

Plant Pathology 602

Plant-Microbe Interactions



Lecture

Gene for gene interactions

Sophien Kamoun
kamoun.1@osu.edu
The Ohio State University
Ohio Agricultural Research & Development Center

Bacterial and fungal host specificity

Lectures 9-11

- Gene for gene interactions (SK)
- Plant disease resistance genes (*R* genes) (SK)
- Genetic engineering/breeding for resistance (SK)

- Fungal/oomycete effector proteins (SK)
- What types of resistance can *R* genes mediate? (SK)
- Discussion

- Bacterial Type III secretion Avr proteins (DC)
- Bacterial pathogenicity islands (DC)

Plant Pathology 602

Plant-Microbe Interactions

Outline - gene for gene interactions

- The gene for gene model
- Plant disease resistance genes (*R* genes)

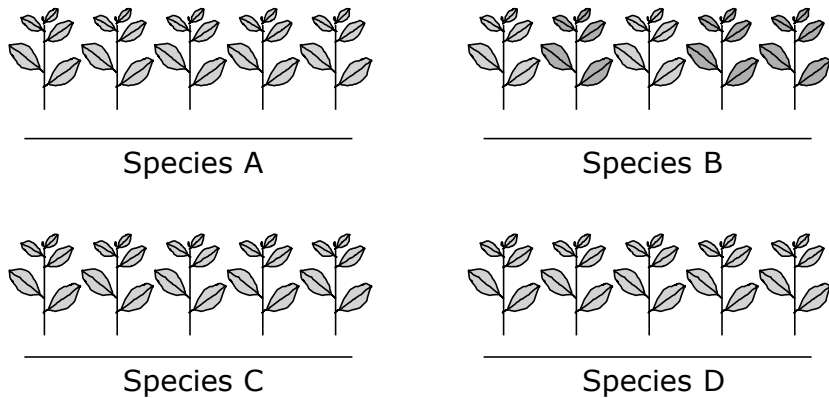
Plant Pathology 602

Plant-Microbe Interactions

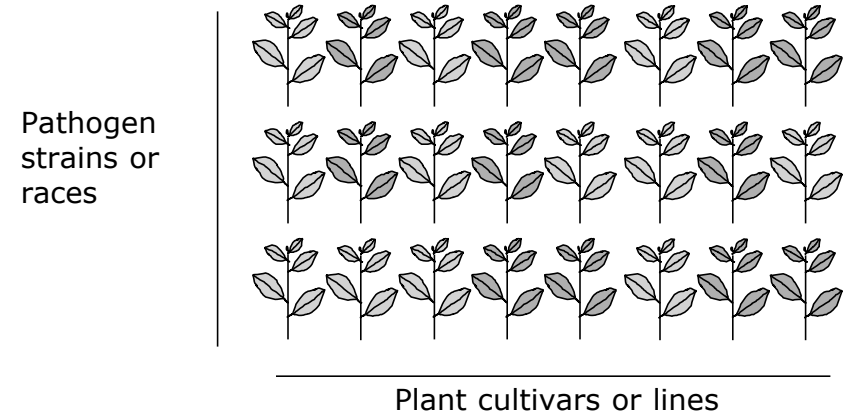
Outline - gene for gene interactions

- The gene for gene model
- Plant disease resistance genes (*R* genes)

Plants are resistant to most pathogens



Plants are resistant to some strains of a pathogen but not to others



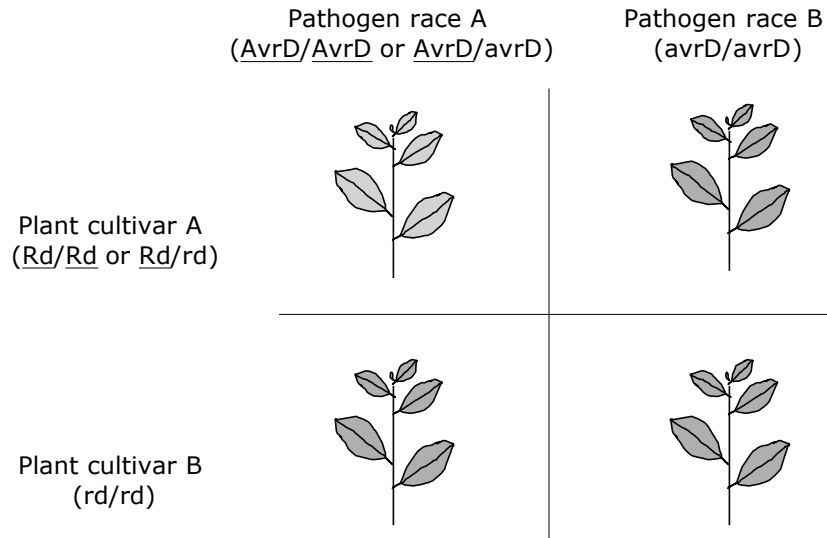
Gene for gene model

- Plant breeders recognized that resistance to disease often segregates as a single dominant or semi-dominant gene (locus). These became known as *R* genes.
- In the 1940s, H.H. Flor performed genetic analyses on both plant and pathogen and defined a model that provides a genetic basis for the variation

