Songklanakarin J. Sci. Technol.
35 (6), 615-621, Nov. - Dec. 2013

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Vegetative compatibility group of *Fusarium solani* pathogenic to tobacco plant in peninsular Malaysia

Mohd Norhafeez bin Jusoh, Nabihah binti Mohd Zin, and Hideyuki Nagao*

School of Biological Sciences, Universiti Sains Malaysia,
11800 Minden, Pulau Pinang, Malaysia.

Received 11 July 2012; Accepted 19 August 2013

Abstract

Five strains of *Fusarium solani* isolated from root rot of tobacco from Kelantan and Terengganu, Malaysia were tested for the pathogenicity on tobacco seedlings by root dipping method. All 5 isolates showed discoloration on the roots. The nitrate non-utilizing (*nit*) mutants were generated from these pathogenic strains of *F. solani* and a compatible *nit1* and NitM pair was obtained in each strain. Vegetative Compatible Groups (VCGs) by *nit* mutants were determined. All 5 strains of *F. solani* were assigned to the independent VCGs. Non-pathogenic strains of *F. solani* previously isolated from root rot of tobacco in Malaysia also generated *nit* mutants and were assigned to 10 different VCGs. However, complementation of *nit* mutants between 5 pathogenic strains and 7 non-pathogenic strains of *F. solani* was not achieved. Both pathogenic and nonpathogenic strains were assigned to the independent VCGs. This suggested that the isolates of *F. solani* pathogenic to tobacco were derived from the progenies of crossing in the field. However, perithecium formation was not observed in their cultures.

Keywords: Tobacco disease, *Fusarium solani,* Malaysia, *nit* mutant, Vegetative compatibility group (VCG)

1. Introduction

Fusarium root rot disease and stem rot disease on tobacco (*Nicotiana tabacum* L.) caused by *Fusarium solani* are well known to cause primary economic disease in Malaysia (Nik Masdek, 1982; Nik Masdek and Abd Karim, 1985; Yusup, 1985; Salleh *et al.,* 1988). *Fusarium solani* was listed as a causal agent of tobacco in Australia (Simmonds, 1966), Brunei (Peregrine and Ahmad, 1982), Sabah, Malaysia (Williams and Liu, 1976), and Papua New Guinea (Shaw, 1984). Azmi (1991) determined that *Fusarium solani* caused root rot disease in tobacco plant in Malaysia. Slanting death disease comprises root rot caused by *F. solani* and Fusarium wilt caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *nicotianae* (J. Johnson) W. C. Snyder and H. N. Hans. This disease is widely distributed throughout the tobacco growing region in peninsular Malaysia mainly Melaka, followed by Kelantan, Terengganu and Negeri Sembilan states (Azmi, 1991).

Puhalla (1985) indicated that the vegetative compatibility group (VCG) are the sub specific group that has the ability that any two strains or hyphae that can anastomose and fuse with each other to form stable heterokaryons. According to Sugimoto *et al.* (2003), hyphal anastomosis and fusion is the way for asexual fungi to exchange genetic information between two isolates. Isolates that are vegetatively compatible with each other are consisted to form a vegetative compatibility group (Glass and Kuldau, 1992). Vegetative compatibility is derived by multiple vegetative incompatibility loci (*vic*, or *het* loci) in many fungi and *vic* loci in *Fusarium* are said to be in allelic form (Leslie and Summerell, 2006). Leslie (1993) indicated that *vic* loci are also responsible

* Corresponding author.
Email address: nagaoh@usm.my

for VCG phenotypes, and the exchang of genetic information via a parasexual process is possible for isolates within a VCG. In case of *F. solani*, since the species have both sexual and asexual cycle stages, the expectation for the types of subdivision induced by VCGs were quite different from those expected for asexual population such as *F. oxysporum*. High level of VCG diversity would be expected in sexually reproducing populations because of the exchange of genetic information compared to asexual populations (Leslie, 1993).

