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Full Length Research Paper

Characterization and intraspecific variation of *Fusarium semitectum* (Berkeley and Ravenel) associated with red-fleshed dragon fruit (*Hylocereus polyrhizus* [Weber] Britton and Rose) in Malaysia

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A total of 79 isolates of *Fusarium semitectum* were characterized by morphological and IGS-RFLP analysis to assess its intraspecific variation. Based on morphological characteristics, the isolates of *F. semitectum* were classified into 2 distinct groups, morphotypes I and II. Morphotype I was characterized by longer macroconidia (3 - septate: 31.03 ± 2.57 µm; 5 - septate: 40.17 ± 1.85 µm), 0 - 7 septate with 5 - septate was the most common, absence of chlamydospores, presence of sporodochia, abundant-floccose mycelium, peach colony appearance, peach to orange pigmentations and fast growing. While isolates of morphotype II produced shorter macroconidia (3 - septate: 24.98 ± 1.87 µm; 5 - septate: 35.24 ± 2.07 µm), 0 - 5 septate with 3 - septate was the most common, with (56%) or without chlamydospores (44%), without sporodochia, abundant-floccose and abundant-powdery mycelium, beige to brown colonies, brown to dark brown pigmentations and slow growing. Corresponding to the morphological characterization, IGS-RFLP analysis indicated that the 79 isolates could be divided into 2 different clusters assigned as RFLP groups I and II. 49 IGS haplotypes were produced by 8 restriction enzymes (*Alu*I, *Bsu*15I, *Bsu*RI, *Eco*881, *Hin*6I, *Msp*I, *Pst*I and *Taq*I) which indicated a high level of intraspecific variation and polymorphism among the 79 isolates. This is the first report of *F. semitectum* associated with *H. polyrhizus*.

Key words: *Fusarium semitectum, Hylocereus polyrhizus,* IGS-RFLP, intraspecific variation, morphology.

INTRODUCTION

Dragon fruit, especially red-fleshed *(Hylocereus polyrhizus),* is a newly introduced fruit crop and now it is being cultivated almost in all states of Malaysia. The suitability of the tropical climate, rainfall, light intensity and soil types (Luders and McMahon, 2006), may contributed to the successful cultivation of this exotic fruit in this country. It is also being considered a potential health crop that contributes to high economic returns. Recently, dragon fruit was reported to be seriously infected with several complex diseases caused by fungi including species of *Fusarium* (Crane and Balerdi, 2005; Wright et al., 2007) and causing serious losses to farmers. Our preliminary study on dragon fruit diseases conducted in 2007 throughout dragon fruit plantations in Malaysia revealed that the highest number of fungal isolates associated with diseased *H. polyrhizus* was *F. semitectum* (Hew et al., 2008; Masratul et al., 2008a, b).

F. semitectum Berkeley and Ravenel [syn: *F. pallidoroseum* (Cooke) Sacc.; syn: *F. incarnatum* (Roberge) Sacc.] is a widespread and common species in the tropic, subtropic and Mediterranean regions and regularly associated with a complex of plant diseases (Leslie and Summerell, 2006). This cosmopolitan species was included in section Arthrosporiella that was proposed by Wollenweber and Reinking (1935) and has no known sexual stage (Burgess et al., 1994). The significant character of this section is the production of polyblastic conidiogenous cell (Booth, 1971). There are 2 varieties of *F. semitectum* that is; vars. *semitectum* and *majus*

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(Gerlach and Nirenberg, 1982). *F. semitectum* var. *semitectum* produced 1 - 5 septate while var. *majus* produced 1 - 7 septate conidia (Booth, 1971; Joffe, 1974; Gerlach and Nirenberg, 1982). Other character to distinguish these varieties is the absence of sporodochia in *F. semitectum* var. *semitectum* while var. *majus* produced sporodochia (Booth, 1971; Joffe, 1974).

One of the methods to observe intraspecific variations is PCR-RFLP analysis of non-coding nuclear ribosomal DNA regions such as the intergenic spacer (IGS) that appear to be the most rapidly evolving spacer regions (Cooke et al., 1996; Hseu et al., 1996). The IGS-RFLP has been used to analyze intraspecific variation of several *Fusarium* species including *F. oxysporum* (Llorens et al., 2005; Alves-Santos, 2007), *F. equiseti* (Kosiak et al., 2004; Jurado et al., 2005), *F. lateritium* (Hyun and Clark, 1998), *F. verticillioides* (Patino et al., 2006) and *F. graminearum* (Carter et al., 2000). As *F. semitectum* was suggested to be a species complex (Leslie and Summerell, 2006), PCR-RFLP of IGS regions (IGS-RFLP) was applied to assess the extent of intraspecific variations within *F. semitectum* isolates associated with *H. polyrhizus*.

Current knowledge and research on *F. semitectum* are surprisingly limited compared to other species of *Fusarium*. Therefore, this study was undertaken to characterize *F. semitectum* isolates associated with *H. polyrhizus* in Malaysia using morphological characteristics and IGS-RFLP, in order to assess intraspecific variation within the isolates.

MATERIALS AND METHODS

Fungal isolates

Fungi were isolated from 3 different parts namely from the stem, fruit and root of diseased *H. polyrhizus* from 9 states (Penang, Perak, Selangor, Melaka, Negeri Sembilan, Johor, Kelantan, Sabah and Sarawak) in Malaysia. Surface sterilization was carried out by cleaning the symptom margins with 70% ethanol and cut into small blocks (ca 1.5 x 1.5 x 1.5 cm), soaked in 1% sodium hypochlorite (NaOCl) for 3 min and rinsed in several changes of sterile distilled water (each 1 min). All sterilized samples were placed onto peptone pentachloronitrobenzene agar (PPA) (Nash and Synder, 1962) and incubated under standard incubation conditions (Salleh and Sulaiman, 1984) whereby the plates were incubated at 25 ± 2°C with 12 h periods of light for 7 days. The light sources were from 40 W cool white fluorescent tubes, 1 UV light tube and one 36 W black light tube. Single conidial isolates were obtained on water agar (WA) (Burgess et al., 1994).

