台灣農業研究 (J. Taiwan Agric. Res.) 66(4):276–285 (2017)
DOI: 10.6156/JTAR/2017.06604.02

Research Article

A Method for the Specific Detection of *Phomopsis destruens* in Sweet Potato by PCR

Ching-Yi Lin[1], Chiao-Wen Huang[1], Hong-Ren Yang[2], Su-Yu Lai[3], and Hui-Fang Ni[4,*]

Abstract

Lin, C. Y., C. W. Huang, H. R. Yang, S. Y. Lai, and H. F. Ni. 2017. A method for the specific detection of *Phomopsis destruens* in sweet potato by PCR. J. Taiwan Agric. Res. 66(4):276–285.

Foot rot caused by *Phomopsis destruens* (Harter) Boerema is a major concern for sweet potato production in Taiwan. The conventional methods for the identification of *P. destruens* are based on culture isolation and morphological analyses, but they are time-consuming and often inconclusive. To assist in a rapid and specific detection of the infection, a polymerase chain reaction (PCR) assay was developed to detect the pathogen from DNA extracted from fungi and plant tissues, respectively. Internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) from *P. destruens* isolates were used for the specific primers design. The amplification of expected 298 bp PCR products was obtained in all *P. destruens* isolates, but not for other fungal isolates from sweet potato storage roots including *Lasiodiplodia theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *Athelia rolfsii* (Sc-3) and *Fusarium* sp. (Fu245). The PCR assay with the primers could detect 1 ng of DNA template. To enhance the sensitivity of detection, a nested PCR was performed and the detection limit was raised up to 10 pg. In addition, the primers proved efficient in detection of the pathogen in stems and storage roots of infected sweet potato. Positive detection was observed in 93% of the naturally infected stems of sweet potato, a rate which constituted a significant improvement in the identification of *P. destruens* in sweet potato as compared to conventional methods. These results indicated that the new PCR assay provided a rapid, specific and reliable diagnosis tool for the detection of sweet potato foot rot pathogen.

Key words: *Ipomoea batatas*, *Phomopsis destruens*, Foot rot, PCR assays.

INTRODUCTION

Sweet potato (*Ipomotea batatas* L.) grows in tropical and subtropical regions and belongs to the family Convolvulaceae. It ranks as the world's seventh most important food crop after wheat, rice, maize, potato, barley and cassava (Food and Agriculture Organization of the United Nations 2011). Sweet potato is an important crop in Taiwan, accounting for a total planted area of 9,819 ha in 2015, and is mainly cultivated in Yunlin County and Changhua County (http://www.afa.gov.tw/Public/Grain-Statistics/2016526104225334.pdf). The most popular sweet potato cultivars for tube produc-

Received: October 19, 2016; Accepted: February 16, 2017.
* Corresponding author, e-mail: hfni@dns.caes.gov.tw
[1] Assistant Research Fellows, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[2] Research Fellow and Director, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[3] Research Assistant, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[4] Associate Research Fellow and Head, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.

tion are 'Tainung 57' ('TNG 57') and 'Tainung 66' ('TNG 66'), while the sweet potato cultivars of 'Tainung 71' ('TNG 71') and 'Taoyuan 2' ('TAY 2') are consumed as a leafy vegetable (Hsing *et al.* 2000; Lai *et al.* 2000).

Sweet potato is susceptible to a wide range of pests and pathogens, the effects of which can cause substantial economic loss. Some of these pests and pathogens have been reported in Taiwan, including bacterial wilt (*Ralstonia solanacearum*), scab (*Elsinoe batatas*), stem rot (*Fusarium oxysporum* f.sp. *batatas*), soft rot (*Rhizopus stolonifer*), Java black rot (*Lasiodiplodia theobromae*), foot rot (*Phomopsis destruens*), witches' broom (a mycoplasma-like organism), and virus diseases (Hsu *et al.* 2002; Chen *et al.* 2012; Huang *et al.* 2012; Shen *et al.* 2013; Huang *et al.* 2016).

Foot rot of sweet potato was first observed in the vicinity of the Dismal Swamp region of Virginia of U.S.A. in 1912, with almost 95% of the sweet potato plants in the area having been infected (Harter 1913a, 1913b; Harter & Weimer 1929). In Brazil, foot rot has been a destructive disease of sweet potato, with yield losses from the disease reaching as high as 80% (Lopes *et al.* 1994). Although the disease has been observed elsewhere in the world for more than ten decades, it was first observed in Taiwan from 2008 through 2011 (Huang *et al.* 2012), during which time it became a major concern of sweet potato producers. In those years, the disease incidence reached as high as 64% and resulted in considerable economic losses in Taiwan (Huang *et al.* 2012).

