Plant Pathology Bulletin 17: 297-305, 2008

Molecular identification of *Uromyces appendiculatus* and *Uromyces vignae* from Taiwan with PCR-based method

Wen-Hsin Chung[1], Wen-Chaun Chung[2], Pen-Fen Ting[1], Hung-Chang Huang[3] and Jenn-Wen Huang[1, 4]

[1] Department of Plant Pathology, National Chung Hsing University, 250, Kuo Kuang Rd., Taichung 402, Taiwan

[2] Taiwan Seed Improvement and Propagation Station, Hsinhse, Taichung 426, Taiwan

[3] Biotechnology Division, Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan

[4] Correspondence author, E-mail: jwhuang@nchu.edu.tw; Fax: +886-4-2285-1676

Accepted for publication: August 12, 2008

ABSTRACT

Chung, W. H., Chung, W. C., Ting, P. F., Huang, H. C., and Huang, J. W. 2008. Molecular identification of *Uromyces appendiculatus* and *Uromyces vignae* from Taiwan with PCR-based method. Plant Pathol. Bull. 17: 297-305.

Bean rust caused by *Uromyces appendiculatus* and cowpea rust caused by *U. vignae* are important diseases of these corps in the world. Identification of these two *Uromyces* species has always been difficult in the past due to confusion in morphological feature and host range of the pathogens. In this study, four primers designed from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA were developed for rapid and reliable identification of the two rust pathogens from Taiwan. Two of the primers, UA-ITSF and UA-ITSR, were highly specific to *U. appendiculatus* on common bean and the others two primers, UV-ITSF and UV-ITSR, were highly specific to *U. vignae* on cowpea. The four primers failed to amplify any of the eight other rust pathogens isolated from non-legume crops.

Key words: *Uromyces appendiculatus*, *U. vignae*, bean, cowpea

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) rust caused by *Uromyces appendiculatus* (Pers.) Unger and cowpea (*Vigna unguiculata* (L.) Walp. ssp. *unguiculata*) rust caused by *Uromyces vignae* Barclay are important diseases of these legume crops in many countries [5, 6, 11, 17, 28]. *U. appendiculatus* was first reported on common bean in Europe in 1795 [cited from 1] and *U. vignae* was first reported on *Vigna vexillate* (L.) A. Rich. in India in 1891 [2]. Separation of these two species of rust fungi is difficult due to contradiction and confusion in nomenclature [1, 9, 14, 15, 19]. Seveal reports [1, 15, 19] indicated that *U. appendiculatus* and *U. vignae* should belong to the same species because of morphological similarity of urediniospores and teliospores between the two species. Cummins [5] reported that urediniospores of *U. vignae* had more superequatorial germ-pores than *U. appendiculatus*. Moreover, cell wall of teliospores of *U. appendiculatus* was warty and *U. vignae* was smooth, respectively. Chung *et al.* [4] confirmed that the position of germ-pores of *U. vignae* and *U.*

appendiculatus in urediniospores was an important morphological characteristic to distinguish this two rust species, however, the characteristics of cell walls between *U. appendiculatus* and *U. vignae* were not significant. Previous reports [9,12,13,17] indicate that host specificity is one of the important criteria for identification of *U. appendiculatus* and *U. vignae*. Elmhirst and Heath [7,8] studied the pathogenicity of *U. vignae* and *U. appendiculatus* on *Phaseolus* spp. and *Vigna* spp., and concluded that *U. appendiculatus* had a wider host range than *U. vignae*. Chung *et al.* [4] conducted a study on molecular phylogenic relationships of *U. vignae* and *U. appendiculatus*, and reported that *U. vignae* and *U. appendiculatus* were in different molecular group.

Molecular biology is a useful tool for resolving problems associated with identification or taxonomical contention of filamentous fungi [26]. Kim *et al.* [20] reported differences in polypeptide patterns between *U. appendiculatus* and *U. vignae*. However, using polypeptide patterns for species identification of fungi is time consuming. The molecular diversity has been used to identify fungal species [3] and fungal isolates showing fungicide-resistance [18,21]. Chung *et al.* [4] reported that *U. appendiculatus* was distinguishable from *U. vignae* based on phylogenetic analysis inferred from internal transcribed spacer (ITS). However, using the phylogenetic analysis must be supported by several computer software packages such as CLUSTAL X sequence alignment software [27], Se-Al sequence alignment editor [24] and PUAP software package, and the procedure of analysis is lengthy and time consuming. The objective of this study was to use the nucleotides diversity on ITS rDNA region for developing species-specific primers for rapid identification of *Uromyces* rust on *Phaseolus* spp. and *Vigna* spp. based on PCR method in the field at Taiwan.

