MPMI Vol. 8, No. 6, 1995, pp. 939–948. © 1995 The American Phytopathological Society

The *PWL* Host Specificity Gene Family in the Blast Fungus *Magnaporthe grisea*

Seogchan Kang, James A. Sweigard, and Barbara Valent

Central Research and Development, E. I. du Pont de Nemours and Co. Inc., Experimental Station, Wilmington, DE 19880-0402 U.S.A.

Received 9 May 1995. Accepted 11 August 1995.

The *PWL2* gene, isolated from a *Magnaporthe grisea* rice pathogen, prevents this fungus from infecting a second host grass, weeping lovegrass. We have investigated the distribution of sequences homologous to *PWL2* in *M. grisea* strains isolated from diverse grass species. Multiple *PWL2* homologs with varying degrees of sequence homology were identified. The presence of *PWL2* homologs does not correlate with an avirulent phenotype on weeping lovegrass in many cases: some strains were fully pathogenic on weeping lovegrass although they carry multiple *PWL2* homologs. Three weakly hybridizing *PWL2* homologs were cloned and characterized. One of these, the *PWL1* gene previously identified by genetic analysis, functioned to prevent infection of weeping lovegrass. Cloned *PWL3* and *PWL4* genes were nonfunctional, although *PWL4* became functional if its expression was driven by either the *PWL1* or the *PWL2* promoter. The *PWL1*, *PWL2*, and *PWL3/PWL4* genes map to different genomic locations. The amino acid sequences of the predicted PWL1, PWL3, and PWL4 proteins have 75, 51, and 57% identity, respectively, to the PWL2 protein. Our studies indicate that *PWL* genes are members of a dynamic, rapidly evolving gene family.

Additional keywords: fungal-plant interactions, gramineous species, repetitive DNA sequences, *Pyricularia grisea,* rice blast.

The filamentous ascomycete *Magnaporthe grisea* (Hebert) Barr is a pathogen of many gramineous species (Ou 1985). Rice blast disease, caused by this pathogen, threatens rice crops worldwide. In addition, this fungus causes disease on crops such as finger millet (*Eleusine coracana*; Kato 1978), pearl millet (*Pennisetum glaucum* L.; Pandotra 1976), barley (*Hordeum vulgare* L.; Yaegashi 1988), wheat (*Triticum aestivum* L.; Urashima et al. 1993) and maize (*Zea mays* L.; Notteghem 1990). Weeping lovegrass (*Eragrostis curvula*), sometimes grown as a forage crop, is highly susceptible to the blast fungus. Many wild gramineous species that grow near crops represent potential inoculum sources for disease on these crops (Borromeo et al. 1993; Mackill and Bonman 1986). Although the collective host range of *M. grisea* is extensive, individual isolates of the fungus are limited to infecting a small number of grass species. Understanding the molecular basis for host specificity will aid in designing methods to control the disease, whether it involves understanding the necessity to control disease on relevant weed plants, or developing successful strategies to engineer resistant crops by transferring resistance between grass species.

The interfertility of *M. grisea* strains that infect different grass species has allowed genetic analysis of specificity at the host species level. Examples of both monogenic and polygenic inheritance of host species specificity have been demonstrated (Notteghem 1990; Leung et al. 1988; Ellingboe et al. 1988; Valent and Chumley 1991). Single genes that determine specificity toward the host weeping lovegrass have been identified in independent crosses (Valent et al. 1986; Valent and Chumley 1991; Yaegashi 1978). *PWL1*, a gene that has a major effect on pathogenicity toward weeping lovegrass, was identified in a cross between the finger millet pathogen WGG-FA40 and the weeping lovegrass pathogen K76-79 (Valent et al. 1986). Crosses involving laboratory strains pathogenic on rice (Valent and Chumley 1991) identified a second gene, *PWL2*, with a similar phenotype to *PWL1*. *PWL2* was not genetically linked to *PWL1*.

Frequent mutation to pathogenicity on weeping lovegrass suggested that the *PWL2* gene functioned to prevent infection of weeping lovegrass, as predicted for classical avirulence genes controlling cultivar specificity within a particular host species. The *PWL2* gene was subsequently cloned by chromosome walking techniques, and was predicted to encode a glycine-rich, hydrophilic protein of 145 amino acids (Sweigard et al. 1995). Most rice pathogens appear to have one or more copies of this gene (Sweigard et al. 1995; Zeigler et al. 1995).

In this work, distribution of sequences homologous to *PWL2* has been investigated in *M. grisea* strains with diverse host specificity. This investigation has led to the discovery of a *PWL* multigene family. No correlation could be found between the presence of *PWL* gene sequences and lack of ability to infect weeping lovegrass. We report the cloning and characterization of the *PWL1* gene, as well as a nonfunctional homolog from each parent of the cross in which *PWL1* was identified. The four cloned genes define three subgroups within the *PWL* gene family, based on sequence homologies and genomic locations. We also report here sequences corre-

Corresponding author: Barbara Valent, Tel: 302-695-2187, Fax: 302-695-8480; E-Mail: Valent@esvax.dnet.dupont.com

Current address of first author: Department of Biology, University of New Mexico, Albuquerque, NM 87131 U.S.A.

Nucleotide and/or amino acid sequence data to be found at GenBank as accessions numbers U36923, U36995, and U36996.

sponding to the middle repetitive DNA elements MGR608 (Hamer et al. 1989) and MGR619 (J. E. Hamer, F. G. Chumley and B. Valent, unpublished results), which are found adjacent to each other near the *PWL1* gene.

Table 1. *Magnaporthe grisea* field isolates used in this study

Strain	Host[a]	Country of origin	Comments[b]
K76-79	Weeping lovegrass	Japan	G-17, H. Yaegashi, 1976
T-5	Wheat	Brazil	S. Igarashi, 1988
T-29	Wheat	Brazil	K. Kmetz, 1989
T-47	Wheat	Brazil	S. Igarashi, 1985
G-158	Feral *Triticale*	Brazil	Infects wheat, K. Kmetz, 1989
G-48	*Setaria italica*	USA	F. M. Latterell, 1982
G-188	*Setaria* sp.	USA	J. A. Sweigard, 1991
G-225	*Setaria italica*	Japan	JP37, J. L. Notteghem
G-160	*Leersia hexandra*	Philippines	Lh-A8401, J. M. Bonman
G-194	*Leersia oryzoides*	Japan	JP36, J. L. Notteghem
G-227	Ginger	Japan	JP41, J. L. Notteghem
G-228	Ginger	Reunion	RN1, J. L. Notteghem
G-229	*Cyperus brevifolus*	Philippines	PH54, J. L. Notteghem
G-231	*Cyperus rotundus*	Philippines	PH52, J. L. Notteghem
G-197	Barley	Thailand	TH2, J. L. Notteghem
WGG-FA40	*Eleusine coracana*	Japan	G-22, H. Yaegashi, 1977
G-172	*Eleusine coracana*	Uganda	UG77-3-2-1, H. Kato
G-176	*Eleusine coracana*	Nepal	NP10-17-4-1-3, H. Kato
G-177	*Eleusine coracana*	India	In77-24-1-1, H. Kato
G-199	*Eleusine coracana*	Rwanda	RW12, J. L. Notteghem
G-26	*Eleusine indica*	Japan	SM81-11, H. Yaegashi
G-77	*Eleusine indica*	Philippines	J. M. Bonman
G-167	*Eleusine indica*	Philippines	Ei-A8309, J. M. Bonman
G-200	*Eleusine indica*	Ivory Coast	CD156, J. L. Notteghem
G-201	*Eleusine indica*	Brazil	BR62, J. L. Notteghem
G-202	*Eleusine indica*	Madagascar	MD112, J. L. Notteghem
G-81	*Pennisetum glaucum*	USA	83P-161, H. Wells
G-123	*Pennisetum glaucum*	USA	84P-19, H. Wells
G-221	*Pennisetum clandestinum*	Japan	JP35, J. L. Notteghem
G-222	*Pennisetum pedicelatum*	Mali	ML30, J. L. Notteghem
G-78	*Pennisetum polystachyon*	Philippines	R. Gopinath
G-161	*Pennisetum purpureum*	Philippines	Pp-A8201, J. M. Bonman
G-223	*Pennisetum typhoideum*	Burkina Faso	BF17, J. L. Notteghem
G-224	*Pennisetum typhoideum*	Ivory Coast	CD86, J. L. Notteghem
G-58	*Panicum repens*	Philippines	Pr-8212, J. M. Bonman
G-220	*Panicum repens*	India	IN3, J. L. Notteghem
G-218	*Panicum coloratum*	Japan	JP31, J. L. Notteghem
G-219	*Panicum maximum*	India	IN5, J. L. Notteghem
G-216	*Paspalum distichum*	Philippines	PH62, J. L. Notteghem
G-66	*Paspalum paspaloides*	Philippines	Pp-8250, J. M. Bonman
G-156	*Digitaria horizontalis*	Brazil	A. S. Prabhu
G-210	*Digitaria horizontalis*	Burkina Faso	BF16, J. L. Notteghem
G-1	*Digitaria sanguinalis*	USA	F. M. Latterell, 1981
G-11	*Digitaria sanguinalis*	Japan	A26, H. Yaegashi
G-211	*Digitaria sanguinalis*	Brazil	BR29, J. L. Notteghem
G-212	*Digitaria sanguinalis*	India	IN6, J. L. Notteghem
G-60	*Digitaria ciliaris*	Philippines	Dc-8217, J. M. Bonman
G-163	*Digitaria ciliaris*	Philippines	DcA8301, J. M. Bonman
G-213	*Digitaria smutsii*	Japan	JP34, J. L. Notteghem
G-209	*Digitaria exilis*	Ivory Coast	CD143, J.L. Notteghem
G-32	*Digitaria* sp.	Japan	H. Kato, 1982
G-189	*Digitaria* sp.	USA	J. A. Sweigard, 1991
G-190	*Digitaria* sp.	USA	A. R. Kubelik, 1991

