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Rapid Detection of *Phytophthora nicotianae* in Infected Tobacco Tissues and Soil Samples Based on Its *Ypt*1 Gene

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Abstract

This paper describes the development of a polymerase chain reaction (PCR) assay for the detection of *Phytophthora nicotianae*, the causal agent of *Phytophthora* blight of tobacco and other plants. The PCR primers were designed based on a Ras-related protein (*Ypt*1) gene, and 115 isolates representing 26 species of *Phytophthora* and 29 fungal species of plant pathogens were used to test the specificity of the primers. PCR amplification with species-specific (Pn) primers resulted in a product of 389 bp only from isolates of *P. nicotianae*. The detection sensitivity with Pn primers was 1 ng of genomic DNA. Using *Ypt*1F/*Ypt*1R as first-round amplification primers, followed by a second round using the primer pair Pn1/Pn2, a nested PCR procedure was developed, which increased the detection sensitivity 100-fold to 10 pg. PCR with the Pn primers could also be used to detect *P. nicotianae* from naturally infected tobacco tissues and soil. The PCR-based methods developed here could simplify both plant disease diagnosis and pathogen monitoring as well as guide plant disease management.

Introduction

Phytophthora nicotianae is a common and destructive pathogen of numerous ornamental, agronomic and horticultural crops, such as tobacco, tomato and citrus. Infected tissues and infested soil are primary sources of inoculum for *Phytophthora* disease in fields. Chlamydospores of *P. nicotianae* can survive in field soil for several years. Infested soil is not only an important source of inoculum but also an efficient means of spreading pathogens from a single site of infection to an entire field. Infested irrigation water is also an important source of inoculum and an efficient means of spreading pathogens from a single site of infection to an entire nursery, greenhouse or farm and from one geographic location to other locations that use the same water system (McIntosh, 1966; Thomson and Allen, 1974; Oudemans, 1999). Rapid, accurate and sensitive detection is important for managing *Phytophthora* diseases and reducing pathogen spread.

DNA-based techniques have become an effective means of identifying plant pathogens (Goodwin et al., 1989; Ersek et al., 1994; Levesque et al., 1998). Because of advantages in speed, sensitivity, specificity and flexibility, the polymerase chain reaction (PCR) technique has been used extensively and offers great promise for future plant pathogen detection systems (Picard et al., 1992; Thomas et al., 1993; Audy et al., 1996; Moricca et al., 1998; Faggian et al., 1999; James, 1999; Schubert et al., 1999). *Phytophthora nicotianae*-specific primers have been used for rapid identification of pure cultures (Ersek et al., 1994) infecting tobacco and tomato plants as well as zoospore suspensions in the laboratory (Lacourt and Duncan, 1997). The two pairs of specific primers are based on the *P. nicotianae* elicitin gene, *parA*1. *parA*1 is a good candidate for designing specific PCR primers due to its high copy number (Kamoun et al., 1993) and its specificity (Lacourt and Duncan, 1997). However, the sequences of the *parA*1 coding and non-coding regions share a high degree of similarity with those of elicitin genes of many other oomycetes including *Pythium* spp. (Gayler et al., 1997; Panabieres et al., 1997),. For this reason, specific primers based on *parA*1 cannot be used for detection of some plant pathogens.

The internal transcribed spacer (ITS) regions of the nuclear-encoded ribosomal RNA genes (rDNA) are the target genes most widely used to identify and detect *Phytophthora* species (Cooke et al., 2000). *Phytophthora nicotianae* species-specific primers based on ITS were developed for detection of *P. nicotianae* in irrigation water (Kong et al., 2003). However, in some other cases, studies found that the ITS sequences were not sufficiently variable making the design of primers for identification and detection of closely related taxa very difficult or impossible (Martin and

