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Analysis of *Phytophthora parasitica* by retrotransposon-derived DNA fingerprinting

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Abstract. *Phytophthora parasitica*, being able to attack a wide variety of plants, is a very important plant pathogen in Taiwan. It has been shown that isolates of *P. parasitica* from tobacco, dieffenbachia, and loquat differed significantly from other isolates in morphology and pathogenicity and were recognized as "atypical" types of the fungus. In the current study, a partial sequence of the reverse transcriptase gene of a Ty1-copia retrotransposon was cloned from *P. parasitica* and used as the probe, designated herein as G2Ty-1, for genomic Southern hybridization analyses of *P. parasitica*. It was demonstrated that G2Ty-1 existed as a moderately repetitive sequence in the genome of *P. parasitica*. The banding pattern of G2Ty-1 was mitotically stable for at least five consecutive asexual generations. DNA fingerprint analysis of *P. parasitica* using G2Ty-1 as the probe demonstrated that the banding patterns of G2Ty-1 were highly polymorphic among the isolates analyzed. "Atypical" strains from tobacco, dieffenbachia, and loquat, nonetheless, displayed host-specific banding patterns. Phylogram generated by cluster analysis demonstrated that isolates from tobacco and dieffenbachia were well separated from each other and from all other isolates of *P. parasitica*. These results provided a genetic basis for distinguishing these isolates from others and validate the use of retrotransposon as a genetic marker to study phylogeny in *Phytophthora parasitica*.

Keywords: DNA fingerprinting; Genotypes; Host specificity, *Phytophthora parasitica*; Retrotransposon.

Introduction

Phytophthora parasitica Dastur (= *Phytophthora nicotianae* Breda de Haan), an oomyceteous fungus, is the most widespread and important species of *Phytophthora* in Taiwan (Ho et al., 1995). It causes root rot, foot rot, leaf blight, and fruit rot in a variety of economically important crops (Ann et al., 1992; Ann, 1992, 2000a, 2000b; Erwin and Ribeiro, 1996). The taxonomy of *Phytophthora* is based mainly on morphological and physiological characteristics (Waterhouse, 1963). Although isolates morphologically identified as *P. parasitica* are pathogenic to a wide range of plant species, considerable evidence shows that some isolates are specifically pathogenic to a few hosts or a single host, such as tobacco (Bonnet et al., 1978, 1994; Matheron and Matejka, 1990; Erwin and Ribeiro, 1996). In support of this view, it was demonstrated in a recent study that isolates from dieffenbachia (*Dieffenbachia maculata* Don) were nonpathogenic or weakly pathogenic to other hosts of *P. parasitica*, nor were isolates from these plants pathogenic to dieffenbachia (Ann, 1992). Besides, some isolates from loquat (*Eriobotrya japonica* (Thunb.) Lindl.), known as "atypical" strains of *P. parasitica*, were found to be highly pathogenic to loquat while displaying reduced virulence toward eggplant fruits, sweet orange, and tomato seedlings as compared to typical strains in the pathogenicity test (Chern et al., 1998). It is not clear if atypical characteristics in pathogenicity observed in these isolates result from differentiation of the fungi at the genetic level. In past years, molecular markers such as isozyme patterns (Oudemans and Coffey, 1991) or mitochondrial restriction fragment length polymorphism (RFLP) (Föster et al., 1990; Lacourt et al., 1994) have been used for analysis of *P. parasitica*. These studies, however, revealed only a low level of polymorphism among the *P. parasitica* isolates analyzed, and no correlation with pathogenicity was observed. As a first attempt to seek molecular support for host specialization in *P. parasitica* isolates, retrotransposon-derived DNA fingerprinting was used to analyze the genotypes of various *P. parasitica* isolates in the current study.

Retrotransposons are mobile genetic elements, which replicate through an RNA intermediate prior to integration into the chromosomal DNA of their host. As a result, they may constitute a substantial portion of the repetitive sequences in an organism, and provide useful markers for analyses of the plant and fungal genomes (Hamer et al., 1989; Purugganan and Wessler, 1995; Flavell et al., 1998). According to the structural organization and the amino acid sequences of the encoded reverse transcriptase and

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integrase, retrotransposons can be classified into three major groups: the Ty1-copia, the Ty3-gypsy, and LINE (long interspersed nuclear elements)-like retrotransposons (Boeke and Sandmeyer, 1991, and references therein). The Ty1-copia and Ty3-gypsy retrotransposons are characterized by the flanking long terminal repeats (LTR). LINE, on the contrary, does not have LTRs. In this report, partial reverse transcriptase sequence of a Ty1-copia retrotransposon was cloned and used as the probe for DNA fingerprint analyses of *P. parasitica*. It was demonstrated that, although the DNA banding patterns of most isolates are highly polymorphic, *P. parasitica* isolated from tobacco, dieffenbachia, and loquat exhibited host-specific DNA banding patterns. These results provide a genetic basis for separating these *P. parasitica* isolates from others.

