

DISTRIBUTION AND DIVERSITY OF *FUSARIUM* SPECIES ASSOCIATED WITH GRASSES IN TEN STATES THROUGHOUT PENINSULAR MALAYSIA

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ABSTRACT

Fusarium is one of the important genera associated with grasses as saprophytes, endophytes and pathogens. A study was carried out on distribution and diversity of *Fusarium* species associated with two groups of grasses in 10 states throughout Peninsular Malaysia *i.e.* agricultural grasses (*Oryza sativa* and *Saccharum officinarum*) and non-agricultural grasses (*Axonopus compressus*, *Centotheca lappacea*, *Chloris barbata*, *Crysopogon aciculatus*, *Cyanadon dactylon*, *Dactyloctenium aegyptium*, *Digitaria ciliaris*, *Echinochloa colona*, *Eleusine indica*, *Eragrostis amabilis*, *Eragrostis malayana*, *Eragrostis uniloides*, *Ischaemum magnum*, *Panicum brevifolium*, *Panicum millaneum*, *Panicum repens*, *Paspalum commersonii*, *Paspalum conjugatum*, *Paspalum orbiculare*, *Pennisetum purpureum*, *Sacciolepis indica*, *Sporobolus diander* and *Sporobolus indicus*). A total of 474 isolates were single-spored and identified by morphological characteristics. *F. semitectum* was frequently isolated (23.6%), followed by *F. sacchari* and *F. fujikuroi* with 15.4% and 14.6%, respectively. The other nine species were *F. solani* (10.3%), *F. proliferatum* (8.9%), *F. oxysporum* (7.4%), *F. subglutinans* (6.5%), *F. equiseti* (5.5%), *F. verticillioides* (3.4%), *F. compactum* (2.5%), *F. chlamydo sporum* (1.1%) and *F. longipes* (0.8%). Based on the Shannon-Weiner Index, *F. solani* was the highest ($H' = 2.62$) isolated from grasses. Species of *Fusarium* from *O. sativa* were widely diverse with 11 species, followed by non-agricultural grasses with nine species and *S. officinarum* with only six species. This is the first report on diversity of *Fusarium* associated with grasses in Malaysia.

Key words: *Oryza sativa*, *Saccharum officinarum*, non-agricultural grass, *Fusarium* species, diversity and Gramineae

INTRODUCTION

Fusarium species are well-known as soil borne fungi and widely distributed and could be associated with any parts of various plants from the deepest roots to the

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highest flowers, plant debris and other organic substrates. It can exist as free-living saprophytic, parasitic, pathogenic or toxigenic fungi (Booth 1971; Nelson *et al.* 1983; Burgess *et al.* 1994). Most of the *Fusarium* species are found in fertile, cultivated and rangeland soils but are relatively uncommon in forest soils (Burgess *et al.* 1994). Several species in this genus have been reported as serious plant pathogens causing various diseases such as crown rot, head blight and scab on grains, vascular wilts on a wide range of horticultural crops, root rots, cankers and other important diseases such as pokkah boeng on sugarcane and bakanae disease on rice (Nelson *et al.* 1983; Webster & Gunnell 1992; Leslie & Summerell 2006).

The family Gramineae consists of two main groups *i.e.* high; hard-stemmed grasses *e.g.* bamboos and sugarcane (*Saccharum officinarum*), and herbaceous grasses that include cereals bearing grain *e.g.* maize (*Zea mays*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*) (Wycherley & Mohd Yusof 1974). Other than economically important crops, true grasses could also be one of the suitable hosts for *Fusarium* species.

In Malaysia as well as in other developed countries, the information on diversity of *Fusarium* associated with plants under family Gramineae are limited. Thus, this study was conducted to elucidate the distribution and diversity of *Fusarium* species associated with selected grasses throughout Peninsular Malaysia.

