View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by UKM Journal Article Repository

Malays. Appl. Biol. (2014) 43(1): 13–20

MORPHOLOGICAL AND GENETIC VARIABILITIES OF *Fusarium* species ISOLATED FROM KENAF

NUR AIN IZZATI, M.Z.*, MOHD AIZAT, Z., SITI NORDAHLIAWATE, M.S. and MAT RASID, I.

Biology Department, Faculty of Science, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.
**E-mail: ainizzati@upm.edu.my*
Telephone: +603-89466642
Fax: +603-86567454

ABSTRACT

The objectives of this study were to identify the *Fusarium* species associated with kenaf based on morphological, pathological and genetic characteristics. Twenty isolates of *Fusarium* were obtained from root, leaf and stem of kenaf in Kuala Terengganu, Malaysia. All *Fusarium* isolates were identified, and classified into four species such as *F. oxysporum*, *F. proliferatum*, *F. semitectum* and *F. solani*. Based on pathogenicity test, *F. oxysporum* was only pathogenic and caused Fusarium wilt disease in kenaf based on disease severity index (DSI), plant height and dry weight of plant. For vegetative compatibility groups (VCGs) study, 217 nitrate non-utilizing (*nit*) mutants were generated with *nit1* was the highest mutants recovered. All the species are genetically diverse reflected from the VCG multiple groups. *F. oxysporum* isolates were grouped into 5 VCGs, *F. proliferatum* in 3 VCGs and *F. semitectum* in 4 VCGs. *Fusarium* species isolated from kenaf were diverse based on morphology and vegetative compatibility, however only *F. oxysporum* caused Fusarium wilt disease.

Key words: disease severity index, *F. oxysporum, F. proliferatum, F. semitectum, F. solani,* vegetative compatibility groups

INTRODUCTION

Kenaf is a member of the Malvaceae family and is closely related to okra and cotton. In Malaysia, the Forestry Research Institute of Malaysia (FRIM), Department of Agriculture (DOA) and Malaysian Agricultural Research and Development Institute (MARDI) previously studied about kenaf. National Kenaf Research and Development Program has been created to make the kenaf as a potential new industrial crop in Malaysia. In the 9th Malaysia Plan (2006–2010), the government has spent about RM12 million for the research and further development of the kenaf-based industries to introduce kenaf as commercially viable crop (New Strait Times, 2008).

The primary causal agents of kenaf diseases are *Selenosporella* sp., causing a root rot and wilts, *Rhizoctonia solani* (stem rot), *Phytium deliense* (root rot), *Phomopsis* sp. (stem spot) and *Fusarium* species (Vawdrey and Peterson, 1990; Gembong *et al.*, 2000). Soilborne fungi, which affect emergence and soil establishment, will attack kenaf in the first few days after planting (William *et al.*, 2000). Anthracnose disease, caused by *Colletotrichum hibisci* is the most widespread and destructive disease attacking kenaf. Stem and seedling rots are also serious disease of kenaf caused by *Macrophomia phaseoli,* especially in Asia. According to Gembong *et al.* (2000), the wilt disease on kenaf is caused by *F. oxysporum,* but there has been no report on the disease in Malaysia.

Fusarium species, which is frequently found as saprophytes on the diseased plants, are *F. proliferatum* and *F. semitectum* on the leaves, *F. poae* and *F. semitectum* on the seeds or cereals, and *F. acuminatum, F. proliferatum, F. oxysporum* and *F. solani* on the stem and root (Summerell *et al.*, 2003). In plant diagnostic laboratories, identification of *Fusarium* isolates that are present in infected plant samples is usually conducted up to species level and sometimes remained an important task to be considered and focused. Morphology and type of conidia are commonly viewed as the most important data when identifying isolates of *Fusarium* into some species. To study the genetic diversity of filamentous fungi including *Fusarium* species, vegetative compatibility (VC) is

* To whom correspondence should be addressed.

widely used. When two hyphae anastomose and fuse to form a stable heterokaryon, the fungi are vegetatively compatible. VC is useful to study the characteristics and genetic diversity of many filamentous fungi including *Fusarium* species. VC is also known as heterokaryon compatibility (HC) (Leslie and Zeller, 1996).

Vegetative compatibility group (VCG) belongs to isolates are vegetatively compatible (Leslie and Summerell, 2006). The isolates considered to be vegetatively incompatible and belong to different VCGs if the hyphae of the two isolates do not fuse (Summerell *et al.*, 2001; Leslie and Summerell, 2006). To force formation of heterokaryons, VCG analyses in *Fusarium* carried out by using nitrate non-utilizing (*nit*) mutants (Sunder, 1998). The differential growth of *nit* mutants on phenotyping media with different nitrogenous compounds as a sole nitrogen source, enabled classification of each *nit* mutants as *nit1*, *nit3* and NitM. The four phenotyping media that differ in their nitrogen sources are minimal medium (MM) with nitrate, MM with nitrite, MM with hypoxanthine and MM with ammonium (Leslie and Summerell, 2006). To force heterokaryons, all *nit* mutants can be used but the mutants in the chlorate resistant, nitrate utilizing (*crn*) class, however, must be discarded (Leslie and Summerell, 2006). Pairings between *nit* mutants derived from different strains are the final practice in VCG analysis and one of the ways to assess genetic variability in *Fusarium* population.

