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Short Communication

Differential gene expression in two potato lines differing in their resistance to *Phytophthora infestans*

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Summary

Horizontal resistance to late blight in the potato is a primary objective of many breeding programs. Knowledge of the physiological and biochemical mechanisms underlying it, however, is scarce. The purpose of the present study was the identification of these physiological and biochemical factors in plant material obtained by crossing a late blight resistant *Solanum phureja* clone with a susceptible dihaploid of *S. tuberosum* subsp. *tuberosum*. The mRNA RT-PCR differential display method was used to compare the gene expression patterns of a resistant hybrid with that of a susceptible one. By sequence homology, we identified several genes with diverse functions, including genes known to be involved in resistance or stress responses and genes known to be involved in primary or secondary metabolism.

Key words: Differential display – gene induction – horizontal resistance – late blight – potato – *Solanum phureja*

Late blight of potato, caused by the oomycete *Phytophthora infestans*, is one of the most serious diseases of the potato, occurring almost everywhere potatoes are grown. The selection for horizontal (polygenic) resistance to *P. infestans* is a challenging task, since it is difficult to predict which genes will provide durable resistance. Indeed, criteria suitable for the identification of such genes are not easy to define (Świeżyński et al. 2000). Late blight resistant potato species originating from South America represent possible sources of new germplasm for resistance breeding. Species like *S. andigena* and *S. phureja* have been described as partially resistant by many authors (Guzmán 1964, Nilsson 1981, De Maine et al. 1993, Cañizares and Forbes 1995).

In the present study we used hybrid plants from a population (hereafter referred to as PD) resulting from a cross between a late blight resistant clone of *S. phureja* (P) and a susceptible dihaploid (D) clone of *S. tuberosum* subsp. *tuberosum*. In this population, resistance to late blight segregates as a quantitative trait. The comparison of genes expressed in

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Nucleotide sequence information for clones dd1 to dd28 presented in table 1 are to be found at the EMBL data base as accession numbers AJ437585 to AJ437600.

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Table 1. Potato cDNA clones showing difference in expression between a resistant and a susceptible hybrid after inoculation with *P. infestans*. * Indicates genes expressed in the susceptible hybrid and not in the resistant one. For sequences not presented in the table, no significant homologies were found. Expect values of best match given in parentheses.

Clone name	Clone length (bp)	Matching sequence from data base	Origin of matching sequence and accession number	Nucleotide sequence % match*	Amino acid sequence % Identity*
dd1	325	EST (flower)	*L. esculentum* AW 737528	96 % (1 e^{-127})	
		Alpha-galactosidase	*L. esculentum* AF 191823		29 % (0.002)
dd3	252*	EST (flower)	*L. esculentum* BG 629467	97 % (1 e^{-97})	
dd5	208	EST (shoot meristem)	*L. esculentum* BG 128340	88 % (7 e^{-59})	
dd6	289	Resistance gene cluster	*S. tuberosum* AF 265664	92 % (2 e^{-12})	
dd9	349	Ubiquitin	*S. tuberosum* Z 11673	98 % (e^{-150})	
dd10	576	Glyceraldehyde–3-phosphate dehydrogenase gene	*Aspergillus nidulans* Z 32524	97 % (0)	
		Methionine sulfoxide reductase	*Fragaria* × *ananassa* AJ 297967	96 % (6 e^{-69})	
		Cutinase	*P. capsici* X 74324	87 % (9 e^{-25})	
dd11	154	mRNA for cold-inducible proteins	*Symplocarpus renifolius* AB 024733		60 % (0.006)
dd13	303*	Pseudouridine synthase	*A. thaliana* AF 234984	83 % (1 e^{-28})	
dd16	227	EST (shoot meristem)	*L. esculentum* BG 123487	92 % (1 e^{-36})	
dd17	480	Senescence-associated protein	*Pisum sativum* AB 049719	96 % (0)	
		Superoxide dismutase	*Zea mays* U 34727	97 % (1 e^{-57})	
dd18	271	EST	*S. tuberosum* BG 598298	95 % (6 e^{-91})	
dd20	571*	Transposable element	*S. tuberosum* X 52387		48 % (1 e^{-20})
		Ribulose–1,5-biphosphate carboxylase	Pea X 00806	89 % (0.067)	
dd21	593	RNA polymerase	*A. thaliana* AB 021119	90 % (4 e^{-33})	
dd25	250*	mRNA for plastid protein	*A. thaliana* Z 86094		66 % (0.006)
dd26	368	Cold-induced gene	*Medicago sativa* L 12462	96 % (1 e^{-4})	
dd27	734	Chlorophyll *a/b* binding protein	*L. esculentum* X 57706	93 % (4 e^{-69})	

a susceptible hybrid upon infection by *P. infestans*, with those expressed in a resistant hybrid from the PD population, could lead to the identification of genes involved in the establishment of the resistance of potato to late blight.

Field and greenhouse resistance evaluations (Ghislain et al. 2001), as well as detached leaflet assays, were performed to assess the resistance of the hybrids. For further analyses we selected a susceptible hybrid (PD60) and a resistant one (PD88).