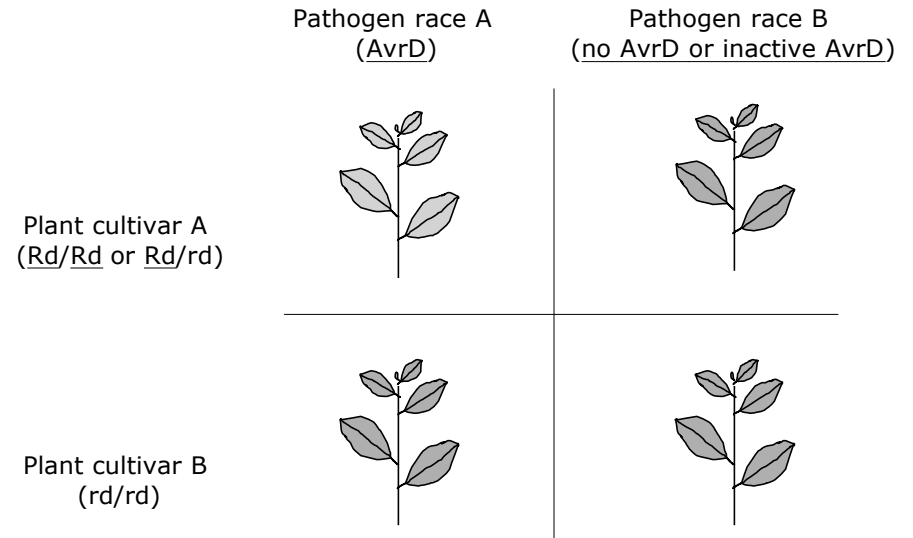
Gene for gene model

- Flor studied the interaction between flax and the fungal pathogen flax rust (*Melampsora lini*)
- He found that whereas flax genes for resistance were dominant, rust genes for virulence were recessive
- This ultimately led to the current view that pathogens contain a variety of molecules, encoded by dominant avirulence (*Avr*) genes, that trigger defense responses in plants carrying the corresponding *R* gene

Gene for gene model- quadratic check



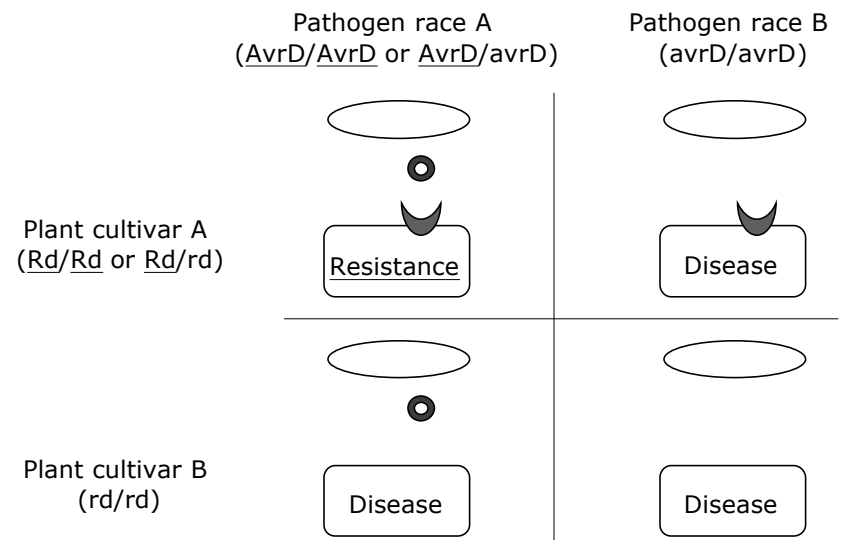
Gene for gene model- haploid pathogen



Gene for gene- direct recognition (elicitor-receptor model)

- One biochemical expression of the gene for gene model is direct recognition
- R genes products are receptors for ligands encoded by Avr genes
- Ligand (Avr) binding to R receptor triggers a signaling cascade leading to inactivation of defense mechanisms including hypersensitive response (HR)

Gene for gene- direct recognition



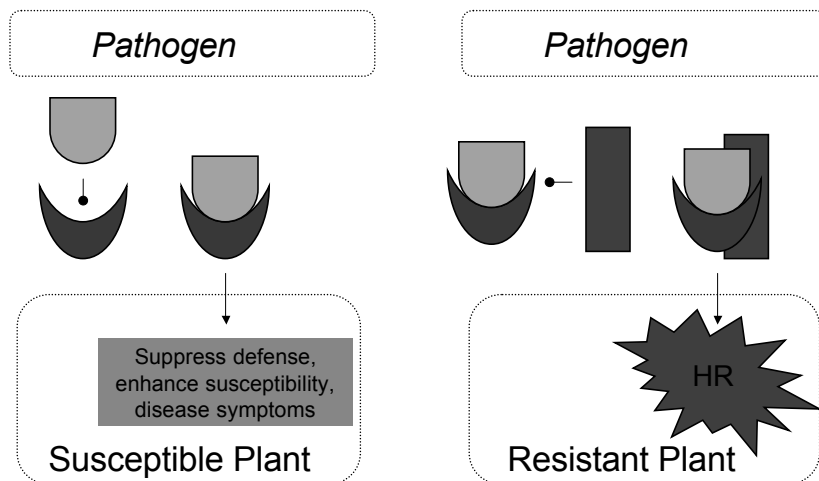
Gene for gene- indirect perception (guard model)

