viral titer and spreading of CymMV at 10 days post inoculation (dpi). Based on symptom formation and progression of virus infection, we further designated the inoculated and adjacent non-inoculated tissues representing late and early stages of infection,

respectively. To decipher defensive-offensive interactions of CymMV-ORSV synergism on *Phalaenopsis* gene networks, we compared *de novo* assembled and annotated transcriptomes from tissues of different infection stages. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses showed terms related to cell wall and endoplasmic reticulum (ER)-associated degradation were enriched in initial stage of infection. Along the progression of viral pathogenesis, biosynthesis pathways of secondary metabolites were highly activated. Also, a group of WRKY transcription factors were up-regulated and in co-expression with arrays of calcium binding proteins, putative receptors, and genes related to reactive oxygen species (ROS) burst or ER stress. These results suggest salicylic acid (SA)- and WRKY-related pathways may play major roles in *Phalaenopsis* in response to viral infection. In addition, through green fluorescent protein (GFP)-based assay [2], we characterized ORSV P126 is the potent viral suppressor of RNA silencing. Co-expression of P126 with CymMV inoculation could increase CymMV accumulation, suggesting P126 as the key determinant of viral synergism, and hindered the potential role of RNA silencing machinery in cross talk with other signaling pathways. In conclusion, this study provides a broad view of regulatory network in aspects of orchid defense, viral pathogenesis and synergism.

References:

[1] T. Mascia and D. Gallitelli. (2016). Plant Science, 242:176.

[1] D. Stephan, C. Slabber, G. George, V. Ninov, K.P. Francis, and J.T. Burger. (2011). Plant Methods, 7:25.

Identification of receptor proteins interacting with HC-Pro encoded by *Potato Virus Y* in stylet of *Myzus persicae*

Yang Gao [a], Jing Peng [a] , Deyong Zhang [a].

[a] *Hunan Plant Protection Institute, Hunan Academy of Agricultural Sciences, Changsha.*

lansetianji519@126.com

Current researches for the interaction between *Potato Virus Y*（PVY）and *Myzus persicae* with non-persistent transmission mode are mostly on Helper Component Proteinase(HC-Pro) that enconded by PVY. The receptor proteins in stylet of *Myzus persicae* has not been reported

previously. In this study, we constructed the cDNA library only using the stylet of *Myzus persicae*, utilized yeast two-hybrid system to capture the receptor proteins which interacting with HC-Pro. More than 30 genes that may interact with HC-Pro were found by yeast two-hybrid system and then analied with the aid of the transcriptome of *Myzus persicae*, and the further validates are in progress. Additionally, we still used Surface Plasmon Resonance (SPR) to find the receptor proteins in the protein solution that prepared with stylet of *Myzus persicae*. The SPR is in progress until now and we will get the result later . This study will help to understand the biological processes of the interaction between HC-Pro and receptor proteins in stylet of *Myzus persicae*.

Identification and functional analysis of interacting proteins with CP of *Ramie mosaic virus* in the whitefly, *Bemisia tabaci* (Homoptera: Ale yrodidae)

Jing Peng [a], Yang Gao [a], Zhang limin [a], Zhang Songbai [a], Liu Yong [a], Deyong Zhang [a].
[a] *Hunan Plant Protection Institute, Hunan Academy of Agricultural Sciences, Changsha.*[b]
pengjing_617@126.com

Abstract: *Ramie mosaic virus* (RaMoV), the genus *Begomovirus* in the family *Geminiviridae*, is transmitted by the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) in a persistent-nonpropagative manner. Although evidence have suggested that the coat protein (CP) of *Geminiviridae* is important in the vector transmission However, How the proteins in the whitefly recognite viral particle to finish its infection route in insect vector is unknown. RaMoV CP gene was amplified by RT-PCR and then inserted into yeast two-hybrid system bait vector pGBKT7. The recombinant was transformed into yeast strain AH109 and the transformant was plated on the different synthetic dropout nutrient medium. The result showed that CP don’t self-activate and has no toxictiy to yeast cell. The total RNA of viruliferous whiteflies were extracted. The double-strand cDNA were synthesized by SMART technology and inserted into modified vector NpGADT7 through suitable enzyme sites, and yeast two-hybrid cDNA librarie of viruliferous whiteflies was constructed. The cDNA libraries plasmids were extracted and transformed into yeast strain AH109 containing bait plasmids pGBK-CP. The transformants were plated on different synthetic dropout nutrient

medium and positive clones which can interact with bait proteins were screened. 9 positive clones were acquired by screening cDNA library using RaMoV CP as bait protein. The full-length gene was amplified by RT-PCR and sequenced. 35588 and 50238 genes were inserted into yeast two-hybrid prey vector pGADT7. The recombinants pGAD-35588 or pGAD-50238 were co-transformed into yeast cell AH109 with bait plasmids pGBK-CP respectively, and then the co-transformants were plated on different synthetic dropout nutrient medium. The results showed that 50238 can interact with CP. The expression of 50238 mRNA in the health and RaMoV-infected whiteflies were examined by Real-Time PCR. The results showed that the expression of 50238 mRNA in the RaMoV-infected whiteflies was up-regulated compared with which in the health vertor.

Key words: *Ramie mosaic virus*, *Bemisia tabaci*, Yeast two-hybrid, Insect proteins

The potential role of miRNAs and target genes in cucumber-*Cucumber green mottle mosaic virus* interactions

Liang Chaoqiong[a,b,c,d], Liu Huawei[e], Luo Laixin[a,b], Barbara Baker[c,d], Li Jianqiang[a,b]

[a]*Department of Plant Pathology / Key laboratory of Plant Pathology Agricultural University, Yuanmingyuan West Road No.2, Beijing*
[b]*Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH) / Beijing Key Laboratory of Seed Disease Testing and Control (BKL-SDTC), Yuanmingyuan West Road No.2, Beijing*
[c] *Plant Gene Expression Center, United States Department of Agriculture, Agricultural Research Service, 800 Buchanan St. Albany, CA, USA*
[d]*Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA*
[e]*Molecular Plant Pathology Laboratory, USDA-ARS, 10300 Baltimore Avenue, Beltsville, MD, USA*

MicroRNAs (miRNAs) play a pivotal role in regulating and fine-tuning gene expression in diverse cellular processes such as development and growth, epigenetic inheritance, and cellular stress responses, including host immunity [1–4]. Cucumber is among the most important greenhouse species in the world [5]. *Cucumber green mottle mosaic virus* (CGMMV) is the serious disease of *Cucurbit* plant in the worldwide. It could be caused most losses production and market values, but there is no effective disease-resistance variety [6]. Thus, to explore the molecular mechanism of cucumber-CGMMV interactions and mining resistance genes, and analysis the function of miRNAs regulatory in vivo of cucumber plant,

this study using high-throughput sequencing and degradome sequencing isolated 8 novel, 23 known miRNAs and their target genes in cucumber responding to CGMMV infection. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis that these miRNAs of their corresponding to target genes were related to metabolism process, genetic information processing, biosynthesis of secondary metabolites and plant-pathogen interaction [7]. Thereinto, target genes of csa-miR159b, csa-miR166a, csa-miR2673a, csa-miR5637 and csa-miRn6-3p are *PBS1*, *CML42*, *CML21*, *WRKY33* and *CPK32* by degradome sequencing. These target genes are involved in plant-pathogen interaction and are potential resistance genes in cucumber. Expression levels of these miRNAs and target genes were altered in CGMMV-infected cucumber by real-time quantitative-PCR. Csa-miR159b, csa-miR166a and csa-miRn6-3p negatively regulate expression of their target mRNAs through guiding corresponding target mRNA cleavage. Our results confirm the importance of miRNAs in cucumber-CGMMV interactions, and suggest more study on miRNAs and targets can enrich the knowledge of miRNA mediated-regulation in cucumber.

References:

[1] J.K. Seo, J. Wu, Y. Lii, Y. Li, H. Jin (2013). Molecular Plant-Microbe Interactions 26:617-625.
[2] D. Staiger, C. Korneli, M. Lummer, L. Navarro (2013). New Phytologist 197:394-404.
[3] A. Weiberg, M. Wang, M. Bellinger, H. Jin (2014). Annual Review of Phytopathology 52:495-516.
[4] L. Yang, H. Huang (2014). Journal of Integrative Plant Biology 56:962-970.
[5] M. Pitrat, M. Chauvet, C. Foury (1999). Acta Horticulturae 492: 21–28.
[6] H.W. Liu, L.X. Luo, J.Q. Li, P.F. Liu, X.Y. Chen, J.J. Hao (2014) Plant Pathology 63(1): 72–77.
[7] H.W. Liu, L.X. Luo, C.Q. Liang, N. Jiang, P.F. Liu, J.Q. Li (2015). PLoS ONE 10(6): e0129002.

Exploring different mutations at a single amino acid position of *Cucumber green mottle mosaic virus* replicase to attain stable symptom attenuation

Liming Liu[a], Bin Peng[a], Zhenwei Zhang[a], Yang Wu[a], Manuel Miras[b], Miguel A. Aranda[b], Qinsheng Gu[a]

[a]*Henan Provincial Key Laboratory of Fruit and cucurbit Biology, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou 450009, Henan.*

[b]*Centro de Edafología y Biología Aplicada del Segura (CEBAS)- CSIC, Apdo. correos 164, 30100 Espinardo, Murcia, Spain*

Correspondence: e-mail: guqinsheng@caas.cn

ABSTRACT

Cucumber green mottle mosaic virus (CGMMV) is a member of the genus *Tobamovirus* (family *Virgaviridae*) which causes serious economic losses in cucurbit crops. A possibility

for CGMMV control is the use of cross-protection, for which stable attenuated isolates are required. In this study, an infectious clone was constructed for the hn isolate of CGMMV. Unexpectedly, this clone carried a non-conserved mutation involving a single nucleotide change resulting in the replacement of Arg to Cys at residue 284 of the replicase protein; this mutation correlated with delayed symptom induction and RNA accumulation, as shown in time course experiments. Sequencing of the viral progeny showed that restoration of wild-type symptoms and increased RNA accumulation correlated with reversion of the mutation to the wild-type sequence, a phenomenon that occurred at around 7 to 10 days post-inoculation. Thus, Arg284 seems to be crucial but not strictly necessary for virus infection. Subsequently, four other mutants in the triplet encoding Arg284 were constructed and assayed. Results showed that symptoms and their timing were diverse for the different mutants, with enhanced pathogenicity and RNA accumulation always correlating with reversion to Arg284. Therefore, the nature of the mutation strongly influenced the genetic stability of the mutant. At least two mutants were identified for which reversion did not occur by 30 days post inoculation, and these were defined as good candidates to attain stable symptom attenuation that could be useful in cross-protection.

Key words: CGMMV, infectious clone, replicase protein, reversion, pathogenicity

A reverse genetics platform for tobacco bushy top disease complex

Xingneng Zhao [a,b #], Lifang Zhang [a,b #], Ping Xu [a,b #], Yanqiong Li [a,b], Zhenyuan Xia [a], Xiyun Qin [a], Hairu Chen [b], Xiaohan Mo [a *]

[a] *Yunnan Academy of Tobacco Agricultural Sciences，Kunming 650031*

[b] *College of Plant Protection, Yunnan Agricultural University，Kunming 650201*

*# Equal contributors, *correspondence: xiaohanmo@foxmail.com*

Tobacco bushy top disease (TBTD) is the only plant disease caused by a luteovirus/umbravirus complex and its causal agent, TBTD complex, is one of the most complicated plant virus complex. The coat proteins of the virions of TBTD complex are encoded by tobacco vein distorting virus (TVDV) only, while there are five distinct viral RNA components being encapsidated in, namely, TVDV, tobacco bushy top virus (TBTV), tobacco vein distorting virus-associated RNA (TVDVaRNA), TBTV satellite RNA, and an unidentified viral RNA.

Agrobacterium-mediated infectious clones of TVDV, TBTV, TVDVaRNA and TBTV satellite RNA were constructed, serving as a reverse genetics platform to investigate the

interactions among these viral components and their roles in the symptom developments and aphid transmission. The preliminary application of this reverse genetics platform shed light on the interactions among different viral agents in the TBTD complex.

TBTV alone was able to replicate and move systematically and cause mild symptoms *in planta* (*Nicotiana tabacum* and *N. benthamiana*). While the satellite RNA was dependent on TBTV for its replication and systematic movement, it boosted the symptom severity of the disease when coinfected with TBTV. TBTV satellite RNA stimulated mildly the accumulation of TBTV genomic RNA in *N. benthamiana*, while in *N. tabacum*, it downregulated the accumulation of TBTV genomic RNA slightly, indicating host specificity in the association of TBTV and its satellite RNA.

TVDV alone could infect *N. benthamiana* systemically without prominent symptoms. The plants developed intensive disease symptoms when coinfected with TVDV and TVDVaRNA, and the two viral components were able to move systemically in the plants, while TVDVaRNA alone could not infect *N. benthamiana* systemically. The results indicated that TVDV could facilitate the systemic movement of TVDVaRNA *in planta,* and TVDVaRNA helped TVDV in boosting the development of disease symptoms in *N. benthamiana*.

Exploiting this reverse genetics platform would facillitate the functional genomics of the viral RNA components in the TBTD complex, and deepen our understanding of the mechanism of plant virus interactions.

Establishing RNA virus resistance in plants by harnessing CRISPR immune system

Tong Zhang[1]*, Qiufeng Zheng[1], Xin Yi[2], Hong An[3], Siqi Ma[1], & Guohui Zhou[1]

[1]*Guangdong Province Key Laboratory of Microbial Signals and Disease Control, College of Agriculture, South China Agricultural University, Guangzhou, Guangdong 510642.*

[2]*Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, College of Agriculture, South China Agricultural University, Guangzhou, Guangdong 510642.*

[3]*Division of Biological Sciences, University of Missouri, Columbia, Missouri, 65211, USA.*

** zhangtong@scau.edu.cn*

Abstract

Recently, CRISPR-Cas (clustered, regularly interspaced short palindromic repeats-CRISPR associated proteins) system has been used to produce plants resistant to DNA virus infections.

However, there is no RNA virus control method in plants that uses CRISPR-Cas system to target the viral genome directly. Here we show that the CRISPR-Cas9 system from *Francisella novicida* can be used to confer molecular immunity against RNA viruses in *Nicotiana benthamiana* and *Arabidopsis* plants. Plants expressing FnCas9 and sgRNA specific for the cucumber mosaic virus (CMV) exhibited significantly attenuated CMV infection symptoms and reduced viral RNA accumulation. These data reveals that the CRISPR/Cas9 system can be used to produce plants resistant to RNA viruses, thereby broadening the use of such technology for virus control in agricultural field.

The C3 protein from *Tomato yellow leaf curl virus* interacts with a plant DNA polymerase

Mengshi Wu, Xue Ding, Liping Wang, Dan Zhang, Rosa Lozano-Duran

Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, No. 3888 Chenhua Road, Shanghai. mswu@psc.ac.cn

Geminiviruses are one of the largest families of plant viruses, with circular single-stranded (ss) DNA genomes and a very characteristic geminate virion particle. *Tomato yellow leaf curl virus* (TYLCV) is a geminivirus species that causes yellow leaf curl disease, one of the most devastating diseases in tomato. TYLCV has a small genome (2.7 Kb) that encodes for six proteins only, none of which is a DNA polymerase. Therefore, TYLCV has to rely on the plant cell machinery to replicate its genetic material. The C3 protein from TYLCV acts as a replication enhancer through its interaction with the viral replication-associated protein, Rep, and with plant proteins. C3 is conserved in geminiviruses, which suggests that this protein must play an essential role in the viral infection. We have found that C3 can interact with a plant DNA polymerase in yeast and *in planta* (in co-immunoprecipitation and bimolecular fluorescence complementation assays), suggesting that C3 might be orchestrating viral DNA replication through direct interaction with this host protein. Our results will help elucidate how geminiviruses manipulate the plant cell machinery to replicate.

Eukaryotic translation initiation factor 2B-beta (*eIF2Bβ*), a new class of plant virus resistance gene

Jannat Shopan[a], John A. Walsh[b], Jinghua Yang[a*], Mingfang Zhang[a]
[a]*Laboratory of Germplasm Innovation and Molecular Breeding, Institute of Vegetable Science, Zhejiang University, Hangzhou,310058*
[b]*School of Life Science, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK*

E-mail of presenting author: yangjinghua@zju.edu.cn

Recessive resistances to plant viruses in the *Potyvirus* genus have been found to be based on mutations in the plant eukaryotic translation initiation factors, *eIF4E* and *eIF4G* or their isoforms. Here we report that natural, monogenic recessive resistance to the potyvirus Turnip mosaic virus (TuMV) has been found in a number of mustard (*Brassica juncea*) accessions. Bulked segregant analysis and sequencing of resistant and susceptible plant lines indicated the resistance is controlled by a single recessive gene, *recessive TuMV resistance 03* (*retr03*), an allele of the *eukaryotic translation initiation factor 2B-beta* (*eIF2Bβ*). Silencing of *eIF2Bβ* in a TuMV-susceptible mustard plant line and expression of *eIF2Bβ* from a TuMV-susceptible line in a TuMV-resistant mustard plant line confirmed the new resistance mechanism. A functional copy of a specific allele of *eIF2Bβ* is required for efficient TuMV infection. *eIF2Bβ* represents a new class of virus resistance gene conferring resistance to any pathogen. eIF2B acts as a guanine nucleotide exchange factor (GEF) for its GTP-binding protein partner eIF2 via interaction with eIF2·GTP at an early step in translation initiation. Further genotyping indicated that a single non-synonymous substitution (A120G) in the N-terminal region of *eIF2Bβ* was responsible for the TuMV resistance. A reproducible marker has been developed, facilitating marker-assisted selection for TuMV resistance in *B. juncea*. We investigated genotypes of *eIF2Bβ* in a large population of *B. juncea* and its ancestor of *B. rapa* indicating the phylogeny and evolutionary relationship of *eIF2Bβ* and TuMV resistance. Our findings provide a new target for seeking natural resistance to potyviruses and new opportunities for the control of potyviruses using genome editing techniques targeted on *eIF2Bβ*.

Acknowledgement: We would like to thank Dr. C.W. Zhang and Prof. I. Jupin for the pTY-S vector.

References:

[1] Shopan J, Mou HP, Zhang LL, Zhang CT, Ma WW, Walsh JA, Hu ZY, Yang JH*, Zhang MF. Eukaryotic translation initiation factor 2B-beta (eIF2Bβ), a new class of plant virus resistance gene. ***The Plant Journal***, 2017, DOI: 10.1111/tpj.13519

Necrotic syndrome associated to mutation of the C-terminus of the RNA replicase NIb of the potyvirus *Plum pox virus*

Mingmin Zhao, Frida Mesel-Casanova，David San León, Juan Antonio García and Carmen Simón-Mateo.

Plant Molecular Genetics, National Centre for Biotechnology (CNB-CSIC), Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain

College of Agriculture, Inner Mongolia Agricultural University, 010019, Hohhot, Inner Mongolia

Abstract:

A mutant of the potyvirus *Tobacco vein mottling virus* (TVMV) that causes only very mild symptoms in *Nicotiana tabacum* contains a 58-nt long "AU" rich element expected to fold as a stem-loop structure at its 3´UTR (TVMV XBS8) (1). To assess the possibility that this RNA fragment may induce the symptom attenuation also in another potyvirus, a *Plum pox virus* (PPV)-TVMV chimeric virus, including a 333-nt long fragment from TVMV XBS8 was inserted between the NIb and CP coding sequences of PPV. The chimera was able to cause a systemic infection in *Nicotiana benthamiana*. Nevertheless, the analysis of the virus progeny from systemically infected leaves showed that a virus variant in which the inserted sequence was deleted was accumulated. This virus variant is predicted to produce an RNA replicase NIb with a modified C-terminus (NIbmut). The systemically infected *N. benthamiana* leaves accumulating this PPV mutant showed a severe necrosis response with the overexpression of PR2 protein and low viral CP accumulation level. Intriguingly, NIbmut was also selected in the systemically infected leaves of *N. clevelandii* plants inoculated with PPV-TVMV and it was also associated with low viral CP accumulation, but not with the PR2-related necrosis response, thus uncoupling both phenotypic traits of the NIb-mutated virus. We have performed a high-throughput transcriptomic analysis by RNA-seq to analyze which genes apart from those encoding PR proteins are misregulated in systemically infected leaves of *N.benthamiana* accumulating the NIbmut variant. These preliminary results suggest that NIbmut allowed PPV-TVMV to escape from a non-identified deleterious effect caused by the TVMV hairpin, and the mutated NIb protein could: i) have a reduced activity, explaining low virus accumulation in infected plants, and ii) interact with the *N. benthamiana* immune system. We propose a speculative scenario based on classical arm race models in which plants have

developed immune responses against a RNA replicase NIb and viruses have evolved to hide them from this defense mechanism although small modifications of the protein structure could unmask it.

(1) Rodriguez-Cerezo E, Klein P G, Shaw J G. (1991). A determinant of disease symptom severity is located in the 3'-terminal noncoding region of the RNA of a plant virus. *Proc. Natl. Acad. Sci. USA* **88:** 9863-9867.

