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Ancient Diversification of the *Pto* Kinase Family Preceded Speciation in *Solanum*

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Recent phylogenetic analyses of the nucleotide binding sites (NBS)–leucine-rich repeats (LRR) class of plant disease resistance (*R*) genes suggest that these genes are ancient and coexist next to susceptibility alleles at resistance loci. Another class of *R* genes encodes serine–threonine protein kinases related to *Pto* that were originally identified from wild relatives of tomato. In this study, we exploit the highly diverse genus *Solanum* to identify *Pto*-like sequences and test various evolutionary scenarios for *Pto*-like genes. Polymerase chain reaction amplifications with the use of primers that were developed on the basis of conserved and variable regions of *Pto* revealed an extensive *Pto* gene family and yielded 32 intact *Pto*-like sequences from six *Solanum* species. Furthermore, *Pto*-like transcripts were detected in the leaf tissue of all tested plants. The kinase consensus and autophosphorylation sites were highly conserved, in contrast to the kinase activation domain, which is involved in ligand recognition in Pto. Phylogenetic analyses distinguished nine classes of *Pto*-like genes and revealed that orthologs were more similar than paralogs, suggesting that the *Pto* gene family evolved through a series of ancient gene duplication events prior to speciation in *Solanum*. Thus, like the NBS-LRR class, the kinase class of *R* genes is highly diverse and ancient.

Additional keywords: evolution, potato, wild *Solanum* spp.

The occurrence of conserved structural features in plant disease resistance (*R*) genes provides great potential for the isolation of novel analogs of these *R* genes when polymerase chain reaction (PCR) is used. In most *R* genes cloned so far, one or more characteristic functional domains are conserved across plant families. Such domains include the leucine-rich repeats (LRR), nucleotide binding sites (NBS), leucine zippers (LZ), and transmembrane (TM) and kinase domains, and suggest that *R* genes encode components of conserved and ubiquitous signal transduction pathways in plants (Hammond-Kosack and Jones 1997). A PCR-based strategy with degenerate primers corresponding to NBS motifs was applied to Arabidopsis, soybean, potato, lettuce, and several other plants, resulting in the cloning of a large collection of resistance gene candidates (RGAs or *R* gene analogs) from the NBS–LRR class (Aarts et al. 1998; Kanazin et al. 1996; Leister et al. 1996; Shen et al. 1998; Yu et al. 1996). *R* genes from other classes such as the LRR, LRR–TM, LRR–TM kinase, and kinase class (Bent 1996; Richter and Ronald 2000) do not possess a NBS domain. Yet, a similar gene discovery approach can be applied with primers on the basis of other conserved domains such as the kinase domain.

The *Pto* gene from tomato confers hypersensitive response (HR)-mediated resistance to *Pseudomonas syringae* pv. *tomato* (Martin et al. 1993a). *Pto* is a member of a multigene family that is clustered within a 400-kb region on chromosome 5 of tomato (*Lycopersicon*) (Martin et al. 1993b). Another member of this family is *Fen*, which confers sensitivity and HR-like lesions to the insecticide fenthion (Martin et al. 1994). Characterization of the Pto and Fen proteins revealed that they are 80% identical and 87% similar, encode active serine–threonine kinases, and participate in the same signal transduction pathway that leads to the HR (Loh and Martin 1995; Martin et al. 1993a; Martin et al. 1994). *Pto* and *Fen* originate from the wild species *Lycopersicon pimpinellifolium* and were introgressed into tomato cultivars by classical breeding approaches (Pitblado and Kerr 1980). Homologs of *Pto* and *Fen* genes, *pto* and *fen*, respectively, were cloned from the cultivated tomato *Lycopersicon esculentum* and encode active kinases but do not confer bacterial speck resistance or fenthion sensitivity (Jia et al. 1997). *Pto* versus *pto* and *Fen* versus *fen* homologs differ only in a few amino acids, which presumably cause conformational changes that affect their ability to interact physically with other proteins (Jia et al. 1997). Pto specifically phosphorylates other kinases such as Pto interacting kinase 1 (Zhou et al. 1995) and interacts physically with the avirulence gene product AvrPto (Scofield et al. 1996; Tang

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et al. 1996). The activation domain (the region between amino acid 182 and 211 of Pto) plays a critical role in recognition. In the Pto–AvrPto interaction, S198 is required for elicitation of the HR (Sessa et al. 2000b), T204 is required for recognition specificity, Y205 plays a subsidiary role in recognition (Frederick et al. 1998), and Y207 influences binding properties (Rathjen et al. 1999). In addition, autophosphorylation sites appear to be required for kinase activity or physical interaction with proteins, including AvrPto and several *Pto* interacting proteins (Sessa et al. 2000b).

Despite tremendous advances in the structural molecular genetics of *R* genes (Baker et al. 1997), the evolution of recognitional specificities remains poorly understood. Genomic sequencing and genetic mapping studies revealed the occurrence of clusters of *R* genes in the genome (Michelmore and Meyers 1998; Richter and Ronald 2000). In tomato, the *Pto* locus evolved through a series of gene duplications and deletions, resulting in five *Pto* homologs (Michelmore and Meyers 1998). Additionally, other *R* gene families such as those at the tomato *Hcr9* loci, which harbor homologs of *Cladosporium fulvum* resistance gene *Cf9*, and the lettuce *Dm3* locus, which contains *Bremia lactucae* resistance genes, occur in duplicated gene clusters (Meyers et al. 1998; Parniske and Jones 1999). A wide diversity of *R* genes of the NBS class occurs in the plant kingdom, and elegant phylogenetic analyses of an extensive set of NBS-containing *R* gene-like sequences pointed to an ancient evolutionary history for these genes (Meyers et al. 1999). In addition, recent population genetic data suggests that *R* genes of the NBS–LRR class coexist next to susceptibility alleles (Stahl et al. 1999). The results of both of these studies conflict with the widely accepted "arms race" model for the rapid evolution of resistance genes as a response to an adapting pathogen. A novel "trench warfare" theory is thus emerging, which encompasses a dynamic process of advances and retreats of ancient resistance alleles to maintain diversity at a disease resistance locus (Michelmore and Meyers 1998; Stahl et al. 1999).

The genus *Solanum* is highly diverse, consisting of approximately 1,100 species (D'Arcy 1991). On the basis of chloroplast DNA (cpDNA) restriction fragment analysis, *Solanum* spp. is thought to form a monophyletic lineage that includes *Lycopersicon* (Spooner et al. 1993); here, we will refer to *Solanum* in the broad sense. The center of origin of *Solanum* is thought to be in Mexico, where species migrated southward and evolved into a separate gene pool in South America (Hawkes 1990). The cultivated potato *Solanum tuberosum* arose in this area, probably from a complex of diploid domesticated *Solanum* species. A selection of wild *Solanum* species has been incorporated into modern breeding programs to introgress resistance against a broad spectrum of potato pathogens. For example, *Solanum demissum* has supplied the 11 known *R* genes that confer HR-mediated resistance to the potato late blight pathogen *Phytophthora infestans*. Several other tuber-bearing *Solanum* species also show HR-mediated resistance, which suggests the involvement of *R* genes in late blight resistance (Vleeshouwers et al. 2000).