Materials and Methods

Phytophthora Isolates and Culture Conditions

Phytophthora parasitica isolates were from one of us (P. J. Ann) (Table 1; Ann, 1992, 1995; Chern et al., 1998; Ann, 2000a, 2000b). Cultures were grown on 5% V-8 juice agar (5% Campbell's V-8 juice, 0.02% $CaCO_3$, and 2% Bacto agar) plates for 3-5 days, cut into small pieces (5×3×2 mm), and soaked in test tubes containing sterile distilled water at 24°C for maintenance. For preparation of DNA, fungal isolates were grown on 5%V-8 juice medium (5% Campbell's V-8 juice and 0.02% $CaCO_3$) at 25°C in darkness for 10 days.

The method described by Hwang et al. (1976) was used for production and isolation of single-zoospore isolates. A few pieces of 3-5-day-old culture blocks (3×3×2 mm) grown on 5% V-8 agar were soaked in a small petri dish (6 cm in diameter) containing 5 ml sterile distilled water at room temperature for releasing zoospores. Single-zoospore isolates obtained from the "parent" isolates were designated as Z1. Subsequently, the second asexual generation was isolated from Z1 by the same method and designated as Z2. A total of five asexual generations (Z1~Z5) were collected from four different "parent" isolates (731-0, 991-3-1, PPPr 1-1, and PPT 2-1), respectively.

Polymerase Chain Reaction and DNA Sequence Analysis

General DNA manipulations including extraction of fungal DNA, preparation of plasmid DNA, DNA ligation, bacterial transformation, and agarose gel electrophoresis were performed as described by Sambrook et al. (1989). Nuclear DNA of *P. parasitica* was further purified by CsCl gradient centrifugation according to Garber and Yoder (1983).

For amplification of the reverse transcriptase domain of the Ty1-copia retrotransposon, two sets of degenerate oligonucleotides, ty1A (5´-ACNGCNTTYYTNCAYGG-3´) and ty1B (5´-ARCATRTCRTCNACRTA-3´) (Flavell et al., 1992), were used to prime the DNA polymerization reaction. The reaction mixture contained 100 ng of fungal genomic DNA, 2.5 μM of oligonucleotide primers, 0.2 mM dNTP, 1X PCR buffer, and 1 U of DyNazyme™ II DNA polymerase (Finnzymes). PCR was performed in a thermocycler (GeneAmp PCR System 2400) programmed for denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 15 sec, 50°C for 15 sec, 72°C for 15 sec, and a final extension at 72°C for 10 min. Following analysis of the amplified products by 1.5% agarose gel electrophoresis, DNA bands of expected size were collected from the agarose gel using the Geneclean III kit (Bio101, Inc.), and cloned into pGemT-easy (Promega). DNA sequencing was performed on both strands of DNA by the dideoxy termination method (Sanger et al., 1977), using the BigDye terminator cycle sequencing ready reaction kit and an automated Applied Biosystem 373 instrument (Applied Biosystems). Sequence was analyzed using programs in the GCG software package (Genetics Computer Group, Wisconsin, Package Version 10.0).

Genomic Southern Hybridization

Phytophthora parasitica DNA was digested with 10 units of restriction enzyme and then separated on a 0.8% agarose gel in 0.5X TBE buffer at 1.2 V cm^{-1} overnight. Blotting was performed according to the standard protocol (Sambrook *et al*., 1989). DNA probe was labeled with digoxigenin (DIG)-11-dUTP by PCR using the PCR DIG probe synthesis kit (Roche). PCR was primed with oligonucleotides 1S and 1A (Figure 1) in order to exclude the conserved reverse transcriptase sequences and thereby to avoid cross hybridization with those of other retrotransposons. This probe was herein designated as G2Ty-1. Prehybridization, hybridization with DIG-labeled probe, and detection of the hybridization signal were performed according to the manufacturer's protocol (Roche).

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          .         .         .         .         .         .
  1 ACGGCGTTCCTGCACGGCTGGCTGGACGAAGACATCTACATGGTGCAGCCGGACGGCTAC  60
    T  A  F  L  H  G  W  L  D  E  D  I  Y  M  V  Q  P  D  G  Y
          .         .         .         .         .         .
 61 GTTGACGAGGCGCACCCGGAATTCGTGTGCAAGCTCAAGCGTTCGCTTTACGGCCTCAAG 120
    V  D  E  A  H  P  E  F  V  C  K  L  K  R  S  L  Y  G  L  K
          .         .         .         .         .         .
121 CAGTCGCCGCGAATGTGGAACCAGACCATCGACAAGTTCATGCTGGAGCTGGGGTTCAAG 180
    Q  S  P  R  M  W  N  Q  T  I  D  K  F  M  L  E  L  G  F  K
          .         .         .         .         .         .
181 AAGTGTGAGGCGGATCACTGCATCTACGTTAAGCGTGATGACCAAGACATGATCTTCGTG 240
    K  C  E  A  D  H  C  I  Y  V  K  R  D  D  Q  D  M  I  F  V
          .         .
241 GCGCTGTACGTGGACGACATGCT 263
    A  L  Y  V  D  D  M
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Figure 1. Partial nucleotide and deduced amino acid sequence of the reverse transcriptase gene of a Ty1-copia retrotransposon from *Phytophthora parasitica*. The sequence (GeneBank accession number AF130855) was cloned by PCR with primers ty1A and ty1B (Flavell et al., 1992). Underlines indicate positions of PCR primers used for synthesis of the DIG-labeled G2Ty-1 probe: 1S (the upstream primer) and 1A (the downstream primer).