Tooley, 2003a,b; Brasier et al., 2004; Kroon et al., 2004). Intergenic regions of the mitochondrial DNA (mtDNA IGS) demonstrated intra- and inter-specific variation (Schena and Cooke, 2006; Wattier et al. 2003), and one of these regions was suited to the development of specific detection methods for *Phytophthora ramorum*, *Phytophthora nemorosa* and *Phytophthora pseudosyringae* (Martin et al. 2004). A limit to the use of the mtDNA-IGS regions is that they are usually very AT/GC rich making the design of effective primers quite difficult (Schena and Cooke, 2006). Another promising target gene for the design of *Phytophthora*-specific detection methods is the Ras-related protein (*Ypt*1) gene (Chen and Roxby, 1996). The non-coding regions of the *Ypt*1 gene showed sufficient variation to enable the development of molecular markers for almost all *Phytophthora* species, but did not contain intra-specific variability (Schena and Cooke, 2006). Based on the *Ypt*1 gene, four pairs of primers were designed for detection of four *Phytophthora* species, including *Phytophthora kernoviae*, *Phytophthora citricola*, *Phytophthora quercina* and *P. ramorum* (Schena et al., 2006). These primers proved to be rapid, reliable, sensitive and cost effective in the detection of target pathogens.

In this study, we developed a species-specific PCR assay for detection of *P. nicotianae* in infected tissues and soil. Our objectives were to design a pair of *P. nicotianae*-specific primers based on *Ypt*1 gene that provided the specificity and sensitivity needed for identifying pathogens in both infected tissues and soil samples. We also developed efficient PCR protocols for evaluating the sensitivity and specificity of primers and sample detection.

Materials and Methods

Phytophthora isolates and fungal isolates: isolates of *Phytophthora* spp., *Pythium* spp. and fungal isolates used in this study are listed in Table 1. A total of 15 isolates of *P. nicotianae* were isolated from different hosts and sites. Five isolates from infected tobacco tissues and four from soil samples were collected from Yunnan province of China. Five diseased citrus tissues were collected from Jiangsu province of China.

All isolates were stored either on lima bean agar slants at 10°C (*Phytophthora* spp. and *Pythium* spp.) or on potato dextrose agar at 4°C (other fungi). The isolates were stored in a collection at the Department of Plant Pathology, Nanjing Agricultural University, China.

Phytophthora isolates were cultured on tomato juice medium (Zheng, 1997) (per liter, 200 ml of tomato juice, 0.1 g of $CaCO_3$ and 15 g of agar mixed with sterile distilled water, sterilized at 120°C for 20 min). Mycelia of each *Phytophthora* and *Pythium* isolate were obtained by growing the isolates in tomato juice broth (Zheng, 1996) at 18 to 25°C (temperature dependent isolates) for at least 5 days. Mycelia of the other fungi were grown in potato dextrose broth (Erwin and Ribeiro, 1996). The mycelia were harvested by filtration and frozen at −20°C. DNA was extracted from the mycelia according to a modified cetyltrimethylammonium bromide procedure (Sambrook et al.,1989). The DNA samples were dried at room temperature and then resuspended in Tris-EDTA buffer(10 mM Tris–HCl and 0.1 mM EDTA, pH 8.0) and stored at −20°C.

Primers design for *Phytophthora nicotianae*

Alignment of the *P. nicotianae Ypt*1 gene with all the related sequences in the NCBI database was used to identify the sequences for the universal primers Ypt1F (5′-CGACCATTGGCGTGGACTTT-3′) and Ypt1R (5′-ACGTTCTCGCAGGCGTATCT-3′). In a similar fashion, the gene primers Pn1 (5′-GACTTTGTAAGTGCCACCATAC-3′) and Pn2 (5′-CTCAGCTCTTTTCCTTGGATCT-3′) were designed to amplify a *P. nicotianae*-specific regions of the *Ypt*1 gene.

Extraction of *Phytophthora nicotianae* DNA from soil

Soil samples were collected from diseased tobacco root in the field of Yuxi city of Yunnan province. DNA samples, which were extracted from soil samples by using a FastDNA® SPIN Kit for Soil of BIO 101® Systems (Q-Biogene Ltd, Morgan Irvine, CA, USA) according to the manufacturer's recommendations were finally stored at −20°C.