The objectives of this study were to (1) determine the pathogenicity of *F. solani* isolates from tobacco, and (2) determine the VCG of pathogenic isolates of *F. solani*.

2. Material and Methods

2.1 Fungal isolate

Twenty stock cultures of *F. solani* isolated from diseased tobacco plants by Azmi (1991) in the Fusarium laboratory, USM, were examined. For new isolates, typical Fusarium root rot symptoms on tobacco plants were sampled from 5 locations in tobacco production areas in Kelantan and Terengganu states of east coast peninsular Malaysia (Table 1). Diseased roots and lower stem parts were washed in running tap water and cut into pieces (ca. 5 × 5 × 5 mm). These pieces were surface-sterirlized by successively dipping in 70% ethanol for 1 min, immersing in 10% commercial bleach solution containing 4.25% NaOCl for 5 min, and rinsing in sterile distilled water thrice each for 1 min. The sterilized pieces were left to air dry on sterile filter paper in laminar flow bench. They were then placed on water agar medium (WA) amended with 10% lactic acid at 1 ml/liter. Plates were incubated at 25°C under daylight condition for 10 to 14 days on the laboratory bench. Colonies of *F. solani* were identified and transferred onto the potato sucrose agar (PSA) medium amended with 10% lactic acid at 1 ml/liter and incubated at 25°C for 5 to 7 days. Single spores of *F. solani* were isolated on PSA before being transferred onto carnation leaf-piece agar (CLA) medium and incubated at 25°C under daylight condition for a period of 10 to 14 days on the laboratory bench. Colonies were observed and identified according to Nelson *et al.* (1983) and preserved in a slant bottle containing potato agar (PA) medium at 20°C. As perithecium formation was not observed in their cultures, all strains

Table 1. Origin of isolates of *Fusarium solani* from tobacco field in Malaysia and the number of *nit* mutants recovered.

Isolate	Description of source of isolation	Location	No. of *nit* mutant recovered			No.of block inoculated
			nit 1	*nit* 3	Nit M	
S01 109025*	Tobacco, root rot	Tg. Pauh, Bachok, Kelantan	3	8	7	240
S02 109026*	Tobacco, stem rot	Beris Tengah, Bachok, Kelantan	7	8	1	320
S05	Tobacco, root rot	Kg. Panjang (B), Bachok, Kelantan	4	0	2	120
S17	Tobacco, root rot	Bukit Keluang, Besut, Terengganu	10	12	6	200
S21	Tobacco, root rot	Beris Tok Fakir (a), Marang, Terengganu	4	14	2	200
216	Tobacco, stem root,	Tok Uban, Kelantan	2	2	1	67
223	Tobacco, stem root,	Tok Uban, Kelantan	6	4	1	105
291	Tobacco, stem root,	Pasir Puteh, Kelantan	3	4	1	81
296	Tobacco, infected leaf	Pasir Puteh, Kelantan	17	4	2	40
301	Tobacco, wilt	Plot (4), Kelantan	13	11	1	40
305	Tobacco, leaf stalk wilt	Telong, Kelantan	6	3	2	59
309	Tobacco, wilt	Telong, Kelantan	3	3	1	99
317	Tobacco, stem root	Kg, Pak Amat, Kelantan	5	2	1	62
360	Tobacco, stem root	Rantau Abang, Terengganu	4	1	0	91
362	Tobacco, stem root	Rantau Abang, Terengganu	3	0	0	60
363	Tobacco, stem root	Rantau Abang, Terengganu	3	0	2	91
364	Tobacco, stem root	Rantau Abang, Terengganu	3	3	2	39
370	Tobacco, stem root	Rantau Abang, Terengganu	4	2	0	114
371	Tobacco, stem root	Rantau Abang, Terengganu	2	4	0	63
372	Tobacco, stem root	Rantau Abang, Terengganu	6	1	1	62
411	Tobacco	Telong, Kelantan	5	3	4	29
494	Tobacco, root rot	Lakluk, Terengganu	4	4	0	63
499	Tobacco, root rot	Merchang, Terengganu	11	11	4	91
600	Tobacco, stem root,	Sintok, Kedah	7	0	3	47
921	Tobacco, slanting death	Kelulut, Marang, Terengganu	5	1	1	86
		Total	140	105	45	2469

*Strain deposited in Biological Resource Center (NBRC), NITE.

seemed to be heterothallic.