Due to the unavailability of information on wilt disease of kenaf in Malaysia, and the limited report in Asia, therefore this study was conducted. The objective of this study were to isolate and identify the *Fusarium* species in kenaf based on the morphological characteristics, to conduct pathogenicity test of *Fusarium* species isolated from kenaf and to examine the variability of *Fusarium* species based on VCGs.

MATERIALS AND METHODS

Fungal Isolation and Identification

Twenty *Fusarium* isolates were obtained from various parts of the kenaf plant including root, leaf and stem from Kuala Terengganu, Malaysia. All isolates were isolated on peptone pentachloronitro-benzene agar (PPA), single-spored and identified. The shape and characteristic of microconidia and present of chlamydospore recorded. For the macroconidia, the characteristics observed were the shape, size, number of septa, apical and basal cell shapes. The *in situ* observation done on carnation leaves agar (CLA) cultures and the type of phialide and the microconidial arrangement were recorded. For the secondary criteria on potato dextrose agar (PDA), the isolate was incubated for about 7 days in room temperature, the colony morphology such as the colony features, and pigmentation recorded.

Pathogenicity Test

Pathogenicity test was conducted in the greenhouse at Ladang 2, Universiti Putra Malaysia (UPM) using two isolates for each of four *Fusarium* species namely *F. oxysporum*, *F. proliferatum, F. semitectum* and *F. solani.* The conidial suspension was prepared by adding 10 ml sterile distilled water onto the 7-day-old PDA culture. The conidial suspension concentration adjusted to 1 x 10^6 conidia/ml. Four seeds of kenaf immersed in 10 ml of conidial suspension of each treatment for 12 hours. The immersed seeds then grown in a polybag that contained soil in a 3:2:1 ratio of clay, silt and sand in four replicates. The plants watered with an automatic watering system for every 2 hours. The growth of kenaf plants were observed and recorded every week. The symptoms were scored based on a disease scale from 0 to 5 (0: No symptoms, 1: slight chlorosis, 2: chlorosis plus curvature of leaf or stem, 3: chlorosis, curvature and wilt, 4: chlorosis, curvature and wilt severe stunting and 5: dead plant). On the 12th weeks after planting, the height and wet weight of the plants were recorded. The small pieces of tissues of all inoculated and non-inoculated (control) plants re-isolated onto PPA and the isolated fungi were re-identified for accomplishment of the Koch's postulate. The kenaf plants were then; dried in the oven for 3 days and the dry weight was recorded. Disease Severity Index (DSI) was calculated and all the data analyzed by using SPSS programme version 17.0.

Generation of *nit* mutants

For the generation of the actively growing colony with dense fungal growth, pure cultures of isolates placed on a complete medium (CM) (Leslie, 1993; Lui and Sundheim, 1996). A mycelial disc (2 mm^2) subcultured onto MM containing 2.5% of $KClO_3$. All the plates then incubated for 7 days until colonies formed chlorate resistant sectors (CRSs) that appeared like sectors or fan. On the slant agar of MM containing $NaNO_3$ as the nitrogen source, the individual sectors from each colony were transferred. *Nit* mutants were identified when the sectors produced thin growth on MM after 4 days of incubation and all the *nit* mutants were stored at 4ºC for phenotyping.

Phenotyping and complementary *nit* mutants

Four phenotyping media, which were sodium nitrate ($NaNO_3$), sodium nitrite ($NaNO_2$), hypoxanthine (HX^-), and ammonium tartrate (NH_4) were prepared and a tiny piece of *nit* mutant from MM was transferred onto phenotyping media. All

the plates incubated in the dark and colony morphology scored after four days of incubation. To identify the phenotypes of *nit* mutants, differentiation was based on colony growth on the phenotyping media. The dense colony growth or wild type growth was scored as positive (+) and the transparent or thin growth was scored as negative (-). Pairing was completed on MM with $NaNO_3$ as the nitrogen source following a procedure described in the Fusarium Laboratory Manual (Leslie and Summerell, 2006). All the isolates were identified as heterokaryon self-compatible (HSC) and paired between *nit* mutants of different isolates to group them into VCG.