In this study, we take advantage of the genetic diversity of the genus *Solanum* to test the coevolutionary arms race hypothesis for the *Pto* gene family. We amplified *Pto*-like sequences from a diverse set of *Solanum* species with primers on the basis of conserved and variable regions of known *Pto*-like genes. Sequencing and Southern blot analyses revealed an extensive *Pto*-like gene family in *Solanum*, and Northern blot analyses showed expression of *Pto*-like genes in leaves. Phylogenetic analyses revealed that orthologs (homologs separated by a speciation process) are more similar than paralogs (homologs generated by a gene-duplication event), suggesting that ancient duplications of the common ancestor of *Pto*-like genes probably occurred prior to *Solanum* speciation.

RESULTS

Pto-like sequences are diverse in *Solanum* spp.

A set of diverse potato cultivars and wild *Solanum* species was selected (Table 1). To explore the presence of *Pto*-like sequences, a Southern blot containing *Eco*RV-digested genomic DNA from the selected *Solanum* plants was hybridized with a probe from the *Pto*-like clone berDF1 from *Solanum berthaultii* (berDF1 was obtained by PCR with primers based on *Pto*; see below) (Fig. 1). In each lane, four to nine bands were detected, revealing the presence of an extensive family of *Pto*-like sequences in *Solanum*. Variation in the signal intensity among the hybridizing bands suggests sequence diversity or the presence of multiple copies (per *Eco*RV fragment).

Table 1. *Solanum* species and hybrids used in this study

Solanum species/hybrids	Code	Accession–origin	Country of collection	Plant clones–cultivars
ABPT-hybrid[a]	ABPT	Plant Breeding, WUR		44
S. arnezii × *hondelmannii*	axh	BGRC 27308[b]	Bolivia	63, 72
S. berthaultii	ber	BGRC 10063	Bolivia	9, 11
S. circaeifolium ssp. *circaeifolium*	crc	BGRC 27058	Bolivia	circ1
S. demissum	dms	CPC[c] 2127	Mexico	PBL[d]
S. microdontum	mcd	BGRC 24981	Argentina	167, 178
S. microdontum var. *gigantophyllum*	mcd	BGRC 18570	Unknown	265
S. nigrum	ngr	Plant Res. Int., WUR	Netherlands	SN18
S. nigrum × *tuberosum*[e]	nxt	Plant Res. Int., WUR		SN18 × Des
S. sucrense	scr	BGRC 27370	Bolivia	23, 71
S. tuberosum	tbr	Potato cultivars		Bintje, Ehud, Estima, Première, Robijn
S. vernei	vrn	BGRC 24733	Argentina	530

[a] Double-bridge hybrid (30 × 33) of *S. acaule, S. bulbocastanum, S. phureja, S. tuberosum* (Hermsen and Ramanna 1973).
[b] Braunschweig Genetic Resource Center (Germany), which moved to Center of Genetic Resources, The Netherlands.
[c] Commonwealth Potato Collection, Scottish Crop Research Institute, U.K.
[d] Plant Breeding Line, obtained from D. J. Huigen, Laboratory of Plant Breeding, Wageningen University.
[e] Hybrid of *S. nigrum*–SN18 × *S. tuberosum* cv. Désirée (Eijlander and Stiekema 1994).

Within species, the hybridization patterns were partly conserved, suggesting conservation of the *Pto*-like sequences. Intraspecific polymorphisms also were revealed for the different *Solanum* species. Hybridization of the same Southern blot with a probe from a distinct member, berDF4 of the *Pto* family (see below), yielded a similar banding pattern with only slight differences in intensity of some bands (data not shown), indicating that most family members share a high level of identity at the DNA level.

Pto-like genes expressed in _Solanum_ leaves.

To examine expression of *Pto* homologs in *Solanum*, a Northern blot was prepared containing poly(A)$^+$ RNA isolated from leaves from a subset of the selected set of plants (Fig. 2). Hybridization with the berDF1 probe revealed the presence of *Pto*-like transcripts in all tested *Solanum* plants. Several bands with slight size differences could be discriminated for individual plant genotypes, suggesting the presence of a mixture of *Pto*-like mRNAs. The same blot was hybridized with a potato tubulin probe, and signals from *Pto*- and tubulin-hybridizing bands were quantified with a phosphorimager. Calculation of the relative amounts of *Pto*-like mRNA revealed similar levels of *Pto*-like mRNA in wild *Solanum* species (data not shown). Within the potato cultivars, the relative mRNA levels also were similar but, overall, were higher than in their wild relatives. In summary, these results suggest that *Pto*-like genes are expressed in all tested *Solanum* plants.

Pto-like sequences from _Solanum_ spp.

An iterative PCR-based approach was adopted to identify *Pto*-like sequences from a diverse set of *Solanum* species. Forward primer F_1 and reverse primers R_1 and R_2 were designed on the basis of sequences that were conserved in *Pto* and *Fen* from *L. pimpinellifolium* but divergent in other kinases (Fig. 3). An initial set of *Solanum* DNA fragments was amplified and sequenced. This sequence information was used to design additional forward primers F_4 and F_5 on the basis of a semiconserved region. In addition, two specific primers were designed: forward primer F_2, based on dmsFD2 and a common 2 amino acid insertion–deletion (in–del), and reverse primer S_1, based on berS1 and a highly variable region in the 3 amino acid position.

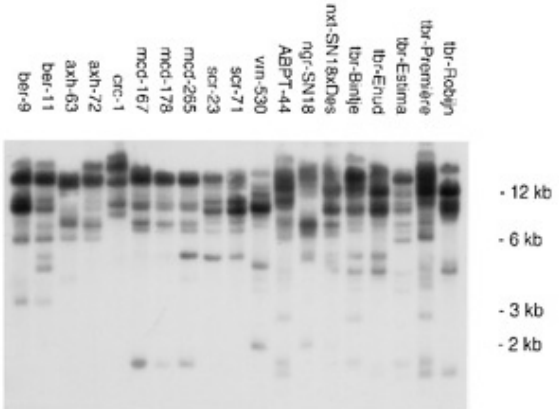

Fig. 1. Multiple *Pto*-like sequences in *Solanum*. Autoradiograph of a Southern blot containing *Eco*RV-digested genomic DNA from *Solanum* plants hybridized with a berDF1 probe. For plant code abbreviations, see Table 1.

PCR with the conserved, semiconserved, and specific primers yielded a large and diverse pool of *Pto*-like sequences in *Solanum*, and 66 clones were sequenced. Of these, 20 contained frame shifts and stop codons and were excluded from further analyses. Among the remaining 46 sequences, 14 were redundant. The 32 remaining *Pto*-like sequences were included for further study (Table 2). Sequence alignments were performed and revealed a higher conservation in the 5′ part of the gene than in the 3′ part.

Phylogenetic analyses of the _Pto_ family.