- Recent data suggest that in many cases no direct physical interactions between R and Avr proteins occur
- This led to the alternative indirect perception model, one example of which is the guard model
- R proteins do not directly detect Avr proteins but rather detect the biochemical perturbations the Avr proteins cause in plant cells

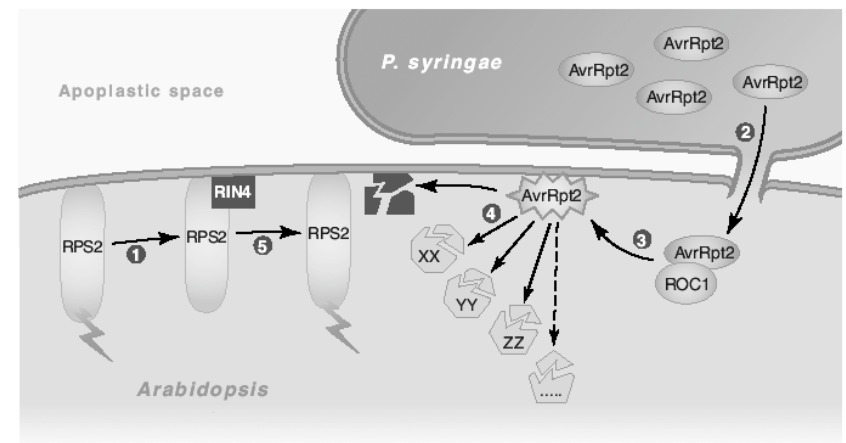
Indirect perception model- Roger Innes Plant Physiol 135:695

The discovery that one's kitchen has been invaded by mice is often made indirectly. The holes chewed in the muesli bag and the teeth marks on the corn flakes box are a dead give away. Although you have not seen the mouse, you deploy your defensive weapons, and if successful, succeed in protecting your valuable goods from the invasion, hopefully before all of your food has been eaten. Recent research results indicate that plants also make use of such indirect surveillance systems to protect themselves from being consumed by pathogens. Rather than wait for a direct observation of the pest, plants appear to activate their defenses as soon as pathogen-induced damage is detected.