[a] The host plant on which the isolate was found.
[b] Other name if applicable, individual providing the strain, and/or date of collection.

RESULTS

The *PWL2* gene is a member of a multigene family.

We have surveyed 52 *M. grisea* strains isolated from diverse grass species other than rice (Table 1) for sequences homologous to the rice pathogen *PWL2* gene, and for ability to infect weeping lovegrass (Fig. 1). The Southern analysis in Figure 1A showed that most *Digitaria* pathogens have multiple bands with strong homology to *PWL2*, in addition to bands with weaker homology. Among the 13 *Digitaria* pathogens analyzed, G-209 was exceptional in having one hybridizing band (Fig. 1A). No correlation was found between *PWL* sequences and ability to cause disease on weeping lovegrass.

Ginger and *Cyperus* pathogens (Fig. 1B) are the only strains that lack homology to *PWL2* under hybridization conditions used in this study. These strains are genetically distant from *M. grisea* strains infecting the other hosts listed and probably should be classified as separate species (Borromeo et al. 1993; S. Kang and B. Valent, unpublished results). All remaining strains analyzed in Figure 1B–D had at least one *Eco*RI fragment hybridizing to the *PWL2* gene. The sizes and numbers of the *Eco*RI fragments were often different even among strains isolated from the same host species. Hybridization intensities ranged from strong to weak, in some cases even among strains infecting the same host plants. For example, *Digitaria* pathogens, *Pennisetum* pathogens, and wheat pathogens were highly variable in the intensity of the bands hybridizing to *PWL2* (Fig. 1A–C).

Magnaporthe grisea strains isolated from *Eleusine* spp. were of particular interest because *PWL1* was originally identified in strain WGG-FA40 isolated from this host species (Fig. 1D). All *Eleusine* pathogens tested contained one or two faintly hybridizing *Eco*RI fragments. One out of five strains (WGG-FA40, G-172, G-176, G-177, and G-199) isolated from finger millet (*E. coracana*) was pathogenic on weeping lovegrass. In contrast, all six strains (the remaining strains shown in Fig. 1D) isolated from goosegrass (*E. indica*) were pathogenic on weeping lovegrass. While all the strains isolated from *Eleusine* spp. had an *Eco*RI fragment in the 4 to 5 kb range, only the nonpathogens of weeping lovegrass contained an *Eco*RI band in the 2 kb range. This result was consistent with the cloning of *PWL1* on a 1.8 kb *Eco*RI fragment (see below).

Cloning and characterization of *PWL2* homologs.

The *Eleusine* pathogen WGG-FA40 has two *Eco*RI fragments, 4.8 kb and 1.8 kb (Fig. 1D), and the weeping lovegrass pathogen K76-79 has a single 4.3 kb *Eco*RI fragment (data not shown), all of which hybridized weakly to *PWL2*. Since *PWL1* was first identified in a cross between these two strains (Valent et al. 1986), we analyzed progeny of this cross for segregation of these *Eco*RI fragments with *PWL1* (data not shown). The 1.8 kb fragment co-segregated with *PWL1* in the members of four tetrads, suggesting that the *PWL2* homolog residing on the 1.8 kb fragment might be the *PWL1* gene. The 4.8 kb and 4.3 kb fragments segregated independently from the *PWL1* gene.

Genomic DNA libraries of WGG-FA40 and K76-79 were screened with the labeled *PWL2* gene. Two λ clones, designated as PL-B and PL-C, respectively, were isolated from

WGG-FA40, and one λ clone, designated as PL-A, was isolated from K76-79 (Fig. 2). Restriction mapping and segregation data suggested that PL-B and PL-C correspond, respectively, to the homologous 4.8 kb and 1.8 kb *Eco*RI fragments from WGG-FA40.

To determine whether these *PWL2* homologs could prevent *M. grisea* from infecting weeping lovegrass, two weeping lovegrass pathogens, CP987 and 4091-5-8, were co-transformed with each PL clone along with a vector conferring hygromycin B resistance. Only transformants obtained with

