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Different Genetic Mechanisms Control Foliar and Tuber Resistance to *Phytophthora infestans* in Wild Potato *Solanum verrucosum*

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Abstract Late blight, caused by the oomycete pathogen, *Phytophthora infestans*, is a devastating disease of potatoes and tomatoes. A key long-term management strategy for combating this disease is to develop potato cultivars with broad-spectrum and durable resistance through identification and integration of major resistance genes. In support of previous results, we have determined that some accessions of the wild potato species *Solanum verrucosum* contain strong foliar resistance to *P. infestans* while others are susceptible. In addition, whole- and wounded-tuber inoculations revealed that most accessions lacked resistance to tuber infection. However, one accession (PI 570643) contained little foliar resistance but expressed tuber resistance, suggesting that expression of these two phenotypes in this species is not correlated.

Resumen El tizón tardío, causado por el patógeno oomycete *Phytophthora infestans*,es una enfermedad devastadora de papas y tomates. Una estrategia clave de manejo a largo plazo para combatir esta enfermedad es el desarrollo de cultivares de papa con un amplio espectro de resistencia durable, mediante la identificación e integración de genes mayores de resistencia. Como respaldo a resultados previos, hemos determinado que algunas introducciones de la especie silvestre de papa *Solanum verrucosum* contiene fuerte resistencia foliar a *P. infestans,* mientras que otras son susceptibles. Además, inoculaciones a tubérculos completos y heridos revelaron que la mayoría de las introducciones carecían de resistencia a la infección de tubérculo. No obstante, una introducción (PI 570643) contenía poca resistencia foliar pero expresó resistencia de tubérculo, sugiriendo que la expresión de estos dos fenotipos en esta especie no está correlacionada.

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Introduction

Late blight disease of potato and tomato has been one of the most serious of all plant diseases worldwide throughout history. The disease is caused by the oomycete pathogen *Phytophthora infestans*, which can cause significant crop losses due to early defoliation and tuber infection in potato. Most commercially grown potato cultivars are susceptible to *P. infestans*. Therefore, fungicides are used intensively to protect crops because outbreaks of late blight are difficult to control and can be remarkably destructive, affecting all parts of potato plants. The late blight pathogen spreads rapidly and within days can lead to total crop loss (Fry 2008).

Two types of resistance to foliar late blight have been described and used in potato breeding (Malcolmson and Black 1966; Simmonds and Wastie 1987). Vertical, or gene-for-gene resistance, is conferred by a series of dominant resistance (*R*) genes that render the host incompatible with specific races of the pathogen. Upon recognition, the host undergoes a predictable series of responses including activation of hypersensitive cell death surrounding the site of ingress, induction of pathogenesis related gene transcription, and initiation of a systemic resistance response (Vleeshouwers et al. 2000). Eleven *R* genes (*R1-R11*) with this resistance phenotype have been identified in the Mexican hexaploid potato species *Solanum demissum* and

were introduced into cultivated potato varieties used worldwide (Malcolmson and Black 1966; Wastie 1991; Umaerus and Umaerus 1994). However, all of these genes have already been overcome by specific *P. infestans* strains, although some still provide moderate resistance in certain regions of the world (Świeżyński et al. 2000). Foliar resistance sources have also been identified in other wild potato species, including *S. bulbocastanum*, *S. microdontum*, *S. berthaultii*, *P. pinnatisectum*, *S. stoloniferum*, *S. papita*, and *S. verrucosum* (Ewing et al. 2000; Naess et al. 2000; Sandbrink et al. 2000; Kuhl et al. 2001; Park et al. 2005a; van der Vossen et al. 2005; Liu and Halterman 2006; Vleeshouwers et al. 2008). The genetics of tuber resistance to late blight is less understood and relatively few sources of resistance have been identified (Collins et al. 1999; Oberhagemann et al. 1999; Park et al. 2005b; Simko et al. 2006).

Our previous study using a *P. infestans* isolate containing only avirulence (*avr*) genes 8 and 9 demonstrated that most of the eight *S. verrucosum* accessions used in these experiments exhibit a high degree of foliar resistance, with the exception of accession PI 570643 which showed moderate susceptibility (Liu and Halterman 2006). Here, we have analyzed tubers of these accessions to assay for the presence or absence of tuber resistance in order to determine whether genetic mechanisms controlling tuber and foliar resistance are correlated in *S. verrucosum*. In addition, we have tested for foliar resistance to a *P. infestans* isolate containing *avr* genes 3, 4, 6, 7, 8, 9, 10, and 11 to better characterize the resistance capabilities of this species.