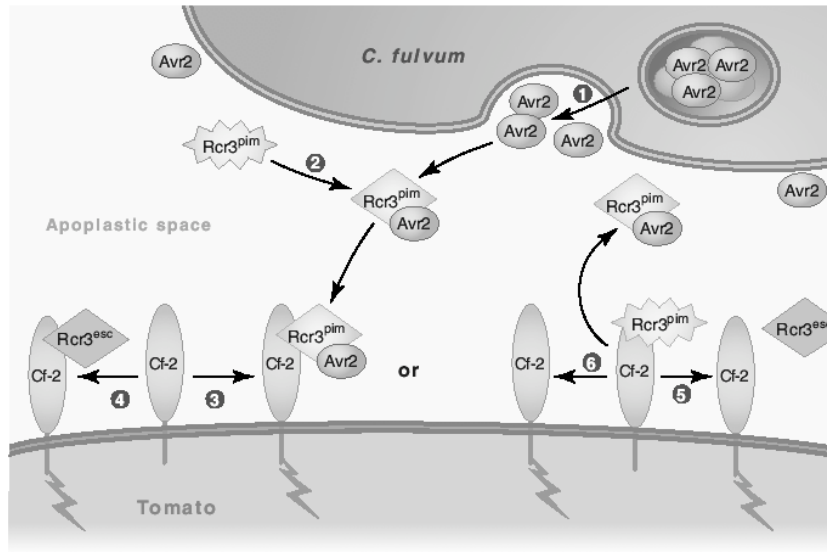
Indirect recognition model



Examples: AvrRpt2 - RIN4 - Rps2



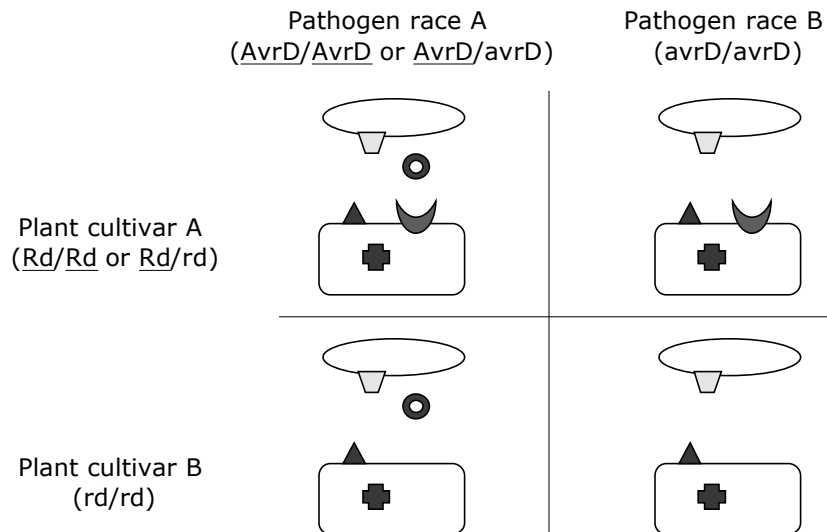
Examples: Avr2 - Rcr3 - Cf-2



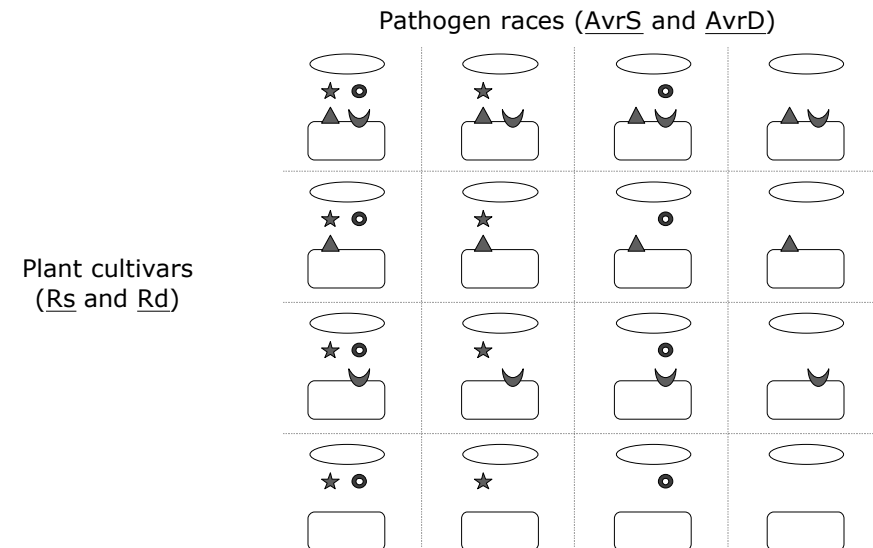
Implications of indirect perception model

- Goes beyond one *Avr* gene-one *R* gene model
- Explains why R proteins can recognize unrelated pathogens (example tomato *Mi* gene recognizes nematodes and aphids)
- Virulence and Avirulence genes are functionally similar. The term avirulence is misleading. Thus, new terminology: virulence or avirulence=effector genes
- What are the effector targets and what is their function in defense?

Gene for gene- other genes are important too!



Genes for genes interactions



Gene for gene interactions

The interaction between:



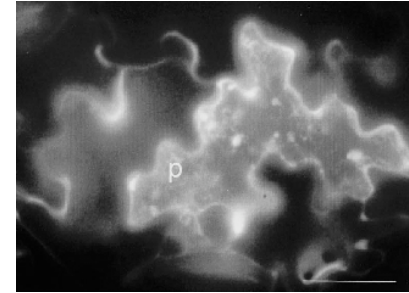
- Pathogen Avirulence (=virulence=effector) genes encoding Avr products



- Plant Effector (=virulence) Targets (ET) that are the cellular targets of pathogen effectors



- Plant Resistance genes encoding Resistance gene products (R proteins) that detect the Effector/Effector Target complexes or the biochemical perturbations caused by the effectors



Hypersensitive response

R gene mediated resistance and the HR

- R gene mediated resistance is often (but not always) associated with a localized cell death response, the hypersensitive response (HR)
- The HR is also accompanied by a complex set of biochemical and physiological changes (defense response)
- In some cases, HR cell death is not observed- example: Extreme resistance to viruses such as with Rx potato resistance to PVX

Gene for gene- recognition vs. response

- The HR and other defense responses are generally not specific to a particular pathogen
- The key to the gene for gene model is that specificity of resistance lies in the recognition of a particular Avr product by a particular R gene product and not in the subsequent defense response

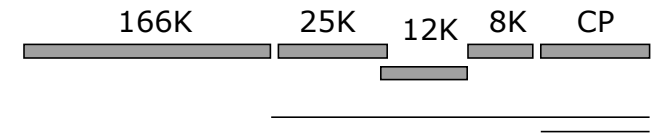
Gene for gene- recognition vs. response

- Can an *Avr* gene from a particular pathogen confer avirulence in a gene for gene manner to an unrelated pathogen?
- Fungal *Avr* gene: *Cladosporium fulvum* *Avr9* transfer to Potato Virus X