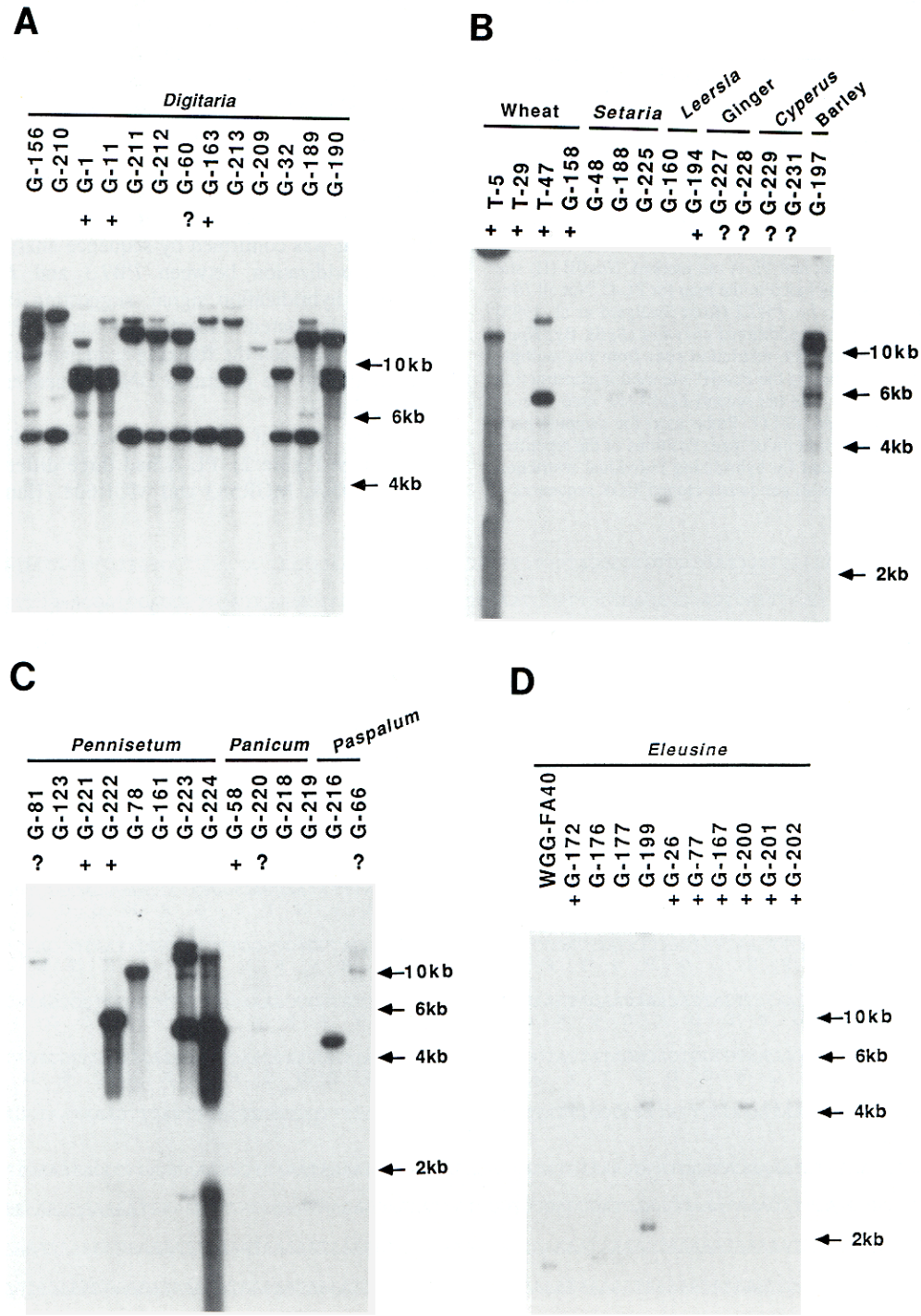

Fig. 1. Distribution pattern of *PWL* gene sequences in *Magnaporthe grisea* pathogens of diverse grass species. Genomic DNAs (1 μg) were digested with *Eco*RI, fractionated on 0.7% agarose gels, transferred to Hybond-N membranes, and probed with the *PWL2* gene (Sweigard et al. 1995) as described in Materials and Methods. Gels (**A–D**) were loaded with equivalent amounts of the digested genomic DNAs. Differences in hybridization intensity reflect differences in homology to the *PWL2* gene probe. Genus (or common name) of host plant from which individual strains (Table 1) were isolated is shown on top of each panel. Virulence of individual strains on weeping lovegrass is recorded as + (virulent), ? (not determined), or blank (avirulent). Size standards included in experiment were 1 kb Ladder Standards (BRL, Gaithersburg, MD).

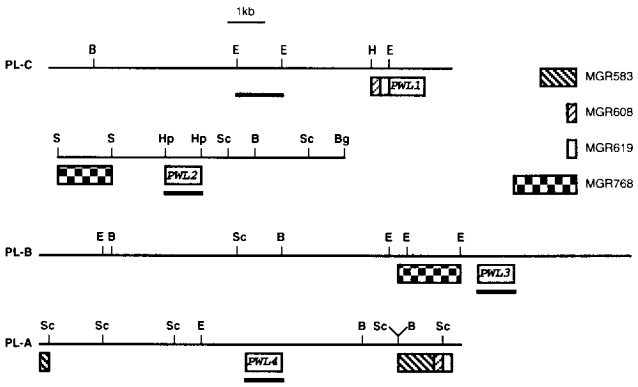

Fig. 2. Restriction maps of *Magnaporthe grisea* genomic DNA sequences flanking the *PWL* genes. Gene locations are indicated. Restriction sites for *Bam*HI (B), *Eco*RI (E), and *Sac*I (Sc) within the insert DNAs for λ clones PL-C, PL-B, and PL-A are marked. *Hin*dIII (H) site used to construct pSK10 was included in the map for PL-C. Not all *Hin*dIII sites are shown. The map for *PWL2* (from Sweigard et al. 1995) includes restriction sites for three additional enzymes (*Bgl*II, Bg; *Hpa*I, Hp; S, *Sal*I). Not indicated here are restriction sites from the multiple cloning site in λGEM-12, including the *Bam*HI site used in construction of the *PWL1* subclone pSK10, and the second *Eco*RI site used in construction of the *PWL3* subclone pSK47. Probe used for mapping each *PWL* gene is marked by bar at bottom of map. Positions of the repetitive DNA sequences were determined by hybridizing individual restriction fragments to a blot that contains all previously cloned MGR sequences.

PL-C were nonpathogenic on weeping lovegrass. These results along with the mapping data indicated that the *PWL2* homolog in PL-C is the *PWL1* gene. Hereafter, we designate the *PWL2* homologs in PL-A, PL-B, and PL-C as *PWL4*, *PWL3*, and *PWL1*, respectively (Fig. 2). A 1.8 kb *Hin*dIII-*Bam*HI fragment of PL-C (*Bam*HI site from the vector), which was able to transform CP987 and 4091-5-8 to nonpathogenicity on weeping lovegrass, was subcloned as pSK10. The 6.1 kb *Sac*I fragment of PL-A and the 4.8 kb *Eco*RI fragment of PL-B (second *Eco*RI site from the vector) were also subcloned, and designated pSK49 and pSK47, respectively.

Cross-hybridization under identical conditions suggested that the sequences of the two finger millet pathogen genes, *PWL1* and *PWL3*, were more diverged from each other than those of the rice pathogen gene *PWL2* and each homolog. This result was confirmed by sequence analyses (see below). Cross-hybridization between *PWL3* and *PWL4* gave the strongest hybridization signal, suggesting that they are the most closely related. Except in the case of *PWL3* and *PWL4*, homology between the flanking sequences of the *PWL* genes was limited to the repetitive DNA sequences present on the clones (Fig. 2).

Several types of repetitive DNA were found in the PL clones (see Figure 2). PL-A had three different types of repetitive DNA, MGR583 and MGR608 (Hamer et al. 1989)

```
TTTTTTTTTTAAAACAAATAAAACGTTGCATGTCCTGTCGCCATGCTCTTTACCGCGCTTGTGAACCTCCCTGCACCTCGTGTTGTGCGTCGTATAGATT   100

CAGGAGCGCCCTGAAATGCTCGAATTTACTCAGGGAGAGTGGTTTTGTAAGTCCGTCTGCAGGCATCAGGCTCAGTCTCCAGGTAGGAAGCTTTAACCCT   200

AAGGCATACTTGGAGAACCTCAGTGTATTAGGCGCTATTAACGAAAATTTTAAAATTGAAGAGAAGAGAGAAATTACCATTAACGACGCGCTGGAGAGAC   300

GCGCTTAAATCTGGACTTAGTGGACCCTTTGTCCGATCCCTGGCTCGGCGTGGAGACATTGTGATTCTGAGGGTAGGTCTGGGGGCCTGATCCTCACATT   400

ATTATTAATATGTTTGCATAGTTATAAGTTTACTTGCATTATTATAATTTTACCTCCACTACTATTAATAACTTTCAATAACTTACTATTAATTTACTTC   500

GATGATTTCTAATTTTATTGCATTGTTACTAATTTTACTGCATTATTGTCGATTTAATTAAGTTTCTATTAGCTCAATTACGTTCTTACTAATTCAATTT   600

CGCTGTTATTGATGCGAATTCCCTTACTAGTTATTTACAAAGTAGTTATATAAAGCTGGCGTATGAAAAACTTTACTTGGAAGTAATAGGAGTGTTGGTT   700

GAGATTTTACAAGTTCCAAAAACCACGATTAAGTTTGCCGAACATTATTCTTACACATTAAAGCTCTTACAATGAAATTCAACAAAACTATCCCTCTCTA   800
                                                                  M  K  F  N  K  T  I  P  L  Y

TATTCTTGCCTTTTTCTCGACCGCTGTAATCGCTGGTGGTCGCAAATGGACTAACAAAGTTATTTATAACGACAAAGGCCCAAGGGAGGGCTCAATATCA   900
 I  L  A  F  F  S  T  A  V  I  A  G  G  R  K  W  T  N  K  V  I  Y  N  D  K  G  P  R  E  G  S  I  S

ATTAGAAAAGGCGCGGAAGGCGACTTCAACTGTGGCCCCGGTTACCCTGGGGGGCCCGACAGGATGGTGCGGGTTCATGAGGATAATGGGAACATTCGCG  1000
I  R  K  G  A  E  G  D  F  N  C  G  P  G  Y  P  G  G  P  D  R  M  V  R  V  H  E  D  N  G  N  I  R  G

GAATGCCCCCGGGCTACCGTTTAGGCCCTGATGACAAAGAAGATAAAGGGGATAACCAGTACTATAGCCGAAACGGATATCATGTTGGCGATGGTCCGGC  1100
  M  P  P  G  Y  R  L  G  P  D  D  K  E  D  K  G  D  N  Q  Y  Y  S  R  N  G  Y  H  V  G  D  G  P  A

CGAGTACCAAAATCACGGTGGAGGACAATGGGGTGACGGATATTATGGACCGCCTGGGCAAATTACCAATCAACACGGGAAACGTCAGGGAGATCAGGGC  1200
 E  Y  Q  N  H  G  G  G  Q  W  G  D  G  Y  Y  G  P  P  G  Q  I  T  N  Q  H  G  K  R  Q  G  D  Q  G

TGCCATATTATGTAAACTGCTGGAATAGGGTTTTATTTGGCGTTTATTTATATTCTAAGCTACTTTGCTACGTCGTTTTACTTGAAATAATGGAGTGGAG  1300
C  H  I  M  End

ATAAATATAAAAAGTACAAGGTGTAAAACATAAGTAGGAAAAAGTAGAAAAGAAATAAACGAAAAATAAAATCCTAAAAGAGACGGAAAAAGTAAATAAA  1400

AGAAAGGAAAACATACGCGTACTTAGAAATAAAGGAAACTAGCGAGCGTGGTTAGTAAAAGGAAAATAATGGATAATAAAATAAAAATAAAAATAAAAAT  1500

AAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAA  1600

ATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAA  1700

AAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAAATAAAATAAGAGTAAAACTAAACACAAAAACAAAAGAAAAGGGATATTAA  1800

TTCCGTCGTGGTAAATCGTTTGCAATCGAAATTGCAACCGTCGTTCTGGACCGCAATAACGCCAACGAAATCGACGTATCCAACCAATGGATACTACAGG  1900

ACCTATTAGCTTTTGCAGCTGTTTATAGGCCTGGGCAGTGCTTTTTTCCATAACGGTCCGGCACTTATAATAATCGGATGGAAAAAAGGATCC        1993
```