The Arabidopsis SUMO E3 ligase SIZ1 mediates the temperature dependent trade-off between plant immunity and growth

Valentin Hammoudi[a], Like Fokkens[a], Bas Beerens[a], Georgios Vlachakis[a,*], Sayantani Chatterjee[a], Manuel Arroyo-Mateos[a], Paul F.K. Wackers[b], Martijs J. Jonker[b], and Harrold A. van den Burg[a]

[a] *Molecular Plant Pathology, SILS, University of Amsterdam, Amsterdam, The Netherlands*

[b] *RNA Biology and Applied Bioinformatics, SILS, University of Amsterdam, Amsterdam, The Netherlands*

H.A.vandenburg@uva.nl

Increased ambient temperature is considered to be inhibitory to basal and effector-triggered plant immunity. For example, Arabidopsis SNC1 gain-of-function mutants show auto-immunity at 22°C, which is fully suppressed at 28°C. The sumoylation mutant *siz1* displays a very similar auto-immune phenotype at 22ºC. Strikingly, *siz1* auto-immunity is sustained at 28°C while still requiring EDS1 and SNC1. Moreover, the rosette size of the *siz1* mutant does not fully recover at 28°C, which is normally the case for SNC1-mediated auto-immunity. We expose that the partial growth recovery is linked to a compromised thermosensory growth repsonse in SUMO mutants, which is independent of well-known immune regulators. Hence, our data reveals a novel link between temperature, growth and immunity.

Identification of novel regulators of pathogen associated molecular pattern (PAMP)-triggered inhibition of growth through an activation-tagging screen in Arabidopsis

Huang Tan [a]*, Tamara Jimenez Góngora [a]*, Dan Zhang [a]*, Moira Walters [b], Tanja Seissler [b], Lena Stransfeld [b], Florian Jupe [b], Chuan Jiang [a], Renyi Liu [a], Cyril Zipfel [b], Rosa Lozano Duran [a]

[a] *Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, No. 3888 Chenhua Road, Shanghai*

[b] *The Sainsbury Laboratory, Norwich Research Park, NR4 7UH, Norwich, United Kingdom*

*These authors contributed equally to this work
tanhuang@sibs.ac.cn, lozano-duran@sibs.ac.cn

Plants utilize common protection strategies like animals, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), to induce signalling cascades to fight against pathogens. However, the identified signaling components of late downstream events following PAMP perception are scarce and how plants balance defense and growth is still unclear. Here, we used a forward genetic screen, based on activation-tagging, to identify mutants that show restoration of seedling growth inhibition (SGI, a late response of PTI) in *bak1-5 bkk1-1*, a mutant highly impaired in PTI signaling at the level of the receptor complex; we called this screen *AMB*, for activation tagging-modifier of *bak1-5 bkk1-1*. Thirty mutants were isolated; following targeted enrichment and sequencing, 38 T-DNA insertions and at least 76 genes which could be potentially affected were identified. Of these, 21 genes were selected as *AMB CANDIDATE GENES* (*ACGs*) for further characterization. To date, we have found that an increase in expression of seven of these *ACGs* in the *bak1-5 bkk1-1* background can partially restore the SGI after PAMP treatment, indicating that they are positive regulators of the PAMP-triggered inhibition of growth and true *AMB* genes. Molecular characterization of these genes is underway.

Photorespiration-mediated redox homeostasis cofers tomato basal defense against *Pseudomonas syringae*

Golam Jalal Ahammed[a], Xin Li[a,b], Guanqun Zhang[a], Jingquan Yu[a], Kai Shi[a]
[a]*Department of Horticulture, Zhejiang University, Hangzhou, 310058*
[b] *Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, 310008*
E-mail of presenting author: ahammed@zju.edu.cn

Photorespiration, an essential process in C_3 plants, has been implicated in the defense response of plants against pathogens. It is considered as a key process that greatly contributes to cellular redox homeostasis. Previously, we have shown that H_2O_2 production and salicylic acid (SA) signaling are involved in the photorespiration-involved defense against *Pseudomonas syringae* pv. *tomato* DC3000 in tomato plants [1]. In this study, we

investigated the involvement of redox state in tomato-*Pseudomonas syringae* pv. *tomato* DC3000 interaction focusing on three photorespiratory genes such as *GLYCOLATE OXIDASE* (*GOX2*), *SERINE GLYOXYLATE AMINOTRANSFERASE* (*SGT*) and *SERINE HYDROXYL METHYLTRANSFERASE* (*SHMT1*). Results showed that although silencing of the *GOX2*, *SGT* or *SHMT1* gene all increased the susceptibility of tomato plants to *P. syringae*, only silencing of the *GOX2* decreased the contents of reduced glutathione (GSH) and ascorbate (ASA) in leaves, leading to decreased ratios of GSH:GSSG and ASA:DHA. Further investigation using exogenous H_2O_2 treatment revealed that H_2O_2 could restore the *GOX2* but not *SGT* or *SHMT1* silencing-caused imbalance in redox state. Since redox signaling pathways play a vital role in SA signaling and biotic stress response, it is plausible that *GOX2*-mediated redox signaling may modulate basal defense against *P. syringae* involving H_2O_2 production in tomato plants.

Acknowledgements:
This work was supported by the National Natural Science Foundation of China (31550110201), and the China Postdoctoral Science Foundation (517000-X91608, 2015M580515).

Reference:

[1] G.J. Ahammed, X. Li, G.Q. Zhang, H. Zhang, J. Shi, C. Pan, J.Q. Yu, K. Shi (2017) Plant, Cell and Environment. doi: 10.1111/pce.12932.

SOBIR1-mediated immunity requires conserved tyrosine residues in its kinase domain

Aranka M. van der Burgh[1], Guozhi Bi[1,3], Jelle Postma[2], Silke Robatzek[2] and Matthieu H. A. J. Joosten[1]

[1]Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands

[2]The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

[3]College of Horticulture, Northeast Agricultural University, Harbin 150030

Aranka.vanderburgh@wur.nl

Receptor-like proteins (RLPs) that sense non-self molecules, induce immune signalling to promote plant resistance against pathogens. Leucine-rich repeat (LRR)-RLPs are transmembrane (TM) proteins without an intracellular kinase domain, which constitutively interact with the LRR receptor-like kinase (RLK) SOBIR1/EVR (SUPPRESSOR OF BIR1-1/EVERSHED) to form signalling-competent receptor complexes. Perception of Avr4 from *Cladosporium fulvum* by the tomato RLP Cf-4 recruits the co-receptor BRI1-ASSOCIATED KINASE 1 (BAK1) to the SOBIR1-Cf-4 complex, thereby activating Cf-4-mediated immunity. Here we identify that SOBIR1 induces auto-immunity in *N. tabacum* and *N. benthamiana* in a BAK1-dependent manner. The SOBIR1 ectodomain, the GxxxGxxxG protein-protein interaction motif located in its TM domain, and a functional kinase domain are all essential for SOBIR1 auto-immune activity. The SOBIR1 kinase domain contains highly conserved tyrosine (Tyr) residues, one of which is located between the N- and C-lobe, and a second Tyr residue which is located just after the activation segment. We determine that the Tyr residue located between the N- and C-lobe is required for both SOBIR1 auto-immunity and the Cf-4-mediated hypersensitive response (HR), whereas the second Tyr residue located close to the C-terminal end of the activation segment is essential for SOBIR1 auto-immunity and plays a minor role in the HR.

LsGRP1 from *Lilium* contributes to plant immunity against *Botrytis* spp. *via* altering plant defense responses

Chia-Hua LIN [a], Chao-Ying CHEN [a]

[a] *Department of Plant Pathology and Microbiology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei, Taiwan.*

d95633001@ntu.edu.tw

Gray mold disease, caused by the fungus *Botrytis elliptica*, severely damages the production of lily (*Lilium* spp.), an economically important bulbous monocot. Defense-related LsGRP1 is a glycine-rich protein with enhanced expression in the leaves of Stargazer lily which exhibits salicylic acid-elicited systemic resistance against gray mold disease [1-3], and the C-terminal region of LsGRP1 is capable of *in vitro* inhibiting fungal

species *via* the mechanism of inducing fungal programmed cell death (PCD) [4]. In this study, the effect of LsGRP1 on plant immunity was investigated using *LsGRP1*-silenced lily and *LsGRP1*-expressing *Arabidopsis* transformants under the challenges of *B. elliptica* and *Botrytis cinerea*, respectively. In *Lilium*, LsGRP1 silencing enhanced the developments of disease symptom and *B. elliptica* population, and meanwhile suppressed defense-related callose deposition. A following assay with callose inhibitor revealed that callose deposition in lily leaves was required for impeding the *in planta* growth and sporulation of *B. elliptica*, revealing that LsGRP1-enhanced callose deposition can protect lily from *B. elliptica* infection. In *Arabidopsis*, *LsGRP1* expression reduced the symptom severity and *B. cinerea* population growth accompanied with the earlier and stronger occurrences of several defense responses including callose deposition, reactive oxygen species accumulation and fungal PCD-induction, as known to be the important defense responses of *Arabidopsis* to fight against *B. cinerea* [5-6]. Thus, the positive effect of LsGRP1 on plant immunity was confirmed in both monocot and dicot plant species, and the defense responses strengthened by LsGRP1 expression not only revealed the common plant mechanisms involving in the combat to *Botrytis* spp., but also pointed out the possible physiological pathway for LsGRP1-activated plant defense.

References:

[1] Y.-Y. Lu, Y.-H. Liu, C.-Y. Chen (2007) Plant Science, 172, 913.
[2] C.-H. Lin, C.-Y. Chen (2014) Phytopathology, 104, 1012.
[3] C.-H. Lin, C.-Y. Chen (2017) Plant Science, 254, 22.
[4] C.-H. Lin, M.-W. Chang, C.-Y. Chen (2014) Phytopathology, 104, 340.
[5] J. Laźniewska, V. Macioszek, C. Lawrence, A. Kononowicz (2010) Acta Physiol. Plant, 32, 1.
[6] N. Shlezinger, A. Minz, Y. Gur, I. Hatam, Y. F. Dagdas, N. J. Talbot, A. Sharon (2011) PLoS Pathog. 7, e1002185.

NLR network mediates immunity to diverse plant pathogens

Chih-Hang Wu [a], Ahmed Abd-El-Haliem [b], Tolga O. Bozkurt[a,c], Khaoula Belhaj[a], Ryohei Terauchi[d,e], Jack H. Vossen[b] and Sophien Kamoun[a]

[a] *The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.*

[b] *Plant Breeding, Wageningen University and Research, Wageningen, The Netherlands.*

[c] *Department of Life Sciences, Imperial College London, London, United Kingdom.*

[d] *Iwate Biotechnology Research Center, Iwate, Japan.*

[e] Kyoto University, Kyoto, Japan.
chih-hang.wu@tsl.ac.uk

Both plants and animals rely on nucleotide-binding domain and leucine-rich repeat-containing (NLR) proteins to respond to invading pathogens and activate immune responses. An emerging concept of NLR function is that "sensor" NLR proteins are paired with "helper" NLRs to mediate immune signaling. However, our fundamental knowledge of sensor/helper NLRs in plants remains limited. In this study, we discovered a complex NLR immune network in which helper NLRs in the NRC (NLR-required for cell death) family are functionally redundant but display distinct specificities toward different sensor NLRs that confer immunity to oomycetes, bacteria, viruses, nematodes, and insects. The helper NLR NRC4 is required for the function of several sensor NLRs including Rpi-blb2, Mi-1.2 and R1, whereas NRC2 and NRC3 are required for the function of the sensor NLR Prf. Interestingly, NRC2, NRC3 and NRC4 redundantly contribute to the immunity mediated by other sensor NLRs including Rx, Bs2, R8 and Sw5. NRC family and NRC-dependent NLRs are phylogenetically related clustering into a well-supported superclade. Using extensive phylogenetic analysis, we discovered that the NRC-superclade has probably emerged over 100 million years ago from an NLR pair that diversified to constitute up to one half of the NLRs of asterids. These results reveal a complex genetic network of NLRs by linking evolutionary history to immune signaling. We propose that this NLR network increases robustness of immune signaling to counteract rapidly evolving plant pathogens.

Pattern recognition receptors and WRKY transcription factors mediate salinity stress tolerance in *Arabidopsis thaliana*

Eliza Po-iian Loo[a], Kohji Yamada[b], Hirotaka Ariga[c], Teruaki Taji[c], and Yusuke Saijo[ad]
[a] *Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan*
[b] *Graduate School of Bioscience and Bioindustry, Tokushima University, Tokushima, Japan*
[c] *Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan*
[d] *Japan Science and Technology Agency (JST), Precursory Research for Embryonic Science and Technology (PRESTO), Kawaguchi, Japan*

E-mail of presenting author: eliza.loo.dx3@bs.naist.jp

A deeper understanding of signaling crosstalk between biotic and abiotic stress responses in plants is needed to efficiently enhance crop stress tolerance. Here, we report that recognition of microbe- and damage-associated molecular patterns (M/DAMPs) via cognate pattern recognition receptors (PRRs) induces salinity stress tolerance in *Arabidopsis thaliana*. Seedlings pre-treated with the bacterial MAMPs flagellin and EF-Tu or the endogenous DAMPs Pep peptides exhibit improved high salinity tolerance. High expression of Pep receptor1/2 (PEPR1/2) also enhances salinity tolerance, without discernible negative effects on plant growth, in transgenic plants. Transcriptome and subsequent genetic analyses reveal that a subfamily of WRKY transcription factors are induced during which and contribute to Pep-induced salinity stress tolerance. Our findings point to a critical role for the signaling module involving PRRs and WRKY transcription factors in plant adaptation to both biotic and abiotic stresses.

High Valued Rice Landrace Mushk Budji :from Conservation to Market

Parray G A, Sofi N.R, Shikari AB, Sheikh FA, Ganai MA

[a] *Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir*

Mountian Research Centre for Field Crops, Khudwani-Anantnag Kashmir

Parray_2005@rediffmail.com

More than 200 landraces of rice have been documented from Kashmir valley suited to different agro-ecological niches and possessed combined adaptive traits for the temperate climate. These landraces mostly belonged to short and bold grained japonica types (*Oryza sativa* ssp *japonica*) and are known for their unique quality features particularly aroma and for desirable taste and texture of the cooked rice besides being early and highly cold tolerant. Some aromatic landraces like *Mushk Budji, Kamad, Nun Beoul, Begum, Mughal, Noormeeri, Zag* etc. are either found in Kashmiri literature or are being conserved and preserved in original form at *Mountain Research Centre for Field Crops,* Khudwani a constituent unit of Sher-e-Kashmir University of Agricultural Sciences and Technology

Kashmir. After the introduction and popularization of high yielding rice varieties in Kashmir valley, these indigenous rice varieties including Mushk Budji and Kamad got replaced and overall area under these heritage rices declined drastically. However, Mushk Budji and Kamad continued to occupy few of the fragmented rice growing areas of the valley.

Mushk Budji is a short bold aromatic rice grown in higher reaches of Kashmir valley. The cooked rice is unique and possesses harmonious blend of taste, aroma and rich organoleptic properties. Although Mushk Budji was conserved and grown by the farmers from generation after generation, but with passage of time, the cultivar had assumed the high levels of admixture at farmers fields to the extent that the original *Mushk Budji* was not even known to custodian farmers. This was where SKUAST-Kashmir realized the gravity of the problem and outlined the program for its purification and revival which was followed by its meticulous execution thereafter. MRCFC, Khudwani took the lead role in first ever genetic purification of Mushkbudji. This was initiated with exhaustive field explorations in order to procure original Mushk Budji genotypes from mixed populations across diversity hot spots in Kashmir. These collections were planted at MRCFC, Khudwani and several cycles of purification were practiced as per ear-to-row method of selection. The rigorous screening, purification and selection of the material lead us to isolate genetically pure versions of Mushk Budji. The stocks thus developed were multiplied under breeders' supervision at Khudwani. In parallel, integrated disease and nutrient management modules were framed for its successful cultivation. The yield performance of purified Mushk Budji along with production and plant protection technology was demonstrated in the farmer's fields in participatory mode during the year 2012 at Sagam area of Tehsil Kokernag. The farmers were highly impressed with the performance of pure Mushk Budji version which was uniform, high yielding and possessed rich aroma as compared to un-improved farmers check. The demonstrations catched farmers attention and triggered huge demand for purified seed of Mushk Budji. Consequently, during 2013, ten ha area was brought under pure Mushk Budji seed in Sagam and about 550 q of pure seed was produced. Hon'ble Governor (Chancellor) of the J&K Sate declared it as the success story brought by the scientists of MRCFC, Khudwani, SKUAST-K on the occasion of 3rd Agriculture Science

Congress, and the first harvest of pure Mushk Budji was released on the occasion.

Deciphering and engineering the RPS4/RRS1 immune receptor pair

Hailong Guo[a], Yan Ma[a], Zane Duxbury[a], Pingtao Ding[a], Sung Un Huh[a], Panagiotis F. Sarris[b], Jonathan D. G. Jones[a]

[a]*The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK*

[b]*Department of Biosciences, College of Life and Environmental Sciences,*

University of Exeter, Exeter EX4 4QD, UK

hailong.guo@tsl.ac.uk

Pathogens secrete effector proteins into plant cells to promote disease. In turn, plants evolved disease resistance genes (R genes) that confer specific recognition of pathogen effectors and activate a strong defense response known as effector-triggered immunity (ETI), which includes rapid transcriptional reprogramming and programmed cell death at sites of infection. Most plant R genes cloned to date encode immune receptors called NLRs, with a conserved nucleotide-binding (NB) domain and a C-terminal leucine-rich repeat (LRR) domain. The adjacent, divergently transcribed, *Arabidopsis Resistance to Ralstonia Solanacearum 1* (*RRS1*) and *Resistance to Pseudomonas Syringae 4* (*RPS4*) genes encode TIR-NB-LRR proteins that function together to recognize at least three unrelated effectors, PopP2, an acetyl-transferase from *Ralstonia solanacearum*, AvrRps4, a coil-coiled protein from *Pseudomonas syringae* pv. *pisi* and a yet unknown effector from *Colletotrichum higginsianum*[1, 2]. How this immune receptor complex recognizes pathogen effectors and subsequently activates defense is poorly understood.

We previously reported that hetero-dimerization of RRS1 and RPS4 TIR domains is required for AvrRps4 recognition and cell death signaling and the WRKY domain integrated into RRS1 acts as an integrated decoy that enables detection of effectors [3, 4]. AvrRps4 interacts with, and PopP2 acetylates, the RRS1 WRKY domain, resulting in activation of the RPS4/RRS1 complex and subsequent defense activation. Recent work from our lab shows that RRS1/RPS4 is negatively regulated pre-activation by the RRS1 WRKY domain through interaction between it and Domain 4 (DOM4). AvrRps4

derepresses RRS1 by disrupting WRKY and DOM4 association, leading to a more open Domain 456(DOM456) conformation and subsequent activation of RPS4. Here we show that we can engineer recognition capacity of the complex through artificially manipulating the conformational state of DOM456. In addition, we show that RRS1 and RPS4 TIR domains are in less proximity upon recognition of AvrRps4 in an EDS1-dependent manner. Understanding such intra- and inter-domain reconfigurations within the RRS1/RPS4 complex that convert effector recognition into complex activation should provide valuable knowledge for future immune receptor engineering.

References:

[1] Narusaka M, Shirasu K, Noutoshi Y, et al., 2009. *Plant J*. 60, 218-26.

[2] Noutoshi Y, Ito T, Seki M, et al., 2005. *Plant J*. 43, 873-88.

[3] Sarris PF, Duxbury Z, Huh SU, et al., 2015. *Cell* 161, 1089-100.

[4] Williams SJ, Sohn KH, Wan L, et al., 2014. *Science* 344, 299-303.

Pattern recognition receptor-mediated control of plasma membrane intrinsic proteins (PIPs) in plant immunity

Taishi Hirase[1], Kohji Yamada[2, 5], Misuzu Yamashita-Yamada[3, 5], Iris Finkemeier[5], Hirofumi Nakagami[4, 5], Christophe Maurel[6] and Yusuke Saijo[1, 5, 7]

[1]Graduate School of Biological Sciences, NAIST, Japan

[2]Department of Bioscience and Bioindustry, Tokushima University, Japan

[3]Fuculty of Life and Environmental Sciences, University of Tsukuba, Japan

[4]RIKEN Center for Sustainable Resource Science, Japan

[5]Max Planck Institute for Plant Breeding Reserch, Germany

[6]Biochimie et Physiologie Moleculaire des Plantes, France

[7]JST, PRESTO, Japan

E-mail of first author: hirase.taishi.hn1@bs.naist.jp

Perception of microbe- and damage- associated molecular patterns (MAMPs and DAMPs) by surface-localized pattern recognition receptors (PRRs) leads to an enhanced state of plant immunity. However, it is still largely unknown how PRR-mediated immune signalings mount effective defenses against pathogens. To reveal the molecular mechanisms

underlying PRR-mediated signaling, we undertook a proteomics approach into the membrane-associated proteins that co-purified with the DAMP receptors PEPR1/PEPR2 in *Arabidopsis*. A mass spectrometry analysis identified plasma membrane intrinsic proteins (PIPs) as novel PEPR-interactor candidates. We found that PIPs were associated with not only PEPR1/ PEPR2 but with the MAMP receptors *in planta*, suggesting the PIPs work downstream of both MAMP/DAMP signaling. Importantly, the PIPs KO plants were impaired in MAMP-induced stomatal closure, which is required to restrict bacterial invasion. Furthermore, the PIPs KO plants were impaired in basal resistance to the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000. These results suggest that PIPs-mediated stomatal closure upon MAMPs perception is required for pre-invasive antibacterial immunity. Further investigation is currently ongoing into the roles of PIPs on PRR-mediated immunity. We will present our progress in these research lines.

Simple DNA extraction with porous ceramic matters (Biocube) from radish and Chinese cabbage

Hyun Jung Kim[a], Hyun-Ah Lee[a], Junseong Shin[b],

[a]*Division of Eco-Friendly Horticulture, Yonam College,Cheonan, Korea*

[b]*Biocube System, Gwacheon, Korea*

hkim@yonam.ac.kr

Numerous methods to easily obtain DNA available for PCR such as heat, microwave, and NaOH mediated extractions have been reported. However, these methods have drawbacks that the extraction efficiency of the genetic material is not uniform, and reproducibility of PCR reaction is low. In this study, a new method for rapidly isolating PCR templates from plants was developed by putting a porous solid phase (Biocube) in contact with a sample, in which the templates of interest are sucked into the pores of Biocube. The Biocube was directly added into a PCR tube as a template without a solvent extraction process. In order to examine the absorption efficiency of the templates, the 3 kinds of Biocube were constructed with different combinations between main component and manufacturing temperature and subjected to evaluate their compatibility for PCR. In the case of total 10

DNAs from Chinese cabbage and radish leaves each showed a higher amplification in the PCR product in 3 kinds of Biocube.