Plants were inoculated by spraying with a suspension of *P. infestans* sporangia (12,000/mL). Mock inoculations were performed by spraying control plants with water. Sampling was performed 24 h after inoculation in order to point at early-induced differences in gene expression (Birch et al. 1999). The pathogen isolate used was of Peruvian origin. It possessed virulence genes 1,2,3,4,5,6,7,9,10,11 and was of the A1 mating type.

RNA extraction was performed using a Qiagen RNA Plant Extraction kit (Westburg, Belgium). Differential display was performed on the DNase-treated RNA samples using the differential display kit from TaKaRa (BioWhittaker, Belgium). PCR products were loaded on horizontal polyacrylamide gels (ETC-Electrophorese Technik, Germany) and non-radioactive detection was performed by staining the gels with silver nitrate according to the manufacturers instructions. Each PCR reaction was repeated twice on two different RNA samples in order to reduce the number of false-positives.

Sequencing was performed through a commercial company (Eurogentec, Belgium). Homology search was done by using the TBLASTx network services at the EMBL Nucleotide Sequence Databases.

Twenty-eight differentially expressed bands, presumably genes, were identified (Table 1). These genes were either expressed in the resistant hybrid (Fig. 1 A), and not in the susceptible one, or vice versa (Fig. 1 B). However, most of the differentially expressed genes (except 5) were expressed in the resistant hybrid.

In order to verify that the differentially expressed genes were originating from the host and not from the pathogen *P. infestans*, dot blot hybridizations were carried out on total DNA from both plants and pathogen. Most clones were confirmed to be of plant origin.

To confirm the differential expression of these genes, a reverse dot blot hybridization procedure was performed. The

Figure 1. Partial view of a differential display gel. The RT-PCR was performed with primers 5′ T_n CG and 5′ TCGATACAGG. Lanes 1 and 2: PD60 (susceptible hybrid). Lanes 3 and 4: PD88 (resistant hybrid). The arrows show a band corresponding to a transcript accumulating only in the resistant hybrid PD88 (A) or only in the susceptible hybrid (B).

reverse transcribed products from the resistant and the susceptible plant RNAs and from the *P. infestans* RNA were fixed onto a membrane and hybridized with the cloned cDNAs (resulting from the fragments cut out from the gels) (Fig. 2). Differential expression was confirmed for seven bands. No difference in the hybridisation signal could be shown for two bands, dd12 (Fig. 2B) and dd29. However, it is possible that the differential expression was masked by the constitutive expression of some members of the same gene family. In the remaining cases, no signal could be observed on the dot blots. This absence of hybridization signal could be due to either false positives during the differential display PCRs, or to a lack of sensitivity of the hybridization procedure. However, as PCR reactions have been repeated 4 times (2 times on 2 different RNA samples) with consistent results, the latter explanation seems more plausible.

Among the seven bands for which differential expression was confirmed, dd1, dd2, dd4, dd5, dd6 and dd7 were expressed only in the resistant hybrid (Fig. 2A).

Homology between cloned cDNA sequences and sequences in databases are presented in Table 1. BLAST scores can be divided into three cases according to Nelson et al. (1997): highly significant ($P<10^{-19}$), moderately significant ($P = 10^{-5}$ to 10^{-19}) and weakly significant ($P = 10^{-2}$ to 10^{-4}). In many cases (dd1, dd3, dd5, dd16 and dd18), important matches were found to expressed sequence tags (ESTs) originating from *Lycopersicon esculentum* or *S. tuberosum*. Interestingly, the cDNA dd1 matched to an alpha-galactosidase from tomato. Alpha-galactosidases (EC 3.2.1.22) are exoglycosidases capable of cleaving alpha-linked galactose residues from glycoconjugates (Ly et al. 2000). To our knowledge, this enzyme has never been described in potato in the context of a plant-pathogen interaction. The band dd6 matched to a resistance gene cluster in potato. Such a resistance gene cluster has also been observed by Van der Vossen et al. (2000) who described a potato hypersensitive resistance gene against potato virus X that maps to a resistance gene cluster on chromosome V. The band dd9 matched with a highly significant score to a potato ubiquitin gene shown to be induced by different stresses (Garbarino et al. 1992). The band dd10 is a gene for which a positive signal on the DNA dot blot hybridization was found for the plants as well as for *P. infestans*, and hence was suspiciously from the pathogen. The homology search confirmed this suspicion with the best match being with a glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) from a fungus. The band dd11 and dd26 showed homologies with genes whose products were reported to be associated with cold-stress (Monroy et al. 1993). In the case of dd13, a highly significant match was observed with a putative pseudouridine synthase, an enzyme catalyzing the isomerization of uridine to pseudouridine. The band dd17 perfectly matched to a *Pisum sativum* senescence-associated protein (Pariasca et al. 2001), and also to a superoxide dismutase from *Zea mays*, a gene related to resistance and playing a role in the defense reaction against toxic oxygen radicals (Ruiz-Lozano et al. 1996). The band dd20 is one of the few differentially expressed genes only expressed in the susceptible hybrid. A high similarity at the protein level was observed with sequences of transposable elements. At the DNA level, a match was found to ribulose-1,5-biphosphate carboxylase, a gene whose expression level is affected by oxidative damage. The band dd21 showed a highly significant match to a RNA polymerase from *Arabidopsis thaliana*. For band dd25, the deduced amino acid sequence showed a 66 % homology to an *A. thaliana* mRNA for a plastid protein. Finally, dd27 matched in a highly significant score to a light harvesting chlorophyll *a/b* binding protein.

