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Does basal *PR* gene expression in *Solanum* species contribute to non-specific resistance to *Phytophthora infestans*?

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Systemic acquired resistance (SAR) occurs in many plant species, including potato. SAR can be induced by various signals, but also basal levels of SAR may vary between plants. In *Arabidopsis* mutants, basal SAR levels positively correlate with pathogen resistance. Here we test whether in 13 wild *Solanum* clones and five potato cultivars, basal expression levels of SAR marker genes correlate with resistance to *Phytophthora infestans*. Most of the examined *Solanum* plants displayed significant and variable levels of race/isolate-non-specific, partial resistance to five *P. infestans* isolates of diverse origin. Constitutive mRNA levels of the pathogenesis-related genes *PR-1*, *PR-2* and *PR-5* in non-infected leaves varied between the *Solanum* clones. However, no correlation between basal *PR* mRNA levels and resistance was observed at the genus level. In contrast, significant correlation was found at the species level in *S. arnezii* × *hondelmannii*, *S. microdontum*, *S. sucrense* and *S. tuberosum*. In *S. tuberosum* cultivars, the levels of *PR* gene expression were the highest in resistant Robijn, intermediate in partially resistant Première, Estima and Ehud, and the lowest in susceptible Bintje. These results suggest that constitutive expression of *PR* genes may contribute to non-specific resistance to *P. infestans* in *Solanum*. Therefore, *PR* mRNAs could serve as molecular markers in potato breeding programs.

Keywords: Late blight; non-specific resistance; partial resistance; pathogenesis-related proteins; glucanase; osmotin; systemic acquired resistance; *Phytophthora infestans*; *Solanum arnezii* × *hondelmannii*; *Solanum berthaultii*; *Solanum circaeifolium*; *Solanum microdontum*; *Solanum nigrum*; *Solanum sucrense*; *Solanum tuberosum*; *Solanum vernei*; wild *Solanum* species; potato.

INTRODUCTION

Resistance of plants to pathogens can be attributed to the action of various resistance mechanisms, each functioning at a certain level and specificity. One such mechanism is systemic acquired resistance (SAR), which generally follows a localized unsuccessful pathogen attack, involves an enhanced state of resistance to a broad spectrum of pathogens and is associated with an increased expression of genes encoding pathogenesis-related (PR) proteins [30]. In other cases, pathogen attack is hampered by another type of resistance, called induced systemic resistance (ISR), which is not associated with increased PR gene expression [28].

SAR has been described in several plant species, including potato, but has best been documented in *Arabidopsis thaliana*. Several *Arabidopsis* mutants altered in SAR have been identified. For example, mutants in the *npr1* gene (*n*on-expresser of *PR* genes) fail to respond to SAR-inducing treatments, and are susceptible to the bacterial pathogen *Pseudomonas syringae* in contrast to wild-type plants [7, 13, 31]. Overexpression of *npr1* led to enhanced resistance to *P. syringae* and the oomycete *Peronospora parasitica* in a dosage-dependent fashion since levels of *npr1* mRNA, NPR1 protein, *PR-1* mRNA and resistance were positively correlated in the transformants [8]. The *cpr* (*c*onstitutive expressers of *PR* genes) [6] and *dnd1* (*d*efense with *n*o cell *d*eath) mutants [47] exhibit constitutive SAR-related phenotypes including elevated mRNA levels for genes encoding *PR* proteins. The

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Abbreviations used in text: IE, infection efficiency; LGR, lesion growth rate; PR, pathogenesis-related; SAR, systemic acquired resistance.

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analyses of these *Arabidopsis* mutants indicate that subtle mutations may affect basal SAR levels, and that higher resistance levels can be reached by manipulating basal levels of SAR.

In solanaceous plants, SAR has also been reported. In potato, SAR could be induced by treatment with hyphal wall components, unsaturated fatty acids and jasmonic acid, resulting in enhanced resistance to the late blight oomycete pathogen *Phytophthora infestans* (Mont.) de Bary [*14*, *9*, *10*]. In engineered potato plants containing a transgene encoding a bacterio-opsin proton pump from *Halobacterium halobium*, the expression of several *PR* genes was increased and resistance to *P. infestans* was enhanced [*2*]. Naturally occurring constitutive SAR was found in hybrids of tobacco and in certain tomato lines in which enhanced resistance to various pathogens was associated with constitutive *PR* gene expression [*3*, *46*, *19*].