To develop a data set for phylogenetic analyses, we used BLAST searches to mine the NCBI and TIGR databases with the *Pto* sequence (Altschul et al. 1997). This resulted in a large set of (putative) kinases, predominantly receptor-like and serine–threonine kinases from various plants, including *Arabidopsis thaliana*, *Catharanthus roseus*, *Zea mays*, and *Oryza sativa*. Other homologous sequences such as the receptor-like kinase homolog from *A. thaliana* (*At*-62020, accession no. CAB62020) and a *L. esculentum* expressed sequence tag clone (*Le*-776077, accession no. AI776077), in addition to the *Pto–Fen* homologs from *Lycopersicon*, *Nicotiana* (*Nt*-HPto), and *Solanum*, PCR fragments were included in a data set (Jia et al. 1997; Martin et al. 1993a; Martin et al. 1994; Martin et al. 1996). *Pto*-like sequences also were reported from *Capsicum annuum* (Pflieger et al. 1999). Because they represent pseudogenes, however, they were not included. Phylogenetic analyses were performed with the neighbor-joining and maximum parsimony algorithms. Statistical significance of the identified groups was evaluated with bootstrapping. Similar clustering of sequences was obtained with both methods (Fig. 4 and data not shown). Nine different classes (I to IX) could be distinguished on the basis of phylogeny and in–del distribution (Table 3).

Class IX includes disparate Solanaceae and Cruciferae spp. sequences, which suggests that this class diverged prior to the radiation of the Solanaceae. In addition, all class IX sequences contain a three amino acid in–del (Table 3) that is conserved in a broader range of serine–threonine receptor-like kinases from *A. thaliana*, *C. roseus*, *Z. mays*, and *O. sativa*. (obtained with BLAST searches with *Pto*; data not shown). Therefore, class IX was used to root the tree.

Features of _Pto_-like sequence classes.

The sequence characteristics of the nine classes of *Pto*-like sequences were carefully analyzed and are summarized in

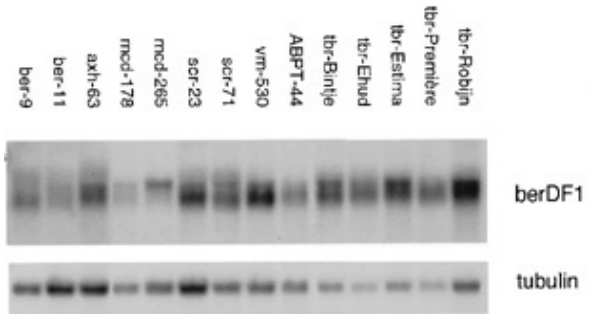

Fig. 2. *Pto*-like transcripts in *Solanum* leaves. Autoradiographs of a poly(A$^+$) Northern blot of *Solanum* leaves hybridized with a berDF1 and a tubulin probe. For plant code abbreviations, see Table 1.

Table 3. Class I contains the two *Pto* alleles from *Lycopersicon* Class II includes the *Fen* alleles from *Lycopersicon* as well as berDF1 from *S. berthaultii*. In class IX, two non-*Solanum* sequences from *Nicotiana* and Arabidopsis cluster together with four *Solanum–Lycopersicon* sequences. All members of this class share a 3 amino acid in–del at position 159 (numbered according to the *Pto* sequence), in contrast to all other *Solanum*. *Pto*-like sequences. An additional multiple amino acids in–del, AVGRY, was evident at position 245 in scr8E, scr8I, scr8B, scr8D, and tbr5A, all of which were classified as class V–VI in the phylogenetic tree. Although these in–dels were excluded from the phylogenetic analysis, their occurrence is consistent with the classification.

In the *Pto*-like sequences from *Solanum*, the 15 amino acid residues of the protein kinase consensus (Hanks and Quinn 1990) were highly conserved (Table 3). Autophosphorylation sites, identified previously in *Pto*, show a high degree of conservation; for example, T38 and T288 are conserved in all sequences. At position 133, the threonine was often replaced by a serine, thereby providing an alternative phosphorylation site. Additionally, T195, S198, and T199 are highly conserved, except for the class V–VI sequences. Furthermore, the kinase activation consensus residues domain appeared well conserved compared with other plant serine–threonine kinases (Sessa et al. 2000a) and showed only one amino acid change in classes V–VI, VII, and VIII sequences. In contrast, there was more variation in the entire activation domain than in the 5′ part of the *Pto*-like sequences (Table 3 and data not shown).

DISCUSSION

In this study, a diverse pool of 32 *Pto*-like sequences was identified and characterized from two potato cultivars and five wild *Solanum* species, revealing a complex family of *Pto*-like genes in *Solanum* spp. Several *Pto*-like transcripts of varying sizes were detected in all tested *Solanum* plants, suggesting that various members of the gene family are expressed in *Solanum* leaves. All amplified *Solanum* sequences displayed fully conserved serine–threonine kinase motifs, suggesting that the uncovered genes are likely to encode active kinases and are members of a large family of *Pto*-like kinases.

Most cloned *R* genes are members of multigene families, indicating that gene duplication and subsequent diversification are common processes in *R* gene evolution (Ronald 1998). For example, the *Xa21* gene family evolved through gene duplication, recombination, and diversification into two distinct classes of genes in rice (Song et al. 1997). In the present study, phylogenetic analyses of the *Pto*-like sequences revealed nine different classes in *Solanum*, several of which occur in a single *Solanum* species and represent paralogous sequences. The observation that orthologs are more similar than paralogs indicates that gene duplications, the most likely source of diversification of *Pto*-like genes, probably occurred prior to *Solanum* speciation.

The phylogenetic analyses provided an insight into the evolution of *Pto*-like genes in the Solanaceae. We used a combination of sequence-based phylogenies and the distribution of in–dels in the various classes and across various plant families in an attempt to reconstruct the evolutionary history of *Pto*-like sequences (Fig. 5). We hypothesize that prior to the divergence of Solanaceae from other plants, at least one gene-duplication event of a common ancestral *Pto* gene occurred. A three amino acid deletion diagnostic of *Pto*-like sequences from the Solanaceae but not from other dicots and monocots may have occurred early in Solanaceae evolution, distinguishing classes I through VIII. Subsequently, several gene-duplication and diversification events gave rise to the nine identified *Pto*-like classes in *Solanum* spp.

Primer	Sequence (5′-3′)	Location on Pto (aa)	Specificity
F1	CAAATTCGATAAATGATGC	9-15	conserved
F2	AGATCTACGTCTTCCCACT	130-136	specific to dmsFD2
F4	TTTAAACTCGAGTTATCGC	16-21	conserved
F5	TTGTATGGATCAGATCTAC	126-132	semi-conserved
R1	CCGAAAGAATAAACATCAG	222-228	conserved
R2	GTGCATACTCCAGTTTCCA	308-313	conserved
S1	CCCTTCTTCATCCAATTCATT	257-262	specific to berS1

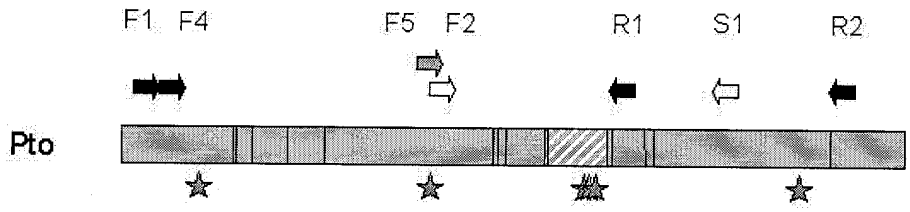

Fig. 3. Primers designed to amplify *Pto*-like sequences from *Solanum* nucleotide sequences, position in *Pto* (Martin et al. 1993a), and degrees of specificity of the primers. In the schematic representation of *Pto*, the orientation of the primers is indicated by arrows, the 15 invariant kinase amino acid residues by vertical bars, the activation domain by dashes, and conserved autophosphorylation sites by asterisks.