Extraction of *Phytophthora nicotianae* DNA from plant tissues

DNA samples from *P. nicotianae*-infected tobacco tissues originating from Yuxi city of Yunnan province and infected citrus tissues collecting from Nanjing, Jiangsu province were prepared from the diseased tissues by using NaOH method for conventional PCR according to Wang (Wang et al.,1993).

PCR

Each reaction for the *Phytophthora* species universal primers *Ypt*1F/*Ypt*1R protocol consisted of 2.5 μl of 10× PCR buffer, 1 μl of 2.5 mM dNTPs, 2.5 μl of 25 mM $MgCl_2$, 0.25 μl each of 20 μM/μl primers and 0.25 μl of *Taq* DNA polymerase (5 units/μl) to which were added template DNA and finally sterile distilled water was added to a final volume of 25 μl. All reactions were performed in a PTC200 PCR instrument (MJ Research, Watertown, MA, USA). The PCR programme was set as following: an initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s and a final extension at 72°C for 10 min. The PCR conditions used with *P. nicotianae* species-specific primers Pn1 and Pn2 were similar to those described above for *Ypt*1F and *Ypt*1R with the exception that the temperature of annealing was 64°C. Negative controls lacking template DNA were performed in each experiment to test for contaminated reagents. All of the reagents used for the PCR amplification were purchased from Takara (Dalian, China).

Table 1
Isolates of fungi and oomycetes used to screen the polymerase chain reaction (PCR)-specific primers (Pn1/Pn2)