Statistical Analysis

Presence (1) or absence (0) of a band at a particular position in the Southern blot (ranging from 2 to 6 kb) were treated as discrete characters, and banding patterns were converted into binary matrices. The total banding number of each isolate and the number of bands shared by every pair of isolates were counted. "Similarity index" (S) was calculated according to the formula: $S_{ab} = 2 \times n_{ab}/(n_a+n_b)$, where n_a and n_b represent the total number of bands present in the DNA fingerprint patterns of fungal isolates a and b, respectively, and n_{ab} is the number of bands shared by isolates a and b (Nei and Li, 1979). Subsequently, pairwise distance (D_{ab}) between isolates a and b was obtained by the following formula: $D_{ab} = 1- S_{ab}$. The resulting distance values were then used as the basis of cluster analysis. Dendrograms were produced by cluster analysis using the UPGMA (unweighted pair group method using arithmetic average) (Sneath and Sokal, 1973) option of the Neighbor-joining program in the PHYLIP (Phylogeny Inference Package) (Version 3.5c; J. Felstein,

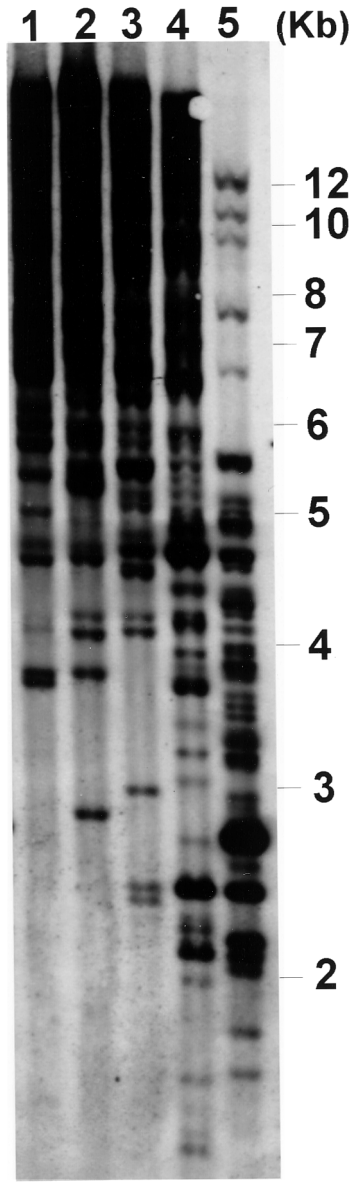

Figure 2. Southern hybridization analysis of the G2Ty-1 sequence in *Phytophthora parasitica*. Fungal DNA (isolate number PPAv3) was cut with *Xho*I (lane 1), *Sac*I (lane 2), *Hind*III (lane 3), *Eco*RI (lane 4), or *Bam*HI (lane 5), and subjected to Southern hybridization analysis using DIG-labeled G2Ty-1 as the probe. Positions of size standards are indicated in kilobases to the right.

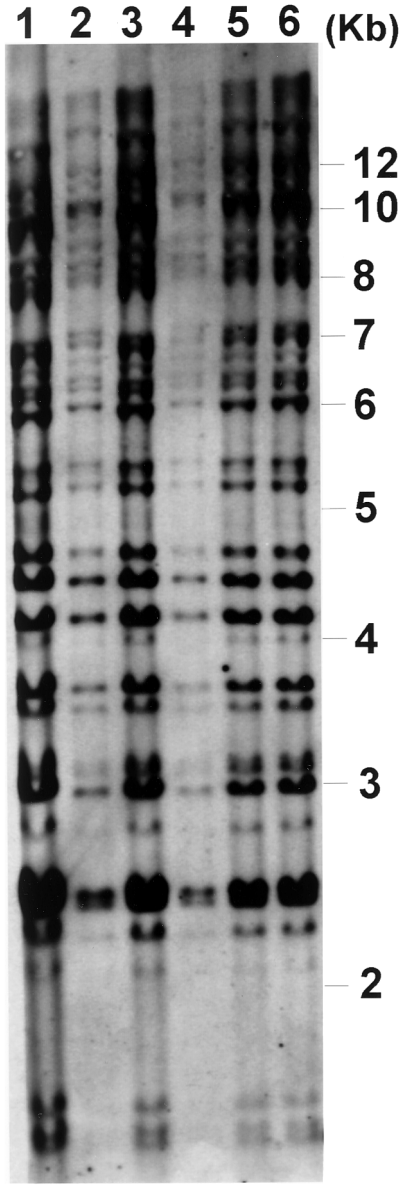

Figure 3. Somatic stability of the G2Ty-1 banding pattern of *Phytophthora parasitica*. DNA from single-zoospore isolates representing five successive asexual generations (Z1 to Z5) (lanes 2~6) from a parent isolate (991-3-1) (lane 1) was cut with *Eco*RI and analyzed by Southern hybridization using DIG-labeled G2Ty-1 as the probe. Positions of size standards are indicated in kilobases to the right.