In addition to realization of full-fledged SAR, the effects of overexpression of single *PR* genes on resistance have been described. Overexpression of *PR-1* in tobacco-increased resistance to *Phytophthora parasitica* and *Peronospora tabacina* [*4*], and overexpression of *PR-5* (osmotin) slightly enhanced resistance to *P. infestans* in potato [*21*, *27*, R. Li and A. Pereira, pers. comm.] and *Solanum commersonii* [*49*]. *PR*-1-like proteins are conserved within the plant, fungal, vertebrate and invertebrate kingdoms. In animals these proteins function as venoms, allergens, or are implicated otherwise in defense [*41*]. The purified PR-1 protein from tomato and tobacco inhibited germination of *P. infestans* zoospores *in vitro* and lesion growth *in vivo* [*25*]. *PR-2* and *PR-3* encode glucanases and chitinases respectively, and these enzymes may play a role in cell wall degradation. However, oomycetes lack chitin in their cell wall and are not expected to be affected by chitinases. Actin-binding studies suggested that a basic chitinase and an osmotin-like protein might be involved in cytoplasmic aggregation, an important event in potato's cellular defense to *P. infestans* [*36*]. In addition, *PR-5* proteins play a role in osmotic stress, freezing tolerance, permeabilization of fungal and oomycetal plasma membranes and pathogen resistance [*45*, *21*, *1*, *49*].

P. infestans is a major pathogen of potato and tomato. In recent years, the severity of this disease has increased dramatically, and a more profound insight in the mechanisms of resistance to *P. infestans* is needed to develop novel control strategies. In the *P. infestans*–potato interaction, the most commonly studied type of resistance is race-specific resistance, which is governed by single dominant resistant genes (*R* genes). Unfortunately, race-specific resistance is only effective against certain strains of the pathogen, and is easily overcome by rapid evolution of the pathogen resulting in a lack of durability in the field. In contrast, race-non-specific resistance is effective against all known strains or races of the pathogen. It is thought to be based on multiple genes, may be durable, and is generally of a partial nature. Several wild *Solanum* species possess varying levels of partial resistance to *P. infestans* [*11*, *44*], and in old potato cultivars, such as cv. Robijn, partial resistance appeared to be durable [*12*]. In a previous study, we cytologically analysed *Solanum* species inoculated with *P. infestans*, and found that defense responses were always associated with the hypersensitive response (HR), a programmed cell death defense response of plants. In partially resistant clones, hyphal escape occurred and growing lesions were established [*18*, *43*]. The growth rate of these lesions varied between different *Solanum* clones, indicating that defense mechanisms other than the HR operate at different levels in the different clones.

Even though a causal link between the accumulation of PR proteins and SAR has not always been established, a correlation between the timing of *PR* gene expression and the onset and duration of SAR is evident from many studies [*30*]. Therefore, measuring expression levels of *PR* genes is an appropriate method for determining levels of SAR [*30*, *22*]. In this study, we determined the variation in basal mRNA levels of SAR marker genes (*PR-1*, *PR-2* and *PR-5*) in *Solanum* plants, and tested whether resistance to *P. infestans* in *Solanum* species is associated with high basal levels of SAR.

MATERIALS AND METHODS

Plant material

The plant material used in this study is listed in Table 2. The origin of the plant material and the *in vitro* propagation was described previously [*44*]. Plants were grown under controlled conditions in climate chambers with a 16 h/8 h day/night regime at 18/15 °C and HPIT (Philips) illumination. The condition of different batches of plants used for the various resistance tests and for the *PR* gene expression analyses was comparable [*44*].

Phytophthora infestans

Phytophthora infestans isolates of different origins were used (Table 1). The propagation of the isolates and the preparation of inoculum was performed following standard procedures [*44*].

