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Potato Homologs of *Arabidopsis thaliana* Genes Functional in Defense Signaling—Identification, Genetic Mapping, and Molecular Cloning

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Defense against pests and pathogens is a fundamental process controlled by similar molecular mechanisms in all flowering plants. Using *Arabidopsis thaliana* as a model, steps of the signal transduction pathways that link pathogen recognition to defense activation have been identified and corresponding genes have been characterized. Defense signaling (DS) genes are functional candidates for controlling natural quantitative variation of resistance to plant pathogens. Nineteen *Arabidopsis* genes operating in defense signaling cascades were selected. *Solanaceae* EST (expressed sequence tag) databases were employed to identify the closest homologs of potato (*Solanum tuberosum*). Sixteen novel DS potato homologs were positioned on the molecular maps. Five DS homologs mapped close to known quantitative resistance loci (QRL) against the oomycete *Phytophthora infestans* causing late blight and the bacterium *Erwinia carotovora* subsp. *atroseptica* causing blackleg of stems and tuber soft rot. The five genes are positional candidates for QRL and are highly sequence related to *Arabidopsis* genes *AtSGT1b, AtPAD4,* and *AtAOS*. Full-length complementary DNA and genomic sequences were obtained for potato genes *StSGT1, StPAD4,* and *StEDS1*, the latter being a putative interactor of *StPAD4*. Our results form the basis for further studies on the contributions of these candidate genes to natural variation of potato disease resistance.

Additional keywords: allene oxide synthase, genomics resources, jasmonic acid, lipases, quantitative trait.

Effective plant defense against pests and pathogens involves recognition and activation of appropriate defenses. Similar underlying mechanisms are likely to control this fundamental process in all flowering plants (McDowell and Woffenden 2003). Therefore, structural and functional analysis of genes involved in plant defense in a model species such as *Arabidopsis thaliana* (L.) Heynh. can facilitate the identification of structural and functional orthologs and their role in disease resistance pathways in other plant species (Glazebrook et al. 1997a; Hammond-Kossack and Parker 2003).

Natural plant populations and breeding populations of crop plants show qualitative and quantitative phenotypic variation for resistance to pests and pathogens. Qualitative resistance is characterized by two distinct phenotype classes, resistant and susceptible, and follows Mendelian inheritance. It is this type of single gene- or resistance (*R*) gene-mediated resistance that has been most thoroughly studied in the context of plant–pathogen recognition and defense signaling (Feys and Parker 2000; Glazebrook 2001; Hulbert et al. 2001; Kombrink and Somssich 1997). In contrast, quantitative resistance is characterized by continuous phenotypic variation ranging from high susceptibility to high resistance among the recombinant individuals within a progeny. Such resistance is controlled by more than one gene and can be strongly influenced by environmental factors. The molecular basis of quantitative resistance is yet to be elucidated. Genetic dissection of quantitative resistance in discrete quantitative trait loci (QTL) and molecular mapping of genes known to function in pathogen defense suggest that allelic variation of some of those genes may be responsible for certain quantitative resistance loci (QRL) (Bormann et al. 2004; Faris et al. 1999; Geffroy et al. 2000; Leonards-Schippers et al. 1994; Pflieger et al. 2001b; Ramalingam et al. 2003; Trognitz et al. 2002). Thus, a candidate gene approach could expedite the identification of QRL compared with map-based QTL cloning approaches alone (Pflieger et al. 2001a). Functional candidates for quantitative resistance are, in principle, all genes operating in pathogen recognition or regulation of defense responses. This potentially large number of genes can by reduced by applying positional criteria to functional candidates. A gene that is causal for a QTL effect is expected to co-localize with that QTL. Molecular variants of the gene (single nucleotide polymorphisms, insertion or deletion polymorphisms, present within the coding sequence or regulatory region) with potential impact on function, accompanied by variation of gene expression or biochemical activity, are expected to be present in the genetic material used to detect and map the QTL. Further validation is achieved when molecular variants of the gene are associated with trait variation in populations of genotypes related by descent. The final confirmation of a candidate gene would require quantitative complementation analysis using different functional alleles (El-Din El-Assal et al. 2001; Fridman et al. 2004; Pflieger et al. 2001a).

Potato (*Solanum tuberosum* L.) is the fourth most important crop grown globally in terms of acreage, yield, and value, with

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Nucleotide sequence data is available in the GenBank database under accession numbers *StAOS3*, AY615276; *StDND1*, AY615277; *StEDS1*, AY679160; *StEDS5*, AY615278; *StHIN1*, AY615279; *StNDR1*, AY615280; *StNPR1*, AY615281; *StPAD4-1*, AY753546; *StPAD4-2*, AY753547; *StPEN1*, AY616763; *StRAR1*, AY615275; *StSGT1-1*, AY615272; *StSGT1-2*, AY615274; *StWRKY1*, AY615273.

annual production exceeding 320 million tons (online FAOSTAT data for 2004). Pests and diseases are a major threat to crop yield, especially to resource-limited farmers in developing countries. In combating late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, the introgression of single-gene resistance into cultivars was not durable (Wastie 1991). In other cases, such as blackleg of stem and tuber soft rot caused by the bacterium *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye, no monogenic resistance is currently available (Zimnoch-Guzowska et al. 2000). Hence, genetic improvement of quantitative resistance is an important target in potato cultivar development. DNA-based markers closely linked or identical to genes that control quantitative resistance would facilitate the selection of genotypes with combinations of superior alleles at several QRL. QTL mapping of potato resistance to various pathogens has been conducted by several research groups and in different genetic backgrounds (Gebhardt and Valkonen 2001). Whereas a number of candidate genes for pathogen recognition and defense responses have been located on the potato molecular maps (Leister et al. 1996; Leonards-Schippers et al. 1994; Trognitz et al 2002; Zimnoch-Guzowska et al. 2000), information on position and linkage to QRL of candidate genes involved in signal transduction pathways is lacking.

We have used knowledge gained from the genetic dissection of *Arabidopsis*-pathogen interactions to select candidate genes involved in various defense signaling pathways. The selection included loci mediating *R* gene-activated defenses, as well as regulators of salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene (ET)-dependent response pathways (Glazebrook 2001, 2005). Our search for potato homologs of *Arabidopsis* genes was facilitated by genomics resources available for the *Solanaceae* family, including extensive expressed sequence tag (EST) databases (Crookshanks et al. 2001; Ronning et al. 2003; Van der Hoeven et al. 2002). We describe the molecular mapping of 16 new potato defense homologs and the identification of positional candidates for QRL against *P. infestans*, *E. carotovora* subsp. *atroseptica*, or both. For three potato defense signaling homologs, for which this information was not available before, we further characterize full-length coding and genomic sequences as prerequisite for complementation analyses. *StSGT1* and *StPAD4* were selected as positional candidates. In addition, *StEDS1* was chosen based on the interaction reported between *At*EDS1 and *At*PAD4 proteins (Feys et al. 2001). This work is the basis for unraveling the potential role of defense signaling genes for quantitative disease resistance in this major crop species.

RESULTS

Database sequence similarity searches.

A total of 19 *Arabidopsis thaliana* genes were selected on the basis of current knowledge of their roles in signal transduction pathways linking pathogen recognition with defense responses (Table 1) (Glazebrook 2001, 2005; Hammond-Kossack and Parker 2003). Complementary DNA (cDNA) sequences corresponding to these genes were subjected to nucleotide BLAST analyses against EST databases of potato, tomato, or tobacco. Twenty-five ESTs with the highest simi-

Table 1. *Arabidopsis thaliana* defense signaling genes selected for searching potato homologs