Fig. 3. DNA sequence of the *PWL1* locus and deduced amino acid sequence of the PWL1 protein. The repetitive DNA sequences MGR608 (202-327) and MGR619 (328-406) are underlined and marked by arrows. The AAAAAT repeats at the 3′ end of the gene are italicized and underlined.

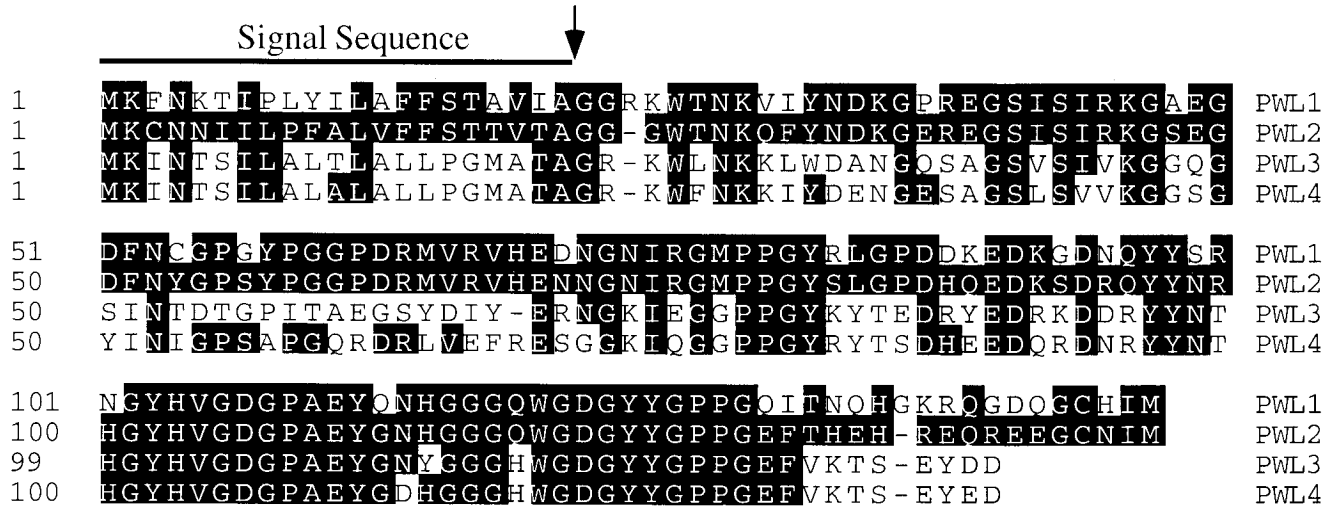

Fig. 4. Amino acid sequence alignment of the PWL proteins. Identities to PWL2 are boxed and shaded. The predicted cleavage site of the putative signal sequence is marked by an arrow.

and MGR619 (J. E. Hamer, F. G. Chumley, and B. Valent, unpublished results). MGR768 (S. Kang, J. A. Sweigard, and B. Valent, unpublished results) was present in PL-B. The *PWL1* gene in PL-C was closely associated with MGR608 and MGR619.

Genetic mapping of the *PWL2* homologs.

The chromosomal locations of *PWL1*, *PWL3*, and *PWL4* were determined by following the segregation of restriction fragment length polymorphisms (RFLPs) in 66 randomly picked ascospores that had been used to construct an RFLP map of *M. grisea* (Sweigard et al. 1993). The *PWL2* gene was previously mapped to chromosome 2c of mapping strain 6043. Since neither parent of the mapping cross contained the *PWL1* gene, flanking sequences (Fig. 2) were used for mapping. The *PWL1* gene probe co-segregated with one of the telomeres (TEL33) of chromosome 2a. The *PWL3* gene was linked to the marker cos125 on chromosome 2c of strain 6043 (two recombinants), at the opposite end of the chromosome from the *PWL2* gene. The *PWL4* gene mapped to the *PWL3* locus, suggesting that the *PWL3* and *PWL4* genes are allelic.

Sequence analysis.

The regions with homology to *PWL2* were sequenced. In all three cases an open reading frame (ORF) was found with homology to ORF3 of *PWL2* (Sweigard et al. 1995). The *PWL1* ORF (Fig. 3) encodes a protein of 147 amino acids (aa) with a molecular mass of 16.2 kDa. The *PWL3* and *PWL4* ORFs would encode proteins with 137 aa (14.9 kDa) and 138 aa (15.0 kDa), respectively. These PWL proteins do not have significant primary amino acid sequence homologies to proteins of known function. Sequence alignment of the PWL proteins is shown in Figure 4.

The PWL proteins have several common characteristics (see Table 2 and Figure 4). First, the amino terminus has features common among the eukaryotic signal sequences (von Heijne 1992). As shown in Figure 4, the first 21 aa residues of the PWL proteins could be divided into a basic N-terminal region, a central hydrophobic region, and a more polar C-terminal region. The putative signal sequences of the PWL proteins would be cleaved between alanine and glycine (residues 21 and 22). Second, many glycine residues (between 17 and 19% of all residues) are evenly distributed throughout the protein, suggesting that few α helices could form. Third, the PWL proteins are hydrophilic (approximately 27% of residues are charged aa). However, the net charges of the proteins are significantly different between PWL1 (+2) and the three others (all –5).

Table 2. Characteristics of the PWL proteins

Protein	Size[a]	Net charge	Composition of amino acids (%)		
			Gly	Pro	Charged aa
PWL1	147	+2	19.0	7.5	26.5
PWL2	145	–5	17.9	6.9	27.6
PWL3	137	–5	17.5	5.1	26.3
PWL4	138	–5	18.1	5.8	27.5

[a] The number of amino acid (aa) residues.

The sequence in Figure 3 includes two previously identified repetitive DNA sequences that occur in the *PWL1* promoter region. The ends of these elements have been defined by sequence comparisons of homologous elements, which are often but not always adjacent to each other, from different genomic regions (S. Kang and B. Valent, unpublished results). MGR619 (J. E. Hamer, F. G. Chumley, and B. Valent, unpublished results), which is approximately 80 bp long, is present 366 bp upstream from the putative start codon of the *PWL1* gene. MGR608 (Hamer et al. 1989), which is approximately 125 bp long, is adjacent to MGR619. At the 3′ end of *PWL1* (approximately 270 bp from the stop codon), a long stretch of a 6 bp repeat (AAAAAT; 45 copies) is found.

Functional analysis of chimeric *PWL* genes.