Resistance assessment

The resistance levels of 18 *Solanum* clones to six *P. infestans* isolates were determined using a routine resistance assay [*44*]. Detached leaves were spot-inoculated (10 μl) with a zoospore suspension of 50 000 spores ml^{-1}, and incubated at high humidity in the dark. On the fourth, fifth and sixth day after inoculation, the largest length and width

TABLE 1. Phytophthora infestans *isolates used in this study*

Isolate	Clonal lineage	Race	Origin	Host of origin	Year	Mating type	Provided by
IPO-0	US-1	0	Unknown	Unknown	1987	A1	Lo Turkensteen, Plant Research Int., Wageningen, The Netherlands
90128	Unknown	1.3.4.6.7.8.10.11	Geldrop, The Netherlands	Potato	1990	A2	Francine Govers, Wageningen University, Wageningen, The Netherlands
Mex580	Unknown	Complex	Toluca Valley, Mexico	*S. demissum*	Unknown	A1	Bill Fry, Cornell University, Ithaca, U.S.A.
BIN-16	US-6	Complex	Northwestern U.S.A.	Unknown	1992	A1	Bill Fry, Cornell University, Ithaca, U.S.A.
ME93-2A	US-8	Complex	Maine, U.S.A.	Potato	1993	A2	Bill Fry, Cornell University, Ithaca, U.S.A.

(perpendicular on the length) of the lesions were measured. The ellipse area ($A = 1/4 \times \pi \times$ length $\times$ width) was calculated, and the lesions were divided into two groups, i.e. "no growing lesion" ($A \leqslant 16$ mm^2), or "growing lesion" ($A > 16$ mm^2). The infection efficiencies (IE) were calculated as the percentage of growing lesions. The areas of the "growing lesion" group were square root transformed, and the average lesion growth rate (LGR) was estimated by linear regression on time. The mean LGRs were analysed with REML using Genstat [16].

Southern and Northern blot analysis

DNA was isolated from leaves of *Solanum* plants and digested with *Eco*RV [33]. The DNA was electrophorized, transferred to Hybond-N$^+$, and the Southern blot was hybridized with various probes. For expression analyses, leaf material (3rd, 4th and 5th fully developed leaf) from healthy, uninoculated plants was harvested and immediately frozen in liquid nitrogen. Two independent RNA isolation [42] series were carried out. For each RNA sample 15 μg was loaded, electrophorized and transferred to Hybond-N$^+$. The Northern blot was hybridized concurrently with the Southern blot with probes representing the *PR-1*, *PR-2*, *PR-5* and tubulin gene at 65, 60, 60 and 65 °C respectively, and the blots were washed at 1, 0·5, 0·5 and 1 × SSC stringency, respectively.

Messenger RNA levels were determined from the Northern blots using a Fujix Bio-Imaging Analyser (BAS 2000). The signals were quantified in photo-stimulated luminescence (PSL) per mm^2. To correct for slight differences in loading the signals for *PR* gene expression were normalized to the constitutively expressed tubulin signal.

DNA probes

PR gene members previously described to be correlated to resistance were selected. As DNA templates for probe synthesis the following fragments were used: a 400 bp *Eco*RI/*Kpn*I fragment of *StPR1-1*, a *PR-1* cDNA clone from potato [39], a 1300 bp *Eco*RI/*Xho*I fragment of an acidic glucanase cDNA clone from tomato [40], a PCR fragment amplified on tobacco genomic DNA for *PR-5* [23], and a 1800 bp *Eco*RI/*Xho*I fragment from cDNA clone pFB19 encoding tubulin from potato.

RESULTS AND DISCUSSION

Specific and non-specific resistance in Solanum

To test the correlation between high levels of SAR and resistance to *P. infestans*, we first carefully determined the resistance levels of a set of 18 *Solanum* plants to five isolates from different clonal lineages, races, geographical origins, hosts of origin, years of isolation and mating types (Table 1). Various types of resistance were noted as illustrated by the mean LGRs and IEs data (Table 2).