Expansion of sesquiterpene biosynthetic gene clusters confers nonhost resistance to the Irish potato famine pathogen in pepper (*Capsicum* spp.)

Hyun-ah Lee [a, b], Sejun Kim [a], Seungill Kim [a], Doil Choi [a]

[a] *Department of Plant Science, Plant Genomics and Breeding Institute, College of Agricultural and Life Sciences, Seoul National University, Seoul 08826, Korea*

[b] *Division of Eco-friendly Horticulture, Yonam College, Cheonan 31005, Korea*

lha1219@yonam.ac.kr

Chemical barriers contribute to nonhost resistance, which is defined as the resistance of an entire plant species to nonadapted pathogen species. However, the molecular basis of metabolic defense in nonhost resistance remains elusive. Here, we report genetic evidence for the essential role of phytoalexin capsidiol in nonhost resistance of pepper (*Capsicum* spp.) to potato late blight *Phytophthora infestans* using transcriptome and genome analyses. Two different genes for capsidiol biosynthesis, 5-epi-aristolochene synthase (*EAS*) and 5-epi-aristolochene-1,3-dihydroxylase (*EAH*), belong to multigene families. However, only a subset of *EAS*/*EAH* gene family members were highly induced upon *P. infestans* infection, which was associated with parallel accumulation of capsidiol in *P. infestans*-infected pepper. Silencing of *EAS* homologs in pepper resulted in a significant decrease in capsidiol accumulation and allowed the growth of nonadapted *P. infestans* that is highly sensitive to capsidiol. Phylogenetic and genomic analyses of *EAS*/*EAH* multigene families revealed that the emergence of pathogen-inducible *EAS*/*EAH* genes in Capsicum-specific genomic regions rendered pepper a nonhost of *P. infestans*. This study provides insights into evolutionary aspects of nonhost resistance based on the combination of a species-specific phytoalexin and sensitivity of nonadapted pathogens.

Functional analysis of SOBIR1 in CLV2-mediated development and defence

Jinbin Wu [a], Guodong Wang [b] and Matthieu H. A. J. Joosten [a]

[a] *Laboratory of Phytopathology, Wageningen University, 6708 PB Wagenignen, the Netherlands*

[b] *Key Laboratory of Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry, College of Life Sciences, Shaanxi Normal University, 710062 Xi'an, Shaanxi*

E-mail: jinbin.wu@wur.nl

Keywords: CLV2, CRN, SOBIR1, developmental signalling, defence signalling

Abstract: Receptor-like proteins (RLPs) and receptor-like kinases (RLKs) are cell surface receptors that are essential for detecting invading pathogens and subsequent activation of plant defence responses. In contrast to RLKs, RLPs lack a cytoplasmic kinase domain to trigger downstream signalling leading to host resistance. The RLK SOBIR1 constitutively interacts with Cf-4 and is required for Cf-4-mediated resistance to *Cladosporium fulvum* [1]. Accumulating evidence shows that SOBIR1 is broadly required for RLP-involved resistance to fungal, oomycete and bacterial pathogens [2]. CLAVATA2 (CLV2) was firstly identified as an RLP in regulating meristem homeostasis in Arabidopsis, tomato and maize [3]. Recent evidence shows that CLV2 also functions in plant-parasitic infection as colonisation by nematodes is reduced in *Atclv2* mutants [4] and such mutants are also more resistant to the bacterial pathogen *Ralstonia solanacearum* [5]. In addition, a pseudo-kinase CORYNE (CRN) interacts with CLV2 and participates in all CLV2-mediated signalling pathways [5]. As the kinase domain of CRN is inactive, the current hypothesis is that the CLV2/CRN heterodimer likely functions together with an unknown RLK [6]. Our preliminary data show that both Arabidopsis and tomato SOBIR1 interact with CLV2, indicating that SOBIR1 might be a good candidate for functionally participating in the CLV2/CRN complex. Further studies will be focused on a possible interaction between SOBIR1 and CRN and the possible involvement of SOBIR1 and the CLV2/CRN heterodimer in the same signalling pathway, including the CLV2/CRN-mediated regulation of stem cell maintenance, susceptibility to nematodes and *R. solanacearum*, and the

requirement of SOBIR1 for resistance to different pathogens.

References:

[1] T. W. H. Liebrand, G. C. M. van den Berg, Z. Zhang, *et al*. (2013). PNAS, 110(24):10010.

[2] T. W. H. Liebrand, H. A. van den Burg, M. H. A. J. Joosten (2014). Trend Plant Sci, 19(2):123.

[3] M. Galli and A. Gallavotti (2016). Trends Genet, 32(6):372.

[4] A. Replogle, J. Wang. A Bleckmann, *et al*. (2011). Plant J, 65(3):430.

[5] M. Hanemian, X. Barlet, C. Sorin, *et al*. (2016). New Phytol, 211(2):502.

[6] M. Somssich, A. Bleckmann and R. Simon, *et al*. (2016). J Exp Bot, 67(16):4901.

Transcriptional and post-transcriptional regulation of plant immunity through mitogen-activated protein kinases

Martin Weyhe, Naheed Tabassum, Manaswita Baruah, Lennart Eschen-Lippold, Pascal Pecher, Dierk Scheel, Justin Lee [a]

[a] *Dept. of Stress & Developmental Biolology, Leibniz Institute of Plant Biochemistry, Weinberg 3, Halle/Saale, D-06120, Germany*

jlee@ipb-halle.de

Upon recognition of conserved pathogen-associated molecular patterns (PAMPs) by cell surface-localized pattern recognition receptors, a network of cellular signaling events is induced This includes a rise in cellular Ca^{2+} levels, reactive oxygen species generation and activation of mitogen-activated protein kinases (MAPKs) including MPK3, MPK4, MPK11 and MPK6. MAPK-mediated phosphorylation of substrates eventually controls cellular immune reactions via changes in defense gene expression. Our research focusses on immunity-related phospho-substrates of MPK3 and MPK6 [1]. A subset of *Arabidopsis thaliana* VQ-motif containing proteins (VQPs) were identified to be MPK3 and MPK6 substrates (therefore renamed as MPK3/6-targeted VQPs or MVQs) and found to control defense gene expression, presumably through interaction with specific WRKY transcription factors [2]. A second set of MPK3/6 substrates is the family of Tandem Zinc Finger (TZF) proteins that are predominantly found in processing bodies, which are cytoplasmic

RNA-protein complexes involved in mRNA decay or translational arrest [3]. We will present here examples of MAPK control of plant immunity via these two family of MAPK substrates at the level of transcriptional and post-transcriptional regulation, respectively.

References:

[1] Lee et al., 2015. Cellular reprogramming through mitogen-activated protein kinases. Front Plant Sci. 6: 940.

[2] Pecher et al., 2014. The Arabidopsis thaliana mitogen-activated protein kinases MPK3 and MPK6 target a subclass of 'VQ-motif'-containing proteins to regulate immune responses. New Phytol. 203: 592.

[3] Maldonado-Bonilla et al., 2014. The Arabidopsis tandem zinc finger 9 protein binds RNA and mediates pathogen-associated molecular pattern-triggered immune response. Plant Cell Physiol. 55: 412.

Characterization of novel transcription factor bHLH27 in the jasmonate pathway

Marciel Pereira Mendes, Richard J. Hickman, Marcel C. Van Verk, Corné M.J. Pieterse and Saskia C.M. Van Wees

Plant-Microbe Interactions, Department of Biology, Utrecht University, P.O. Box 800.56, 3508 TB, Utrecht, the Netherlands

Email: m.pereiramendes@uu.nl

The phytohormone jasmonic acid (JA) is a critical regulator of plant growth and defense. To better understand the architecture and dynamics of JA gene regulatory network, we performed high-resolution RNA-Seq time series analysis of methyl JA-treated *Arabidopsis thaliana*. Our analysis showed in detail the chronology of events that occur during the early and later phases of the JA response. Several transcription factors showed quick transcriptional induction by MeJA treatment. Among these transcription factors was well-characterized MYC2, but also several unknowns were induced, one of which was transcription factor bHLH27. To uncover the role of bHLH27 in JA-dependent defense

responses, knockout lines of bHLH27 were challenged with pathogens and showed enhanced susceptibility to necrotrophic while its resistance to caterpillar was enhanced. RNA-Seq analysis of the *bhlh27* mutant revealed a significant overlap with the MeJA-responsive gene regulatory network, confirming a role of bHLH27 in regulation of JA responses. In Y2H assays SAMDC showed up as an interacting protein. SAMDC can convert S-adenosylmethione, a precursor of ethylene, to spermine. In accordance, ethylene levels were found to be 3-fold lower in *bhlh27* compared with WT plants. For this reason, we speculate that bHLH27 is involved in steering the JA pathway towards the ET-coregulated ERF-branch, which antagonizes the ABA-coregulated MYC-branch. The involvement of bHLH27 in production of ET supports our finding that *bhlh27* has enhanced susceptibility to *Botrytis cinerea* and enhanced resistance to *Mamestra brassicae*. Our work characterized a novel early JA-induced transcription factor with a function in regulation of the balance between the ERF- and MYC-branch of the JA pathway, possibly via an effect on ET production.

The Role of Plant Class II Glycine-rich Protein LsGRP1 in Plant Immunity

Ye, Nai-Hua [a], Chen, Chao-Ying [a]

[a] *Department of Plant Pathology and Microbiology, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei, Taiwan*

r04633007@ntu.edu.tw

LsGRP1, which is classified into the plant class II glycine-rich protein (GRP), involves in the defense of *Lilium* cv. Star Gazer against pathogen attack. [1] Since plant class II GRPs have been shown capable of activating plant defense *via* protein-protein interactions, we intend to investigate how LsGRP1 involves in and the relationship of its interacting proteins to plant defense. [2,3] Firstly, the experiment with *Pseudomonase syringae* pv. *tomato* DC3000 and *Pst* DC3000 *hrcC*$^-$ mutant (type III secretion system mutant) strongly

suggested that *LsGRP1*-overexpressed *A. thaliana* might own stronger pattern-triggered immunity (PTI) than the wild-type plants. The effects of LsGRP1 on PTI were then studied by using flg22 and chitohexaose as bacterial and fungal molecular patterns, respectively. Higher level of reactive oxygen species and callose were significantly induced in *LsGRP1*-overexpressed *A. thaliana*. The CDPK-related gene was induced as well. Thus, the presence of LsGRP1 conducts stronger PTI responses on plants indeed, and its interacting protein candidates are explored by co-immunoprecipitation method on *Arabidopsis* to decipher the action mode of LsGRP1 on plant immunity.

References:

[1] Y.Y. Lu, C.Y. Chen. (2005). Plant Sci. 169, 1.

[2] A.R. Park, S.K. Cho, U.J. Yun, M.Y. Jin, S.H. Lee, G. Sachetto-Martins, *et al*. (2001). J. Biol. Chem. 276, 26688.

[3] S. Ueki, V. Citovsky (2005). Proc. Nat. Acad. Sci. USA 102, 12089.

RBOHD is indispensable for LPS-triggered ROS burst

Nang Myint Phyu Sin Htwe, Quiying Li, Min Wang, Keke Shang-Guan, Yan Liang*

*Department of Plant Protection, Institute of Biotechnology, Zhejiang University, Hangzhou, 310058, nmphyusinhtwe@gmail.com, *yanliang@zju.edu.cn*

Lipopolysaccharide (LPS), a major component of the Gram-negative bacterial outer membrane, is an important microbe-associated molecular pattern (MAMP), which triggers immune responses in both plants and animals. In plants, relatively little is known about plant LPS recognition and subsequent signal transduction. We found LPS induces a very strong long lasting second ROS burst produced from intracellular organs of cell (Poster of Keke Shang-guan in this meeting). In this study, We have performed a genetic screen to identify mutants that are defective in LPS-triggered ROS burst (*delt*) using ethylmethylsulfonate (EMS) mutagenized Arabidopsis seedlings. One of these mutants, *delt8*, was characterized in details. Genetic analysis by backcrossing of the *delt8* mutant with wild-type plants indicated that *delt8* was a recessive mutation in a single nuclear gene. Map-based cloning and whole-genome sequence revealed a G to A mutation at nucleotide

TGG of *At5g47910*, which encodes RESPIRATORY BURST OXIDASE HOMOLOGUE D (RBOHD), a plant NADPH oxidase. The *delt8* mutation cause the substitution of a tryptophan at amino acid 246 to a stop codon. It has been well studied that PAMP-triggered apoplast ROS burst is mainly mediated by RBOHD. As expected, *delt8* mutants exhibit no ROS burst after treatment with other PAMP elicitors, including flagallin, Elongation factor Tu, and chitin oligosaccharides. Take together, our results suggest that RBOHD mediates not only the apoplast, but also the intracellular ROS production.

Characterization of novel transcription factor bHLH27 function in the jasmonate pathway

Marciel Pereira Mendes, Richard J. Hickman, Marcel C. Van Verk, Corné M.J. Pieterse and Saskia C.M. Van Wees

Plant-Microbe Interactions, Department of Biology, Utrecht University, P.O. Box 800.56, 3508 TB, Utrecht, the Netherlands

Email: m.pereiramendes@uunl

The phytohormone jasmonic acid (JA) is a critical regulator of plant growth and defense. To better understand the architecture and dynamics of JA gene regulatory network, we performed high-resolution RNA-Seq time series analysis of methyl JA-treated *Arabidopsis thaliana*. Our analysis showed in detail the chronology of events that occur during the early and later phases of the JA response. Several transcription factors were quickly transcriptionally induced by MeJA treatment, among which well-characterized MYC2, but also several unknowns of which one was bHLH27. To uncover the role of bHLH27 in JA-dependent defense responses, knockout lines of bHLH27 were assayed and found to be enhanced susceptible to necrotrophic pathogen *Botrytis cinerea*, while resistance to chewing caterpillars of *Mamestra brassicae* was enhanced. RNA-Seq analysis of the *bhlh27* mutant revealed a significant overlap with the MeJA-responsive gene regulatory network, confirming a role of bHLH27 in regulation of JA responses. In Y2H assays,

SAMDC showed up as an interacting protein. SAMDC can convert S-adenosylmethione, a precursor of ethylene, to spermine. In accordance, ethylene levels were found to be 3-fold lower in *bhlh27* compared with WT plants. For this reason, we speculate that bHLH27 is involved in steering the JA pathway towards the ET-coregulated ERF-branch, that is effective against necrotrophic pathogens and which antagonizes the anti-herbivore, ABA-coregulated MYC-branch. Our work characterizes a novel early JA-induced transcription factor that is involved in regulation of the balance between the ERF- and MYC-branch of the JA pathway, possibly via an effect on ET production.

Comparative Analysis of Signaling Pathways Triggered by Different Pattern-recognition Receptor-types

Wei-Lin Wan [a], Maricris Zaidem [b], Joachim Kilian [a], Detlef Weigel [b], Andrea A. Gust [a], Thorsten Nürnberger [a]

[a] *Center for Plant Molecular Biology and Plant Biochemistry, University of Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany*

[b] *Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tuebingen, Germany*

wei-lin.wan@zmbp.uni-tuebingen.de

Plant cell surface receptors sense microbial pathogens by recognizing microbial structures called pathogen or microbe-associated molecular patterns (PAMPs/MAMPs). There are two major types of plant pattern recognition receptors: 1. Leucine-rich repeat receptor proteins (LRR-RP) and leucine-rich repeat receptor kinases (LRR-RK) and 2. Plant receptor proteins and receptor kinases carrying ectopic lysin motifs (LysM-RP and LysM-RK). Although many studies focused on the signal pathways triggered by these receptors individually, the exact overlap and the differences, respectively, between these pathways remain unknown. We use three different PAMPs, flg22, nlp20, chitin (chitohexaose), and their corresponding receptor types, FLS2 (RLK), RLP23 (RLP), CERK1 (LYM-RK) for our comparisons. By systematic

analyses of defense responses such as transcriptome changes, ROS burst and accumulated hormones, we found that flg22 triggers faster and stronger early responses, and also causes more extensive transcriptome changes. On the other hand, only nlp20-treatment results in high amounts of salicylic acid and camalexin accumulation. After mutant lines screening we found that BIK1 may play different roles in signaling after flg22 and nlp20 treatments. We will present a more complete picture of PAMP-triggered immunity and to discover new key components participating in plant immunity.

Molecular regulation of the AAA ATPase LRD6-6 on cell death and immunity in rice[1]

Xiao-Bo Zhu, Rui-Hong Liang, Mu Ze, Jun-Jie Yin, Wen-Ming Wang* and Xue-Wei Chen*

State Key Laboratory of Hybrid Rice, Key Laboratory of Major Crop Diseases & Collaborative Innovation Center for Hybrid Rice in Yangtze River Basin, Rice Research Institute, Sichuan Agricultural University at Wenjiang, Chengdu, Sichuan

E-mails: Wen-Ming Wang (j316wenmingwang@163.com) and Xuewei Chen (xwchen88@163.com).

The endosomal sorting complex required for transport (ESCRT) machinery has been reported to paly essential roles in the formation of multivesicular bodies (MVBs) which mediate the delivery of cargo destined for degradation to the vacuole or lysosome [2]. The ESCRT pathway is comprised of five complexes, ESCRTs-0 (plants lack orthologs of this subunits), -I, -II, and -III, and the AAA ATPase VPS4/SKD1 [2]. Previous studies have shown that MVBs-mediated vesicular trafficking may play key roles in plant immunity and cell death [3]. However, the molecular regulation is poorly understood in rice. Here we report the identification and characterization of a MVBs-localized AAA ATPase LRD6-6 in rice. Disruption of LRD6-6 leads to enhanced immunity and cell death in rice. The ATPase activity and self-association of LRD6-6 is essential for its regulation on plant immunity and cell death. An ATPase inactive mutation (LRD6-6^{E315Q}) leads to dominant-negative inhibition in plants. The LRD6-6 protein co-localizes with the MVBs marker protein RabF1/ARA6 and interacts with ESCRT-III components OsSNF7 and OsVPS2. Further

analysis reveals that LRD6-6 is required for MVBs-mediated vesicular trafficking and inhibits the biosynthesis of antimicrobial compounds. Collectively, our data indicate that the AAA ATPase LRD6-6 inhibits plant immunity and cell death most likely through modulating MVBs-mediated vesicular trafficking in rice.

References:

[1] Zhu, X., Yin, J., Liang, S., Liang, R., Zhou, X., & Chen, Z., et al. (2016). The multivesicular bodies (MVBs)-localized aaa atpase lrd6-6 inhibits immunity and cell death likely through regulating mvbs-mediated vesicular trafficking in rice. Plos Genetics, 12(9), e1006311.
[2] Henne, W.M., N.J. Buchkovich, and S.D. Emr (2011). The ESCRT pathway. Dev Cell, **21**(1): p. 77-91.
[3] Inada N, Ueda T (2014). Membrane trafficking pathways and their roles in plant-microbe interactions. Plant & Cell Physiology, 55(4):672-86.

Interplay of MAPK signaling and calcium-regulated transcription in plant innate immunity

Xiyuan Jiang [a], Justin Lee [a], Dierk Scheel [a]
[a] *Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Weinberg 3, Halle (Saale), Germany*
xjiang@ipb-halle.de

Plant innate immune responses against pathogenic microorganisms are initiated after recognition of pathogen-derived molecules by pattern recognition receptors (PRRs), and this recognition elicits a complex signal transmission network that includes early signaling events, such as activation of MAP kinase cascades and other calcium-dependent pathway. It has been reported that the *Arabidopsis thaliana* Calmodulin (CaM)-binding transcription factor 3 (designated CAMTA3) negatively regulates plant defense pathway. Many defense-related genes are upregulated in *camta3* mutants, according to microarray data analysis [1]. CAMTA3 appears to be a direct repressor of the expression of the *EDS1*, *NDR1* and *EIN3* genes by directly binding to their promoter region in a sequence specific manner [2,3]. And recently, phosphoproteomics studies suggest that CAMTA3 is a potential phospho-target of pathogen-induced MAPKs [4]. It is, however, not clear how

phosphorylation of CAMTA3 by MAPKs affects plant defense regulation. In this project, we successfully showed that CAMTA3 can interact with pathogen-induced MAPKs, MPK3, MPK4, and MPK6 *in vivo*. And meanwhile, all these MAPKs directly phosphorylate CAMTA3 *in vitro*, and MPK3 and MPK6 phosphorylate CAMTA3 *in vivo* as well. We also could show that flg22- and MAPKs-induced phosphorylation results in CAMTA3 degradation via proteasome-mediated pathway. We propose a model where the negative role of CAMTA3 in plant innate immune pathway, is regulated by pathogen-induced MAPKs phosphorylation, which can induce its degradation, and therefore releasing CAMTA3 from defense-related gene promoters, and enabling expression of downstream defense genes.