Morphological characterization

All single-spored isolates of *Fusarium* that were successfully isolated were identified morphologically based on the microscopic and macroscopic characteristics. For species determination, the descriptions by Wollenweber and Reinking (1935), Booth (1971), Joffe (1974), Gerlach and Nirenberg (1982), Nelson et al. (1983), Burgess et al. (1994) and Leslie and Summerell (2006) were adopted.

Microscopic characteristics

For microscopic characteristics, the structure of conidiophores, the shape of conidia and the presence or absence of chlamydospores and sporodochia were observed (Wollenweber and Reinking, 1935; Booth, 1971; Joffe, 1974; Gerlach and Nirenberg, 1982; Nelson et al., 1983; Burgess et al., 1994; Leslie and Summerell, 2006). The length and width of conidia from a sample of 50 conidia were measured and the frequency of conidial septation was determined. Each isolate was cultured onto carnation leaf-pieces agar (CLA) (Fisher et al., 1982) and soil agar (SA) (Klotz et al., 1988) to enhance the formation of chlamydospores and incubated at 25 ± 2°C for 2 weeks.

Macroscopic characteristics

The macroscopic characteristics such as colony appearances (texture and colour of aerial mycelium), pigmentations and growth rates were examined. A mycelial disc of 6 mm diameter was transferred and inoculated centrally onto PDA plates (90 mm diameter) in 5 replicates for each isolate and incubated at 25 ± 2°C. The colony appearances and pigmentations were assessed after 2 weeks of incubation while growth rate was measured daily until fully grown (6 - 9 days). The Methuen handbook of colour chart (Kornerup and Wancher, 1978) was used for pigmentation analysis.

Statistical analysis

The data on the length and width of conidia and frequency of conidial septation were analyzed by 2 - sample T-test by using MINITAB® statistical software version 15.

DNA extraction

Each isolate of *Fusarium* was grown on PDA with sterile dialysis membranes (Lui et al., 2000) and incubated at 25 ± 2°C for 3 days. The mycelium grown over the dialysis membranes was harvested and ground to a fine powder in a sterile mortar with liquid nitrogen. DNA was extracted by using the DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer's instructions.

PCR amplification

IGS region was amplified by using primers CNL12 (5' - CTGAACGCCTCTAAGTCAG - 3') and CNS1 (5' – GAGACAAGCATATGACTACTG - 3') (Appel and Gordon, 1995). Amplification reactions were carried out in a total volume of 25 µl containing 5 µl 5X buffer (Promega, Madison, WI, USA), 1.25 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTP) (Promega), 0.8 µM each primer, 4 ng of template DNA and 0.625 units of DNA polymerase (Promega). Each reaction was overlaid with 20 µl of mineral oil to prevent evaporation.

PCR was performed in a Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA) with the following conditions: an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 35 s, annealing at 59°C for 55 s, extension at 72°C for 2 min and final extension at 72°C for 7 min. The PCR products were electrophoresed on 1% agarose gel in TBE buffer for 90 min at 90 V, 400 mA and visualized under UV light by ethidium bromide staining. The size of the amplified IGS fragment was estimated based on comparison with 1 kb DNA ladder (GeneRulers™, Fermentas).

Table 1. Morphological characteristics of morphotypes ('M' should be in small capital letter) I and II of *F. semitectum* associated with *H. polyrhizus* in Malaysia.

Morphological characteristic	*F. semitectum*	
	Morphotype I (27 isolates)	**Morphotype II (52 isolates)**
Microscopic characteristics		
Length of macroconidia (µm)	3-septate = 31.03 ± 2.57 a 5-septate = 40.17 ± 1.85 a	3-septate = 24.98 ± 1.87 b 5-septate = 35.24 ± 2.07 b
Width of macroconidia (µm)	3-septate = 4.86 ± 0.30 a 5-septate = 5.04 ± 0.35 a	3-septate = 4.74 ± 0.23 a 5-septate = 4.93 ± 0.29 a
Conidial septation	0-7 septate	0-5 septate
Common conidial septation	5-septate (34%)	3-septate (32%)
Chlamydospores	Absent	Present (56%) /absent (44%)
Sporodochia	Present	Absent
Macroscopic characteristics		
Colony texture	Abundant-floccose	Abundant-floccose and abundant-powdery
Colony colour	Peach	Beige to brown
Pigmentation	Peach to orange	Brown to dark brown
Growth rate	Fast growing (4.0 - 5.99 cm) (Groups C and D)	Slow growing (2.0 - 3.99 cm) (Groups A and B)

- Mean values of 50 random conidia ± standard deviation. In each row, numbers followed by the same letter were not significantly different at $p < 0.05$ according to 2-Sample T-Test by using MINITAB® statistical software.
- Conidial septation and common conidial septation are shown in Figure 2.
- Colony texture and colony colour were referred to the upper surface of the colony.
- Pigmentation was referred to the lower surface of the colony.
- Growth rates were classified into four distinct groups: (A) 2.0 - 2.99 cm; (B) 3.0 - 3.99 cm; (C) 4.0 - 4.99 cm; (D) 5.0-5.99 cm after 3 days of incubation at 25°C.

IGS-RFLP analysis

Aliquots of 12 µl of PCR products were digested with 10 units restriction enzymes *Alu*I, *Bsu*15I, *Bsu*RI, *Eco*88I, *Hin*6I, *Msp*I, *Pst*I and *Taq*I according to the manufacturer's instructions (Fermentas). The restriction fragments were separated on 2.5% agarose gel, run for 140 min at 80 V, 400 mA and stained with ethidium bromide. The restriction fragments were visualized under UV and 100 bp DNA ladder (GeneRulers™, Fermentas) was used to estimate the size of the restriction fragments. The restriction analysis was repeated twice.