Statistical analyses revealed a highly significant interaction ($P < 0{\cdot}001$) between *Solanum* clones and *P. infestans* isolates, indicating that race-specific resistance occurs. In potato cv. Ehud (*R1*), race-specific resistance was evident since inoculation with race 0 strain IPO-0 resulted in complete resistance (IE = 0, LGR = 0), whereas inoculation with isolate 90128 (virulent on *R1* plants), resulted in high IEs and LGRs (Table 2). In Estima and Première (*R10*), the race-specific response was less pronounced, as a considerable percentage of growing lesions was noted in the interaction with IPO-0. This is in line with previous findings that *R1* functions as a "strong" *R* gene and *R10* as a "weak" *R* gene [38, 43]. Statistical analyses of LGR

TABLE 2. *Resistance levels of* Solanum *clones to* P. infestans *isolates IPO-0, 90128, Mex580, BIN-16 and ME93-2A*

		IPO-0		90128		Mex580		BIN-16		ME93-2A	
Solanum	Clone	LGR	IE	LGR	IE	LGR	IE	LGR	IE	LGR	IE
Solanum berthaultii	ber-9	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0
S. berthaultii	ber-11	1·6	25	2·4	10	1·9	18	0·0	0	0·0	0
S. arnezii × *hondelmannii*	axh-63	2·0	60	1·9	95	2·2	88	1·6	30	1·8	25
S. arnezii × *hondelmannii*	axh-72	2·9	88	3·3	75	2·6	65	2·3	10	1·8	5
S. circaeifolium ssp. *circaeifolium*	circ1	0·0	0	0·0	0	0·7	3	0·0	0	0·6	25
S. microdontum	mcd-167	0·0	0	3·2	45	1·8	5	1·5	3	0·0	0
S. microdontum	mcd-178	1·3	18	2·7	25	1·1	8	2·4	15	1·4	15
S. microdontum var. *gigantophyllum*	mcd-265	4·6	100	5·4	90	4·1	95	3·2	100	2·0	65
S. sucrense	scr-23	3·4	88	5·0	45	3·0	85	4·1	15	3·1	15
S. sucrense	scr-71	3·5	43	4·1	100	2·2	55	2·1	30	2·9	20
S. vernei	vrn-530	1·4	28	3·8	80	2·9	53	4·5	18	1·0	10
ABPT (30 × 33) hybrid	ABPT-44	2·3	13	2·6	80	2·0	10	1·7	23	3·4	25
S. nigrum	ngr-SN18	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0
S. tuberosum	Bintje	4·1	100	5·0	75	3·7	98	2·7	85	4·0	35
S. tuberosum	Ehud (*R1*)	0·0	0	4·2	100	3·7	100	3·0	83	3·3	35
S. tuberosum	Estima (*R10*)	3·6	80	4·8	100	3·5	33	2·8	53	0·8	10
S. tuberosum	Première (*R10*)	3·1	45	3·5	90	3·8	33	3·3	28	4·0	15
S. tuberosum	Robijn	0·9	18	2·9	100	1·7	50	1·6	25	2·0	40

The resistances are expressed as mean lesion growth rate (LGR, in mm day^{-1}) and infection efficiency (IE, in percentage), and are based on two experiments for IPO-0, Mex580, BIN-16, and one experiment for 90128 and ME93-2A.
$LSD_{LGR} = 1·3$.
$LSD_{IE} = 24$.

and IE between *Solanum* clones and *P. infestans* isolates also revealed a highly significant interaction ($P < 0·001$) when cultivars bearing *R1* and *R10* were excluded, suggesting that novel undefined *R* genes may occur in the examined set of *Solanum* plants. However, strong isolate-specific resistance [*43*] comparable to *R1* was not evident. In contrast, weaker isolate-specific responses were common. For example, *S. microdontum*-167 was more susceptible to isolate 90128 than to the other isolates. *S. sucrense*-23 was more often infected by IPO-0 and Mex580 (IE = 88 and 85 % respectively) than by other isolates (IE = 15–45 %), although high LGRs were noted in all interactions. Potato cv. Estima and Première exhibited similar IEs and LGRs to isolates IPO-0, 90128, Mex580 and BIN-16, but Estima was remarkably resistant to isolate ME93-2A.

Low LGR values are considered as indicators of non-specific, partial resistance [*12*]. In addition to isolate-specific responses, *Solanum* clones differed in LGRs independently of the isolate tested. For example, potato cv. Robijn showed lower LGR values than Bintje with all tested isolates. Cultivar Estima and Première, and to a lesser extent cv. Ehud, showed intermediate LGRs, indicating a certain level of partial resistance for these cultivars. In *S. microdontum*, clone 167 and 178 displayed lower LGRs than clone 265, and in *S. arnezii* × *hondelmannii*, clone 63 generally displayed slightly lower LGRs than clone 72, independently of the isolate. This suggests that most of the examined *Solanum* plants display significant and variable levels of race/isolate-non-specific, partial resistance to five *P. infestans* isolates of diverse origin.