References:

[1] Y. Galon, R. Nave, J. M. Boyce, D. Nachmias, M. R. Knight, H. Fromm (2008). FEBS Letters, 582, 943-948

[2] L. Du, G. S. Ali, K. A. Simons, J. Hou, T. Yang, A. S. N. Reddy, B. W. Poovaiah (2009). Nature, 457, 1154-1158

[3] H. Nie, C. Zhao, G. Wu, Y. Wu, Y. Chen, D. Tang (2012). Plant Physiology, 158, 1847-1859

[4] W. Hoehenwarter, M. Thomas, E. Nukarinen, V. Egelhofer, H. Röhrig, W. Weckwerth, U. Conrath, G. J. M. Beckers (2013). Molecular & Cellular Proteomics, 12(2), 369-380

Genome-wide epigenetic and transcriptional profiling of *Arabidopsis* EDM2

Yan Lai [a,b], Xueqing Maggie Lu [c], Jianqiang Wang [a], Tokuji Tsuchiya [d], Karine G. Le Roch [c] and Thomas Eulgem [a]

[a] *Department of Botany & Plant Sciences, Institute for Integrative Genome Biology, Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA*

[b] *College of Life Sciences, Fujian Agriculture and Forestry University, No.15 Shangxiadian Road, Cangshan District, Fuzhou, Fujian, 350002*

[c] *Department of Cell Biology and Neuroscience, Institute for Integrative Genome Biology, Center of Disease Vector Research, University of California, Riverside, CA 92521, USA*

[d]*Department of Applied Biological Sciences, College of Bioresource Science, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*

yan.lai@ucr.edu

The *Arabidopsis thaliana* EDM2 (enhanced downy mildew2) is a nuclear-localized protein including $2^{1/2}$ PHD-finger-like epigenetic reader domain and one cytosine specific DNA methyltransferase domain. Previous studies showed that EDM2 is required for several developmental process and race-specific immunity against oomycete *Hyaloperonospora arabidopsidis* (*Hpa*) mediated by the disease resistance gene *RPP7*. In addition, EDM2 controls levels of H3K9me2, H3K27me1 and CHG methylation at some loci. EDM2 recognizes the intronic heterochromatin region of *RPP7* and blocks the proximal polyadenylation site selection by maintaining the high H3K9me2 levels around this site, resulting in the synthesis of full length RPP7 coding transcripts. By genome wide epigenetic and transcriptional profiling we show EDM2 to have a broad role in controlling a set of genes involving in DNA or RNA metabolism or other biological process. Over 93% of differential genes in *edm2-2* are hypermethylated. Several NBS-LRR immune receptors regulated by EDM2 were also identified. New results on the roles of EDM2 will be presented at the meeting.

Reference:

[1] T, Eulgem, et al, (2007). The Plant Journal, 49, 829

[2] T, Tsuchiya, T, Eulgem (2010). The Plant Journal, 62, 518

[3] T, Tsuchiya, T, Eulgem (2013). Sci Rep, 3, 1701

[4] T, Tsuchiya, T, Eulgem (2013). Proc Natl Acad Sci USA, 110, E3535

PMCK1 associates with FLS2 to regulate plant innate immunity

Yunhe Jiang, Baoda Han, Kiruthiga G.Mariappan, HeribertHirt

Desert Agriculture Initiative, 4700 King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia.

yunhe.jiang@kaust.edu.sa

The first layer in plant innate immunity is the recognition of pathogen-associated molecular patterns (PAMPs) such as bacterial flagellin or fungal chitin by cell-surface pattern-recognition receptors (PRRs) (1, 2). In Arabidopsis, flg22, a synthetic 22-amino

acid flagellin peptide, is perceived by the PRR complex FLAGELLIN-SENSING 2 (FLS2) together with BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) which ultimately activates a signaling cascade resulting in the establishment of PAMP-triggered immunity (PTI) (3).Here, we show that the plasma membrane/cytoplasmic kinase 1 (PMCK1) associates with FLS2to regulate plant innate immunity. PMCK1 is an active kinase andlocalizes to the cytosol and plasma membrane. PMCK1 physically interacts with FLS2 and the association is not altered upon flg22 treatment. The flg22-induced reactive oxygen species (ROS) production is decreased in *pmck1* plants, and the flg22-induced expression of the PTI marker gene *FRK1* is also affected in *pmck1* plants, indicating that PMCK1 regulates flg22 triggered immunity response. Consistently, global transcriptome analysis by RNA sequencing shows that the overall response to flg22 is decreased in *pmck1*. Furthermore, *pmck1* plantsshow enhanced susceptibility to *Pst* DC3000 *hrcC*.Taken together, PMCK1 associates with FLS2 toregulate PTI.

References:

[1] J. D. G. Jones, J. L. Dangl (2006). The plant immune system. Nature 444, 323-329

[2] D. Couto, C. Zipfel (2016). Regulation of pattern recognition receptor signalling in plants. Nat Rev Immunol 16, 537-552

[3] T. Boller, G. Felix (2009). A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. Annual review of plant biology 60, 379-406

Osa-miR166 negatively regulates rice immunity against the rice blast fungus *Pyricularia oryzae*

Xiao-Long Cao, Zhi-Xue Zhao, Yong Zhu, Yan Li, Wen-Ming Wang

Rice Research Institute, Sichuan Agricultural University, Chengdu, 611130

1113641883@qq.com

MicroRNAs (MiRNAs) are 20-24 nt non-coding RNAs that play important roles in plant

growth and development. Previous studies have demonstrated that miR166 regulates development and stress-induced responses through five target genes (*PHB*, *PHV*, *REV*, *ATHB8* and *ATHB15*) of the *HD-ZIP III* transcription factor family [1,2]. However, the potential role of miR166 in regulating rice immunity against *Pyricularia oryzae* is unclear. Our previous deep sequencing data showed that miR166 is up-regulated upon *P. oryzae* infection in a susceptible accession LTH and a resistant accession IRBLkm[3]. Here, we showed that miR166 may negatively regulate rice immunity against *P. oryzae*. Transgenic rice lines overexpressing miR166a exhibited more susceptible than wild type. On the contrary, transgenic lines overexpressing an artificial target mimicry that sponges miR166a displayed enhanced resistance to *P. oryzae*.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (grant 31430072 to W-MW).

References

[1] Xiaoyun Jia, Na Ding, et.al. (2014). Plant Science, 233, 11.

[2] Hui Zhang, Jinshan Zhang, et.al. (2017). Proceedings of the National Academy of Sciences, 114, 5277.

[3] Yan Li, Yuangeng Lu, et.al. (2014). Plant Physiology, 164, 1077.

OsCJ1 encoding a cytoplasmic kinase negatively regulates rice resistance to bacterial blight

Jie Chen, Shiping Wang, *

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070

**E-mail of Corresponding author*: swang@mail.hzau.edu.cn

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a serious bacterial disease in rice worldwide. Cytoplasmic protein kinases play critical roles in the defense response in plants. However, the roles of cytoplasmic protein kinases in rice-bacterium interations are not well understood. Here, we identified a putative cytoplasmic protein kinase OsCJ1, whose expression was induced by *Xoo*. *OsCJ1* knockdown plants showed a broad-spectrum resistance to *Xoo*. By yeast two hybrid analysis, we identified protein

OsIP1, which interacted with OsCJ1. *OsIP1*'s expression was also induced by *Xoo*. In addition, OsCJ1 could phosphorylate OsIP1 *in vitro*. Furthermore, *OsIP1*-overexpressing plants showed enhanced resistance to *Xoo*. These results suggest OsCJ1 may function as a negative regulator in rice-Xoo interaction.

Key Words: rice-*Xoo* interaction; cytoplasmic kinase.

Identification of a novel rice bacterial blight resistance gene derived from a mutant H120

Shen Chen, Congying Wang, Wenjuan Wang, Jing Su, Aiqing Feng, Liexian Zeng, Jianyuan Yang, Xiaoyuan Zhu*

Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640

**Corresponding author. E-mail: ricedisease@tom.com*

Rice bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious disease in rice produced countries. Resistance breeding is always the most effective method to control BB disease. The mutant line H120 derived from the *japonica* line Lijiangxintuanheigu which induced by space flight is resistant to all Chinese BB races, including regnant pathotype Ⅳ. To identify and map the BB resistance gene(s) involved in Chinese *Xoo* races, the association assay of phenotypic and genotypic variations was examined in two F_2 populations derived from the crosses between H120/JG30 and H120/IR24. The segregation ratios of F_2 individuals from the crosses of H120/CO39 and H120/IR24 were 1960 resistant : 608 susceptible and 952 resistant : 311 susceptible, respectively, which is consistent with the expected allelic frequency of a 3:1 ratio, which suggested that H120 resistance is controlled by a dominant resistance gene, which temporarily nominated *Xa41*(t). Genetic analysis and graphical mapping indicated that *Xa41*(t) was located between the flanking markers RM26981 and RM26984 within an approximately 75-kb region on chromosome 11. The eight candidate genes functionally predicted were included in the target region. The identification of *Xa41*(t) conferring resistance to pathotype Ⅳ is of significant value to rice breeding programs for marker-assisted breeding and gene pyramiding strategies. Further characterization of *Xa41* (t) at the protein level will be helpful to elucidate the mechanisms of resistance.

Spatio-temporal orientation of microtubules controls conical cell shape in *Arabidopsis thaliana* petals

Huibo Ren[a, b], Xie Dang[b], Deshu Lin[a, b*]

[a] *Basic Forestry and Proteomics Research Center, Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou*

[b] *Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou*

xiap127@126.com

The physiological functions of epidermal cells are largely determined by their diverse morphologies. Most flowering plants have special conical-shaped petal epidermal cells that are thought to influence light capture and reflectance, and provide pollinator grips, but the molecular mechanisms controlling conical cell shape remain largely unknown. Here, we developed a live-confocal imaging approach to quantify geometric parameters of conical cells in *Arabidopsis thaliana* (*A. thaliana*). Through genetic screens, we identified *katanin* (*KTN1*) mutants showing a phenotype of decreased tip sharpening of conical cells. Furthermore, we demonstrated that SPIKE1 and Rho of Plants (ROP) GTPases were required for the final shape formation of conical cells, as KTN1 does. Live-cell imaging showed that wild-type cells exhibited random orientation of cortical microtubule arrays at early developmental stages but displayed a well-ordered circumferential orientation of microtubule arrays at later stages. By contrast, loss of KTN1 prevented random microtubule networks from shifting into well-ordered arrays. We further showed that the filamentous actin cap, which is a typical feature of several plant epidermal cell types including root hairs and leaf trichomes, was not observed in the growth apexes of conical cells during cell development. Moreover, our genetic and pharmacological data suggested that microtubules but not actin are required for conical cell shaping. Together, our results provide a novel imaging approach for studying petal conical cell morphogenesis and suggest that the spatio-temporal organization of microtubule arrays plays crucial roles in controlling conical cell shape.

References:

H.B. Ren, X Dang, Y.Q. Yang, D.Q. Huang, M.T. Liu, X.W. Gao, D.S. Lin (2016). Plant Physiol 172: 358-371.

Proteomic Analysis of Differentially Expressed Proteins of *Nicotiana benthamiana* Triggered by INF1 Elicitin from *Phytophthora infestans*

Juan Du, Xiao Guo, Lin Chen, Conghua Xie, Jun Liu

Huazhong Agricultural University, Wuhan, 430070

Email: juandu@mail.hzau.edu.cn

Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is considered to be durable, given that PAMPs are conserved in entire classes of microbes. Elicitins are structurally conserved extracellular proteins in oomycete species and are well characterized as having features of PAMPs. INF1 is an elicitin protein secreted by the late blight pathogen *Phytophthora infestans*. A cell surface receptor-like protein that mediates INF1 response was recently cloned in potato. In addition, some other genes are reportedly involved in INF1-triggered immune responses; however, the molecular mechanisms of INF1-triggered immunity remain poorly understood. Here, we used isobaric tags for relative and absolute quantification-based quantitative proteomics to analyze proteins involved in INF1-triggered cell death responses in *Nicotiana benthamiana*. Our approach identified 2964 proteins, 32 of which were significantly altered in abundance after INF1 induction. Two of eight selected upregulated proteins, namely, ATP dependent transporter and 60S ribosomal protein L15 were shown to be essential in INF1-triggered cell death responses by virus-induced gene silencing analysis. This study represents the first proteomic analysis of INF1-triggered cell death responses in plants and provides the basis for further work to elucidate molecular mechanisms into oomycete PTI in host plants.

References:

[1] Du J, Guo X, Chen L, Xie C, Liu J*. Proteomic analysis of differentially expressed proteins of *Nicotiana benthamiana* triggered by INF1 elicitin from *Phytophthora infestans*. Journal of General

Plant Pathology, 2017, 83(2): 66-77

A receptor like kinase gene *SBRR1* positively regulates resistance to sheath blight in rice

Zhiming Feng, Yafang Zhang, Yu Wang, Huimin Zhang, Wenlei Cao, Zongxiang Chen, Xuebiao Pan and Shimin Zuo*

Key Laboratory of Crop Genetics and Physiology/Co-Innovation Center for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009

**Corresponding author: smzuo@yzu.edu.cn*

Presenting author: fengzhiming88@163.com

Abstract: Sheath blight (SB), caused by the necrotrophic fungal pathogen *Rhizoctonia solani* Kühn, poses a great threat to the rice grain yield as one of the most serious rice diseases. Receptor like kinases play important roles in plant defense responses to disease. However, receptor like kinases associated with rice SB have not been reported. We analyzed the differential gene expression profiles of the rice variety YSBR1 with high resistance to SB before and after inoculation with *R. solani*, and isolated a rice receptor like kinase gene *SBRR1* (*sheath blight-related RLK gene 1*) induced by *R. solani*. *SBRR1* is preferentially expressed in leaf sheaths and leaves, which is consistent with the main infective site of *R. solani*, implying its role in regulating rice resistance to SB. The *SBRR1* loss-of-function mutant *sbrr1* is more susceptible to SB compared with its wild type. Moreover, knockdown of *SBRR1* expression by RNAi reduces the resistance of rice to SB. These results demonstrated that *SBRR1* positively regulates resistance to SB. We further identified an SBRR1-interaction protein SIP1 (SBRR1 interaction protein 1) that encodes an ankyrin repeat protein via yeast two hybrid screening. *SIP1* is also induced by *R. solani* and preferentially expressed in leaf sheaths and leaves. Our results enrich the knowledge of the molecular mechanism of resistance to rice SB, which will provide the theories basis for

establishing a new strategy to prevent and control this disease.

Exploration and optimization of the preparation conditions of protoplasts in *Oryza sativa*

Chen yue, Fu Jian, Wang lingxian, Yin fuyou, Wang bo, Zhong qiaofang, Cheng zaiquan

Biotechnology & Genetic Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650223, Yunnan

E-mail of presenting author: 358455454@qq.com

Recent years, protoplasts have been widely used in functional genomics research. *Oryza sativa* protoplasts were obtained from embryogenic suspension cells by cell walls enzymatic hydrolysis. However, protoplast isolation, transformation and downstream analyses in rice are often hampered by a number of factors, such as time-consuming, cost-intensive and low transformation efficiency. In this study, the preparation process of embryogenic suspension cell line was optimized in *Oryza sativa*. The results showed that: (1) the initial dosage of the suspension cell should be inoculated with 0.4 g callus per 25 mL liquid medium， and the cell mass should be uniform and bright color ; (2) 0.01% FDA activity test and examination showed that the *Oryza sativa* status was better, the cell debris was less and the protoplast activity was higher in microscope treated by 0.6 M mannitol; (3)before enzymatic hydrolysis, large particles suspended cells must be filtered with 60 mesh stainless steel mesh filter. In this step, parts of dead cells will be put away and the time and efficiency of enzymatic hydrolysis was improved obviously; (4) the enzyme mixture was optimized as 1.5% cellulase + 0.3% pectinase + 0.6 M mannitol + 5 mM MES + 5 mM $CaCl_2$. Using this enzyme mixture, the yield of protoplast was up to 1.4×10^6, the activity reached to 89.4 %. Finally the enzymolysis time was reduced from 6 hours to 2 hours. This study, a high-efficiency and reliable technology of protoplasts preparation was established in *Oryza sativa*. It will be a useful tool in functional genomics research in *Oryza sativa*.

A potato field resistance against late blight in QTL dPI09c is conferred by the major resistance gene as revealed by map-based cloning and diagnostic RenSeq

Rui Jiang[a], Zhendong Tian, Juan Du, Fanye Meng, Huan He, Jun Zhou, Merideth Bonierbale, Ingo Hein, Hannele Lindqvist-Kreuze, Conghua Xie

[a] *Potato Biology and Biotechnology Key Laboratory of Ministry of Agriculture; Huazhong Agricultural University, Wuhan, 430070*

jiangrui828@webmail.hzau.edu.cn

Potato late blight caused by *Phytophthora infestans* (Mont.) is one of the most destructive crop diseases and seriously threatens potato production. We previously identified a major QTL, dPI09c, against late blight disease on potato Chromosome 9[1]. The present research was conducted to clone the gene(s) conferring dPI09c. An expanded population encompassing 4000 progenies ($B3C1HP_{4000}$) was used to fine-map the interval of *dPI09c*. Recombinants were obtained by using previously published flanking markers, and revealed a 1:1 segregation both in the field and greenhouse late blight resistance evaluations, suggesting a single dominant gene may be responsible for the resistance QTL dPI09c. Additional PCR markers for the end of Chromosome 9 were developed and 3 recombinants identified that enabled the fine-mapping of dPI09c. For cloning the gene, a BAC library was constructed and the sequencing of the selected clones revealed that the QTL dPI09c resides in a 186kb DNA stretch. To identify the gene conferring the resistance in QTL dPI09c, the parents and the resistant and susceptible bulks of the $B3C1HP_{4000}$ progenies were utilized for resistance gene enrichment sequencing (RenSeq and dRenSeq). The results showed that only a single gene conferred resistance. Our data further demonstrate that this gene is also found in specific *Solanum demissum accession,* in diverse breeding lines, as well as in cultivars, which present high levels of resistance to a set of diverse *P. infestans* isolates.

References:

[1] Li, J., Lindqvist-Kreuze, H., Tian, Z., Liu, J., Song, B., Landeo, J., Portal, L., Gastelo, M., Frisancho, J. and Sanchez, L. (2012) Conditional QTL underlying resistance to late blight in a

diploid potato population. Theoretical and Applied Genetics, 124, 1339-1350.

Evaluation of adzuki bean germplasm resistance against rust and histological observation of rust infection on different cultivars

Xiwang Ke [a], Dong Han [a], Lihua Yin [a], Yongbo Li [b], Zhenxing Zhang [c], Yuhu Zuo [a#]

[a] *Heilongjiang Bayi Agricultural University, XinFeng Road, Daqing*

[b] *Plant protection and inspection station of Baoquanling bureau, Heilongjiang Land Reclamation, Hegang*

[c] *Agricultural Technology Extension Center of Qiang'an county, Qing'an, Suihua*

kexylh@163.com

Adzuki bean (*Vigna angularis*) is one of the important legume crops of the Ceratotropis subgenus in Asian, under the papilionoid subfamily of the Fabaceae. Due to its sweet taste with high protein and starch content, adzuki bean is widely cultivated and used as traditional food material in East Asia and other areas. However, adzuki bean rust caused by the fungus *Uromyces vignae* is one of the major diseases in adzuki bean production, and the use of resistant varieties is an economically effective management strategy. Unfortunately, major adzuki bean cultivars used presently were susceptible to the rust.

In current study, 85 adzuki bean accessions were evaluated for rust resistance. Subsequently, histological observation of the rust infection on different cultivars was conducted to reveal the resistance mechanism. Results showed that there were 2 immunity cultivars, 16 high resistance cultivars, 10 resistant cultivars, 26 medium susceptible cultivars and 31 high susceptible cultivars. The symptoms and uredia on the resistant ones delayed for 2-3 d compared to the susceptible cultivars. Additionally, lower density and smaller size of uredia generated on resistant cultivars were also observed. Histological investigation of the rust infection on resistant cultivar, via fluorescence microscopy and staining with calcoflour white, showed a decrease in ratio of urediospore germination and infection, cell wall deposits and cell death for preventing the fungus spreading.

These results indicated that there were abundant resistance resources in adzuki bean accessions, and the resistant adzuki bean cultivars mainly prevent rust disease through inhibiting germ tube invasion and mycelium spreading.

Acknowledgement: Financial support by National Natural Science Foundation of China

(31501629), China Postdoctoral Science Foundation (2014M561378), Scientific research project of agricultural reclamation regional central (HNK125B-08-08A, HNK135-02-10-3, HNK135-02-10-5) and Heilongjiang college students innovative entrepreneurship training program (2017nxy05).

Molecular Marker Mapping and Cloning of the Resistance Gene of Blister Rust in *Pinus armandii* Franch

LI Cui-xin, HE De

Department of biology science, Southwest Forestry University, Kunming city

If three templates can be all amplificated with clear bands in ISSR-PCR, the primer will be selected. There were 18 primers were selected totally. After ISSR-PCR were carried out by the 18 primers, special bands were produced in ISSR with three primers between the resistant samples and the diseased samples of *Pinus armandii* Franch, which may be related with the resistant gene of blister rust. Only primer SONG-10 can not be amplificated only the special band about 250bp-500bp between above two kinds of samples, but also the special band can be located at the site h13 (between h9 and h15) of c1 linkage group in genetic linkage map of *P. armandii*, which showed that the specific band amplificated with SONG-10 was an ISSR marker which associated with resistant gene of blister rust.

After the special band was purificated, a light and single DNA band was obtained. The DNA fragment was linked with vetors and brought into competent cells. A few or more colonies of bacteria appeared on the culture plate in the second day. Using the single bacteria colony as template to do colony PCR, the result of agarose gel showed that it was the aim band. After sequencing for the DNA band, the BLAST result of its sequence showed 84％ similarity with *Pinus taeda*, which suggested that the ISSR marker be linked to resistant gene of blister rust in *P. armandii*.

Our work provided the basis for deeply studying on resistant genes related with blister rust of *P. armandii*.

References:

[1] Hu Hong-li. The research situation of domestic pine blister rust [J]. Journal of Southwest Forestry University, 2004,24 (4): 73-78.