Data analysis

Each fragment was scored on the basis of the presence (1) or absence (0) of particular fragments. A data matrix was constructed based on the presence or absence of the fragments and converted to a similarity matrix. The similarity matrix was then subjected to the unweighted pair group method with arithmetical mean (UPGMA) cluster analysis based on simple matching coefficient (SMC) (Romesburg, 1984). The data analysis was performed by using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc, version 2.1) (Rohlf, 2000) to analyze the relationship among all isolates of *F. semitectum.*

RESULTS

Morphological characterization

All 79 isolates obtained from diseased *H. polyrhizus* from 9 states (Penang, Perak, Selangor, Melaka, Negeri Sembilan, Johor, Kelantan, Sabah and Sarawak) in Malaysia were identified as *F. semitectum* based on the presence of monophialides and polyphialides, pyriform microconidia, sickle- and spindle-shaped macroconidia and mesoconidia appeared as rabbit-ears *in situ*. Some isolates of *F. semitectum* produced chlamydospores singly, in pairs or in chains. Formation of orange sporodochia was also observed (Figure 1). In general, isolates of *F. semitectum* could be recognized by peach and beige colony appearances, orange and brown pigmentations and growth rate between 2 - 6 cm for 3 days of incubation. The results indicated that all isolates of *F. semitectum* can be divided into 2 different groups assigned as morphotypes I and II, comprised 27 and 52 isolates of *F. semitectum*, respectively. The morphological characteristics of both morphotypes of *F. semitectum* associated with *H. polyrhizus* in Malaysia are summarized in Table 1.

Microscopic characteristics

For microscopic characteristics, the marked difference between isolates in morphotypes I and II was the length of macroconidia (Figure 1). Longer macroconidia (3 - septate: 31.03 ± 2.57 µm; 5 - septate: 40.17 ± 1.85 µm) were showed by isolates in morphotype I while isolates in morphotype II produced shorter macroconidia (3 - septate: 24.98 ± 1.87 µm; 5 - septate: 35.24 ± 2.07 µm)

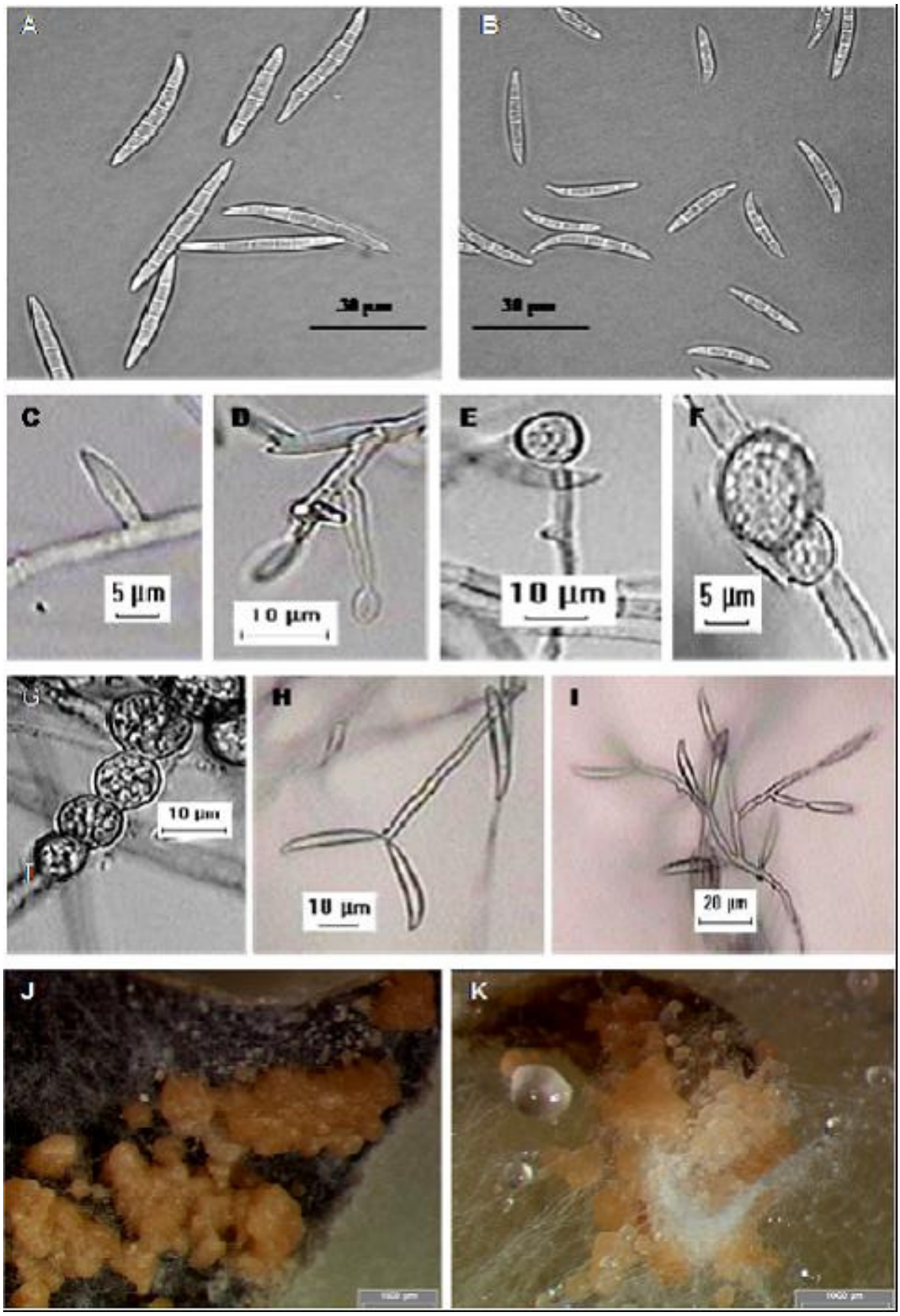

Figure 1. Microscopic characteristics showed by *F. semitectum* associated with *H. polyrhizus* in Malaysia. (A) Conidia belong to morphotype I; (B) Conidia belong to morphotype II; (C) Monophialide; (D) Polyphialides; (E) Chlamydospore singly; (F) Chlamydospores in pair; (G) Chlamydospores in chain; (H) Rabbit-ears appearance; (I) Mesoconidia *in situ*; (J) Sporodochia on carnation leaf; (K) Sporodochia on CLA.