MATERIALS AND METHODS

Sample collection, DNA extraction, PCR amplification, purification and sequencing of ITS region

The urediniospores of *U. appendiculatus* (isolate CH0001), collected from rust pustules of a diseased plant of common bean (*P. vulgaris*) at Hsinshe, Taichung, Taiwan, in 11 December 2006 and *U. vignae* (isolate CH0002), collected from rust pustules of a diseased plant of cowpea (*V. unguiculata* ssp. *unguciculata*) at Tungshih, Taichung, Taiwan, in 26 August 2006 were identified based on morphological characteristics [4]. For amplifying ITS and 5.8S rDNA regions, genomic DNAs of CH0001 and CH0002 isolates were extracted from a single uredinium, using the methods described by Virtudazo *et al.* [29]. From this crude extract of each species, 2 to 3 μl was used directly for each PCR amplification. Amplification was done using 25 μl PCR reaction mixture, including 0.2 μM of each primer, 1 unit of DNA polymerase (GeneMark, Taiwan), and commercial dNTP mixture (containing 2.5 mM of each of dNTP) and Taq reaction buffer of GeneMark supplied with 2 mM Mg^{2+}. The primers used for amplifying ITS and 5.8 S regions were ITS5-u and ITS4-u [23]. PCR was carried out using a P×2 Thermal Cycler (Thermo, USA) under the conditions described by Pfunder *et al.* [23]. PCR products were run in 1.5% agarose gels containing 0.5 μg/ml ethidium bromide in TAE (Tris-acetate, EDTA) buffers. PCR products were purified by spin columns (PCR Clean-Up Kit, GeneMark) and reacted with BigDye Terminator v3.0 Cycle Sequencing (Applied Biosystems, USA) under the following conditions: 25 cycles of denaturation at 96℃ for 10 sec, annealing at 50℃ for 5 sec, and extension at 60℃ for 4 min. Cycle sequencing reaction products were purified by ethanol precipitation, and then analyzed by ABI PRISM 3100 automated sequencers (Applied Biosystems, Ramsey, USA).

