European Journal of Plant Pathology **110:** 435–439, 2004.

Short communication

Erwinia carotovora infection enhances the expression of two novel abiotic stress-inducible genes in potato

Anita Sós-Hegedüs, Donatas Žvingila*, Zsófia Bánfalvi and Géza Dallmann**
*Agricultural Biotechnology Center, H-2101 Gödöllő, P.O. Box 411, Hungary; *Present address: Department of Botany and Genetics, Vilnius University, M.K. Čiurlionio Street 21, LT-2009, Vilnius, Lithuania; **Author for correspondence (Phone: 36 28 526100; Fax: 36 28 526165; E-mail: dallmann@abc.hu)*

Accepted 7 November 2003

Key words: abscisic acid, dehydration, differential display, pathogenesis-related genes, wounding

Abstract

In this study, cDNAs of two *Erwinia carotovora*-induced potato genes, designated *Solanum tuberosum–Erwinia-induced-1* and *2* (*Stei1* and *Stei2*) were isolated by differential display technique. *Stei1* and *Stei2* were detected in low copy number in the potato genome and found to encode putative proteins with no significant homology to any genes with known function. Treatment of the leaves with salicylic acid, methyl jasmonate and ethylene elevated neither *Stei1* nor *Stei2* mRNA levels. However, *Stei1* and *Stei2* expression were induced not only by *E. carotovora* but also by infiltration of water in leaves, albeit to a lesser extent. In addition, *Stei2* was up-regulated by NaCl, wounding, dehydration and abscisic acid. Thus *Stei1* and *Stei2* define novel genes belonging to the family of those pathogenesis-related genes whose expression can be induced both by biotic and abiotic stresses.

Erwinia carotovora is a gram-negative soil bacterium. It causes potato soft rot and infects a wide variety of crops (Pérombelon and Kelman, 1980). Genetically defined resistance to *E. carotovora* has not been described and the pathogen does not contain typical avirulence genes. The main virulence factors of *E. carotovora* are plant cell-wall-degrading enzymes including pectinases and cellulases. These enzymes function not only as pathogen virulence factors (Collmer and Keen, 1986; Kotoujansky, 1987) but they also trigger plant defence responses probably by releasing cell wall fragments (Norman et al., 1999; Palva et al., 1993; Vidal et al., 1998; Weber et al., 1996).

In the case of the *E. carotovora–Arabidopsis* interaction, the plant possesses two types of defence pathway against *Erwinia*. One of them is jasmonic acid (JA) dependent and is inhibited by salicylic acid (SA) and ethylene. In the other one, ethylene and JA act synergistically and SA enhances the process (Norman-Setterblad et al., 2000). In tobacco, SA is not the signal molecule leading to the early response to *Erwinia*, but an antagonist of the induction of pathogenesis-related (PR) genes elicited by *Erwinia* (Vidal et al., 1997).

Up-regulation of genes encoding enzymes involved in protection against environmental stresses, such as phenylalanine–ammonia–lyase (PAL), an isogene of the 3-hydroxy-methyl-3-methylglutaryl coenzyme A reductase (HMGR) family and a catalase gene (*Cat2St*), have been detected in potato (Niebel et al., 1995; Rumeau et al., 1990; Yang et al., 1991). Genes induced early upon infection with *E. carotovora* have been isolated recently by suppression subtractive hybridisation. These genes include a *WRKY*-like transcription factor (Dellagi et al., 2000a) and *erg-1*, a gene with strong similarity to *phi-1* induced in phosphate-starved tobacco cells (Dellagi et al., 2000b). Potato genes responsive to cell-wall-degrading enzymes after 1 h of treatment with *E. carotovora* culture filtrate or short oligogalacturonides have also been isolated and identified as putative serine–threonine protein kinases

(*PRK 1–4*) constituting a family of related receptors (Montesano et al., 2001).

Here we report the isolation of two novel potato genes whose expression is also characteristic for the early stages of *E. carotovora* infection.