At gene	Full name	At locus	Annotation
ACD11	Accelerated cell death 11	At2g34690.1	Transporter of the glycolipid precursor sphingosine between membranes (Brodersen et al. 2002).
AOS	Allene oxide synthase	At5g42650.1	Allene oxide synthase, catalyses dehydration of the hydroperoxide to an unstable allene oxide in the jasmonic acid biosynthetic pathway (Laudert et al. 1996).
CEV1	Constitutive expression of *vsp1* 1	At5g05170.1	Cellulose synthase CeSA3 (Ellis et al. 2002).
COI1	Coronatine insensitive 1	At2g39940.1	An F-box protein required for response to jasmonates, which regulate defense against insects and pathogens, wound healing, and pollen fertility (Xie et al. 1998).
CPR5	Constitutive expressor of pathogenesis-related genes 5	At5g64930.1	A transmembrane protein regulating expression of pathogenesis-related (PR) genes. Participates in signal transduction pathways involved in plant defense (systemic acquired resistance [SAR]) (Clarke et al. 2001).
DND1	Defense, no death 1	At5g15410.1	Cyclic nucleotide-gated ion channel, also known as *CNGC2* (Clough et al. 2000).
EDR1	Enhanced disease resistance 1	At1g08720.1	A mitogen-activated protein kinase kinase kinase (MAPKKK) that confers resistance to powdery mildew disease caused by fungus *Erysiphe cichoracearum* (Frye et al. 2001).
EDS1	Enhanced disease susceptibility 1	At3g48090.1	Component of resistance (*R*) gene-mediated disease resistance in *A. thaliana* with homology to eukaryotic lipases (Falk et al. 1999; Parker et al. 1996).
EDS5	Enhanced disease susceptibility 5	At4g39030.1	Member of the MATE-transporter family, essential for salicylic acid-dependent signaling during defense responses, also known as *SID1* (Nawrath et al. 2002).
ETR1	Ethylene receptor 1	At1g66340.1	A putative ethylene receptor containing a histidine kinase and a response regulator domain, membrane component capable of ethylene binding, also known as *EIN1* (Chang et al. 1993).
JAR1	Jasmonate response 1	At2g46370.1	An auxin-induced gene encoding a cytoplasmic localized phytochrome A signaling component protein similar to the GH3 family of proteins (Staswick et al. 2002).
HIN1	Harpin-induced 1	At5g06320.1	An *NDR1*-like gene, potentially functional in plant response to pathogens downstream of signal recognition (Gopalan et al. 1996; Varet et al. 2002).
NDR1	Non-race-specific disease resistance 1	At3g20600.1	Required for non-race-specific resistance to bacterial and fungal pathogens; mediates SAR response (Century et al. 1995).
NPR1	Non-expressor of PR genes 1	At5g45110.1	Adaptor molecule containing ankyrin repeats, controls SAR, also known as *NIM1* and *SAI1* (Cao et al. 1997).
PAD4	Phytoalexin-deficient 4	At3g52430.1	A lipase-like gene important for salicylic acid signaling (Glazebrook et al. 1997b; Jirage et al. 1999).
PEN1	Penetration 1	At3g11820.1	Plant syntaxin *AtSYP121* (Collins et al. 2003; Sanderfoot et al. 2000, 2001).
RAR1	Required for Ml*a*12 resistance 1	At5g51700.1	Resistance signaling gene, encodes a protein with two zinc binding (CHORD) domains that are highly conserved across eukaryotic phyla, also known as *PBS2*, *RPR1* (Azevedo et al. 2002; Shirasu et al. 1999).
SGT1	Suppressor of G-two allele of SKP1	At4g23570.1	Component of the ubiquitin ligase complex, phosphatase-like protein, required for *Peronospora parasitica* resistance in *Arabidopsis* (Austin et al. 2002).
WRKY75	WRKY-domain (Trp-Arg-Lys-Tyr)	At5g13080.1	Transcription factor from the WRKY superfamily (group IIc), carrying a zinc-finger-like motif and binding specifically to the W-box (Eulgem et al. 2000).

larity to the *Arabidopsis* genes were retrieved independently from different databases. In all, 23 ESTs were from potato and corresponded to 18 *Arabidopsis* genes included in the search. For *AtNDR1*, only a tomato-derived EST sequence was present in the databases. In four cases, multiple hits with highly significant similarities to the target *Arabidopsis* gene were found: for *StHIN1,* a potato and a tobacco EST were retrieved, and identification of *StRAR1* and *StNPR1* was supported equally by potato and tomato ESTs. For *AtAOS*, three different potato ESTs were identified, annotated as putative members of a gene family. Primer pairs were designed for these ESTs and polymerase chain reactions (PCR) were performed using genomic DNA of three diploid *S. tuberosum* genotypes as templates (*results not shown*). For seven genes (*AtACD11*, *AtCEV1*, *AtCOI1*, *AtCPR5*, *AtEDR1*, *AtETR1,* and *AtJAR1*), no homologous gene fragments were obtained from potato DNA. No PCR product was obtained for the potato ESTs homologous to *AtCOI1, AtETR1,* and *AtJAR1*. Several primers designed to amplify *StACD11, StCEV1, StCPR5,* and *StEDR1* generated multiple PCR products (*StCEV1*, *StEDR1*) or a smear (*StACD11, StCPR5*), most likely due to the presence of multicopy gene families in the potato genome. For the 12 remaining *Arabidopsis* genes, singular potato genomic fragments were amplified that could be sequenced and compared with the sequence databases. All deduced amino acid sequences of potato exhibited a high level of sequence conservation with the dicotyledonous species *Arabidopsis* sp., tomato, and soybean, and the monocotyledonous species barley and rice (Table 2). Potato, tomato, and tobacco EST accession numbers (TIGR), *e* values for amino acid similarities, and the GenBank accession numbers of the potato genomic fragments described in this article are listed in Table 2.

Development of PCR-based markers and genetic mapping. Cleaved amplified polymorphic sequence (CAPS) or single-strand conformation polymorphism (SSCP) markers, which segregated in at least one of three mapping populations considered, were developed for 16 potato DS homologous genes (Fig. 1). All genes were mapped relative to restriction fragment length polymorphism (RFLP) or amplified fragment length polymorphism (AFLP) loci of known map position (Gebhardt et al. 2003; Leister et al. 1996; Schäfer-Pregl et al. 1998; Zimnoch-Guzowska et al. 2000). One locus was identified for each gene, except *StSGT1*, for which two unlinked loci were identified, and *StPAD4*, where two closely linked, highly sequence-related loci were found (see below). The 16 candidate loci were distributed on 10 of the 12 potato chromosomes. With the exception of *StPAD4-1*, *StPAD4-2, StSGT1-1, StSGT1-2,* and *StAOS2,* the DS loci were not closely linked to markers known to detect QRL in different mapping populations (Fig. 2). Genes *StPAD4-1* and *StPAD4-2* both mapped to the short arm of chromosome II, 4 cM distal to *GP23*. The marker *GP23* tags QRL *Eca2A* for resistance to *E. carotovora* subsp. *atroseptica* (Zimnoch-Guzowska et al. 2000) and *Pin2A* for resistance to late blight (Bormann et al. 2004, Oberhagemann et al. 1999). *StSGT1-1* on chromosome III mapped to a region tagged by markers *GP1-a*, *GP25*, and *CP6* (Fig. 2). This region is, depending on the mapping population considered, between 5 and 20 cM long. Markers in this region detected the late blight QRL *Pin3B* in three different mapping populations (Bormann et al. 2004; Leonards-Schippers et al. 1994; Oberhagemann et al. 1999). *StSGT1-2* on chromosome VI was located within a map segment of approximately 10 cM tagged by markers *GP79* and *St3.3.13-c*, which are linked to QRL *Pin6A* (Oberhagemann et al. 1999) and *Eca6A* (Zimnoch-Guzowska et al. 2000), respectively. Finally, *StAOS2* mapped 1 cM proximal to the marker *St1.2.1-a* on chromosome XI. Markers *St1.2.1-a*, *GP250*, and *GP185* (Fig. 2) all are located within the distal 10 cM of the short arm of chromosome XI, and were linked to QRL *Eca11A* and *Pin11A* (Oberhagemann et al. 1999, Zimnoch-Guzowska et al. 2000).

Table 2. GenBank accession numbers of *Solanaceae* expressed sequence tags (ESTs) with the highest similarity to *Arabidopsis* defense response genes, and percent amino acid sequence similarities of the potato homologs to the most closely related genes of *Arabidopsis*, tomato, soybean, rice, and barley[a]

					Amino acid similarities (%)[b]				
***At* gene**	**EST acc. no.[c]**	***E* value[d]**	***St* gene**	**Potato acc. no.[e]**	***At***	**Tomato**	**Soybean**	**Rice**	**Barley**
AOS	NP451990	1.5e-126	*StAOS1*	AJ457080[f]	78	83	83	77	71
AOS	TC128063	5.9e-128	*StAOS2*	AJ457081[f]	69	91	77	67	69
AOS	TC12	1.2e-22	*StAOS3*	AY615276	65	97	73	64	65
DND1	TC129362	4.0e-89	*StDND1*	AY615277	54	80	…	59	59
EDS1	TC111810	1.6e-10	*StEDS1*	AY679160[f]	57	86	67	61	58
EDS5	TC132023	8.0e-40	*StEDS5*	AY615278	78	…	…	76	69
HIN1	TC116297	5.8e-15							
	TC1573[g]	8.1e-18	*StHIN1*	AY615279	60	91	67	55	51
NDR1	BF114006[h]	3e-008	*StNDR1*	AY615280	62	95	66	…	…
NPR1	TC116672	4.4e-30							
	TC164925[h]	1.5e-29	*StNPR1*	AY615281	73	94	84	80	78
PAD4	TC118477	7.5e-98	*StPAD4-1*	AY753546[f]	61	87	77	56	68
PAD4	TC118477	3.2e-99	*StPAD4-2*	AY753547[f]	62	86	77	52	67
PEN1	TC122378	1.4e-76	*StPEN1*	AY616763	79	91	83	65	62
RAR1	TC121848	1.2e-41							
	TC159170[h]	2.7e-41	*StRAR1*	AY615275	79	95	81	73	74
SGT1b	TC115479	7.3e-108	*StSGT1-1*	AY615272[f]	66	92	76	70	70
SGT1b	TC115479	3.8e-79	*StSGT1-2*	AY615274	67	96	69	67	69
WRKY75	TC121153	9.5e-41	*StWRKY1*	AY615273	97	98	98	90	91
	…	…	…	…	(69)	(91)	(77)	(67)	(68)

[a] *At* = *Arabidopsis thaliana* and *St* = *Solanum tuberosum.*
[b] … Indicates no corresponding EST was found; numbers in parentheses = mean.
[c] EST accession number (TIGR).
[d] *E* value for amino acid similarity.
[e] GenBank accession number of potato homolog.
[f] Complete deduced amino acid sequence was used for the comparison.
[g] Tobacco EST.
[h] Tomato EST.