RESULTS AND DISCUSSION

Morphological characteristics of *Fusarium* species isolated from kenaf

From the present study, 20 isolates of *Fusarium* species obtained from the roots, stems and leaves of kenaf. *F. oxysporum* was the most abundant of *Fusarium* species isolated from the kenaf samples with 9 isolates, followed by *F. proliferatum* and *F. semitectum* with 4 isolates each and *F. solani* with 3 isolates. *F. oxysporum* was identified from the root of kenaf with 2 isolates. *F. proliferatum*, *F. semitectum* and *F. solani* have a single isolate. For the leaves part, four isolates of *F. oxysporum* was isolated, *F. semitectum* (3 isolates), *F. proliferatum* (2 isolates) and a single isolate of *F. solani.*

The identification from the stem part of kenaf also showed that *F. oxysporum* was isolated with three isolates and a single isolate of *F. proliferatum* and *F. solani*. According to Leslie and Summerell (2006), *F. oxysporum* is regularly recovered from root and stem bases of diseases plant as saprophytes. *F. proliferatum* is also easily recovered from the roots, stem bases, leaves and aerial parts of the plants. At the leaves, aerial parts, flowers, seeds and grains. *F. semitectum* and *F. solani* are commonly recovered from the roots and stem bases.

F. oxysporum produced a dark violet pigment on PDA. The mycelia were floccose and abundant where the colors of colony ranged from white or pale orange to violet (Fig. 1A). In the central spore mass, abundant pale violet macroconidia were produced. The growth rate of *F. oxysporum* was 6.38 ± 1.45mm/day. The aerial mycelia of *F. proliferatum* was white but may become purple with age (Fig. 1B), and produced a dark violet pigment in the agar. It was grown uniformly on plate with growth rate at 7.50 ± 0.83 mm/day. *F. semitectum* produced abundant dense aerial mycelia that initially was white, became brown with age (Fig. 1C), and produced brown pigment in PDA. The growth rate for this species was 7.88 ± 1.15 mm/day. *F. solani* produced cream and slightly light yellow pigments (Fig. 1D). The colony was white to cream with sparse mycelia. The growth rate for this species was 4.00 ± 0.25 mm/day.

Micromorphological characteristics were examined on CLA, *F. oxysporum* produced slender and thin-walled macroconidia. Microconidia was oval shaped or slightly sickle-shaped, thin walled and delicate with an attenuated apical cell and a foot-shaped basal cell with size 4.42-6.83µm x 15.98-30.79µm (Fig. 1E). Microconidia of *F. proliferatum* were club shaped, thin walled and flattened base without septate (Fig. 1F) with size 2.22-3.83µm x 5.98-15.80µm. Conidiophore were monophialide and polyphialides. Microconidia produced by *F. semitectum* were pyriform to obovate and usually 1-septate (Fig. 1G) and most common in older cultures. However, *F. solani* produced oval with 0-1 septate microconidia with size 2.10-3.87µm x 4.20-7.95µm (Fig. 1H). Microconidia were formed in false heads.

On CLA, the apical cell of macroconidia of *F. oxysporum* was slightly curved and generally has 3-5 septate (Fig. 1I). The size of macroconidia was 7.67-8.87µm x 44.50-86.53µm. *F. proliferatum* produced slender and thin-walled macroconidia (Fig. 1J). The apical cell was slightly curved and generally have 3-5 septate. The size of macroconidia was 4.57-3.32µm x 27.50-46.03µm. Some cultures produced mesoconidia with 1-septate. *F. semitectum* produced slender with curved dorsal surface and a straighter ventral surface of macroconidia (Fig. 1K). The basal was foot shaped, the apical cell curved, and tapering to a point generally has 3-5 septate. The size of macroconidia was 4.10-5.16µm x 26.58-63.73µm. The macroconidia of *F. solani* were relatively slender with a curved dorsal surface and a straighter ventral surface and have 3-5 septate (Fig. 1L). The size of macroconidia was 4.60-7.10µm x 31.58-55.47µm.

Some of the *Fusarium* species produced chlamydospores as a survival spore, *F. oxysporum* produced chlamydospores in pair intercalary (Fig. 1M), terminal intercalary (Fig. 1N), and singly intercalary. The conidiophore of this species was monophialides without polyphialides. Microconidia were formed in false heads on short monophialides (Fig. 1O). *F. proliferatum* have no chlamydospore and the conidia were borne in chains of branched-monophialides varying length and in false heads (Fig. 1P). Most of the isolates of *F. semitectum* produced singly intercalary chlamydospore. The conidiophore of this species was monophialides. Microconidia were formed in singly or pair on short monophialides (Fig. 1Q). In *F. solani*, the long monophialide and terminal chlamydospore were observed.