The symptoms of the disease on an infected sweet potato include black stem lesions at the soil level, vine wilting, storage root rot and, finally, plant death due to girdling of the base of the stem. Several plant diseases such as fusarium wilt and fusarium root rot show similar symptoms. The conventional methods used to identify pathogens often rely on visual symptoms, culturing isolation and morphological characteristics, and these methods are time-consuming and often inaccurate. In addition, *P. destruens* is a slow-growing fungus that may take at least 21 d to produce a noticeable colony with a diameter of 8.5 cm on a culture medium at 25℃ (Huang *et al.* 2012), and often resulting in a lack of clarity in identification due to the simultaneous growth of other nonpathogenic fungi that cover the colony of *P. destruens*. Therefore, it is necessary to develop a rapid and specific detection method for *P. destruens* that can facilitate the screening of sweet potato samples.

A rapid and specific detection and identification method is required for plant pathogen diagnosis and disease management. Molecular techniques based on polymerase chain reaction (PCR) amplification offer a specific and faster means of pathogen diagnosis (Ferrer *et al.* 2001; McCartney *et al.* 2003; Rickerts *et al.* 2007), and PCR-based methods for identifying *Phomopsis* spp. from plant tissues have previously been reported (Shishido *et al.* 2010; Jayaramaiah *et al.* 2013; Shirahatti *et al.* 2015). However, none of those methods are available for *P. destruens*. As such, the objective of this study was to develop a rapid and specific PCR-based identification method for detecting the presence of *P. destruens* within sweet potato tissue.

MATERIALS AND METHODS

Plants and fungal materials

Six fungi commonly found in sweet potato were selected to test the new set of specific primers. All the fungi including three isolates of *P. destruens* (SPPD-1, 5, and 7, which were isolated from Chiayi County, Changhua County and Nantou County in Taiwan, respectively), *Lasiodiplodia theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *Athelia rolfsii* (Sc-03) and *Fusarium* sp. (Fu245) were lab collected and cultured separately on potato dextrose agar (PDA) mediun. The sweet potato plants naturally infected with *P. destruens* were obtained from a field in Chiayi County, Taiwan.

DNA extraction

The mycelia of *P. destruens* (SPPD-1, 5, 7), *L. theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *A. rolfsii* (Sc-03) and *Fusarium* sp. (Fu245) were grown in potato dextrose broth (PDB) at 25℃ for 7 d, respectively. The mycelia of the different types of fungi were then harvested by centrifugation (2,500× *g* for 5 min). The genomic DNA was extracted from these samples using a commercial MasterPure yeast DNA purification kit (Epicentre, Madison, WI) according to manufacturer's instructions. The extracted DNA from the fungal cultures was then quantified spectrophotometrically at OD260/280 nm with ratios between 1.7 to 1.8, and the sensitivity of the specific primers was evaluated by PCR amplification with serial diluted concentrations (100 ng–10 fg) of purified genomic DNA isolated from the fungal samples as described previously.

A NaOH-based method was used for plant DNA extraction. Basically, the NaOH extraction followed the protocol of Wang *et al.* (1993) with some modifications. The diseased tissue (0.2 cm × 1 cm) of sweet potato was homogenized using a sterilized plastic pestle in a microcentrifuge tube with 150 µl 0.5 N NaOH. The samples were centrifuged at 16,200× *g* for 5 min and the supernatant was diluted 1:10 in 100 mM Tris-HCl (pH 8).

Primer design

The 18S rRNA gene sequences of *P. destruens* (accession no. JN848791, JX421687 and JX421688), and two other pathogens of sweet potato including *L. theobrome* (B2225) (accession no. KX345935) and *A. rolfsii* (Sc-03) (accession no. KX186998), and one fungus commonly isolated from sweet potato: *Fusarium* sp. (Fu245) (accession no. KX345936) was obtained from the NCBI GenBank database and aligned to determine the pattern of sequences. The specific primers for foot rot of sweet potato were designed based on the regions that were conserved in the *P. destruens* isolates, but not conserved with other fungi. The specific primers for *P. destruens* were SPPD-3F: 5'-TCTCTGCTGAGGCCCCCGGAGA-3' and SPPD-3R: 5'-AAGGCAGTGCCCCATCACCAAGCCAG-3'. The fungal ribosomal 18S rRNA gene and internal transcribed spacer (ITS) region with primer binding locations are shown in Figure 1.