Design of specific PCR primers for *U. appendiculatus* and *U. vignae*

ITS sequences were aligned using Clustal X v.1.8 [27]. Further visual alignments were done in Sequence Alignment (Se-Al) Editor v.2.0 [24]. Specimen sequences were analyzed together with other specimens of *U. appendiculatus*, AB115740 and AB115741, and *U. vignae*, AB115718 and AB115720, sequences found in GenBank database. For comparing the sequence homology with related *Uromyces species*, *U. fabae* (AB08192), *U. pisi* (AF180191), and *U. striatus* (AF180162) also were aligned with *U. appendiculatus* and *U. vignae* in Taiwan. Thus, variable nucleotides between the sequences of *U. appendiculatus* and *U. vignae* (Fig. 1) were used to design

```
CH0001    (UA)    GGTTTTTGCCATTGCACTCAGGTAAACGTAACA-CTTTTGTGTTACATTACCCCCCTCCC
AB115741  (UA)    GGTTTTTGCCATTGCACTCAGGTAAACGTAACA-CTTTTGTGTTACATTACCCCCCTCCC
AB115740  (UA)    GGTTTTTGCCATTGCACTCAGGTAAACGTAACA-CTTTTGTGTTACATTACCCCCCTCCC
CH0002    (UV)    CATCTTTGCCATTGCACTCAGGTATACGTAACA-TTTTTGTGTTACATT-ACCCCCTCCC
AB115720  (UV)    CATCTTTGCCATTGCACTCAGGTATACGTAACA-TTTTTGTGTTACATTTACCCCCTCCC
AB115718  (UV)    CATCTTTGCCATTGCACTCAGGTATACGTAACA-TTTTTGTGTTACATT-ACCCCCTCCC
AB085192  (UF)    CATTTATGCCATTGCACTCAGGTATACGTAACA-TTTATTTGTTACATTATCCCCCTCCC
AF180162  (US)    CATTTTTGTCATTGCACTCAGGTAGACGTAACAATTTTTTTTGGTACATT-ATCCCCTCCC
AF180191  (UP)    GATCATTAT--TTAAAACCAAATGAGTGCACT--TTATTGTGGCTCAAACTCTTTTATCT
                    *   *    **  *  **  *    * *     *  * **   **

CH0001    (UA)    CAAT-------TTTTTTTTTTTTTATAAAACACATGTTGAAA-TAAGAATGTAA---TATA
AB115741  (UA)    CAAT-------TTTTTTTTTTTTTATAAAACACATGTTGAAA-TAAGAATGTAA---TATA
AB115740  (UA)    CAAT-------TTTTTTTTTTTTTATAAAACACATGTTGAAA-TAAGAATGTAA---TATA
CH0002    (UV)    CAATATTTTCTTTTTTTTTTTATTATTAAACACAAGTGGAAT-TAAGAATGTAA---CATA
AB115720  (UV)    CAATATTTTCTTTTTTTTTTTATTATTAAACACAAGTGGAAT-TAAGAATGTAA---CATA
AB115718  (UV)    CAATATTTTCTTTTTTTTTTTATTATTAAACACAAGTGGAAT-TAAGAATGTAA---CATA
AB085192  (UF)    AA-----------TTTTTTTTTTAT-AAACACAAGTTGAATCTAAGAATGTAAA--CCCT
AF180162  (US)    AATT-----TTTTTTTTTTTTTTTTTTAAACACAAGTTGAAT-TAAGAATGTAAAAACCTT
AF180191  (UP)    CACC---CCCTTTTTTTTTTTTGTTTGTAACACAAGTTGAAT-TAAGAATGTAAAAAACCT
                   *           *******  *    ****** ** ***  ***********

CH0001    (UA)    TATATTTTGAAAATAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
AB115741  (UA)    TATATTTTGAAAATAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
AB115740  (UA)    TATTTTTTGAAAATAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
CH0002    (UV)    TTTATTTTTAAA-TAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
AB115720  (UV)    TTTATTTTTAAA-TAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
AB115718  (UV)    TTTATTTTTAAA-TAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
AB085192  (UF)    TTTATTTGAAAAATAACTTTTAACAATGGATCTCTAGGCTCTCATATCGATGAAGAACAC
AF180162  (US)    TTTATTTTGAAA-TAACTTTTAACAATGGATCTCTTGGCTCTCATATCGATGAAGAACAC
AF180191  (UP)    TTTATTTTGAAAATAACTTTTAACAATGGATCTCTTGGCTCTCATATCGATGAAGAACAC
                  * *****  *** ********************** ************************

CH0001    (UA)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AB115741  (UA)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AB115740  (UA)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
CH0002    (UV)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AB115720  (UV)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AB115718  (UV)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AB085192  (UF)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AF180162  (US)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
AF180191  (UP)    AGTGAAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACG
                  ************************************************************

CH0001    (UA)    CACCTTGCATCTTTTGGTATTCCAAAAGGTATGCCTGTTTGAGTGTCATGAAAATCTCTC
AB115741  (UA)    CACCTTGCATCTTTTGGTATTCCAAAAGGTATGCCTGTTTGAGTGTCATGAAAATCTCTC
```

Fig. 1. The nucleotide sequence variation at ITS1 and ITS2 regions in *Uromyces appendiculatus* (UA) AB115740, AB115741 and CH0001 and *U. vignae* (UV) AB115718, AB115720 and CH0002. The underlined sequences represent the regions designed to identify *U. appendiculatus* and *U. vignae*. In addition sequences alignment were *U. fabae* (AB08192, UF), *U. striatus* (AF180162, US), and *U. pisi* (AF180191, UP). All of accession numbers on DDBJ/NCBI/GenBank. (* indicated sequences consensus.)

primers potentially specific to the two rust fungi. In present study, four specific primers, UA-ITSF, UA-ITSR, UV-ITSF and UV-ITSR, were designed to identify *U. appendiculatus* and *U. vignae* (Table 1).

Detection of *U. appendiculatus* and *U. vignae* by specific primers

A total of 11 fresh urediniospore specimens were collected from diseased plants in Taiwan, including four specimens of *U. appendiculatus* from common bean (*P. vulgaris*) at Hsinshe and seven specimens of *U. vignae* from cowpea (*V. unguiculata* ssp. *unguiculata*) at Hsinshe, Tungshih and Jian (Table 2). Genomic DNAs of *U. appendiculatus* and *U. vignae* were extracted using the methods described by Virtudazo *et al.*[29]. Amplification was done using 25 μl PCR reaction mixture, including 0.2 μM of each primer, 1 unit of DNA polymerase, and commercial dNTP mixture (containing 2.5 mM of each of dNTP) and Taq reaction buffer containing 2 mM Mg^{2+}. PCR was carried out using a P×2 Thermal Cycler under the following conditions: initial denaturation at 95℃ for 3 min, followed by 35 cycle of denaturation at 95℃ for 30s, annealing at 53℃ for 1 min, extension at 72℃ for 1 min, followed by a final extension phase at 72℃ for 10 min. PCR products were run in 1.5% agarose gels containing 0.5 μg/ml ethidium bromide in TAE buffers.