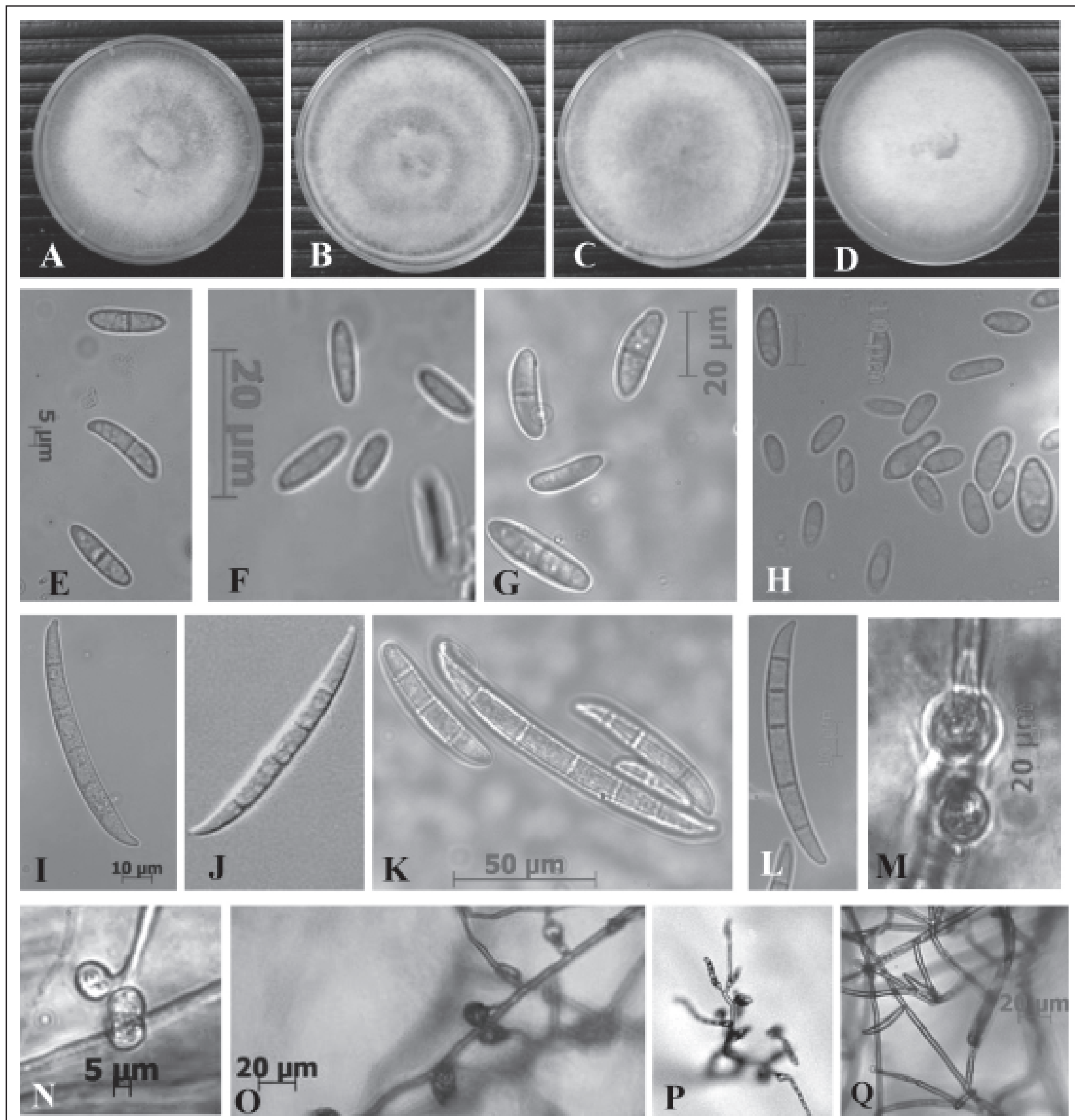

Fig. 1. Morphological characteristic of *Fusarium* species isolated from kenaf. Colony features of *F. oxysporum* (A), *F. proliferatum* (B), *F. semitectum* (C) and *F. solani* (D) on PDA; club-shaped microconidia of *F. oxysporum* (E); 0-1 septate oval microconidia of *F. proliferatum* (F), *F. semitectum* (G) and *F. solani* (H); 5-septate macroconidia of *F. oxysporum* (I), *F. proliferatum* (J), *F. semitectum* (K) and *F. solani* (L); pair intercalary (M) dan terminal chlamydospores (N) produced by *F. oxysporum* cultures; Conidia of *F. oxysporum* in false heads; Microconidia of *F. proliferatum* in chain (P); and conidia of *F. semitectum* in pair and singly (Q).

Microconidia of *F. oxysporum* formed in false heads on short phialides of hyphae. The production of chlamydospores and the shapes of the macroconidia and the microconidia are some critical morphological features of *F. oxysporum*. *F. oxysporum* includes many representatives that are pathogenic to plants and often causing vascular wilt diseases, damping-off problems and crown and root rots (Summerell & Rugg, 1992). An isolate originally identified as a tomato pathogen can also cause disseminated infection of immunodepressed in mice (Ortoneda *et al*., 2004). The vascular wilt diseases of plants often result when xylem vessels are blocked, with the blockage in at least some cases being due to gels composed of neutral sugars commonly found in the host plant's cell wall (Shi *et al*., 1992). Host resistance, numerous biological and chemical control methods were recommended as disease reduction measures. Economically important plant pathogenic strains can be recovered from non-host, usually native, plants (Wang *et al*., 2004). *F. oxysporum* can be dispersed by many different means including wind and in soil, seeds, or infected planting materials (Garibaldi *et al*., 2004).