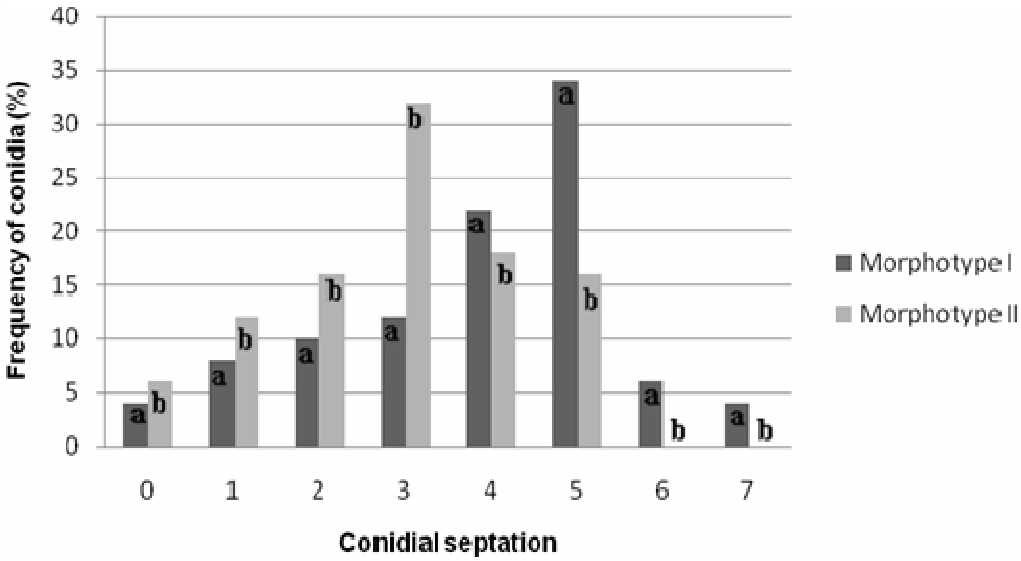

Figure 2. Frequency of conidial septation of *F. semitectum* of morphotypes I and II. For each conidial septation, frequency of conidia with the different letter are significantly different at $p < 0.05$ according to 2 - sample T-test.

(Table 1). There was a significant difference ($p < 0.05$) on the length of 3 - and 5 -septate macroconidia between isolates of morphotypes I and II. However, no significant difference was observed on the width of 3 - and 5 - septate macroconidia between the 2 morphotypes.

Additionally, isolates of morphotype I produced 0 - 7 septate conidia but isolates of morphotype II only produced 0 - 5 septate conidia (Table 1 and Figure 2). The obvious difference of conidial septation was observed in 3 - and 5 - septate conidia (Figure 2) where isolates of morphotype I produced mostly 5 - septate (34%) macroconidia, whereas isolates of morphotype II, produced mostly 3 - septate (32%) macroconidia. Each group of conidial septation (0 - 7 septate) was statistically different ($p < 0.05$) for both morphotypes I and II (Figure 2). Other dissimilarities between morphotypes I and II were isolates in morphotype I exhibited the absence of chlamydospores and presence of sporodochia whereas isolates in morphotype II were with (56%) or without chlamydospores (44%) and without sporodochia (Figure 1, Table 1).

Macroscopic characteristics

For macroscopic characteristics, isolates of *F. semitectum* in morphotype I produced abundant-floccose mycelium, peach colony appearance, peach to orange pigmentations and fast growing (Group C: 4.0 - 4.99 cm; Group D: 5.0-5.99 cm) whereas isolates of *F. semitectum* in morphotype II produced abundant-floccose and abundant-powdery mycelium, beige to brown colony appearances, brown to dark brown pigmentations and slow growing (Group A: 2.0 - 2.99 cm; Group B: 3.0 - 3.99 cm) (Table 1 and Figure 3).

Molecular characterization

An approximately 2200 bp fragment of IGS region was amplified for all isolates of *F. semitectum*. All restriction enzymes used were able to digest the IGS fragment which indicated that the enzymes had one or more restriction sites within the IGS region. Restriction fragments less than 90 bp were not taken into consideration because the fragments were not clearly resolved by 2.5% agarose gel electrophoresis. The size of IGS fragment for each isolate was estimated by summing up the sizes of constituent restriction fragments which varied depending on the restriction enzymes used to generate the fragments. Within a pattern, fragments that showed stronger intensity than fragments of higher molecular weight were considered as 2 restriction fragments of the similar size.

