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Identification and Pathogenicity of *Fusarium* Species Associated with Root Rot and Stem Rot of *Dendrobium*

Z. Latiffah, M.Z. Nur Hayati, S. Baharuddin and Z. Maziah
School of Biological Sciences, Universiti Sains Malaysia,
11800 Minden, Penang, Malaysia

Abstract: Three *Fusarium* species, *F. oxysporum*, *F. proliferatum* and *F. solani* were isolated from root and stem rot of *Dendrobium* orchid. Their pathogenicity on *Dendrobium* was studied and the inoculation test showed that the three *Fusarium* species were pathogenic, causing root and stem rot on the orchid. Molecular characterization using PCR-RFLP of ITS+5.8S regions showed that the isolates from the same species produced similar patterns and UPGMA cluster analysis of PCR-RFLP of ITS+5.8S clearly grouped *F. oxysporum*, *F. proliferatum* and *F. solani* into separate clusters. The present study showed that three *Fusarium* species, *F oxysporum*, *F. proliferatum* and *F. solani* were associated with root and stem rot of *Dendrobium* orchid.

Key words: *Fusarium*, root rot, stem rot, *Dendrobium*, PCR-RFLP

INTRODUCTION

Floriculture industry in Malaysia is relatively new compared to other agriculture commodities. With the introduction of Malaysian National Agriculture Policy (1992-2010), cut flowers have been identified as one of priority groups of commercial crops, as local and foreign demand of cut flowers have increased. One of the most important cut flowers planted in Malaysia is orchid, which contributed about 40% of total production of cut flowers and the most popular orchid cultivated is *Dendrobium* (Lim *et al*., 1998). With an increased of orchid cultivation to meet the demand of local and foreign markets, disease incidence especially in nurseries have also increased.

During a disease survey, root and stem rot disease of *Dendrobium* were observed in a few nurseries in Penang Island and Taiping, Perak, Peninsula Malaysia. Typical symptom on the root was discolorations, which indicate rotting of the tissues. Infected stem showed yellowish discolouration with water soaked appearance and very friable. In preliminary studies, *Fusarium* species were frequently isolated from infected orchid in nurseries in Penang Island. In the late 1999, *F. proliferatum* has been isolated from stem rot of orchid during disease survey in nurseries in Kuala Lumpur.

Several *Fusarium* species have been reported to infect orchidaceous plant in several parts of the world. However, in Malaysia the information on disease occurrence and the causal agent is limited. Thus, the study was conducted to identify the *Fusarium* species associated with the root and stem rot of *Dendrobium* orchid using morphological characteristics and PCR-RFLP of ITS+5.8S region and to examine their pathogenicity on the orchid.

MATERIALS AND METHODS

Isolation and Identification of *Fusarium* Isolates

Dendrobium orchids with symptoms of root and stem rot were collected from orchid nurseries in northern states of Peninsula Malaysia in 2006. The samples were collected from an orchid nursery at Agriculture Center, Relau, Penang and from two commercials orchid nurseries in Taiping, Perak.

Corresponding Author: Z. Latiffah, School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia Tel: 604-6533506 Fax: 604-6565125

All diseased roots and stems were cut about 3 mm and surface sterilized with 1% natrium hypochloride (NaOCl) for 2 min. Then the cuttings were rinsed immediately in distilled water, blotted dry and plated onto peptone-pentachloro-nitrobenze agar (PPA) and incubated at 27±1°C for 7 days. Mycelium developed from the tissue cuttings was sub-cultured onto Potato Sucrose Agar (PSA) and single spore technique was used to purify the *Fusarium* isolates.

For identification of *Fusarium* species, Carnation Leaf Agar (CLA) and Potato Dextrose Agar (PDA) were used for morphological characteristics observation. Species descriptions were based on the description of Nelson *et al*. (1983) and *Fusarium* Laboratory Manual (Leslie and Summerell, 2006).

Pathogenicity Test

Pathogenicity tests were conducted using Koch Postulate to confirm the *Fusarium* species as the causal agent of root and stem rot of *Dendrobium* orchid. The plants used were 3 years old and showed no disease symptoms.