PCR amplification

The PCR reaction was performed in 25 µL volumes containing 1 µL of the DNA, 5 µL 5× PCR reaction buffer, 1.25 mM dNTPs, 7.5 mM $MgCl_2$, 5 pmol of each primer (SPPD-3F/SPPD-3R) and 0.75 U Tag DNA polymerase (Protech, Taiwan). PCR amplification was carried out in a thermal cycler (SensoQuest LabCycler 011-101, Göttingen, Germany) with initial denaturation at 94℃ for 4 min, followed by 30 cycles of denaturation at 94℃ for 20 s, annealing at 60℃ for 30 s and extension at 72℃ for 40 s. The thermal cycles were terminated by a final extension at 72℃ for 7 min. Ten microliters of PCR products were separated by electrophoresis in 2% agarose gel in 0.5 TBE buffer. Gels were photographed under UV light. For nested PCR, the universal primers ITS1/ITS4 (White *et al.* 1990) were used in a first-round of fungus-specific amplification. The resulting PCR products were diluted 1/100 with 1× Tris EDTA (TE) buffer and subsequently used as template DNA in a second PCR amplification using the specific primers SPPD-3F/SPPD-3R.

A comparison of methods for the diagnosis and identification of *P. destruens*

The sweet potato plants showed the symptom of vine blackening around the soil level, and the black pycnidia produced near the surface of the naturally infected vines were collected as samples for the identification of the presence of *P. destruens* by developed PCR-based method and the traditional method of using the medium to isolate fungi from the diseased tissue. The PCR-based method was performed as described previously. For the traditional method, the fresh diseased vines

Fig. 1. Schematic of the fungal ribosomal 18S rRNA gene and ITS regions. (A) The positions of primers and the sizes of amplified fragments are indicated. ITS, internal transcribed spacer region; (B) Sequence alignment of *Phomopsis destruens* and other three sweet potato fungi [*Lasiodiplodia theobrome* (B2225, accession no. KX345935), *Fusarium* sp. (Fu245, accession no. KX345936) and *Athelia rolfsii* (Sc-03, accession no. KX186998)]. The positions and sequences of the primers are indicated as boxed and bold capital letters.

were surface-sterilized with 75% ethanol and cut with a sterile scalpel into approximately 3 mm pieces. Surface-sterilized vine cuttings were then transferred onto the PDA medium containing 0.25% lactic acid and incubated at 25℃ until fungal hyphae emerged from the plant tissue. Based on morphological differences (such as difference in the color and shape of mycelia), fungal cultures were picked and cultured on PDA plates.

RESULTS

Primer design

After alignment of the conserved regions of *P. destruens* and the other three sweet potato fungi [*L. theobrome* (B2225), *Fusarium* sp. (Fu245) and *A. rolfsii* (Sc-03)], the specific primers for *P. destruens* SPPD-3F (location: nt 103–125 of *P. destruens* accession no. JN848791) and SPPD-3R (location: nt 375–400 of *P. destruens* accession no. JN848791) were selected and used (Fig. 1B). The SPPD-3F/SPPD-3R primer pair was predicted to yield a PCR product of 298 bp (Fig. 1A)

PCR specificity evaluation

To determine whether cross-amplification would occur, DNA samples from other fungi of sweet potato including *L. theobrome* (B2225),

Phomopsis sp. (Ph735), *Rhizopus* sp., *A. rolfsii* (Sc-03) and *Fusarium* sp. (Fu245) were tested for the primer set. The results indicated that the 298 bp fragment was amplified with the primers SPPD-3F/SPPD-3R from all three isolates of *P. destruens*. No amplification fragment was observed with the genomic DNA isolated from the other five fungi (Fig. 2). The specificity of the 298 bp fragments produced was verified by direct DNA sequencing, and the fragment was matched with the *P. destruens* DNA sequences (data not shown).