Table 2 shows the IGS haplotypes and restriction patterns of *F. semitectum* isolates associated with *H. polyrhizus* in Malaysia. Isolates were scored for the patterns revealed by the restriction patterns produced and each unique 8 letter code was regarded as different IGS haplotypes. A total of 49 IGS haplotypes were assigned among the 79 isolates of *F. semitectum* examined (Table 2). Depending on the restriction enzymes and the isolates, 2 - 18 distinct restriction patterns were resolved. Digestion with *Msp*I, produced the highest variability with 18 distinct patterns, A-R (Table 2; Figure 4), while *Bsu*15I yielded only 2 patterns, A-B. Both *Alu*I and *Taq*I, yielded 14 distinct patterns. For *Bsu*RI, 9 restriction patterns were recognized (Figure 5), for *Pst*I,

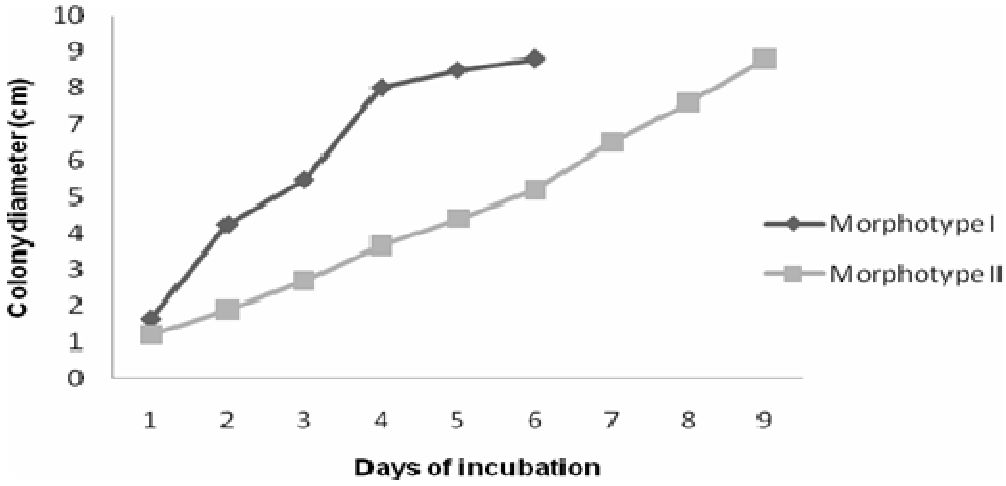

Figure 3. Growth rates of isolates of *F. semitectum* in morphotypes I and II (The observation was stopped on day 6 and 9 for morphotypes I and II, respectively as the petri dish was fully colonized).

there was 7 patterns and *Eco*88I and *Hin*6I, showed 6 and 3 restriction patterns, respectively.

The similarity among the 79 isolates of *F. semitectum* ranged from 75 - 100%. From UPGMA cluster analysis, a dendogram clearly divided *F. semitectum* isolates into 2 distinct clusters, designated as RFLP groups I and II (Figure 6). RFLP groups I and II comprised 27 and 52 isolates of *F. semitectum,* respectively which were in accordance with morphological characterization. The similarity coefficient between RFLP groups I and II was 0.87. The dendogram also reflected that isolates of *F. semitectum* showed high levels of intraspecific variation and polymorphism in IGS region.

DISCUSSION

From current descriptions of *F. semitectum,* there were 2 varieties with different morphological types, vars. *semitectum* and *majus*. In the present study, 2 morphological varieties, morphotypes I and II were distinguished among *F. semitectum* isolates associated with *H. polyrhizus* in Malaysia based on the microscopic characteristics. Majority of the isolates produced shorter macroconidia which fulfilled the criteria of *F. semitectum* var. *semitectum* while isolates that produced longer macroconidia, were suggested to be *F. semitectum* var. *majus* (Booth, 1971; Joffe, 1974; Gerlach and Nirenberg, 1982). This finding is in agreement with an earlier study by Zaccardelli et al. (2006) who reported the existence of 2 different morphological types of *F. semitectum* associated with *Medicago sativa* in northern Italy.

Similar to microscopic analysis, isolates of *F. semitectum* can also be classified into 2 distinct groups based on macroscopic characteristics. Abd-Elsalam et al. (2003) reported that isolates of *F. semitectum* associated with a seedling disease of cotton in Egypt also produced abundant-floccose and abundant-powdery aerial mycelium with whitish, buff, ochreous and peachy colour aerial mycelium. Similar study by Zaccardelli et al. (2006) also indicated that a vast difference in the growth rates of isolates of *F. semitectum* were observed which can be divided into 2 types namely, the fast growing (10 - 11 mm / day) and the slow growing (4 - 6 mm /day).

Results of molecular characterization indicated that restriction enzymes with shorter recognition sequences produced smaller fragments, as there are more recognition sites at the target fragment, which in turned produced variability of the restriction patterns. This statement was supported by the results of RFLP analysis in *F. lateritium* (Hyun and Clark, 1998), *F. equiseti* (Kosiak et al., 2004), *F. culmorum, F. graminearum, F. cerealis, F. poae, F. oxysporum* and *F. fujikuroi* (Llorens et al., 2005) and *F. verticillioides* (Patino et al., 2006). From IGS-RFLP analysis, 49 distinct haplotypes were identified among the 79 isolates of *F. semitectum* and separated into 2 distinct clusters (RFLP groups I and II) with similarity of 87%. The UPGMA cluster of IGS-RFLP analysis was in accordance with morphological characterization in which 2 distinct morphological groups, morphotypes I and II were recognized.

The results also indicated that high levels of intraspecific variability existed within *F. semitectum* isolates obtained from a single host *(H. polyrhizus)* propagated only by a vegetative mean. The high level of intraspecific variability could be due to point mutations (Mishra et al., 2002). Point mutations at recognition sites were responsible in changing the restriction patterns. IGS-RFLP analysis of several *Fusarium* species has also indicated intraspecific variability among the isolates such

Table 2. IGS haplotypes and restriction patterns of *F. semitectum* isolates associated with *H. polyrhizus* in Malaysia.