2.2 Pathogenicity test

Tobacco seeds were disinfected in 10% commercial bleach solution containing 4.25% NaOCl for 5 min and rinsed under running tap water for 24 hour. The seeds were sown in sterilized soil (black soil: field soil, 3:4) and left to germinate until first leaf appeared in approximately 14 to 20 days in plant house condition. Seedlings were watered every day in early morning and late evening. Organic fertilizer (HB-101, YK Flora (M) Sdn.Bhd.) was sprayed and pesticide (Sumithion®) applied twice a week. The seedlings were uprooted gently from the nursery soil and dipped into spore suspension (1×10^6 cfu/ml) for a period of 10 minutes. Ten uninoculated seedlings were dipped into sterile distilled water as control. Observation and disease diagnosis were done every day and after 4 weeks period, all seedlings were uprooted and the data on disease discoloration of seedlings root were recorded. Disease index was rated as 0 = no root discoloration, 1 = 1-25% root discoloration, 2 = 26-50 % root discoloration, 3 = 51-75 % root discoloration and 4 = 76-100% root discoloration.

2.3 *Nit* mutant generation

In VCG experiment, 20 stock cultures of *F. solani* in Fusarium laboratory, USM, and newly isolated 5 strains of *F. solani* were examined. Nitrate non-utilizing mutant (*nit*) was generated to determine the VCG for the isolates (Puhalla, 1985; Correll *et al.*, 1987). Each mycelial plug of *F. solani* was inoculated on minimal medium (MM) amended with 1.5% $KClO_3$ (MMC) to generate the *nit* mutant sectors to be selected for phenotypic identification and heterokaryon compatibility pairing. Around 10-20 sectors were generated on MM+$KClO_3$ with 5 mycelial plugs for each plate. Isolates which did not efficiently generate *nit* mutants with 1.5% $KClO_3$ were further examined in increasing concentrations from 2.5 to 6.0% $KClO_3$ in order to generate the mutant sectors. Water agar chlorate medium (WAC) (Korolev and Katan, 1997) and Corn meal agar chlorate medium (CMC) (Correll *et al.,* 1988) were additionally used for the recovery of *nit* mutants. Thin and extensive growth colonies without aerial mycelium were selected as *nit* mutants (Larkin *et al.*, 1990; Puhalla, 1985). Each of the *nit* mutants was transferred onto four nitrogen-source phenotype media; MM, MM+ Hypoxanthine (HX), MM+ Nitrate (NO_2), MM+ Ammonium (NH_4), to determine the phenotypes (Correll *et al.,* 1987). Single spore isolation was done to all of the *nit* mutants gained.

2.4 VCG analysis

Both *nit1* and NitM mutants from each *F. solani* strain were paired with intra-strain and the representative *nit1* and NitM strains were selected for each strain. Inter-strain compatibility was tested with all possible combinations even if only a single *nit1* or NitM representing that strain was obtained. Tests were carried out on MM under room temperature (27°C) for 14 days. Pairing was made by placing small mycelial blocks (ca. 3 mm × 3 mm × 2 mm) from each of *nit* mutants 2 to 3 cm apart on MM. For the strain that did not generate NitM, only *nit1* representative of that strain was examined for complementation in inter-strain pairing with the representative NitM mutants of other strains. Each pairing was repeated twice. Heterokaryon complementation was scored according to the degree of mycelial growth along the confronting colonies. Four categories of mycelial reactions were defined: ++, strong wild type growth or line was formed more than 1 cm width; ±, patches or dots of mycelial tufts was formed; +, weak wild type growth, line was less than 1 cm and slow growth; –, no reaction.