Materials and Methods

Foliar Late Blight Disease Resistance Screening

The Mexican *P. infestans* isolate MX980085 was provided by W. E. Fry (Cornell University) and was grown on Rye A medium in the dark at 15°C for 14 days (Caten and Jinks 1968). This isolate contains avirulence genes 3, 4, 6, 7, 8, 9, 10, and 11 (Becktell et al. 2006). Sporangia were harvested by washing the plates with sterile distilled water. Washes of multiple plates were combined to obtain a sporangial suspension of approximately 75,000 sporangia/ml. Sporangial suspensions were placed at 12°C for 1.5–3 h to induce zoospore release.

Seeds for *S. verrucosum* accessions (PI numbers 161173, 275256, 275258, 275260, 310966, 365404, 558485, and 570643) were obtained from the National Research Support Program (NRSP)-6 potato GeneBank in Sturgeon Bay, Wisconsin. Seedlings were grown under greenhouse conditions (23°C day/15°C night temperatures with 14 h of light) and watered as needed.

Whole-plant disease resistance assays were replicated five times on different dates. Three plants of each accession and controls were inoculated and scored within each replication. One to 3 h before inoculation, 8-week old seedlings were placed in a closed greenhouse with a misting system that maintains greater than 90% relative humidity. Greenhouse temperatures were set at 23°C during the day and 15°C at night with 14 h of light. Plants were inoculated in the early evening near the time the lights were turned off and the temperature began to drop to night-time levels. Each plant was sprayed with ~3 ml of a suspension containing both sporangia and zoospores of *P. infestans*, on both the underside and topside of the leaves.

Plants were scored 10 days after inoculation. The late blight resistance score was determined by visual inspection of the plants using the scale given by Colton et al. (2006) as follows: 0 = 100% infected tissue; 1 = >90%; 2 = 81–90%; 3 = 71–80%; 4 = 61–70%; 5 = 41–60%; 6 = 26–40%; 7 = 11–25%; 8 = <10%. Late blight resistance score means were separated using an analysis of variance (ANOVA).

Tuber Resistance Screening

Wounding method: Ten tubers, harvested from greenhouse-grown plants, were washed with 0.6% sodium hypochlorite, rinsed with distilled water three times, and allowed to dry. Each tuber was punctured one to three times (at least 2 cm apart) depending on the size of the tuber. The punctures were 2 mm wide by 6 mm deep and arrayed on one half of the tuber. Tubers were inoculated with 20 µl of a zoospore/sporangial suspension containing 44,000 sporangia/ml of *P. infestans* isolate MX 980085 at each puncture site. Inoculated tubers were placed within incubation chambers lined with wet paper towels and placed inside heavy duty plastic bags to maintain nearly 100% humidity. These chambers remained at room temperature (~22°C) for 70 h and were then transferred to a dark, 15°C incubator for an additional 10 days. Tubers were sliced at the inoculation sites and the depth of the lesions extending below the inoculation site was measured. Each inoculation was considered a separate interaction and individual lesion measurements on each tuber were analyzed individually.

Non-wounding method: Four tubers from each *S. verrucosum* accession and *S. tuberosum* cv. 'Katahdin' were harvested from greenhouse-grown plants, washed with 0.6% sodium hypochlorite, rinsed with distilled water, and allowed to dry. Tubers were sprayed with a zoospore/sporangial suspension containing 75,000 sporangia/ml in

order to completely cover the tuber. Incubation chambers containing inoculated tubers were lined with wet paper towels and placed inside heavy duty plastic bags to maintain nearly 100% humidity. Chambers were incubated at 12°C for 34 days before scoring.

Late blight symptoms were assessed by shallow skinning of the entire tuber using a knife. The percentage of tubers exhibiting late blight symptoms were recorded. Data from the four replicated individuals were averaged.