PDA cultures of *F. proliferatum* and *F. oxysporum* often appear similar, but these species easily distinguished by the presence of

microconidia in chains for *F. proliferatum* and the presence of chlamydospores and microconidia in false heads for *F. oxysporum*. *F. proliferatum* has been recovered from numerous environments worldwide. It is a cause of root rot of pine seedlings (Ocamb *et al*., 2002), Fusarium crown and root rot of asparagus (Elmer, 1995) and date palm decline (Abdalla *et al*., 2000). *F. semitectum* commonly isolated from soil as reported by Leslie *et al.* (1990) and has been reported to cause a canker of walnut (Seta *et al*., 2004), a blight of the kangaroo paw ornamental plant (Singh *et al.*, 1992) and is one of the dominant fungi on the grain of pearl millet (Wilson, 2002). However, in this study, *F. proliferatum, F. semitectum* and *F. solani* most probably exist as saprophytes. *F. solani* is widely distributed in numerous soils from temperate to tropical regions (Burgess and Summerell, 1992; Leslie and Summerell, 2006).

Pathogenicity test

Out of four *Fusarium* species used in the pathogenicity test, only *F. oxysporum* was pathogenic to kenaf. All seeds inoculated with both isolates of *F. oxysporum* developed disease symptoms similar to those in the field. Disease symptoms seen were stunted growth with yellowish and chlorosis leaves. The means of DSI of *F. oxysporum* isolates T261k and T262k were significantly different from other species and the control plants at DSI 0.44 (Table 1). The other species namely *F. proliferatum, F. semitectum* and *F. solani* were not pathogenic to kenaf and the mean of DSI of the kenaf infected by these three species ranged from 0 until 0.063and not significantly different at $p \leq 0.05$ from the control plants.

There were significantly different between the mean of kenaf height that inoculated with *F. oxysporum* isolate T261k and control (Table 1). The infected plant showed abnormalities with chlorosis symptom and stunted plant when compared with control plant. Dry weight of kenaf inoculated with *F. oxysporum* isolates T261k and T262k was significantly different from the control. This indicated that *F. oxysporum* gave greater effects towards the kenaf (Table 1). Other isolates of *F. proliferatum, F. semitectum* and *F. solani* have slight effects towards the kenaf, but not significantly different with control plants, therefore they were not pathogenic. Vascular browning symptom was apparent in the petioles of the detached leaves showing the early symptoms of chlorosis. The stunted height and lowest dry weight of kenaf plants inoculated with *F. oxysporum* showed that it did not only cause the chlorosis on leaves but also disturb the growth of kenaf.

The development of wilt symptoms in the inoculated kenaf plant was observed, and the same inoculated *F. oxysporum* isolate was successfully reisolated from the infected plant materials. This was confirmed the Koch's postulate that *F. oxysporum* was pathogenic in kenaf. Effort was also made to revitalize the non-pathogenic isolates and re-checked for their pathogenicity by re-inoculating the new kenaf seed with non-pathogenic isolates, but similar result were observed.

Vegetative Compatibility Groups (VCGs)

About 133 *nit* mutants of *F. oxysporum* were successfully recovered from all 9 isolates on MMC. The majority of the *nit* mutants recovered from MMC was *nit1* mutants with 67.58% from the total *nit* mutants recovered, NitM was 24.54% and the lowest was *nit3* with only 9.72%. Consequently, the relative frequency of the *nit1* was considerably highest on MMC. Vegetative compatible *nit* mutants complemented to one another by forming a heterokaryon on MM. The heterokaryon formation was more robust in the pairings of NitM and *nit1* or NitM and *nit3* mutants as compared to the pairings

Table 1. Disease Severity Index (DSI) of kenaf for control and after inoculated with different species of *Fusarium*

Fusarium species	Isolates	DSI	Height (cm)	Dry weight (g)
F. oxysporum	T262k	0.438^{a}	100.04^{c}	15.03^{c}
	T261k	0.375^{a}	85.63^{c}	12.88^{c}
F. proliferatum	T263k	0.063^{b}	160.50^{a}	29.75^{ab}
	T258k	0^{b}	149.88^{ab}	26.00^{ab}
F. semitectum	T257k	0.063^{b}	153.13^{ab}	22.93^{ab}
	T273k	0^{b}	131.25^{b}	21.78^{b}
F. solani	T280k	0.063^{b}	159.38^{ab}	24.23^{ab}
	T259k	0^{b}	141.75^{ab}	19.13^{ab}
Control (distilled water)		0^{b}	179.45^{a}	31.73^{a}

Means for respective fungus with same letter in same column are not significantly different at $p \leq 0.05$

between *nit1* and *nit3* mutants, which formed weak appearance of heterokaryon (Fig. 2). Nine isolates of *F. oxysporum* identified as HSC and were grouped in 5 VCGs which consisted of 1-5 isolate member(s) (Table 2). The VCG A01 had the highest numbers of 5 isolates whilst the other groups only consisted of only single isolate. VCG A01 consisted of isolates obtained from the various kenaf plant parts, which are from roots, leaves and stems. VCG A02 consisted of isolates from kenaf root, VCG A03, VCG A04 consisted isolates from kenaf leaves, and isolates in VCG 5 obtained from kenaf stems.