3. Results

3.1 Pathogenicity test

A total 5 strains of *F. solani* was recovered from field sampling. All strains were able to infect and cause discoloration on the roots. Strain S17 was able to cause 60% rate of discoloration while the other 4 strains; S01, S02, S05, and S21, were able to cause 70% of discoloration in the pathogenicity test. The highest disease index value was scored by strain S01 at 3.4. Those of strain S02, S05, S21, and S17 were 3.3, 3.3, 2.9, and 2.7, respectively. In one month cultivation period for the test, around 70% of plants in each isolate's pots exhibited wilting and another 30 % indicated stunting. These symptoms were reproduced as seen in the tobacco fields.

3.2 *Nit* mutant generation

Amended concentration of chlorate in MMC varied for both stock and field strains (Table 1). Initially *nit* mutants were generated on MMC with 1.5%, 2.0%, 2.5%, 3.0% and 3.5% chlorate. On 1.5% and 2.0% chlorate concentration, the chlorate did not greatly restrict the growth rate of certain isolates. The colony developed abundant aerial mycelium with little and no sector formation. The growth of the mycelium was quite fast (2 to 3 days) like wild type growth on MMC 1.5% and 2.0%. Sectors were abundantly formed on 2.5% and 3.0% of chlorate concentration. Therefore, these concentrations of chlorate were chosen to generate *nit* mutants. The concentration of chlorate was increased to 4.0%, 5.0% and 6.0% for generating the *nit* mutants on three strains, 216, 360 and 499. For certain strains that did not generate NitM, induction of NitM mutant was tried on MMC with 6.0% chlorate concentration. The duration for the formation of chlorate resistant sectors depended on the concentration of chlorate in the MMC. The higher the percentage of chlorate, the more days it took to form chlorate-resistant sectors. Usually the formation of sectors could be observed after one

week of incubation. Finally, spontaneous chlorate-resistant sectors were recovered from all of the strains but the total of *nit* mutants recovered was quite low even though the amount of inoculation per one isolate was up to 114 inoculations (Table 1).

Sometimes, the colony inoculated on MMC also produced red to brown pigmentation on the agar and the colony morphology of strains also changed. WAC containing chlorate 2.0% was tried to generate the *nit* for all of the 20 stock strains. However, the percentage of sectors formation was quite low with only 14.0% on WAC. Chlorate-resistant sectors appeared as thin, fast-growing fan-like sectors after 5 days of inoculation. Although CMC with 6.0% chlorate was used to generate *nit* mutants of all of the isolates, the majority of the colonies developed abundant aerial mycelium with no clear sector formation. Only 1.3% of colonies developed fast-growing sector formation. A total of 202 *nit* mutants of *F. solani* was generated on all of the chlorate media used. From these three chlorate media, MMC with 2.5% and 3.0% yielded the highest sector formation compared to WAC and CMC (Table 2). Phenotyping of the entire *nit* mutants was done on the four different nitrogen-source media. Most *nit* mutants generated were *nit1* followed by *nit3* and NitM in the stock strains. Five stock strains, 360, 362, 370, 371, and 494, were unable to generate NitM mutants (Table 1). Strain 362 did not generate either *nit3* or NitM. Strain 363 and 600 on the other hand did not generate *nit3*. The frequencies of *nit1, nit3* and NitM phenotype from the stock cultures were 30.1%, 16.9% and 7.3% respectively (Table 3). Strain 223 was unable to form heterokaryon and was considered to be heterokaryon self-incompatible (HSI).