For each experiment, potato plants were grown in pots in a greenhouse. *Erwinia carotovora* SCC3193 strain (Pirhonen et al., 1988) was cultured overnight at 28 °C in LB medium. Bacteria were harvested by centrifugation (15 min, 4000*g*). Following the method of Brader et al. (2001) and Montesano et al. (2001), the cells were re-suspended in 0.9% NaCl at 1.5×10^8 CFU/ml (10× dilution) and used for leaf infiltration. Differences between *Erwinia*-treated and untreated leaf mRNA populations isolated at 2 and 4 h after infection were detected by differential display (Liang and Pardee, 1992) using the Differential Display Kit of Display Systems Biotech (Vista, CA, USA). cDNAs of 69 genes showing different expression at both time points were isolated and 43 of them were cloned into the pBluescript SK vector (Stratagene). The clones were sequenced and tested by northern hybridisation. This experiment resulted in detection of six cDNA fragments whose expressions were induced by *Erwinia* treatment. Full-length cDNAs of two of them, designated *Solanum tuberosum–Erwinia-induced-1* and *2* (*Stei1* and *Stei2*), were isolated from a cDNA library established from *Erwinia*-treated leaves. Based on DNA sequence analysis *Stei1* consists of 1266 bps with an open reading frame extending from nucleotide 61 to 1125 that encodes a polypeptide of 354 amino acids (accession no.: AY187625). *Stei2* is 735 bps, its open reading frame starts at nucleotide 59 and extends to nucleotide 453 coding for a putative polypeptide of 131 amino acids (accession no.: AY187626). *Stei1* and *Stei2* show 47% and 76% identities with *Arabidopsis* proteins of accession numbers NP565406 and NP197221, respectively. The function of the proteins is unknown.

Southern blot analysis was used to identify the copy number of *Stei1* and *Stei2* genes in *Solanum tuberosum* cv. Désirée. Genomic DNAs were digested with *Eco*RI, *Hind*III and *Xba*I, and hybridised with the full-length *Stei1* and *Stei2* cDNAs. Two to three hybridising bands were detected in all cases. These findings suggest that the copy number of *Stei1* and *Stei2* is low in *S. tuberosum* cv. Désirée (Figure 1).

Expression of *Stei1* and *Stei2* was analysed by northern hybridisation in potato leaves infiltrated with *E. carotovora* in 0.9% NaCl solution, and with water and 0.9% NaCl as controls. Figure 2 shows that

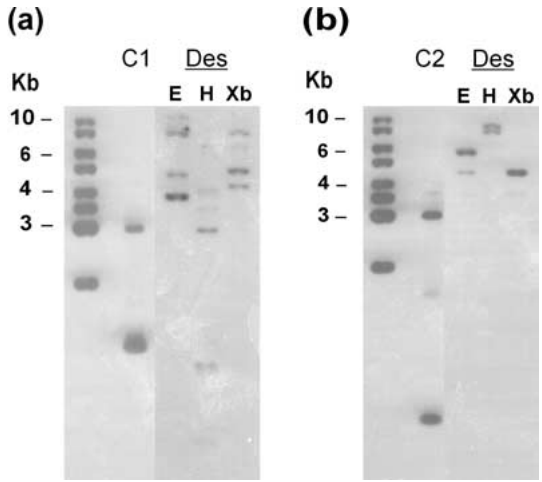

Figure 1. Detection of *Stei1* and *Stei2* genes in *S. tuberosum* cv. Désirée. Genomic DNA was digested with *Eco*RI (E), *Hind*III (H) and *Xba*I (Xb), and hybridised with *Stei1* (a) and *Stei2* (b) probes. C1 marks *Stei1* cDNA digested with *Eco*RI and *Xho*I. C2 labels *Stei2* cDNA also digested with *Eco*RI and *Xho*I. The full-length coding regions of the *Stei1* and *Stei2* cDNAs were used for labelling reactions. The same conditions described for northern analysis (Lovas et al., 2003) were applied for southern hybridisations.

expression of both genes is weakly induced by infiltration of water. *Stei2* but not *Stei1* mRNA level is slightly higher by infiltration of NaCl solution than by infiltration of water. In the presence of *E. carotovora*, elevated expression of both genes was detected after 2–4 h incubations. Since *E. carotovora* mostly infects the potato tubers, expression of *Stei1* and *Stei2* were also analysed in this organ. Microtubers were induced from axillary buds of potato stem segments, *in vitro* (Bánfalvi et al., 1996). Bacteria suspended in 0.9% NaCl were dropped on microtuber slices and incubated at room temperature. After 4 h incubation both *Stei1* and *Stei2* showed enhanced expression in *Erwinia*-treated microtuber slices compared to NaCl-treated ones (Figure 2). However, the expression of *Stei1* was much weaker than that of the *Stei2*. Northern blots probed with *Stei1* were exposed twice as much longer time than those hybridised with *Stei2*.