Specificity test of primers

To confirm specificity of the primers developed in this study, eight specimens of rust fungi collected from non-legume plants were used. These eight non-legume hosts of rust fungi were: citronella grass (*Cymbopogon nardus* (L.) Rendle), perilla (*Perilla ocymoides* L. var. *crispa* Benth), ma bamboo (*Dendrocalamus latiforus* Munro), willow (*Salix babylonica* L.), Chinese chive (*Allium odorum* L.), fig (*Ficus carica* L.), mint (*Mentha* sp.), and peanut (*Arachis hypogaea* L.) (Table 3). The methods of DNA extraction and PCR amplification of ITS region were conducted by the same procedure previously described for rusts of common bean and cowpea.

RESULTS AND DISCUSSION

According the morphological observation, the urediniospores of *U. appendiculatus* (CH0001) on common bean have 2 germ pores on equatorial area, and *U. vignae* (CH0002) on cowpea have 2 germ pores on superequatorial area. Of the four primers tested, UA-ITSF and UA-ITSR, showed specificity to *U. appendiculatus*, and the primers, UV-ITSF and UV-ITSR, showed specificity to *U. vignae*. These primers could amplify about 500 bp in the ITS regions of *U. appendiculatus* and *U. vignae* (Fig. 2). Moreover, eleven specimens on bean

Table 1. Species-specific PCR primers designed from the ITS rDNA region for the identification of *Uromyces* species

Uromyces species	Primers	Primer sequence
U. appendiculatus	UA-ITSF	5'-GGTTTTTGCCATTGCACTCAG-3'
	UA-ITSR	5'-CATTTGTTTAGGAGTCCTGAC-3'
U. vignae	UV-ITSF	5'-CATCTTTGCCATTGCACTCAG-3'
	UV-ITSR	5'-AGTTAGTTTAGGAGTTCTAAC-3'

Table 2. PCR reaction of *Uromyces* species from common bean and cowpea

Host plants	Reaction of primers		Location
	UA-ITSF/UA-ITSR	UV-ITSF/UV-ITSR	
Phaseolus vulgaris	+	−	Hsinshe, Taichung
P. vulgaris	+	−	Hsinshe, Taichung
P. vulgaris	+	−	Hsinshe, Taichung
P. vulgaris	+	−	Hsinshe, Taichung
Vigna unguiculata	−	+	Tungshih, Taichung
V. unguiculata	−	+	Hsinshe, Taichung
V. unguiculata	−	+	Hsinshe, Taichung
V. unguiculata	−	+	Hsinshe, Taichung
V. unguiculata	−	+	Hsinshe, Taichung
V. unguiculata	−	+	Jian, Hualien
V. unguiculata	−	+	Jian, Hualien

Table 3. The rust fungi collected from non-legume host plants used for testing specificity to primers from *Uromyces appendiculatus* and *U. vignae*

Host plants	Rust species	Location	Date of Collection
Allium odorum	*Puccinia allii* (DC) Rud.	Hsinshe, Taichung	2006/10/18
Arahis hypogaea	*Puccinia arachidis* Speg.	Yijhu, Chiayi	2006/11/12
Cymbopogon nardus	*Puccinia nakanishikii* Diet.	Hsinshe, Taichung	2006/10/18
Ficus carica	*Phakopsora fici* Nishida	Hsinshe, Taichung	2006/10/18
Perilla ocymoides var. *crispa*	*Coleosporium perillae* Syd.	Hsinshe, Taichung	2006/10/18
Salix babylonica	*Melampsora coleosporioides* Diet.	Hsinshe, Taichung	2006/10/18
Mentha sp.	*Puccinia menthae* Pers.	Hsinshe, Taichung	2006/10/18
Dendrocalamus latiflorus	*Uredo ditissima* Cumm.	Dounan, Yulin	2006/11/16

and cowpea collected from different area showed that these four primers were highly specific to detect *U. appendiculatus* and *U. vignae* (Table 2).The result also suggested that *U. appendiculatus* and *U. vignae* were highly related with bean and cowpea, respectively, in Taiwan (Table 2). In addition, the test of a single uredinium randomly obtained from bean and cowpea indicated that *U. appendiculatus* and *U. vignae* had no cross-infection on these two hosts (Fig. 3). Chung *et al.*[4] indicated that *U. vignae* could be on *Vigna* spp., *P. vulgaris* and *Lablab purpureus* (L.) Sweet. However, we did not find *U. vignae* on *P. vulgaris* in Taiwan. For testing primers specificity, eight other rust fungi from non-legume plants were tested and the results showed that all the four primers did not react with the eight rust specimens tested (Table 3). The results reveal that these primers designed from ITS rDNA regions are highly specific to *U. appendiculatus* and *U. vignae* (Fig. 4). Previous reports indicate that ITS rDNA regions are highly variable for studying the phylogeny of rust fungi[4, 29, 30, 31]. The specificity of the designed primers on the rust pathogens of common bean and cowpea suggests that the diversity of ITS rDNA regions is useful to find molecular markers for rapidly separating different rust species.