Species[a]	Isolation/origin		No. isolates	PCR product[c]	
	Host/substrate	Source[b]		ITS1/ITS4	Pn1/Pn2
Phytophthora nicotianae	*Nicotiana tabacum*	YN	8	+	+
	Citrus	JS	1	+	+
	Lycopersicum esculentum	JS	1	+	+
	Sophora chinensis	JS	1	+	+
	Cucurbitaceae	FJ	1	+	+
	Fragaria ananassa	FJ	1	+	+
	Salvia splendens	JS	2	+	+
Phytophthora boehmeriae	*Gossypium*	JS	2	+	–
	Beohmeria niver	JS	2	+	–
Phytophthora brassicae	*Brassica*	CBS178.87	1	+	–
Phytophthora cambivora	*Castanea sativa*	CBS 248.60	1	+	–
Phytophthora cactorum	*Malus pumila*	W.H.Ko	1	+	–
	Rosa chinensis	FJ	1	+	–
Phytophthora capsici	*Capsicum annuum*	JS	5	+	–
	Lycopersicon esculentum	YN	1	+	–
Phytophthora cinnamomi	*Cedrus deodara*	JS	1	+	–
Phytophthora colocasiae	Unknown	CBS	1	+	–
Phytophthora cryptogea	*Gerbera jamesonii*	JS	5	+	–
Phytophthora drechsleri	*Beta vulgaris var. altissima*	CBS 292.35	1	+	–
Phytophthora erythroseptica	*Solanum tuberosum*	CBS 129.23	1	+	–
Phytophthora fragariae var. *rubi*	*Raspberry*	CBS 967.95	1	+	–
Phytophthora hibernalis	*Cirrus sinensis*	CBS 270.31	1	+	–
Phytophthora idaei	*Raspberry*	CBS 968.95	1	+	–
Phytophthora infestans	*Solanum tuberosum*	FJ	2	+	–
Phytophthora medicaginis	*Medicago sativa*	ATCC	1	+	–
Phytophthora megasperma	*Matthiola incana*	CBS305.36	1	+	–
	Rosa chinensis	FJ	1	+	–
Phytophthora melonis	*Cucumis sativus*	JS	2	+	–
	Cucumis sativus	FJ	2	+	–
Phytophthora palmivora	*Iridaceae*	YN	1	+	–
	Scindapsus aureus	FJ	1	+	–
	Cymbidium sinense	FJ	1	+	–
Phytophthora phaseoli	Unknown	CBS 556.88	1	+	–
Phytophthora primulae	*Malus sylvestris*	CBS 275.74	1	+	–
Phytophthora quercina	*Quercus petraea*	CBS 781.95	1	+	–
Phytophthora sojae	*Glycine max*	USA	2	+	–
	Glycine max	HLJ	1	+	–
Phytophthora syringae	Unknown	CBS 132.23	1	+	–
Phytophthora tentaculata	*Aucklandia lappa*	YN	1	+	–
Phytophthora tropicalis	*Leucospermum*	W.H.Ko	1	+	–
Pythium ultimum	Irrigation water	JS	1	+	–
Phytophthora aphanidermatum	*Cucumis sativus*	ZJ	2	+	–
	Glycine max	JS	1	+	–
Bremia lactucae	*Lactuca sativa*	JS	1	+	–
Fusarium solani	*Gossypium*	JS	3	+	–
	Glycine max	JS	5	+	–
Fusarium oxysporum f. sp. *vasinfectum*	*Gossypium*	JS	2	+	–
F. oxysporum f. sp. *cucumerinum*	*Cucumis sativus*	JS	3	+	–
F. oxysporum f. sp. *cubense*	*Musa sapientum*	FJ	1	+	–
Fusarium graminearum	*Triticum aestivum*	JS	1	+	–
Fusarium equiseti	unknown	CGMCC	1	+	–
Fusarium avenaceum	unknown	CGMCC	1	+	–
Fusarium nivale	unknown	CGMCC	1	+	–
Fusarium sambucinum	unknown	CGMCC	1	+	–
Fusarium culmorum	unknown	CGMCC	1	+	–
Fusarium moniliforme	*Oryza sativa*	L.F.Chen	1	+	–
	Gossypium	L.F. Chen	1	+	
Fusarium sp.	*Gossypium*	JS	2	+	–
Macrophoma kawatsukai	*Malus pumila*	JS	1	+	–
Alternaria alternata	Soil	JS	1	+	–
Alternaria sp.	*Glycine max*	JS	1	+	–
Botrytis cinerea	*Cucumis sativus*	ZJ	1	+	–
Endothia parasitica	*Castanea mollissima*	JS	3	+	–
Magnaporthe grisea	*Oryza sativa*	YN	3	+	–
Colletotrichum g ossypii	*Gossypium*	JS	1	+	–
Colletotrichum glycines	*Glycine max*	JS	1	+	–
Colletotrichum orbiculare	*Citrullus lanatus*	JS	1	+	–
Rhizoctonia solani	*Gossypium*	JS	2	+	–
	Glycine max	SD	3	+	–

Table 1
Continued

Species[a]	Isolation/origin		No. isolates	PCR product[c]	
	Host/substrate	Source[b]		ITS1/ITS4	Pn1/Pn2
Ascochyta gossypii	*Gossypium*	JS	1	+	–
Verticilium dahaliae	*Gossypium*	JS	3	+	–
Verticilium albo-atrum	*Medicago sativa*	JS	1	+	–
Tilletia indica	*Triticum aestivum*	JS	1	+	–

[a]All isolates of *Phytophthora nicotianae* and other *Phytophthora* species were maintained in the collection of Nanjing Agricultural University. All other fungi genera were collected from the wild for this study.
[b]Isolates were collected from the following: JS, Jiangsu Province; SD, Shandong Province; YN, Yunnan Province; HN, Hainan Province; ZJ, Zhejiang Province; FJ, Fujian Province; HLJ, Heilongjiang Province; Q.H.Chen, Dr Q.H. Chen of Fujian Academy of Agricultural Sciences; W.H.Ko, Dr W.H. Ko of Hawaii University; L.F.Chen, Dr L.F. Chen of Nanjing Agricultural University; CGMCC, China General Microbiological Culture Collection Center in Beijing.
[c]PCR product: '+'a significant PCR product band; '–' no PCR product band.