Some pathogen-induced genes are induced by abiotic stresses (Timmusk and Wagner, 1999; Borsics and Lados, 2002). The effect of drought and that of the mediator abscisic acid (ABA) (Shinozaki and Yamaguchi-Shinozaki, 2000) on *Stei1* and *Stei2* expression was analysed by northern hybridisation. Water-loss of leaves was achieved in two ways. Detached leaves were dried until they lost 30% of their

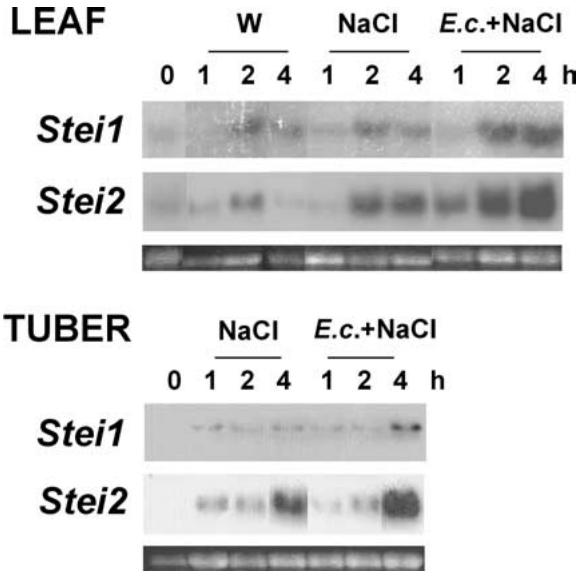

Figure 2. Expression of potato genes *Stei1* and *Stei2* in leaves and microtubers in response to *E. carotovora* (E.c.) infection. Leaves of intact plants grown in pots were infiltrated while microtuber slices were treated with drops of *Erwinias* suspended in 0.9% NaCl. As a control, plants were infiltrated with water (W) and 0.9% NaCl. Samples were collected at time points indicated. Ethidium-bromide-stained 25S rRNA bands are shown in the lower panels as loading controls. Hybridisations were carried out to 20 μg of RNA in each lane using the full-length *Stei1* and *Stei2* cDNAs as probes. RNA isolation and hybridisation conditions were the same described by Lovas et al. (2003).

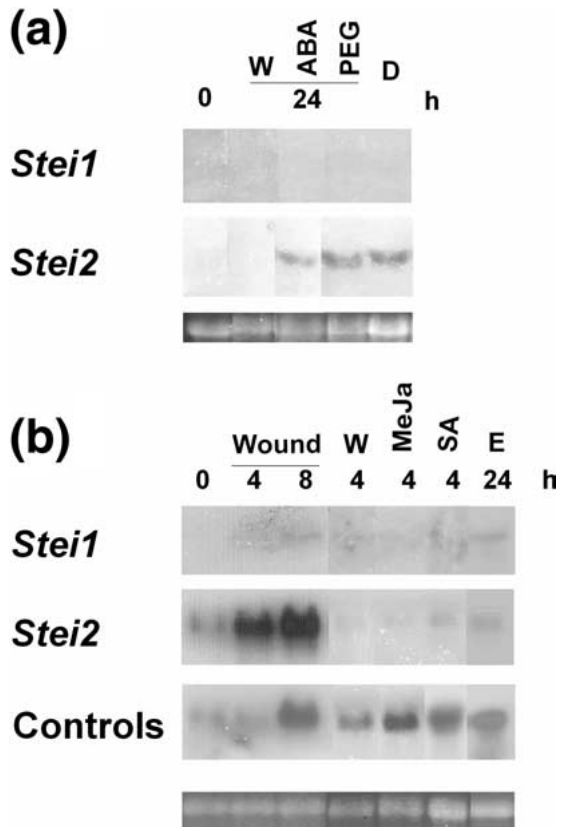

Figure 3. Effect of abiotic stresses and plant hormones on *Stei1* and *Stei2* expression. (a) PEG (20%) and ABA (200 μM, in darkness) treatments were carried out by placing leaves into the appropriate solutions for 24 h. As a control, detached leaves were supplied with water (W). Leaves were dried (D) until they lost 30% of their weight. (b) Leaves were wounded as described by Stanford et al. (1989) or treated with water (W), MeJa (50 μM) and SA (5 mM) for the times indicated. For ethylene treatment (E) intact plants were sprayed (~15 ml/plant) by 0.4% etefon solution and kept in the greenhouse for 24 h. *Win1* (Cleveland et al., 1987) was used as a treatment control of wounding and MeJa. Acidic chitinase (*ChtA*) (Büchter et al., 1997) and *PAL* (Rickey and Belknap, 1991) were the controls of SA and ethylene treatment, respectively. The controls were hybridised to the same filter as *Stei1* and *Stei2*. Ethidium-bromide-stained 25S rRNA bands are shown in the lower panels as loading controls. Hybridisations were carried out to 20 μg of RNA in each lane using the full-length *Stei1*, *Stei2* cDNAs as probes. RNA isolation and hybridisation conditions were the same described by Lovas et al. (2003).

