Contribution to the Knowledge of Diversity of *Fusarium* Associated with Maize in Malaysia

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Abstract

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The *Fusarium* species associated with maize are widely distributed in Malaysia. Eight *Fusarium* species were obtained in this country. A series of field samplings was conducted from 2006 to 2008, when 167 *Fusarium* isolates were obtained from maize plants in seven locations throughout Malaysia. The determination was based on micro- and macromorphological features (growth rates, colony features, mode of production of microconidia, macroconidia, conidiophores, and chlamydospores). *F. proliferatum* (29.9% isolates), *F. semitectum* (22.2% isolates), *F. verticillioides* (13.7% isolates), and *F. subglutinans* (12.6% isolates) were found out most frequently. *F. equiseti, F. pseudograminearum, F. oxysporum,* and *F. solani* were also isolated. This is the first report on the occurrence of *F. equiseti, F. pseudograminearum*, and *F. subglutinans* associated with maize plants in Malaysia.

Keywords: *Fusarium* species; distribution; morphology; *Zea mays* L.

Fusarium species are extensively distributed worldwide from temperate to tropical regions (Leslie & Summerell 2006). The species are also ubiquitous fungi that emerge as saprophytes, endophytes or pathogens of plants, animals as well as humans. Generally, they are pathogens of a wide range of plants in natural habitats, i.e. tomato, legumes, sorghum, maize, pine, pineapple, wheat, barley, oats, carnation, coffee, banana, rice, sugarcane, mango, asparagus, and grasses (Nelson *et al.* 1983; Burgess *et al.* 1994). Besides being pathogens of plants, *Fusarium* species may also produce secondary metabolites that involve mycotoxins (beauvericin, fumonisin and moniliformin) or phytotoxins (fusaric acid and gibberellic acid) (Booth 1971; Summerell *et al.* 2003). Maize (*Zea mays*) belongs to economically important plants that are well-known hosts of *Fusarium* species. Maize, which is an important and valuable crop, is a dicotyledonous angiosperm plant and a member of the grass family Poaceae (Park 2001).

Nowadays, the majority of research projects that were focused on the diversity of *Fusarium* species isolated from maize were frequently carried out in temperate areas (Leslie & Summerell 2006). However, in Malaysia and also in other coun-

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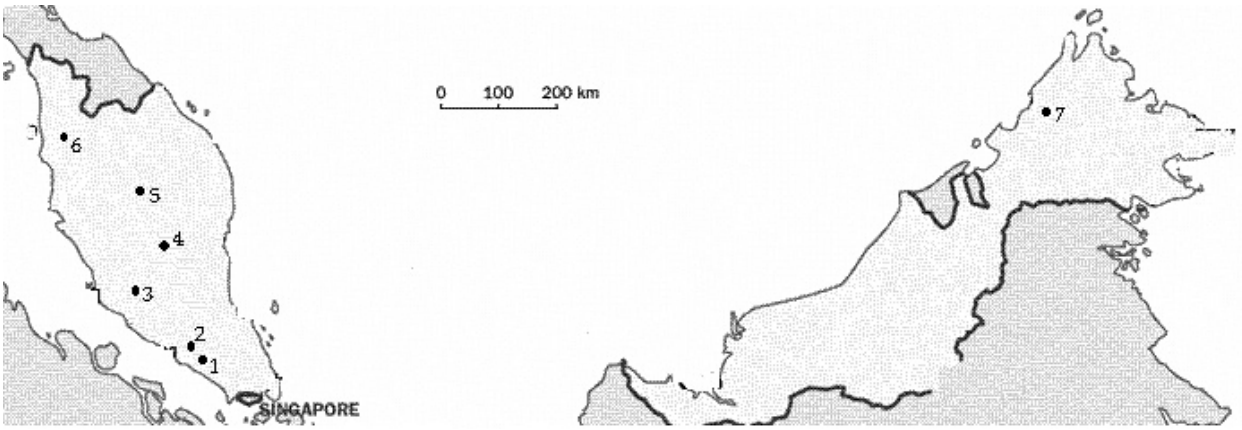
tries in the Southeast Asia region these studies are limited. Recently, *Fusarium* species such as *F. chlamydosporum, F. fujikuroi, F. graminearum, F. proliferatum, F. pseudoanthophilum, F. oxysporum, F. nygamai, F. sacchari, F. semitectum, F. solani, F. thapsinum,* and *F. verticillioides* were successfully isolated from maize showing typical symptoms of ear rot disease in Indonesia and four states of Malaysia, i.e. Perlis, Pulau Pinang, Sabah, and Sarawak (Darnetty *et al.* 2008). However, no report is available on the distribution and diversity of *Fusarium* species obtained from the west coast, east coast and southern areas of Peninsular Malaysia. Therefore, a study on the diversity of *Fusarium* species associated with maize plants in these regions was conducted due to the importance of the findings to supplement such information. The objectives of the present study were to investigate the distribution and morphological characteristics of *Fusarium* species associated with maize in Malaysia.