Specificity and sensitivity of primers Pn1 and Pn2

The specificity of the Pn primers was evaluated using DNA from 115 isolates of oomycetes and fungi. The sensitivity of Pn1 and Pn2 was tested by using different concentrations of the primers ranging from 20 ng to 1 fg of *P. nicotianae* isolate purified DNA as a template. The sensitivity of the Pn primers was further tested by crude DNA extracted from zoospores. To extract crude DNA from zoospores, 100 zoospores were suspended in 100 μl of double-distilled water, 0.5 g of silica was added, and the mixture was vortexed for 1 min, after which, 1–10 μl of suspension solutions were added to the PCR reactions respectively. DNA was extracted using a FastDNA® SPIN Kit from soil according to the manufacturer's recommendations. DNA samples extracted from 0.5 g of soil were suspended in 50 μl of ultrapure water after which 1 and 10 μl of DNA template was added to the PCR reactions.

The amplified products were electrophoresed on 1% agarose gels containing ethidium bromide (0.5 μg/ml) in 1× Tris-acetate-EDTA running buffer. A 2000 bp DNA ladder (TaKaRa, Dalian) was used on each gel as a molecular size standard. The experiments were repeated for at least three times.

Results

Specificity of primers Pn1 and Pn2

The specificity of the *P. nicotianae* primers Pn1/Pn2 evaluated using 100 other isolates of fungi and oomycetes (Table 1). All of the DNA preparations from other oomycetes were amplified with the *Phytophthora* species universal primers *Ypt*1F/*Ypt*1R and DNA extracted from fungi were amplified with the ITS universal primers ITS1/ITS4. All of the isolates tested gave a positive PCR reaction, demonstrating that they were of sufficient quality as PCR templates. The primer set Pn1/Pn2 was able to amplify a unique DNA fragment of 389 bp from all isolates of *P. nicotianae* originating from different regions and host plants in China. However, the 100 isolates of other oomycetes and fungi tested yielded no amplification products (Fig. 1).

Sensitivity of primers Pn1 and Pn2

The sensitivity of Pn1 and Pn2 was tested using different concentrations of the primers ranging from 20 ng to 1 fg of *P. nicotianae* isolate purified DNA as a template. The primers Pn1 and Pn2 can detect 1 ng purified DNA per 25-μl reaction volume (Fig. 2a). To increase the sensitivity of the molecular detection assays, purified DNA templates were amplified using nested PCR combining a first amplification with the *Phytophthora* species primers *Ypt*1F and *Ypt*1R, and a second amplification using 1-μl amplified product from the first round as the template and the species-specific primers Pn1 and Pn2. Nested PCR increased the sensitivity of the assay at least 100-fold to 10 pg per 25-μl reaction volume (Fig. 2b).

Crude DNA from zoospores was used as the template for testing the sensitivity of the primers Pn1 and Pn2. In a 25-μl reaction volume, DNA from 1 to 10 μl of zoospore extract all resulted in the amplification of the 389-bp DNA band by nested PCR (Fig. 3).

Detection in diseased field soil

DNA extracted from four infested soil samples was subjected to PCR using primers *Ypt*1F/*Ypt*1R for the first round of amplification followed by a second

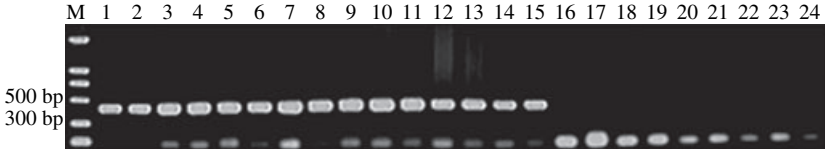

Fig. 1 Agarose gel electrophoresis of PCR-amplified products using the specific primers Pn1/Pn2; M, 2000 bp DNA marker; lanes 1–15, *Phytophthora nicotianae* isolates; lanes 16–23, other oomycete and fungal isolates; lane 24, negative control