weight, or were treated for 24 h with 20% of polyethylene glycol (PEG), a compound that mimics the drought stress conditions. No expression of *Stei1* was detectable when the leaves were dried, treated with PEG or ABA (200 μM, 24 h). In contrast, *Stei2* was up-regulated in desiccated, PEG-treated and also in ABA-treated leaves (Figure 3a).

There are pathogen response genes that are inducible by wounding (Norman et al., 1999; Sturm and Chrispeels, 1990). Figure 3b shows that *Stei2* belongs to this category since it was strongly up-regulated by wounding while *Stei1* was not, or very weakly expressed in wounded leaves as compared to the known wound-inducible gene, *win1*, used as a control. The mediator of *win1* expression is methyl jasmonate (MeJa) (Cleveland et al., 1987). Induction of *Stei2* expression, however, could not be elicited by MeJa. In addition, neither SA nor ethylene appeared to have a role in activation of *Stei1* and *Stei2*. The lack of induction by these compounds was also described in the case of the *Erwinia*-induced *WRKY*-like potato gene, *St-WRKY* (Dellagi et al., 2000a) and the *Phytophtora*-induced *Arabidopsis* gene, *PAD2* (Roetschi et al., 2001).

To summarise our results, two *E. carotovora*-induced potato genes, *Stei1* and *Stei2*, were isolated and characterised. These genes have no homology

with known genes. *Stei1* and *Stei2* are low copy number genes in *S. tuberosum* cv. Désirée. Expression of both genes was induced moderately by water. *Stei2* expression was triggered also by NaCl, dehydration, wounding and ABA. Thus *Stei1* and especially *Stei2* resemble some PR genes that are induced both by biotic and abiotic stresses, as for example *ERD15* of *Arabidopsis* (Timmusk and Wagner, 1999) and *PPRG2* of alfalfa (Borsics and Lados, 2002). Our data illustrate the complexity of signalling in plant–pathogen interactions and existence of common factors in biotic and abiotic stress responses.

Acknowledgement

We are grateful to Mrs. E. Marinka and M. Kiss for technical assistance. We thank E. Kombrink (MPI für Züchtungsforschung, Köln, Germany) for the *ChtA* clone and G.D. Lyon (Scottish Crop Research Institute, Invergowrie, Dundee, UK) for critical reading of the manuscript. D. Žvingila was awarded an UNESCO/BETCEN fellowship. This work was supported by EU (contract no. ERBIC-15-CT-960908).

References

Bánfalvi Z, Molnár A, Molnár G, Lakatos L and Szabó L (1996) Starch synthesis, and tuber storage protein genes are differently expressed in *Solanum tuberosum* and in *Solanum brevidens*. FEBS Letters 383: 159–164

Borsics T and Lados M (2002) Dodder infection induces the expression of a pathogenesis-related gene of the family *PR-10* in alfalfa. Journal of Experimental Botany 53: 1831–1832

Brader G, Tas E and Palva ET (2001) Jasmonate-dependent induction of indole glucosinolates in *Arabidopsis* by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiology 126: 849–860

Büchter R, Strömberg A, Schmelzer E and Kombrink E (1997) Primary structure and expression of acidic (class II) chitinase in potato. Plant Molecular Biology 38: 749–761

Cleveland TE, Thornburg RW and Ryan CA (1987) Molecular characterization of wound-inducible inhibitor I gene from potato and the processing of its mRNA and protein. Plant Molecular Biology 8: 199–207

Collmer A and Keen NT (1986) The role of pectic enzymes in plant pathogenesis. Annual Review of Phytopathology 24: 383–409

Dellagi A, Heilbronn J, Avrova AO, Montesano M, Palva ET, Stewart HE, Toth KI, Cooke DEL, Lyon GD and Birch PRJ (2000a) A potato gene encoding a WRKY-like transcription factor is induced in interactions with *Erwinia carotovora* subsp. *atroseptica* and *Phytophthora infestans* and is coregulated with class I endochitinase expression. Molecular Plant–Microbe Interaction 13: 1092–1101