In the phylogenetic tree, a diverse set of *Solanum* species is represented in the sequences in class III (Fig. 4). The clustering of these sequences is in agreement with the geographical distribution, current evolutionary hypotheses (Hawkes 1990), and cpDNA-based phylogeny (Spooner and Castillo 1997) of *Solanum*. In contrast to the *Lycopersicon* species, *Solanum* species are outbreeders, highly heterozygous, and different alleles can be amplified by PCR. The two, three, and four sequences from the diploid *S. berthaultii,* tetraploid *S. tuberosum*, and tetraploid *Solanum sucrense*, respectively, could be the different alleles, although genetic analyses are required to prove this. The *S. berthaultii, Solanum hondelmannii, S. tuberosum, Solanum vernei*, and *S. sucrense* orthologs cluster together, which is in line with their taxonomic classification in the Tuberosa series (Hawkes 1990; Spooner and Castillo 1997). These species all evolved as diploid populations in the central Andes, except for *S. sucrense*, which evolved in a population of polyploid species at higher altitudes. *S. demissum,* a member of the polyploid demissa series, may have arose following a return migration to Mexico and subsequent amphipolyploidizations with primitive Mexican ancestors (Hawkes 1990). In line with this migration hypothesis, the *S. demissum* sequence clustered more distantly. We cannot exclude at this stage, however, the possibility that some recent duplication occurred in these species and that subsets of the *Pto*-like sequence classes occur in some species.

Recently, D. O. Lavelle and colleagues submitted the full sequence of *Pto* loci of *L. pimpinellifolium* and *L. esculentum* to GenBank, accession nos. AF220602 and AF220603, thereby revealing two sets of three novel paralogs, in addition to *Pto* and *Fen*. Adding these sequences to our data set did not significantly alter the phylogenetic analyses. Clustering in sets of two paralogs (orthologs from the two *Lycopersicon* species) in classes I, II, and IV was obtained, confirming the robustness of the tree and the observation that orthologs are more similar than paralogs (data not shown). In addition, the fact that these paralogs cluster with a subset of classes suggests that genes from these classes also may be localized at a syntenous position on chromosome V in *Solanum*. Other classes of *Pto*-like kinases may be located at different chromosomal positions, as described in *Capsicum* spp. (Pflieger et al. 1999), and additional studies are needed to test whether more than one cluster of *Pto*-like genes occurs in *Solanum*.

The *Pto*-like kinases appear to be encoded by members of an ancient gene family because in *Solanum* (this study) and *Lycopersicon* (Michelmore and Meyers 1998), *Pto* orthologs are more similar than paralogs. Similar conclusions also were reported for other classes of *R* genes. These observations raise questions about the arms race hypothesis for the evolution of *R* gene specificity (Michelmore and Meyers 1998). For example, although *Cf* paralogs of the LRR–TM class have been subjected to unequal crossing over and gene conversion (Parniske et al. 1997), these events did not occur frequently enough to homogenize them within a haplotype (Michelmore and Meyers 1998). Individual NBS–LRR *R* genes are thought

Table 2. Overview of *Pto*-like sequences obtained by polymerase chain reaction (PCR). *Solanum* clone from which the template DNA was derived, primers used for amplification, size of the PCR fragment, and GenBank accession numbers are indicated

***Pto*-like sequence**	***Solanum* clone**	**Primers[a]**	**Size (bp)**	**GenBank accession no.**
axhBF1	*S. arnezii* × *hondelmannii*-63	F1–R1	614	AF288538
ber2H	*S. berthaultii*-9	F1–R2	871	AF288539
ber7A	*S. berthaultii*-9	F2–S1	362	AF288540
berBD3	*S. berthaultii*-9	F1–R1	614	AF288541
berDF1	*S. berthaultii*-9	F5–R2	512	AF288542
berDF4	*S. berthaultii*-9	F5–R2	530	AF288543
berDJ2	*S. berthaultii*-9	F4–R1	595	AF288544
berS1	*S. berthaultii*-9	F2–R2	516	AF288545
dmsFD2	*S. demissum*-PBL	F1–R2	877	AF288546
scr1A	*S. sucrense*-71	F1–R2	871	AF288547
scr1C	*S. sucrense*-71	F1–R2	871	AF288548
scr1G	*S. sucrense*-71	F1–R2	871	AF288549
scr8A	*S. sucrense*-71	F2–S1	362	AF288550
scr8B	*S. sucrense*-71	F2–S1	377	AF288551
scr8C	*S. sucrense*-71	F2–S1	362	AF288552
scr8D	*S. sucrense*-71	F2–S1	377	AF288553
scr8E	*S. sucrense*-71	F2–S1	377	AF288554
scr8I	*S. sucrense*-71	F2–S1	377	AF288555
scrDG1	*S. sucrense*-71	F5–R2	521	AF288556
scrDG4	*S. sucrense*-71	F5–R2	530	AF288557
scrDK1	*S. sucrense*-71	F4–R1	595	AF288558
scrY10	*S. sucrense*-71	F1–R1	614	AF288559
tbrBA3	*S. tuberosum*-Ehud	F1–R1	614	AF288560
tbr4A	*S. tuberosum*-Robijn	F1–R2	871	AF288561
tbr4D	*S. tuberosum*-Robijn	F1–R2	871	AF288562
tbr5A	*S. tuberosum*-Robijn	F2–S1	377	AF288563
tbrDI2	*S. tuberosum*-Robijn	F5–R2	521	AF288564
tbrDI4	*S. tuberosum*-Robijn	F5–R2	530	AF288565
tbrW1	*S. tuberosum*-Robijn	F1–R1	614	AF288566
vrn6A	*S. vernei*-530	F2–S1	386	AF288567
vrnBB1	*S. vernei*-530	F1–R1	614	AF288568
vrnDL2	*S. vernei*-530	F4–R1	595	AF288569

[a] See Figure 3.

to have evolved mainly by divergent evolution on the basis of a birth-to-death process similar to that envisaged for the vertebrate major histocompatibility complex (Michelmore and Meyers 1998; Nei et al. 1997). In addition, minimal sequence changes in LRRs drastically altered recognition specificity in the flax *L* gene (Ellis et al. 1999), and diversifying selection appears to have created extreme variation in rice *Xa21* (Wang et al. 1998) and tomato *Cf* genes (Parniske et al. 1997). This suggests that the *R* gene evolution toward recognitional specificity occurs relatively rapidly within regions involved in recognition, as in LRR or possibly the activation domain for kinase *R* genes (Grube et al. 2000; Pan et al. 2000a), and a balance is established, creating new specificities and conserving old ones (Stahl and Bishop 2000; Young 2000). Likewise, the evolution to create novel specificities in the activation domain may have occurred relatively rapidly for the *Pto*-like genes, but their ancient origin and strong conservation of kinase characteristics implies that they may not have evolved following the arms race model. This assessment is consistent with the view that emerged for the NBS–LRR *R* genes, which suggests that these genes occur in reservoirs of ancient and highly diverse families, with fine-tuning of recognition specificity occurring at various layers of antagonistic coevolution (Meyers et al. 1999; Pan et al. 2000b; Stahl and Bishop 2000).