MATERIALS AND METHODS

Isolation of** Fusarium **isolates. A total of 265 samples of maize ears and 133 samples of maize bracts were obtained from seven maize-growing areas in the states of Johor (31 samples), Pahang (119 samples), Pulau Pinang (86 samples), Sabah (19 samples), and Selangor (143 samples) in Malaysia (Figure 1). All samples were surface sterilized with 0.5% of sodium hypochlorite for three minutes and rinsed twice in sterile water. The sterile samples were plated on a selective medium (PPA, Peptone Pentachloronitrobenzene Agar) as described by Nash and Snyder (1962) and incubated for 7 days under standard conditions (12 h under fluorescence and nUV lights, 12 h dark at a temperature of 28 ± 2°C) (Salleh & Sulaiman 1984).

Monospore isolation. From the fungal colonies grown on the selective medium, 5-mm sections of the mycelium were sub-cultured onto potato dextrose agar (PDA). The single-spore culture of the fungus was done on water agar (WA) by streaking technique.

Species identification and morphological characteristics. The cultures on PDA were used for the observation of macroscopic characteristics such as colony features, growth rate and pigmentation. For microscopic characteristics, pure cultures were transferred on carnation leaf agar (CLA), and the mycelium was inoculated close to sterile carnation leaf pieces. After 10 days of growth, the morphological characteristics were observed and evaluated according to Burgess *et al.* (1994) and Leslie and Summerell (2006) using a light microscope (Olympus model BX-50F4) and photographed using a JVC camera model KY-F55BE with an image analyser-SIS programme. The morphological characteristics of the fungi were also observed from slide cultures and *in situ* observation on CLA. Publications of Nelson *et al.* (1983), Burgess *et al.* (1994), and Leslie and Summerell (2006) were used for the identification of *Fusarium* isolates on a species level by morphological features.



1 = Seri Medan, Johor; 2 = Senggarang, Johor; 3= University Agricultural Park (TPU), Universiti Putra Malaysia, Selangor; 4 = Lanchang, Pahang; 5 = Cameron Highlands, Pahang; 6= Malaysian Agricultural Research and Development Institute (MARDI), Pulau Pinang; 7 = Kota Kinabalu, Sabah

Figure 1. The seven study sites in Malaysia

RESULTS AND DISCUSSION

Out of the 398 samples cultured, 167 isolates of *Fusarium* species (49 isolates from bracts and 118 isolates from maize ears) were obtained, monospore cultured and identified into eight species. Ninety-three of the *Fusarium* isolates were classified into three species in the section Liseola, i.e. *F. proliferatum* (50 isolates), *F. subglutinans* (21 isolates) and *F. verticillioides* (23 isolates) (Table 1). *F. proliferatum* was the most frequently occurring in all study sites. *F. equiseti, F. pseudograminearum, F. oxysporum, F. semitectum*, and *F. solani*, which belong to different sections, were also isolated and identified. *F. pseudograminearum* (19 isolates) was obtained only from samples from Cameron Highlands, Pahang. The distribution of *Fusarium* species isolated from maize has been widely studied in Africa (Fandohan *et al.* 2003) and in European countries, maize samples showed especially the Fusarium ear rot disease (Longrieco *et al.* 2002). In Europe, comprehensive study on the pathogen of ear rot of maize, *F. graminearum*, was conducted including Austria (Krska *et al.* 1996), Poland (Lew *et al.* 1996) and the Czech Republic (Nedelnik 2000).

In Malaysia *F. proliferatum* has been isolated from samples of maize the most frequently. Through experience working with morphological characteristics of *Fusarium* spp. in the section Liseola and other sections, considerable mistakes can be made by many, even experienced researchers, particularly in distinguishing *F. proliferatum* and *F. verticillioides.* However, both species can be distinguished by observing the conidiophore formation. Conidia of *F. proliferatum* arise from the monophialides and polyphialides conidiophores, while conidia of *F. verticillioides* are produced only from monophialides (Leslie & Summerell 2006). *F. verticillioides* was reported to be a pathogen on maize and can be found throughout the world wherever the plant is cultivated including Africa (Fandohan *et al.* 2003). With respect to morphological characteristics, *F. verticillioides* is also intermediate between *F. nygamai* and *F. thapsinum* and the macroconidia of *F. verticillioides* could be mistaken as these two species (Leslie & Summerell 2006). However, *F. verticillioides* and *F. thapsinum* do not produce chlamydospores (Nelson *et al.* 1983; Leslie & Summerell 2006). Thus, this character was used to distinguish between *F. verticillioides* and *F. nygamai.* However, differentiation between