In the present study, we used the mRNA RT-PCR differential display technique to identify genes that are differentially expressed between a late blight resistant and a late blight susceptible hybrid. Some genes (dd9, dd17 and dd20), previously shown to be expressed in the race-specific resistance of potato to late blight by Birch et al. (1999) were identified indicating that there could be several pathways in common between vertical and horizontal resistance. This has already been shown by Coffey and Gees (1991) and Kamoun et al. (1999), among others. Some genes are known to be induced

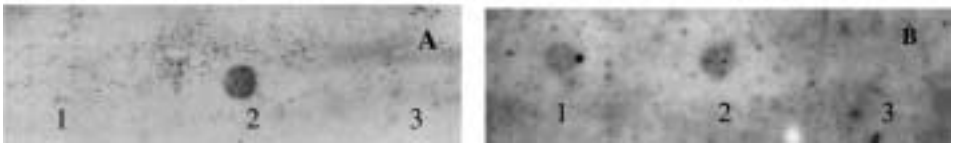

Figure 2. cDNA dot-blot, hybridized with the fluorescently labeled probe dd1 (A) and dd12 (B). (1) cDNA from PD60; (2) cDNA from PD88; (3) cDNA from *P. infestans*. Hybridization was carried out using DNA probes labeled with the AlkPhos Direct labeling system from Amersham Pharmacia Biotech.

by other types of stresses which could point to additional common mechanisms of defense to various types of stresses. This concept has been well described by Leshem and Kuiper (1996) as the so-called ‹general adaptation syndrome›. Susceptible plants could be less able to adapt to the stress conditions induced by the late blight infection, compared to the resistant plants. Thus, the partial resistance exhibited by some plants could be due to a better capacity to perceive the first signals of pathogen infection or to react to it.

In conclusion, different cDNAs with enhanced or decreased expression were identified. The isolation of the corresponding full-length cDNA, their time-course expression, their mapping on the potato genetic map and their expression upon other stresses will allow corroboration of these results. Moreover, time-course expression studies will allow one to see whether similar responses take place in susceptible plants, but at a later time.

References

Birch PRJ, Avrova AO, Duncan JM, Lyon GD, Toth RL (1999) Mol Plant Micr Interact 12: 356–361

Cañizares CA, Forbes GA (1995) Potato Res 38: 3–10

Coffey MD, Gees R (1991) In: Ingram DS, Williams PH (eds) Advances in Plant Pathology. *Phytophthora infestans*, The Cause of Late Blight in Potato. University Press, Cambridge pp 31–51

De Maine MJ, Carrol CP, Stewart HE, Solomon RM, Wastie RL (1993) Potato Res 36: 21–28

Garbarino JE, Rockhold DR, Belknap WR (1992) Plant Mol Biol 20: 235–244

Ghislain M, Trognitz B, Del R Herrera M, Solis J, Casallo G, Vásquez C, Hurtado O, Castillo R, Portal L, Orrillo M (2001) Theoret Appl Gen 103: 462–468

Guzmán J (1964) Phytopathol 9: 291–294

Kamoun S, Huitema E, Vleeshouwers VGAA (1999) Trends Plant Sci 4: 196–200

Leshem YY, Kuiper PJC (1996) Biol Plant 38: 1–18

Ly HD, Howard S, Shum K, He SM, Zhu A, Withers SG (2000) Carbohydr Res 329: 539–547

Monroy AF, Castonguay Y, Laberge S, Srhan F, Vezina LP, Dhindsa RS (1993) Plant Physiol 102: 873–879

Nelson MA, Kang S, Braun EL, Crawford ME, Dolan PL, Leonhard PM, Mitchell J, Armijo AM, Bean L, Blueyes E, Cushing T, Errett A, Fleharty M, Gorman M, Judson K, Miller R, Ortega J, Pavlova I, Perea J, Todisco S, Trujillo R, Valentine J, Wells A, Werner-Washburne M, Yazzie S, Natvig D (1997) Fung Gen Biol 21: 348–363

Nilsson BA (1981) Potato Res 24: 239–244

Pariasca JAT, Sunaga A, Miyazaki T, Hisaka H, Sonoda M, Nakagawa H, Sato T (2001) Postharv Biol Technol 22: 239–247

Ruiz-Lozano JM, Azcon R, Palma JM (1996) New Phytol 134: 327–333

Świeżyński KM, Domański L, Zarzycka H, Zimnoch-Guzowska E (2000) Plant Breed 119: 119–126

Van der Vossen EAG, Rouppe van der Voort JNAM, Kanyuka K, Bendahmane A, Sandbrink H, Baulcombe DC, Bakker J, Stiekema WJ, Klein-Lankhorst RM (2000) Plant J 23: 567–576