Dellagi A, Birch PRJ, Heilbronn J, Avrova AO, Montesano M, Palva ET and Lyon GD (2000b) A potato gene, *erg-1*, is rapidly induced by *Erwinia carotovora* ssp. *atroseptica*, *Phytophthora infestans*, ethylene and salicylic acid. Journal of Plant Physiology 157: 201–205

Kotoujansky A (1987) Molecular genetics of pathogenesis by soft-rot Erwinias. Annual Review of Phythopathology 25: 405–430

Liang P and Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257: 967–971

Lovas A, Bimbó A, Szabó L and Bánfalvi Z (2003) Antisense repression of StubGAL83 affects root and tuber development in potato. The Plant Journal 33: 139–147

Montesano M, Koiv V, Mae A and Palva ET (2001) Novel receptor-like protein kinases induced by *Erwinia carotovora* and short oligogalacturonides in potato. Molecular Plant Pathology 2: 339–345

Niebel A, Heungens K, Barthels N, Inzé D, Van Montagu M and Gheysen G (1995) Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. Molecular Plant–Microbe Interaction 8: 371–378

Norman C, Vidal S and Palva ET (1999) Oligogalacturonide-mediated induction of a gene involved in jasmonic acid synthesis in response to the cell wall-degrading enzymes of the plant pathogen *Erwinia carotovora.* Molecular Plant–Microbe Interaction 12: 640–644

Norman-Setterblad C, Vidal S and Palva ET (2000) Interacting signal pathways control defense gene expression in *Arabidopsis* in response to cell wall-degrading enzymes from *Erwinia carotovora*. Molecular Plant–Microbe Interaction 13: 430–438

Palva TK, Holmström K-O, Heino P and Palva ET (1993) Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. Molecular Plant–Microbe Interaction 6: 190–196

Pérombelon MCM and Kelman A (1980) Ecology of soft rot erwinias. Annual Review of Phytopathology 18: 361–387

Pirhonen M, Heino P, Helander I, Harju P and Palva ET (1988) Bacteriophage T4 resistant mutants of the plant pathogen *Erwinia carotovora*. Microbial Pathogenesis 4: 359–367

Rickey TM and Belknap WR (1991) Comparison of the expression of several stress-responsive genes in potato tubers. Plant Molecular Biology 16: 1009–1018

Roetschi A, Si-Ammour A, Belbahri L, Mauch F and Mauch-Mani B (2001) Characterization of an *Arabidopsis–Phytophthora* pathosystem: Resistance requires a functional *PAD2* gene and is independent of salicylic acid, ethylene and jasmonic acid signaling. The Plant Journal 28: 293–305

Rumeau D, Maher EA, Kelman A and Showalter AM (1990) Extension and phenylalanine ammonia-lyase gene expression altered in potato tubers in response to wounding, hypoxia, and *Erwinia carotovora* infection. Plant Physiology 93: 1134–1139

Shinozaki K and Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: Differences

and cross-talk between two stress signalling pathways. Current Opinion in Plant Biology 3: 217–223

Stanford AC, Bevan MW and Northcote DH (1989) Differential expression within a family of novel wound induced genes in potato. Molecular and General Genetics 215: 200–208

Sturm A and Chrispeels MJ (1990) cDNA cloning of carrot extracellular fructosidase and its expression in response to wounding and bacterial infection. Plant Cell 2: 1107–1119

Timmusk S and Wagner EGH (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: Possible connection between biotic and abiotic stress responses. Molecular Plant–Microbe Interaction 12: 951–959

Yang Z, Park H, Lacy GH and Cramer CL (1991) Differential activation of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes by wounding and pathogen challenge. Plant Cell 3: 397–405

Vidal S, Ponce de León I, Denecke J and Palva ET (1997) Salicylic acid and plant pathogen *Erwinia carotovora* induce defense genes via antagonistic pathways. The Plant Journal 11: 115–123

Vidal S, Eriksson ARB, Montesano M, Denecke J and Palva ET (1998) Cell wall-degrading enzymes from *Erwinia carotovora* cooperate in the salicylic acid-independent induction of a plant defense response. Molecular Plant–Microbe Interaction 1: 23–32

Weber J, Olsen O, Wegener C and Wettstein von D (1996) Digalacturonates from pectin degradation induce tissue responses against potato soft rot. Physiological and Molecular Plant Pathology 48: 389–401