Classification of rust fungi is still a problem due to complex morphology, life cycle and putative host. Moreover, most rust fungi are obligate parasite and incapable of growing on culture media. Thus, the extraction of DNA is not convenient. There are some reports of molecular research focusing on rust fungi, including species diagnosis[20], ecological study[23], fungicide-resistance analysis[10], and phylogenetic relationship[4, 22, 29, 30, 31]. The DNA extraction method with sterile glass slides reported by Suyama *et al.*[25] was used in

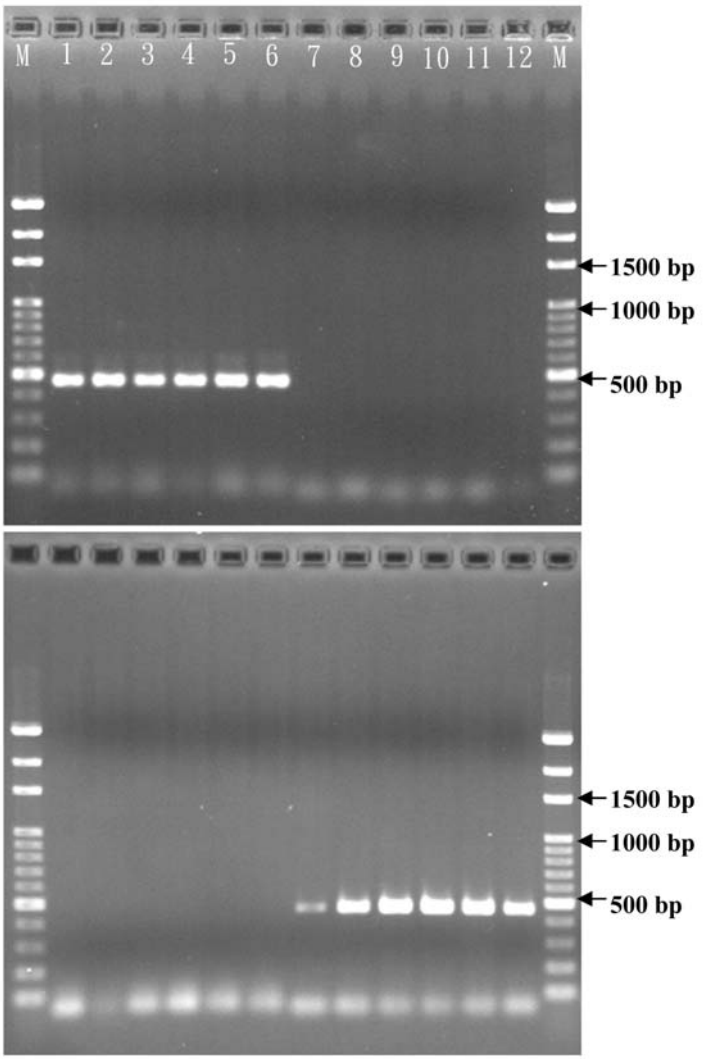

Fig. 2. The polymerase chain reaction (PCR) of species-specific primers UV-ITSF+UV-ITSR (top) and UA-ITSF+UA-ITSR (bottom) for identification of *Uromyces vignae* (Lane 1~6) and *U. appendiculatus* (Lane 7~12). The amplified band of DNA is 500 bp. M: marker (100 bp ladder)

the present study. Virtudazo *et al.*[29] and Chung *et al.*[4] successfully used this method and cooperated with PCR to study the phylogenetic relationship between different rust species. In this study, the specific primers which we designed could rapidly classify *U. appendiculatus* and *U. vignae*. *U. appendiculatus* and *U. vignae* apparently have high host specificity on *P. vulgaris* and *V. unguiculata*, respectively, although their pathogenicity may vary depending on environmental factors and host plants[7,8].