MATERIALS AND METHOD

Identification of the grasses

In this study, rice (*O. sativa*), sugarcane (*S. officinarum*) and 25 non-agricultural grasses *i.e.* *Axonopus compressus* (Broadleaf carpet grass), *Centotheca lappacea*, *Chloris barbata* (Swollen finger grass), *Crypsogon aciculatus*, *Cyanodon dactylon* (Bermuda grass), *Dactyloctenium aegyptium* (Durban crowfoot grass), *Digitaria ciliaris* (Henry's crab grass), *Echinochloa colona* (Jungle rice), *Eleusine indica* (Goose grass), *Eragrostis amabilis* (Japanese love grass), *Eragrostis malayana*, *Eragrostis uniloides*, *Ischaemum magnum*, *Panicum brevifolium*, *Panicum millaneum*, *Panicum repens* (Torpedo grass), *Paspalum vaginatum* (Seashore paspalum), *Paspalum conjugatum* (Hilo grass), *Paspalum orbiculare* (Rice grass paspalum), *Pennisetum purpureum* (Elephant grass), *Sacciolepis indica* (Glenwood grass), *Sporobolus diander* (Tussock Dropseed) and *Sporobolus indicus* (Smut grass) were collected throughout 10 states of Peninsular Malaysia (Johor, Kedah, Kelantan, Melaka, Pahang, Perak, Perlis, Pulau Pinang, Selangor and Terengganu). The grasses were identified into species level based on morphological characteristics following Gilliland *et al.* (1971) and Harada *et al.* (1996).

Sampling and Isolation of *Fusarium* isolates

Sampling was done in various areas within Peninsular Malaysia from 2004 until 2006 and part of the leaves and roots were taken. The samples were maintained in a cool dry state during transit to minimize the growth of saprophytic fungi and bacteria. The technique used was indirectly that involved plating plant tissue segments on agar media. First, samples were surface-sterilized with 0.5% sodium hypochlorite (NaOCl), then washed three times of sterile water and placed on peptone

pentachloronitrobenzene agar (PPA) (Nash & Snyder 1964). The cultures were incubated under the standard growth conditions at room temperature for 7 days and then purified by employing the single-spore isolation technique (Burgess *et al.* 1994) and incubated at standard incubation conditions (Salleh & Sulaiman 1984). Single conidia cultures were plated onto potato dextrose agar (PDA) and carnation leaf agar (CLA) for further species delimitation. The pure cultures were used and maintained on water agar (WA) (Burgess & Liddell 1983) as a short-term working cultures, and preserved in 15% sterile glycerol at -80°C on a sterile filter papers.

Identification of *Fusarium* species

Colony features and pigmentations were recorded after single-spored isolates were cultured on PDA for 7 days. The Methuen handbook of colour chart (Kornerup & Wachter 1978) was used as a reference for describing the pigmentations. *Fusarium* isolates were transferred onto CLA plates (Fisher *et al.* 1982) and incubated for 10 day under the standard growth conditions. The morphological characteristics were observed from slide by using a light microscope (Olympus model BX-50F4) and photographed by using a camera (JVC model KY-F55BE) with an image analyzer-SIS programme. The identification of *Fusarium* species were carried out based on the taxonomic guidelines by Booth (1971), Burgess and Liddell (1983), Nelson *et al.* (1983), Burgess *et al.* (1994), Nirenberg and O'Donnell (1998) and Leslie and Summerell (2006).

Measurement of Species Diversity

All the *Fusarium* species that had been verified where then calculated by using the Shannon-Weiner Index (Spellerberg 2008) for species diversity within the grasses family as formula below:

$$H' = -\sum_{i=1}^s p_i \ln p_i$$

where: \sum refers to "the sum of"

there are s species in the community

p_i = is the relative abundance (proportion) of the i species in the community

\ln = natural log

RESULTS AND DISCUSSION

A total of 474 isolates of *Fusarium* were isolated, singled-spored and identified from parts of grass samples collected during sampling periods from year 2004 - 2006. As can be derived in Figure 1 and Table 1, 12 species of *Fusarium* were successfully isolated, and were belonged to six sections *i.e.* section Arthrosporiella (*F. semitectum*), section Elegans (*F. oxysporum*), section Gibbosum and their allied (*F. compactum*, *F. equiseti* and *F. longipes*), section Liseola and their allied (*F. fujikuroi*, *F. proliferatum*, *F. sacchari*, *F. subglutinans*, and *F. verticillioides*), section Martiella and Ventricosum (*F. solani*), and section Sporothrichiella (*F. Chlamydosporum*).