Results

Most accessions of *S. verrucosum* displayed high levels of foliar resistance when compared to the susceptible *S. tuberosum* cv. 'Katahdin' control (Table 1). *S. verrucosum* PIs 161173, 275256, 275260, 365404, and 558485 consistently exhibited the strongest resistance to *P. infestans* and were significantly ($P<0.05$) more resistant than 'Katahdin' and other *S. verrucosum* accessions. The resistance scores of PIs 275258, and 310966 were not significantly different than that of the susceptible control cv. 'Katahdin'. PI 570643 was significantly ($P<0.05$) more susceptible than cv. 'Katahdin' and all other *S. verrucosum* accessions. As expected, transgenic cv. 'Katahdin' leaves with *RB* were resistant to *P. infestans*, although some disease symptoms were observed, which is consistent with the partial resistance phenotype observed previously (Song et al. 2003; van der Vossen et al. 2003; Halterman et al. 2008).

Inoculation of *P. infestans* into wounded tubers resulted in the development of late blight symptoms in all accessions tested. Only tubers of PIs 161173, 275258, and 570643 exhibited less disease severity than cv. 'Katahdin', with lesion depths (in millimeters) of 2.2±0.8, 3.7±1.0, and 0.9±0.5, respectively, compared to 4.5±0.3 for cv. 'Katahdin' (Table 1). Tubers of other accessions contained deeper disease lesions at the inoculation sites. PIs 310966, 365404, and 558485 contained the deepest lesions, with depths of 9.4±1.3, 12.3±2.3, and 11.7±2.3, respectively. Disease severity in tubers from these three accessions was significantly different from the three most resistant *S. verrucosum* accessions ($P<0.05$). Consistent with previous research with cv. 'Katahdin' tubers containing *RB* (Halterman et al. 2008), transgenic tubers were moderately susceptible with an average lesion depth of 4.2±0.5.

Overall, non-wounded tubers showed very little disease severity (Table 1). Only tubers of PIs 161173 and 558485 had an incidence of disease greater than 10%. Tubers from other PIs and cv. 'Katahdin' exhibited only minor disease symptoms under the periderm.

Conclusions

Previously, we assayed eight accessions of *S. verrucosum* for foliar resistance to late blight using a *P. infestans* isolate containing avirulence genes 8 and 9 and found a considerable amount of variation in the resistance phenotypes (Liu

Table 1 Results of greenhouse inoculations with *P. infestans* isolate MX 980085 containing *Avr* genes 3, 4, 6, 7, 8, 9, 10, and 11

Accession	Foliar late blight score[a]	Tuber late blight symptoms	
		Wounded tuber severity[b]	Non-wounded tuber incidence[c]
PI 161173	7.9±0.1 A	2.2±0.8 A	25%
PI 275260	7.8±0.1 A	5.9±0.9 AB	<10%
'Katahdin' + *RB*	7.7±0.2 A	4.2±0.5 AB	<10%
PI 365404	7.6±0.2 A	12.3±2.3 B	<10%
PI 558485	7.4±0.3 A	11.7±2.3 B	25%
PI 275256	7.2±0.3 A	5.3±1.5 AB	<10%
'Katahdin'	5.0±0.3 B	4.5±0.3 AB	<10%
PI 275258	4.8±0.9 B	3.7±1.0 A	<10%
PI 310966	4.0±0.8 B	9.4±1.3 B	<10%
PI 570643	1.3±0.2 C	0.9±0.5 A	<10%

[a] Late blight resistance score calculated based on the percentage of diseased leaf tissue: 0 = 100% diseased tissue, 8 = <10% diseased tissue. Scores are the means of five replications, each containing three plants. Accessions are ordered from highest (most resistant) to lowest (most susceptible) foliar late blight score. Late blight scores followed by different letters indicate that they are significantly different ($P<0.05$)

[b] Severity of late blight based on wounded tubers (depth of lesions). The mean depth of lesions below the wounding site (± std. error) was calculated for at least 10 inoculation sites. Severity values followed by different letters indicate that they are significantly different ($P<0.05$)

[c] Incidence of late blight on non-wounded tubers. Inoculated tubers were skinned and the percentage of tubers with late blight symptoms was recorded

and Halterman 2006). Foliar resistance in *S. verrucosum* is, in part, conferred by the presence of a functional homolog of the *RB* resistance gene (Liu and Halterman 2006). In *S. bulbocastanum*-derived and *RB*-transgenic materials, the *RB* gene confers broad-spectrum foliar resistance to multiple *P. infestans* isolates. Here, using a *P. infestans* isolate containing a different set of *avr* genes, we have found that the *S. verrucosum* accessions maintained their resistance phenotype. While we know that foliar resistance in this species can be conferred by a functional homolog of the *RB* resistance gene, we cannot rule out the possibility of functional *R8* or *R9* genes in *S. verrucosum* that would confer resistance to isolates containing the corresponding *avr8* and *avr9* genes.