The *Fusarium* isolates used were the representative of isolates, which was successfully isolated and identified from root and stem rot of *Dendrobium*. Two representatives from each of the *Fusarium* species were tested for their pathogenicity on apparently healthy *Dendrobium* and separate plants were used for inoculation tests on root and stem.

The root and stem of the *Dendrobium* were washed and surface sterilized before inoculation. For inoculation, spore suspensions (adjusted to 1×10^6 spores mL^{-1}) were prepared. Twenty milliliter of the spore suspensions for each *Fusarium* species were sprayed on the root and stem of the plants. The control plants were sprayed with 20 mL of sterile distilled water.

The experiment was conducted in a Completely Randomized Design (CRD) with five replicates for each *Fusarium s*pecies and control and grown in a plant house until disease symptoms were observed. The plants were position randomly on the bench with temperature about 28±1°C and watered daily.

Disease rating was based on a scale of 0-5 as described by Benyon *et al*. (1996). For assessment of pathogenicity, four roots per plant were selected randomly by cutting 3 cm section from behind the root tip. For stem, about 2-3 cm section from the margin of infected tissues were cut. After surface sterilization using 1% natrium hypochloride (NaOCl) for 2 min, rinsed in distilled water and blotted dry, the infected plant parts were plated onto PPA for reisolation of *Fusarium* species.

Analyses of differences between treatments were analysed by using Duncan Multiple Range Test (DMRT) and analysis of variance (ANOVA).

PCR-RFLP of ITS+5.8S

A total of 40 *Fusarium* isolates from root and stem rot of orchid were used in this study (Table 1). Twenty nine isolates were recovered from root and stem rot of *Dendrobium* and eight isolates namely, *F. proliferatum* (1325, 1374, 1377, 1378, 1380 and 13811), *F. solani* (1257) and *F. oxysporum* (1493) were obtained from *Fusarium* Culture Collection, Universiti Sains Malaysia, Penang, Malaysia. These cultures were isolated from stem rot of orchid in a disease survey conducted in the late 1999. Another three isolates comprising *F. oxysporum* (AP7 and AP9) and *F. solani* (AP8) were from root rot of *Oncidium* orchid obtained during this study.

For DNA extraction, mycelium was grown on Potato Sucrose Agar (PSA) and incubated for 6 days. DNA was extracted using DNeasy Plant Mini Kit (Qiagen) according to the manufacturers' instructions. The extracted DNA was stored at -20°C until used.

The ITS+5.8S were amplified using ITS1 (5TCCGTAGGTGAACCTGCGG3) and ITS2 (TCCTCCGCTTATTGATATGC3) primers (White *et al*., 1990). Amplification reactions were conducted in a 25 μL 10X PCR buffer (Promega), 2.5 μL $MgCl_2$, 0.8 mM dNTP mix, 0.4 μM each primers, 1 unit *Taq* polymerase (Promega) and approximately 5 ng of template DNA. Each reaction