Reasons for *PWL3* and *PWL4* being nonfunctional as host specificity genes could be improper expression of the gene and/or a protein that fails to function in conferring nonpathogenicity toward weeping lovegrass. We constructed chimeric

genes to investigate if the *PWL3* and *PWL4* genes failed to function due to problems with expression. The coding sequences of the *PWL3* and *PWL4* genes were fused to the promoters of the *PWL1* and *PWL2* genes. Based on subcloning results, the *PWL1* and *PWL2* promoter regions, approximately 670 bp and 850 bp, respectively, were amplified by polymerase chain reaction (PCR) and subcloned into pCB1004 to produce pWL1P and pWL2P, respectively. The ORF and 3′-end flanking sequences (approximately 320 bp after the stop codon) of the *PWL3* and *PWL4* genes were amplified and each was cloned into pWL1P and pWL2P. The *PWL2* gene and the four chimeric constructs were each transformed into weeping lovegrass pathogen 4091-5-8, and the resulting transformants (at least 10 transformants for each plasmid) were assayed for pathogenicity. Both *PWL1*::*PWL4* and *PWL2*::*PWL4* constructs transformed 4091-5-8 to nonpathogenicity on weeping lovegrass as efficiently as did the wild-type *PWL2* gene (>70% of the transformants tested), suggesting that the *PWL4* gene failed to function because it was not properly expressed. In contrast, neither *PWL3* construct transformed 4091-5-8 to nonpathogenicity (from a total of 25 transformants tested), suggesting that the *PWL3* coding sequence does not encode a protein that confers avirulence toward weeping lovegrass.

Sequence comparisons among the *PWL* genes.

Based on homologies in DNA and aa sequences and on genomic locations, the *PWL* genes could be divided into three groups: *PWL1*, *PWL2*, and (*PWL3* + *PWL4*). The aa sequences of the PWL proteins diverged more than the DNA sequences (Table 3). The PWL1 protein showed 75% identity to the PWL2 protein, but only 46% and 50% identity to the PWL3 and PWL4 proteins, respectively. The PWL2 protein showed 51 and 57% identities to the PWL3 and PWL4 proteins, respectively. The amino acid sequence identity between the PWL3 and PWL4 proteins was 72%.

Table 3. Sequence homology between *PWL* genes

	Identity (%)	
	Amino acids	DNA
PWL1-PWL2	75	78
PWL1-PWL3	46	57
PWL1-PWL4	50	58
PWL2-PWL3	51	62.5
PWL2-PWL4	57	65
PWL3-PWL4	72	85

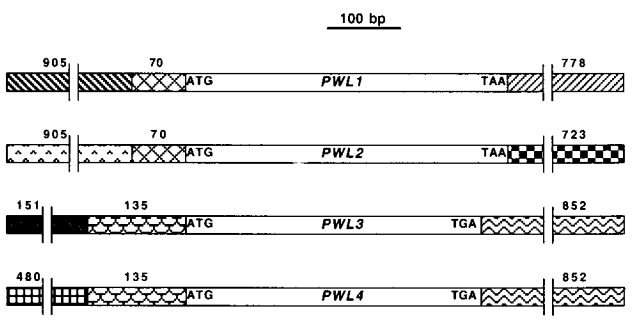

Fig. 5. Schematic diagrams of DNA sequences of the *PWL* genes. Different patterns represent completely different sequences. Numbers on the bar correspond to base pairs of sequence. Open reading frame of each gene is marked by the start codon and the corresponding stop codon.

At the DNA level, the homology between genes in different groups was limited to the ORF, except for the case of *PWL1* and *PWL2* where a short 5′ flanking sequence was also conserved (Fig. 5). The homology between *PWL1* and *PWL2* (78% nucleotide identity) began 70 bp upstream from the initiation codon of *PWL1* and ended immediately after the stop codon. Beyond the conserved region, the sequences of the two genes were completely unrelated. Homology between *PWL3* or *PWL4* and *PWL1* or *PWL2* (57 to 65%) was apparent only in a part of the ORF: the homology begins at the initiation codon and ends around 30 bp upstream from the stop codons of the *PWL1* and *PWL2* genes. The homology between *PWL3* and *PWL4* extended beyond the ORFs in both directions (135 bp from the 5′ end and >800 bp from the 3′ end). Surprisingly, the homology between noncoding sequences (>98% identity) was significantly higher than that between ORFs (85%). For the *PWL3* and *PWL4* ORFs, the middle one-third of the ORF was most diverged (Fig. 6).

DISCUSSION

Members of a multigene family confer avirulence toward weeping lovegrass.

Segregation analysis previously identified two genes, *PWL1* and *PWL2*, that have an all-or-nothing effect on the ability of *M. grisea* strains to infect weeping lovegrass (Valent and Chumley 1991). These genes have now been cloned; *PWL2* by map-based cloning (Sweigard et al. 1995), and *PWL1* from its limited homology to *PWL2* (this study). *PWL1*, from the finger millet pathogen WGG-FA40, and *PWL2*, from the rice pathogen Guy11, map to different genomic locations, as predicted by the genetic analysis. Both genes have a mode of action that is analogous to that of classical avirulence (*AVR*) genes that determine cultivar specificity. That is, fungal strains expressing either gene are blocked from infecting a specific host, weeping lovegrass. Heath et al. (1990) reported that the presence of the *PWL1* gene in an invading fungal strain correlated with browning of weeping lovegrass cells around developing colony margins. The fungus was not able to grow beyond these brown cells. We hypothesize that a specific resistance mechanism in weeping lovegrass recognizes the products or by-products of active *PWL* genes and subsequently initiates a defense response, leading to the browning of plant cells.

Some members of the *PWL* gene family fail to function in conferring nonpathogenicity toward weeping lovegrass. For instance, some strains isolated from *Digitaria* and *Eleusine* and all strains isolated from wheat were fully pathogenic, even though they carry at least one restriction fragment with sequences homologous to *PWL2* (Fig. 1). Our results suggest that the field isolate K76-79 is able to infect weeping lovegrass because its *PWL4* gene is not properly expressed. The *PWL4* gene becomes functional in preventing infection of weeping lovegrass if its expression is driven by either the *PWL1* or the *PWL2* promoter. Several *PWL* genes isolated from *Digitaria* pathogens and *Pennisetum* pathogens also appear to be improperly expressed (S. Kang, unpublished results). In contrast, the *PWL3* gene, a second cloned gene from strain WGG-FA40, does not become functional when its

coding sequence is fused to either the *PWL1* or the *PWL2* promoter. The *PWL3* gene may encode a protein that fails to function in triggering a defense response in weeping lovegrass.

Magnaporthe grisea has evolved to parasitize a wide range of grasses, yet individual isolates of the fungus have a very narrow host range. Thus, specificity determinant(s) for all grasses may be widely distributed in *M. grisea*. The *PWL* gene family is large and highly diverse, and nearly ubiquitous. Indeed, lower stringency hybridizations might detect even more diversity. This gene family may represent broad specificity determinants within *M. grisea*, and thus members of this family may interact with a wide range of resistance (*R*) genes in monocots. Three types of information would support this view: (i) Cloned *R* genes corresponding to different pathogens in different plant species show a surprising degree of structural similarity (Bent et al. 1994; Jones et al. 1994; Mindrinos et al. 1994; Whitham et al. 1994), suggesting that pathogen molecules with diverse structures are recognized through common biochemical mechanisms in diverse plant species. Sequences homologous to the *Pto* gene, an *R* gene in tomato, have been identified in a variety of dicots as well as in a number of monocots (Martin et al. 1993); (ii) Many bacterial *AVR* genes function as specificity determinants toward both host and nonhost species, suggesting that nonhost species also carry *R* genes corresponding to these *AVR* genes (Fillingham et al. 1992; Kobayashi et al. 1989; Swarup et al. 1992; Whalen et al. 1988 and 1991); and (iii) Surprising conservation of gene composition and colinearity of gene order on chromosomes have been demonstrated within monocots (Bennetzen and Freeling 1993).

Cloning of a broad collection of *PWL* genes now permits testing of the possibility that some *PWL* genes act as specificity determinants toward other hosts as well. Multiple hosts can be easily screened for resistance correlating with *PWL* genes by transforming *M. grisea* pathogens of various grasses with the cloned *PWL* genes. Alterations in host specificity in the fungal transformants will suggest the presence of a corresponding *R* gene in the host plant.