For the field isolates, concentration of chlorate used was ranging from 1.5% to 3.0 % (Table 2). Field isolates were able to generate *ni1*, nit3 and NitM mutants (Table 3). Most *nit* mutants generated were *nit1* followed by *nit3* and NitM in the stock strains.

3.3 VCG analysis

Nineteen stock cultures were assigned to 10 VCGs. Six of these VCGs, VCG 1, VCG 5, VCG 7, VCG 8, VCG 9, and VCG 10, were composed of a single strain while the other four, VCG 2, VCG 3, VCG 4, and VCG 6, were of 2 to 5 isolates (Table 4). Strain 301 reacted as a bridging strain between VCG 3 and VCG 4 testers.

Five field strains were assigned to 5 VCGs (Table 5). All VCGs were composed of a single strain. *Nit* mutants of stock strains were examined in inter-strain complementation with 5 field strains. However, 3 strains and their *nit* mutants could not revive. None of the *nit1* from strains 301, 309, and 317, and none of the NitM from strains 363, 600, and 921 revived. Therefore, the recovered *nit1* and/or NitM mutants from 7 stock strains and both nit mutants of 5 field strains were paired for intra-strain complementation test on MM. No heterokaryon formation occurred among them (Table 5).

4. Discussion

Hawthorne and Rees-George (1996) reported that strains of *F. solani* were insufficiently inhibited on 1.5% chlorate concentration. In this study, chlorate concentration ranging from 1.5% and up to 6.0% on MMC was used to generate *nit* mutants since chlorate did not greatly restrict the growth of many strains of *F. solani* especially at 1.5% and 2.0% chlorate concentration. Different isolates showed different suitable concentration of chlorate to restrict the growth of the colony to form sectors. Even though sector formation appeared frequently on the colony, *crn* mutants were more easily recovered than *nit* mutants. On MMC with 6.0% chlorate concentration, more NitM were produced.

Table 2. Percentage of sector formation on different ClO_3^- concentration media.

Type of medium	Concentration of chlorate (%)	Percentage of sectors form (%)	
		Stock strain	Field isolate
Minimal Medium Chlorate (MMC)			
	1.5	9.3	10.8
	2.0	13.4	NT*
	2.5	23.2	20.7
	3.0	22.0	18.8
	3.5	17.4	NT
	4.0	7.1	NT
	5.0	3.8	NT
	6.0	3.8	NT
Water Agar Chlorate (WAC)	2.0	14.0	NT
Corn Meal Agar Chlorate (CMC)	6.0	1.3	NT

*Not tested.

Table 3. Frequency of phenotypic classes of *nit* mutants from 20 stock strains and 5 field isolates of *Fusarium solani* on chlorate containing media.

Phenotypic classes	Number of *nit* mutant		Percentage of *nit* mutant (%)	
	Stock strain	Field isolate	Stock strain	Field isolate
nit1	112	28	30.1	2.5
nit3	63	42	16.9	3.8
Nit M	27	18	7.3	1.6
Wild type	170	1029	45.7	92.1
Total	372	1117	100	100

Table 4. Complementation test among the stock strains of *F. solani*.

NitM		216	291	296	411	499	301	305	317	309	372	363	364	600	921
nit1	VCG	1	2	2	3	3	3	4	4	5	6	7	8	9	10
216	1	++	-	-	-	-	-	-	-	-	-	-	-	-	-
494	2	-	++	±	-	-	-	-	-	-	-	-	-	-	-
291	2	-	++	++	-	-	-	-	-	-	-	-	-	-	-
296	2	-	++	++	-	-	-	-	-	-	-	-	-	-	-
411	3	-	-	-	++	++	±	-	-	-	-	-	-	-	-
499	3	-	-	-	++	++	±	-	-	-	-	-	-	-	-
301	3	-	-	-	++	++	++	-	±	-	-	-	-	-	-
305	4	-	-	-	-	-	-	++	±	-	-	-	-	-	-
317	4	-	-	-	-	-	-	++	++	-	-	-	-	-	-
309	5	-	-	-	-	-	-	-	-	++	-	-	-	-	-
360	6	-	-	-	-	-	-	-	-	-	++	-	-	-	-
362	6	-	-	-	-	-	-	-	-	-	±	-	-	-	-
370	6	-	-	-	-	-	-	-	-	-	++	-	-	-	-
371	6	-	-	-	-	-	-	-	-	-	++	-	-	-	-
372	6	-	-	-	-	-	-	-	-	-	++	-	-	-	-
363	7	-	-	-	-	-	-	-	-	-	-	++	-	-	-
364	8	-	-	-	-	-	-	-	-	-	-	-	++	-	-
600	9	-	-	-	-	-	-	-	-	-	-	-	-	++	-
921	10	-	-	-	-	-	-	-	-	-	-	-	-	-	++