Ten *Uromyces* species on legumes have been reported from Taiwan[16]. Only *U. appendiculatus*, *U. vignae*, *U. viciae-fabae* and *U. striatus* were on cultivated legumes. However, *U. vicae-fabae* (=*U. fabae* de Bary) and *U. striatus* have not been found in the fields during the past 10 years. Although, we did not test the specificity of these four primers on other *Uromyces* spp. on cultivated legumes, the alignment result showed that ITS rDNA sequences of *U. appendiculatus* and *U. vignae* on Taiwan were highly different from those of *U. viciae-fabae* and *U.*

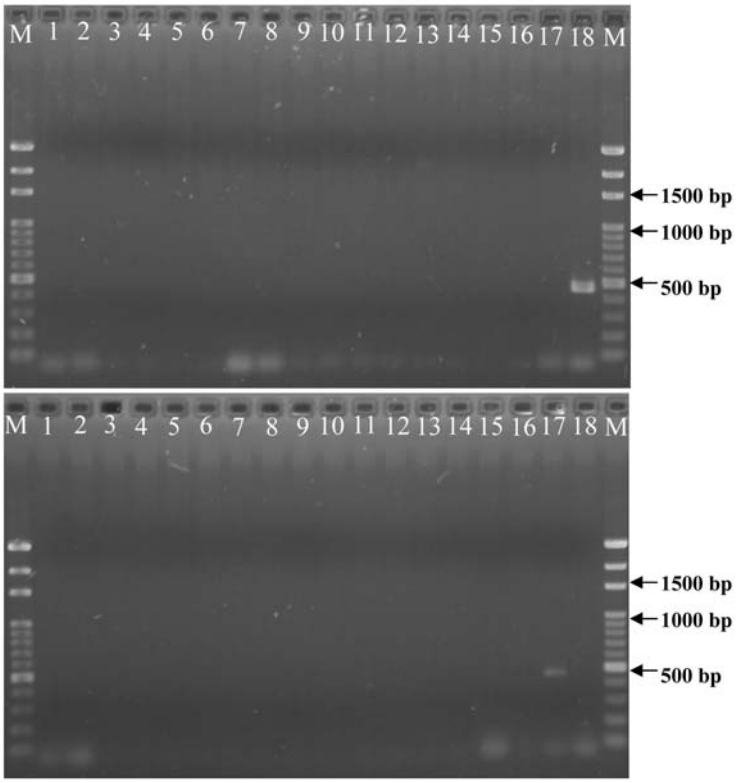

Fig. 4. The polymerase chain reaction (PCR) of species-specific primers UA-ITSF+UA-ITSR (top) and UV-ITSF+UV-ITSR (bottom) for testing the specificity of different rust fungi. Lanes 1-2: *Puccinia nakanishikii*; Lanes 3-4: *Puccinia phyllostachydis*; Lanes 5-6: *Coleosporium perillae*; Lanes 7-8: *Melampsora coleosporioides*; Lanes 9-10: *Puccinia allii*; Lanes 11-12: *Phakopsora fici*; Lanes 13-14: *Puccinia menthae*; Lanes 15-16: *Puccinia arachidis*; Lane 17: *Uromyces vignae*; Lane 18: *U. appendiculatus*; M: marker (100 bp ladder). The amplified band of DNA is 500 bp.