[2] Lerceleau E, Plomion C, Anderason B. AFLP mapping and detection of quantitative trait

loci(QTLs) for economically important traits in Pinus sylceatris:a preliminary study[J].Molecular Breeding ,2000,(6):451-458.
[3] CHEN Yan-ping， MENG Qing-chang， YUAN Jian-hua. Identification of the Molecular Markers Linked to the MRDV-resistance Locus in Maize Using SSR-BSA Technique [J]. Jiangshu J. Agr．Sci．，2008，24(5)：590—594.
[4] GU Ai-xing. Analysis of vertcallium wilt resistance and selection of molecular markers in cotton. Wulumuqi: Xinjiang agricultural university. 2013
[5] Lerceleau E,Plomion C,Anderason B.AFLP mapping and detection of quantitative trait loci(QTLs) for economically important traits in Pinus sylceatris:a preliminary study[J].Molecular Breeding ,2000,(6):451-458.
[6] WANG Ling-ping．WU Xiao-hua，WANG Bao-gen, et al. SCAR marker linked to resistance gene of powdery mildew in bottle gourd [Lagenaria siceraria(Molina) Standl.] breeding line J083[J]．Journal of Zhejiang University (Agric. & Life Sci.)，2011，37(2)：119-124.
[7] QIAN Yi-liang Genetic Analysis and QTL Detection of bacterial wilt resistance in tobacco (*N. tabacum* L.)[D]. Hefei: Anhui Agricultural University.2013.

Construction of ISSR Molecular Genetic Map of *Pinus armandii* Franch

HE De, LI Cui-xin

Department of biology science, Southwest Forestry University, Kunming city

A molecular genetic linkage map will be put a high value on studies designed to analyze genetic variation，to identify qualitative character，to locate quantitative character loci，and to perform marker-assisted selection in plants. *Pinus armandii* Franch is a special aiphyllium with high economic value in China. In the paper, three linkage group of *P. armandii* were gotten by ISSR-PCR based on 115 endosperm samples. The linkage groups contains1702.4cM, the longest linkage group incluing 19 ISSR sites is 801.0cM; and the shortest linkage group incluing 9 ISSR sites is 442.4cM.The average distance of three linkage group is 567.5cM.The longest distance between marks is 167.3cM; and the shortest distance between markers is 9.2cM.The average distance between markers is 44.8cM. The genetic map supplies basis to deep research on *P. armandii*.

References:

[1] Lerceleau E,Plomion C,Anderason B.AFLP mapping and detection of quantitative trait loci(QTLs) for economically important traits in Pinus sylceatris:a preliminary study[J].Molecular

Breeding, 2000, (6):451-458.
[2] Kubisa KTL, Stine M,Nelson CD,et al.Comparative mapping in a single long lesf pine [C]. SANDIEGO, CA: PLANT GENOME Ⅲ.1995:223-239.
[3] He Z-X, Lin G-Z, Shi J-S. The application of mapmaker 3.0 (for PC) and the development of a companion program [D]. Nanjing : Nanjing Forestry Univisity, 1999.
[4] Godwin I D,Aitken E A,Smith L W. Application of inter-simple sequence repeat(ISSR) markers to plant genetics[J]. Electrophoresis, 1997, 18: 1524 -1528.

Tomato LysM receptor-like kinases mediate symbiosis and immunity

Dehua Liao, Yan Liang
Institute of Biotechnology, Zhejiang University, Hangzhou

Many plants have the ability to establish symbiosis with arbuscular mycorrhizal (AM) fungi, which improves the growth of host plants. The AM symbiosis is initiated by the signal communication between partners. Myc factors, consisting of short-chain chitin backbone with various substitutions, are the major signaling molecules secrected by AM fungi. The key step in establishing the AM symbiosis is the plant recognition of fungal signal molecules. However, how plants recognize Myc factors is still unclear. It has been hypothesized that Myc factor recognition might have evolved from the receptor of chitin, which is the major component of fungal cell wall and acts as a MAMP (Microbe-Associated Molecular Patterns) inducing plant innate immune responses. Phylogenetic analysis indicates that four LysM receptor-like kinases are clustered into one group with Arabidopsis chitin receptor, AtCERK1, in tomato. After silencing these genes individually by virus-induced gene silencing (VIGS) approach. We found that *SlLYK1* is essential for chitin-triggered immunity, but not for the AM symbiosis, whereas *SlLYK12* is required for the AM symbiosis, but not for chitin-triggered immunity. Our results suggest that *SILYK1* maintained the ancestral role in the chitin recognition, anda gene duplication occurs in tomato, preceding the origin of the Myc factor recognition.

A NIN and NIP1 complex mediates nitrate inhibition of nodulation in *Medicago truncatula*

Jie-shun Lin[1, 2], Xiaolin Li[1], Kirankumar S. Mysore[3], Jiangqi Wen[3] and Fang Xie[1]

[1] *National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

[2] *University of the Chinese Academy of Sciences*

[3] *Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, Oklahoma 73401*

Nitrate is a major macronutrient and signaling molecule for plant growth and development. Legume plants can assimilate inorganic nitrate from the soil and get ammonia through symbiotic interaction with nitrogen-fixing rhizobia. Symbiotic nitrogen fixation is an energy-consuming process and is strongly inhibited when sufficient levels of fixed nitrogen are available, but the molecular mechanisms governing this regulation are largely unknown. The transcription factor NIN (Nodule Inception) is strictly required for nodulation, whose induction by Nod Factor (NF) is block by nitrate through AON pathway. Here, we find that NIP1 (NIN Interaction Protein 1) interact with NIN and that down-regulation of *NIP1* expression in *M. truncatula* prevents nitrate inhibition of nodulation. Similarly, *nip1* mutants displayed resistance to nitrate inhibition of nodulation including rhizobial infection, nitrogenase activity and nodule development. In line with this, *nip1* showed a nitrate-starved phenotype in nitrate-replete conditions as indicated by increased expression of genes for nitrate uptake and other starvation markers. Moreover, we find nitrate triggers NIP1 re-localization from the cytosol to the nucleus. In addition, we find that NIP1 suppresses NIN activation of *CRE1* expression in *N. benthamiana*. Finally, using a root graft assay we show that NIP1 regulation of nitrate tolerance for nodule formation and lateral root formation is root-determined. Our findings highlight that nitrate sensing in legume plants triggers NIP1 translocation to the nucleus where it forms heterodimer with NIN thereby suppressing NIN activation of target genes expression.

A novel spontaneous nodule formation mutant *spd19* in *Medicago truncatula*

Haiyue Liu , Fang Xie

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai

Hyliu2015@sibs.ac.cn

Root nodule is a specific organ for rhizobia to fixed nitrogen in host legumes. Nodule organogenesis was triggered by rhizobial, but the legume host plant control the developmental program responsible for building the nodule tissues and for regulating the process. Previous studies shown that *snf1*[1] and *snf2*[2] in *Lotus japonicus,* which can spontaneously generated nodule-like tissues without rhizobium infection by continuous activated CCaMK and cytokinin receptor LHK1. Moreover, lateral root was another lateral root organ, but the molecular mechanisms governing the identity and maintenance of this organ are still poorly understood, and what's the difference of root nodule and lateral root are elusive.

In this study, we isolate a spontaneous nodule mutant *spd19* in an EMS mutagenesis screening. This mutant formed white nodules without rhizobia infection, and defective in lateral root development as well. This phenotype suggested this mutant may involve in regulation of nodule organogenesis and lateral root formation. Genetic analysis suggested that the *spd19* is a single gene controlled dominant mutant. The causative mutation was studied by map-based cloning, and this research would highlight the molecular mechanism between the initiation of nodule primordium and lateral root development.

References:

[1]Gleason C, Chaudhuri S, Oldroyd GE et al(2006). Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature, 441, 1149–1152.

[2]Suzaki, T., Yano, K., Ito, M., Umehara, Y., Suganuma, N., and Kawaguchi, M. (2012). Positive and negative regulation of cortical cell division during root nodule development in Lotus japonicus is accompanied by auxin response. Development 139: 3997–4006.

Effect of Exogenous Salicylic Acid on Antioxidase and Pathogenesis-related Proteins in Cucumber under the Infection of *Acidovorax citrulli*

LIU Jun[a], ZHANG Meng-yang[a]

[a] *College of Forestry and Horticulture, Xinjiang Agricultural University, No 311 Nongda East Road, Urumqi, Xinjiang*

liujem@126.com

In order to investigate the inductive effect of exogenous salicylic acid (SA) on cucumber against *Acidovorax citrulli*, using variety ‘Changchun Mici’ as material, the changes of antioxidases and pathogenesis-related proteins in cotyledons of cucumber were studied. After spraying with 7 $mmol \cdot L^{-1}$SA on 6 d cucumber seedlings, cotyledons were wounded inoculated with *Acidovorax citrulli*, and then sampled and tested the activities of antioxidase (SOD, POD and CAT), pathogenesis-related enzymes (PAL and PPO) and the content of reactive oxygen species ($O_2^{-} \cdot$and H_2O_2) on day 0 to 6 post inoculation. The results showed that the treatment of 7 $mmol \cdot L^{-1}$SA could significantly increase the activity of SOD, POD, CAT, PAL and PPO in cotyledons at the early stage (2 d) of interaction between cucumber and bacteria. But activities of these enzymes in cotyledons decreased in different degree along with the development of disease, especially the activity of POD and CAT reduced greatly, however the content of $O_2^{-} \cdot$and H_2O_2 were significantly increased at the same time. It indicated that SA could induce resistance in cucumber seedlings against *Acidovorax citrulli* at certain extent by raising the activities of antioxidase and pathogenesis-related enzymes, but the induction effect of SA in cucumber seedlings had timeliness.

References:

[1]Aicha Belkadhi, Antonio De Haro, Pilar Soengas, Sara Obregon, Maria Elena Cartea, Wahbi Djebali,and Wided Chaib(2013). Salicylic Acid Improves Root Antioxidant Defense System and Total Antioxidant Capacities of Flax Subjected to Cadmium, OMICS A Journal of Integrative Biology, 17,398

[2]Raskin I(1992). Salicylic acid, A New Plant Hormone, Plant Physiology，99,799

[3] Vanhoof Thram , Alblass W , Derijkr H(1986). Induction by Salicylic Acid of Pathogenesis-related Proteins and Resistance to Alfalfa Mosaic Virus Infection in Various Plant Species. Journal of General Virology,67,2143

The calcium sensor TaCBL4 and its interacting protein kinase TaCIPK5 positively regulate wheat resistance against *Puccinia striiformis* f. sp. *tritici*

Peng Liu, Yinghui Duan, Qinghe Xue, Jia Guo, Tuo Qi, Zhensheng Kang* and Jun Guo*

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100

E-mail:wood319@126.com; guojunwgq@nwafu.edu.cn; kangzs@nwafu.edu.cn

Calcineurin B-like proteins (CBLs) act as Ca^{2+} sensors to activate specific protein kinases, CBL-interacting protein kinases (CIPKs). Recent research has demonstrated that the CBL-CIPK complex is not only required for abiotic stress signaling, but also is likely involved in biotic stress perception. However, a full understanding of the role of this complex in immune signaling, including pathogen perception, is lacking. To this end, we isolated the key signaling components of the TaCBL-TaCIPK complex and characterized their role in wheat disease responses. Specifically, a wheat *CBL* gene, *TaCBL4*, was identified and characterized. *TaCBL4* encodes 219 amino acid containing four EF-hands. During the interaction between wheat and *Pst*, among all *TaCBLs*, *TaCBL4* mRNA accumulation showed a marked increase post-infection. Silencing of *TaCBL4* resulted in enhanced susceptibility to the avirulent race, CYR23. In a cDNA library which construct from infected leaves by *Pst*, TaCIPK5 was screening out as the interaction partner of TaCBL4. The interaction between TaCBL4 and TaCIPK5 was further confirmed by BiFC and Co-IP assay. In addition, accumulation of *TaCIPK5* mRNA was significantly induced by CYR23. Knock down of expression of *TaCIPK5* decreased the resistance of wheat to CYR23. In *TaCBL4*- or *TaCIPK5*-knockdown plants, reactive oxygen species (ROS) accumulation and hypersensitive response (HR) were greatly reduced. The expression of

two ROS scavenging genes *TaCAT* and *TaSOD* were significantly decreased, as were two pathogenesis-related proteins *TaPR1* and *TaPR2*. Thus, we inferred that TaCBL4-TaCIPK5 complex positively modulates wheat resistance to *Pst* in an ROS-dependent manner.

Acknowledgement: This work was supported by National Basic Research Program of China (No. 2013CB127700) and National Natural Science Foundation of China (No. 31371889；NO. 31171795).

Resistance of Wild Rice (*O. meyeriana*) to rice blast

Jing Li, Yun-qian Wang, Qiong Luo

State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Ministry of Education Key Laboratory of Agriculture Biodiversity for Plant Disease Management, Yunnan Agricultural University, Kunming 650201

E-mail of presenting author: qiongbf@aliyun.com

The wild species of *Oryza* contains mumerous genes of economic importance and are being used as an important cultivated rice gene pool to expand the genetic background, improve yield and quality, and increase the ability of resistance or tolerance to pests and bioic and abiotic stresses[1-3]. *Oryza meyeriana* (*O. meyeriana*) is one of three native wild rice species in China, mainly distributed in Yunnan province[4]. To further understand the disease resistance to rice blast, we carried out a systematic investigation of rice blast resistance of *O. meyeriana*. First, *O. Meyeriana* were inoculated with multiple blast strains from different rice planting areas by injection method. Then, we performed the cloning and functional identification of *Pid2* [5]and *Pid3*[6] orthologous genes in *O. meyeriana*. Our results showed that *O. Meyeriana* is sensitive to all blast isolates used in this experiment. We found that the sequences of DNA and amino are significant different between *Pid2* and its orthologous in *O. Meyeriana*, as *Pid3* and its orthologous. The Nipponbare plants overexpressed the orthologous genes of *Pid2* and *Pid3* showed mildly enhanced the susceptible to *Magnaporth oryzae*. We speculated that the orthologous of blast resistant genes harbored in *O. meyeriana* may be susceptible alleles, *O. meyeriana* exhibited the resistance to rice blast under the condition of natural inoculation was likely to be xeromorphic leaf structure may reduce susceptibility to *Magnaporth oryzae*,

characterization of related genes will help to breed elit rice. This hypothesis still needs further investigation to clarify.

References:

[1] C.Q. Yan, K.X. Qian, Q.S. Yan, et al. (2004). Plant Cell Report, 22,569-575.

[2] H.J. Li, X.H. Li, J.H. Xiao, et al. (2011). Molecular Plant, 5,281-290.

[3] Q.M. Lv, X. Xu, J.J. Shang, et al. (2013). Phytopathology, 103,594-599.

[4] Y.Yong,Y.S. Han(2014).Jurnal of Plant Gnentic Resources,15,472-476.

[5] X. W. Chen, J.J. Shang, D. X .Chen, et al. (2006). Journal of Plant, 46,794-804.

ER stress, a potential key mechanism regulating RTP1-mediated plant resistance to *Phytophthora parasitica*.

Xiaoyu Qiang [a], Qing Zheng [b,1] , Hong Zhao [b,1], Qiurui Zhang, Weixing Shan [a,*]

[a] *College of Agronomy, State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, Shaanxi 712100*

[b] *College of Plant Protection, State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, Shaanxi 712100*

[1] *These authors contribute equally*

Presenting author: qiangxiaoyu@nwafu.edu.cn

Oomycetes, particularly the *Phytophthora* species, cause destructive diseases on agriculturally crops, forests and natural ecosystems, such as potato late blight mediated by *P. infestans* (Nowicki et al., 2012), *Phytophthora* root and stem rot by *P. sojae* (Tyler et al., 2006). To investigate the host factors that participate in compatible plant–*Phytophthora* interactions, we have taken advantage of the compatible interaction between *P. parasitica* and the model plant *Arabidopsis* (Wang et al., 2011) to uncover the genetic and molecular basis of plant susceptibility to oomycete pathogens. Recently, we have identified a novel gene, *RTP1*, which might negatively regulate resistance to a broad range of biotrophic pathogens but not necrotrophs (Pan et al., 2016). The *RTP1* encodes a nodulin MtN21 family protein, which localizes in ER. To deepen the molecular mechanisms of RTP1-mediated resistance, we have established a set of molecular, biochemical and cytological studies on WT and *rtp1* knockout mutant. Our preliminary results indicated that

rtp1 mutant showed hypersensitivity to ER stress and RTP1 might function in tunicamycin-induced ER stress. The kinetic analyses in WT roots demonstrated that ER stress signaling was significantly regulated upon infection by *P. parasitica*, as most UPR marker genes exhibited induction by *P. parasitica*. In comparison, the induction of several key UPR marker genes was impaired or partially impaired in *rtp1* mutant. Furthermore, our biochemical studies implied that RTP1 might be required for protein secretion. In sum, we present data indicating that RTP1 might play an important role in sensing ER stress and regulating ER stress-mediated plant immunity upon infection by *Phytophthora*.

Acknowledgement: This work was supported by the 111 Project from the Ministry of Education of China to WS and NWAFU research funding (Z111021309) to XQ.

References:

[1] Wang Y, Meng YL, Zhang M, Tong XM, Wang QH, Sun YY, Quan JL, Govers F, Shan WX. (2011) Infection of *Arabidopsis thaliana* by *Phytophthora parasitica* and identification of variation in host specificity. *Molecular Plant Pathology* 12: 187–201.

[2] Pan, Q. N., Cui, B. M., Deng, F. Y., Quan, J. L., Loake, G, and Shan*, W. (2016) RTP1 encodes a novel ER-localized protein in *Arabidopsis* and negatively regulates resistance against biotrophic pathogens. *New Phytol*. 209: 1641-1654.

Lipopolysaccharide triggers a burst of reactive oxygen species in chloroplasts

Keke Shang-Guan [a], Min Wang [a], Nang Myint Phyu Sin Htwe [a], and Yan Liang [a] *

[a] *College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, 310058*

*kekeshangguan@zju.edu.cn , *yanliang@zju.edu.cn*

Lipopolysaccharide (LPS) triggers immune responses in both plants and mammals. In mammals, LPS is recognized at the plasma membrane by Toll-like receptor 4 (TLR4) but also in the cytoplasm. In plants, the plasma membrane-localized receptor for LPS was identified, LIPOPOLYSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE). However, there is no clue whether a cytoplasmic receptor for LPS is present in plants. In this study, we found LPS triggered a biphasic production of reactive oxygen species (ROS) in Arabidopsis. LPS-triggered second elevation of ROS burst is dose dependent, which is

conserved in dicot and monocot. The first transient burst was similar to that induced by flagellin, whereas the second, long-lasting burst was unique to LPS. Microscope observation revealed that the second ROS burst was produced in chloroplasts. LPS can impair chloroplast development and function. We also found LPS increases Botrytis-Induced Kinase 1 (BIK), a central immune regulator, protein accumulation and LPS-triggered ROS level was significantly reduced in *bik1* mutants. Interestingly, the LPS-triggered chloroplast ROS burst was only partially dependent on the LORE receptor, suggesting that, similar to mammals, LPS recognition in plants may occur both at the plasma membrane but also within the cytoplasm.

High efficient genome editing in allotetraploid cotton (Gossypium hirsutum) using CRISPR/Cas9 system

Lin Sun, Hangping Rui, Lei Qin, Pengcheng Wang, Jun Zhang,, Shuangxia Jin,[*] and Xianlong Zhang [*]

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei

[*] *Corresponding author: jsx@mail.hzau.edu.cn and xlzhang@mail.hzau.edu.cn*

Abstract

The CRISPR/Cas9 system has widely used in plant genome editing. In our previous study, we successfully utilized a CRISPR/Cas9 system to edit the allotetraploid cotton(Wang et al., 2017). An exogenously transformed gene Discosoma red fluorescent protein2(*DsRed2*) and an endogenous gene *GhCLA1* were chosen as targets. The *DsRed2*-edited plants in T0 generation reverted its traits to wild type, with vanished red fluorescence the whole plants. Besides, the mutated phenotype and genotype were inherited to their T1 progenies. For the endogenous gene *GhCLA1*, 75% of regenerated plants exhibited albino phenotype with obvious nucleotides and DNA fragments deletion. The efficiency of gene editing at each target site is 66.7–100%. The mutation genotype was checked for both genes with Sanger sequencing. Barcode-based high-throughput sequencing, which could be highly efficient

for genotyping to a population of mutants, was conducted in *GhCLA1*-edited T0 plants and it matched well with Sanger sequencing results. No off-target editing was detected at the potential off-target sites. The high efficiency of CRISPR/Cas9 system in cotton prompts us to further exert its great potential for functional genomic research. The *Gossypium hirsutum* is an allotetraploid with a genome size of 2.5 Gb, has 76943 annotated genes. On the other hand the cotton genetic transformation is very time and labour consumed . Therefore, it is hard to create large-scale mutant library by traditional T-DNA insertion strategy. Recently, we identified more 1000 candidate genes in cotton host related with insect-resistant by transcriptome and proteomics analysis. Based on our previous CRISPR/Cas9, we have constructed a sgRNAs library containing 2000 sgRNAs targeting to these candidate insect-resistant genes to create midi-size mutant library for cotton functional genomic research.

Keywords: cotton, genome editing, CRISPR/Cas9, mutant library.