Table 1: *Fusarium* isolates used in molecular analysis, their host and location

Isolates	Host and plant part	Location
F. oxysporum		
AP1	Root (*Dendrobium*)	Relau, Penang
AP2	Root (*Dendrobium*)	Relau, Penang
AP3	Root (*Dendrobium*)	Relau, Penang
AP5	Root (*Dendrobium*)	Relau, Penang
AT14	Root (*Dendrobium*)	Taiping, Perak
AT15	Root (*Dendrobium*)	Taiping, Perak
AP7	Root (*Oncidium*)	Relau, Penang
AP9	Root (*Oncidium*)	Relau, Penang
BP1	Stem (*Dendrobium*)	Relau, Penang
BP2	Stem (*Dendrobium*)	Relau, Penang
BP3	Stem (*Dendrobium*)	Relau, Penang
BP4	Stem (*Dendrobium*)	Relau, Penang
BT6	Stem (*Dendrobium*)	Taiping, Perak
BT8	Stem (*Dendrobium*)	Taiping, Perak
BT9	Stem (*Dendrobium*)	Taiping, Perak
1493	Stem (stock culture)	Kuala Lumpur
F. solani		
AP4	Root (*Dendrobium*)	Relau, Penang
AT13	Root (*Dendrobium*)	Taiping, Perak
AT16	Root (*Dendrobium*)	Taiping, Perak
AT17	Root (*Dendrobium*)	Taiping, Perak
AP8	Root (*Oncidium*)	Relau, Penang
BP5	Stem (*Dendrobium*)	Relau, Penang
BT7	Stem (*Dendrobium*)	Taiping, Perak
BT13	Stem (*Dendrobium*)	Taiping, Perak
1257	Stem (stock culture)	Kuala Lumpur
F. proliferatum		
AP6	Root (*Dendrobium*)	Relau, Penang
AP10	Root (*Dendrobium*)	Relau, Penang
AP11	Root (*Dendrobium*)	Relau, Penang
AP12	Root (*Dendrobium*)	Taiping, Perak
BT10	Stem (*Dendrobium*)	Taiping, Perak
BT11	Stem (*Dendrobium*)	Taiping, Perak
BT12	Stem (*Dendrobium*)	Taiping, Perak
BT14	Stem (*Dendrobium*)	Taiping, Perak
BT15	Stem (*Dendrobium*)	Taiping, Perak
1325	Stem (Stock culture)	Kuala Lumpur
1374	Stem (Stock culture)	Kuala Lumpur
1377	Stem (Stock culture)	Kuala Lumpur
1378	Stem (Stock culture)	Kuala Lumpur
1380	Stem (Stock culture)	Kuala Lumpur
1381	Stem (Stock culture)	Kuala Lumpur

was overlaid with 25 μL of mineral oil. Amplification was performed in a programmable thermal cycler (DNA Engine Peltier Thermal Cycler model PTC-100) as follows, initial denaturation of 95°C for 2 min followed by 35 cycles of denaturation at 30 sec, annealing at 63°C for 1 min and extension at 72°C for 2 min. After 35 cycles, additional extension was performed at 72°C for 10 min. The PCR product was run on 1.7% agarose gel and stained with ethidium bromide. The estimated size of the amplified ITS+5.8S was based on comparison with 100 bp marker (Promega).

The PCR products were digested with four restriction enzymes namely, *Eco*RI, *Eco*881, *Bsu*I and *Msp*I (Fermentas). Ten microliter of the PCR products were digested with the restriction enzymes according to the manufacturer's instructions. The digested PCR products were separated on 1.7% agarose gel and stained with ethidium bromide. The estimated bands were based on 1 kb DNA marker (GeneRuler, Fermentas).

Restriction bands were scored based on presence (1) and absence (0) of a particular band with reference to the standard markers. A binary matrix was compiled and subjected to cluster analysis. A

genetic similarity was constructed using Simple Matching Coefficient. The Numerical Taxonomy System of Multivariate Program (NT-SYS) software package version 2.0 (Rohlf, 2000) was used to analyse the data. A dendrogram was constructed using unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis to infer the relationships within and between the *Fusarium* isolates from root and stem rot of orchid.

RESULTS

A total of 29 *Fusarium* isolates were isolated from root and stem rot of *Dendrobium* in which 14 isolates from the root and 15 isolates from the stem. Based on morphological characteristics described by Nelson *et al*. (1993) and Leslie and Summerell (2006), three *Fusarium* species were identified namely, *F. oxysporum*, *F. solani* and *F. proliferatum*.

The species identified from root rot of *Dendrobium* were *F. oxysporum* (six isolates) and four isolates each for *F. solani* and *F. proliferatum*. Seven *F. oxysporum* isolates, three *F. solani* and five *F. proliferatum* were recovered from stem rot of *Dendrobium*.

Twenty days after inoculation, disease symptoms were observed on the roots. Initially, the roots showed brownish discolouration and water-soaked appearance. The brownish discolouration eventually turned to dark colour which indicates rotting of the root tissues. The disease symptoms observed corresponding to the symptoms observed in the nurseries.

For infection on the *Dendrobium* root, *F. solani* (AT4) shows the highest infection with a disease rating of 3.70. There were no significant differences between the two *F. solani* isolates used in the test (Table 2). For *F. oxysporum* isolates (AT14 and AP1) there were no significant differences of disease infection between the two isolates. Only *F. proliferatum* isolates (AP11 and AT10) showed significant difference of infection on the root.