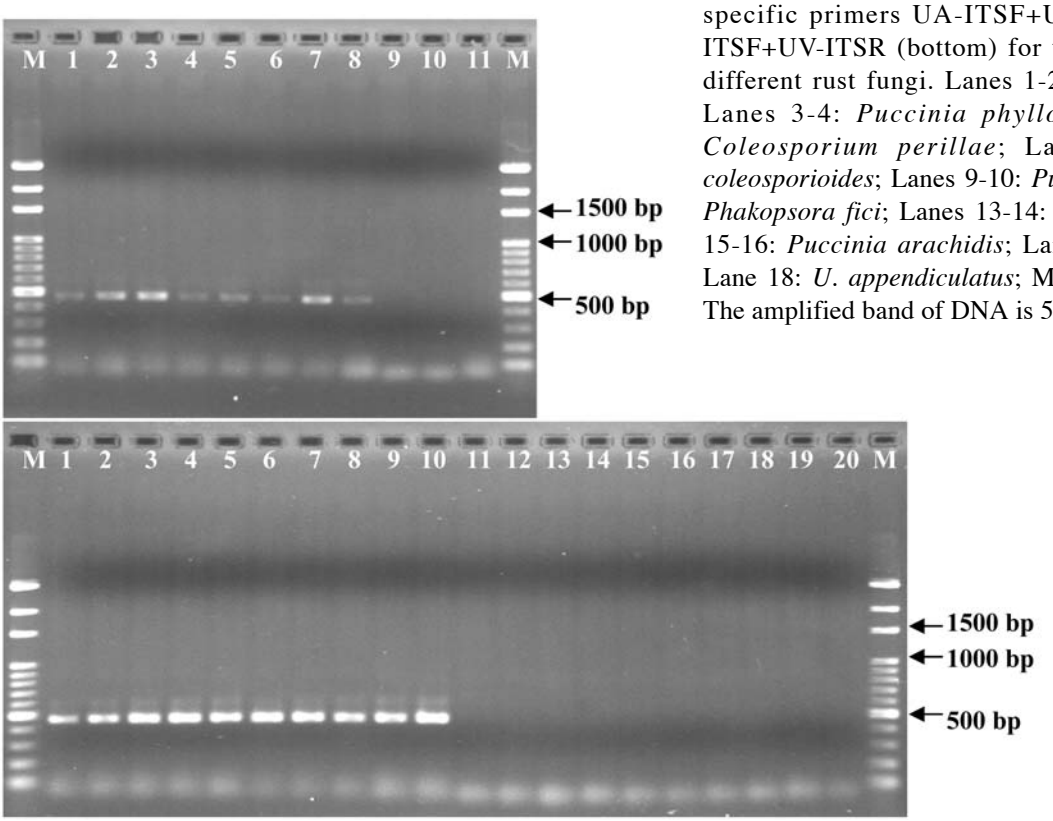

Fig. 3. The polymerase chain reaction (PCR) of specific primers UA-ITSF+UA-ITSR (top) and UV-ITSF+UV-ITSR (bottom) for testing the reaction of a single uredium of *Uromyces* spp. randomly obtained from one bean or cowpea leaf. Lanes 1-8 of top were *Uromyces* uredium obtained from bean, and lanes 9-11 were *Uromyces* uredium obtained from cowpea. Lanes 1-10 of bottom were *Uromyces* uredium obtained from cowpea, and lanes 11-20 were *Uromyces* uredinium obtained from bean. The amplified band of DNA is 500 bp. M: marker (100 bp ladder).

striatus from GenBank (Fig. 1). Thus, our specific primers perhaps will not amplify the ITS rDNA region of other *Uromyces* spp. on cultivated legumes in Taiwan. These primers designed from ITS rDNA region should also be useful for monitoring dynamic ecology of U. *appendiculatus* and *U. vignae* in fields.

ACKNOWLEDGMENTS

This research was funded by the Grant No. NSC 95-2313-B005-020 from the National Science and Technology Program for Agricultural Biotechnology, National Science Council, Taiwan, ROC.

LITERATURE CITED

1. Arthur, J. C. 1934. Manual of the Rusts in United States and Canada. Hafner Publishing Company, New York, 438pp.
2. Barclay, A. 1891. Additional Uredineae from the neighbourhood of Simla. J. Asiatic Soc. Bengal. 60: 211-212.
3. Burnett, J. 2003. Fungal Populations and Species. Oxford University Press Inc., New York, 348pp.
4. Chung, W. H., Tsukiboshi, T., Ono, Y., and Kakishima, M. 2004. Morphological and phylogenetic analyses of *Uromyces appendiculatus* and *U. vignae* on legumes in Japan. Mycoscience 45: 233-244.
5. Cummins, G. B. 1978. Rust Fungi on Legumes and Composites in North America. University of Arizona Press. Tuscon Arizona, 424pp.
6. Duke, J. A. 1981. Handbook of Legumes of World Economic Importance. Plenum Press, New York and London, 345pp.
7. Elmhirst, J. F., and Heath, M. C. 1987. Interactions of the bean rust and cowpea rust fungi with species of the *Phaseolus-Vigna* plant complex. I. Fungal growth and development. Can. J. Bot. 65: 1096-1107.
8. Elmhirst, J. F., and Heath, M. C. 1989. Interactions of the bean rust and cowpea rust fungi with species of the *Phaseolus-Vigna* Plant complex. II. Histological responses to infection in heat-treated and untreated leaves. Can. J. Bot. 67: 58-72.
9. Fromme, F. D. 1924. The rust of cowpeas. Phytopathology 2: 67-79.
10. Grasso, V., Sierotzki, H., Garibaldi, A., and Gisi, U. 2006. Characterization of the cytochrome b gene fragment of *Puccinia* species responsible for the binding site of QoI fungicides. Pest. Bio. Physiol. 84: 72-82.
11. Guo, L., and Wang, Y. C. 1986. Taxonomic study of the genus *Uromyces* from China. Acta. Mycology Sinica Suppl., pp. 107-108.
12. Heath, M. C. 1980. Effects of infection by compatible species or injection of tissue extracts on the susceptibility of nonhost plants to rust fungi. Phytopathology 70: 356-360.
13. Heath, M. C. 1984. Relationship between heat-induced fungal death and plant necrosis in compatible and incompatible interactions involving the bean and cowpea rust fungi. Phytopathology 74: 1370-1376.
14. Hiratsuka, N. 1937. Notes on Japanese species of *Uromyces*. Jap. J. Bot. 13: 29-47.
15. Hiratsuka, N. 1937. Revision of taxonomy of the genus *Uromyces* in the Japanese Archipelago. Rept. Tottori Mycol. Inst. 10:1-98.
16. Hiratsuka, N., and Chen, Z. C. 1991. A list of Uredinales collected from Taiwan. Trans. Mycol. Soc. Japan 32: 3-22.
17. Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T., Harada, Y., Hiratsuka, T., and Nakayama, K. 1992. The Rust Flora of Japan. Tsukuba Shuppankai, Ibaraki, 1205pp.
18. Ishii, H. 2002. DNA-based approaches for diagnosis of fungicide resistance. Pages 242-259 *in*: Agrochemical Resistance: Extent, Mechanism, and Detection. J. M. Clark and I. Yamaguchi (eds). American Chemical Society, Washington, DC.
19. Ito, S. 1922. Uromyces of Japan. J. Coll. Agric, Hokkaido Imp. Uni. 11: 211-287.
20. Kim, W. K., Heath, M. C., and Rohringer, R. 1985. Comparative analysis of proteins of *Uromyces phaseoli* var. *typical*, *U. phaseoli* var. *vignae*, and *U. viciae-fabae*: polypeptide mapping by two-dimensional electrophoresis. Can. J. Bot. 63: 2144-2149.
21. Ma, Z., and Michailides, T. J. 2004. An allele-specific PCR assay for detecting azoxystrobin-resistant *Alternaria* isolates from pistachio in California. J. Phytopathol. 152: 118-121.
22. Maier, W., Begerow, D., WeiB, M., and Oberwinkler, F. 2003. Phylogeny of the rust fungi: an approach using nuclear large subunit ribosomal DNA sequences. Can. J. Bot. 81: 12-23.
23. Pfunder, M., Schürch, S., and Roy, B. A. 2001. Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. Mycol. Res. 105: 57-66.
24. Rambaut, A. 2000. Se-Al; Sequence alignment editor. Department of Zoology, University of Oxford, Osford, UK.
25. Suyama, Y., Kawamuro, K., Kinoshita, I., Yoshimura,