Notes: ++; Strong wild type growth, line more than 1 cm. ±; patches or dots of mycelial tufts. -; no reaction.

Sugimoto *et al.* (2003) reported that more *nit* mutants of *V. lecanii* were recovered on WAC containing chlorate 2.0% than on MMC. However, sector formation on WAC was detected as thin, fast growing fan-like sectors after one week of inoculation. As the growth of the colony on the WAC is very thin, the sector formation was hardly to be recognized. Moreover, the frequency of sector formation was quite low, only 14.0%, on WAC in our experiments. Therefore, WAC was not a suitable medium to generate *nit* mutants for *F. solani.* Correll *et al.* (1988) recovered *nit* mutants of *Verticillium albo-atrum* on CMC. Korolev and Katan (1997) also reported that CMC was another chlorate media that could be used to induce *nit* mutants on *Verticillium dahliae.* The majority of the colonies of *F. solani* developed abundant aerial mycelium with no clear sectors formation on CMC with 6.0% chlorate. Only 1.3% developed fast-growing sector formation. Recovery of *nit* mutants of *F. solani* on CMC was definitely not a good choice since this medium did not restrict the growth of the isolates.

A total of 202 *nit* mutants of *F. solani* were generated on all of the chlorate media used. From these three chlorate media, MMC with 2.5% and 3.0% yielded the highest sector formation compared to WAC and CMC (Table 2). Based on the low recovery of *nit* mutants of *F. solani* in this study and the study by Hawthorne and Rees-George (1996) on *F. solani*, *Fusarium solani* was clearly more tolerant of chlorate than most of the other species such as *F. oxysporum* and *F. moniliforme* of which the colonies were severely restricted on 1.5% chlorate (Correll *et al.,* 1987; Klittich and Leslie, 1988).

Nit1 mutants were the most frequently recovered compared to NitM and *nit3* in the stock strains. *Nit 3* mutants were the most frequent one in the field strains. Hawthorne and Rees-George (1996) also reported that *nit1* and *nit3* mutants were readily recovered and *nit1* mutants were of higher frequency than *nit3* from all 89 strains in *F. solani* on MMC containing 3.0% $KClO_3$. Klittich and Leslie (1988) also recorded that in *F. moniliforme*, *nit1* mutants were most frequently recovered. Klittich and Leslie (1989) suggested that some loci may contain sequences which are mutational "hot spots", lead them to be more susceptible to mutation. Certain isolates in this study did not produce *nit3* or NitM even were induced in 6.0% minimal medium chlorate concentration.

Sometimes the generated *nit* mutants reverted to the wild type. *Nit3* and NitM showed a high frequency of reversion compared to *nit1*. Puhalla (1985) describe this phenomenon in his study saying that ca. 2 to 4% of the *nit* mutants developed small patches of heavy growth after prolonged incubation on MM. Therefore, to avoid this occurence, the mutants were kept in bottles at -5°C. Klittich and Leslie (1988) found that *nit* mutant types differed in their suitability for VCG standards. Complementation between *nit1* and NitM mutants was chosen in this complementation test. Correll *et al.* (1987) reported that in most strains of *F. oxysporum*, *nit1* and NitM mutants derived from the same parental strain were able to complement to each other. This phenomenon may be the same and can be applied to *F. solani*. The strength of a heterokaryon not only depends on the compatibility but also depends on biochemical complementation between the mutation involved (Katan *et al.,* 1991). Therefore, we had to generate as many *nit* mutants as possible so that the most suitable compatible mutants that produced maximal heterokaryotic growth were chosen.