How frequently is host range determined by the presence of host specificity genes similar to the *PWL* genes? Since at least one pathogen strain isolated from each plant genus has the ability to infect weeping lovegrass (Fig. 1), *M. grisea* must have basic compatibility (Heath 1981) with this host. Therefore, the *PWL* genes or some yet-to-be-identified *AVR*-like host specificity genes are likely to determine the fate of weeping lovegrass infections by *M. grisea* pathogens of diverse grass species. However, it does not appear that specificity of *M. grisea* toward all potential hosts is controlled exclusively by *AVR* genes like those in the *PWL* gene family. Extensive analysis of the genetic differences between the weeping lovegrass pathogen 4091-5-8 and the rice pathogen O-135 showed that these two parental strains differ in two types of genes that control ability to infect rice (Valent et al. 1991). In addition to *AVR* genes that control specificity toward particular rice cultivars, these two strains differ in polygenic factors that determine the extent of lesion development by those progeny that infect rice. It seems likely that these particular quantitative rice specificity genes are involved in determining basic compatibility toward rice.

Magnaporthe grisea has another multigene family of host specificity genes. *AVR2-YAMO*, an avirulence gene that prevents the fungus from infecting the rice cultivar Yashiromochi, also belongs to a multigene family (S. Kang and B. Valent, unpublished results). Although the members of this gene family are not as widely distributed as those of the *PWL* gene family, many grass pathogens that are unable to infect rice, such as *Digitaria* pathogens and *Pennisetum* pathogens,

```
1   ACTTAAGTTCATTGAGTCCTTGTGACAACAGAGTACTGCTCCCAGCTGCATCCTCTGGAACATGACAATAGTAACATTAC  PWL3
1   ACTTAAGTTCATTGAGTCCTTGTGACAACAGAGTACTGCTCCCAGCTGCATCCTCTGGAACATGACAATAGTAACATTAC  PWL4

                                                          Start
81  ATTAAATTTCTTGACATTTCCTCTCTAAACAATATCGCAACAACTTTTCGCCATGAAAATTAATACCAGCATCCTCGCTC  PWL3
81  ATTAAATTTCTTGACATTTTCTCTCTAAACAATATCGCAACAGCTTTTCGCCATGAAAATTAATACCAGCATCCTCGCTC  PWL4

161 TTACTTTGGCGCTGCTCCCGGGCATGGCCACCGCCGGTCGAAAATGGGTCAATAAAAAGTTATGGGATGCCAATGGTCAA  PWL3
161 TTGCTTTGGCGCTGCTCCCGGGCATGGCCACCGCCGGTCGAAAATGGTTCAATAAAAAGATATATGATGAGAACGGTGAA  PWL4

241 AGCGCCGGCTCAGTGTCTATTGTAAAGGGCGGGCAAGGCTGTATAAACACCGACACGGGTCCCATCACTGCCGAAGGCAG  PWL3
241 AGCGCCGGCTCATTGTCTGTCGTAAAGGGCGGGTCAGGCTATATAAACATCGGTCCCAGCGCTCCTGGTCAGCGTGATAG  PWL4

321 ---TTATGACATCTATGAGCGTAAATGGCAAAATCGAAGGTGGGCCCCCGGGTTACAAATATACGGAAGACCGCTACGAAG  PWL3
321 ACTTGTTGAATTCCGTGAGAGCGGTGGCAAAATCCAAGGGGGGCCCCCGGGTTACAGATATACGAGCGACCACGAGGAAG  PWL4

398 ATCGAAAGGATGATCGCTATTACAACACCCACGGATATCACGTCGGCGATGGACCAGCCGAATATGGAAATTATGGAGGT  PWL3
401 ATCAAAGGGATAATCGCTACTACAACACCCACGGATATCACGTCGGCGATGGACCAGCCGAATATGGAGATCATGGAGGT  PWL4

                                                                  Stop
478 GGGCATTGGGGCGATGGGTACTATGGTCCTCCAGGGGAGTTTGTAAAGACCAGCGAATACGACGACTGAATAGGATGGAT  PWL3
481 GGGCATTGGGGCGATGGGTACTATGGTCCTCCAGGGGAGTTTGTAAAGACCAGCGAATATGAAGACTGAATAGGATGGAT  PWL4

558 ACTATGGTCGTCAAGGGCAGTCTACAAACAACAGACGACACGGACATGGCGGAAACGATTGCACCTTTGATGTGAATGGTA  PWL3
561 ACTATGGTCATCAAGGGCAGTCTACAAACAACAGACGACACGGACATGGTGGAAACGATTGCACCTTTATGTGAATGGTA  PWL4

638 TTGCTCCGTCAAGTTGAGACAGCTGTCAAAGGCGCGAGGGTGCC                                      PWL3
641 TTGCTCCGTCAAGTTGAGACAGCTGTCAAAGGCGCGAGGGTGCC                                      PWL4
```

Fig. 6. DNA sequence alignment between the *PWL3* and *PWL4* genes. Start and stop codons of the gene are boxed and labeled. Sequences of the *PWL3* gene that differ from the *PWL4* gene are boxed and shaded.

carry functional family members. The presence of *AVR-YAMO* genes in pathogens of diverse plant hosts supports the suggestion that host specificity genes of *M. grisea* are widely distributed. Results from genetic analyses also support this possibility: nonpathogens of rice have been shown to carry several *AVR* genes that elicit rice cultivar specific responses (Yaegashi and Asaga 1981; Valent et al. 1991).

The *PWL* gene family appears to have rapidly diverged.

The number of *PWL* genes and the degree of their sequence homology to *PWL2* are variable even among the isolates from the same host species, suggesting that this gene family is highly dynamic. Sequence comparison between members of the *PWL* gene family shows unusual patterns of sequence evolution. First, sequence identity at the amino acid level is consistently lower than that at the DNA level (Table 3). Secondly, some *PWL* genes have undergone faster sequence changes than the rest of the genome, as shown in the *PWL3* and *PWL4* genes. The *PWL3* and *PWL4* genes isolated from WGG-FA40 and K76-79, respectively, appear allelic based on their genomic locations and the presence of nearly identical sequences at the flanking regions. The genomes of these two fungal strains are believed to be highly related based on RFLPs in the mitochondrial genome (A. R. Kubelik, J. A. Sweigard, F. G. Chumley, and B. Valent, unpublished results), RFLPs and sequences in the ribosomal DNA cistron (Biju-Duval 1994), and polymorphisms associated with single copy regions of the nuclear genome (S. Kang and B. Valent, unpublished results). As expected, the sequences flanking these *PWL* ORFs are highly conserved (>98% identity), and another 400 bp, within 2 kb of these *PWL* genes, is 100% identical between two strains (S. Kang, unpublished results). However, the coding regions of *PWL3* and *PWL4* are significantly diverged: the DNA sequence identity within the *PWL3* and *PWL4* ORFs is only 85%. Differences include a 3-bp deletion and multiple nucleotide changes, mainly clustered in the middle third of the ORF (see Figure 6). Highly localized sequence changes between *PWL3* and *PWL4* suggest that sequence divergence among some members of the *PWL* gene family might not be the consequence of early gene divergence during evolution.

How and why did the sequence of these *PWL* genes change so rapidly? A clue to these intriguing questions probably lies in the function of this gene family in the fungus. It is likely that the *PWL* genes have alternative function(s) other than as a trigger for defense responses in the host. If the sole function of this gene family is simply to prevent the fungus from infecting hosts carrying the corresponding resistance gene, it is difficult to explain why, during evolution, *M. grisea* has not only maintained but also amplified the *PWL* gene family. This gene family has probably been maintained in the population because the putative benefits of its presence outweigh the penalties. Roles for host specificity genes as fitness factors have been demonstrated. Four bacterial host specificity genes, *avrBs2* from *X. campestris* pv. *vesicatoria* (Kearney and Staskawicz 1990), *pthA* from *X. citri* (Swarup et al. 1992), and *avrA* and *avrE* from *P. syringae* pv. tomato (Lorang et al. 1994), have been shown to be necessary for the fitness of the pathogen.

If the *PWL* genes have an alternative function that is necessary for the fitness of the fungus, it would benefit the fungus to rapidly change this gene product in such a way that it can no longer be recognized by the host, but maintains the other function. The cluster of sequence changes in the middle third of the ORF might suggest that this highly variable portion of the protein is responsible for triggering the host defense response. Host selection has been suggested as a driving force for sequence variation in a host specificity gene family in the genus *Xanthomonas* (Swarup et al. 1992; Hopkins et al. 1992; De Feyter et al. 1993). Members of this gene family are highly homologous in DNA sequence, but internal 102-bp direct repeats, which determine race specificity, are "highly" variable (Herbers et al. 1992; Yang et al. 1994).