The first symptoms on *Dendrobium* stems were observed 30 days after inoculation. The infected stems turned yellowish with water-soaked appearance and very friable. Severe infection could cause the plant to collapse. It was also observed that there were some degrees of rotting on a few leaves close to the stem base. However, not all the leaves showed the symptoms.

The symptom on the stem resembled those observed in the nurseries. *F. oxysporum* (BP1) gave the highest disease rating, but there were no significant differences between the two *F. oxysporum* (BP1 and BP2) isolates (Table 3). There were also no significant differences between *F. solani* isolates (BP5 and BT7) and *F. proliferatum* isolates (BT10 and BT15).

Table 2: Mean disease rating on *Dendrobium* root of *F. oxysporum*, *F. solani* and *F. proliferatum*

Fusarium species	Isolates	Disease rating
Control	-	0^{a}
F. oxysporum	AT14	2.46^{b}
F. oxysporum	AP1	2.86^{c}
F. solani	AP4	3.70^{e}
F. solani	AT13	3.62^{e}
F. proliferatum	AT11	3.27^{d}
F. proliferatum	AT10	2.79^{c}

Mean values in the same row followed by different superscripts are significantly different, $p<0.05$

Table 3: Mean disease rating on *Dendrobium* stem of *F. oxysporum*, *F. solani* and *F. proliferatum*

Fusarium species	Isolates	Disease rating
Control	-	0^{a}
F. oxysporum	BP1	3.48^{c}
F. oxysporum	BP2	3.45^{c}
F. solani	BP5	2.38^{b}
F. solani	BT7	2.46^{b}
F. proliferatum	BT10	2.56^{b}
F. proliferatum	BT15	2.56^{b}

Mean values in the same row followed by different superscripts are significantly different, $p<0.05$

Table 4: Estimated sizes of restriction bands of *F. oxysporum*, *F. solani* and *F. proliferatum* using *Eco*881, *Msp*1, *Bsu*RI dan *Eco*R1

Isolates	Size of PCR product (bp)	Estimated sizes of restriction bands (bp)			
		*Eco*881	*Msp*1	*Bsu*RI	*Eco*R1
F. oxysporum					
AP1	550	550	100, 450	100, 350	300, 250
AP2	550	550	100, 450	100, 350	300, 250
AP3	550	550	100, 450	100, 350	300, 250
AP5	550	550	100, 450	100, 350	300, 250
AP7	550	550	100, 450	200, 350	300, 250
AP9	550	550	100, 450	200, 350	300, 250
AT14	550	550	100, 450	200, 350	300, 250
AT15	550	550	100, 450	200, 350	300, 250
BP1	550	550	100, 450	100, 400	300, 250
BP2	550	550	100, 450	100, 400	300, 250
BP3	550	550	100, 450	100, 400	300, 250
BP4	550	550	100, 450	100, 400	300, 250
BT6	550	550	100, 450	100, 400	300, 250
BT8	550	550	100, 450	100, 400	300, 250
BT9	550	550	100, 450	100, 400	300, 250
1493	550	550	100, 450	100, 400	300, 250
F. solani					
AP4	590	210, 370	200, 350	150, 400	290, 300
AP8	590	220, 370	200, 350	150, 250	280, 300
AT13	590	220, 370	200, 350	150, 350	290, 300
AT16	590	220, 370	200, 350	150, 350	290, 300
AT17	590	220, 370	200, 350	150, 350	290, 300
BP5	590	210, 370	200, 350	100, 200	280, 300
BT7	590	210, 370	200, 350	100, 200	280, 300
BT13	590	210, 370	200, 350	100, 200	280, 300
1257	590	210, 370	200, 350	100, 200	280, 300
F. proliferatum					
AP6	590	210, 370	100, 180, 250	150, 300	280, 310
AP10	590	210, 370,	100, 180, 250	150, 300	280, 310
AP11	590	210, 370	100, 180, 250	150, 300	280, 310
AT12	590	210, 370	100, 180, 250	150, 300	280, 310
BT10	590	210, 370	100, 180, 250	200, 300	280, 310
BT11	590	210, 370	100, 180, 300	200, 300	280, 310
BT12	590	210, 370	100, 180, 300	200, 300	280, 310
BT14	590	210, 370	100, 180, 300	200, 300	280, 310
BT15	590	210, 370	100, 180, 300	200, 300	280, 310
1325	590	210, 370	100, 180, 250	200, 300	280, 310
1374	590	210, 370	100, 180, 250	200, 300	280, 310
1377	590	210, 370	100, 180, 250	200, 300	280, 310
1378	590	210, 370	100, 180, 250	200, 300	280, 310
1380	590	210, 370	100, 180, 250	200, 300	280, 310
1381	590	210, 370	100, 180, 250	200, 300	280, 310