Four isolates of *F. proliferatum* resulted in 3 VCGs. VCG B01 consisted of single isolates of *F. proliferatum* from kenaf root, the isolates from leaves was grouped in VCG B02. VCG B03 was derived from kenaf stem. Three VCGs with every isolate of *F. semitectum* was placed in their own group (Table 2). VCG C01, VCG C02 and VCG C03 consisted of isolates obtained from kenaf leaves. Only one *F. semitectum* isolate, T257k obtained from the kenaf root sample but failed to produce a heterokaryon and therefore was classified as heterokaryon self-incompatible (HSI).

F. oxysporum isolates were grouped into five VCGs, *F. proliferatum* in 3 VCGs and *F. semitectum* in 3 VCGs. VCG diversity can be calculated by dividing the number of total VCG by the total number of strains (Smith-White *et al*., 2001). In the present study, the overall VCG diversity for *F. oxysporum* was 55%, *F. proliferatum* was 75% and *F. semitectum* was 100%. For comparison, in *F. oxysporum,* where 29 VCGs were identified among 100 strains of *F. oxysporum* collected from soil in the San Joaquin Valley in 1988, the ratio of VCGs to isolates was 29% (Gordon and Okamoto, 1991). This phenomena took place was probably due to the effects of the small numbers and sources of *Fusarium* strains.

The previous study by Gembong *et al.* (2000) also indicated that the *F. oxysporum* is pathogenic to kenaf and caused the wilt disease. The other *Fusarium* species isolated from kenaf such as *F. proliferatum*, *F. semitectum* and *F. solani* were classified as the secondary colonizers or saprophytes of kenaf because they showed no significant effect towards the kenaf. CRSs were recovered from 9 isolates of *F. oxysporum* and 4 isolates of *F. proliferatum* and *F. semitectum*. Fast-growing fanlike sectors with a thin texture of CRSs grew because that species have the ability to utilize nitrate as a source of nitrogen by the internal reduction of chlorate into ammonium form via nitrate and nitrite reductase. The Basidiomycetes, the Saprolegniaceae and the Blastocladiales are unable to utilize nitrate because they cannot synthesize nitrate reductase. Chlorate toxicity in this fungi resulted from the reduction of chlorate to chlorite by nitrate reductase. Some isolates are very sensitive to $KClO_3$ and may neither form sector nor grow if the $KClO_3$ level is too high (Leslie, 1993).

Most fungi have the ability to utilize nitrate as a source of nitrogen under normal growth condition in the internal reduction of chlorate into the ammonium form via nitrate and nitrite reductase. However, when they are grown on media containing chlorate the isolates were unstable and the colonies were unable to reduce chlorate to chlorite. This type of unstable growth on chlorate medium was previously observed in *Fusarium* spp. such as *F. poae* (Lui and Sundheim, 1996) and *F. proliferatum* (Elmer *et al.*, 1999).

The *nit* mutant phenotypes were determined based on colony morphology when grown on media containing one of the four different nitrogen sources. These classes of *nit* mutants presumably reflect mutations at a nitrate reductase structural locus (*nit1*), a nitrate assimilation pathway-specific regulatory locus (*nit3*) and loci (at least five) that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM). The dense growth or wild-type growth, which

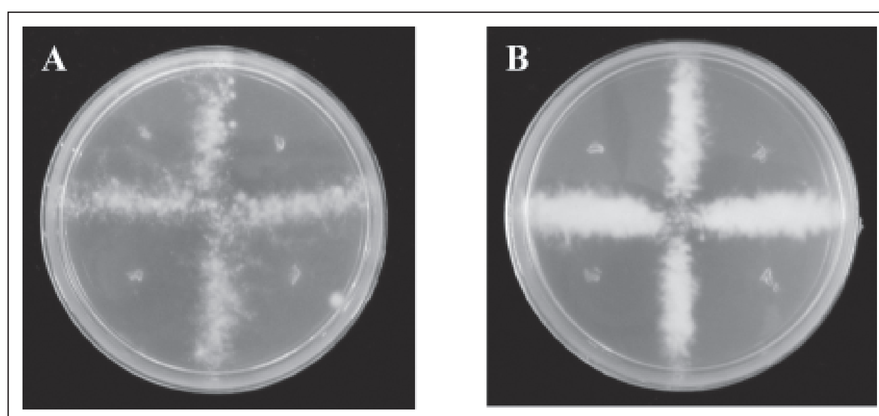

Fig. 2. Comparison between weak heterokaryon (A; *nit1* and *nit3*) and, robust heterokaryon (B; *nit1* and NitM).