Rapid amplification of cDNA ends PCR-based isolation of the potato candidate genes.

Of the five selected candidate loci, full-length genomic and cDNA sequences are available in the database for *StAOS2* only (accession AJ457081). Hence, potato genes homologous to *AtPAD4*, *AtEDS1,* and *AtSGT1b* were further sequence characterized.

Rapid amplification of cDNA ends (RACE) amplification and cloning of *StSGT1* resulted in a 1,113-bp full-length cDNA. Sequencing revealed a high level of conservation with *SGT1* genes

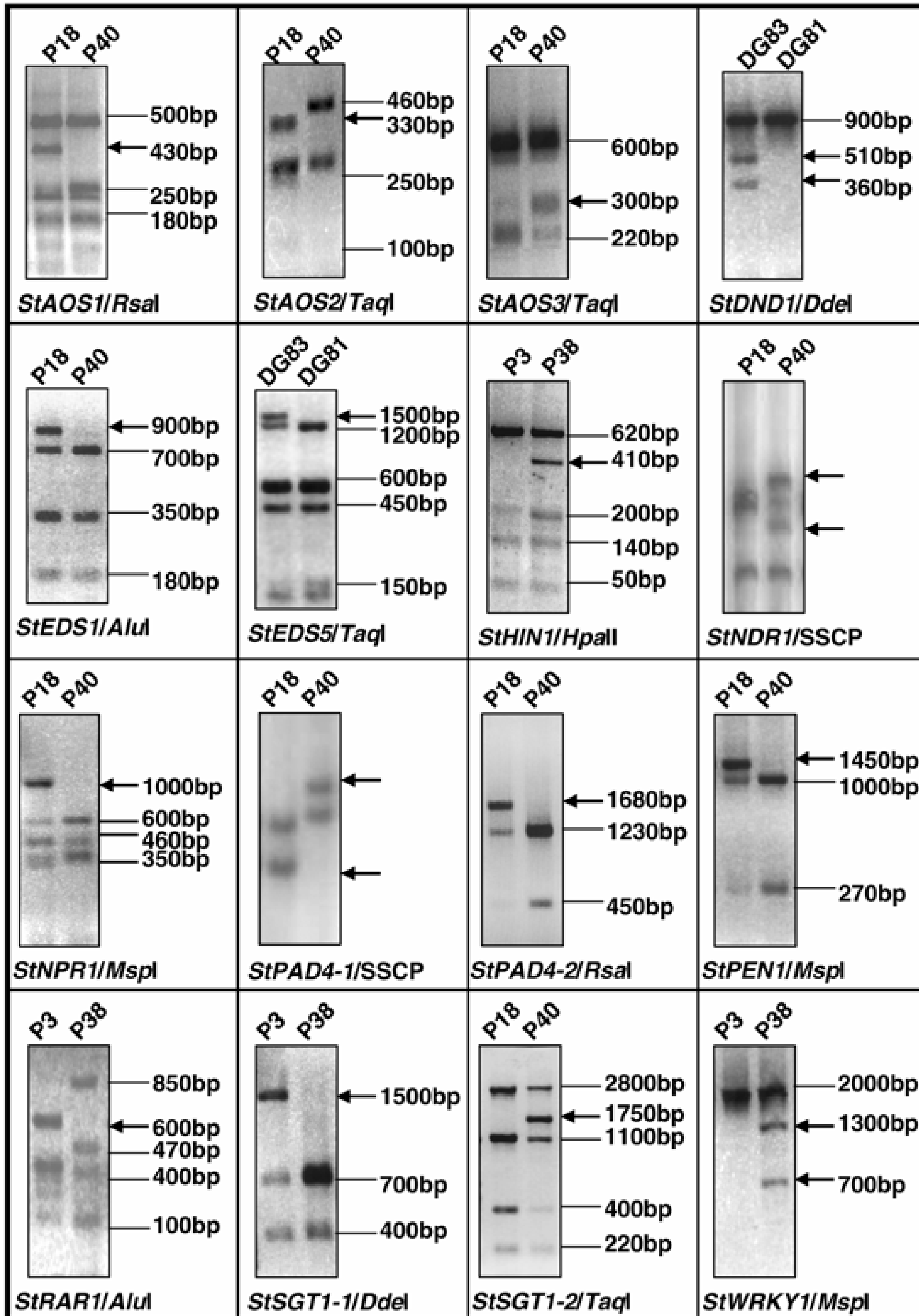

Fig. 1. Cleaved amplified polymorphic sequence (CAPS) or single-strand conformation polymorphism (SSCP) marker phenotypes. Sixteen markers polymorphic between the parental genotypes of three mapping populations were developed. Parents of the F1840 population: P18 (line H82.337/49, seed parent) and P40 (line H80.696/4, pollen parent) (Gebhardt et al. 1989, 2003). Parents of the K31 population: P3 (line H80.577/1, seed parent) and P38 (line H80.576/16, pollen parent) (Gebhardt et al. 1989; Oberhagemann et al. 1999). Parents of the "Erwinia" population: DG83 (line DG 83-2025, seed parent), and DG81 (line DG 81-68, pollen parent) (Zimnoch-Guzowska et al. 2000). Estimated sizes of restricted CAPS fragments are shown to the right. For loci *StNDR1* and *StPAD4-1* mapped as SSCP markers, no information regarding the size of segregating fragments is included, because the polymorphism is based on nucleotide composition and, consequently, conformation of undigested polymerase chain reaction products. Arrows indicate the polymorphic fragment or fragments used for scoring the marker in the entire population.

from other plants. The isolated potato genomic fragment was 4,482 bp long (accession AY615272). Ten exons encoded a putative protein of 370 amino acids (aa) with a predicted molecular mass of 41.2 kDa and an isoelectric point (pI) of 5.11. Alignment of *StSGT1* with other *SGT1* genes supported the predicted open reading frame (Fig. 3). No signal peptide or transmembrane domains were identified when using the prediction programs for subcellular localization, whereas typical motifs pres-