The three *Fusarium* species were successfully recovered from the inoculated root and stem which indicated that the three *Fusarium* species were able to cause root and stem rot of *Dendrobium* orchid.

Using primer pairs of ITS1 and ITS4, a PCR product was amplified from the isolates of the three *Fusarium* species. *Fusarium oxysporum* produced approximately 550 bp band; *F. solani* and *F. proliferatum* approximately 590 bp bands.

Table 4 shows estimated sizes of the restriction bands produced after digestion of the ITS+5.8S using *Eco*881, *Msp*I, *Bsu*RI and *Eco*RI for *F. oxysporum*, *F. solani* and *F. proliferatum*. Generally, the restriction patterns produced by the restriction enzymes could differentiate between the three *Fusarium* species.

After digestion with *Eco*881, the PCR products of *F. oxysporum* isolates were undigested as band of 550 bp was observed which indicated that there's no restriction sites for the restriction enzyme

within the ITS+5.8S of the isolates. Digestion with *Msp*I and *Eco*RI produced the same restriction patterns for all the isolates. *Bsu*I restriction patterns were variable in which three restriction patterns were generated. The similarity values based on the restriction bands ranged from 91.7-100%.

Digestion of *F. solani* PCR products with *Eco*881, *Bsu*I and *Eco*RI produced variable restriction patterns. Digestion with *Msp*I generated the same restriction patterns for all *F. solani* isolates from *Dendrobium* root and stem rot as well as from *Oncidium* root rot and the stock culture (1257). The similarity value for all the isolates ranged from 75-100%.

Fusarium proliferatum isolates from *Dendrobium* stem and root rot as well as from the stock culture showed similar restriction patterns when digested using *Eco*881, *Msp*I and *Eco*RI. Only *Bsu*RI restriction patterns produced variable patterns. The similarity values of the *F. proliferatum* isolates ranged from 91.7-100%.

Cluster analysis based on the restriction bands formed two major clusters, I and II (Fig. 1). The cluster analysis clearly discriminate the three *Fusarium* species into three separate clusters in which *F. solani* isolates were clustered in sub-cluster A in major cluster I, *F. oxysporum* isolates in sub-cluster B and *F. proliferatum* isolates in major cluster II.