Isolate number	Origin	IGS haplotype	Restriction patterns							
			***Alu*I**	***Bsu*15I**	***Bsu*RI**	***Eco*88I**	***Hin*6I**	***Msp*I**	***Pst*I**	***Taq*I**
[a]P4001π	Penang	1	A	A	A	A	A	A	A	A
B4115π	Selangor	2	A	A	A	E	A	D	C	A
A4443π	Perak	3	D	A	C	A	A	A	A	A
P4014π	Penang	4	A	A	E	A	A	A	A	A
Q4095π	Sarawak	4	A	A	E	A	A	A	A	A
Q4101π	Sarawak	4	A	A	E	A	A	A	A	A
B4114π	Selangor	4	A	A	E	A	A	A	A	A
P4007π	Penang	5	D	A	D	A	A	D	C	E
N4036π	Negeri Sembilan	6	E	A	C	A	A	D	C	E
P4008π	Penang	7	D	A	C	A	A	D	D	E
P4009π	Penang	8	A	A	C	A	A	A	E	E
A4024π	Perak	9	E	A	C	A	A	A	E	E
A4442π	Perak	9	E	A	C	A	A	A	E	E
M4082π	Melaka	9	E	A	C	A	A	A	E	E
D4067π	Kelantan	10	E	A	C	A	A	A	B	E
A4117π	Perak	10	E	A	C	A	A	A	B	E
M4076π	Melaka	10	E	A	C	A	A	A	B	E
M4078π	Melaka	10	E	A	C	A	A	A	B	E
Q4097π	Sarawak	10	E	A	C	A	A	A	B	E
N4047π	Negeri Sembilan	11	E	A	C	E	A	A	B	E
N4041π	Negeri Sembilan	12	I	A	C	A	A	A	B	E
J4454π	Johor	13	I	A	C	A	A	A	E	E
P4012π	Penang	14	I	A	C	E	A	D	F	E
M4074π	Melaka	15	A	A	H	A	A	A	A	E
M4081π	Melaka	16	E	A	H	A	A	A	A	H
M4083π	Melaka	16	E	A	H	A	A	A	A	H
S4107π	Sabah	17	E	A	H	A	A	M	B	I
D4062π	Kelantan	18	E	B	C	A	A	K	E	G
D4063π	Kelantan	18	E	B	C	A	A	K	E	G
D4068π	Kelantan	18	E	B	C	A	A	K	E	G
B4003π	Selangor	19	B	A	B	A	A	B	B	B
S4104π	Sabah	20	B	A	B	A	A	B	F	B
S4103π	Sabah	21	B	A	B	E	A	L	F	B
P4010π	Penang	22	E	A	C	A	B	A	B	E
A4025π	Perak	23	E	A	C	A	B	D	F	E
P4015π	Penang	24	F	A	C	A	B	E	B	E
P4020π	Penang	24	F	A	C	A	B	E	B	E
Q4099π	Sarawak	24	F	A	C	A	B	E	B	E
B4110π	Selangor	24	F	A	C	A	B	E	B	E
P4018π	Penang	25	F	A	C	A	B	E	F	E
P4019π	Penang	25	F	A	C	A	B	E	F	E
J4056π	Johor	26	J	A	G	A	B	E	B	G
B4112π	Selangor	27	J	A	C	B	B	N	B	G
N4034π	Negeri Sembilan	28	H	A	C	A	B	H	E	E
M4075π	Melaka	28	H	A	C	A	B	H	E	E
S4102π	Sabah	28	H	A	C	A	B	H	E	E

Table 2. Contd.

Isolate number	Origin	IGS haplotype	Restriction patterns							
			*Alu*I	*Bsu*15I	*Bsu*RI	*Eco*88I	*Hin*6I	*Msp*I	*Pst*I	*Taq*I
J4448π	Johor	28	H	A	C	A	B	H	E	E
B4004π	Selangor	29	C	A	C	B	A	C	B	C
N4038π	Negeri Sembilan	30	C	A	C	B	A	I	B	C
N4035π	Negeri Sembilan	31	C	A	C	B	A	I	B	F
J4057π	Johor	31	C	A	C	B	A	I	B	F
D4070π	Kelantan	32	J	A	C	D	A	G	B	C
D4071π	Kelantan	32	J	A	C	D	A	G	B	C
P4006π	Penang	33	C	A	C	C	A	A	B	D
M4080π	Melaka	33	C	A	C	C	A	A	B	D
P4005π	Penang	33	C	A	C	C	A	A	B	D
S4106π	Sabah	34	B	A	C	C	A	A	B	D
S4109π	Sabah	35	K	A	C	C	A	E	B	J
P4013π	Penang	36	C	A	C	C	A	E	B	C
J4457π	Johor	37	J	A	C	C	A	E	B	C
P4016π	Penang	38	C	A	C	C	A	F	B	C
P4017π	Penang	38	C	A	C	C	A	F	B	C
Q4096π	Sarawak	38	C	A	C	C	A	F	B	C
J4451π	Johor	38	C	A	C	C	A	F	B	C
P4021π	Penang	39	C	A	C	C	A	F	F	C
A4028π	Perak	40	C	A	F	C	A	C	B	C
A4031π	Perak	41	G	A	C	C	A	G	B	C
M4048π	Melaka	42	B	A	C	A	A	J	E	G
P4011π	Penang	43	L	A	C	C	A	O	A	K
M4072π	Melaka	44	L	A	C	C	A	O	A	A
S4105π	Sabah	44	L	A	C	C	A	O	A	A
Q4100π	Sarawak	44	L	A	C	C	A	O	A	A
Q4092π	Sarawak	44	L	A	C	C	A	O	A	A
J4452π	Johor	44	L	A	C	C	A	O	A	A
N4039π	Negeri Sembilan	45	N	A	C	F	A	P	A	L
A4445π	Perak	46	L	A	I	C	B	Q	A	M
D4064π	Kelantan	47	L	A	I	C	B	R	G	M
M4077π	Melaka	48	L	A	C	C	C	O	A	N
M4049π	Melaka	49	M	A	C	F	C	D	F	C