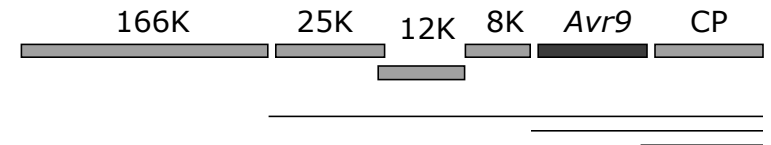
Kamoun et al. 1999 MPMI 12:459

Potato Virus X (PVX)

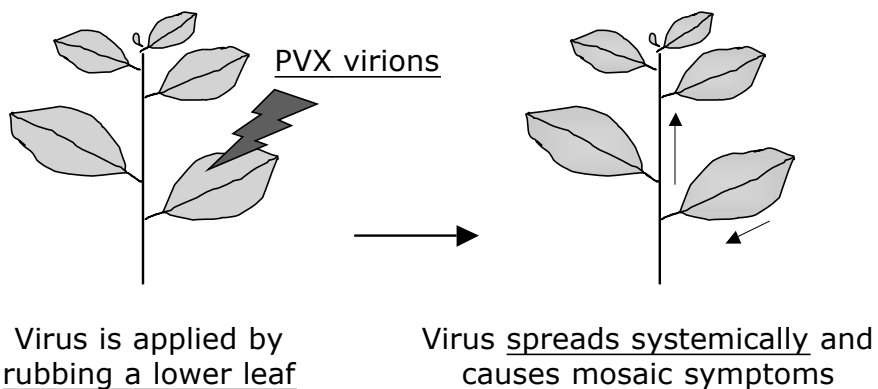
PVX



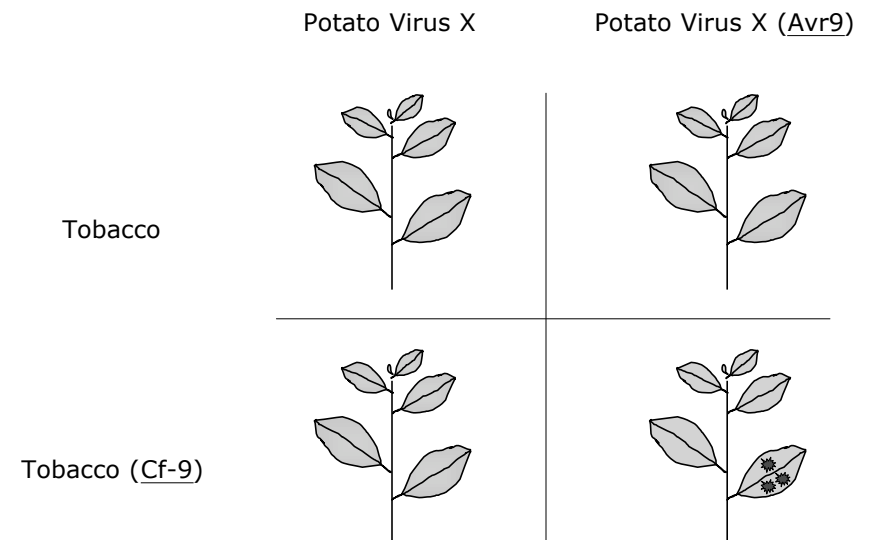
PVX-Avr



Potato Virus X (PVX)



Gene for gene model- specificity is the key!



Gene for gene model- specificity is the key!



Potato Virus X



Potato Virus X (Avr9)

Gene for gene- recognition vs. response

- Fungal gene *Avr9* gene confers specific avirulence to Potato Virus X only on tobacco plants that contain the corresponding Cf-9 R gene
- Similar results obtained with bacterial *AvrPto*, oomycete *inf1* avirulence genes, etc...
- Gene for gene interactions can be transferred to other organisms

Gene for gene- recognition vs. response

- HR and other related defense responses triggered by specific Avr elicitors are equally effective against viruses, bacteria, fungi, oomycetes, nematodes and insects

Plant Pathology 602 Plant-Microbe Interactions

Outline - gene for gene interactions

- The gene for gene model
- Plant disease resistance genes (*R* genes)

Plant disease resistance genes

Properties of *R* genes and their products

- Pathogen recognition
- Activation of signaling cascade leading to HR and defense response
- Rapid evolution of new recognition specificities

Plant disease resistance genes

Cloning

- Dozens of *R* genes have now been cloned from a diversity of plants
- These *R* genes recognize viruses, bacteria, fungi, oomycetes, nematodes and insects
- Transposon-based gene tagging used to clone *R* genes from maize, tobacco, tomato, and flax
- Map-based positional cloning used for tomato, barley, rice and *Arabidopsis*

Plant disease resistance genes

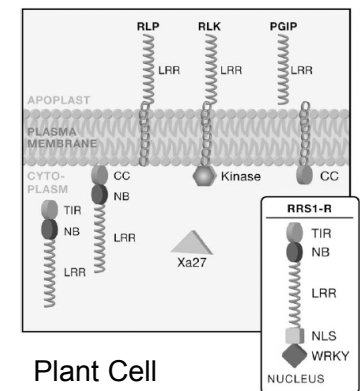
Molecular characterization

- Several classes of *R* genes are known
- *R* genes from diverse plants share similar structural features independently of the pathogens they target
- *R* genes often occur in complex gene clusters