In this experiment, the result form VCG analysis showed that 5 pathogenic and 7 non-pathogenic strains were assigned to different VCGs (Table 5). The differences in VCGs indicated that there were differences in genetic basis for both pathogenic and non-pathogenic populations of *F.solani* in peninsular Malaysia. Previous study of *F. oxysporum* strains indicated that the pathogenic strains were normally grouped in less or fewer VCGs compared to non-pathogenic strains (Correll *et al.,* 1987; Lori *et al.*, 2004). Sexual reproduction of *Fusarium* spp. was suggested as contributing to the degree of genotypic diversity. The indigenous populations of *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasass f. sp. *pini* (= *Gibberella circinata* Nirenberg & O'Donnell (anamorph *Fusarium circinatum* Nirenberg & O'Donnell) were very diverse and resulted in a large number of VCGs. This great number of VCGs can be associated with the strains that reproduce sexually in nature. Segregation of genes during meiosis would generate genetically diverse genotypes in nature (Britz *et al.,* 2005; Viljoen *et al.,* 1997). While our results suggest that the isolates of *F. solani* pathogenic to tobacco were derived from the progenies of crossing in the field, perithecium formation should be examined among these isolates.

In this experiment, we can conclude that there was no correlation between the pathogenicity and the particular VCG

Acknowledgements

We thank Ministry of Higher Education, Malaysia, for financial support by the Fundamental Research Grant Scheme 203/PBIOLOGI/6711134.

References

Azmi, A.R. 1991. Kajian mati senget yang disebabkan oleh *F. oxysporum* dan *F. solani* pada tembakau. M. Sc. Thesis, School of Biological Sciences, Universiti Sains Malaysia, Malaysia. pp.1-125.

Table 5. Complementation test among the stock strains and field isolates of *F. solani*.

Nit M			S01	S02	S05	S17	S21	301 109031*	317 109032*	309 109033*	372 109027*
nit1		VCG	A	B	C	D	E	3	4	5	6
S01		A	++	-	-	-	-	-	-	-	-
S02		B	-	++	-	-	-	-	-	-	-
S05		C	-	-	++	-	-	-	-	-	-
S17		D	-	-	-	++	-	-	-	-	-
S21		E	-	-	-	-	++	-	-	-	-
372	109034*	6	-	-	-	-	-	-	-	-	++
363	109028*	7	-	-	-	-	-	-	-	-	-
600	109029*	9	-	-	-	-	-	-	-	-	-
921	109030*	10	-	-	-	-	-	-	-	-	-

Notes: ++; Strong wild type growth, line more than 1 cm. ±; patches or dots of mycelial tufts. -; no reaction.
*Strain deposited in Biological Resource Center (NBRC), NITE.

Britz, H., Coutinho, T.A., Wingfield, B.D., Marasas, W.F.O. and Wingfield, M.J. 2005. Diversity and differentiation in two populations of *Gibberella circinata* in South Africa. Plant Pathology. 54, 46-52.

Correll, J.C., Gordon, T.R. and McCain, A.H. 1988. Vegetative compatibility and pathogenecity of *Verticulium alboatrum*. Phytopathology. 78, 1017-1021.

Correll, J.C., Klittich, C.J.R. and Leslie, J.F. 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility test. Phytopathology. 77, 1640-1646.