Dept. Genetics, University of Washington, Seattle). The TREEVIEW program was used to draw the resulting phylogenetic tree (Page, 1996).

Results

Cloning and Analysis of Partial Reverse Transcriptase Sequence of Ty1-Copia Retrotransposons from P. parasitica

PCR with ty1A and ty1B, which were designed according to the conserved reverse transcriptase sequence of Ty1-copia retrotransposons (Flavell et al., 1992), gave rise to a DNA fragment of the expected size, approximately 260 bp in length (data not shown). This fragment was eluted from the agarose gel, cloned into a T-vector, and subjected to DNA sequence analysis. Of the ten recombinant clones being analyzed, one contained a full-length ORF which was obviously irrelevant to the reverse transcriptase sequence of the copia retrotransposon, and three contained short ORFs and stop codons. Thus, they were neglected in the subsequent analysis. The remaining six clones contained ORFs with an identical deduced amino acid sequence. Analysis of the amino acid sequence revealed the presence of TAFLH and YVDDM on the N- and C-termini of each ORF, which corresponded to the sequences of the PCR primers (Figure 1). Analysis by BLAST indicated that it is very similar to the reverse transcriptase sequences of *Phytophthora infestans* (AF262230), *Alstroemeria inodora* (AJ223608), and *Picea abies* (AJ290661), as well as to other Ty1-copia retrotransposons from plants. Moreover, a conserved reverse transcriptase