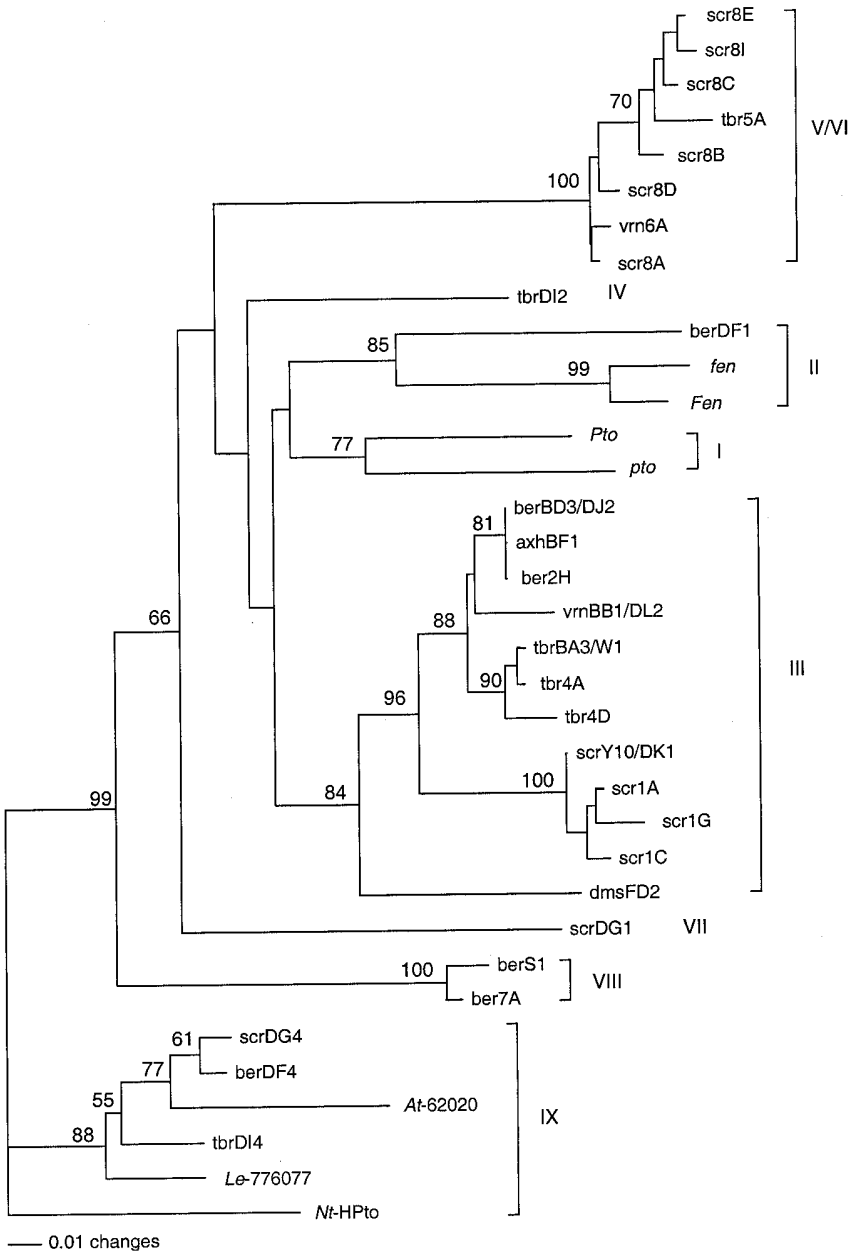

Fig. 4. Phylogenetic tree of *Pto*-like sequences. The tree was constructed by the Neighbor-Joining method (Saitou and Nei 1987) with the aligned amino acid sequences. The tree was rooted with class IX. For sequence codes, see Table 2 and text. Branch lengths are proportional to average substitutions per site, as indicated by the scale. Gaps were treated as missing characters. Bootstrap values are indicated at nodes supported with > 50% of 100 replicates. Branches were supported by the phylogenetic tree generated by parsimony analysis (data not shown).

Further characterization of the genetic structure of *Pto*-like gene clusters in representative *Solanum* species should help test the various evolutionary scenarios.

More than 3,000 plant kinases have now been deposited in GenBank. Sequence comparison of *Pto*-like sequences with those of other kinases suggest that *Pto* forms a unique family of kinases in plants. In almost all *Pto*-like sequences, the invariant residues characteristic of functional protein kinases are fully conserved (Hanks and Quinn 1990), suggesting that these *Pto*-like genes are likely to encode active kinases. The activation domain, which in Pto is involved in ligand binding, shows significant conservation of the plant serine–threonine kinase consensus (Sessa et al. 2000a) but considerable variation in other residues. For example, in this region, the AvrPto unresponsive *Pto* allele from *L. esculentum* shows aberrant signature sequences compared with *Pto* and all other *Solanum*. *Pto*-like sequences such as the gap at positions 196 to 198 and QLY appearing instead of ELD at positions 191 to 193. A particularly attractive hypothesis is that each member of the family might be interacting with different ligands, whether they are pathogen elicitors and/or signal transduction pathway components. The *Lycopersicon* genes *Pto* and *Fen* act in the same signal transduction pathway, leading to HR and defense responses, but are activated by different signals, i.e., AvrPto

Table 3. Characteristics of Pto-like sequences[a]