Reference:

Wang, Pengcheng; Zhang, Jun; Sun, Lin; Ma, Yizan; Xu, Jiao; Liang, Sijia; Deng, Jinwu; Tan, Jiafu; Zhang, Qing; Tu, Lili; Daniell, Henry; Jin, Shuangxia*; Zhang, Xianlong*. High efficient multi-sites genome editing in allotetraploid cotton using CRISPR/Cas9 system, ***Plant Biotechnology Journal***, 2017, DOI: 10.1111/pbi.12755

Transiently Expressed Tomato Resistance-Related Gene SlHin1 inhibited the accumulation and spread of TMV in *N. benthamiana*

PENG HaoRan, XUE Yang, WEI ZhouLing, QING Ling, SUN XianChao

College of Plant Protection, Southwest University, Chongqing, 400716

sunxianchao@163.com

In order to analyze the bioinformatics characters, tissue expression and subcellular localization of tomato resistance-related gene SlHin1 and evaluate its anti-role in the process of the infection and movement of *Tobacco mosaic virus* (TMV), we used RT-PCR method to clone the tomato Hin1 from tomato (*Solanum lycopersicon* cv. Ailsa Craig)

leaves. Results showed that SlHin1(GenBank number: KU195820) was 675 bp in length and was predicted to encode a protein with 225 amino acid residues, a molecular weight of 26.1 kD and a theoretical isoelectric point of 9.35. SlHin1 contains the LEA-14 domains structure, doesn't have transmembrane segments and locates on chromosome 10 (Solyc10g081980). Sequence analysis and phylogenetic tree analysis showed that SlHIN1 shared approximately 80% similarity with HIN1 from other solanaceae plants and was close to that of rice and sorghum monocotyledons. Subcellular localization showed that SlHIN1 distributed on the plasma membrane of the leaf epidermis cell of *N. benthamiana*, which is consistent with the predicted results. The results of qRT-PCR showed that the SlHin1 was of tissue-specificity, whose expression decreased from tomato roots, leaves to stems. The SlHIN1 was transiently expressed in the N. benthamiana leaves by agro-infiltration, and the SlHIN1 expressed leaves were inoculated with TMV-GFP. After 4 days, there was no green fluorescence observed in the SlHIN1 expressed leaves under UV light, but the green fluorescence could be observed in the control group. With the passage of the inoculation, the sporadic fluorescence on the leaves of the treated group was slightly enlarged after 7 days post inoculation, and the green fluorescence of the control group had spread to the leaf. The spread of virus was inhibited and the time that the virus needed to reach the lobus cardiacus was longer, the content of the virions was less than that of control group through indirect ELISA test result. Our results indicated that transiently expressing SlHIN1 could inhibit the accumulation of the virions and spread of the virus, which can reflect that it may be involved in the resistance reaction of Solanaceae plants to TMV causing them to have resistance.

Acknowledgement: This study was partly supported by the National Natural Science Foundation of China (31670148), the Fundamental Research Funds for the Central Universities (XDJK2016A009, XDJK2017C015).

References:

[1] Takahashi Y, Berberich T, Yamashita K, Uehara Y, Miyazaki A, Kusano T. (2004). Identification of tobacco HIN1 and two closely related genes as spermine-responsive genes and their differential expression during the Tobacco mosaic virus-induced hypersensitive response and during leaf- and flower-senescence. Plant Molecular Biology, 54: 613-622.

Transcriptional Characteristics of *xa34*(t) -mediated Defense Responses in Rice

Congying Wang, Shen Chen, Aiqing Feng, Wenjuan Wang, Jing Su, Jianyuan Yang,,
Liexian Zeng, Xiaoyuan Zhu

Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640

E-mail of presenting author: adee117@163.com; phone: +86-20-87597562.

Bacterial leaf blight which caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is considered one of the most destructive bacterial diseases in rice-producing countries worldwide. The novel recessive gene *xa34*(t) confers resistance to a broad spectrum of *Xoo* races. To understand the molecular mechanism of broad spectrum resistance mediated by *xa34*(t), we used the whole-genome transcriptome sequencing (RNA-Seq) to identify differentially expressed transcripts between resistant cultivar BG1222 carrying *xa34*(t) and susceptible cultivar JG30 against Chinese race V. A total of 992 differentially expressed genes (DEGs) were identified. Based on their functional annotations, the DEGs were assigned to 17 categories, including defense-related, hormone signaling, transcriptional regulators, cell wall, lipid and secondary metabolism. Most of the defense-related genes belonged to the pathogenesis-related gene family, which was induced dramatically at 3 and 5 days post inoculation. Two differentially expressed annotated genes, including Cytochrome P450 family gene (Os01g0377000) and Pathogenesis-related gene (Os01g0382400), and two novel transcripts, designated as novel00007 and novel00008, were located in the fine-mapping region harboring *xa34*(t). Further characterization and functional analysis of these candidate genes will enhance our understanding of the molecular mechanism of broad-spectrum resistance in rice.

Acknowledgement: This research is supported by grants from National Natural Science Foundation (31401724), Natural Science Foundation of Guangdong Province (2015A030310420), and Guangzhou Scientific Research Project (201510010064).

Plant Disease Management Strategies in Organic Agriculture from the View of Plant Pathogenesis

Chao Wang, Gang Li, Wei Tian, Lei Wang, Yunguan Xi, Xingji Xiao
Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection, Nanjing
wcofrcc@126.com

Worldwide, organic agriculture (OA) has increased tremendously in recent decades. As an ecologically, economically and socially responsible production system, OA provides an enduring supply of safe and healthy food and fibers, with the least possible losses of nutrients and energy, and the least negative impacts on the environment. OA relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects, and its crop production is hampered by several limiting factors, of which diseases caused by microbial pathogens, including bacteria, oomycetes, fungi, phytoplasmas, viruses and viroids, still remain a formidable one. Plant disease management in OA is largely based on the maintenance of biological diversity and soil health by balanced crop rotations, as the use of synthetic pesticides is prohibited. Pathogenesis in plant-pathogen interaction can be viewed as a battle between a plant and a pathogen refereed by the environment. Each plant-pathogen interaction involves a two-way invasive and defensive dynamic communication. In nature, environmental referees, chiefly climatic conditions, physical and chemical properties of soils, and the plant surface microflora, are constantly interacting with each other and with the plant and pathogen to determine the course of pathogenesis. Thus, four strategies for disease management in OA would be: (1) preventing pathogen introduction by preplant measures; (2) limiting pathogen entry by minimizing initial inoculum; (3) regulation of pathogen establishment by minimizing the suitability of the host and its environment for infection and reproduction; (4) controlling pathogen by employing curative means that limit further spread. These strategies are not directed at controlling possible pathogens directly, but at management of the environment such that plants are able to withstand potential attacks, via preventing the establishment of pathogen's infection, restricting the disease development, or eliminating the pathogens.

Acknowledgement: This research was supported by the National Science and Technology

Major Project of the Ministry of Science and Technology of China (2014ZX07206001).

References:

[1] Birkhofer K, Bezemer TM, Bloem J, et al. (2008). Soil Biol Biochem, 40, 2297.

[2] Chaparro J, Sheflin A, Manter D, et al. (2012). Biol Fert Soils, 48, 489.

[3] Gomiero T, Pimentel D and Paoletti MG (2011). Crit Rev Plant Sci, 30, 95.

[4] Mansour A, Al-Banna L, Salem N et al. (2014). Crop Prot, 60, 48.

[5] Souza AGC, Maffia LA, Silva EF, et al. (2015). Plant Pathol, 64, 157.

Lignin biosynthesis pathway plays important role in maize defense response

Guan-Feng Wang [a] , Mengjie Liu [a], Yang Sun [a], Peter J. Balint-Kurti [b]

[a] *School of Life Science, Shandong University, Jinan, Shandong 250100*

[b] *Department of Plant Pathology, NC State University, Raleigh, NC 27695, USA;*

gfwang@sdu.edu.cn

Disease resistance (*R*) genes encode nucleotide binding leucine-rich-repeat (NLR) proteins that confer resistance to specific pathogens. Upon pathogen recognition they trigger a defense response that usually includes a so-called hypersensitive response (HR), a rapid localized cell death at the site of pathogen infection. Intragenic recombination between two maize NLRs, Rp1-D and Rp1-dp2, resulted in the formation of a hybrid NLR, Rp1-D21, which confers an autoactive HR in the absence of pathogen infection. From a previous QTL and genome wide association study, we identified genes encoding two key enzymes in lignin biosynthesis, hydroxycinnamoyltransferase, HCT and caffeoyl CoA *O*-methyltransferase, CCoAOMT, adjacent to the SNPs which were highly associated with variation in the severity of Rp1-D21-induced HR. We provide evidence that maize HCT and CCoAOMT, suppress the HR conferred by Rp1-D21 in a heterologous system. We also demonstrate that CCoAOMT, HCT and Rp1 proteins form the same complex(es). The metabolic activities of HCT and CCoAOMT are unlikely to be necessary for their roles in suppressing HR. We show that the lignin biosynthesis pathway is activated by Rp1-D21 at both the transcriptional and metabolic levels. We are investigating the role of other enzymes in lignin pathway in maize defense response. A model is derived to explain the

roles of HCT and CCoAOMT in Rp1-mediated defense resistance.

References:

[1] Guan-Feng Wang#, Jiabing Ji, Farid EI-Kasmi, Jeffery L. Dangl, Guri Johal, Peter Balint-Kurti#. (2015). Plos Pathogens 11: e1004674 .

[2] Guan-Feng Wang#, Yijian He, Renee Strauch, Bode A Olukolu, Dahlia Nielsen, Xu Li, Peter Balint-Kurti#. (2015). Plant Physiology 169: 2230-2243.

[3] Guan-Feng Wang#, Peter Balint-Kurti. (2016). Plant Physiology 171: pp.00224.2016.

[4] Bode A Olukolu*, Guan-Feng Wang*, Vijay Vontimitta*, … Peter J Balint-Kurti, Gurmukh Johal. (2014). Plos Genetics 10: e1004562.

[5] Guan-Feng Wang#, Peter Balint-Kurti#. (2015). MPMI 28: 1023-1031.

Map-based Cloning of a Stripe Rust Resistance Gene in *Triticum urartu*

Huan Wang [a,b], Shenghao Zou [e], Yongfang Chen [a], Dingzhong Tang [a,c,d]

[a] *State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Development Biology, Chinese Academy of Sciences, Beijing 100101*

[b] *University of Chinese Academy of Sciences, Beijing 100049*

[c] *State Key Laboratory of Ecological Control of Fujian-Taiwan Crop Pests, Fujian Agriculture and Forestry University, Fuzhou 350002*

[d] *Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou 350002*

[e] *State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101*

E-mail of presenting author: dztang@genetics.ac.cn

Wheat stripe rust, caused by *Puccinia striiformis f.sp.tritici*, is one of the most devastating diseases that caused major production losses in hexaploid wheat (*Triticum aestivum*) around the world[1]. Exploiting the resistant genes and developing resistant varieties is the most cost-effective and environmentally friendly method of controlling wheat stripe rust. *Triticum urartu* is diploid and A genome donor of common wheat. Because of the completion of genomic sequencing, cloning resistance genes in *Triticum urartu* is less difficult than in hexaploid wheat[2]. We mapped and cloned a stripe rust resistance gene

(*Yrtu*) in *Triticum urartu*. The genetic distances between *Yrtu* and the closest flanking markers were 0.034 cM and 0.068 cM , respectively. Four genes, *CG1*,*CG2*,*CG3*,*CG4* in this region are the candidate genes. Two of these candidate genes are *CNLs*. We analysed the expression patterns of *CG1*, *CG2*, *CG3*, *CG4* in Tu-51 and G1812 after inoculated with CY33. Expression of *CG1* decreased and expression of *CG2* remained unchanged after inoculation. However, we could not detect expression of *CG3* or *CG4* before and after inoculation. The *CG1* and *CG2* gene were introduced into the stripe rust susceptible wheat cultivar Bob White by stable transformation. Both *CG1* and *CG2* genes enhanced disease resistance to CY33, indicating those two genes are functional.

References:

[1] Chen, X.M. (2010). Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can. J. Plant Pathol. *27*, 314-337.

[2] Ling, H.Q., Zhao, S.C., Liu, D.C., Wang, J.Y., Sun, H., Zhang, C., Fan, H.J., Li, D., Dong, L.L., Tao, Y., *et al.* (2013). Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature *496*, 87-90.

Two related receptor kinases SYR1 and SYR2 of tomato act as high and low affinity receptors for the plant peptide hormone systemin

Lei Wang [a], Elias Einig [a], Markus Albert [a], Judith Fliegmann [a], Axel Mithoefer [b], Hubert Kalbacher [c], Georg Felix [a]

[a] *Center for Plant Molecular Biology, University of Tuebingen, Tuebingen, Germany*

[b] *Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany*

[c] *Interfaculty Institute of Biochemistry, University of Tuebingen, Tuebingen, Germany*

lei.wang@zmbp.uni-tuebingen.de; markus.albert@zmbp.uni-tuebingen.de; georg.felix@zmbp.uni-tuebingen.de

Plant peptides play important roles regulating growth, development and interaction with other organisms [1]. Discovered more than a quarter-century ago as the first plant peptide hormone, systemin was shown to be critical for systemic wound response and anti-herbivore defense in tomato [2, 3]. The receptor for this peptide hormone remained mysterious since the receptor SR160 proposed earlier [4] is a tomato homolog of the

brassinosteroid receptor BRI1 and its role as systemin receptor could not be corroborated in later work [5, 6]. Starting with the observation that the wild tomato *S. pennellii*, in contrast to the cultivated tomato *S. lycopersicum*, lacks sensitivity to systemin, we mapped the trait responsible for systemin responsiveness by using a collection of introgression lines between these two species [7] and cloned two closely related leucine-rich repeat receptor like kinases (LRR-RLKs) that defined sensitivity to systemin. Heterologous expression of these receptors, named Systemin Receptor 1 (SYR1) and Systemin Receptor 2 (SYR2), confered systemin responsiveness to *Nicotiana benthamiana* and *Arabidopsis thaliana*, corroborating their role as systemin receptors. SYR1 exhibited specific, high-affinity binding for systemin whereas SYR2 acted as a low-affinity receptor. Complementing SYR1 into the introgression line lacking systemin receptors showed that presence of this receptor, although not decisive for local and systemic wound responses, was important for defense against the generalist insect herbivores *Spodoptera littoralis*.

References:

[1] P. Tavormina, et al. (2015). The Plant Cell, 27(8), 2095-2118.

[2] G. Pearce, D. Strydom, S. Johnson, C. A. Ryan (1991) Science, 253(5022), 895-899.

[3] C. A. Ryan, G. Pearce (1998). Annual review of cell and developmental biology, 14(1), 1-17.

[4] J. M. Scheer, C. A. Ryan (2002). Proceedings of the National Academy of Sciences, 99(14), 9585-9590.

[5] N. Holton, et al. (2007). The Plant Cell, 19(5), 1709-1717.

[6] R. Malinowski, et al. (2009). Plant molecular biology, 70(5), 603-616.

[7] Y. Eshed, D. Zamir (1995). Genetics, *141*(3), 1147-1162.

TIR-NBS2, a truncated NLR protein, interacts with a intracellular NLR receptor in Arabidopsis

Wei Wang[a,c,d], Na Liu[a,b,c], Lu Rui[a,b,c], Ting Zhao[c], Dingzhong Tang[a,b,c]*

[a]*Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou 350002*

[b]*State Key Laboratory of Ecological Control of Fujian-Taiwan Crop Pests, Fujian Agriculture and*

Forestry University, Fuzhou 350002

[c]The State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101

[d]University of Chinese Academy of Sciences, Beijing 100049

[*] E-mail: dztang@genetics.ac.cn

As a evolutionarily conserved component of exocytosis, exocyst complex plays an important role in the plant immune response. Previously, we showed that loss of the function of EXO70B1, a subunit of exocyst complex in Arabidopsis, results in activated defense responses and enhanced resistance to a range of pathogens. Furthermore, we showed EXO70B1 physically associates with TIR-NBS2(TN2), a truncated NLR protein, and the enhanced disease resistance and cell death in the *exo70B1* mutant are dependent on TN2[1]. In further investigation, we find TN2 can interact with a intracellular NLR receptor(TNL). Then, we generated *exo70B1-3tnl* double mutant using CRISPR/Cas9 system. Interestingly, it is the enhanced resistance to *pto* DC3000, but not the spontaneous cell death in *exo70B1-3* that can be suppressed by the *tnl* mutation. Therefore, we suppose there is another intracellular NLR receptor which function redundancy to TNL in Arabidopsis. Although the phenotype need to be further validated, our data suggest that EXO70B1 may be guarded by TN2-associated immune complex.

References:

[1] Zhao, T., Rui, L., Li, J., Nishimura, M. T., Vogel, J. P., Liu, N., ... & Tang, D. (2015). A truncated NLR protein, TIR-NBS2, is required for activated defense responses in the exo70B1 mutant. *PLoS Genet*, *11*(1), e1004945.

A sheath blight resistance QTL *qSB-11*[LE] encodes a receptor like protein involved in rice early immune response to *Rhizoctonia solani*

Xiang Xue, Yu Wang, Zhiming Feng, Huimin Zhang, Wenfeng Shan, Wenlei Cao, Yafang Zhang, Zongxiang Chen, Xuebiao Pan and ShiminZuo*

Key Laboratory of Crop Genetics and Physiology/Co-Innovation Center for Modern Production Technologyof Grain Crops, Key Laboratory of Plant FunctionalGenomics of the Ministry of Education, YangzhouUniversity, Yangzhou 225009

**Corresponding author: smzuo@yzu.edu.cn*

Presenting author: 861911991@qq.com

Sheath blight (SB) is one of the three most serious diseases, which leads to severe grain loss annually. Rice resistance to SB disease is a typical of quantitative trait controlled by polygenes or quantitative trait loci (QTLs). Lots of QTLs for SB resistance have been reported while none of them was isolated so far, which significantly hinders the elucidation of resistant mechanism. Previously, we have mapped an SB resistance QTL on chromosome 11 into a 78kb region, in which the resistant allele (here after called *qSB-11*^{LE}) is from Lemont (LE).Here, through a series of experiments like RNA interference, overexpression and complementation tests, we confirmed that a receptor-like protein lack of intracellular domain in the fine mapping region is *qSB-11*^{LE}. RT-PCR data showed that *qSB-11*^{LE} transcription was significantly induced by SB pathogen *Rhizoctonia solani* but not by rice blight pathogen *XOO*. The *qSB-11*^{LE} is highly expressed in leaf sheath at the booting stage. *Subcellular localization* assay indicated that *qSB-11*^{LE} protein localizes in both *nucleus and* membrane. Through the treatments of known pathogen associated molecular patterns (PAMPs), chitin and flg22, we found that knock down of *qSB-11*^{LE} significantly affected rice innate immune response to chitin. Some known receptor/co-receptor like kinase proteins were found no interaction with *qSB-11*^{LE} protein in *vitro*. Upon the infection of SB fungus, the synthesis of hormone ethylene (ET) was apparently induced in wild plants, while it was only weakly increased in *qSB-11*^{LE} RNAi plants as well as in susceptible near isogenic line (NIL). Spray of ET was able to recover the resistance phenotype of *qSB-11*^{LE} RNAi and NIL plants. Collectively, we conclude that *qSB-11*^{LE} is involved in rice early immune response to SB fungus and regulates ET synthesis upon SB pathogen infection to activate ET-dependent defense response.

Capping protein coordinates actin remodeling in response to reactive oxygen species signaling during innate immunity

Jiejie Li, Lingyan Cao, Christopher J. Staiger

Department of Biological Sciences and The Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907-2064

Reactive oxygen species (ROS) play a key signal transduction role in cells. During innate immunity, rapid ROS production is activated by recognition of microbe-associated molecular patterns (MAMPs) through pattern recognition receptors (PRRs) to coordinate antimicrobial defense. The actin cytoskeleton has been suggested as a central component for innate immune signaling and associated cellular responses. However, whether ROS signaling impinges on cytoskeletal rearrangements and what are the underlying molecular mechanisms remain poorly understood. Using high performance, live-cell imaging approaches, we find that epidermal pavement cells in *Arabidopsis* respond to a diverse array of MAMPs and DAMPs, by significantly elevating the density of actin filament arrays and enhancing the overall dynamicity. Genetic analyses demonstrate that actin remodeling requires perception of MAMPs via cognate PRR components. Additionally, actin rearrangement depends upon ROS production by the NADPH oxidase, RBOHD, and can be recapitulated with exogenous addition of H_2O_2. We further demonstrate that capping protein (CP) is a key downstream target of ROS signaling during innate immunity. Finally, disrupting actin dynamics by LatB or regulation of CP leads to enhanced ROS production, suggesting a feedback loop between ROS signaling and actin remodeling during innate immunity. Collectively, our data provide compelling genetic evidence that CP is a key transducer of ROS signaling into changes of actin dynamics during plant immunity.

GhLA1 from *Gossypium hirsutum* modulates broad-spectrum resistance to biotic stress in cotton

Qin Hu[1], Longfu Zhu[1], Xianlong Zhang[1]

[1]*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei*

E-mail: xlzhang@mail.hzau.edu.cn; lfzhu@mail.hzau.edu.cn

Plants are constantly challenged by a multitude of pathogens and pests, which cause massive yield and quality losses annually [1,2]. A potentially valuable approach to improve plant resistance to biotic stresses is to enhance the capabilities of the plant's own immune system [3]. Here we demonstrate that transgenic manipulation of the expression of the *GhLA1* gene of cotton (*Gossypium hirsutum*) can confer an enhanced defence response to both pathogens and pests. While over-expression of this gene leads to increased lignification associated with increased tolerance to the fungal pathogen *Verticillium dahliae* and to the insect pests cotton bollworm (*Helicoverpa armigera*) and cotton aphid (*Aphis gosypii*). While, the knock-down lines of *GhLA1* with decreased lignin content but enhanced JA level and constitutively activated JA signalling pathway, which results in enhanced defence response to *Verticillium dahliae* and cotton bollworm, but show more susceptible to cotton aphid. We demonstrate that the mechanisms underlying the broad-spectrum resistance in transgenic over-expression and knock-down lines are different due to *GhLA1*-mediated differential redirection of the metabolic flux in the phenylpropanoid pathway, the biosynthesis of JA, and the balance of JA-SA defence responses.

References:

1. Lacombe S, Rougon-Cardoso A, Sherwood E, Peeters N, Dahlbeck D, et al. (2010) Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. Nat Biotechnol 28: 365-369.

2. Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59: 41-66.