Occurrence of PR genes in Solanum

To check whether sufficient cross-hybridization occurs between the *PR* gene probes and the selected plants, a Southern blot containing genomic DNA from the different *Solanum* species was hybridized with probes from a potato *PR-1* gene, a tomato *PR-2* gene, and a tobacco *PR-5* gene. As shown in Fig. 1, the three probes cross-hybridized with DNA from all *Solanum* clones. In all cases multiple hybridizing bands were detected revealing the presence of multi-gene families for *PR-1*, *PR-2* and *PR-5* in *Solanum* species. In addition, there was variation in signal intensity among the hybridizing bands, suggesting sequence diversity or differences in copy number among the family members. Multi-gene families have been reported for *PR-2* in *S. tuberosum* [*5*], for *PR-5* in *S. commersonii* [*48*], and for *PR-1*, *PR-2* and *PR-5* in tobacco [*41*].

For each of the three *PR* genes, the hybridization pattern within the species was reasonably conserved. Between species however, hybridization patterns were quite diverse. For *PR-2*, a certain specificity can be noted, as the hybridization patterns of the closely related species

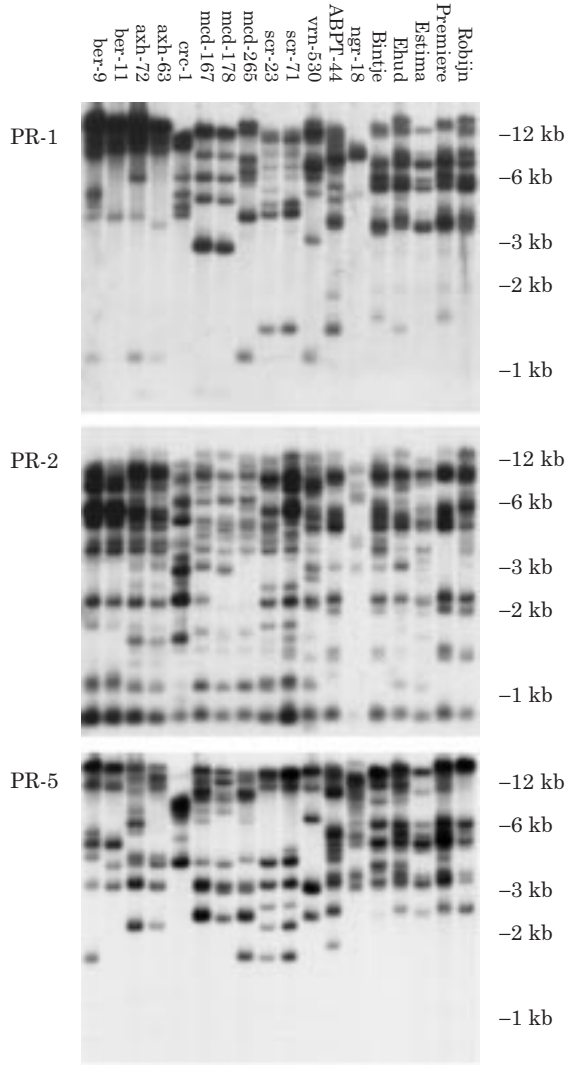

FIG. 1. Occurrence of *PR* genes in *Solanum* clones (for abbreviation of clones see Table 2). A Southern blot containing *Eco*RV digested genomic DNA isolated from *Solanum* clones was sequentially hybridized with a *PR-1* probe from potato, a *PR-2* probe from tomato, and a *PR-5* probe from tobacco.