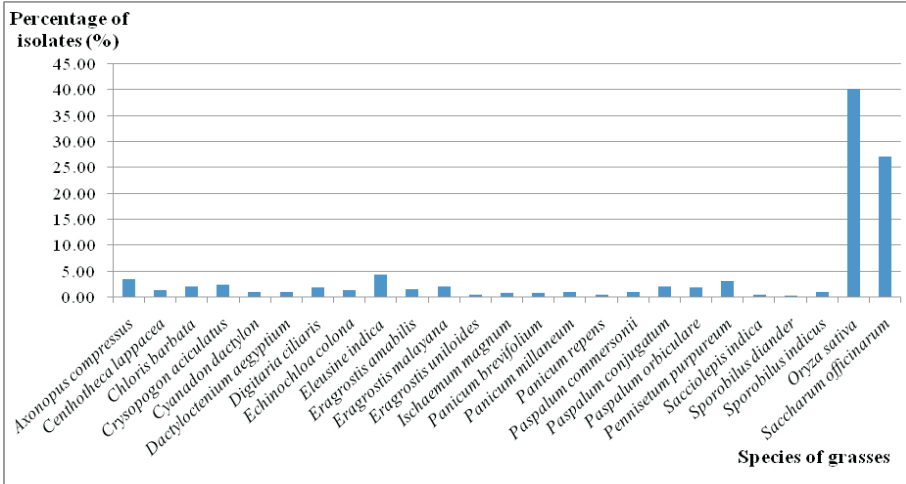


Figure 1. The probability of *Fusarium* isolates obtained from each species of grasses

Table 1. Frequency of *Fusarium* isolates belongs to 12 *Fusarium* species associated with several species of grasses

Species of grasses	^a <i>Fusarium</i> species											
	<i>F. semitectum</i>	<i>F. sacchari</i>	<i>F. fijiense</i>	<i>F. solani</i>	<i>F. proliferatum</i>	<i>F. oxysporum</i>	<i>F. subglutinans</i>	<i>F. equiseti</i>	<i>F. verticillioides</i>	<i>F. compactum</i>	<i>F. dlanysporum</i>	<i>F. longipes</i>
^b <i>Axonopus compressus</i>	3	-	-	3	1	-	-	6	-	3	-	-
^b <i>Centotheca lappacea</i>	1	-	-	1	1	1	1	-	-	1	-	-
^b <i>Chloris barbata</i>	1	-	-	3	-	-	-	4	-	1	-	-
^b <i>Cryspogon aciculatus</i>	4	1	-	4	-	1	-	-	-	1	-	-
^b <i>Cyanadon dactylon</i>	2	-	-	1	-	-	-	-	-	1	-	-
^b <i>Dactyloctenium aegyptium</i>	1	-	-	3	-	-	-	-	-	-	-	-
^b <i>Digitaria ciliaris</i>	4	-	-	2	-	-	-	2	-	-	-	-
^b <i>Echinochloa colona</i>	2	-	-	1	1	-	2	-	-	-	-	-
^b <i>Eleusine indica</i>	10	-	-	3	2	-	-	3	-	-	2	-
^b <i>Eragrostis amabilis</i>	-	-	-	4	-	1	-	1	-	1	-	-
^b <i>Eragrostis malayana</i>	1	-	-	2	1	2	-	3	-	-	-	-
^b <i>Eragrostis uniloides</i>	1	-	-	-	-	1	-	-	-	-	-	-
^b <i>Ischaemum magnum</i>	-	-	-	1	-	-	-	-	-	2	-	-
^b <i>Panicum brevifolium</i>	2	-	-	-	-	-	1	-	-	-	-	-
^b <i>Panicum millaneum</i>	2	-	-	1	1	-	-	-	-	-	-	-
^b <i>Panicum repens</i>	-	-	-	-	1	1	-	-	-	-	-	-
^b <i>Paspalum commersonii</i>	2	-	-	-	-	1	1	-	-	-	-	-
^b <i>Paspalum conjugatum</i>	2	3	-	2	-	-	-	1	-	1	-	-
^b <i>Paspalum orbiculare</i>	4	-	-	2	-	1	-	-	-	1	-	-
^b <i>Pennisetum purpureum</i>	8	-	-	2	2	-	-	1	-	-	1	-
^b <i>Sacciolepis indica</i>	1	-	-	-	1	-	-	-	-	-	-	-
^b <i>Sporobolus diander</i>	1	-	-	-	-	-	-	-	-	-	-	-
^b <i>Sporobolus indicus</i>	3	-	-	-	-	-	1	-	-	-	-	-
<i>Oryza sativa</i>	33	11	69	11	13	26	4	1	16	-	2	4
<i>Saccharum officinarum</i>	24	58	-	3	18	-	21	4	-	-	-	-
Percentage (%)	23.6	15.4	14.6	10.3	8.9	7.4	6.5	5.5	3.4	2.5	1.1	0.8
Total of isolates	112	73	69	49	42	35	31	26	16	12	5	4