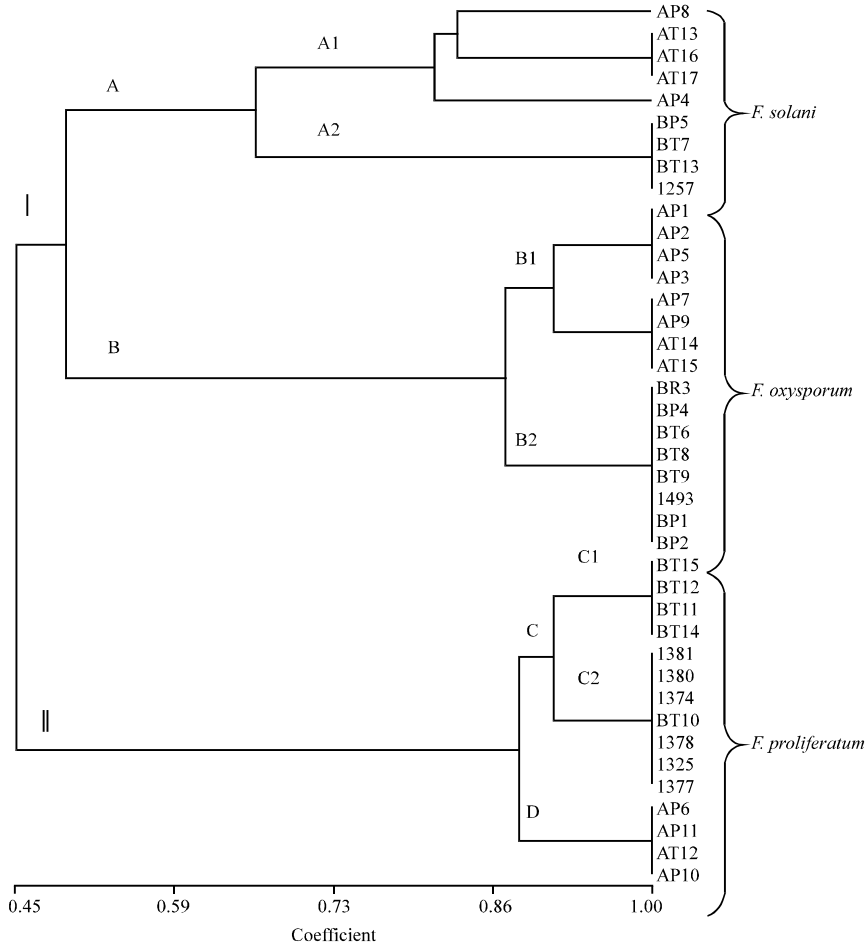

Fig. 1: Dendrogram from UPGMA clusters analysis using simple matching coefficient based on restriction bands of *F. oxysporum*, *F. proliferatum* and *F. solani* from root and stem rot of orchid

DISCUSSION

From pathogenicity test, *F. oxysporum, F. solani* and *F. proliferatum* were recovered from the inoculated root and stem which indicated that the three *Fusarium* species were able to cause root and stem rot of *Dendrobium* orchid. Although *F. oxysporum* was the most frequent isolates isolated from the root and stem rot of *Dendrobium*, based on the mean disease rating, *F. oxysporum* gave the highest rating on the stem and *F. solani* on the root. *F. proliferatum* could also cause root and stem rot of *Dendrobium*. The results indicated that a complex of *Fusarium* species could be pathogenic to the orchid and suggested that *F. oxysporum* could be the main causal agent of stem rot and *F. solani*, the main causal agent of root rot.

The three *Fusarium* species have been reported to be pathogenic to orchidaceous plant in several countries. *Fusarium oxysporum*, *F. solani* and *F. proliferatum* have been associated with root rot of *Cymbidium* orchid in Australia (Benyon *et al*., 1996), dry rot of *Cymbidium* (Lee *et al*., 2002) and root rot of moth orchid (*Phalaenopsis* spp.) (Kim *et al*., 2002) in Korea. *Fusarium proliferatum* has also been reported to cause yellow spot disease in *Cymbidium* in Japan by Ichikawa and Aoki (2000) and leaf spot of *Cymbidium hybrida* in Korea (Kim *et al*., 2002). *Fusarium solani* has been recorded as root pathogen on orchids by Burnett (1975). Although *F. subglutinans* was isolated and associated with root rot and yellow spot disease of orchidaceous plants (Benyon *et al*., 1996; Ichikawa and Aoki, 2000), however in this study, *F. subglutinans* was not recovered.

PCR-RFLP of ITS+5.8S have been used by Hyun and Clark (1998) on characterization of *F. lateritium* from sweet potato, Suga *et al*. (2000) in distinguishing *F. solani* forma speciales and Lee *et al*. (2000) on comparison of genetic relationship of 12 *Fusarium* species from different sections.