S. berthaultii and *S. arnezii* × *hondelmannii* [35] were quite similar. In addition, the hybridization of the tomato *PR-2* probe to *S. nigrum* DNA was exceptionally weak, whereas hybridization of the same blot with the *PR-1* and *PR-5* probes resulted in stronger signal intensities. This suggests that the *PR-2* genes from *S. nigrum* are quite divergent from those of the other tested *Solanum* species. This is not surprising since *S. nigrum* is the most distantly related species in the examined set. *S. nigrum* is classified in the subgenus *Solanum*, whereas the other tested *Solanum* species and tomato belong to the subgenus *Potatoe* [34]. In summary, the Southern blot hybridizations showed that the heterologous *PR* probes are suitable for analysing expression of *PR-1*, *PR-2* and *PR-5* genes in the *Solanum* plants.

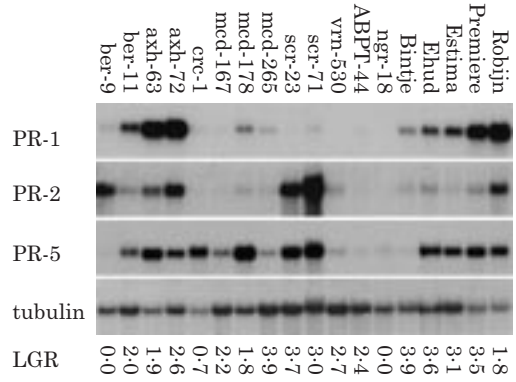

FIG. 2. Expression analyses of *PR* genes in uninoculated *Solanum* plants (for abbreviation of clones see Table 2). A Northern blot containing total RNA from *Solanum* clones was sequentially hybridized with *PR-1*, *PR-2*, *PR-5* and tubulin probes. The average LGRs (mm day^{-1}) (based on *P. infestans* isolates IPO-0, 90128, Mex580, BIN-16, ME93-2A) are indicated for each clone (LGR = 0 excluded).

Expression analysis of Solanum PR genes

To monitor constitutive SAR in the *Solanum* plants, the basal expression levels of the *PR-1*, *PR-2* and *PR-5* genes were determined in two independent experiments using uninoculated plants cultivated under defined conditions in growth chambers. The results of the first experiment are shown in Fig. 2. The autoradiographs showed that *PR-1*, *PR-2* and *PR-5* mRNAs were present at detectable levels in the majority of the tested plants. Interestingly, there is variation in *PR* mRNA levels between the different clones. In addition, within the entire *Solanum* set, the patterns of mRNA levels of the three *PR* genes were not identical. This is in line with the separation of *PR-1*, *PR-2* and *PR-5* gene activation pathways previously shown for *Arabidopsis* *e*nhanced *d*isease *s*usceptibility mutants *eds5* [29]. The absence of coordinated regulation between the different *PR* genes was also observed in *s*alicylic acid *i*nduction *d*eficient mutants *sid1* and *sid2*, in which the pathway leading to *PR-1* expression was blocked, whereas *PR-2* and *PR-5* were expressed at wild-type levels [24]. Thus unequal mRNA accumulation of the different *PR* genes could reflect different pathways of regulations for individual members of the three *PR* gene families, perhaps reflecting the wide range of physiological responses in which these proteins are involved [37, 26, 48].

PR mRNA levels were quite similar in the two experiments. However, in potato cv. Ehud and *S. vernei*-530, the mRNA levels observed for all three *PR* genes were higher in the second experiment compared to the first experiment. We assume that despite the precautions we took the Ehud and *S. vernei* plants in the second experiment may have been in a stressed state. In other experiments we noted high *PR-1* mRNA levels in plants

grown in the greenhouse (data not shown), where they were exposed to heat and drought stress.

Does PR *gene expression correlate with resistance?*

To test whether isolate-non-specific, partial late blight resistance in *Solanum* is associated with a constitutive SAR, the obtained LGRs were compared to basal mRNA levels of *PR* genes. *PR-1*, *PR-2* and *PR-5* mRNA levels were quantified and normalized using tubulin mRNA levels as a reference. At the genus level, there was no indication for a correlation between non-specific resistance and *PR* expression levels. This may be explained by the different genetic background of the different plant species and the noted complexity of the three *PR* gene families. In contrast, significant correlation was observed at the species level. In *S. arnezii* × *hondelmannii*, *S. microdontum*, *S. sucrense* and *S. tuberosum*, partially resistant clones exhibited higher levels of *PR* mRNA than more susceptible ones. In *S. sucrense* and *S. arnezii* × *hondelmannii*, this correlation was evident for all three *PR* genes in the two independent experiments. In *S. circaeifolium*, *S. vernei*, ABPT and *S. nigrum*, only one clone was used, and thus correlations at the species level could not be tested. No correlation was observed between *PR* mRNA levels and resistance in *S. berthaultii*. However, the full resistance observed in clone 9 is HR-mediated [*43*] and suspected to operate through a novel *R* gene as recently observed for another accession of *S. berthaultii* [*15*].