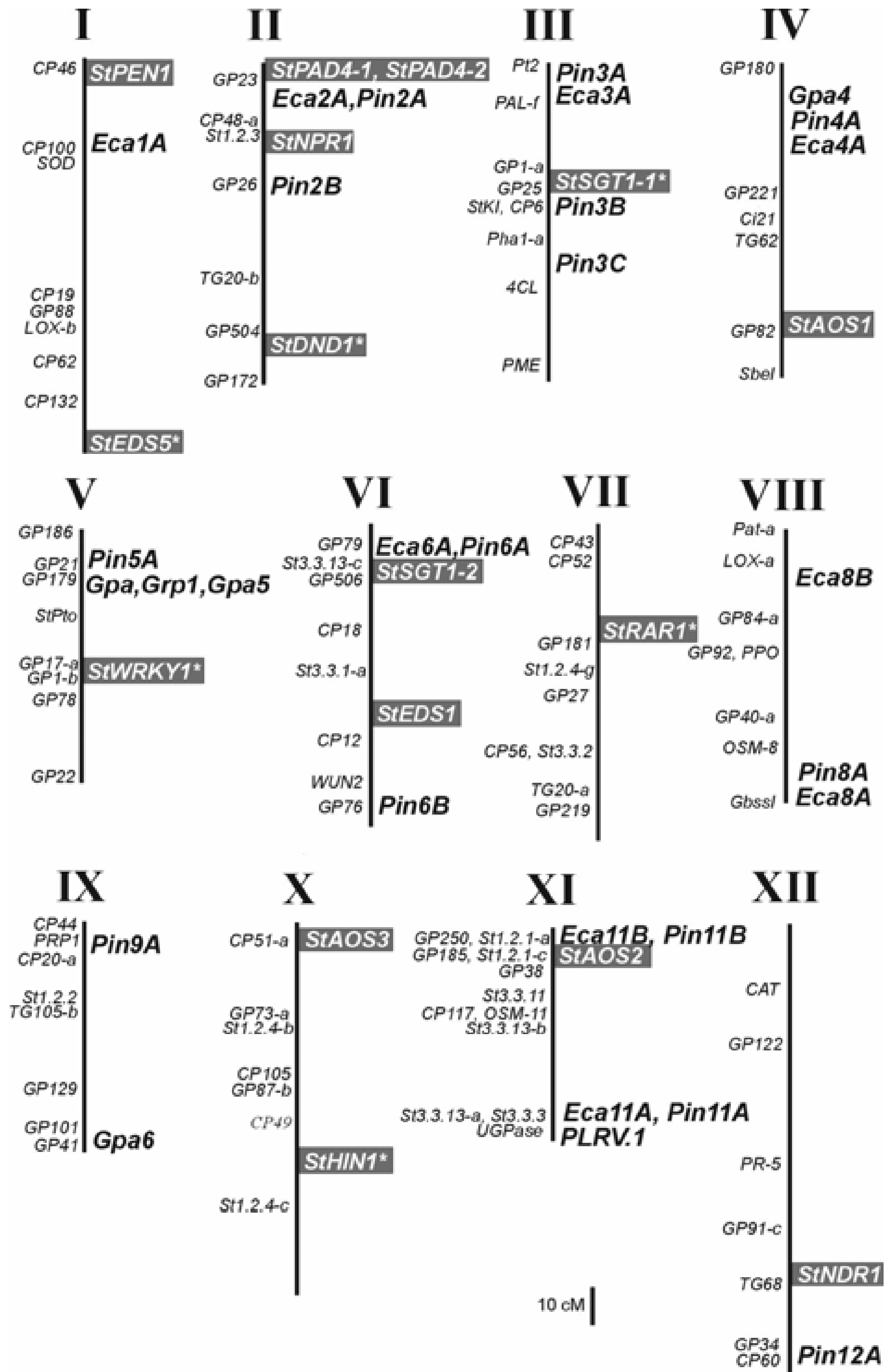

Fig. 2. Map positions of defense signaling (DS) loci in potato. The 12 linkage groups of the F1840 mapping population are shown, displaying to the left of each linkage group a subset of all restriction fragment length polymorphism (RFLP) loci (GABI PoMaMo Database). RFLP loci *St1.2.1, St1.2.2, St1.2.3, St1.2.4, St3.3.1, St3.3.2, St3.3.11, St3.3.13,* and *StPto* are sequence related to known resistance (*R*) genes (Leister et al. 1996). The RFLP loci detected by known defense-related genes in population F1840 are: *SOD* = superoxide dismutase, *LOX* = lipoxygenase, *PAL* = phenylalanine ammonia-lyase, *StKI* = Kunitz-type proteinase inhibitor, *4CL* = 4-coumaryl CoA ligase, *WUN2* = wound induced, *PPO* = polyphenol oxidase, *OSM-8* and *OSM-11* = basic osmotin-like, *PRP1* = pathogenesis-related glutathione S-transferase 1, *CAT* = catalase, *PR-5* = acidic osmotin-like (Castillo Ruiz et al. in press). Lowercase extensions of RFLP loci indicate that the same probe detected more than one RFLP locus. The newly mapped DS loci are shown to the right of the linkage groups. DS loci that were mapped in populations other than F1840 are labeled with *. They were positioned relative to the closest anchor RFLP markers shared between the maps. Map segments having quantitative resistance loci (QRL) are indicated to the right of the linkage groups: *Pin*** = QRL to *Phytophthora infestans* (Bormann et al. 2004; Leonards-Schippers et al. 1994; Oberhagemann et al. 1999), *Eca*** = QRL to *Erwinia carotovora* subsp. *atroseptica* (Zimnoch-Guzowska et al. 2000), *Gpa** and *Grp1* = QRL to root cyst nematodes *Globodera pallida* or *Globodera rostochiensis* (Kreike et al. 1994; Rouppe van der Voort et al. 1998, 2000), *PLRV.1* = QRL to *Potato leafroll virus* (Marczewski et al. 2001).

ent in previously described SGT1 proteins could be found: an N-terminal tetratricopeptide repeat (TPR) protein-protein interaction domain; a central cysteine and histidine-rich domain (CHORD)-specific (CS) domain involved in interactions with CHORD domain-bearing proteins and Hsp90; and a so-called SGT1-specific motif (SGS) that, in yeast, mediates interaction with the leucine-rich repeat (LRR) domain of adenylyl cyclase and also is found in calcyclin binding proteins (Breen and Tang 2003; Muskett and Parker 2003; Schulze-Lefert 2004; Shirasu and Schulze-Lefert 2003) (Fig. 3). The *StSGT1-1* locus on potato chromosome III was identified with primers designed from exons 2 and 5 of the full-length gene, whereas locus *StSGT1-2* on chromosome VI was identified with different primers corresponding to the original EST sequence (Table 3). This indicates that there are at least two *SGT1* genes in the potato genome and that the fully sequenced gene *StSGT1-1* likely is encoded at the locus on potato chromosome III. For gene *StSGT1-2,* the complete genomic sequence was not determined.

A full-length, 1,737-bp cDNA of *StPAD4* was obtained by RACE amplification. Sequencing of three cDNA clones revealed two highly similar but distinct *StPAD4* transcripts. PCR using the same set of primers, performed on genomic DNA template, resulted in two amplicons of 4,637 and 5,300 bp (accessions AY753546 and AY753547, respectively). Both products contained a gene composed of four exons and three introns, the first exon being small (25 bp) and the second intron large (2,309 and 2,985 bp in *StPAD4-1* and *StPAD4-2*, respectively). Differences between both genes mostly were confined to the introns with numerous insertions or deletions up to 700 bp and few single base pair exchanges present in the coding sequence. Based on sequence analysis, both genes are functional *StPAD4* copies, having defined start and stop codons and uninterrupted reading frames. The cDNA sequences deduced from the genomic clones perfectly matched the cDNAs obtained from RACE experiments, confirming the presence of at least two *PAD4* genes in the potato genome. The genes were named *StPAD4-1* (shorter) and *StPAD4-2* (longer). Genetic mapping, performed with the help of gene-specific primers, revealed two genetically closely linked loci on chromosome II (Fig. 2). This could classify them as paralogs that arose from gene duplication. The deduced polypeptides of both *St*PAD4 sequences comprise 578 aa and share 98% identity with a predicted molecular mass of 65 kDa and a pI of 6.49 (Fig. 4). Domain searches identified a class 3 lipase motif in the N-terminal part, a catalytically active serine (S^{129})

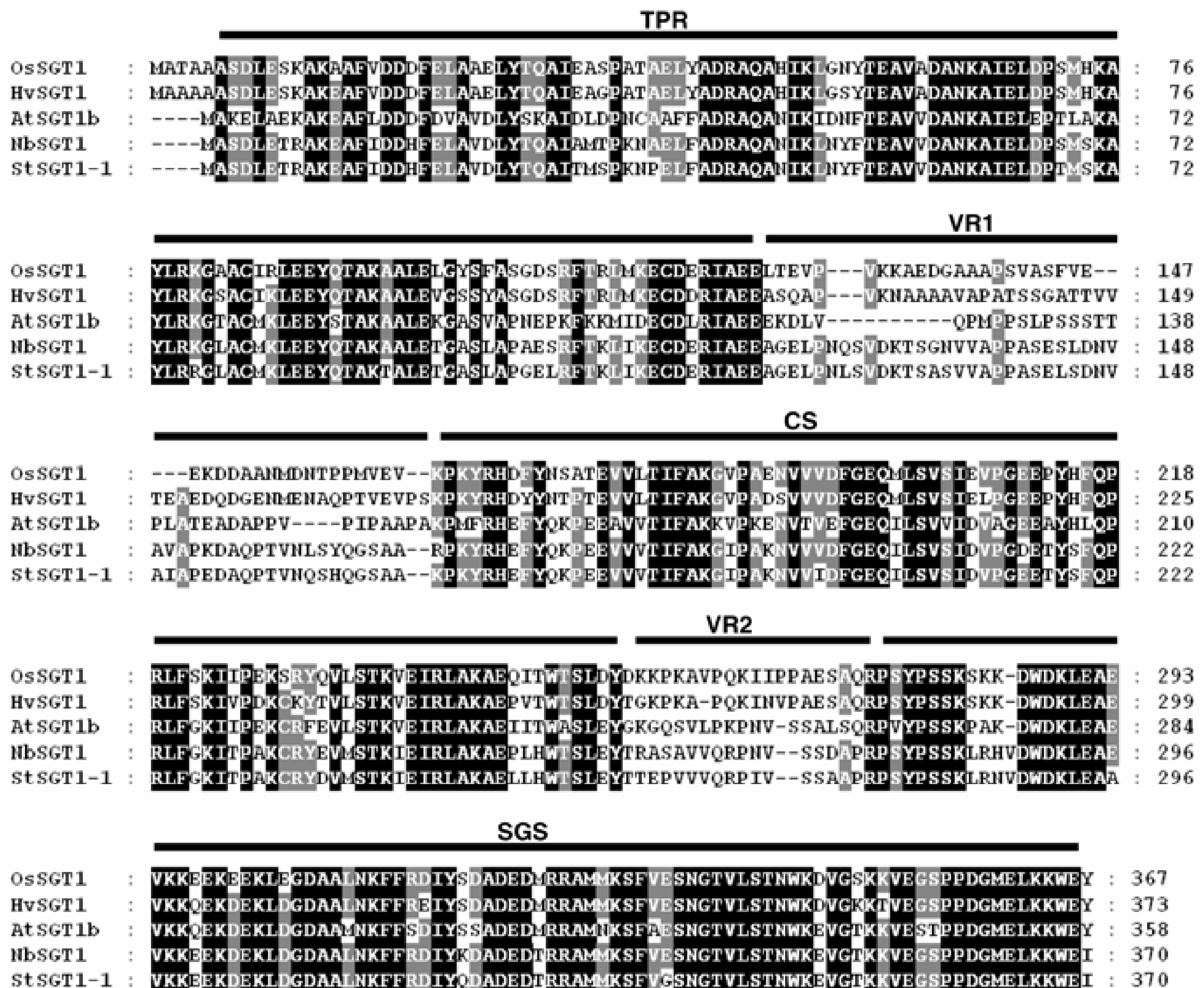

Fig. 3. Alignment of deduced amino acid sequence of *St*SGT1-1 with rice (*Os*), barley (*Hv*), *Arabidopsis thaliana* (*At*), and *Nicotiana benthamiana* (*Nb*) SGT1 proteins. GenBank accession numbers: *Os*SGT1, AAF18438; *Hv*SGT1, AF439974; *At*SGT1b, AAL33612; *Nb*SGT1, AAO85509; and *St*SGT1-1, AY615272. The black and gray boxes represent 100 and 70% amino acid sequence conservation, respectively, between the deduced proteins compared. TPR: tetratricopeptide repeat domain, VR1 and VR2: variable region 1 and 2, CS: CHORD protein and SGT1-specific motif, SGS: SGT1 specific domain.