Several mechanisms might be responsible for the diversification of the *PWL* gene family. All the characterized *PWL* genes are associated with repetitive DNA (this study; Sweigard et al. 1995; S. Kang and B. Valent, unpublished results). Recombination between homologous repetitive DNA sequences could generate deletions, inversions, duplications, and translocations of the *PWL* genes depending on the relative orientation and position of the recombining sequences. Successive unequal sister chromatid exchanges via recombination between homologous repetitive DNA present on both sides of a *PWL* gene appear to be responsible for the generation of three tandem copies of the gene in a strain isolated from *Pennisetum* (S. Kang, unpublished results).

Retrotransposition is another potential source of novel genes in different locations, at times producing nonfunctional pseudogenes in eukaryotes (Weiner et al. 1986). The location and sequence organization of the *PWL* genes suggest that retrotransposition might have had a role in the amplification of the *PWL* gene family. In the Guy11-type genome (represented by strain 6043 in the RFLP map), *PWL1* is on a different chromosome from the *PWL2*, *PWL3* and *PWL4* genes, and these latter three genes occur in two different locations on the same chromosome. Most importantly, sequences flanking the coding regions of the *PWL1*, *PWL2*, and *PWL3/PWL4* genes are different, suggesting that only the coding sequences have been translocated. In addition, several *PWL* genes isolated from other grass pathogens also have 5′ and 3′ flanking sequences completely different from those of the *PWL* genes described in this study (S. Kang, unpublished results). It is unlikely that DNA-mediated translocations are responsible for such genetic changes. *Magnaporthe grisea* contains multiple defined and potential retrotransposable elements that could supply reverse transcriptase for retrotransposition (Dobinson et al. 1993; S. Kang and B. Valent, unpublished results; M. H. Lebrun, F. G. Chumley, and B. Valent, unpublished results; Valent and Chumley 1991). However, none of the sequenced *PWL* genes appears to have poly(A) tracts at the 3′ end of the gene, one of the characteristics commonly associated with retrotransposed genes (Weiner et al. 1986). It remains to be seen whether retrotransposition has had a role in the evolution of the *PWL* gene family.

MATERIALS AND METHODS

Strains, transformation, and infection assays.

Magnaporthe grisea strains used in this study are listed in Table 1. The laboratory strains CP987 and 4091-5-8, which are pathogenic on weeping lovegrass, were used as transformation recipients to test the function of cloned *PWL* genes.

Transformation of *M. grisea* was performed as described previously (Kang et al. 1994). Co-transformation, in which unselected plasmid containing *PWL* gene sequences was co-transformed along with the selected plasmid carrying the *HPH* gene conferring resistance to hygromycin B, was described in Sweigard et al. (1995). Both transformation procedures result in >50% nonpathogenic transformants with an active *PWL* gene. *Escherichia coli* DH5α was used for maintaining plasmids (Hanahan 1983). Infection assays on weeping lovegrass were performed as described (Sweigard et al. 1995).

DNA analyses.

Genomic DNAs of *M. grisea* were prepared from mycelia grown in complete media (Valent et al. 1986) as described (Hamer et al. 1989) except that DNA was purified by CsCl gradient centrifugation. The λ phage DNA was prepared using the plate lysate method (Sambrook et al. 1989). Southern analysis was performed with Hybond-N membrane as recommended by the manufacturer (Amersham, Arlington Heights, IL). Labeled DNA probes were prepared by random priming (Feinberg and Vogelstein 1983). All nucleic acid hybridizations were conducted at 65° C in 6× SSPE (1 × SSPE is 0.15 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [ph 7.4]), 0.5% sodium dodecyl sulfate (SDS), and 5× Denhardt's solution (Sambrook et al. 1989). The blots were subsequently washed twice with 2× SSPE and 0.1% SDS for 30 min each at 65°C. Membranes in Figure 1A–D were probed with the 1-kb *Hpa*I fragment containing the *PWL2* gene (Sweigard et al. 1995). However, patterns identical to those in Figure 1 were obtained using only *PWL2* coding sequences as a hybridization probe, ruling out the possibility that the 1-kb *Hpa*I fragment contains a low copy repetitive DNA sequence in addition to the *PWL2* gene (data not shown). Computer analyses of the sequences were carried out with programs from the University of Wisconsin Genetics Computer Group (Devereux et al. 1984) and Lasergene (DNASTAR, Madison, WI). The GenBank accession numbers for the *PWL1, PWL3,* and *PWL4* sequences are U36923, U36995, and U36996, respectively.

Clones containing the *PWL2* homologs were isolated from genomic DNA libraries of K76-79 and WGG-FA40 constructed using the λGEM12 vector (Promega, Madison, WI). High molecular weight genomic DNA was partially digested with *Mbo*I to result in an average size of 15 kb. To fill-in the first two nucleotides of the 5′ overhangings generated by *Mbo*I, partially digested genomic DNA was treated with DNA polymerase I large fragment in the presence of dATP and dGTP. Partially filled-in genomic DNA (1 μg) was ligated to the same amount of λGEM12 vector arms that were partially filled-in following *Xho*I digestion. Gigapack II Gold packaging extract was used for packaging the λ library as instructed by the manufacturer (Stratagene, La Jolla, CA). The titers of the K76-79 and WGG-FA40 libraries were 1.7×10^5 and 1.5×10^5 PFU/ml, respectively. The libraries were screened as described previously (Sambrook et al. 1989).

Construction of chimeras between the coding sequence of the *PWL3* or *PWL4* gene and the promoter of the *PWL1* or *PWL2* gene.

The oligonucleotides used to amplify the *PWL1* and *PWL2* promoters are shown in Table 4. These oligos contain one to three extra nucleotide(s) in the middle in order to produce a *Kpn*I site at the 5′ end of the promoter and a *Cla*I site at the 3′ end of the promoter after PCR (see Table 4). The *PWL1* promoter region (sequences between 51 and 765; see Figure 3) was amplified with the oligos P1-1 and P1-2 in 50 μl of PCR mixture (80 mM Tris-HCl, pH 9, 20 mM NH_4SO_4, 1.5 mM $MgCl_2$, each dNTP at 0.15 mM, 0.1 μg of each primer, and 1 unit of *Taq* polymerase) using the GeneAmp PCR system 9600 (Perkin-Elmer Cetus, Norwalk, CT). The mixture was subjected to 1 cycle of amplification at low annealing temperature (1 min at 94°C, 1 min at 40°C, and 1 min at 72°C) followed by 25 cycles of amplification at a higher annealing temperature (40 s at 94°C, 1 min at 55°C, and 1 min at 72°C) and a final 3 min extension at 72°C. The 848 bp *PWL2* promoter region (Sweigard et al. 1995) was amplified using the oligos P2-1 and P2-2 under the same conditions described above. Amplified DNA was digested with *Kpn*I and *Cla*I, separated on a 0.7% agarose gel, and the expected size fragment was eluted. The eluted DNA was cloned into pCB1004 (Carroll et al. 1994) resulting in pWL1P containing the *PWL1* promoter and pWL2P containing the *PWL2* promoter. The ORF and 3′ flanking sequences of the *PWL3* and *PWL4* genes were amplified using P3-1 and P3-2 (see Table 4) as described above. These oligos were designed to produce a *Cla*I site and a *Sac*II at the 5′ end and 3′ end, respectively, of amplified *PWL3* and *PWL4* coding sequences. Amplified DNA was digested with *Cla*I and *Sac*II and was cloned into the *Cla*I-*Sac*II-digested pWL1P and pWL2P plasmids. The resulting constructs were sequenced to confirm that no mutations were generated by PCR.

Table 4. Oligonucleotides used in this study

Name	Sequence[a]
P1-1	5′-TGCTCTT*GGTACC*GCGCTTGTG-3′
P1-2	5′-TTA*ATCGAT*GGTTTTTGGAAC-3′
P2-1	5′-ACGG*GGTACC*GCGTCAGTGAAC-3′
P2-2	5′-AAAAAG*ATCGAT*TTTCCGAGCT-3′
P3-1	5′-ATTAC*ATCGAT*AAATTTCTTGACA-3′
P3-2	5′-CGGTTC*CCGCGG*CAATCGACG-3′

[a] Nucleotide(s) added to create a new restriction site are italicized. The resulting restriction site is underlined.

ACKNOWLEDGMENTS

We wish to thank all individuals listed in Table 1 for sharing pathogen strains. We are also grateful to Anne M. Carroll and Leonard Farrall for assisting in the infection assays. We are indebted to Jeff Groteluesch en at the University of Wisconsin for preparing Figures 4 and 6. We would like to thank John E. Hamer of Purdue University for critical reading of the manuscript.