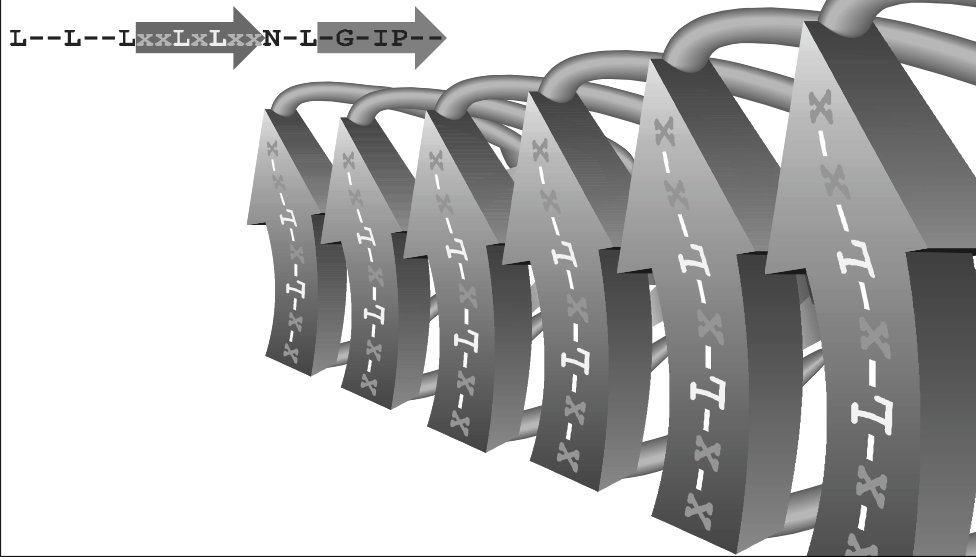
Plant disease resistance genes

Major protein motifs found in various classes of products of *R* genes (*R* proteins)

- Coil-coil motifs (CC)
- Toll-Interleukin Domain (TIR)
- Nucleotide Binding Sites (NB)
- Leucine Rich Repeats (LRR)
- Kinase
- Nuclear localization signal (NLS)
- DNA binding (WRKY)

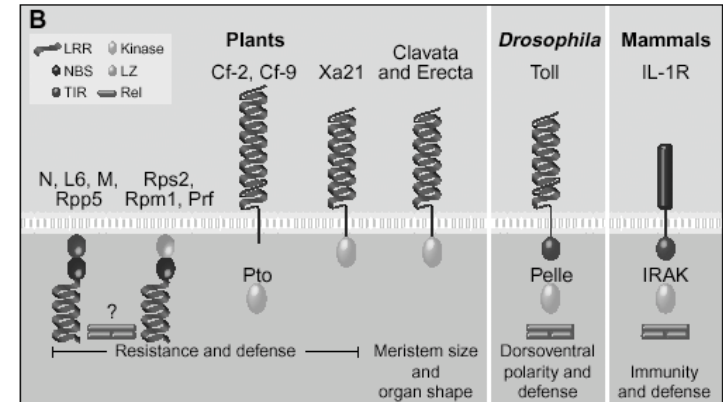


Leucine-Rich Repeats



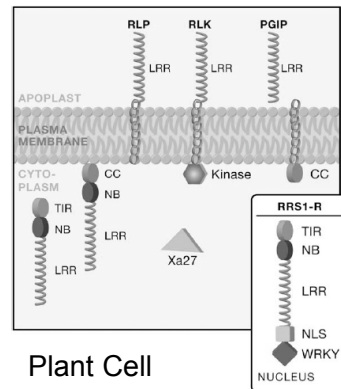
Plant disease resistance genes

Major protein motifs found in various classes of products of *R* genes (*R* proteins) are also found in animal defense response proteins



Plant disease resistance genes

- Resistance proteins are receptor-like proteins that localize in different cellular compartments:
 - apoplast (surface of the plant)
 - cytoplasm
 - nucleus



Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)
1	Pto	Tomato	<i>Pseudomonas syringae</i> (B)	AvrPto, AvrPtoB
2	Bs2	Pepper	<i>Xanthomonas campestris</i> (B)	AvrBs2
	Dm3	Lettuce	<i>Bremia lactucae</i> (F)	
	Gpa2 ^a	Potato	<i>Globodera pallida</i> (N)	
	Hero	Potato	<i>G. rostochiensis</i> , <i>G. pallida</i> (N)	
	HRT ^b	<i>Arabidopsis</i>	Turnip Crinkle Virus	Coat Protein
	I2	Tomato	<i>Fusarium oxysporum</i> (F)	
	Mi	Tomato	<i>Meloidogyne incognita</i> (N)	
	Mi	Tomato	<i>Macrosiphum euphorbiae</i> (I)	
	Mla	Barley	<i>Blumeria graminis</i> (F)	
	Pib	Rice	<i>Magnaporthe grisea</i> (F)	
	Pi-ta	Rice	<i>M. grisea</i> (F)	AVR-Pita
	R1	Potato	<i>Phytophthora infestans</i> (O)	