PCR sensitivity evaluation

The sensitivity was evaluated using two PCR-based methods. The genomic DNA was extracted from fungal cultures and diluted subsequently. For PCR amplification with the primers SPPD-3F/SPPD-3R, the sensitivity was found to range from 1 ng to 100 pg (Fig. 3A). For nested PCR, the universal primers ITS1/ITS4 were used in the first PCR amplification and followed by the specific primers SPPD-3F/SPPD-3R of *P. destruens*. The result indicated that the sensitivity was thereby improved to 10 pg (Fig. 3B).

Detection of *P. destruens* in sweet potato by PCR

DNA was extracted from infected root tubers and vines of sweet potato using a NaOH-based method and then used as the template for PCR with the specific primers SPPD-3F/SPPD-3R. The 298 bp PCR fragment was observed in all the tested infected samples (Fig. 4).

A comparison of methods for diagnosis and identification of *P. destruens*

Two methods, one of which consisted of a PCR-based detection method with the specific primers SPPD-3F/SPPD-3R and the other of which was a conventional method consisting of fungi culture isolation, were utilized to evaluate the efficiency of diagnosis and identification of *P. destruens*. In each experiment, 18 or 19 samples out of 20 tested plant tissues (Fig. 5A) showed a positive reaction in PCR detection with the primers SPPD-3F/SPPD-3R. On average, the presence of *P. destruens* was detected in 18.6 of 20 plant tissues (93%) (Table 1). For the conventional method of using the medium to isolate fungi from the plant tissues, the orange-yellow color hyphae of *P. destruens* emerged for 4 to 11 out of the 20 tested plant tissues (Fig. 5B), and the fungal cultures were isolated and cultured on PDA plates (Fig. 5D). On average, *P. destruens* isolates were isolated from 7.3 of 20 plant tissues (38%) (Table 1). Colonies of non-target fungi such as sapro-

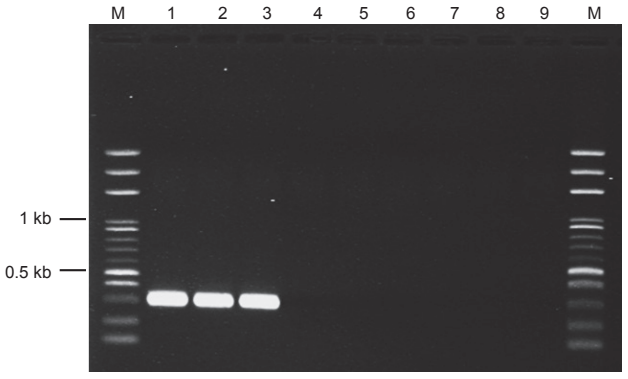

Fig. 2. Specificity of PCR amplification by primers SPPD-3F/SPPD-3R from various samples of genomic DNA. Genomic DNA was extracted from the fungal mycelia of sweet potato fungi and amplified with specific primers for foot rot of sweet potato. Lanes 1–3, *Phomopsis destruens* SPPD-1, 5, and 7; lanes 4–8, *Lasiodiplodia theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *Athelia rolfsii* (Sc-3) and *Fusarium* sp. (Fu245); lane 9, negative control (without genomic DNA); M, molecular weight markers.