A total of 474 isolates of *Fusarium* species

^bTrue grasses

The most dominant species was *F. semitectum* (112 isolates; 23.6%) that belonged to the section Arthrosporiella. This species was the most prevalent species associated with the grasses; being present in 94% of the grass samples. *F. semitectum* is cosmopolitan (Nelson *et al.* 1983); regularly found as secondary invaders in diseased tissues (Summerell *et al.* 2003) and frequently isolated on aerial plant parts in subtropical and tropical regions (Leslie & Summerell 2006), commonly isolated from soils (Burgess *et al.* 1994; Nik Mohd Izham *et al.* 2005) and from diverse aerial parts of several plants, namely asparagus (Al-Amodi 2006), kangaroo paw (Satou *et al.* 2001), potatoes (Kim *et al.* 1995) and some grasses (Nor Azliza *et al.* 2005). *F. semitectum* is not considered as an important pathogen although there were reports of this species being associated with several plant diseases such as corky dry rot of melons (Carter 1979) and storage rot of tropical crops (Booth 1971).

The second highest isolates were *F. sacchari* with 73 isolates (15.4%), followed by *F. fujikuroi* with 69 isolates (14.6%). Both later species were frequently isolated from sugarcane (*S. officinarum*) and rice (*Oryza sativa*), respectively. *F. sacchari* was found in Asia on sugarcane as the cause of pokkah boeng disease (Nirenberg & O'Donnell 1998; Leslie & Summerell 2006). *F. sacchari* has been isolated from sugarcane with pokkah boeng disease (Siti Nordahliawate *et al.* 2008) and was also isolated from the true grass samples at sugarcane plantations of Padang Terap, Kedah *i.e.* *Eleusine indica* (Goose grass) and *Dactyloctenium aegyptium* (Durban crowfoot grass). Thus, both collected grasses were assumed to be the secondary hosts for this fungus prior to their invasion into the sugarcane. *F. sacchari* also has been isolated from sorghum, maize and orchids (Leslie & Summerell 2006).

F. fujikuroi was also noted as the most dominant species isolated, mainly from the rice. This species has been reported as a causal agent for bakanae disease on rice (Webster & Gunnell 1992; Nur Ain Izzati *et al.* 2008). *F. verticillioides* was reported as a pathogen on maize and can be found worldwide wherever maize is cultivated (Leslie & Summerell 2006). This species causes epidemics called maize ear rot in Africa, Europe, North and South America, and Asia (Chulze *et al.* 2000; Danielsen *et al.* 1998; Desjardins *et al.* 2000; Kedera *et al.* 1999; Leslie 1995; Mulè *et al.* 2004; Munkvold & Desjardins 1997).