Class	Pto-like sequence[b]	In–del[c] 159	In–del[c] 245	Kinase consensus[d]	Phosph[e] ppp	Activation domain[f] p pp[g]
I	Pto			GGVKEDKNDGPEDGR	TTT	DFGISKKGTELDQTHLST-VVKGTLGYIDPE
	pto			GGVKEDKNDGPEDGR	TST	DFGLSKTRPQLYQT---T-DVKGTFGYIDPE
II	Fen			GGVKEDKNDGPEDGR	TST	DFGISKTMPELDQTHLST-VVRGNIGYIAPE
	fen			GGVKEDKNDGPEDGR	TTT	DFGISKTMPELDLTHLST-VVRGNIGYIAPE
	berDF1			?????DKNDGPEDGR	?ST	DFGISKTIPELDQTHFST-TVEGSIGYIDPE
III	berBD3/DJ2			GGVKEDKNDGPE???	TS?	DFGLSKKGTELDQTHLST-LVQGTIGYLDPE
	axhBF1			GGVKEDKNDGPE???	TS?	DFGLSKKGTELDQTHLST-LVQGTIGYLDPE
	vrnBB1/DL2			GGVKEDKNDGPE???	TT?	DFGLSKKGTELDETHLST-LVQGTIGYLDPE
	tbrBA3/W1			GGVKEDKNDGPE???	TT?	DFGISKKGTELDQTHLST-LVQGTIGYLDPE
	scrY10/DK1			GGVKEDKNDGPE???	TS?	DFGLSKKRTELDQTHLST-LVQGTLGYLDPE
	scr1A			GGVKEDKNDGPEDGR	TST	DFGLSKKRTELDQTHLST-LVQGTLGYLDPE
	scr1G			GGVKEDKNDGPEDGR	TST	DFGLSKKRTELDQTHLST-LVQGTLGYLDPE
	scr1C			GGVKEDKNDGPEDGR	TST	DFGLSKKRTELDQTHLST-FVQGTLGYLDPE
	tbr4A			GGVKEDKNDGPEDGR	TTT	DFGISKKGTELDQTHLST-LVQGTIGYLDPE
	tbr4D			GGVKEDKNDGPEDGR	TTT	DFGISKKGTELDQTHLST-LVQGTIGYLDPE
	ber2H			GGVKEDKNDGPEDGR	TST	DFGLSKKGTELDQTHLST-LVQGTIGYLDPE
	dmsFD2			GGVKEDKNDGPEDGR	TTT	DFGISKTMSELDQTHLST-TVKGSIGYIDPE
IV	tbrDI2			?????DKNDGPEDGR	?TT	DFGLSKIRTELDQTHVST-VVKGTLGYLDPE
V	scr8E		AVGRY	?????DKNDGPEDG?	???	DFGLFKKGPELDRIQTTK-IMKGTMGYLDPE
	scr8I		AVGRY	?????DKNDGPEDG?	???	DFGLFKKGAELDRIQTTK-IMKGTMGYLDPE
	scr8B		AVGRY	?????DKNDGPEGG?	???	DFGLSKKGPELDRIQTTK-IMKGTMGYLDPE
	tbr5A		AVGRY	?????DKNDGPEDG?	???	DFGLFKKGPELDRIQTTK-IMKGTMGYLDPE
	scr8D		AVGRY	?????DKNDGPEDG?	???	DFGLSKKGPELDRIHTTK-LMKGTMGYLDPE
VI	scr8C			?????DKNDGPEDG?	???	DFGLFKKGAELDRIQTTK-IMKGTMGYLDPE
	vrn6A			?????DKNDGPEDG?	???	DFGLSKKGPEL-RIHTTKVMMKGTMGYLDPE
	scr8A			?????DKNDGPEDG?	???	DFGLSKKGPELDRIHTTK-LMKGTMGYLDPE
VII	scrDG1			?????DKNDGPEDGR	?ST	DFGISKKGTEPDQTRVTT-NVKGTVPYLDPE
VIII	berS1			?????DKNDGIEDGR	??T	DFGLSKTRTELDQTHVST-DVKGSFGYLDIE
	ber7A			?????DKNDGIEDG?	???	DFGLSKTRTELDQTHVST-DVKGSFGYLDIE
IX	scrDG4	YAK		?????DKNDGPEDGR	?TT	DFGLSKTGPELDQTHVST-AVKGSFGYLDPE
	berDF4	YAK		?????DKNDGPEDGR	?ST	DFGLSKTGPELDQTHVST-AVKGSFGYLDPE
	tbrDI4	DAK		?????DKNDGPEDGR	?ST	DFGLSKTGPELDQTHVST-AVKGSFGYLDPE
	Le-776077	YAK		?????DKNDGPEDG?	?S?	DFGLSKAGPELDQTHVST-AVKGSFGYLDPE
	Nt-HPto	YAN		GGVKEDKNDGPEDGR	TST	DFGLSKTRLELDQTHTST-VVKGTFGYLDPE
	At-62020	DSK		GGVKEDKNDGPEDGR	TST	DFGLSKTGPELDQTHVST-AVKGSFGYLDPE
Consensus[h]						DF T G GY PE

[a] Two multiple amino acid in–del sites (positions 159 and 245), the kinase consensus sequence, autophosphorylation sites (marked with p), and the activation domain are shown. Conserved consensus residues are in bold, residues with similar or different physicochemical properties (Grantham 1974) to the consensus residue are underlined or plain text, respectively. Numbering of amino acid positions corresponds to the *Pto* sequence (Martin et al. 1993). Question marks indicate unknown amino acid residues. Dashes indicate gaps introduced to maximize alignment.

[b] See Table 2.

[c] In–del: insertion–deletion.

[d] Kinase consensus sequence at positions 48, 50, 55, 69, 84, 164, 166, 169, 182, 184, 210, 211, 223, 228, and 300 (Hanks and Quinn 1990).

[e] Pto autophosphorylation sites, outside (positions 38, 133, and 288) the activation domain (Sessa et al. 2000b).

[f] Kinase activation domain between positions 182–211 of *Pto* (Hanks and Quinn 1990).

[g] Pto autophosphorylation sites, inside (positions 195, 198, and 199) the activation domain (Sessa et al. 2000b).

[h] Consensus of the activation domain for plant serine–threonine kinases (Sessa et al. 2000a).

and fenthion, respectively (Martin et al. 1994; Ronald et al. 1992). Another characteristic of protein kinases is autophosphorylation sites, which fulfill an important regulatory function. The autophosphorylation sites, which are essential for kinase activity or AvrPto–Pto-mediated HR induction (Sessa et al. 2000b), are highly conserved in the *Solanum* Pto-like sequences, except for class V–VI sequences, which show weaker conservation in the rest of the activation domain. In summary, the conservation of kinase consensus residues and autophosphorylation sites suggest that the uncovered genes are likely to encode active kinases. It remains to be determined whether all members of the *Pto* family are involved in similar signal transduction pathways, leading to disease resistance. Functional assays to test whether the *Pto*-like sequences are involved in resistance can be performed by complementation, particularly with virus or *Agrobacterium*-mediated transient assays (Bendahmane et al. 2000; Sessa et al. 2000b) or by performing loss-of-function experiments, e.g., through virus-induced gene silencing (Baulcombe 1999).

We used amplification with conserved primers to obtain a great diversity of sequences, identifying novel classes of *Pto*-like sequences. Increasing the specificity of the PCR primers by designing them on the basis of variable domains biased the amplification of certain classes. For example, all class V–VI sequences were amplified with the F_2–S_1 primer set. Such specific primers may be applied in resistance breeding such as cloning specific candidate resistance genes or following such genes in a breeding progeny. In pepper, quantitative trait loci mapped close to *Pto* homologs obtained by PCR (Pflieger et al. 1999). These sequences were pseudogenes, although *R* gene-like sequences with uninterrupted reading frames, as obtained in our study, are putative resistance gene candidates with unknown specificity. PCR-based cloning of *Pto*-like sequences proved successful in *Solanum* spp., suggesting that cloning novel *R* genes by this approach has great potential.

MATERIALS AND METHODS

Plant material.

The *Solanum* clones used in this study are presented in Table 1. Wild *Solanum* spp. accessions contain resistance to various bacterial, viral, nematode, fungal, and oomycete pathogens (Hoekstra and Seidewitz 1987; Vleeshouwers et al. 2000).

Southern and Northern blot analyses.