Glass, N.L. and Kuldau, G.A. 1992. Mating type and vegetative incompatibility in filamentous ascomycetes. Annual Review Phytopathology. 30, 210-224.

Hawthorne, B.T. and Rees-George, J. 1996. Use of nitrate non-utilizing mutants to study vegetative incompatibility in *Fusarium solani* (*Nectria haematococca*) especially members of mating populations I, V and VI. Mycological Research. 100, 1075-1081.

Katan, T., Zamir, D., Sarfatti, M. and Katan, J. 1991. Vegetative compatibility grouping in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from the UK, the Netherlands, Belgium and France. Plant Pathology. 48, 541-549.

Klittich, C. J. R., and Leslie, J. F. 1988. Nitrate reduction mutants of *Fusarium moniliforme (Gibberella fujikuroi)*. Genetics. 118, 417-423.

Klittich, C. J. R., and Leslie, J. F. 1989. Chlorate-resistant, nitrate-utilizing mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Journal of General Microbiology. 135, 721-727.

Korolev, N. and Katan, T. 1997. Improved medium for selecting nitrate non-utilizing (*nit*) mutants of *Verticillium dahlia*. Phytopathology. 87, 1067-1070.

Larkin, R.P., Hopkins, D.L. and Martin, F.N. 1990. Vegetative compatibility within *Fusarium oxysporum* f. sp. *niveum* and its relationship to virulence, aggressiveness and race. Canadian Journal of Microbiology. 36, 352-358.

Leslie, J.F. 1993. Fungal vegetative compatibility. Annual Review Phytopathology. 31, 127-150.

Leslie, J.F. and Summerell, B.A. 2006. The Fusarium laboratory manual, Blackwell Publishing, Oxford, UK., pp. 31-56.

Lori, G., Edel-Hermann, V., Gautheron, N. and Alabouvette, C. 2004. Genetic diversity of pathogenic and nonpathogenic populations of *Fusarium oxysporum* isolated from carnation fields in Argentina. Phytopathology. 94, 661-668.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park, Pennsylvania, U.S.A., p. 193.

Nik Masdek, H. 1982. Tinjauan penyakit-penyakit tembakau di Bachok, Kelantan. Teknologi Pertanian. 3, 36-45.

Nik Masdek, H. and Abd Karim, S. 1985. Incidence of some tobacco disease in Kelantan. Teknologi Tembakau. 1, 41-45.

Peregrine, W.T.H. and Ahmad, K.B. 1982. A first annotated list of plant diseases and associated organisms. Phytopathological Paper. 27, 1-87.

Puhalla, J.E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Canadian Journal of Botany. 63, 179-183.

Salleh, B. Azmi, A.R. and Din, I. 1988. Taburan dan insiden penyakit tembakau tularan tanah di Utara Semenanjung Malaysia, Paper read at Simposium Biologi Kebangsaan ke III, Selangor, Malaysia, November 22-24, 1988.

Shaw, D.E. 1984. Microorganisms in Papua New Guinea. Department of Primary Industries Research Bulltin. 33, 1-344.

Simmonds, J.H. 1966. Host index of plant diseases in Queensland. Queensland Department of Primary Industries, Brisbane, 111.

Sugimoto, M., Koike, M., Nagao, H., Okumura, K., Tani, M. and Kuramochi, K. 2003. Genetic diversity of the entomopathogen *Verticillium lecanii* on the basis of vegetative compatibility. Phytoparasitica. 31, 450-457.

Viljoen, A., Wingfield, M.J., Gordon, T.R. and Marasas, W.F.O. 1997. Genotypic diversity in a South Africa population of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. Plant Pathology. 46, 590-593.

Williams, T.H., and Liu, P.S.W. 1976. A host list of plant diseases in Sabah, Malaysia. Phytopathological Paper. 19, 1-67.

Yusup, S. 1985. Penyakit utama tembakau serta pengawalannya di Malaysia Barat. Teknologi Tembakau. 1, 40-44.