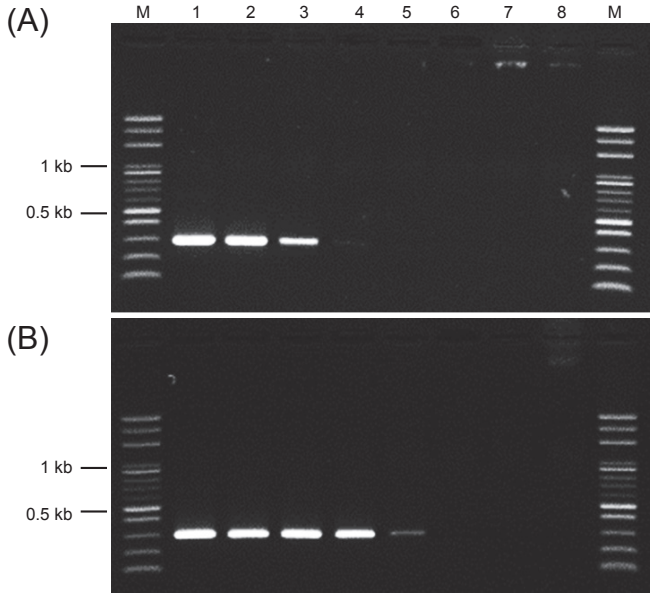

Fig. 3. Sensitivity of PCR amplification at the DNA level using 10-fold dilutions of *Phomopsis destruens* DNA. (A) Genomic DNA was extracted from the mycelia of *Phomopsis destruens* and amplified with the specific primers SPPD-3F/SPPD-3R; (B) Nested PCR using the ITS universal primers ITS1/ITS4 was followed by the specific primers SPPD-3F/SPPD-3R. Lane 1: 100 ng; lane 2: 10 ng; lane 3: 1 ng; lane 4: 100 pg; lane 5: 10 pg; lane 6: 1 pg; Lane 7: 100 fg; lane 8: 10 fg; M, molecular weight markers.

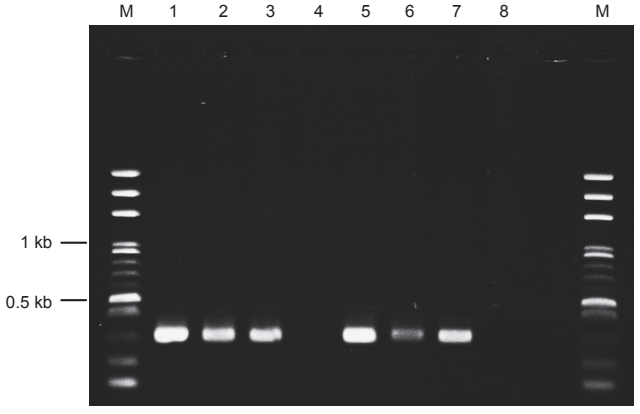

Fig. 4. Detection of *Phomopsis destruens* in sweet potato samples using the specific primers SPPD-3F/SPPD-3R. The DNA from the root tubers or vines of infected sweet potato plants was extracted with NaOH, and then used as the template for PCR. Lanes 1–3, the DNA from the infected sweet potato storage roots; lane 4, the DNA from healthy sweet potato storage roots; lanes 5–7, the DNA from the infected sweet potato stems; lane 8, healthy sweet potato stem; M, molecular weight markers.

phytic fungi were also observed on the residue of the culture plates (Fig. 5C).

DISCUSSION

In this study, the specific primers SPPD-3F/SPPD-3R were designed according to the ITS region sequences of *P. destruens* and were evaluated to develop a rapid and specific PCR method for detection of *P. destruens*. The results indicated that the developed PCR method could specifically and effectively detect the