In PCR-RFLP of ITS+5.8S, restriction patterns produced by *Bsu*RI revealed variations within *F. oxysporum*, *F. solani* and *F. proliferatum* isolates as well as *Msp*I restriction patterns of *F. proliferatum* isolates and *Eco*RI restriction patterns for *F. solani* isolates. Intraspecies variations could be due to minor changes in nucleotide composition within the ITS+5.8S which may lead to different restriction patterns. Similar results were obtained by Konstantinova and Yli Mattila (2004) in their studies using PCR-RFLP of intergenic spacer to analyse *Fusarium* species in Section Sporotrichiella. Further studies using ITS+5.8S sequence analysis would be necessary to compare the genetic variations observed in *Fusarium* isolates from root and stem rot of orchid.

In conclusion, three *Fusarium* species were isolated from root and stem rot of *Dendrobium*. Based on morphological characteristics, the *Fusarium* species were identified as *F. oxysporum*, *F. solani* and *F. proliferatum*. From pathogenicity test, the three *Fusarium* species were found to be pathogenic, causing root and stem rot on *Dendrobium*. PCR-RFLP of ITS+5.8S analysis, offers a convenient tool for characterization and analyzing variations of *Fusarium* species associated with root and stem rot of *Dendrobium* orchid.

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REFERENCES

Benyon, F., B.A. Summerell and L.W. Burgess, 1996. Association of *Fusarium* species with root rot of *Cymbidium* orchids. Aust. Plant. Pathol., 25: 226-228.

Burnett, H.C., 1975. Diseases Caused by Fungi and Bacteria. Handbook of Orchid Pests and Diseases. 1st Edn., American Orchid Society, Cambridge Massachusetts, pp: 15-36.

Chang, M., I.K. Hyun, Y.H. Lee and D.H. Lee, 1998. Leaf spot of *Cymbidium hybrida* caused by *Fusarium proliferatum*. Korean J. Plant. Pathol., 14: 664-667.

Hyun, J.W. and C.A. Clark, 1998. Analysis of *Fusarium lateritium* using RAPD and rDNA RFLP techniques. Mycol. Res., 102: 1259-1264.

Ichikawa, K. and T. Aoki, 2000. New leaf spot disease of *Cymbidium* species caused *Fusarium subglutinans* and *Fusarium proliferatum*. J. Gen. Plant Pathol., 66: 213-218.

Kim, W.G., B.D. Lee, W.S. Kim and W.D. Cho, 2002. Root rot of moth orchid caused by *Fusarium* species. Plant Pathol. J., 18: 225-227.

Konstantinova, P. and T. Yli-Mattila, 2004. IGS-RFLP analysis and development of molecular markers for identification of *Fusarium poae*, *Fusarium langethiae*, *Fusarium sporotrichioides* and *Fusarium kyushuense*. Int. J. Food Microbiol., 95: 321-331.

Lee, B.D., W.G. Kim, W.D. Cho and J.M. Sung, 2002. Occurrence of dry rot on *Cymbidium orchids* caused by *Fusarium* spp. in Korea. Plant. Pathol. J., 18: 156-160.

Lee, Y.M., L.Y. Choi and B.R. Min, 2000. PCR-RFLP and sequence analysis of the rDNA ITS region in the *Fusarium* spp. J. Microbiol., 38: 66-73.

Leslie, J.F. and B.A. Summerell, 2006. The *Fusarium* Laboratory Manual. 1st Edn., Blackwell Publishing, Iowa, USA., ISBN: 0-8138-1919-9.

Lim, H.J., M.S.M. Ridzuan and H.N. Auni, 1998. Cut Flower Production in Malaysia. In: Cut Flower Production in Asia, Papademetriou M.K. and N.K. Dadlani (Eds). FAO/RAP Publication 1998/14, Bangkok, Thailand.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1993. *Fusarium* species: An Illustrated Manual for Identification. 1st Edn. Pennsylvania State University Press, University Park, New York, ISBN: 0-271-00349-9.

Rohlf, F.J., 1998. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Version 2.2, Exeter Publications Setauket, New York.

Suga, H., T. Hasegawa, H. Mitsui, K. Kageyama and M. Hyakumachi, 2000. Phylogenetic analysis of the phytopathogenic fungus *Fusarium solani* based on the rDNA-ITS region. Mycol. Res., 104: 1175-1183.

White, T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocol, Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White (Eds). Academic Press, San Diego, ISBN: 0-12-372181, pp: 315-322.