F. solani was also a common species that was isolated from all genera of grasses but the frequency level was less than those of earlier species with 49 isolates (10.3%). Meanwhile, *F. chlamydosporum* and *F. longipes* were noted as the least frequent species with 5 isolates (1.1%) and 4 isolates (0.8%), respectively. The other six species, *i.e.* *F. compactum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. subglutinans*, and *F. verticillioides*. *F. semitectum* and *F. solani* were unevenly distributed in all 10 states throughout Peninsular Malaysia (Fig. 2). In contrast, *F. longipes* which is known as cosmopolitan soil inhabitant that has been recovered from many parts of the world, but in this study, this species only recovered in Pahang from *O. sativa* samples.

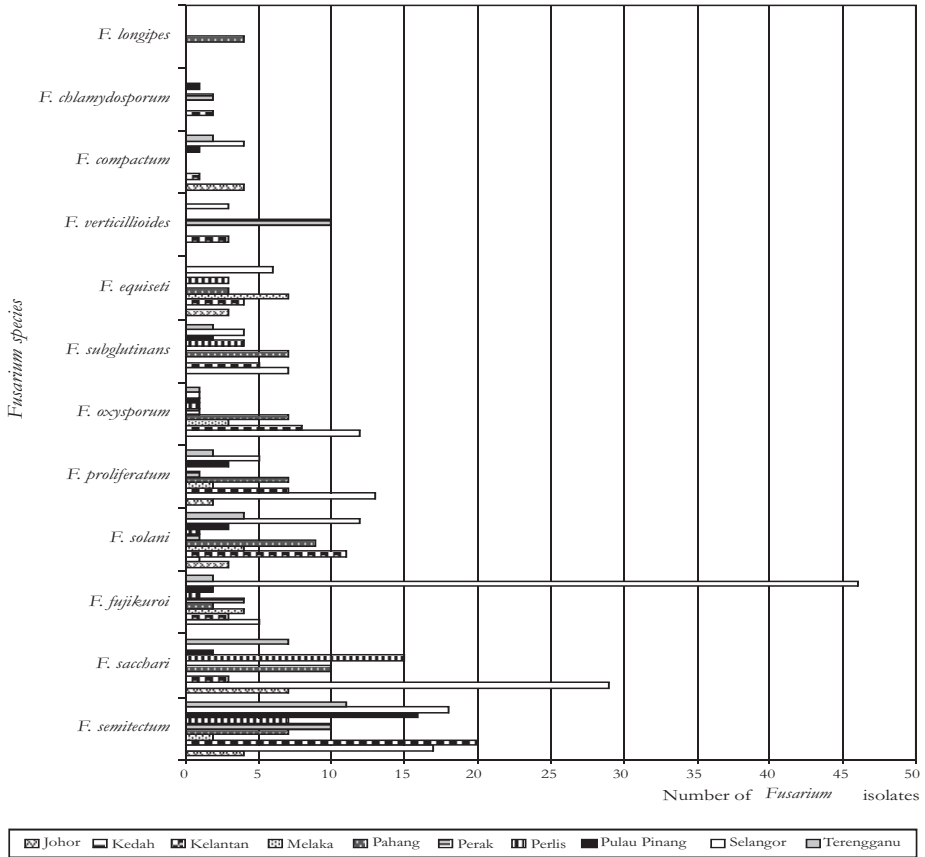


Figure 2. Distribution of *Fusarium* species associated with grasses in Peninsular Malaysia

F. solani is cosmopolitan on a wide-range of substrates (Nelson *et al.* 1983); frequently isolated from soils (Nik Mohd Izham *et al.* 2005; Leslie & Summerell 2006) and a pathogen to a large number of plant species, especially trees that show cankers and dieback symptoms (Nelson *et al.* 1983). However, no reports have been published in showing that *F. solani* is pathogenic to grasses.