[a]The first letter in the isolate number denotes a particular state in Malaysia and pie symbol at the last isolate number denotes the host i.e. red-fleshed dragon fruit *(Hylocereus polyrhizus).*

as 9 IGS haplotypes were identified among 22 isolates of *F. oxysporum* (Kim et al., 2001), 29 IGS haplotypes among 75 isolates of *F. culmorum* (Mishra et al., 2002), 4 IGS haplotypes among 27 isolates of *F. equiseti* (Kosiak et al., 2004) and 14 IGS haplotypes among 33 isolates of *F. verticillioides* (Patino et al., 2006). Another reasonable cause to the high variability of IGS region may be due to recombination phenomenon. The IGS region was a part of rDNA repeat unit which occurs in a tandem array on one or more chromosomes (Fekete et al., 1993; Boehm et al., 1994). Through concerted evolution by means of unequal chromatid exchange and biased gene conversion, the multiple copies of IGS were homogenized within an individual and fixation of this region occur within populations of sexually reproducing species (Dover, 1982; Hillis and Dixon, 1991). The intraspecific variation observed in the IGS region may reflect the slow rate of concerted evolution particularly at a population level, perhaps due to low levels of sexual recombination. Although, sexual reproduction may be absent or infrequent within *F. semitectum*, the tandem repeat units were still likely to be homogenized within an individual through mitotic gene conversion (Jackson and Fink, 1981; Klein and Petes, 1981; Appel and Gordon, 1995) or transloca-

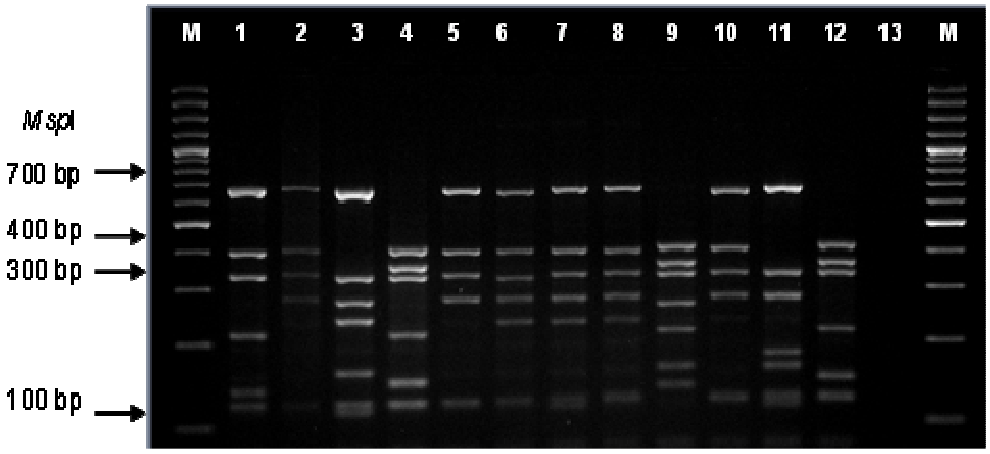

Figure 4. Restriction patterns generated from digestion with *Msp*I (M = DNA size marker of 100 bp ladder; Lane 1= P4001π; 2= B4115π; 3= A4443π; 4= Q4095π; 5= N4036π; 6= D4067π; 7= M4076π; 8= J4454π; 9= P4012π; 10 = M4074π; 11 = Q4101π; 12 = S4107π; 13 = Control).

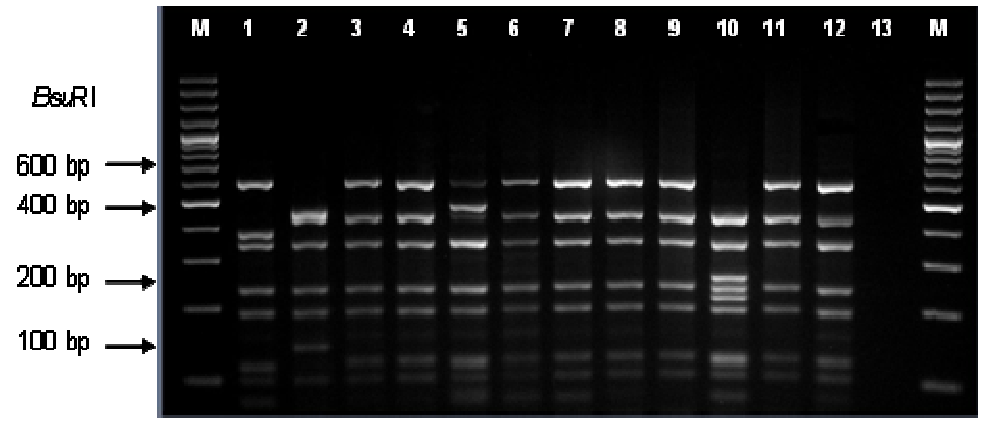

Figure 5. Restriction patterns generated from digestion with *Bsu*RI (M = DNA size marker of 100 bp ladder; Lane 1 = D4062; 2 = D4068π; 3 = S4104π; 4 = A4025π; 5 = P4018π; 6 = J4056π; 7 = B4112π; 8 = N4038π; 9 = M4080π; 10 = J4457π; 11 = Q4096π; 12 = P4011π; 13 = Control).

tions during mitotic divisions (Szostak and Wu, 1979; Appel and Gordon, 1995).

Genetic variation of *F. semitectum* isolates was also observed using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) analyses (Abd-Elsalam et al., 2003; Zaccardelli et al., 2006). A study by Abd-Elsalam et al. (2003) of *F. semitectum* from seedling disease of cotton revealed intraspecific variation among the isolates from different locations. In their study, a good correlation was also obtained between genomic groups of RAPD and AFLP and cultural characteristics. Similar results was also obtained by Zaccardelli et al. (2006) in which the clustering of *F. semitectum* from alfalfa (*Medicago sativa*) were divided into 2 distinct AFLP clusters and the clustering corresponds to the morphological characteristics.