residue surrounded by the L3 consensus sequence (amino acids 123 to 132), and the two other residues of the catalytic triad of serine hydrolases: an aspartate (D^{188}) and a histidine (H^{275}) (Falk et al. 1999, Jirage 1999). In the C-terminal region (amino acids 378 to 505), high similarity was detected with the EDS1- and PAD4-specific (EP) domain of *Arabidopsis* PAD4, which is shared with *At*EDS1 and *At*SAG101 and may direct interactions between these proteins (Feys et al. 2001).

Molecular cloning of *StEDS1* resulted in the isolation of a single 1,824-bp cDNA clone with high similarity to *Arabidopsis* and tobacco *EDS1* genes. The genomic sequence of *StEDS1* is composed of three exons and two introns with a total size of 2,597 bp. The gene encodes a putative 607-aa protein of 69 kDa and pI = 6.72. Like PAD4 proteins, *St*EDS1 possesses characteristic features of a class 3 lipase with a defined catalytic triad: S^{125} surrounded by the L3 consensus between amino acids 119 and 128, D^{190}, and H^{325} (Falk et al. 1999). The EP interaction domain was detected in the region of residues 426 to 544 and contains a "KNEDT" motif specific to EDS1 sequences identified so far (amino acids 499 to 503), with a conserved substitution of threonine to serine (Peart et al. 2002) (Fig. 5).

DISCUSSION

Comprehensive EST databases available for several Solanaceous species provide access to sequence information covering a significant proportion of the gene content in potato. At least one EST with high sequence similarity was found in silico for each of the 19 targeted defense signaling genes from *A. thaliana*. Based on potato, tomato, or tobacco EST sequences, PCR-based marker assays were developed that allowed detection and mapping of 16 novel loci on the potato molecular linkage maps. These loci encode genes that may have important functions in defense signaling in potato. They represent a third class of candidate loci for controlling quantitative resistance to pathogens, in addition to loci coding for genes with similarity to *R* genes or defense response genes that have been detected and mapped previously (Gebhardt and Valkonen 2001). Due to their potential functional relevance, the PCR-based markers identifying these loci have added value as anchors for resistance QTL mapping in potato and other Solanaceous species and may be useful in marker-assisted selection experiments.

Map-based selection of candidate genes for QRL.

In our study, we took advantage of the detailed and extensive characterization of numerous *Arabidopsis* mutants showing defects in defense signaling. The picture emerging from these studies shows that impaired signaling can lead to drastically increased pathogen susceptibility by disabling basal resistance or *R* gene-triggered defenses (Glazebrook 2001, 2005; Hammond-Kossack and Parker 2003). Of all genes that function in defense signaling, only a subset may be relevant for natural variation of pathogen resistance, when selective constraints reduce or prevent allelic variation of functionally essential genes. Therefore, a positional criterion was used to identify candidate genes that are most promising for further functional and structural characterization in the context of quantitative pathogen resistance in potato. Of 16 putative DS genes, 5 were located in the same genome segments as known potato QRL. These five positional candidate genes were *StSGT1-1, StSGT1-2*, *StPAD4-1*, *StPAD4-2,* and *StAOS2*. The 11 DS loci that were not positional candidates for known QRL are still the most closely related potato homologs of *Arabidopsis* defense genes, and we anticipate that they have conserved functions in potato. Overlapping positions of QRL and candidate genes are observed either by chance or because there is a causal relationship between allelic variation of the candidate gene and the observed QRL. Two DS genes, *StSGT1-2* and *StAOS2*, also were closely linked to *R* gene-like loci (Fig. 2), indicating that there are several candidates for the same QRL. Similarly, *StSGT1-1* also was linked to the defense response locus *StKI* encoding Kunitz-type proteinase inhibitor (Fig. 2). Whether these loci are, in fact, responsible for the QRL cannot be resolved in the populations used for linkage mapping. Further studies, such as association mapping and quantitative complementation analysis, are necessary.

Structural and functional relationships of selected candidate genes with pathogen resistance.

To further analyze the structure and function of the potato candidate genes, full-length cDNA and genomic fragments of potato *SGT1*, *PAD4,* and *EDS1* were cloned by PCR-based approaches and sequenced. For *StAOS2*, this information is available in the GenBank database (accession AJ457081). Although not a positional candidate itself, *EDS1* was included because *At*EDS1 and *At*PAD4 are known to interact directly and cooperate in expression of basal and *R* gene-mediated resistance (Feys et al. 2001). If *St*PAD4 and *St*EDS1 proteins also interact in potato, allelic variation of the interacting proteins could be the molecular basis of an interaction QRL that was detected by markers linked to the *StPAD4* and *StEDS1* loci in progeny derived from crossing the potato cultivars Escort and Leyla (Bormann et al. 2004). This possibility requires further investigation.

Table 3. Primers used for amplification of the candidate genes and markers for genetic mapping

Candidate gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Annealing temperature (°C)	Product size (bp)	Mapping population	Marker
StAOS1	aattcatcgtctcatcgtgttag	gcttcatcaagaacggaagttg	57.5	860	F1840	CAPS/*Rsa*I
StAOS2	tctcttcctcttccttctc	gaccgagagtgagtacagg	58.5	741	F1840	CAPS/*Taq*I
StAOS3	accaaagactcataccacat	atatccttcatggagttatag	59	803	F1840	CAPS/*Taq*I
StDND1	tccctcacatgcattattatgttgcc	agcgatctctcgacacgtaagc	63.5	874	Erwinia	CAPS/*Dde*I
StEDS1	actcatttcctctacatttcatcc	ttcagattacatgcagcatagc	59	1,288	F1840	CAPS/*Alu*I
StEDS5	ggacctttgatgagtcttattg	catgccaagcctcgaatctg	60.5	1,787	Erwinia	CAPS/*Taq*I
StHIN1	cggagcctattatggtccatcc	gatctgccactggactccaaag	60.5	624	K31	CAPS/*Hpa*II
StNDR1	tctcaggcttaacagctctc	tttataatctcgtcgtaacg	59	198	F1840	SSCP direct
StNPR1	gagcgagcttctcactcattgcgttg	agggaccaataatcgtgcaaatgcc	63.5	1,452	F1840	CAPS/*Msp*I
StPAD4-1	gaattttatgcaatttgaattttc	cggcatggaccattgccggatc	63	323	F1840	SSCP direct
StPAD4-2	tgttgaaaaaatatgttatactag	taaactggaaagaacatgatgggg	53.5	1,676	F1840	CAPS/*Rsa*I
StPEN1	atgggagataccggtggtgtc	ttgtaattgttgagcacctcctc	61.5	1,239	F1840	CAPS/*Msp*I
StRAR1	caatggagagacttcgatgtcag	acaaaagaatccctggtggcatc	58.5	1,415	K31	CAPS/*Alu*I
StSGT1-1	gccgttgacctctacactc	ccacctcctctggtttctg	62	2,864	K31	CAPS/*Taq*I
StSGT1-2	ttctatatcatgtgcatgaatctcg	acattagattagcccatgttctcc	59	1,887	F1840	CAPS/*Dde*I
StWRKY1	gccgggttcttgggactaatgg	tcaatgggatgtgaatgcatgccttc	65	2,010	K31	CAPS/*Msp*I

The potato genes *StSGT1-1* and *StSGT1-2* both are highly sequence related to *AtSGT1b*, a functional ortholog of yeast SGT1 (Austin et al. 2002; Azevedo et al. 2002). In yeast, SGT1 originally was described as a regulator of centromere and kinetochore function in cell cycle progression as well as in ubiquitin-mediated proteolysis (Kitagawa et al. 1999). Current data reveal multiple sites of action of plant, yeast, and human SGT1 as a co-chaperone of Hsp90 in assembly and activation of protein complexes (Muskett and Parker 2003; Schulze-Lefert 2004). These include plant R protein complexes governing resistance to bacterial, viral, and fungal pathogens. So far, only one full-length cDNA for *SGT1* has been cloned from another Solanaceous species, *Nicotiana benthamiana* (Liu et al. 2002), and no information has been reported regarding its copy number and chromosomal position in tobacco. *SGT1* is present in at least two copies in the potato genome which are located on chromosomes III and VI. Interestingly, both copies of *StSGT1* mapped to segments of the potato genome, where QRL were identified previously. The RACE-PCR-based approach identified only one *StSGT1* transcript in uninfected leaf tissue. The second gene might be expressed at different developmental stages, in other tissues, or under environmental conditions which were not tested.