Three species *i.e.* *F. equiseti*, *F. compactum* and *F. longipes* that belong to section Gibbosum were recovered particularly from the true grass samples. These species are cosmopolitan soil inhabitants that have been recovered from many parts of the world in cool and temperate to hot and arid regions, primarily as saprophytes or secondary invaders (Nelson *et al.* 1983; Summerell *et al.* 2003; Leslie & Summerell 2006) and were also isolated from soils (Nelson *et al.* 1983; Leslie & Summerell 2006). However, no report has shown these species are pathogenic to rice, sugarcane, and true grasses.

F. oxysporum is the most widely distributed and as an important vascular wilts pathogen on many plants, and a common soil saprophyte (Nelson *et al.* 1983; Leslie &

Summerell 2006). *F. oxysporum* has been reported as a serious pathogen on various plants but the species was avirulent to cereals and grasses despite that they are colonized by this species (Kommendahl *et al.* 1979; Leslie *et al.* 1990; Opperman & Wehner 1994). Extensive surveys on ear blight of barley, wheat, maize, oats and rye in temperate areas showed no disease symptoms particularly vascular wilts disease even though *F. oxysporum* has been isolated from these crops (Bottalico & Perrone 2002; Logrieco *et al.* 2002). This was also experienced in all of our grass samples (rice, sugarcane and true grasses) *i.e.* no symptoms of vascular wilts were observed during the study.

F. chlamydosporum is commonly found in soils and as a saprophyte or endophyte on a variety of substrates (Leslie & Summerell 2006). This fungus is ubiquitous in arid and semi arid areas (Burgess & Summerell 1992; Sangalang *et al.* 1995). The species is likely to colonize the plants as a secondary invader. *F. chlamydosporum* was believed to be non-pathogenic to the most plants as well as on rice, sugarcane, and true grasses (Salleh & Strange 1988). Hence, the etiology of this species was rarely discussed by plant pathologists (Desjardins 2006; Leslie & Summerell 2006).

Based on the Shannon-Weiner Index, *F. solani* ($H' = 2.62$) > *F. semitectum* ($H' = 2.45$) > *F. equiseti* ($H' = 2.13$) > *F. compactum* ($H' = 2.06$) > *F. proliferatum* ($H' = 1.57$) > *F. oxysporum* ($H' = 1.13$) > *F. subglutinans* ($H' = 1.12$) > *F. chlamydosporum* ($H' = 1.06$) > *F. sacchari* ($H' = 0.65$) > *F. longipes* ($H' = 0.00$) > *F. verticillioides* ($H' = 0.00$) > *F. fujikuroi* ($H' = 0.00$). Therefore, the diversity of *F. solani* was the highest isolated and fairly common in the grasses Family than others *Fusarium* species.

There have been considerable assumptions about expected differences in fungal populations and species compositions between agricultural and non-agricultural ecosystems. The agricultural ecosystems are characterized by host populations that are relatively uniform genetically, numerically and spatially (Burdon *et al.* 1989). As shown in Table 1, *F. fujikuroi* was the most prevalent species isolated (14.6%) from *O. sativa* whereas *F. sacchari* was most frequently isolated (12.2%) from the sugarcane (*S. officinarum*) planting areas. These data were in accordance with previous authors (Nirenberg & O'Donnell 1998; Desjardins *et al.* 1997; Semangun 1991; Tan 1989). Meanwhile, a less uniform pattern of species distribution and dispersion could be observed in non-agricultural ecosystems as no specific *Fusarium* species colonized each of the grass samples.

CONCLUSION

As a conclusion, the occurrence of *Fusarium* species on agricultural and non agricultural grass samples in Peninsular Malaysia was considerably variable in terms of their diversity and distribution.

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