Overall, very few disease symptoms were observed in non-wounded tubers in our experiments. Since it is difficult to draw conclusions from this data, we place greater emphasis on results from our wounded tuber experiments. However, for resistance screening during long-term storage conditions, non-wounded tuber assays may also be worthwhile, since wounding circumvents some basal pathogen protection. Wounded tubers of *S. verrucosum* accessions PI 161173, PI 275258, and PI 570643 all expressed high levels of resistance to *P. infestans*. Interestingly, tubers of PI 570643, which exhibited the least foliar resistance to *P. infestans*, were quite resistant suggesting different mechanisms involved in foliar and tuber resistance to late blight in *S. verrucosum*. While notable, this is not surprising since it has been suggested that any correlation between foliar and tuber resistance is dependent on the gene(s) involved (Park et al. 2005b). Some genetic evidence indicates that there may be little or no correlation between foliar and tuber resistance (Stewart et al. 1992; Kirk et al. 2001; Simko et al. 2006), while other results indicate some resistance genes have the ability to confer both foliar and tuber resistance (Stewart et al. 1994; Platt and Tai 1998; Park et al. 2005b). Although some accessions exhibited moderate levels of both foliar and tuber resistance, our results using *S. verrucosum* support a model where foliar and tuber resistance mechanisms are controlled by different genetic mechanisms. Therefore, when using *S. verrucosum* as a source for resistance to late blight in breeding programs, multiple accessions should be included in order to incorporate both foliar and tuber resistance phenotypes.

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References

Becktell, M.C., C.D. Smart, C.H. Haney, and W.E. Fry. 2006. Host-pathogen interactions between *Phytophthora infestans* and the Solanaceous hosts *Calibrachoa* × *hybridus*, *Petunia* × *hybrida*, and *Nicotiana benthamiana*. *Plant Disease* 90: 24–32.

Caten, C.E. and J.L. Jinks. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. *Canadian Journal of Botany* 46: 329–347.

Collins, A., D. Milbourne, L. Ramsay, R. Meyer, C. Chatot-Balandras, P. Oberhagemann, W. De Jong, C. Gebhardt, E. Bonnel, and R. Waugh. 1999. QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. *Molecular Breeding* 5: 387–398.

Colton, L.M., H.I. Groza, S.M. Wielgus, and J. Jiang. 2006. Marker-assisted selection for the broad-spectrum potato late blight resistance conferred by gene *RB* derived from a wild potato species. *Crop Science* 46: 589–594.

Ewing, E.E., I. Simko, C.D. Smart, M.W. Bonierbale, E.S.G. Mizubuti, G.D. May, and W.E. Fry. 2000. Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. *Molecular Breeding* 6: 25–36.

Fry, W.E. 2008. *Phytophthora infestans*: the plant (and *R* gene) destroyer. *Molecular Plant Pathology* 9: 385–402.

Halterman, D., L.C. Kramer, S. Weilgus and J. Jiang. 2008. Performance of transgenic potato containing the late blight resistance gene *RB*. *Plant Disease*.

Kirk, W.W., K.J. Felcher, D.S. Douches, B.A. Niemira, and R. Hammerschmidt. 2001. Susceptibility of potato (*Solanum tuberosum* L.) foliage and tubers to the US8 genotype of *Phytophthora infestans*. *American Journal of Potato Research* 78: 319–322.

Kuhl, J.C., R.E. Hanneman Jr., and M.J. Havey. 2001. Characterization and mapping of Rpi1, a late-blight resistance locus from diploid (1EBN) Mexican Solanum pinnatisectum. *Molecular Genetics and Genomics* 265: 977–985.

Liu, Z. and D. Halterman. 2006. Identification and characterization of *RB* orthologous genes from the late blight resistant wild potato species *Solanum verrucosum*. *Physiological and Molecular Plant Pathology* 69: 230–239.

Malcolmson, J.F. and W. Black. 1966. New *R* genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* 15: 199–203.

Naess, S.K., J.M. Bradeen, S.M. Wielgus, G.T. Haberlach, J.M. McGrath, and J.P. Helgeson. 2000. Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. *Theoretical and Applied Genetics* 101: 697–704.