K., Tsumura, Y., and Takahara. H. 1996. DNA sequence from a fossil pollen of *Abies* spp. from Pleistocene peat. Gene Genet. Syst. 71: 145-149.
26. Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibett, D. S., and Fisher, M. C. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Bio. 31: 21-32.
27. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876-4882.
28. Thurston, H. D. 1998. Tropical Plant Diseases (Second Edition). APS Press, St Paul, USA, 208pp.
29. Virtudazo, E. V., Nakamura, H., and Kakishima, M. 2001. Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8S rDNA and D1/D2 regions of LSU rDNA. J. Gen. Plant Pathol. 67: 28-36.
30. Weber, R. W. S., Webster, J., and Engel, G. 2003. Phylogenetic analysis of *Puccinia distincta* and *P. lagenophorae*, two closely related rust fungi causing epidemics on Asteraceae in Europe. Mycol. Res. 107: 15-24.
31. Zambino, P. J., and Szabo, L. J. 1993. Phylogenetic relationships of selected cereal and grass rusts based on rDNA sequence analysis. Mycologia 85: 401-414.

摘 要

鍾文鑫[1]、鍾文全[2]、丁姵分[1]、黃鴻章[3]、黃振文[1,4]. 2008. 以PCR 分生技術鑑定台灣菜豆與豇豆銹病菌. 植病會刊 17: 297-305. ([1] 國立中興大學植物病理系；[2] 行政院農業委員會種苗改良繁殖場；[3] 行政院農業委員會農業試驗所生物技術組；[4] 聯絡作者，電子郵件：jwhuang@nchu.edu.tw；傳眞：+886-4-2285-1676)

菜豆銹病菌 (*Uromyces appendiculatus*) 與豇豆銹病菌 (*U. vignae*) 所引起的豆科銹病爲世界性重要病害，然過去由於此兩種銹菌之形態學與寄主範圍的混淆不清，因而增加此兩種銹病菌在鑑定上的困難。本研究利用核糖體基因之內轉錄間隔區 (Internal transcribed spacer, ITS rDNA) 的多樣性設計四個專一性引子，可快速且正確的鑑定台灣產菜豆銹病菌與豇豆銹病菌。其中UA-ITSF 與UA-ITSR 引子，對菜豆銹病菌具有非常高的專一性；而UV-ITSF 與UV-ITSR 引子，則對豇豆銹病菌具非常高專一性。此外，本研究中所設計的四個引子，對八個來自非豆科作物上的銹病菌均無法增幅到任何DNA 條帶。

關鍵詞：菜豆銹病菌、豇豆銹病菌、菜豆、豇豆