3. Xu G, Yuan M, Ai C, Liu L, Zhuang E, et al. (2017) uORF-mediated translation allows engineered

plant disease resistance without fitness costs. Nature 545: 491-494.

Multi-Omics provide insights to understand cotton responsive to *Verticillium dahliae*

Longfu Zhu[1], Li Xu[1], Wei Gao[1], Lian Xu[1], Chao Li[1], Longqin Sun[1], Weifeng Guo[1], Xianlong Zhang[1]

[1] *National Key Laboratory of Crop Genetic Improvement，Huazhong Agricultural University，Wuhan, Hubei 430070*

E-mail: lfzhu@mail.hzau.edu.cn

Due to no high resistant germplasm in upland cotton (*Gossypium hirsutum*), Verticillium wilt has become the most serious disease for cotton production in China. Through whole genome gene expression analysis by RNA sequence, we found the expression level of lignin synthesis-related genes and the activity of corresponding enzymes induced more quickly in resistant cultivar ‘7124’ (*G. barbasense*) [1]. Recently, an ethylene response-related factor, GbERF1-like, from '7124' was proved as a positive regulator in lignin synthesis and contributed substantially to resistance to *V. dahliae* [2]. And suppression the expression level of a cotton P450 gene, SILENCE-INDUCED STEM NECROSIS (*SSN*), causes a lesion mimic phenotype in cotton. Further study shows that SSN silencing causes an imbalance in LOX (lipoxygenase) expression and excessive hydroperoxide fatty acid accumulation. We also show that an unknown oxylipin-derived factor is a putative mobile signal required for systemic cell death [3]. Complex phytohormones interaction was also found involving in cotton responsive to *V. dahliae* [4]. BR and JA signal pathways play essential roles in interaction of cotton and *V. dahliae* [4, 5]. Furthermore, we identify the key data from genomics and proteomics with a data-mining strategy accompanied by VIGS and heterologous expression [6]. GbWRKY1 is one of the key candidate genes and has been proved with as a critical regulator mediating the plant defense-to-development transition during *V. dahliae* infection by activating JAZ1 expression [7]. Overall, our study provides highlights to understand the mechanism of cotton resistance to *V. dahlia* at molecular level.

References:

1. Xu L, Zhu L, Tu L, Liu L, Yuan D, Jin L, Long L, Zhang X: Lignin metabolism has a central role in the resistance of cotton to the wilt fungus Verticillium dahliae as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. *J Exp Bot* 2011, 62(15):5607-5621.
2. Guo W, Jin L, Miao Y, He X, Hu Q, Guo K, Zhu L, Zhang X: An ethylene response-related factor, GbERF1-like, from Gossypium barbadense improves resistance to Verticillium dahliae via activating lignin synthesis. *Plant Mol Biol* 2016, 91(3):305-318.
3. Sun L, Zhu L, Xu L, Yuan D, Min L, Zhang X: Cotton cytochrome P450 CYP82D regulates systemic cell death by modulating the octadecanoid pathway. *Nat Commun* 2014, 5:5372.
4. Gao W, Long L, Zhu LF, Xu L, Gao WH, Sun LQ, Liu LL, Zhang XL: Proteomic and virus-induced gene silencing (VIGS) Analyses reveal that gossypol, brassinosteroids, and jasmonic acid contribute to the resistance of cotton to Verticillium dahliae. *Mol Cell Proteomics* 2013, 12(12):3690-3703.
5. Gao W, Long L, Xu L, Lindsey K, Zhang X, Zhu L: Suppression of the homeobox gene HDTF1 enhances resistance to Verticillium dahliae and Botrytis cinerea in cotton. *Journal of Integrative Plant Biology* 2016, 58(5):503-513.
6. Xu L, Zhang W, He X, Liu M, Zhang K, Shaban M, Sun L, Zhu J, Luo Y, Yuan D *et al*: Functional characterization of cotton genes responsive to Verticillium dahliae through bioinformatics and reverse genetics strategies. *J Exp Bot* 2014, 65(22):6679-6692.
7. Li C, He X, Luo X, Xu L, Liu L, Min L, Jin L, Zhu L, Zhang X: Cotton WRKY1 Mediates the Plant Defense-to-Development Transition during Infection of Cotton by Verticillium dahliae by Activating JASMONATE ZIM-DOMAIN1 Expression. *Plant Physiology* 2014, 166(4):2179-2194.

A comparative analysis of long non-coding RNAs provides novel insights into cotton resistance against *Verticillium dahliae*

Lin Zhang[a], Longfu Zhu[a] and Xianlong Zhang[a]

[a] *National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, Hubei*

E-mail of presenting author: linzhang@webmail.hzau.edu.cn

Long non-coding RNAs (lncRNAs) play important roles in plant development, but their function in disease response remains largely unresolved [1,2,3]. In this study, lncRNAs were characterized in two main cotton cultivars after infection of *Verticillium dahliae*. Compared with Gossypium hirsutum, more lncRNAs with anther, root and fiber specific

expression patterns were identified in G. barbardense. Many differentially induced lncRNAs, from the Dt subgenome, may contribute to the cotton resistance. GhNAT1 and GhNAT2 silenced seedings displayed an enhanced resistance, in which all their neighbor protein-coding genes were differentially up-regulated. This study builds up a foundation for characterizing functional lncRNAs responding to *V. dahliae* in cotton.

References:

[1] J. Ding, Q. Lu, Y. Ouyang, H. Mao, P. Zhang, J. Yao, C. Xu, X. Li, J. Xiao, Q. Zhang (2012). Proc Natl Acad Sci USA 109: 2654-2659.

[2] J.B. Heo, S. Sung (2011). Science 331: 76-79.

[3] Q.H. Zhu, S. Stephen, J. Taylor, C.A. Helliwell, M.B. Wang (2014). New Phytol 201: 574-584.

BSK1 Directly Associates with Mitogen-Activated Protein Kinase Kinase Kinase (MAPKKK) n to Regulate Plant Immunity in *Arabidopsis*

Haojie Yan[a,b,#], Yaofei Zhao[a,b,#], Hua Shi[c,d], Juan Li[a], Dingzhong Tang[a,c,d,*]

[a]*State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101*

[b]*University of Chinese Academy of Sciences, Beijng 100049*

[c]*Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou 350002*

[d]*State Key Laboratory of Ecological Control of Fujian-Taiwan Crop Pests, Fujian Agriculture and Forestry University, Fuzhou 350002*

[*] *E-mail of presenting author: Dingzhong Tang (dztang@genetics.ac.cn)*

[#] *These authors contributed equally to this work.*

Upon flagellin perception, FLS2 rapidly forms a complex to activate PTI, including production of reactive oxygen species (ROS), transcriptional induction of a large suite of defense-related genes, and activation of the mitogen-activated protein kinase (MAPK) cascades [1]. The receptor-like cytoplasmic kinase BSK1 can interact with FLS2 *in vivo* and dissociate from the complex in response to flg22 [2]. Nevertheless, how does BSK1 transduce signals to induce the final responses remains almost unknown. Here we show that BSK1 can not only interact with but also phosphorylate mitogen-activated protein kinase

kinase kinase n (MAPKKKn) to regulate the plant immunity. Knockout mutants of *MAPKKKn* are more susceptible to the powdery mildew pathogen *Golovinomyces cichoracerum* which is a biotrophic pathogen and also to virulent bacterium *Pseudomonas syringae* pv *tomato* (*Pto*) DC3000 and the avirulent *Pto* DC3000 strains than the wild type. Overall, these results highlight a direct regulatory mode of signaling from the BSK1 to the downstream.

References:

[1] Monaghan, J., and Zipfel, C. (2012). Plant pattern recognition receptor complexes at the plasma membrane. Curr Opin Plant Biol 15:349-357.

[2] Shi, H., Shen, Q., Qi, Y., Yan, H., Nie, H., Chen, Y., Zhao, T., Katagiri, F., and Tang, D. (2013). BR-SIGNALING KINASE1 physically associates with FLAGELLIN SENSING2 and regulates plant innate immunity in Arabidopsis. Plant Cell 25:1143-1157.

Towards dissecting the mechanism of *PigmR*-mediated broad spectrum resistance to rice blast

Dongyong Yang, Yiwen Deng and Zuhua He

Shanghai Institute of Plant Physiology & Ecology, Chinese Academy of Sciences

Email:dyyang@sibs.ac.cn

Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is a devastating disease of rice world-wide. Utilization of broad-spectrum resistance gene is the economic and effective strategy of control the rice blast. Recently the rice *Pigm* locus from an indigenous landrace containing a nucleotide-binding leucine-rich repeat (NLR) receptor cluster confers durable and broad-spectrum resistance to rice blast has been cloned. However the mechanism of *Pigm*-mediated resistance is unclear. Next we performed the yeast two-hybrid system (Y2H) to screen the PigmR-interacting proteins (PIPs) to investigate the downstream signal transduction. Interestingly, we found a gene *PIP4*, encoding the conserved vesicular transport protein in diverse plant species, and identified that PIP4 physically interacted with the full-length and the conserved coil-coil (CC) domain of PigmR *in vivo* dependent on BiFC and co-IP technique. Meanwhile knock out of *PIP4* in

the variety NIL-Pigm significantly decreased *PigmR*-mediated resistance against blast isolates. PIP4 was mainly distributed in the endoplasmic reticulum, implying that it functioned in vesicle-trafficking events. We further identified PigmR was characterized of dot-shaped location on the plasma membrane in wild type variety, but gathered in the cytoplasm in the transgenic *pip4* lines. Hence we deduced that PIP4 was involved in *PigmR*-mediated resistance pathway dependent on controlling accumulation of PigmR on the plasma membrane.

References:

[1] Deng, Y., Zhu, X., Shen, Y., and He, Z. (2006). Theoretical and applied genetics. 113:705-713.

[2] Deng, Y., Zhai, K., Xie, Z., Yang, D., Zhu, X., Liu, J., Wang, X., Qin, P., Yang, Y., Zhang, G., Li, Q., Zhang, J., Wu, S., Milazzo, J., Mao, B., Wang, E., Xie, H., Tharreau, D., and He, Z. (2017). Science, 355:962-965.

Design for broad-spectrum resistance: the knowledge of barley that counteracts bymoviruses

Ping Yang[1,2*], Antje Habekuß[3], Bernhard J. Hofinger[4], Kostya Kanyuka[4], Benjamin Kilian[2], Andreas Graner[2], Frank Ordon[3], Nils Stein[2]

1, Centre of Crop Germplasm Resource, Institute of Crop Research, Chinese Academy of Agricultural Science, No, 80, South Street of Xue Yuan, Hai Dian District, Beijing; 2, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), OT Gatersleben, Corrensstr. 3, D-06466 Stadt Seeland, Germany; 3, Institute for Resistance Research and Stress Tolerance, Julius Kuehn Institute (JKI), Federal Research Centre for Cultivated Plants, Erwin-Baur-Str. 27, D-06484 Quedlinburg, Germany; 4, Plant Biology and Crop Science Department, Rothamsted Research, Harpenden AL5 2JQ, UK

Correspondence: Tel: 010-82107467, E-mail: yangping@caas.cn

Plant pathogens are constantly challenging plant fitness and driving resistance gene evolution in host species. Little is known about the evolution of sequence diversity in host recessive resistance genes that interact with plant viruses. By map-based approaches we identified two barley host factor genes *HvPDIL5-1* and *HvEIF4E* as the causal genes of

recessive resistance loci *rym1/11* and *rym4/5*, respectively, that confer reliable resistance to the agriculturally important *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV). We further analyzed natural variation in a broad collection of wild (*H. spontaneum*) and domesticated barley (*Hordeum vulgare*) by re-sequencing of the two host factor genes. Interestingly, two types of gene evolution conferred by sequence variation in domesticated barley, but not in wild barley were observed. Whereas resistance conferring alleles of *HvEIF4E* exclusively contained non-synonymous amino acid substitutions, loss-of-function alleles were predominantly responsible for the *HvPDIL5-1* conferred bymovirus resistance. Moreover, a strong correlation between the geographic origin and the frequency of barley accessions carrying resistance conferring alleles was found in East Asia. Thus, domestication along with the expansion of barley cultivation into Asia likely resulted in a post domestication burst of novel resistance alleles in cultivated barley, which provided the basis for precision breeding for BaMMV/BaYMV resistance.

Key words: Plant virus, host factor, *HvDPIL5-1*, *HvEIF4E*, resistance, adaptation

Transcriptome Analysis and Functional Identification of Orthologs Resistance to Bacterial Blight and Blast in *O. granulata*

Rui Yang, Xingyu An, Xiaoxuan Zho, Qiong Luo

State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan

Ministry of Education Key Laboratory of Agriculture Biodiversity for Plant Disease Management,

Yunnan Agricultural University, Kunming, 650201

E-mail of presenting author: qiongbf@aliyun.com

O. granulata is one of the two perennial diploid wild species under *O. meyeriana* complex with GG genome[1,2]. It preserves many important genes, such as resistance to bacterial blight and blast, tolerance to drought, and is a reservoir of useful genes for cultivated rice improvement[3-5]. But so far no any genetic resource is available for studying *O. granulata*. Here we report 91,562 *de novo* assembled high quality transcripts of *O. granulata*, and multiple resistance-related genes identified in *O. granulata*. We performed the

characterization of *OgXa13* (ortholgous gene of rice bacterial blight *R* gene *xa13*)[6] and *OgPita* (ortholgous gene of rice blast *R* gene *Pita*) in *O. granulata*[7]. Our results showed that overexpression of *OgXa13* in susceptible rice variety Nipponbare was immunity to bacterial blight contrary to suppressing expressing of dominant or recessive allele of rice *xa13* enhanced the resistance. *OgPita* was highly expressed in root of *O. granulata* and is a new transcript variant encoding a 1024 amino acids polypeptide with a C-terminal thioredoxin (TRX) domain, overexpression of *OgPita* in susceptible rice variety Nipponbare mildly enhanced resistance to rice blast. We suspected that *OgPita* might play more important roles in plant defenses against various environmental stresses than response to fungus *M. oryzea* in *O. granulata*. Comparative transcriptome analysis revealed that 1,311 single-copy orthologs pairs shared by *O. granulata* and *O. meyeriana* (another wild species under *O. meyeriana* complex) with a Ka/Ks ratio >0.5 may have been subjected to adaptative evolution, and genome level differences between *O. granulata* and *O. meyeriana*. Our study provides an important resource for functional and evolutionary studies in genus *Oryza*.

References:

[1] K.K. Jena (2010). Breeding Science, 60,518-523.
[2] X.H. Zou, F.M. Zhang, J.G. Zhang, L.L. Zang, et al. (2008). Genome Biology, 9:R49
[3] C.Q. Yan, K.X. Qian, Q.S. Yan, X.Q. Zhang, et al. (2004). Plant Cell Report, 22,569-575.
[4] H.J. Li, X.H. Li, J.H. Xiao, R.A. Wing, S.P. Wang (2011). Molecular Plant, 5,281-290.
[5] Q.M. Lv, X. Xu, J.J. Shang, G.H. Jiang, et al. (2013). Phytopathology, 103,594-599.
[6]Z.H. Chu, M. Yuan, J.L. Yao, X.J. Ge, et al.(2006). Genes & Development, 20,1250-1255.
[7] G.T. Bryan, K.S. Wu, L. Farrall, Y. Jia,. et al(2000). The Plant Cell, 12,2033-2046.

Sphingolipids facilitate adaptation to environmental stresses by promoting autophagy in Arabidopsis

Ping Zheng[a], Jian-Xin Wu[a], Sunil Kumar Sahu[a], Hong-Yun Zeng[a], Li-Qun Huang[a], Nan Yao[a]

[a] *State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resource, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275*

yaonan@mail.sysu.edu.cn

Sphingolipids, a class of bioactive lipids found in cell membranes, can modulate the biophysical properties of the membranes and play a critical role in plant development [1] and responses to biotic or abiotic stresses [2,3]. Sphingolipids have been reported to be involved in autophagy in humans and yeast, but their role in autophagy in plants is not well understood. In the present study, we found that nitrogen deprivation influenced the profile of sphingolipids in *Arabidopsis thaliana*, especially the long-chain bases (LCBs), which increased significantly. Furthermore, application of exogenous LCB and an increase in the level of endogenous LCB induced autophagy in Arabidopsis. Moreover, the loss of AtACER, an alkaline ceramidase that hydrolyzes ceramide to LCB, accelerated leaf senescence and increased sensitivity to nutrient deprivation and environmental stress. Further empirical data confirmed that ACER is involved in autophagy in Arabidopsis, as the loss of AtACER inhibited autophagy and AtACER overexpression promoted autophagy, regardless of whether the plant was under nutrient or environmental stress. Taken together, our findings revealed that LCBs induce autophagy and AtACER may be involved in autophagy in Arabidopsis, thus playing a crucial role in the maintenance of cellular homeostasis under various environmental stresses. Possible mechanisms will be further discussed.

References:

[1] Li J., Yin J., Rong C., Li K-E., Wu J-X., Huang L-Q., Zeng H-Y., Sahu S.K., Yao N. (2016) Orosomucoid proteins interact with the small subunit of serine palmitoyltransferase and contribute to sphingolipid homeostasis and stress responses in Arabidopsis. Plant Cell, 28: 3038.

[2] Bi, F-C., Liu, Z., Wu, J-X., Liang, H., Xi, X-L., Fang, C., Sun, T-J., Yin, J., Dai, G-Y., Rong, C., Greenberg, J.T., Su, W-W., and Yao, N. (2014) Loss of ceramide kinase in Arabidopsis impairs defenses and promotes ceramide accumulation and mitochondrial H_2O_2 burst. Plant Cell, 26:3449.

[3] Wu, J-X, Li, J., Liu, Z., Yin, J., Chang, Z-Y., Rong, C., Wu, J-L., Bi, F-C. and Yao, N. (2015) The Arabidopsis ceramidase AtACER functions in disease resistance and salt tolerance. Plant Journal, 81, 767.

Pre-inoculation of rhizobium mitigates salt injury to cultivated and wild soybean seedlings by reducing Cl^-/NO_3^- and Na^+/K^+ ratios

Jiashu Guo, Wei Deng, Xiaojiang Li, Bingjun Yu*

Lab of Plant Stress Biology, College of Life Sciences, Nanjing Agricultural University, Nanjing

The cultivated soybean cultivars Lee68 and Jackson and wild soybean accessions BB52 and N23227 with different salt tolerance were used as the experimental materials, the effects of pre-inoculation of rhizobium on salt injury mitigation and its physiological mechanisms were investigated through measurement of contents of Cl^-, NO_3^-, Na^+ and K^+, and other physiological parameters of soybean seedlings under 100 mmol·L^{-1} NaCl stress. The results showed that:

Under salt stress, growth of the four tested soybean seedlings was inhibited and displayed salt injury symptoms, among them, Lee68 and BB52 seedlings suffered from relatively weaker injury. After pre-inoculation of rhizobium, the mitigating effects of salt damage on Lee68 and BB52 plants with lower or not significant salt-induced drop of root nodule number per plant were more obvious than Jackson and N23227 seedlings with both significant decline of root nodule number per plant. When the contents of Cl^-, NO_3^-, Na^+ and K^+ in roots, stems and leaves of four soybean materials were analyzed, which could indicate that, the mitigating difference on salt-stressed different soybeans resulted from pre-inoculation of rhizobium might be related to the rise of NO_3^- content and drop of Cl^- content and then maintenance of lower Cl^-/NO_3^- ratio in roots, stems and leaves of Lee68 and BB52. Moreover, pre-inoculation of rhizobium also possessed the same effects on the contents of Na^+ and K^+, and Na^+/K^+ ratio in roots, stems and leaves of NaCl-treated soybean seedlings, although not more obvious than the change of NO_3^- and Cl^- levels. The results of the study on wild soybean (BB52 and N23227) further showed that, pre-inoculation of rhizobium could increase the chlorophyll content in leaves, decrease the relative electrolytic leakage in roots and improve the root activity under salt stress.

Funding project: NSFC No.31671604.

***Correspondence author**, E-mail: bjyu@njau.edu.cn.

Thus, it is concluded that, pre-inoculation of rhizobium for enhancing nitrogen supply level can effectively alleviate salt stress on the cultivated and wild soybean seedlings with different salt tolerance, and the effects on the salt-tolerant species are more profound. This may have a connection with the obvious reduction of Cl^-/NO_3^- and Na^+/K^+ ratios in roots, stems and leaves of soybean seedlings under salt stress, especially for Cl^-/NO_3^- ratio. It also indicates both can be adopted as the important indexes for estimating plant or crop salt tolerance.

DNA marker-based pyramiding of QTLs for leaf rust-resistance in wheat

Y. B. Zhang, Y. Li, Z. Wang, J. H. Wang, Z.J. Zhang

Department of Plant Pathology, China Agricultural University, 100193, Beijing

zhangyibincau@163.com

Leaf rust (caused by Puccinia triticina), occurring worldwide, often becomes a severe threat to wheat production. In China, leaf rust becomes escalated in recent years as exemplified by the devastating epidemics in the years 2012 and 2015. Utilization of wheat cultivars that possess resistance to leaf rust can reduce the use of fungicides, thus, it is an economical and environmentally-sound strategy for controlling leaf rust. A great number of observations and studies indicate that certain quantitative trait locus (QTL), as represented by the leaf rust-resistance gene Lr34, confer a type of incomplete but durable resistance. Wheat species is likely considerably rich in QTL for resistance to leaf rust. However, an individual QTL is usually unable to give a level of resistance that is high enough as needed in commercial fields in the cases of high disease pressures. Thus, efforts are required for both screening new QTL and pyramiding two or multiple QTLs. Our laboratory previously reported that the wheat cultivars "Luke" and "AQ" illustrate some degree of quantitative resistance to leaf rust. The Luke×AQ recombinant inbred line (RIL) population harbors Lr34 and at least another leaf rust-resistance QTL (QLr.cau-1AL) as well.