Table 1. The frequency of occurrence of the *Fusarium* species obtained from maize in Malaysia

Fusarium species	Maize-growing areas (%)							Percentage	Total isolates obtained from maize bracts	Total isolates obtained from maize ear
	Lanchang, Pahang	Cameron Highlands, Pahang	TPU, UPM, Selangor	Seri Medan, Johor	Senggarang, Johor	MARDI, Pulau Pinang	Kota Kinabalu, Sabah			
F. equiseti	–	0.6	–	–	–	–	–	0.6	1	–
F. pseudograminearum	–	11.4	–	–	–	–	–	11.4	10	9
F. oxysporum	–	5.9	1.2	–	–	1.2	–	8.4	12	2
F. proliferatum	1.8	–	12.6	2.9	1.8	10.8	–	29.9	7	43
F. semitectum	–	2.4	10.8	–	0.6	3.6	4.8	22.2	8	29
F. solani	–	–	–	–	–	1.2	–	1.2	-	2
F. subglutinans	6.6	0.6	4.8	–	–	0.6	–	12.6	5	16
F. verticillioides	–	0.6	6.6	0.6	1.8	4.2		13.7	6	17
Total number of isolates	14	36	60	6	7	36	8	167 isolates	49	118
Number of samples	34	85	143	14	17	86	19	398 samples	133	263 samples

TPU – University Agricultural Park; UPM – Universiti Putra Malaysia; MARDI – Malaysian Agricultural Research and Development Institute

F. verticillioides and *F. thapsinum* could be accomplished only by observing the pigmentation and on the basis of the mating population study (Leslie & Summerell 2006).

F. equiseti is a cosmopolitan soil inhabitant that has been recovered from many parts of the world from cool and temperate to hot and arid regions, primarily as a saprophyte or secondary invader (Leslie & Summerell 2006). This species has been isolated from pumpkin (Elmer 1996), cucurbit fruits (Adams *et al*. 1987) and date palms (Abbas *et al.* 1991). However, no report has shown that the species is pathogenic to maize plants. In the present study, *F. equiseti* was obtained only from Cameron Highlands, Pahang.

F. pseudograminearum was found in maize plant from the site Cameron Highlands, Pahang. This is possibly due to the related species *F. graminearum*, which was also reported to grow well in cool climate such as in that place (Darnetty *et al.* 2008). The Malaysian climate is typically hot and humid, with average day temperature of 33°C and average night temperature of 25°C. Compared to that of Cameron Highlands, the average day temperature is 23°C and average night temperature is 10°C, and is the optimal temperature for the good growth of *F. pseudograminearum.* The presence of *F. pseudograminearum* associated with maize is the first report in Malaysia.

A total of 14 isolates of *F. oxysporum* were obtained from Pahang, Selangor and Pulau Pinang. Previously, the species was reported as a saprophyte on maize in Malaysia and Indonesia (Darnetty *et al.* 2008), however it is also known as an important vascular wilt pathogen on various plants, namely cotton (Khalil *et al.* 2003), tomato (Larkin & Fravel 1998), potato (Kim *et al.* 1995), soybean (Helbig & Carroll 1984), asparagus (Al-Amodi 2006) and banana (Fernández-Falcón *et al.* 2003).

F. semitectum is a cosmopolitan species (Nelson *et al.* 1983). In the present study, the species is the second most frequently isolated *Fusarium* species from maize samples from Malaysia. The species is regularly found as secondary invader on diseased tissues (Summerell *et al.* 2003) and frequently isolated from aerial plant parts in subtropical and tropical regions (Leslie & Summerell 2006). It is frequently isolated from soils (Burgess *et al.* 1994) 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). Besides that, *F. semitectum* has also been reported as an important plant pathogen causing pod and corky dry rot of melons (Carter 1979).

F. solani is also a cosmopolitan species on a wide range of substrates (Nelson *et al.* 1983). It is frequently recorded from soils (Leslie & Summerell 2006) and it is known as the pathogen of a large number of plant species, especially trees that showed canker and dieback symptoms (Nelson *et al.* 1983). Darnetty *et al.* (2008) reported that *F. solani* associated with Fusarium ear rot diseases of maize were obtained from Perlis, Malaysia and West Sumatra, Indonesia. In addition, the present study shows that the two isolates of *F. solani* were only obtained from Malaysian Agricultural Research and Development Institute, Pulau Pinang.

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