DNA was isolated from leaves of *Solanum* plants (Shure et al. 1983). The DNA was digested with *Eco*RV, electrophoresed, and transferred to Hybond-N^+. For expression analyses, leaf material was harvested and RNA was isolated (Verwoerd et al. 1989). Poly(A^+) RNA was extracted from 500 µg of total RNA with the Oligotex mRNA kit (Qiagen, Valencia, CA, U.S.A.). For Northern blot analyses, the poly(A^+) RNA was denatured at 50ºC in 1 M glyoxal, 54% (vol/vol) dimethyl sulfoxide, and 10 mM sodium phosphate buffer (pH 7.0), then electrophoresed and transferred to Hybond-N^+. Fragments used for DNA templates for probe synthesis were a 600-bp *Sac*II–*Pst*I of berDF1, 600-bp *Sac*II–*Pst*I of berDF4, and 1,800-bp *Eco*RI–*Xho*I from cDNA clone pFB19 encoding tubulin from potato. The Southern and poly(A^+) Northern blot were hybridized with ^{32}P-labeled probes at 65ºC and washed at 0.1 or 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) stringency for the berDF1 and tubulin hybridization, respectively. Messenger RNA levels were determined with a Fujix bio-imaging analyzer (BAS 2000; Nikon, Tokyo, Japan), and the signals were quantified in photostimulated luminescence per mm^2.

Primer design, PCR amplification, and DNA sequencing.

The primers used in this study are presented in Figure 3. For primer combinations F1-R1, F4-R1, F2-R2, and F5-R2, PCR amplification was performed on 60 ng of genomic DNA in 50- or 100-µl reaction volumes. The templates were denatured by heating to 94ºC for 5 min, followed by 35 cycles of 1 min at 94ºC, 1 min at 55ºC, and 1 min at 72ºC, with a final extension of 10 min at 72ºC. For primer combinations F1–R2 and F2–S1, the PCR was conducted in 25-µl reaction volumes and denaturation was performed at 94ºC for 7 min, followed by 45 cycles of 30 s at 94ºC, 30 s at 50ºC, and 1 min at 72ºC, with a final extension of 5 min at 72ºC.

PCR products were cloned in the pGEM-T or pGEM-T Easy (Promega, Madison, WI, U.S.A.). Recombinants were selected with X-Gal in the selection medium or by colony hybridization at 65ºC with a radiolabeled PCR product as probe. After the selection of positive clones, plasmid DNA was isolated and digested with *Sac*II–*Pst*I to estimate the size of the cloned insert. Alternatively, the insert sizes were checked by PCR. The DNA sequences were determined by automated DNA sequencing.

Phylogenetic analyses.

Sequence data were evaluated with the DNA-Star software package (Lasergene, Madison, WI, U.S.A.). The sequences were aligned in ClustalX 1.64b (Thompson et al. 1997). Ambiguous regions in the alignment were removed from the data set. Phylogenetic trees were constructed in PAUP (phylogenetic analysis using parsimony) 4.0b4a (Swofford; Sinauer Associates, Sunderland, MA, U.S.A.) by the neighbor-joining method (Saitou and Nei 1987) with default settings. Heuristic bootstrapping was performed to evaluate the degree of support for grouping in the neighbor-joining analyses.

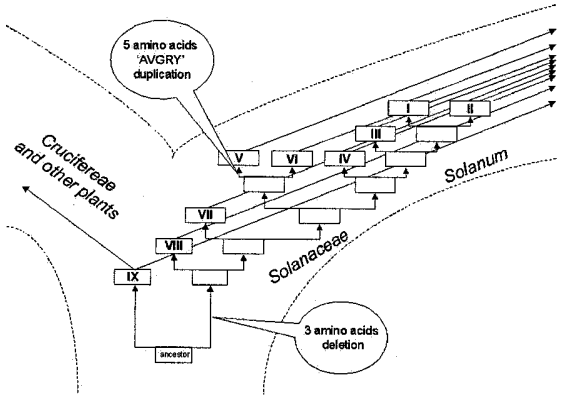

Fig. 5. Hypothetical model for the evolution of the *Pto* superfamily. Model illustrates a series of gene duplications that could explain the radiation of *Pto*-like genes in *Solanum*. The occurrence of insertion–deletions is indicated for the three amino acid deletion (position 159) and the five amino acid duplication (AVGRY at position 245). Numbers in the boxes refer to the nine classes, depicted in Figure 4 and Table 3.

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LITERATURE CITED

Aarts, M. G. M., Hekkert, B. T. L., Holub, E. B., Beynon, J. L., Stiekema, W. J., and Pereira, A. 1998. Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 11:251-258.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S. P. 1997. Signaling in plant–microbe interactions. Science 276:726-733.

Baulcombe, D. C. 1999. Fast forward genetics based on virus-induced gene silencing. Curr. Opin. Plant Biol. 2:109-113.

Bendahmane, A., Querci, M., Kanyuka, K., and Baulcombe, D. C. 2000. *Agrobacterium* transient expression system as a tool for the isolation of disease resistance genes: Application to the *Rx2* locus in potato. Plant J. 21:73-81.

Bent, A. F. 1996. Plant disease resistance genes: Function meets structure. Plant Cell 8:1757-1771.

D'Arcy, W. G. 1991. The Solanaceae since 1976, with a review of its biogeography. Pages 75-137 in: Solanaceae III: Taxonomy, Chemistry, Evolution. J. G. Hawkes, R. N. Lester, M. Nee and N. Estrada, eds. Royal Botanical Gardens, Kew, U.K.

Eijlander, R., and Stiekema, W. J. 1994. Biological containment of potato (*Solanum tuberosum*): Outcrossing to the related wild species black nightshad (*Solanum nigrum*) and bittersweet (*Solanum dulcamara*). Sex. Plant Reprod. 7:29-40.

Ellis, J. G., Lawrence, G. J., Luck, J. E., and Dodds, P. N. 1999. Identification of regions in alleles of the flax rust resistance gene *L* that determine differences in gene-for-gene specificity. Plant Cell 11:495-506.

Frederick, R. D., Thilmony, R. L., Sessa, G., and Martin, G. B. 1998. Recognition specificity for the bacterial avirulence protein AvrPto is determined by Thr-204 in the activation loop of the tomato Pto kinase. Mol. Cell 2:241-245.

Grantham, R. 1974. Amino acid difference formula to help explain protein evolution. Science 185:862-864.

Grube, R. C., Radwanski, E. R., and Jahn, M. 2000. Comparative genetics of disease resistance within Solanaceae. Genetics 155:873-887.

Hammond-Kosack, K. E., and Jones, J. D. G. 1997. Plant disease resistance genes. Annu. Rev. Plant Physiol. Mol. Biol. 48:575-607.

Hanks, S. K., and Quinn, A. M. 1990. Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. Methods Enzymol. 200:38-62.

Hawkes, J. 1990. The Potato: Evolution, Biodiversity, and Genetic Resources. Belhaven Press, London.

Hermsen, J. G. T., and Ramanna, M. S. 1973. Double-bridge hybrids of *Solanum bulbocastanum* and cultivars of *Solanum tuberosum*. Euphytica 22:457-466.

Hoekstra, R., and Seidewitz, L. 1987. Evaluation data on tuber-bearing *Solanum* species. Page 202 in: German–Dutch Curatorium for Plant Genetic Resources. Brauschweig, Federal Republic of Germany.

Jia, Y., Loh, Y. T., Zhou, J., and Martin, G. B. 1997. Alleles of Pto and Fen occur in bacterial speck-susceptible and fenthion-insensitive tomato cultivars and encode active protein kinases. Plant Cell 9:61-73.