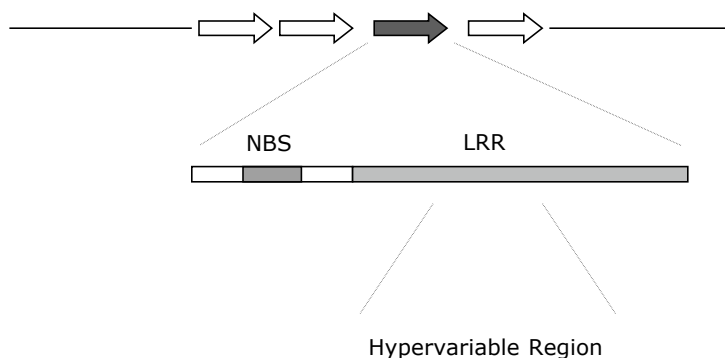
Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)
3	L	Flax	<i>Melampsora lini</i> (F)	Helicase
	M	Flax	<i>M. lini</i> (F)	
	N	Tobacco	Tobacco Mosaic Virus	
	P	Flax	<i>M. lini</i> (F)	
	RPP1	<i>Arabidopsis</i>	<i>P. parasitica</i> (O)	
	RPP4	<i>Arabidopsis</i>	<i>P. parasitica</i> (O)	
	RPP5	<i>Arabidopsis</i>	<i>P. parasitica</i> (O)	
	RPS4	<i>Arabidopsis</i>	<i>P. syringae</i> (B)	
4	Cf-2 ^c	Tomato	<i>Cladosporium fulvum</i> (F)	Avr2
	Cf-4 ^d	Tomato	<i>C. fulvum</i> (F)	Avr4
	Cf-5 ^c	Tomato	<i>C. fulvum</i> (F)	Avr9
	Cf-9 ^d	Tomato	<i>C. fulvum</i> (F)	
5	Xa21	Rice	<i>Xanthomonas oryzae</i> (B)	

Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)
6	Hm1	Maize	<i>Cochliobolus carbonum</i> (F)	
	HS1 ^{pro-1}	Beet	<i>Heterodera schachtii</i> (N)	
	mlo	Barley	<i>B. graminis</i> (F)	
	Rpg1	Barley	<i>Puccinia graminis</i> (F)	
	RPW8	<i>Arabidopsis</i>	<i>Erysiphe chicoracearum</i> (F)	
	RRS1-R	<i>Arabidopsis</i>	<i>Ralstonia solanacearum</i> (B)	
	RTM1	<i>Arabidopsis</i>	Tobacco Etch Virus	
	RTM2	<i>Arabidopsis</i>	Tobacco Etch Virus	
	Ve1 ^e , Ve2 ^e	Tomato	<i>Verticillium alboatrum</i> (F)	

*Shown are R proteins characterized to date, sorted by structural class (see text). The host plant and corresponding pathogen(s) or pest(s) and effector proteins, where known, are given for each. Except for viruses, pathogen or pest type is indicated in parentheses, abbreviated as: B, bacterium; F, fungus; I, insect; N, nematode, O, oomycete. The bottommost proteins do not fit in any of the designated structural classes and are discussed briefly in the text. Highly similar members of the same gene cluster are identified by matching superscript letters.

Plant disease resistance genes

Chromosomal organisation of an R gene cluster



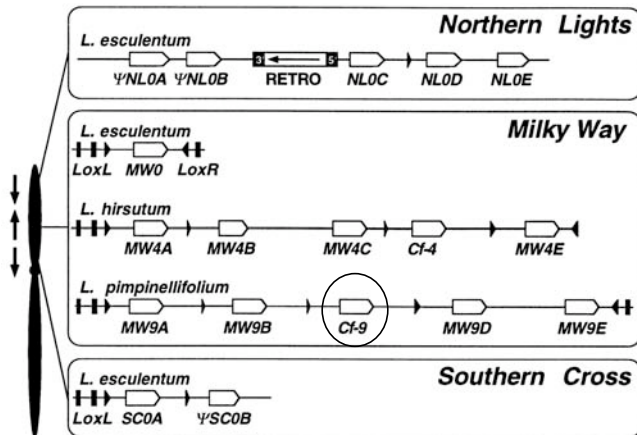
Plant disease resistance genes

Chromosomal organisation of an *R* gene cluster in resistant and susceptible lines: The *Cf-4/9* cluster

- Introgressed into tomato from the wild species relatives: *Lycopersicon pimpinellifolium* (*Cf-9*) and *L. hirsutum* (*Cf-4*)
- Complex clusters of *Hcr9* genes (homologs of *Cf-9*)
- Located in chromosome 1
- At least five functional *Hcr9* genes have been identified encoding at least four distinct recognition specificities