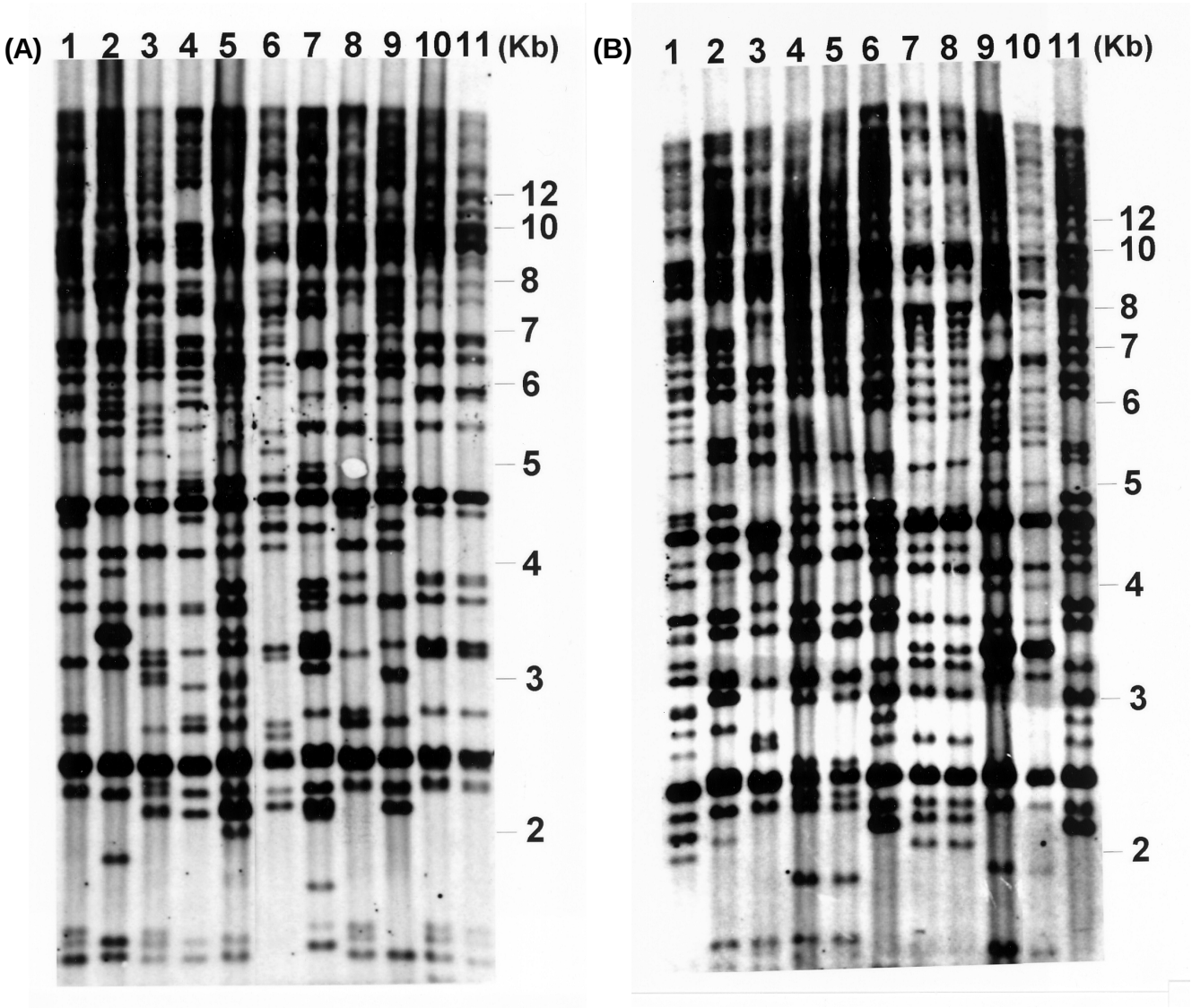

Figure 4. Nuclear polymorphisms of *Phytophthora parasitica* based on the G2Ty-1 probe hybridization patterns. After digestion with *Eco*RI, fungal DNA was subjected to Southern hybridization analysis using DIG-labeled G2Ty-1 as the probe. Positions of size standards are indicated in kilobases to the right. Fungal isolates demonstrated in panel A included PPFa1 (lane 1), PPT1-1 (lane 2), PP3-2 (lane 3), PPEe1 (lane 4), PPPj1 (lane 5), PPPp1 (lane 6), PPPp3 (lane 7), PPL10 (lane 8), PPCc3 (lane 9), PPCr1-1 (lane 10), PPCr1-5 and (lane 11). Panel B included 731-0 (lane 1), 991-3-1 (lane 2), PPPr1 (lane 3), PPD1 (lane 4), PPD2 (lane 5), PPLo5 (lane 6), PPLo1 (lane 7), PPLo2 (lane 8), PPT1-2 (lane 9), PPT4 (lane 10), and PPT5 (lane 11).

motif for the Ty1-copia retrotransposons, SLYXLKQAXRXW (Flavell et al., 1992; Flavell et al., 1998; Xiong and Eickbush, 1990), was also identified in the sequence, indicating that it was amplified from the reverse transcriptase gene of a Ty1-copia retrotransposon.

Hybridization Patterns of the G2Ty-1 Probe

To better understand the nature of the cloned sequence, genomic Southern hybridization was performed following digestion of fungal DNA (isolate PPAV3-1) with restriction enzymes *Xho*I, *Sac*I, *Hind*III, *Eco*RI or *Bam*HI, using G2Ty-1 as the probe. As shown in Figure 2, the DIG-labeled G2Ty-1 probe hybridized to multiple DNA fragments in the genome of *P. parasitica*. The best resolution of banding pattern was obtained with the *Eco*RI digests (lane 4, Figure 2). Thus, genomic Southern hybridization was performed with *Eco*RI digest in the following analysis.

In order to verify the somatic stability of the banding patterns produced by G2Ty-1, single-zoospore isolates were prepared from four different "parent" fungal isolates and analyzed by genomic Southern hybridization using fungal DNA cut with *Eco*RI. The results indicated that, no matter which isolate was analyzed, the hybridization patterns of the single-zoospore progeny from Z1 to Z5, which encompassed a total of five consecutive asexual generations, were identical to their parent isolate (Figure 3). Thus, the DNA banding patterns produced by the G2Ty-1 probe were mitotically stable in *P. parasitica*.

Analysis of P. parasitica by G2Ty-1 DNA Fingerprinting

A total of 42 isolates of *P. parasitica* (Table 1) were studied. Among them, isolates from tobacco, dieffenbachia, and loquat, as well as one isolate from pothos vine (97036) were of special interest due to their characteristics in morphology and/or pathogenicity (Ann, 1995; Chern et al., 1998). Other isolates, on the contrary, were collected from a wide variety of host plants and were recognized as "typical common" strains of *P. parasitica* (Ann, 1995, 2000a, 2000b). Nuclear DNA of each isolate was digested with *Eco*R1, blotted, and hybridized with DIG-labeled probes. As shown in the representative Southern blots (Figure 4A and 4B), the banding patterns lit up by G2Ty-1 were highly polymorphic among the *P. parasitica* isolates being analyzed. Interestingly, some isolates displayed identical DNA banding patterns, and were classified into eight groups designated as types A~H. They included 731-0, PPSp1, and PPPj1 of type A, PPAv2, PPAg1, and PPCa75 of type B, PPLo5, PPBo1 and PPT5 of type E, PPEe1 and PPx1 of type G, and PPFa1, PPL10, and PPPr1 of type H (Table 1). In addition, of the seven tobacco isolates analyzed, six (PPT1-1, PPT1-2, PPT2-1, PPT2-4, PPT3, and PPT4) displayed an identical banding pattern and were designated as type F (Figure 4B). The banding pattern of PPT5 placed it in type E, the same as PPLo1 and PPBo1, isolates from loquat and bougainvillea (*Bougainvillea* sp.), respectively. Isolates from dieffenbachia, collected from Taitong (PPD1) and Changhua (PPD2), respectively, also displayed a unique banding pattern, designated as type C (Figure 4B, lanes 4 and 5). Two isolates from loquat, PPLo1 and PPLo2, known as 'atypical' strains of *P. parasitica* (Chern et al., 1998), exhibited a characteristic banding pattern designated as type D (Figure 4B, lanes 7 and 8).