SGT1 proteins have a remarkably conserved structure across distantly related plant species such as barley, rice, and *Arabidopsis*, and even across kingdoms, consistent with an ancient evolutionary origin and conserved function (Shirasu and Schulze-Lefert 2003). The potato equivalent described in this article is no exception. *StSGT1-1* shares with *Arabidopsis AtSGT1a* and *AtSGT1b* the 10 exons and the exon-intron boundaries, protein molecular mass, amino acid composition, and domain architecture.

A slight (twofold) upregulation of transcript levels upon infection of potato leaves with a compatible strain of *P. infestans* has been recorded in expression arrays, which included the *StSGT1-1* and *StSGT1-2* homologous EST probe STMEP46 (TIGR Solanaceae Gene Expression Database, study ID 62). Similarly, increased levels of *SGT1* homologous transcripts were detected by the same probe in a late blight field infection experiment of a population which segregated for quantitative resistance to late blight and in a defense signaling experiment (study IDs 50 and 64, respectively).

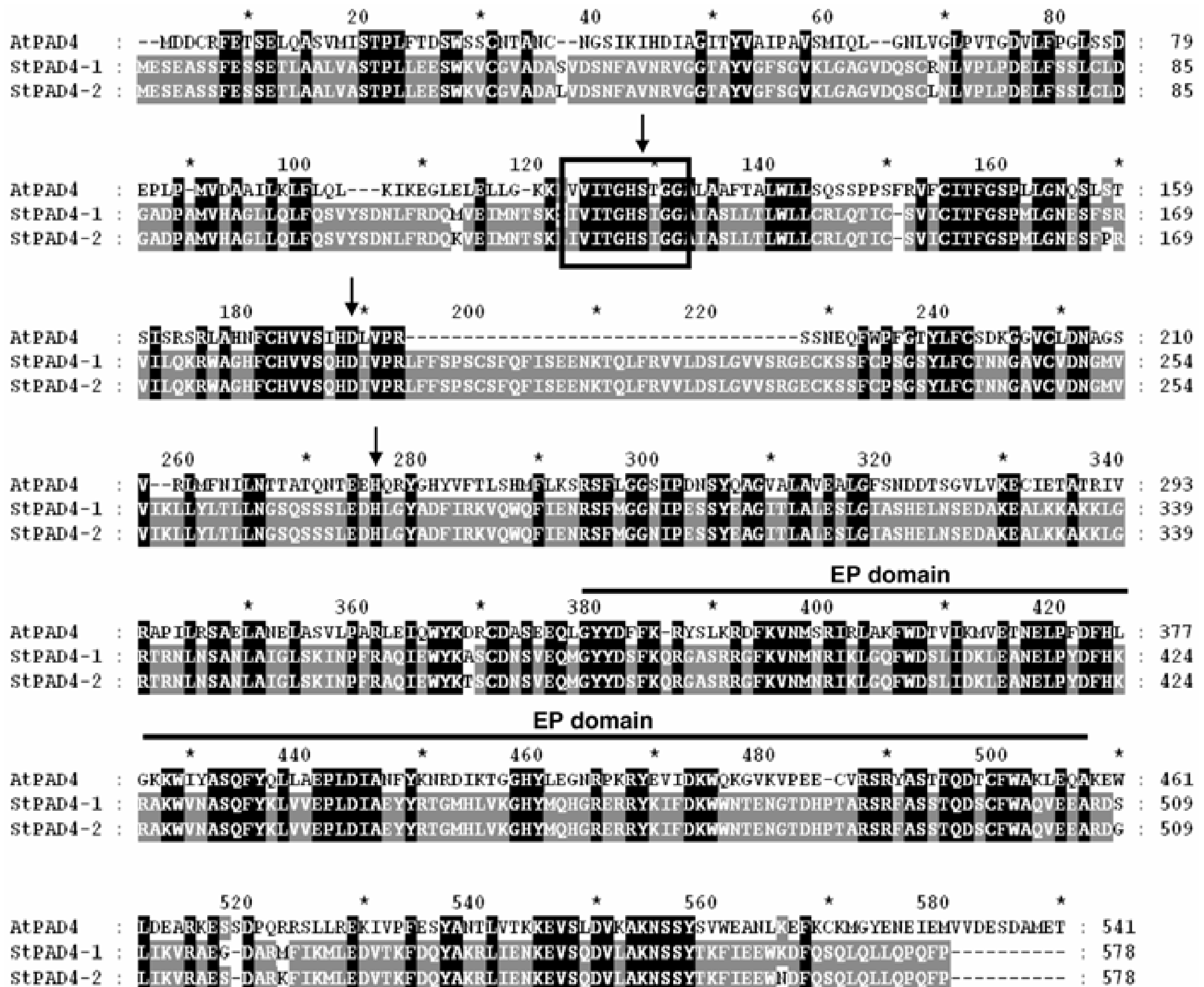

Fig. 4. Alignment of deduced amino acid sequences of *St*PAD4-1 and *St*PAD4-2 with *Arabidopsis thaliana* (*At*) PAD4 protein. The black and gray boxes represent 100 and 70% amino acid sequence conservation, respectively, between the deduced proteins compared. GenBank accession numbers: *At*PAD4, AAF09479; *St*PAD4-1, AY753546; and *St*PAD4-2, AY753547. The class 3 lipase consensus sequence around the predicted catalytic serine (S) is boxed. The three predicted lipase catalytic residues, a serine (S), an aspartate (D), and a histidine (H), are indicated by arrows. The EDS1- and PAD4-specific (EP) domain lies between amino acids 332 and 457 in *A. thaliana* and between amino acids 378 and 505 in potato.

The two *StPAD4* genes found in this study are homologous to *AtPAD4*, a gene originally identified as a necessary component of basal resistance to the oomycete pathogen *Peronospora parasitica* (Glazebrook et al. 1997b). *AtPAD4* also is required for resistance conditioned by TIR-type nucleotide-binding LRR proteins (Feys et al. 2001). Although *Arabidopsis pad4* first was identified in a screen for phytoalexin-deficient mutants, *At*PAD4 is not involved in the biosynthesis of camalexin, the phytoalexin of *Arabidopsis*, because the *pad4* mutant accumulated camalexin in response to infection by the fungus