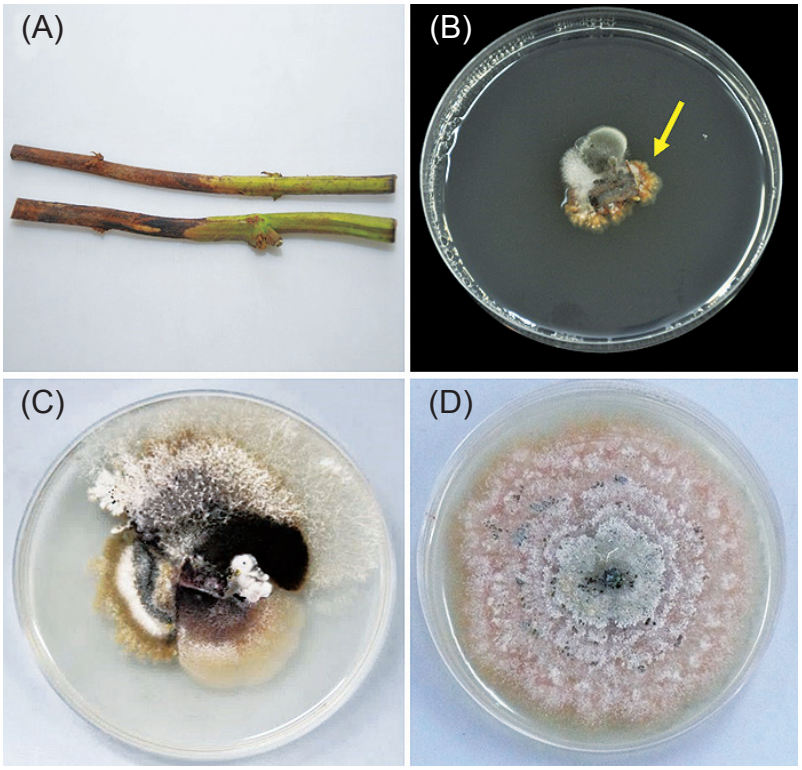

Fig. 5. Isolation of *Phomopsis destruens* from sweet potato with conventional method. (A) Sweet potato with foot rot symptoms; (B) *Phomopsis destruens* isolation on a lactic acid PDA (APDA) medium, with the arrow indicating a fungal colony of *Phomopsis destruens*; (C) *Phomopsis destruens* isolation on an APDA medium, but with the colony covered with non-pathogen fungus; (D) Pure culture of *Phomopsis destruens* on a PDA medium.

Table 1. Detection of *Phomopsis destruens* in sweet potato plants using different methods.

		No. of detected samples	
Experiment No.	*N*[z]	PCR method[y]	Conventional method[x]
1	20	18.0	10.0
2	20	18.0	7.0
3	20	19.0	11.0
4	20	19.0	6.0
5	20	19.0	4.0
avg.	20	18.6 (93%)	7.6 (38%)

[z] *N*, the number of sweet potato test samples with symptoms.
[y] Positive reaction in PCR amplification with specific primers SPPD-3F/ SPPD-3R.
[x] *Phomopsis destruens* was culture-isolated on PDA mediums.

presence of *P. destruens* from pure culture as well as from symptomatic sweet potato tissues and could provide a rapid and reliable diagnosis tool for sweet potato foot rot pathogen.

For PCR-based methods of species-specific detection, ribosomal DNA (rDNA) sequences such as the translation elongation factor 1-α (EF1-α) gene (Shirahatti *et al.* 2015) and ITS region sequences have been used for fungal species-specific detection, and the ITS se-

quences are widely utilized due to their characterization to the species level (Terashima *et al.* 2002; Zhao *et al.* 2007; Shishido *et al.* 2010; Santos *et al.* 2011). Therefore, the ITS regions of *P. destruens* was utilized to construct the SPPD-3F/SPPD-3R pair of PCR primers in this study. The primers successfully amplified the target DNA sequences of three isolates of *P. destruens* from different areas in Taiwan and were able to discriminate *P. destruens* from five species of plant pathogens of sweet potato including one other *Phomopsis* species (Fig. 2). These results indicated the specificity of the primers and confirmed the use of the primers in diagnostic applications.

The primers SPPD-3F/SPPD-3R were evaluated for their potential at low concentrations of the template and the results showed that the PCR products could be amplified from 1 ng to 100 pg of *P. destruens* DNA. The sensitivity of the PCR was promising when a nested PCR was performed even when the presence of pathogen DNA was at a concentration as low as 10 pg. The first PCR reaction used the universal primers ITS1/ITS4 (White *et al.* 1990) to enrich the fungal DNA template, thereby increasing the amplification efficiency of the *P. destruens* DNA at low concentrations.

The primers were used to detect the pathogen in plant tissues consisting of the stems and storage roots of sweet potato exhibiting typical symptoms, and the presence of the pathogen could be detected in both stems and roots. In addition, a simple, rapid and inexpensive NaOH-based method (Wang *et al.* 1993) for plant DNA extraction was used in this study, and only a small segment of sweet potato plant tissue (0.2 cm × 1 cm) was needed for such plant DNA extraction. The entire procedure could be completed in 4 h : 1.5 h for DNA extraction, 2 h for PCR amplification and 0.5 h for electrophoresis. This specific and rapid detection method for *P. destruens* had great practical importance for disease management as this method could also be used to identify disease-free seedlings in commercial nurseries.

One of the most common conventional methods for the diagnosis of fungi is culture isolation from a sample of diseased plant tissue. In this study, *P. destruens* was identified in only 38% of the tested infected vines using this method, and the method required at least 14 d for final identification, because of the slow growth of the *P. destruens* on culture medium (Huang *et al.* 2012). Some of non-pathogenic or saprophytic fungi that grew faster than *P. destruens* on medium affected the diagnosis and resulted in inaccurate identification (Fig. 5D). Relatedly, a selective medium for *P. destruens* is not available at present. These reasons may explain the low diagnosis efficiency of culture isolation in identifying *P. destruens*. While using the PCR method developed in this study, positive detection was observed for 93% of the tested infected vines. These results demonstrated the high specificity and sensitivity of the PCR method compared with the conventional methods.In summary, the results of the present study have demonstrated that the primer pair designed is effective in the detection and identification of *P. destruens*. Compared with conventional culture isolation and morphological methods, the developed method provides a specific and reliable diagnosis tool with significantly increased accuracy in identifying sweet potato foot rot infections, as well as required significantly less time to complete than those conventional methods.