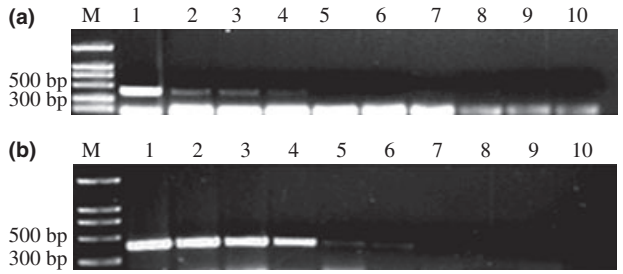

Fig. 2 Sensitivity of (a) polymerase chain reaction (PCR) with primers Pn1/Pn2 using different concentrations of DNA for the detection of *Phytophthora nicotianae*; M, 2000 bp DNA marker; lanes 1–9, amplified products using DNA at concentrations of 20 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg in a 25 μl PCR reaction volume; lane 10, negative control; (b) nested PCR using primers *Ypt*1F/*Ypt*1R for the first round of amplification and primers Pn1/Pn2 for the second round; M, 2000 bp DNA marker; lanes 2–9, 20 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg in a 25 μl PCR reaction volume; lane 10, negative control. The same results were obtained in three replicates

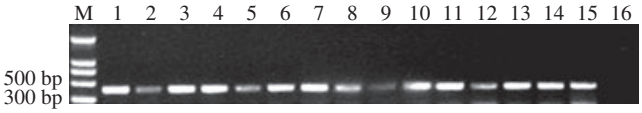

Fig. 3 Sensitivity of nested polymerase chain reaction with primers Pn1/Pn2 for the detection of *Phytophthora nicotianae* zoospores. Products amplified from crude zoospore DNA extracted from inoculated water samples; M , 2000 bp DNA marker; lane 1, positive control; lanes 2–11, amplified products using crude DNA from10, 9, 8, 7, 6, 5, 4, 3, 2 and 1 zoospores; lane 12, negative control

round of amplification using the amplified products from the first round as the template. All four of the samples produced 389-bp bands amplified by Pn1/Pn2 using nested PCR. In contrast, no PCR products were amplified from uninfected soil samples (Fig. 4).

Detection in plant tissue

A 389-bp DNA band was amplified from five different blight-infected tobacco samples (Fig. 5). The presence of *P. nicotianae* was confirmed by isolating the oomycete from the tissue into pure culture. In contrast, there was no band when healthy tobacco tissue DNA was used as a PCR template. Same result was observed when detecting diseased citrus tissues (Fig. 6).

Discussion

In the present paper, we have described a PCR-based strategy for the specific detection of *P. nicotianae*, which is one of the most important pathogens of tobacco and some other vegetable crops. The species-specific primers can amplify a 389-bp DNA band in target species, but not from any other species of *Phytophthora* and fungi. The validity of this strategy was demonstrated by designing universal primers to amplify the target gene in *Phytophthora* and fungi, and showing that only the species-specific primers amplified a product in *P. nicotianae*. Moreover, the primers proved to be effective in isolating pathogen DNA from both infected tissue and soil samples. For most species,

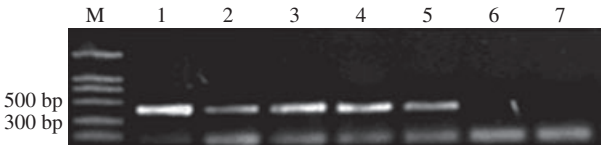

Fig. 4 Nested polymerase chain reaction amplification of DNA extracted from soil samples using the *Ypt*1F/*Ypt*1R primers for the first round of amplification and primers Pn1/Pn2 for the second round; M, 2000 bp DNA marker; lane 1, positive control; lanes 2–5, amplified products using DNA from soil samples collected from diseased tobacco roots; lane 6, uninfected soil sample; lane 7, negative control