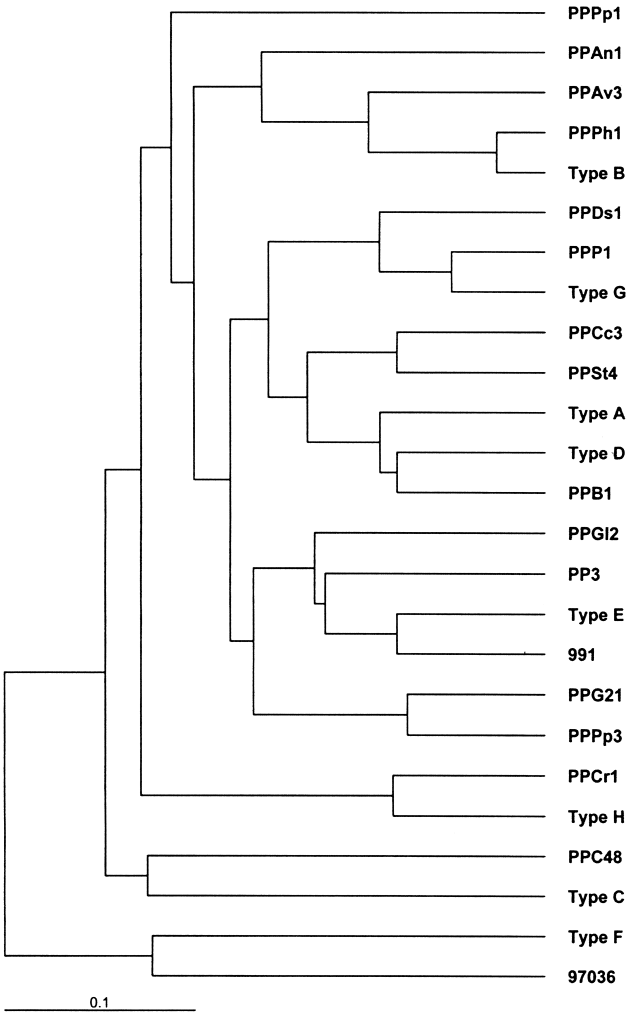

Figure 5. Phylogram generated by UPGMA cluster analysis of the distance values. Types A~H indicated genotypes of *Phytophthora parasitica* as defined in the text (Table 1). Data were compiled as a (0/1) matrix from the DNA fingerprint patterns revealed by the G2Ty-1 probe. Pairwise genetic distance was calculated and cluster analysis was performed as described in Materials and Methods.

To analyze the relationship between the fungal isolates based on the G2Ty-1 banding patterns, a phylogram was derived from the fingerprints of the fungal isolates by cluster analysis using the UPGMA option of the Neighbor-joining program in PHYLIP. As shown in Figure 5, the 42 isolates tested were divided into three groups. Isolate 97036 was clustered with type F, which represented most of the tobacco isolates (Table 1), while isolate PPC48 was clustered with type C, which represented isolates from dieffenbachia. These two clusters were widely separated from each other and from all other fungal isolates. Type

D, which represented "atypical" strains isolated from loquat was, in contrast, closest to isolate PPB1 and comprised part of the main cluster.

Discussions

Retrotransposons are mobile genetic elements, which replicate by successive transcription, reverse transcription, and insertion of the new cDNA copies back into the genome. Insertion of retrotransposon in a new site may be detected by the appearance of a new DNA banding in a genomic Southern blot, and thus is useful for establishment of phylogenies (Ellis et al., 1998; Kalendar et al., 1999). In our study, partial reverse transcriptase sequence of a Ty1-copia group retrotransposon was cloned by genomic PCR. This is the first indication that the genome of *P. parasitica* contains Ty1-copia retrotransposon. Molecular cloning and characterization of the full-length retrotransposon is now being performed in our laboratory.

Genomic Southern hybridization using G2Ty-1 as the probe revealed that this sequence exists as moderately repetitive sequences in the genome of *P. parasitica*. Furthermore, the G2Ty-1 hybridization patterns are highly polymorphic among the fungal isolates being analyzed. Since the presence of a reverse transcriptase sequence is

Table 1. *Phytophthora parasitica* used for analysis in the experiment.