ACKNOWLEDGMENTS

We wish to express our appreciation to Sui-Li Hsu and the staff of the Department of Plant Protection for their technical assistance.

REFERENCES

Chen, Y. J., Y. S. Lin, and W. H. Chung. 2012. Bacterial wilt of sweet potato caused by *Ralstonia solanacearum* in Taiwan. J. Gen. Plant Pathol. 78:80–84.

Ferrer, C., F. Colom, S. Frasés, E. Mulet, J. L. Abad, and J. L. Alió. 2001. Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8S ribosomal DNA typing in ocular infections. J. Clin. Microbiol. 39:2873–2879.

Food and Agriculture Organization of the United Nations. 2011. FAOSTAT database. http://faostat.fao.org/ (visit on 6/27/2016)

Harter, L. L. 1913a. Foot rot, a new disease of the sweet potato. Phytopathology 3:243–245.

Harter, L. L. 1913b. The foot-rot of the sweet potato. J. Agric. Res. 1:251–273.

Harter, L. L. and J. L. Weimer. 1929. A Monographic Study of Sweet Potato Diseases and Their Control. U.S. Department of Agriculture, Technical Bulletin. Washington. 118 pp.

Hsing, C. W., W. H. Lin, J. L. Jiang, T. L. Kung, and W. N. Peng. 2000. Breeding of a new vegetable sweet potato cultivar 'Taoyuan 2'. Bull. Taoyuan Dist. Agric. Improve. Stn. 40:1–6. (in Chinese with English abstract)

Hsu, S. T., T. T. Chang, C. A. Chang, J. L. Tsai, and T. T. Tsay. 2002. List of Plant Diseases in Taiwan. 4th ed. Taiwan Phytopathology Society. Taichung, Taiwan. 386 pp. (in Chinese)

Huang, C. W., M. F. Chuang, S. S. Tzean, H. R. Yang, and H. F. Ni. 2012. Occurrence of foot rot disease of sweet potato caused by *Phomopsis destruens* in Taiwan. Plant Pathol. Bull. 21:47–52. (in Chinese with English abstract)

Huang, C. W., H. R. Yang, C. Y. Lin, S. L. Hsu, S. Y. Lai, and H. F. Ni. 2016. The study of physiological characteristics and control of *Phomopsis destruens* causing foot rot of sweet potato. J. Taiwan Agric. Res. 65:45–53. (in Chinese with English abstract)

Jayaramaiah, K. M., S. Mahadevakumar, A. P. Charith Raj, and G. R. Janardhana. 2013. PCR based detection of *Phomopsis vexans* (Sacc. & Syd.)- The causative agent of leaf blight and fruit rot disease of Brinjal (*Solanum melongena* L.). Intl. J. Life Sci. 7:17–20.

Lai, Y. C., H. C. Lee, and Y. S. Chen. 2000. Development of leafy sweet potato variety Tainung 71. J. Agric. Res. China. 49:14–27. (in Chinese with English abstract)

Lopes, C. A., P. Boff, and V. Duarte. 1994. Foot rot of sweet potato in Brazil. Pesqui. Agropecu. Bras. 29:1407–1410.

McCartney, H. A., S. J. Foster, B. A. Fraaije, and E. Ward. 2003. Molecular diagnostics for fungal plant pathogens. Pest Manag. Sci. 59:129–142.

Rickerts, V., S. Mousset, E. Lambrecht, K. Tintelnot, R. Schwerdtfeger, E. Presterl, V. Jacobi, G. Just-Nubling, and R. Bialek. 2007. Comparison of histopathological analysis, culture, and polymerase chain reaction assays to detect invasive mold infections from biopsy specimens. Clin. Infect. Dis. 44:1078–1083.

Santos, J. M., K. Vrande čić, J. Ćosić, T. Duvnjak, and A. J. L. Phillips. 2011. Resolving the *Diaporthe* species occurring on soybean in Croatia. Persoonia 27:9–19.