Genetic variation of *F. semitectum* was also shown by using VCG analysis. Masratul Hawa et al. (unpublished data) reported that the 79 isolates of *F. semitectum* associated with *H. polyrhizus* in Malaysia were grouped into 69 distinct vegetative compatibility groups (VCGs). However, based on the present study, IGS-RFLP haplotypes were not VCG specific. Similar results were also obtained by Clark et al. (1995) and Hyun and Clark (1998) for *F. lateritium* from sweet potato. VCG suggests greater diversity among isolates of *F. semitectum* associated with *H. polyrhizus* than was revealed by IGS-RFLP analysis. This phenomenon may be due to the vegetative compatibility was thought to be controlled by vegetative incompatibility (*vic*) loci in a homogenic manner (Correll, 1991). Therefore, mutation at a single *vic* locus could result in otherwise closely related isolates becoming vegetatively incompatible.

In summary, morphological characterization corres-

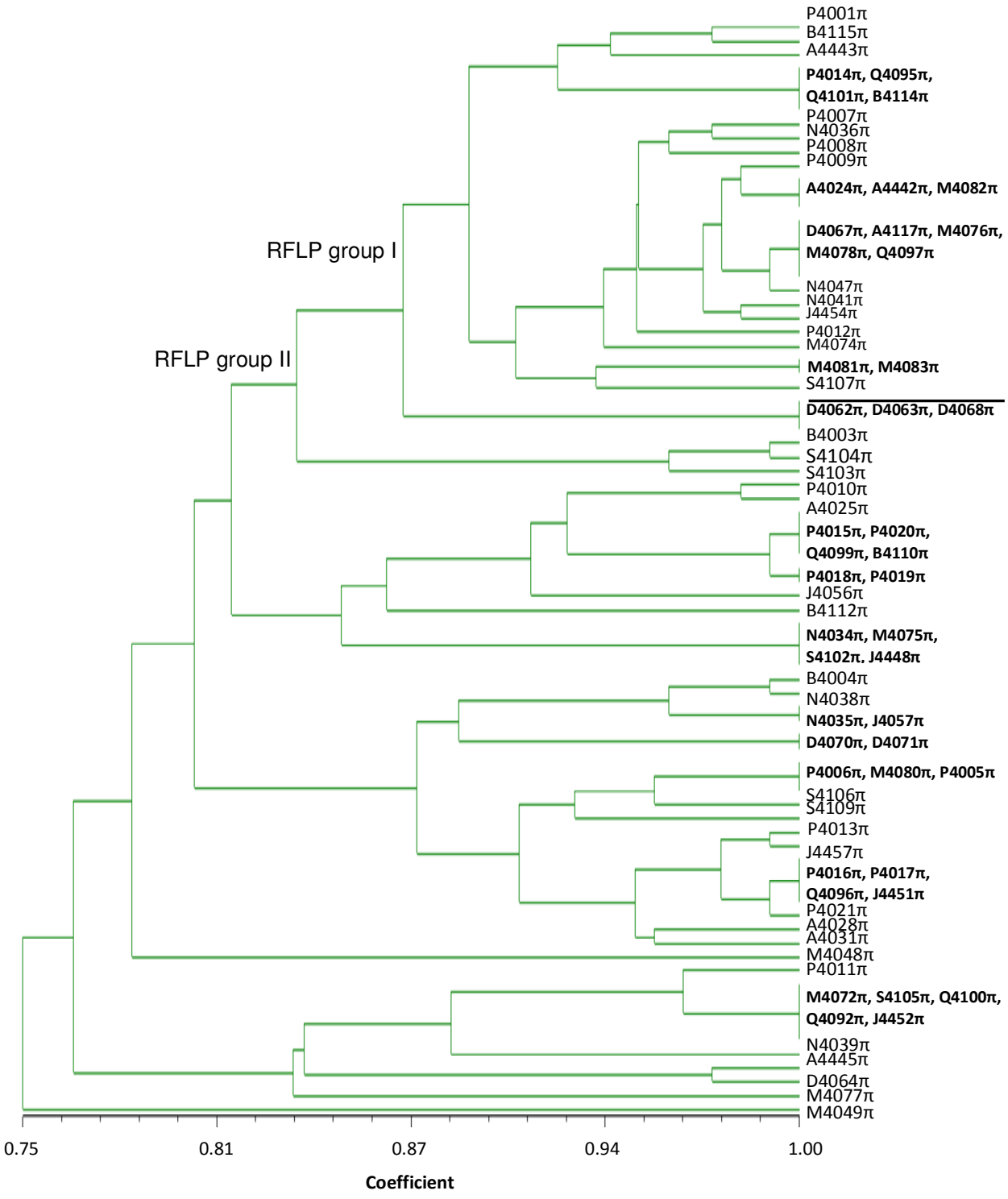

Figure 6. UPGMA dendogram obtained by IGS-RFLP analysis of *F. semitectum* isolates associated with *H. polyrhizus* in Malaysia. RFLP groups I and II represented in 2 distinct clusters. The isolate numbers in bold showed 100% similarity.

ponds with IGS-RFLP analysis. From IGS-RFLP analysis, high level of intraspecific variation and polymorphism within *F. semitectum* isolates were observed which suggested that *F. semitectum* as a species complex. However, further analysis with a larger number of isolates would be desirable in order to properly characterize *F. semitectum* and to clarify its taxonomy to support the existence of 2 distinct taxa within this species. Data from

coding regions such as alpha-elongation, beta-tubulin or histone gene regions would be useful for further in-depth study of *F. semitectum* diversity and phylogeny. This is the first report of *F. semitectum* associated with *H. polyrhizus*.

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