Isolate no.	Location of isolation	Host	Mating type[a]	Type [b]	Genotype[c]
991-3-1	United States	*Citrus* spp. (Citrus)	A^1	TC	
731-0	United States	*Citrus* spp. (Citrus)	A^2	TC	A
PPC48, 88011	Chiayi	*Citrus* spp. (Citrus)	A^1	TC	
PPSp1, 92169	Chiayi	*Spathiphylium* sp. (Spathiphylium)	A^2	TC	A
PPPj1, 90101	Chiayi	*Dianthus japonicus*	A^2	TC	A
PPAv3, 92033	Taitung	*Saintpulia ionantha* (African violet)	A^2	TC	
PPAv2, 91055	Chiayi	*Saintpulia ionantha* (African violet)	A^1	TC	B
PPAg1, 90158	Tianwei, Changhwa	*Aglaonema commutatum* (Aglaonema)	A^1	TC	B
PPCa75, 92081	Chiayi	*Dianthus caryophyllus* (Carnation)	A^1	TC	B
PPD1, 90190	Tianwei, Changhwa	*Diefferenbachia maculata* (Diefferenbachia)	A^2	A	C
PPD2, 92041	Taitung	*Diefferenbachia maculata* (Diefferenbachia)	A^2	A	C
PPLo1, 95023	Wufeng, Taichung	*Eriobotrya japonica* (Loquat)	$H \rightarrow A^2$	A	D
PPLo2, 95034	Wufeng, Taichung	*Eriobotrya japonica* (Loquat)	$H \rightarrow A^2$	A	D
PPLo5, 96085	Yungching, Changhwa	*Eriobotrya japonica* (Loquat)	A^2	TC	E
PPBo1, 96047	Wufeng, Taichung	*Bougainvillea* sp. (Bougainvillea)	A^2	TC	E
PPT5, 94069	Wufeng, Taichung	*Nicotiana tabaccum* (Tobacco)	A^2	T	E
PPT1-1, 93122	Fanlu, Chiayi	*Nicotiana tabaccum* (Tobacco)	A^2	T	F
PPT1-2, 93123	Fanlu, Chiayi	*Nicotiana tabaccum* (Tobacco)	A^2	T	F
PPT2-1, 93149	Chungpu, Chiayi	*Nicotiana tabaccum* (Tobacco)	A^2	T	F
PPT2-4, 93152	Chungpu, Chiayi	*Nicotiana tabaccum* (Tobacco)	A^2	T	F
PPT3, 93153	Chungpu, Chiayi	*Nicotiana tabaccum* (Tobacco)	A^2	T	F
PPT4, 93156	Chungpu, Chiayi	*Nicotiana tabaccum* (Tobacco)	A^2	T	F
PPEe1, 89202	Ieanshsuei, Tainan	*Dracaena deremensis* (Compact dracaena)	A^1	TC	G
PPx1, 98151	Wufeng, Taichung	*Pandanus odorus*	A^2	TC	G
PPFa1, 90162	Puli, Nantow	*Fastia polycarpa* (Fatsia)	A^2	TC	H
PPL10, 91064	Taichung	*Lilum oriental* hybrid "casablanca"	A^2	TC	H
PPPr1, 92172	Chiayi	*Peperomia serpens* (Peperomia)	A^1	TC	H
PPPp3, 89112	Nemen, Kaohsiung	*Carica papaya* (Papaya)	A^1	TC	
PPPp1, 87001	Lutsau, Chiayi	*Carica papaya* (Papaya)	A^2	TC	
PPB1, 92016	Chiayi	*Gypsophila paniculata* (Baby's breath)	A^2	TC	
PPAn1, 90174	Chungpu, Taichun	*Anthrium andraeanum* (Anthrium)	A^2	TC	
PPCr1-1, 96096	Yungching, Changhwa	*Codiaeum variegatum* (Croton)	A^2	TC	
PPCr1-5, 90100	Yungching, Changhwa	*Codiaeum variegatum* (Croton)	A^2	TC	
PP3-2	Chiayi	*Hibiscus sabdoriffa* (Rozelle)	A^2	TC	
PPG2-1-1, ATCC6133-1	Yungching, Changhwa	*Psidium puajava* (Guava)	$A^2(A^1)$	TC	
PPGl2, 92145	Chungpu, Chiayi	*Sinningia speciosa* (Gloxinia)	A^1	TC	
PPDs1, 98161	Wufeng, Taichung	*Adenium obesum* (Desert rose)	A^1	TC	
PPPh1, 90150	Tianwei, Changhwa	*Philodendron* spp. (Parlor ivy)	A^1	TC	
PPSt4, 99009	Tungshiau, Taichung	*Fragaria chiloensis* (Strawberry)	A^2	TC	
PPCc3, 94023	Chiayi	*Schlumbergera bridgesii* (Christmas cactus)	A^2	TC	
PPP1, 96055	Chiayi	*Coleus blumei* (Painted nettle)	A^2	TC	
97036	Ilan	*Epipremnum aureum* (Pothos vine)	A^0	A	

[a] $H \rightarrow A^2$ indicates a few oospores produced in the single culture but a large amount of oospores formed in the dual-culture with A^1 isolate. Parentheses indicate the original mating type. A^0 indicates oospore production was not observed after pairing with either A^1 or A^2 isolate.

[b] TC: Typical common type; A: Atypical type; T: Tobacco type. Determination of "typical" or "atypical" type was based on the descriptions of *P. parasitica* given by Tucker in 1931. All tobacco isolates were pathogenic to the host plants of the typical common strain in the pathogenicity test, but not vice versa.