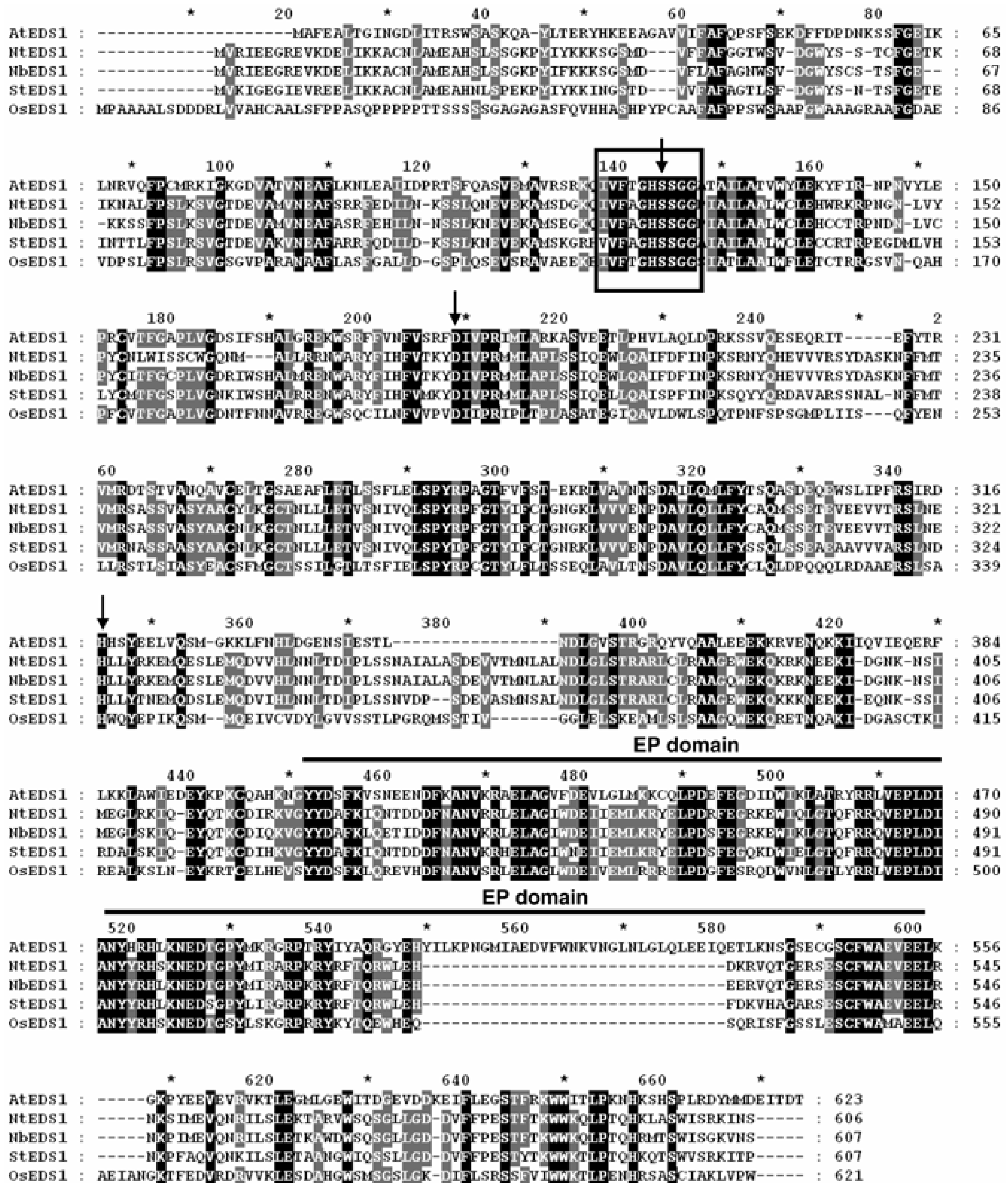

Fig. 5. Alignment of deduced amino acid sequence of *St*EDS1 with *Arabidopsis thaliana* (*At*), tobacco (*Nt*), *Nicotiana benthamiana* (*Nb*), and rice (*Os*) EDS1 proteins. The black and gray boxes represent 100 and 70% amino acid sequence conservation, respectively, between the deduced proteins compared. GenBank accession numbers: *At*EDS1, NP_190392; *Nt*EDS1, AAM62411; *Nb*EDS1, AAL85347; *St*EDS1, AY679160; and *Os*EDS1, XP_450883. The class 3 lipase consensus sequence around the predicted catalytic serine (S) is boxed. The three predicted lipase catalytic residues, a serine (S), an aspartate (D), and a histidine (H) are indicated by arrows. The EDS1- and PAD4-specific (EP) domain lies between amino acids 405 and 554 in *A. thaliana* and between amino acids 426 and 544 in potato.

Cochliobolus carbonum, nonpathogenic on *Arabidopsis* (Glazebrook et al. 1997b). The production of the potato phytoalexins rishitin and lubimin could be controlled in an analogous way, indirectly promoted by *PAD4* homologous genes upon challenge with an appropriate pathogen.

Structurally, the *At*PAD4 protein shows high similarity to class 3 triacyl glycerol lipases, although lipase enzymatic activity has not been demonstrated (Jirage et al. 1999). The three catalytic residues, a serine, an aspartate, and a histidine, embedded within the N-terminal lipase domain, were found in both potato homologs. To our knowledge, *StPAD4-1* and *StPAD4-2* are the first *AtPAD4* homologous genes of Solanaceous plants to be mapped and cloned. Comparison of the genomic sequences revealed structural differences between potato and *Arabidopsis PAD4*. The potato *PAD4* homologs have four exons whereas *Arabidopsis PAD4* is composed of two exons.

The potato EST for *PAD4* that was used as template for primer design in this study originates from a library generated from potato leaves challenged with an incompatible strain of *Phytophthora infestans* (TIGR Solanaceae Gene Expression Database, SGEdb). Moreover, an up to fourfold increase in *StPAD4* transcript was detected in leaf tissue after infection with a compatible strain of *P. infestans*, in the QTL study for late blight disease development in the field (natural infection) and in the defense signaling experiment (SGEdb, study IDs 50, 62, and 64, probes STMER19 and STMEN29). Furthermore, in the field infection experiment (study ID 50), *StPAD4* transcript levels remained elevated even 21 days after infection. The predicted function of both *StPAD4* genes, expression profiles, and localization within QRL for *P. infestans* resistance all support a role of *StPAD4* genes in the late blight defense responses. *StPAD4-1* and *StPAD4-2* share substantial sequence homology at the transcript level and are genetically tightly linked. At this stage, it cannot be resolved which of the two *StPAD4* transcripts is up regulated upon pathogen attack or which is involved in defense signaling by another mechanism. Both genes might be required for the establishment of defense responses. The possibility of duplicated loci underlying a QTL was postulated by Szalma and associates (2002) for maize *whp1* and *c2*, both encoding chalcone synthase. A plausible hypothesis would be the existence of *StPAD4-1* and *StPAD4-2* functional alleles that vary in spatial or temporal regulation of expression, enzymatic activity, or protein stability.

Yeast two-hybrid and in planta co-immunoprecipitation experiments revealed a direct physical interaction between *At*PAD4 and *At*EDS1, another lipase-like protein (Feys et al. 2001). At their C-termini, EDS1 and PAD4 from diverse plant species share an EP domain that also is present in the potato PAD4 and EDS1 homologs. *St*EDS1, like *St*PAD4, has all the features of a class 3 lipase. EST probes corresponding to *StEDS1,* identified in this study, detected a twofold upregulation of the transcript in the compatible interaction with *P. infestans* and in the defense signaling experiment (SGEdb, study IDs 62 and 64, probes STMIX37, STMCD71, and STMDT13); however, without any clear trend in the time course. Recently, the orthologous *EDS1* gene of the closely related tomato (*Solanum lycopersicum* L.) was mapped to a corresponding segment of chromosome VI and was shown to be required for resistance to pathogens mediated by certain types of *R* genes as well as for the basal defense (Hu et al. 2005). We conclude that *St*PAD4-1, *St*PAD4-2, and *St*EDS1 are likely to be the functional potato equivalents of these two *Arabidopsis* disease signaling components.

The fourth candidate locus, *StAOS2*, encodes a putative allene oxide synthase (AOS), a member of the cytochrome P450 superfamily. AOS acts upstream in the JA biosynthesis pathway, catalyzing dehydration of the 13-hydroperoxylinolenic acid to an unstable allene oxide. Jasmonate responses are triggered rapidly upon wounding in *Arabidopsis* (Park et al. 2002) and tomato plants (Sivasankar et al. 2000). Cohen and associates (1993) found that induction of the jasmonate response using methyl jasmonate resulted in increased resistance of potato to *P. infestans*. Consistent with these observations, recent experiments on the jasmonate-deficient *def-1* tomato mutant revealed that plants lacking JA show significantly increased susceptibility to a number of pathogens, including *P. infestans* (Thaler et al. 2004). A similar study in *Arabidopsis* indicated that the jasmonate-insensitive *coi1* mutant displayed enhanced susceptibility to necrotrophs, including *E. carotovora* subsp. *carotovora* (Thomma et al. 2001; Vijayan et al. 1998), which is a close relative of *E. carotovora* subsp. *atroseptica*, the pathogen of interest in our study. *AOS* is a single-copy gene in *Arabidopsis.* Whereas, in other plants, more than one enzyme with AOS activity can be found. Both monocots and dicots appear to contain small families of *AOS* genes (Howe et al. 2000; Itoh et al. 2002; Maucher et al. 2000). In potato, the *AOS* gene family has at least three members, located on three different chromosomes, and only *StAOS2* is a positional candidate at present. To date, no information has been reported regarding the role of any *StAOS* gene in planta. Based on partial information on *StAOS2* expression, transcript levels remained unaffected 3 days post inoculation with a compatible strain of *P. infestans* when comparing challenged plants to the healthy control (TIGR SGEdb, study ID 62, probe STMCR05). In this case, the postulated QTL effects of *StAOS2* alleles may result from variation in enzyme activity, post-translational regulation, or protein stability, rather than gene expression.

MATERIALS AND METHODS

Plant material.

Genomic DNA (purified according to Gebhardt and associates 1989) of three diploid potato genotypes (H79.691/37, H80.601/4, and H83.385/14) was used for initial PCR amplification of potato genomic fragments with high sequence similarity to *A. thaliana* genes. These genotypes were not parents of mapping populations. For mapping of the candidate genes, genomic DNA of parents and progeny of three diploid potato mapping populations were used. Populations F1840 and K31 consisted of 100 and 113 individuals, respectively. Molecular maps based on RFLP and CAPS markers have been constructed for the 12 chromosomes of both parents in these populations (Chen et al. 2001; Gebhardt et al. 2003; Leister et al. 1996; Schäfer-Pregl et al. 1998). In the K31 population, QTL for resistance to late blight caused by *P. infestans* have been mapped (Oberhagemann et al. 1999). The “Erwinia” population, consisting of 158 individuals, was developed in collaboration with the Plant Breeding and Acclimatization Institute in Młochów, Poland. Molecular maps of maternal and paternal chromosomes were constructed based on AFLP and RFLP markers. QTL for resistance to tuber soft rot and blackleg disease caused by *E. carotovora* subsp. *atroseptica* have been mapped using this population (Zimnoch-Guzowska et al. 2000).

PCR and DNA sequencing.