LITERATURE CITED

Bennetzen, J. L., and Freeling, M. 1993. Grasses as a single genetic system: genome composition, colinearity, and compatibility. Trends Genet. 9:259-261.

Bent, A. F., Kunkel, B. N., Dahlbeck, D., Brown, K. L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B. J. 1994. *RPS2* of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. Science 265:1856-1860.

Biju-Duval, M. P. 1994. Etude de la structure genetique des populations du champignon pathogene des graminees, *Magnaporthe grisea*. Ph.D. thesis. University of Paris, Orsay.

Borromeo, E. S., Nelson, R. J., Bonman, J. M., and Leung, H. 1993. Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. Phytopathology 83:393-399.

Carroll, A. M., Sweigard, J. A., and Valent, B. 1994. Improved vectors for selecting resistance to hygromycin. Fungal Genet. Newsl. 41:22.

De Feyter, R., Yang, Y., and Gabriel, D. W. 1993. Gene-for-genes interactions between cotton *R* genes and *Xanthomonas campestris* pv. *malvacearum avr* genes. Mol. Plant-Microbe Interact. 6:225-237.

Devereux, J. R., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387-395.

Dobinson, K. F., Harris, R. E., and Hamer, J. E. 1993. *Grasshopper*, a long terminal repeat (LTR) retroelement in the phytopathogenic fungus *Magnaporthe grisea*. Mol. Plant-Microbe Interact. 6:114-126.

Ellingboe, A. H., Wu, B.-C., and Robertson, W. 1988. Fertility and pathogenicity to rice in *Magnaporthe grisea*. Pages 235-240 in: Molecular Genetics of Plant Microbe Interactions. R. Palacios and D. P. Verma, eds. American Phytopathological Society, St. Paul, MN.

Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Fillingham, A. J., Wood, J., Bevan, J. R., Crute, I. R., Mansfield, J. W., Taylor, J. D., and Vivian, A. 1992. Avirulence genes from *Pseudomonas syringae* pathovars *phaseolicola* and *pisi* confer specificity toward both host and non-host species. Physiol. Mol. Plant Pathol. 40:1-15.

Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA 86:9981-9985.

Hanahan, D. 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166: 557-580.

Heath, M. C. 1981. A generalized concept of host-parasite specificity. Phytopathology 71:1121-1123.

Heath, M. C., Valent, B., Howard, R. J., and Chumley, F. G. 1990. Correlations between cytologically detected plant-fungal interactions and pathogenicity of *Magnaporthe grisea* toward weeping lovegrass. Phytopathology 80:1382-1386.

Herbers, K., Conrads-Strauch, J., and Bonas, U. 1992. Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. Nature 356:172-174.

Hopkins, C. M., White, F. F., Choi, S.-H., Guo, A., and Leach, J. E. 1992. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. Mol. Plant-Microbe Interact. 5:451-459.

Jones, D. A., Thomas, C. M., Hammond-Kosack, K. E., Balint-Kurti, P. J., and Jones, J. D. G. 1994. Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266: 789-793.

Kang, S., Chumley, F. G., and Valent, B. 1994. Isolation of the mating-type genes of the phytopathogenic fungus *Magnaporthe grisea* using genomic subtraction. Genetics 138:289-296.

Kato, H. 1978. Biological and genetic aspects in the perfect state of rice blast fungus, *Pyricularia oryzae* Cav. and its allies. Gamma Field Symp. 17:1-22.

Kearney, B., and Staskawicz, B. J. 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. Nature 346:385-386.

Kobayashi, D. Y., Tamaki, S. J., and Keen, N. T. 1989. Cloned avirulence genes from the tomato pathogen *Pseudomonas syringae* pv. *tomato* confer cultivar specificity on soybean. Proc. Natl. Acad. Sci. USA 86:157-161.

Leung, H., Borromeo, E. S., Bernardo, M. A., and Notteghem, J. L. 1988. Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. Phytopathology 78:1227-1233.

Lorang, J. M., Shen, H., Kobayashi, D., Cooksey, D., and Keen, N. T. 1994. *avrA* and *avrE* in *Pseudomonas syringae* pv. *tomato* PT23 play a role in virulence on tomato plants. Mol. Plant-Microbe Interact. 7: 508-515.

Mackill, A. O., and Bonman, J. M. 1986. New hosts of *Pyricularia oryzae*. Plant Dis. 70:125-127.

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

Mindrinos, M., Katagiri, F., Yu, G.-L., and Ausubel, F. M. 1994. The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78:1089-1099.

Notteghem, J. L. 1990. Results and orientation of EC projects on rice resistance to blast. Summa Phytopathol. 16:57-72.

Ou, S. H. 1985. Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Surrey, England.

Pandotra, V. R. 1976. Note on the occurrence of *Pyricularia* leaf spot of pearl millet in Jammu. Indian J. Agric. Sci. 46:387-388.

Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Swarup, S., Yang, Y., Kingsley, M. T., and Gabriel, D. W. 1992. An *Xanthomonas citri* pathogenicity gene, *pthA*, pleiotropically encodes gratuitous avirulence on nonhosts. Mol. Plant-Microbe Interact. 5: 204-213.

Sweigard, J. A., Carroll, A. M., Kang, S., Farrall, L., Chumley, F. G., and Valent, B. 1995. Identification, cloning and characterization of *PWL2*, a gene for host-species specificity in the rice blast fungus. Plant Cell 7:1221-1233.

Sweigard, J. A., Valent, B., Orbach, M. J., Walter, A. M., Rafalski, A., and Chumley, F. G. 1993. A genetic map of the rice blast fungus *Magnaporthe grisea*. Pages 3.112-3.115 in: Genetic Maps. 6th ed. S. J. O'Brien, ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Urashima, A. S., Igarashi, S., and Kato, H. 1993. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Dis. 77:1211-1216.

Valent, B., and Chumley, F. G. 1991. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. Annu. Rev. Phytopathol. 29: 443-467.

Valent, B., Crawford, M. S., Weaver, C. G., and Chumley, F. G. 1986. Genetic studies of fertility and pathogenicity in *Magnaporthe grisea* (*Pyricularia oryzae*). Iowa State J. Res. 60:569-594.

Valent, B., Farrall, L. and Chumley, F. G. 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. Genetics 127:87-101.

von Heijne, G. 1992. Cleavage-site motifs in protein targeting sequences. Pages 1-11 in: Genetic Engineering, Principles and Methods, Vol. 14. J. K. Setlow, ed. Plenum Press, New York.

Weiner, A. M., Deininger, P. L., and Efstratiadis, A. 1986. Nonviral retroposons: genes, pseudogenes, and transposable elements generated by the reverse flow of genetic information. Ann. Rev. Biochem. 55: 631-661.

Whalen, M. C., Stall, R. E., and Staskawicz, B. J. 1988. Characterization of a gene from a tomato pathogen determining hypersensitive resistance in a non-host species and genetic analysis of this resistance in bean. Proc. Natl. Acad. Sci. USA 85:6743-6747.

Whalen, M. C., Innes, R. W., Bent, A. F., and Staskawicz, B. J. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell 3:49-59.

Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C., and Baker, B. 1994. The product of the tobacco mosaic virus resistance gene *N*: Similarity to Toll and the interleukin-1 receptor. Cell 78: 1101-1115.

Yaegashi, H. 1978. Inheritance of pathogenicity in crosses of *Pyricularia* isolates from weeping lovegrass and finger millet. Ann. Phytopathol. Soc. Jpn. 44:626-632.

Yaegashi, H. 1988. Inheritance of blast resistance in two-rowed barley. Plant Dis. 72: 608-610.

Yaegashi, H., and Asaga, K. 1981. Further studies on the inheritance of pathogenicity in crosses of *Pyricularia oryzae* with *Pyricularia* sp. from finger millet. Ann. Phytopathol. Soc. Jpn. 47:677-679.

Yang, Y., De Feyter, R., and Gabriel, D. W. 1994. Host-specific symptoms and increased release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from leaves are determined by the 102-bp tandem repeats of *pthA* and *avrb6*, respectively. Mol. Plant-Microbe Interact. 7:345-355.

Zeigler, R. S., Cuoc, L. X., Scott, R. P., Bernardo. M. A., Chen, D. H., Valent, B., and Nelson, R. J. 1995. The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. Phytopathology 85:443-451.