[c] Genotypes were determined by DNA fingerprinting using G2Ty-1 as the probe and classified into types A~H based on the DNA banding patterns.

indicative of the existence of retrotransposons, it is possible that the DNA polymorphism observed in our study was caused by active transposition of a retrotransposon. Analysis of single-zoospore progeny, which encompass five successive asexual generations from four different "parent" isolates, however, indicates that the G2Ty-1 banding patterns are mitotically stable, at least to the extent genomic Southern hybridization analysis may detect. Analysis by Northern blot and reverse transcriptase-PCR also confirmed absence of the corresponding RNA message in *P. parasitica* (data not shown). It is thus very likely that the highly polymorphic banding patterns of G2Ty-1 revealed in the current study resulted from a once very active retrotransposon which is unable to transpose anymore. This may provide an excellent basis for the development of marker systems in *P. parasitica*. Previous studies using mitochondrial RFLP (Föster et al., 1990; Lacourt et al., 1994) or isozyme patterns (Oudemans and Coffey, 1991) as genetic markers revealed only a low level of polymorphism, and thus lower intraspecific diversity in *P. parasitica* than other *Phytophthora* species. Analysis by a retrotransposon-derived marker system, as demonstrated in this study, may provide new insights into the genetic diversity of intraspecific taxa of *P. parasitica* isolates.

In our study, *P. parasitica* isolates were analyzed by G2Ty-1-based DNA fingerprinting. The results demonstrated that, although the fingerprints of most isolates were highly polymorphic, some isolates displayed identical banding patterns. In particular, "atypical" strains from loquat, dieffenbachia, and tobacco displayed host-specific DNA fingerprints. These isolates are known to differ significantly from others in regard to morphology, virulence, and host range (Ann, 1992, 1995; Chern et al., 1998). "Atypical" strains of *P. parasitica* isolated from loquat (PPLo1 and PPLo2) displayed characteristic banding patterns of type D, which was distinct from that of PPLo5, also an isolate from loquat but known as a "typical common" strain of the fungus. Dieffenbachia isolates from Changhwa (Tianwei) or Taitung, known to be nonpathogenic or weakly pathogenic to other hosts of *P. parasitica*, showed identical banding patterns of type C. Isolates from tobacco, with one exception, displayed host-specific banding patterns of type F. Furthermore, a phylogram generated by cluster analyses of the G2Ty-1 banding patterns demonstrated that isolates from tobacco (type F) and dieffenbachia (type C) were widely separated from each other and all other fungal isolates. These results provide a genetic basis for distinguishing these fungal isolates from others and illustrate the potential of retrotransposon-derived DNA fingerprints for phylogeny analysis in *Phytophthora parasitica*. In support of our result, a recent study by Colas et al. (1998) also demonstrated that black shank isolates could be distinguished from other *P. parasitica* isolates on the basis of nuclear RFLP. It is thus very likely that the tobacco and dieffenbachia isolates have evolved from separate clonal origins in the course of fungal evolution, and host specialization as observed in these isolates may have a genetic basis.

It is intriguing to ask why characteristics of *P. parasitica*, such as host specialization, are correlated with the insertion site polymorphism of a retrotransposon and why type C is clustered most closely with PPC48, a "typical common" strain isolated from citrus. Since activity of transposable elements may cause mutation and thereby lead to pathogenic specialization in plant pathogenic fungi (McHale et al., 1992; Shull and Hamer, 1995; He et al., 1996), it is not surprising that transposition of a retrotransposon in the genome of *P. parasitica* can result in changes of its phenotypes as well. What is really relevant is the insertion sites of the retrotransposon, namely the genes that have been disrupted by the retrotransposon in individual fungal isolate. In plants, retrotransposons are frequently found in the regions flanking functional genes (White et al., 1994). Nothing is known, however, about their insertion sites in *P. parasitica*. Analysis of sequences flanking the retrotransposons may help elucidate the mechanisms underlying host specialization as well as other characteristics of this fungus.

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以反轉位子局部序列為探針進行疫病菌 DNA 指紋分析

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本研究以 Ty1-copia 反轉位子局部序列為探針進行疫病菌 DNA 指紋分析。疫病菌可危害之寄主植物種類繁多，重要作物包括柑橘、百合、蘭花及多種觀賞植物都是其危害對象，是台灣重要植物病原真菌。研究顯示自菸草、萬年青及枇杷所分離到之疫病菌菌株，不論在型態或病原性都與一般典型疫病菌有顯著差異，為了探討這種差異與疫病菌遺傳變異之關連性，本研究自疫病菌選殖 Ty1-copia 反轉位子之反轉錄酶基因的局部序列，以之製備探針（G2Ty-1）進行南方雜合分析的研究結果發現，G2Ty-1 為一中度重複性序列，其雜合圖譜跨五個無性世代仍維持一致，因此有相當潛力可應用為分子標記。以 G2Ty-1 為探針進行疫病菌DNA指紋分析之結果顯示，雖然不同菌株之 G2Ty-1 雜合圖譜呈現高度多型性，但不論採集地點是哪裏，自菸草、萬年青及枇杷所分離到之“非典型”疫病菌菌株各顯現出寄主專一性圖譜。進一步根據 DNA 指紋進行 UPGMA 群叢分析後發現，可將供試菌株分成三群，其中菸草與萬年青分離株各自形成一個群叢，與其他供試菌株間有明顯的區隔，顯示這些菌株的病原性特化現象有其遺傳基礎，值得進一步深入探討。

關鍵詞：反轉位子；疫病菌；基因型；DNA指紋分析；寄主專一性。