Kanazin, V., Marek, L. F., and Shoemaker, R. C. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. USA 93:11746-11750.

Leister, D., Ballvora, A., Salamini, F., and Gebhardt, C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genet. 14:421-429.

Loh, Y. T., and Martin, G. B. 1995. The *Pto* bacterial resistance gene and the *Fen* insecticide sensitivity gene encode functional protein kinases with serine/threonine specificity. Plant Physiol. 108:1735-1739.

Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., Frary, A., Ganal, M. W., Spivey, R., Wu, T., Earle, E. D., and Tanksley, S. D. 1993a. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432-1436.

Martin, G. B., De Vicente, M. C., and Tanksley, S. D. 1993b. High-resolution linkage analysis and physical characterization of the *Pto* bacterial resistance locus in tomato. Mol. Plant-Microbe Interact. 6:26-34.

Martin, G. B., Frary, A., Wu, T., Brommonschenkel, S., Chunwongse, J., Earle, E. D., and Tanksley, S. D. 1994. A member of the tomato *Pto* gene family confers sensitivity to fenthion resulting in rapid cell death. Plant Cell 6:1543-1552.

Martin, G. B., Frederick, R., Thilmony, R., and Zhou, J. 1996. Signal transduction events involved in bacterial speck disease resistance. Pages 163-176 in: Molecular Aspects of Pathogenicity and Resistance: Requirement for Signal Transduction. D. Mills, H. Kunoh, and N. T. Keen, eds. American Phytopathological Society, St. Paul, MN, U.S.A.

Meyers, B. C., Chin, D. B., Shen, K. A., Sivaramakrishnan, S., Lavelle, D. O., Zhang, Z., and Michelmore, R. W. 1998. The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell 10:1817-1832.

Meyers, B. C., Dickerman, A. W., Michelmore, R. W., Sivaramakrishnan, S., Sobral, B. W., and Young, N. D. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J. 20:317-332.

Michelmore, R. W., and Meyers, B. C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8:1113-1130.

Nei, M., Gu, X., and Sitnikova, T. 1997. Evolution by the birth-and-death process in multigene families of the vertebrate immune system. Proc. Natl. Acad. Sci. USA 94:7799-7806.

Pan, Q., Liu, Y. S., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D., and Fluhr, R. 2000a. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologs in the genomes of two dicotyledons: Tomato and *Arabidopsis*. Genetics 155:309-322.

Pan, Q., Wendel, J., and Fluhr, R. 2000b. Divergent evolution of plant NBS-LRR resistance gene homologs in dicot and cereal genomes. J. Mol. Evol. 50:203-213.

Parniske, M., and Jones, J. D. G. 1999. Recombination between diverged clusters of the tomato Cf-9 plant disease resistance gene family. Proc. Natl. Acad. Sci. USA 96:5850-5855.

Parniske, M., Hammond-Kosack, K. E., Golstein, C., Thomas, C. M., Jones, D. A., Harrison, K., Wulff, B. B., and Jones, J. D. 1997. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato. Cell 91:821-832.

Pflieger, S., Lefebvre, V., Caranta, C., Blattes, A., Goffinet, B., and Palloix, A. 1999. Disease resistance gene analogs as candidates for QTLs involved in pepper-pathogen interactions. Genome 42:1100-1110.

Pitblado, R. E., and Kerr, E. A. 1980. Resistance to bacterial speck (*Pseudomonas tomato*) in tomato. Acta Hortic. 100:379-382.

Rathjen, J. P., Chang, J. H., Staskawicz, B. J., and Michelmore, R. W. 1999. Constitutively active *Pto* induces a *Prf*-dependent hypersensitive response in the absence of *avrPto*. EMBO J. 18:3232-3240.

Richter, T. E., and Ronald, P. C. 2000. The evolution of disease resistance genes. Plant Mol. Biol. 42:195-204.

Ronald, P. C. 1998. Resistance gene evolution. Curr. Opin. Plant Biol. 1:294-298.

Ronald, P. C., Salmeron, J. M., Carland, F. M., and Staskawicz, B. J. 1992. The cloned avirulence gene *avrPto* induces disease resistance in tomato cultivars containing the *Pto* resistance gene. J. Bacteriol. 174:1604-1611.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Scofield, S. R., Tobias, C. M., Rathjen, J., Chang, J. H., Lavelle, D. T.,

Michelmore, R. W., and Staskawicz, B. J. 1996. Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. Science 274:2063-2065.

Sessa, G., D'Ascenzo, M., and Martin, G. B. 2000a. The major site of the Pti1 kinase phosphorylated by the Pto kinase is located in the activation domain and is required for Pto–Pti1 physical interaction. Eur. J. Biochem. 267:171-178.

Sessa, G., D'Ascenzo, M., and Martin, G. B. 2000b. Thr38 and Ser198 are Pto autophosphorylation sites required for the AvrPto–Pto-mediated hypersensitive response. EMBO J. 19:2257-2269.

Shen, K. A., Meyers, B. C., Islam-Faradi, M. N., Chin, D. B., Stelly, D. M., and Michelmore, R. W. 1998. Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol. Plant-Microbe Interact. 11:815-823.

Shure, M., Wessler, S., and Fedoroff, N. 1983. Molecular identification and isolation of the *Waxy* locus in maize. Cell 35:225-233.

Song, W. Y., Pi, L. Y., Wang, G. L., Gardner, J., Holsten, T., and Ronald, P. C. 1997. Evolution of the rice *Xa21* disease resistance gene family. Plant Cell 9:1279-1287.

Spooner, D. M., and Castillo, T. R. 1997. Reexamination of series relationships of South American wild potatoes (Solanaceae: Solanum sect. Petota): Evidence from chloroplast DNA restriction site variation. Am. J. Bot. 84:671-685.

Spooner, D. M., Anderson, G. J., and Jansen, R. K. 1993. Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). Am. J. Bot. 80:676-688.

Stahl, E. A., and Bishop, J. G. 2000. Plant-pathogen arms races at the molecular level. Curr. Opin. Plant Biol. 3:299-304.

Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J. 1999. Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. Nature 400:667-671.

Tang, X., Frederick, R. D., Zhou, J., Halterman, D. A., Jia, Y., and Martin, G. B. 1996. Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. Science 274:2060-2063.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.

Verwoerd, T. C., Dekker, B. M. M., and Hoekema, A. 1989. A small scale-procedure for the rapid isolation of plant RNAs. Nucleic Acids Res. 17:2362.

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

Wang, G. L., Ruan, D. L., Song, W. Y., Sideris, S., Chen, L., Pi, L. Y., Zhang, S., Zhang, Z., Fauquet, C., Gaut, B. S., Whalen, M. C., and Ronald, P. C. 1998. *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10:765-779.

Young, N. D. 2000. The genetic structure of resistance. Curr. Opin. Plant Biol. 3:285-290.

Yu, Y. G., Buss, G. R., and Maroof, M. A. S. 1996. Isolation of a superfamily of candidate disease resistance genes in soybean based on a conserved nucleotide binding site. Proc. Natl. Acad. Sci. USA 93:11751-11756.

Zhou, J. M., Loh, Y. T., Bressan, R. A., and Martin, G. B. 1995. The tomato gene *Pti1* encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. Cell 83:925-935.