Table 2. VCGs of *Fusarium* species isolated from kenaf showing wilt disease symptoms

Fusarium	VCGs	Isolates number	Part of kenaf	No. of *nit* mutants	*Nit* mutant classes (%)		
					nit1	*nit3*	NitM
F. oxysporum	A01	T261k	root	3	33.3	0	66.6
		T266k	leaf	32	93.6	20	0
		T267k	leaf	2	50	0	50
		T276k	stem	40	97.6	0	2.4
		T281k	stem	5	60	0	40
	A02	T262k	root	7	71.4	0	28.6
	A03	T265k	leaf	24	83.3	20	0
	A04	T271k	leaf	9	55.5	11.1	33.3
	A05	T278k	stem	11	63.6	36.4	0
F. proliferatum	B01	T263k	leaf	12	41.7	0	58.3
		T274k	leaf	29	31	69	0
	B02	T258k	root	6	50	50	0
	B03	T277k	stem	4	75	0	25
F. semitectum	C01	T269k	leaf	9	66.7	33.3	0
	C02	T273k	leaf	7	28.6	0	71.4
	CO3	T275k	leaf	7	42.9	57.1	0
	HSI	T257k	root	10	50	50	0

derived from chlorate-resistance mutants were classified as *crn* mutants. The differences between loci in susceptibility to mutation could be related to the physical size of the gene, with larger genes respecting larger targets as in *nit1* mutation (Leslie, 1993; Leslie and Zeller, 1996; Lui and Sundheim, 1996).

The development of dense aerial growth where the mycelia of the *nit* mutant colonies came into contact and anastomosed to form a heterokaryon indicated that the physiological complementation between *nit* mutants with different mutations occurred. Complementation occurred more rapidly and growth of the resulting heterokaryon was more robust in pairings of NitM with *nit1* or *nit3* mutants that in pairings of *nit1* with *nit3* mutants. The complementation usually occurred after 2-3 weeks when *nit1* and *nit3* were paired and very rapid and robust with dense aerial mycelium formation if the complementation reaction occurred between different NitM mutants. Therefore, in each strain the NitM should be recovered as it produces better heterokaryon when paired and some NitM mutants were able to complement with one another.

Single isolate from *F. semitectum* isolate T257k was unable to form heterokaryon. This strain was classified as heterokaryon self-incompatible. According to Leslie (1993), isolates or strains that carry mutations prevent them to form heterokaryons even with themselves, and have been identified in field population of *F. oxysporum, F. moniliforme* and *F. subglutinans.* For *F. solani* isolates, Leslie & Summerell (2006) reported that it was difficult to generate *nit* mutants and the high proportion of the isolate was heterokaryon self-incompatible. When the isolates were genetically compatible, the heterokaryon was observed in this study. The presence of identical alleles at all *het* loci present in both strains, were the heterokaryotic growth depends. Vegetatively incompatible considered when the strain from different VCGs failed to fuse after pairing with one another's *nit* mutants. This phenomenon occurs when strains from different VCGs fuse to form heterokaryotic cells, but with different degrees of cytoplasmic incompatibility, the heterokaryon produced is unstable and the cytoplasm of the fused cells dies (Deacon, 2006; Leslie and Summerell, 2006). The molecular traits of the isolates in the same VCG is similar and is different from isolates in other VCGs of the same species (Deacon, 2006; Leslie and Summerell, 2006).

In VCG analysis, the variation could be because of a single base change with compatible loci, which may divide into two almost identical strains from separate groups (Smith-White *et al*., 2001). Pathological and physiological traits of isolates were shared in a same VCG as well as geographical origins (Swift *et al*., 2002). In this study, these attributes cannot be seen in *F. oxysporum* isolates*, F. proliferatum* isolates and *F. semitectum* isolates in which the VCGs were grouped according to the kenaf plant parts although all the strains were derived from the same location. This could be that

loci and alleles of VCG are selectively neutral with respect to traits such as pathogenicity and vegetative viability.

It is therefore concluded that the pathogen of Fusarium wilt disease in kenaf identified as *F. oxysporum* and three other species isolated from the infected samples were nonpathogenic. All the species were diverse based on morphological and vegetative compatibility properties.

REFERENCES

Abdalla, M.Y., Al-Rokibah, A., Moretti, A. and Mule, G. 2000. Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. *Plant Dis.,* **84**: 321-324.