In the present study, the advanced (>F10) Luke×AQ RILs were used as host materials, and the P. triticina races THTT and FHTR were used as pathogen materials. More than 910

RILs were evaluated for resistance to leaf rust, and 341 RILs were genotyped at 56 SSR-DNA marker loci. The analyses based these genotypic and phenotypic experimental data indicate that: (1) a novel QTL for resistance to leaf rust was detected and mapped to the short arm of chromosome 3B, being distal to the centromere by 46.5 cM (cent-Morgan). No leaf rust-resistance gene or QTL has yet been reported in this site. This new QTL was named as QLr.cau-3BS following the rules used internationally. The effect magnitude of QLr.cau-3BS is near to that of Lr34. The resistance allele is contributed by AQ; (2) a SSR-DNA marker (barc092) was identified that is tightly linked with QLr.cau-3BS; (3) selection based the DNA markers cssfr5, gpw2246, and barc092 resulted in the identification of a RIL that was then proved to be highly resistant to the P. triticina races THTT and FHTR. The three QTLs, as represented by the DNA markers cssfr5, gpw2246, and barc092, respectively, acted in an additive manner without undesired epistasis, having a potential application to breeding for resistance to leaf rust.

References:

[1]Z.Y. Du, M.Z. Che. Theoretical and Applied Genetics, 2015, 128(8):1-16

[2]S.G. Krattinger, E.S. Lagudah. Science, 2009, 323:1360-1363

Phytochrome-associated protein phosphatase type 2C plays a central role in RPW8.2-mediated defence against powdery mildew

Jing-Hao Zhao, He Wang, Jun-Hua Lu, Yan Li, Jing Fan, Wen-Ming Wang

Rice Research Institute, Sichuan Agriculture University, Chengdu 611130

ayanami_rei2@163.com

Many fungal and oomycete pathogens differentiate a feeding structure named the haustorium to extract nutrients from their plant hosts. The atypical resistance (R) protein RPW8.2 specifically targets to haustorium, where it activates salicylic acid (SA) pathway-dependent resistance to *Golovinomyces spp*.. However, how RPW8.2 activates defense remains largely uncharacterized. Here, we found that a powdery mildew protein RNA polymerase Ⅱ transcription factor-like protein B subunit 3 (TFB3) interacts with RPW8.2 and the phytochrome-associated protein phosphatase type 2C (PAPP2C) in yeast and in planta. Previously, PAPP2C was found to be associated with RPW8.2 and negatively regulates SA-signaling pathway[1]. In this study, we found that PAPP2C also associated

with the basic helix-loop-helix (bHLH) transcription factor HOMEODOMAIN PROTEIN FROM ARABIDOIPSIS THALIANA 5 (HAT5) in the nucleus. HAT5 binds to the DNA sequence 5'-CAAT[AT]ATTG-3'[2] which is rich in the *RPW8.2* promoter. We further demonstrated that the *RPW8.2* promotor could be activated by either Myc-PAPP2C or Flag-HAT5. In addition, transient expression of RPW8.2-Myc can also activate the *RPW8.2* promoter, indicating a self-amplification feedback loop. Taken together, our data indicate that TFB3 is functionally connected with RPW8.2-mediated defense, and PAPP2C plays a center role in RPW8.2-mediated defence by regulating the function of RPW8.2 at transcriptional and protein level.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (grants 31371931 and 31672090 to W-MW).

References

[1] Wenming Wang, XianFeng Ma, et al. (2012). Molecular plant, 5, 1125.

[2] Takashi Aoyama, Chun-Hai Dong, et al. (1995). The Plant cell, 7, 1773.

Transport of Receptor Like Kinase-A-a positive regulator in plant basal immunity-is modulated by EDR4 in *Arabidopsis*

Yaofei Zhao [a,b], Guangheng Wu [e], Dingzhong Tang [a,c,d,*]

[a] *State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Development Biology, Chinese Academy of Sciences, Beijing 100101*

[b] *University of Chinese Academy of Sciences, Beijing, 100049*

[c] *State Key Laboratory of Ecological Control of Fujian-Taiwan Crop Pests, Fujian Agriculture and Forestry University, Fuzhou 350002*

[d] *Key Laboratoryof Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou350002*

[e] *College of Ecology and Resources Engineering, Wuyi University, Wuyishan 354300*

**E-mail of correspondent: dztang@genetics.ac.cn*

E-mail of presenting author: yfzhao@genetics.ac.cn

Plants employ surface-localized receptor-like kinases (RLKs) or receptor-like proteins (RLPs) for rapid detection of the potential danger and subsequent signal transduction. EDR4 modulates plant immunity by participating in vesicle trafficking and affecting the in-site accumulation of EDR1, which negatively regulates plant resistance to powdery mildew. Recently, we found Receptor Like Kinase-A (RLK-A), a member of leucine-rich repeat receptor-like kinases (LRR-RLKs), interacted with EDR4 and CHC2. RLK-A mostly located at the plasma membrane and it could be translocated into cytoplasm by protein trafficking which was depended on both EDR4 and CHC2. Through further research, it was revealed that both *edr4-1* mutant and *rlk-a* mutant showed enhanced susceptibility to the bacterial pathogen *Pseudomonas syringae* pv tomato DC3000. *rlk-a* mutant showed defects in flg22-induced basal defense and ABA dependent stomatal closure. Therefore, RLK-A might be a positive regulator in pathogenic bacteria induced plant immunity and mainly functioned in PTI responses. EDR4 performed opposite function in bacteria and fungi induced plant immunity, just as CHC2, by modulating the localization or accumulation of multiple defense-related factors.

References:

[1] G. Wu, S. Liu, Y. Zhao, W. Wang, Z. Kong, D. Tang, (2015). Plant Cell, 27, 857-873.

[2] H. Shi, Q. Shen, Y. Qi, H. Yan, H. Nie, Y. Chen, T. Zhao, F. Katagiri, D. Tang (2013). Plant Cell, 25,1143-1157.

[3] M. Mbengue, G. Bourdais, F. Gervasi, M. Beck, J. Zhou, T. Spallek, S. Bartels, T. Boller, T. Ueda,
H. Kuhn, S. Robatzek (2016) PNAS, 113, 11034-11039

[4] A. Guzel Deger, S. Scherzer, M. Nuhkat, J. Kedzierska, H. Kollist, M. Brosche, S. Unyayar, M. Boudsocq, R. Hedrich, M. R. Roelfsema, (2015) The New phytologist 208, 162-173

Jasmonate suppresses etiolation growth in Arabidopsis

Ziqiang Zhu

College of Life Sciences, Nanjing Normal University, Nanjing, 210023

Email: zqzhu@njnu.edu.cn

A germinating seedling undergoes skotomorphogenesis to emerge from the soil and reach for light. During this phase, the cotyledons are closed, and the hypocotyl elongates. Upon exposure to light, the seedling rapidly switches to photomorphogenesis by opening its

cotyledons and suppressing hypocotyl elongation. The E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) is critical for maintaining skotomorphogenesis. Here, we report that jasmonate (JA) suppresses hypocotyl elongation and stimulates cotyledon opening in etiolated seedlings, partially phenocopying *cop1* mutants in the dark. We also find that JA stabilizes several COP1-targeted transcription factors in a COP1-dependent manner. RNA-seq analysis further defines a JA-light co-modulated and *cop1*-dependent transcriptome, which is enriched for auxin-responsive genes and genes participating in cell wall modification. JA suppresses COP1 activity through at least two distinct mechanisms: decreasing COP1 protein accumulation in the nucleus and reducing the physical interaction between COP1 and its activator, SUPPRESSOR OF PHYTOCHROME A-105 1 (SPA1). Our work reveals that JA suppresses COP1 activity to stabilize COP1 targets, thereby inhibiting hypocotyl elongation and stimulating cotyledon unfolding in etiolated *Arabidopsis* seedlings.

Identification and characterization of *PmUb*, a powdery mildew resistance gene in *Triticum urartu*

Shenghao Zou [a], Huan Wang [a], Yunwei Zhang [a], Dingzhong Tang [a,b,c]

[a] *State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing*

[b] *Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou*

[c] *State Key Laboratory of Ecological Control of Fujian-Taiwan Crop Pests, Fujian Agriculture and Forestry University, Fuzhou*

Wheat breeding needs to be offered many high-efficient powdery mildew resistant genes, but map-based cloning them from wheat or the wild relatives has great difficulties. By designing abundant molecular markers according to the scaffolds of *Triticum urartu* accession G1812 and performing RNA-seq in resistant accession *Tu-51*, a *Blumeria graminis forma specialis tritici* E09 (*Bgt* E09) resistance locus with two candidate genes, *PmUa* and *PmUb*, was mapped in *Tu-51*.

PmUb, a CC-NBS-LRR protein, has been validated to be quite effective for stimulating resistance response through transformation, single cell transient expression and virus-induced gene silencing assays. Over-expression of its full length in *Nicotiana benthamiana* leaves could induce hypersensitive response, but the CC domain is insufficient to work unlike some other NLR proteins. PmUb-2 and PmUb-5 are allelic variants of PmUb from another two resistant accessions， with a motif less and more, could also induce HR. PmUb-5 has also been verified to be functional against *Bgt* E09.

PmUb, map-based cloned from *Triticum urartu*, is effective in defending wheat powdery mildew. However the functional distinctions among the variants are unclear now. In addition, obvious interaction exists between the CC domains of PmUa and PmUb, but the roles they take in powdery mildew resistance are unknown so far.

References:

[1] Bai S, Liu J, Chang C, *et al*. Structure-Function Analysis of Barley NLR Immune Receptor MLA10 Reveals Its Cell Compartment Specific Activity in Cell Death and Disease Resistance (2012). PLoS Pathog, 8, e1002752

[2] Ling H, Zhao S, Liu D, *et al* (2013). Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature, 496, 87

Plant-Response Elicitor Technology (PRE*tec*) Platform for Crop Production

Zhongmin Wei

zwei@planthealthcare.com

Abstract

In nature plant response elicitors derived from various sources can have profound effect on crop resistance and productivity. Innutus 3G, the first family of peptide based elicitor has been characterized from the PRE*tec* platform. In this presentation we will show you how diverse peptide molecules are created to meet the different performance targets such as yield increase, enhanced drought tolerance and resistance to nematode infections. Several leading peptides from the Innatus 3G families are being evaluated for their commercial potential in corn and soybean productions. Mechanisms of how the signal peptides activate innate plant systems or pathways

through specific bindings to plant proteins and consequential gene regulation leading to improved crop yield and health will also be also discussed.

Biography

Dr. Zhongmin Wei currently serves as the Chief Scientific Officer and Senior Vice President of R&D in Plant Health Care Inc. Dr. Wei received his B.S from Zhejiang University (Zhejiang Agricultural University) and M.S and PHD from Nanjing Agricultural University. He discovered the Harpin protein while serving as a Post-Doctoral Associate at Cornell University, has been studying the synthesis of protein-based biological for over 20 years and has published scientific papers in number of peer reviewed prestigious journals such as Science, PNAS and have been named as the inventor of number of patents related to biological products. He is the winner of 2001 Presidential Green Chemistry Challenge Award.

From 1996 to 2007, he served as Chief Scientific Officer and Vice President of Eden BioScience, a plant technology company once publically traded in NASDAQ. Dr. Wei has worked in a variety of roles involved in the development of PRE*tec* platform and has overseen the expansion and operation research facilities and staff in Seattle, Washington.

Natural variation in a rice immune receptor interface extends response to pathogen effectors

Juan Carlos De la Concepcion, Marina Franceschetti and Mark J. Banfield

[a]*Department of Biological Chemistry, John Innes Centre, Norwich, UK.*

Juan.De-La-Concepcion@jic.ac.uk

Pathogens deliver an array of molecules called effectors to supress plant immunity and to manipulate host cellular processes. Perception of effectors inside cells largely relies on intracellular immune receptors from the NLR (Nucleotide-binding, Leucine-rich Repeat) family. Recognition of effectors by NLRs triggers a response leading to cell death. This process creates a high selection pressure in the pathogen, driving the emergence of new effector variants that escape NLR recognition. On the host side, plants also evolve alleles of immune receptors with broader recognition for these effector variants. Here, we unravelled the molecular details of differential recognition specificities in the rice resistant protein Pik for the effector AVR-Pik.

The rice NLR Pikp recognizes the rice blast pathogen effector AVR-PikD through direct binding to an unconventional integrated Heavy Metal Associated (HMA) domain, resulting in disease resistance. However, polymorphic effector variants AVR-PikE, A and C have lower binding affinity for Pikp-HMA and evade recognition in plants [1]. Some rice varieties carry the NLR allele Pikm and recognize AVR-PikD, E and A [2]. Almost all the amino acid polymorphisms between the Pikp and Pikm proteins are located within the HMA domain. Biochemical characterization of the Pikm-HMA domain, showed that it has higher binding affinity than Pikp-HMA for the AVR-Pik variants E and A. These differences in binding correlate with the differential immune response that occurs in plants. Furthermore, we crystallized and solved the structures of Pikm-HMA complexed with AVR-PikD, E and A, respectively. Comparison with the crystal structures of Pikp-HMA in complex with AVR-PikD and E provides an explanation for the extended recognition specificity by the rice Pikm allele. This study shows for the first time the structural basis of natural variation in pathogen recognition by NLRs, and will allow structure-guided protein engineering with the potential to deliver improved disease resistance in crops.

References:

[1] A. Maqbool, H. Saitoh, *et al*. (2015). Elife, 4, e08709.

[2] H. Kanzaki, *et al*. (2012). Plant Journal, 72(6), 894.

Understanding *Cf2/Rcr3* Pathogen Perception System: From Natural Variation to Evolution in Solanaceae

Shivani Malik[a], Jiorgos Kourelis[a], Judith K. Paulus[a] , Daniela J. Sueldo[a] and Renier A. L. van der Hoorn[a]

[a]*The Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, United Kingdom.*

shivani.malik@plants.ox.ac.uk

During colonization of leaf extracellular space, certain pathogens secrete inhibitors to modulate disease resistance components of the host plant. The tomato *Cf2* resistance gene, encoding a transmembrane receptor-like protein (RLP), confer indirect recognition of Avr2, a protease inhibitor secreted by the fungus *Cladosporium fulvum*. Rcr3 is a papain-like cysteine protease in

tomato that Avr2 first targets and the Rcr3-Avr2 complex is hypothesized to eventually get recognized by Cf2. This interaction triggers a hypersensitive cell death response (HR) leading to restriction of fungal invasion of host plant. Inhibitors from diverse pathogens, such as EpiC1 and EpiC2B from *Phytopthora infestans* and Cip1 from *Pseudomonas syringae* can also bind to Rcr3, but somehow escape recognition by Cf2. *Rcr3* and *Cf2* were introgressed from *Solanum pimpinellifolium*, the closest wild relative of currant tomato. We aim to identify natural sequence variation in *Cf2* and *Rcr3*, understand their pattern of evolution and functional consequence on resistance in Solanaceous plants. To screen several wild populations, we have performed heterologous expression of Avr2, EpiC1, EpiC2B, Cip1 and their inactive mutants in *E. coli*. Plants responding with HR upon infiltration of Avr2, EpiC1, EpiC2B and or Cip1 were selected for further analysis by PCR-based genotyping. Our results implicate polymorphisms in response within and among populations in the presence or absence of *Cf2*. By knocking down *Cf2* and *Rcr3,* we investigate if these genes are involved in mediating the perception of unrelated pathogens. Our study will provide novel insights into natural variation and evolution of *Cf2*/*Rcr3* system in Solanaceae.

References:

[1] A.C. Hòrger, M. Ilyas, W. Stephan, A. Tellier, R.A.L. van der Hoorn and L.E. Rose (2012). PLoS Genet., 8, e1002813.

[2] A.L. Caicedo and B.A. Schaal (2004). PNAS, 101, 17444-17449.

[3] H.C.E. Rooney, J.W. van't Klooster, R.A.L van der Hoorn, M.H.A.J. Joosten, J.D.G. Jones and P.J.G.M. De Wit (2005). Science, 308, 1783-1789.

[4] H.P. van Esse, J.W. van't Klooster, M.D. Bolton, K.A. Yadeta, P. van Baarlen, S. Boeren, J. Vervoort, P.J.G.M. de Wit and B.P.H.J. Thomma (2008). Plant Cell, 20, 1948-1963.

[5] J. Song, J. Win, M. Tian, S. Schornack, F. Kaschani, M. Ilyas, R.A.L. van der Hoorn and S. Kamoun (2009). PNAS, 106, 1654-1659.

[6] M. Ilyas, A.C. Hòrger, T.O. Bozkurt, H.A. van den Burg, F. Kaschani, M. Kaiser, K. Belhaj, M. Smoker, M.H.A.J. Joosten, S. Kamoun and R.A.L. van der Hoorn (2015). Current Biol. 25, 2300-2306.

[7] R.A.L. van der Hoorn, M. Kruijt, R. Roth, B.F. Brandwagt, M.H.A.J. Joosten and P.J.G.M. De Wit (2001). PNAS, 98, 10493-10498.

[8] T. Shindo, F. Kaschani, F. Yang, J. Kovács, F. Tian, J. Kourelis, T.N. Hong, T. Colby, M. Shabab, R. Chawla, S. Kumari, M. Ilyas, A.C. Hòrger, J.R. Alfano and R.A.L. van der Hoorn (2016). PLoS Pathog., 12, e1005874.

Transfer of tomato immune receptor Ve1 confers Ave1-dependent *Verticillium* resistance in tobacco and cotton

Yin Song[1], Yidong Wang[1], Linlin Liu[2], Dirk-Jan Valkenburg[1], Xianlong Zhang[2], Longfu Zhu[2] and Bart P.H.J. Thomma[1]*

[1]*Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.*

[2]*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070*

E-mail: yin.song@wur.nl

Verticillium wilts caused by soil-borne fungal species of the *Verticillium* genus are economically important plant diseases that affect a wide range of host plants, and are notoriously difficult to combat. Perception of pathogen (-induced) ligands by plant immune receptors is a key component of plant innate immunity. Race-specific resistance to Verticillium wilt in tomato is governed by the tomato cell surface-localized immune receptor Ve1 through recognition of the effector protein Ave1 that is secreted by race 1 strains of *Verticillium*. It has been previously demonstrated that transgenic expression of tomato *Ve1* in the cruciferous model plant *Arabidopsis thaliana* results in Verticillium wilt resistance. Here, we investigated whether tomato *Ve1* can confer *Verticillium* resistance when stably expressed in the crop species tobacco (*Nicotiana tabcum*) and cotton (*Gossypium hirsutum*). We show that transgenic tobacco and cotton plants constitutively expressing tomato *Ve1* exhibit enhanced resistance against Verticillium wilt in an Ave1-dependent manner. Thus, we demonstrate that the functionality of tomato *Ve1* in *Verticillum* resistance through recognition of the *Verticillium* effector Ave1 is retained after transfer to tobacco and cotton, implying that the Ve1-mediated immune signalling pathway is evolutionary conserved across these plant species. Moreover, our results suggest that transfer of tomato *Ve1* across sexually incompatible plant species can be exploited in breeding programmes to engineer Verticillium wilt resistance in crops.

Softwired signaling in MAMP triggered immunity

Elwira Smakowska-Luzan [a], G. Adam Mott [b], Katarzyna Parys [a], Martin Stegmann [c], Timothy C Howton [d], Mehdi Layeghifard [b], Jixiang Kong [a], Karin Grunwald [a], Natascha Weinberger [a], Santosh B. Satbhai [e], Wolfgang Busch [e], Nicholas J. Provart [b], M. Shahid Mukhtar [d], Cyril Zipfel [c], Darrell Desveaux [b], David S. Guttman [b], Youssef Belkhadir [a]

[a] *Gregor Mendel Institute, Austrian Academy of Sciences, Vienna Biocenter, Dr Bohr Gasse 3, Vienna, 1030, Austria.* [b] *Department of Cell & Systems Biology, University of Toronto, 25 Willcocks St., Toronto, Ontario, Canada .* [c] *The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK.* [d] *Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama, USA.* [e] *Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA*

youssef.belkhadir@gmi.oeaw.ac.at

Leucine-rich repeat receptor kinases (LRR-RKs) can act as pattern recognition receptors (PRRs) that perceive microbe-associated molecular patterns (MAMPs) to trigger immunity. The extracellular domains of >220 LRR-RKs serve either as ligand interaction platforms or as regulatory modules of receptor activation. Understanding how interactions between extracellular domains produce signal-competent receptor complexes is very challenging because of their transient nature and low biochemical tractability. While the principles governing LRR-RK signaling activation are emerging, the systems-level organization of this family of proteins is totally unexplored. To address this, we interrogated 40,000 potential extracellular domain interactions via a sensitized high-throughput interaction assay, and produced an LRR-based Cell Surface Interactome network (CSI^{LRR}) that revealed an enormous potential for variation built into its circuitry. We leveraged CSI^{LRR} by using community detection algorithms and defined the design principles and self-assembling properties of four independent, yet interconnected, LRR-RK interaction spaces. We identified a ‘DEFENSE’ interaction space that was densely populated with PRRs or co-receptors of PRRs. We then predicted and validated the function of uncharacterized LRR-RKs in the modulation of flg22-triggered immunity. In addition, we show that CSI^{LRR} operates as a unified regulatory network in which the LRR-RKs most critical for its overall structure are required to prevent aberrant immune responses of receptors that are at several network-steps away. Thus, plants make unified regulatory decisions by processing immune signals at the single receptor level and by distributing the information to distally-located receptors in the network for carefully balanced immune responses.

Contents

Academic Committee……………………………………………………1

Organizing Committee…………………………………………………..1

Local Organizers………………………………………………………..2

Scientific Program……………………………………………………….3

Abstracts of Invited Speakers…………………………….……………15

Abstracts of Participants……………………………………….……180

Sponsors…………………………………………………………………..403