Shen, Y. M., H. S. Liu, and C. H. Chao. 2013. Analyses for the causal agent of sweet potato foot rot disease and its susceptibility on six sweet potato cultivars. Plant Prot. Bull. 55:25–34. (in Chinese with English abstract)

Shirahatti, P. S., R. Ramu, C. R. Adkar-Purushothama, and M. N. Nagendra-Prasad. 2015. Development of a simple and reliable species-specific detection of *Phomopsis azadirachtae*, using the translation elongation factor 1-alpha gene. Eur. J. Plant Pathol. 141:769–778.

Shishido, M., K. Sato, N. Yoshida, R. Tsukui, and T. Usami. 2010. PCR-based assays to detect and quantify *Phomopsis sclerotioides* in plants and soil. J. Gen. Plant Pathol. 76:21–30.

Terashima, Y., K. Ogiwara, M. Kojima, C. Kubo, A. Seki, and A. Fujiie. 2002. Primers based on specific ITS sequences of rDNAs for PCR detection of two fairy ring fungi of turfgrass *Vascellum pratense* and *Lycoperdon pusillum*. Mycoscience 43:261–265.

Wang, H., M. Qi, and A. J. Cutler. 1993. A simple method of preparing plant samples for PCR. Nucleic Acids Res. 21:4153–4154.

White, T. J., T. Burns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. *in*: PCR Protocols: A Guide to Methods and Applications. (Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.) Academic Press. San Diego, CA. 482 pp.

Zhao, J., X. J. Wang, C. Q. Chen, L. L. Huang, and Z. S. Kang. 2007. A PCR based assay for detection of *Puccinia striiformis* f. sp. *tritici* in wheat. Plant Dis. 91:1669–1674.

甘藷基腐病菌專一性 PCR 檢測技術之研發

林靜宜[1] 黃巧雯[1] 楊宏仁[2] 賴素玉[3] 倪蕙芳[4,*]

摘要

林靜宜、黃巧雯、楊宏仁、賴素玉、倪蕙芳。2017。甘藷基腐病菌專一性 PCR 檢測技術之研發。台灣農業研究 66(4):276–285。

甘藷基腐病 (foot rot) 是由 *Phomopsis destruens* 所引起，為近年來台灣甘藷生產的主要限制因子之一。目前 *P. destruens* 之鑑定仍依賴傳統方法，以病原菌之組織分離及形態特徵為主，不但耗時費力且成功分離出病原菌之比率不高。本研究依據 *P. destruens* 之去氧核醣核酸的內轉錄區 (internal transcribed spacer; ITS) 序列設計專一性引子對，並利用聚合酶連鎖反應 (polymerase chain reaction; PCR) 建立甘藷基腐病菌之檢測技術。結果顯示，本研究開發之檢測方法可專一性檢測 *P. destruens*，且對經常可於甘藷塊根上分離之 *Lasiodiplodia theobrome* (B2225)、*Phomopsis* sp. (Ph735)、*Rhizopus* sp.、*Athelia rolfsii* (Sc-3) 及 *Fusarium* sp. (Fu245) 等其他真菌並無誤檢反應。此法之靈敏度可達 1 ng 之菌絲 DNA，若利用巢式 PCR (nested PCR) 則靈敏度可提高至 10 pg 之菌絲 DNA。此外，本檢測方法亦可運用於分析受感染之甘藷莖部與塊根組織。而此技術於感染植物組織之檢測成效良好，93% 之受感染植物樣本可檢測出病原菌，相較於傳統鑑定法之 38%，可大幅提升其檢測成功率。此專一性 PCR 檢測法具有快速、專一性及靈敏度高之優點，未來可將此技術應用於檢測甘藷是否感染基腐病。

關鍵詞：甘藷、甘藷基腐病菌、甘藷基腐病、PCR 分析。

投稿日期：2016 年 10 月 19 日；接受日期：2017 年 02 月 16 日。

* 通訊作者：hfni@dns.caes.gov.tw
[1] 農委會農業試驗所嘉義農業試驗分所植物保護系助理研究員。台灣 嘉義市。
[2] 農委會農業試驗所嘉義農業試驗分所研究員兼分所長。台灣 嘉義市。
[3] 農委會農業試驗所嘉義農業試驗分所植物保護系研究助理。台灣 嘉義市。
[4] 農委會農業試驗所嘉義農業試驗分所植物保護系副研究員兼系主任。台灣 嘉義市。