Burgess, L.W. and Summerell, B.A. 1992. Mycogeography of *Fusarium*: Survey of *Fusarium* species from sub-tropical and semi-arid grassland soils from Queensland, Australia. *Mycol. Res.,* **96**: 780-784.

Elmer, W.H. 1995. A single mating population of *Gibberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachusetts, and Michigan. *Mycologia,* **87**: 68-71.

Elmer, W.H., Summerell, B.A., Burgess, L.W. and Nigh, J.E.L. 1999. Vegetative compatibility groups in *Fusarium proliferatum* from asparagus in Australia. *Mycologia,* **91**: 650- 654.

Garibaldi, A., Giraldi, G. and Guillino, M.L. 2004. Seed transmission of *Fusarium oxysporum* f. sp. *lactucae*. *Phytoparasitica*, **32**: 61-65.

Gembong, D., Cece, S. and Supriyono, S. 2000. Evaluasi ketahanan aksesi kenaf (*Hibiscus cannabinus* L.) terhadap penyakit layu *Fusarium oxysporum* schlect. *Jurnal Penelitian Tanaman Industri,* **6**(2): 29-32.

Gordon, T.R. and Kamoto, D.O. 1991. Vegetative compatibility groupings in a local population of *Fusarium oxysporum*. *Can. J. Bot.,* **69**: 168-172.

Leslie, J.F. 1993. Fungal vegetative compatibility. *Annu. Rev. Phytopathol.,* **31**: 127-151.

Leslie, J.F. and Summerell, B.A. 2006. *The Fusarium Laboratory Manual.* United Kingdom: Blackwell Publishing Ltd. pp 81-250.

Leslie, J.F. and Zeller, K.A. 1996. Heterokaryon compatibility in fungi-More than just another way to die. *J. Gen.,* **75**: 415-424.

Leslie, J.F., Pearson, C.A.S., Nelson, P.E. and Toussoun, T.A. 1990. *Fusarium* species from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathol.,* **80**: 343-350.

Liu, W. and Sundheim, L. 1996. Nitrate nonutilizing mutants and vegetative compatibility groups in *Fusarium poae*. *Fungal Genet. Biol.*, **20**: 12-17.

New Strait Times. 10 April 2008. Page 17.

Ocamb, C., Juzwik, M.J. and Martin, F.B. 2002. *Fusarium* spp. and *Pinus strobus* seedlings: Root disease pathogens and taxa associated with seed. *New Forests,* **24**: 67-79.

Ortoneda, M.J., Guarro, M.P., Madrid, Z., Caracuel, M.I.G., Roncero, E. Mayayo, and di Pietro, A. 2004. *Fusarium oxysporum* as a multihost model for thegenetic dissection of fungal virulence in plants and mammals. *Infection and Immunity,* **72**: 1760-1766.

Seta, S., Gonzalez, M. and Lori, G. 2004. First report of walnut canker caused by *Fusarium incarnatum* in Argentina. *Plant Pathol.,* **53**: 248.

Shi, J., Mueller, W.C. and Beckman, C.H. 1992. Vessel occlusion and secretory activities of vessel contact cells in resistant or susceptible cotton plantsinfected with *Fusarium oxysporum* f. sp. *vasinfectum*. *Physiol. Mol. Plant Pathol.,* **40**: 133-147.

Singh, A., Kumar, P.K.R. and Schuegerl, K. 1992. Dxylose fermentation and catabolism in *Fusarium oxysporum*. *Biochemistry Int.,* **27**: 831-839.

Smith-White, J.L., Gunn, L.V. and Summerell, B.A. 2001. Analysis of diversity within *Fusarium oxysporum* populations using molecular and vegetative compatibility grouping. *Aus. Plant Pathol.,* **30**: 153-157.

Summerell, B.A. and Rugg, C.A. 1992. Vascular wilt of *Helichrysum* species caused by *Fusarium oxysporum*. *Aus. Plant Pathol.,* **21**: 18-19.

Summerell, B.A., Burgess, L.W., Backhouse, D., Bullock, S. and Swan, L.J. 2001. Natural occurrence of perithecia of *Gibberella coronicola* on wheat plants with crown rot in Australia. *Aus. Plant Pathol,* **30**: 353-356.

Summerell, B.A., Salleh, B. and Leslie, J.F. 2003. A utilitarian approach to *Fusarium* identification. *Plant Dis.,* **87**: 117-128.

Sunder, S.S. 1998. Vegetative compatibility, biosynthesis of GA3 and virulence of *Fusarium moniliforme* isolates from bakanae disease of rice. *Plant Pathol.,* **47**: 767-772.

Swift, C.E., Wickliffe, E.R. and Schwartz, H.F. 2002. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cepae* from onion in Colorado. *Plant Dis.,* **86**: 606- 610.

Vawdrey, L.L. and Peterson, R.A. 1990. Diseases of kenaf (*Hibiscus cannabinus*) in the Burdekin River Irrigation area. *Aus. Plant Pathol.* **19**: 34-35.