Within *S. tuberosum*, the relationship between non-specific resistance and *PR* mRNA levels was examined for the five tested cultivars (Fig. 3). Highly resistant cv. Robijn accumulated the highest levels of *PR-1*, *PR-2* and *PR-5* mRNA, whereas partially resistant Ehud, Estima and Première displayed intermediate levels, and susceptible Bintje very low levels. These results suggest a correlation between *PR* gene expression levels and resistance levels in potato. These results are consistent with those obtained with *S. arnezii* × *hondelmannii*, *S. microdontum*, and *S. sucrense* suggesting that an enhanced constitutive expression of SAR may be a component of the partial/non-specific resistance noted in *Solanum* species.

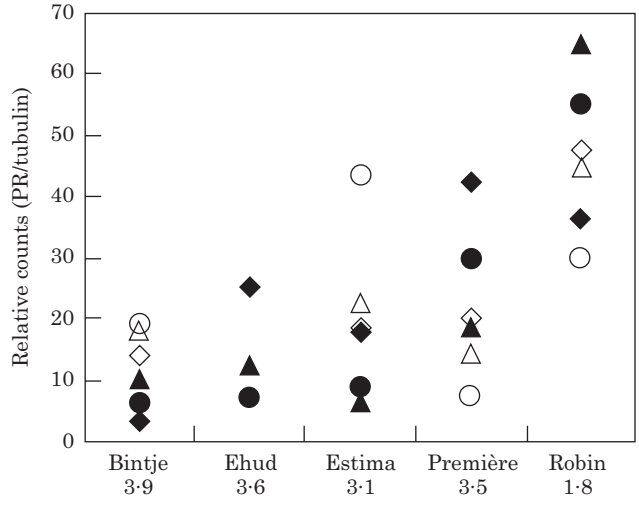

FIG. 3. Messenger RNA levels of *PR* genes and isolate-non-specific resistance in *S. tuberosum*. *PR-1* (●) *PR-2* (▲) and *PR-5* (◆) mRNA levels from uninoculated leaves from potato cultivars Bintje, Ehud, Estima, Première and Robijn were quantified on a phospho-imager, and the PR signals were normalized using the tubulin mRNA level as reference. Messenger RNA levels were determined in two independent experiments (first experiment closed, second open symbols); the data from Ehud in the second experiment were excluded. On the X-axis, average LGRs (mm day^{-1}, see Table 2) determined after inoculation with *P. infestans* isolates IPO-0, 90128, Mex580, BIN-16, ME93-2A are indicated for each cultivar (LGR = 0 excluded).

Concluding remarks

Basal SAR may function as an independent resistance mechanism, but more likely contributes to the complex network of defense reactions that take place following pathogen attack [*17*, *28*]. Resistance in the *Solanum*–*P. infestans* interaction often exhibits a quantitative nature, which can be explained by the extent of an ambiguous HR. Per infection event, a fine balance between invading hyphae and plant cells exhibiting an HR, determines whether infection will be aborted [*43*]. Basal level of SAR may increase the sensitivity of plant cells to HR elicitation [*32*], or may slow pathogen invasion by creating physiological conditions that limit pathogen growth.

The identification of molecular markers linked to partial resistance to *P. infestans* in *Solanum* species, is of great value for potato late blight resistance breeding. High levels of *PR* mRNAs could serve as useful molecular markers for screening breeding populations and germplasm. Assays based on quantitative reverse transcriptase (RT-PCR) of *PR* genes could be developed to assist potato breeders and geneticists in identifying promising genotypes and could supplement other assays based on quantitative trait loci (QTL) [*20*]. Using marker genes for known resistance mechanisms such as SAR may provide a novel prospective for marker-assisted breeding.

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