PCR were carried out according to a standard protocol. Genomic DNA (50 ng) was amplified in 30 µl of 20 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dATP, dGTP, dCTP, and dTTP (Carl Roth & Co. KG, Karlsruhe, Germany), 0.25 µM each primer (Qiagen Operon Biotechnologies, Cologne, Germany), and *Taq* DNA polymerase (Invitrogen, Life Technologies, Karlsruhe, Germany) at 0.03 U/µl. Reaction conditions were as follows: initial sample denaturation (3 min, 95ºC), 35 cycles of denaturation (20 s, 94ºC), primer annealing

(40 s, annealing temperatures listed in Table 3), and elongation (1 min per 1 kb, 72ºC), terminated by final elongation (72ºC, 10 min). PCR products were purified using Qiagen PCR fragments purification kit or Qiagen gel extraction kit (Qiagen, Hilden, Germany) and sequenced to confirm product specificity. DNA sequences were determined by the DNA core facility (ADIS) of Max-Planck Institute for Plant Breeding Research on Abi Prism 377, 3100 or 3730 sequencers (Applied Biosystems, Weiterstadt, Germany) using BigDye-terminator (v3.1) chemistry. Premixed reagents were from Applied Biosystems.

Gene sequence analyses.

BLAST searches with specific gene sequences of *A. thaliana* (Table 1) were performed on the websites of the Institute of Genomic Research (TIGR) and of the Solanaceae Genomic Network (SGN) containing sequences of approximately 125,000 and 80,000 potato ESTs, respectively. ESTs originated from various potato tissues, including healthy leaf tissue and leaves after challenge with *P. infestans* (compatible and incompatible interactions) (Ronning et al. 2003). Exon–intron boundaries of potato genomic fragments were predicted using the DNASTAR software package (DNASTAR, Madison, WI, U.S.A.). Sequence multialignments were computed online with the MultAlin software (Corpet; INRA Toulouse, France) and shaded in GeneDoc (Nicholas; Pittsburgh Supercomputing Center, PA, U.S.A.). Amino acid sequences of cloned genes, molecular masses, and pIs of deduced polypeptides were obtained using the ExPASy Translate and ProtParam Tools. Profile searches were made using the InterProScan Sequence Search tool on the website of EMBL-EBI. The subcellular localization was predicted using PSORT and TargetP.

Genetic mapping of candidate genes.

PCR were performed using gene-specific primers (Table 3) and genomic DNA of parents and progeny as template. PCR products from parents of the mapping population that were polymorphic for a given gene marker were resequenced in order to confirm their identity and specificity. CAPS markers were developed for 14 candidate genes that segregated in at least one mapping population. PCR product (7 µl; sizes varying from 624 to 2,864 bp) were digested with two units of the appropriate restriction enzyme (Table 3) (New England Biolabs GmbH, Frankfurt, Germany) for 4 h, according to the supplier's instructions. Restriction enzyme-digested PCR-generated DNA fragments were separated on ethidium bromide-stained, 2.5% agarose gels (Invitrogen). Two genes (*StNDR1* and *StPAD4-1*) were mapped as SSCP markers. SSCP analysis was performed as described (Bormann et al. 2004). Segregating CAPS and SSCP fragments were scored as present (1) or absent (0). Fragments that could not be scored reliably were declared as missing values (2). The fragments were mapped relative to the existing marker database using the MAPRF software package (Ritter et al. 1990).

Cloning of cDNA and genomic DNA of candidate genes.

To obtain full-length cDNA and genomic sequences of *StSGT1, StPAD4,* and *StEDS1*, RACE-PCR was employed. Total RNA was isolated from 100 mg of fresh, healthy leaf tissue of potato plants (cv. Desirée) grown in the greenhouse. The tissue was flash frozen and ground in liquid nitrogen. Total RNA was extracted with 1 ml of RNAwiz extraction reagent (Ambion, Huntingdon, Cambridgeshire, U.K.) following the supplier's protocol. Poly(A)$^+$ RNA was purified using Dynabeads Oligo $(dT)_{25}$ (Dynal Biotech GmbH, Hamburg, Germany) according to the supplier's instructions. Poly(A)$^+$ RNA was eluted in 20 µl of diethylpyrocarbonate-treated water. The BD SMART RACE cDNA Amplification Kit (BD Biosciences Clontech, East Meadow Circle, CA, U.S.A.) was used for the synthesis of RACE-ready cDNA and for the subsequent RACE experiments. Two independent populations of 5′-RACE-Ready and 3′-RACE-Ready cDNAs were synthesized, each using 250 ng of poly(A)$^+$ RNA, according to the supplier's protocol. RACE-PCR reactions were performed on the RACE-ready cDNA templates, using the Universal Primer Mix provided in the kit, and the following gene-specific primers: *StPAD4*-5′RACE: 5′gcagacggcagagaagccagagag3′, *StPAD4*-3′RACE: 5′cgccgttgctggaggagtcatggaag3′, *StEDS1*-5′RACE: 5′gtgctca ggccaaggtcattcagtgctg3′, *StEDS1*-3′RACE: 5′ggcagctctctggtgt ctggaatggtgc3′, *StSGT1*-5′RACE: 5′gctcttctggtgtcctcgtcagcatc3′, and *StSGT1-1*-3′RACE: 5′cctcagctagtgtcgttgcacctcctgc3′. The RACE-PCR Touch Down program was as follows: 5 cycles of 94ºC for 30 s and 72ºC for 3 min; 5 cycles of 94ºC for 30 s, 70ºC for 30 s, and 72ºC for 3 min; and 23 cycles of 94ºC for 30 s, 68ºC for 30 s, and 72ºC for 3 min. Each PCR reaction (6 µl) was loaded on 1.5% agarose gel and, in each case a clear, single product was observed, except for *StSGT1* 3′RACE, where two fragments were amplified. RACE products were cloned into the pCR2.1-TOPO vector (TOPO TA Cloning Kit; Invitrogen) and sequenced. 5′RACE and 3′RACE sequences were assembled in silico to obtain the full-length cDNA sequence for *StPAD4*, *StEDS1,* and *StSGT1*. Only the larger 3′RACE product of *StSGT1* (approximately 950 bp) was found to be specific. Gateway Technology (Invitrogen) was used to generate full-length cDNA clones that could be used as universal Entry clones in subsequent experiments (e.g., helpful for quick generation of constructs for complementation or over-expression analyses). Gateway Technology-compatible primers flanking the deduced full-length cDNA sequences were designed as follows: *StSGT1* forward: 5′(GWF)taatggcgt ccgatctggagactag3′, *StSGT1* reverse (Δstop): 5′(GWR)cgatctc ccatttcttcagctccatg3′, *StEDS1* forward: 5′(GWF)taatggtgaaaatt ggagaaggaattg3′, *StEDS1* reverse (Δstop): 5′(GWR)caggagttatttt ccttgatacccaag3′, *StPAD4-1* and *StPAD4-2* forward: 5′(GWF)ta atggaatcggaagcttcatcgttc3′, and *StPAD4-1* and *StPAD4-2* reverse (Δstop): 5′(GWR) caggaaactgaggttggagcagctg3′. The universal Gateway-compatible extensions for the BP recombination reactions (between an attB-flanked PCR product and a donor vector containing attP sites to create an entry clone) were GWF (attB1) 5′ggggacaagtttgtacaaaaaagcaggctta3′ and GWR (attB2) 5′ggggaccactttgtacaagaaagctgggtc3′. Oligonucleotides were purchased from Invitrogen. Full-length cDNA sequences were amplified on 50 ng of 5′-RACE-ready cDNA template using high fidelity proof reading TAKARA LA *Taq* polymerase (Takara, Seta 3-4-1; Otsu, Shiga 520-2193, Japan), with the following PCR protocol: initial denaturation for 2 min at 93ºC, 29 cycles of denaturation (15 s at 93ºC), primer annealing (30 s at 64ºC), and elongation (1 min per 1 kb of expected product size at 68ºC), terminated by final elongation (68ºC for 5 min). PCR Master Mix was prepared according to the supplier's protocol. PCR products were cloned into the pDONR201 Gateway vector (Invitrogen) and three positive entry clones of each gene were resequenced to confirm product specificity and obtain a consensus sequence. The same conditions were used for PCR reactions with potato genomic DNA (cv. Desirée) to amplify full-length genomic fragments. Similarly, PCR products were cloned into the pDONR201 vector (Invitrogen) and sequenced on both strands as described above.

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AUTHOR-RECOMMENDED INTERNET RESOURCES

EMBL-European Bioinformatics Institute InterProScan sequence search webpage: www.ebi.ac.uk/InterProScan
The ExPASy (Expert Protein Analysis System) proteomics translate and ProtParam tools: www.expasy.org/tools
Food and Agriculture Organization of United Nations statistical database (FAOSTAT): faostat.fao.org
Genomanalyse im biologischen System Planze (GABI) PoMaMo (Potato Maps and More) database: gabi.rzpd.de/PoMaMo.html
PSORT, a program for the prediction of protein localization sites psort.ims.u-tokyo.ac.jp
Solanaceae Genomics Network (SGN) BLAST interface: www.sgn.cornell.edu/cgi-bin/tools/blast/simple.pl
The TIGR Gene Indices: tigrblast.tigr.org/tgi
TIGR Solanaceae Gene Expression database (SGEdb): www.tigr.org/tigr-scripts/tdb/potato/study/potato_expression.pl
Technical University of Denmark's Center for Biological Sequence (CBS) TargetP program for predicting the subcellular location of eukaryotic proteins: www.cbs.dtu.dk/services/TargetP