Oberhagemann, P., C. Chatot-Balandras, R. Schäfer-Pregl, D. Wegener, C. Palomino, F. Salamini, E. Bonnel, and C. Gebhardt. 1999. A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. *Molecular Breeding* 5: 399–415.

Park, T.-H., J. Gros, A. Sikkema, V.G.A.A. Vleeshouwers, M. Muskens, S. Allefs, E. Jacobsen, R.G.F. Visser, and E. van der Vossen. 2005a. The late blight resistance locus *Rpi-blb3* from *Solanum bulbocastanum* belongs to a major late blight *R* gene cluster on chromosome 4 of potato. *Molecular Plant-Microbe Interactions* 7: 722–729.

Park, T.-H., V.G.A.A. Vleeshouwers, J.-B. Kim, R.C.B. Hutten, and R.G.F. Visser. 2005b. Dissection of foliage and tuber late blight resistance in mapping populations of potato. *Euphytica* 143: 75–83.

Platt, H.W. and G.H. Tai. 1998. Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. *American Journal of Potato Research* 75: 173–178.

Sandbrink, J.M., L.T. Colon, P.J.C.C. Wolters, and W.J. Stiekema. 2000. Two related genotypes of *Solanum microdontum* carry different segregating alleles for field resistance to *Phytophthora infestans*. *Molecular Breeding* 6: 215–225.

Simko, I., S. Costanzo, V. Ramanjulu, B.J. Christ, and K.G. Haynes. 2006. Mapping polygenes for tuber resistance to late blight in a diploid *Solanum phureja* X *S. stenotomum* hybrid population. *Plant Breeding* 125: 385–389.

Simmonds, N.W. and R.L. Wastie. 1987. Assessment of horizontal resistance to late blight of potatoes. *Annals of Applied Biology* 111: 213–221.

Song, J.Q., J.M. Bradeen, S.K. Naess, J.A. Raasch, S.M. Wielgus, G. T. Haberlach, J. Liu, H.H. Kuang, S. Austin-Phillips, C.R. Buell, J.P. Helgeson, and J.M. Jiang. 2003. Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences of the United States of America* 100: 9128–9133.

Stewart, H.E., R.L. Wastie, J.E. Bradshaw, and J. Brown. 1992. Inheritance of resistance to late blight in foliage and tubers of progenies from parents differing in resistance. *Potato Research* 35: 313–319.

Stewart, H.E., J.E. Bradshaw, and R.L. Wastie. 1994. Correlation between resistance to late blight in foliage and tubers in potato clones from parents of contrasting resistance. *Potato Research* 37: 429–434.

Świeżyński, K.M., L. Domański, H. Zarzycka, and E. Zimnoch-Guzowska. 2000. The reaction of potato differentials to *Phytophthora infestans* isolates collected in nature. *Plant Breeding* 119: 119–126.

Umaerus, V. and M. Umaerus. 1994. Inheritance of resistance to late blight. In *Potato genetics*, ed. J.E. Bradshaw and G.R. Mackay, 365–401. Wallingford: CAB International.

van der Vossen, E., A. Sikkema, B.T.L. Hekkert, J. Gros, P. Stevens, M. Muskens, D. Wouters, A. Pereira, W. Stiekema, and S. Allefs. 2003. An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. *Plant Journal* 36: 867–882.

van der Vossen, E.A.G., J. Gros, A. Sikkema, M. Muskens, D. Wouters, P. Wolters, A. Pereira, and S. Allefs. 2005. The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. *Plant Journal* 44: 208–222.

Vleeshouwers, V.G., W. van Dooijeweert, F. Govers, S. Kamoun, and L.T. Colon. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* 210: 853–864.

Vleeshouwers, V.G.A.A., H. Rietman, P. Krenek, N. Champouret, C. Young, S.-K. Oh, M. Wang, K. Bouwmeester, B. Vosman, R.G.F. Visser, E. Jacobsen, F. Govers, S. Kamoun, and E.A.G. Van der Vossen. 2008. Effector Genomics Accelerates Discovery and Functional Profiling of Potato Disease Resistance and *Phytophthora Infestans* Avirulence Genes. *PLoS ONE* 3: e2875.

Wastie, R.L. 1991. Breeding for resistance. In *Phytophthora infestans, The cause of late blight in Potato, Advances in plant pathology*, ed. D.S. Ingram and P.H. Williams, 193–224. London: Academic.