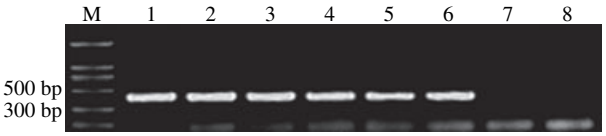

Fig. 5 Polymerase chain reaction amplification of DNA extracted from infected tobacco tissues; M, 2000 bp DNA marker; lane 1, positive control; lanes 2–6, amplified products using DNA from infected tobacco tissues; lane 7, healthy tobacco tissue; lane 8, negative control

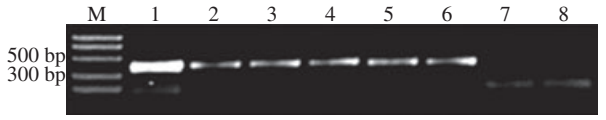

Fig. 6 Polymerase chain reaction amplification of DNA extracted from infected citrus tissues; M, 2000 bp DNA marker; lane 1, positive control; lanes 2–6, amplified products using DNA from infected tobacco tissues; lane 7, healthy tobacco tissue; lane 8, negative control

the ITS regions contains sufficient variability to enable the design of species-specific primers, but this is not the case for *Phytophthora* species. Among alternative targets gene proposed as the basis of PCR, the Ras-related protein gene *Ypt*1 (Chen and Roxby, 1996) possesses conserved exons and variable introns for almost all *Phytophthora* species (Schena and Cooke, 2006). *Ypt*1 gene has been used as a molecular marker to identify *P. fragariae* (Ioos et al., 2006) as well as the detection of *P. ramorum*, *P. kernoviae*, *P. citricola* and *P. quercina* (Schena et al., 2006). Based on the 30 unique *Phytophthora Ypt*1 gene sequences, we designed a pair of *P. nicotianae* species-specific primers and demonstrated their specificity for *P. nicotianae*.

Tests to assess, the sensitivity of the detection system showed a detection limit of 1 ng when using a single round amplification strategy. Unlike rDNA genes, the *Ypt*1 gene exists as only a single copy (Chen and Roxby, 1996). Single round amplification in our test has a detection limit of 1 ng pure *P. nicotianae* DNA per 25 μl PCR reaction volume. In soil samples and irrigation water, there are very low levels of pathogen DNA, so high sensitivity is essential for the detection assay. To increase the sensitivity of detection, a pair of *Phytophthora* universal primers *Ypt*1-F/*Ypt*1R was used as the first round amplification

primers for nested PCR, the products of which were used as templates for the second round with the species-specific primer pair. This method increased detection sensitivity from 1 ng to 10 pg of DNA. This is especially important when the target concentration is low or PCR inhibitory substances are present.

Zoospores are a primary infection propagule of healthy crops. The PCR primers Pn1/Pn2 can detect 1 pure zoospore per 25-μl PCR reaction volume by nested PCR. This high level of sensitivity is useful for detecting pathogens in irrigation water. When combined with the sample DNA extraction method, the PCR assay based on the method used here does not require organic solvents or the use of liquid nitrogen for sample homogenization. Thus, this assay methodology is very suitable for quick detection in field.

The PCR assay is sufficiently sensitive for the detection of *P. nicotianae* in soil samples. For soil samples, the success of PCR -based detection depends on obtaining high yields of target DNA from samples (Cullen et al., 1999) and avoiding the presence of inhibitors. In our tests, we used the FastDNA® SPIN Kit to obtain high -quality DNA from soil samples. With this kit, we can process soil samples in < 0.5 h.

In conclusion, the PCR assay based on the primer pair Pn1/Pn2 is specific and sensitive enough to detect target species in infected tissues, soil samples and irrigation water. This assay is valuable for detailed surveys of *P. nicotianae* in a variety of habitats and for tracking its movement locally in soil and irrigation water, as well as further afield in plants.

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