Plant disease resistance genes

Chromosomal organisation of an *R* gene cluster in the resistant and susceptible lines: The *Cf-4/9* cluster



Parniske and Jones 1999
PNAS 96:5850

Plant disease resistance genes

Chromosomal organisation of an *R* gene cluster in the resistant and susceptible lines: The *Cf-4/9* cluster

- Complex patterns of separation and rare recombination between members of the clusters and between members of different clusters allow high mutation rates and occasional reshuffling of genes
- This probably contributes to the emergence of new specificities

Plant disease resistance genes

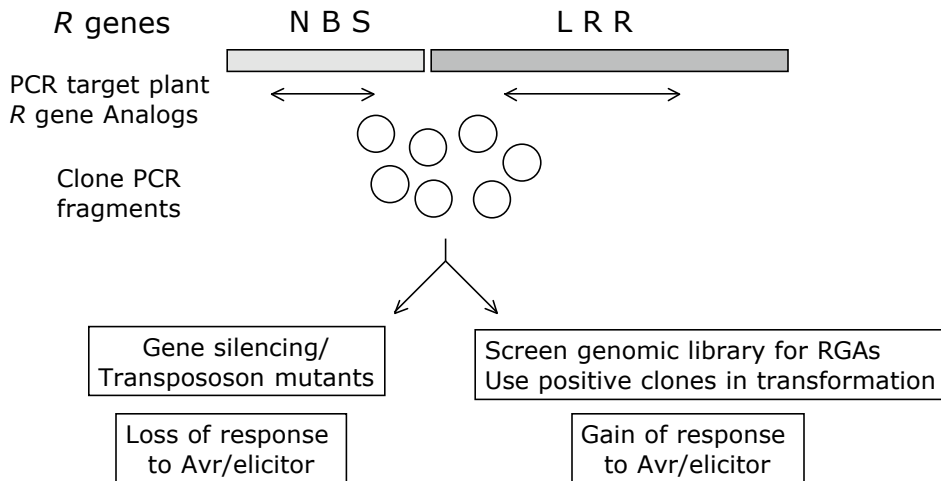
Summary

- Recognition specificity probably lies in LRR domain in a typical R protein
- Signal transduction is probably initiated by CC/NBS region and/or kinase domain
- R protein associate with co-receptors (resistasome)
- High mutation rates and gene rearrangements in complex clusters probably allow rapid evolution

Exploiting *R* genes to engineer resistant plants

- Cloning of *R* genes allow direct transfer of genes to cultivars of interest and from one crop to another
- Great diversity of *R* genes in plants
- Challenge is to identify novel *R* genes and their corresponding targets
- Heterologous transfer of *R* genes works best within the same botanical family
- Strategies based on “artificial” or “engineered” HR

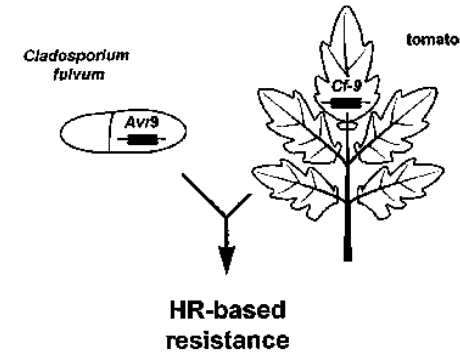
Resistance Gene Analogs (RGAs)- Rapid cloning of R genes



Genetic engineering for resistance

Natural resistance in the gene for gene system

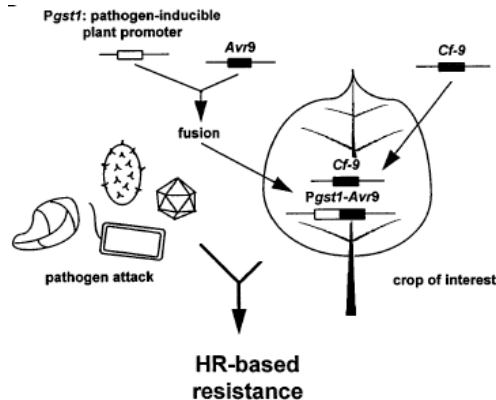
The *Avr9-Cf9* pair determines resistance in the interaction between *Cladosporium fulvum* and tomato



Genetic engineering for resistance

Engineered resistance: the two component system

Both *Avr9* and *Cf9* are introduced in a crop plant under the control of a pathogen inducible promoter. HR occurs upon infection by pathogen



Summary

- *R* genes are the central players in plant innate immunity or the ability of plants to defend against pathogen infection
- The gene for gene model and its modern illustration the guard model define *R* gene mediated resistance
- *R* gene mediated resistance typically functions through activation of the Hypersensitive Response, a ubiquitous defense response
- *R* genes are structurally diverse and complex
- *R* genes can be exploited to engineer resistant plants