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Isolation and Identification of *Fusarium* Species Associated with Fusarium Ear Rot Disease of Corn

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ABSTRACT

Fusarium species associated with corn (*Zea mays*) are very diverse and widely distributed throughout Malaysia. Out of 657 samples cultured, a total of 220 *Fusarium* isolates were obtained from corn plants showing the typical symptoms of Fusarium ear rot in 12 locations throughout Malaysia. All the isolates were identified into 10 species based on the morphological characteristics that emphasized on growth rates, colony features and microscopic characteristics. A total of 117 *Fusarium* isolates were classified into four species in the section Liseola and their allied, tentatively identified as *F. proliferatum* (58), *F. subglutinans* (34), *F. verticillioides* (24), and *F. nygamai* (1). Meanwhile, *F. proliferatum* was the most prevalent species in all the sampling areas. 103 isolates, which were classified into six other *Fusarium* species belonging to different sections, were also isolated and identified, and these included *F. semitectum* (47), *F. oxysporum* (20), *F. pseudograminearum* (19), *F. solani* (15), *F. equiseti* (1), and *F. longipes* (1). *F. semitectum* was the highest among other common saprophytic fungi in corn. *F. pseudograminearum* was only isolated from the samples obtained from Cameron Highlands, Pahang. In term of species diversity, *Fusarium* species was the highest obtained in Semenyih, Selangor, with H`=1.72.

Keywords: *Fusarium* species, Fusarium ear rot, diversity, corn, morphology

INTRODUCTION

Genus *Fusarium* is classified under phylum Ascomycota and ubiquitous fungi that are extensively distributed worldwide, from the temperate to the tropical regions (Leslie & Summerell, 2006). *Fusarium* species are commonly reported as endophytes, saprophytes, and pathogens of various plants, especially economically important crops, including corn (Nelson *et al.*, 1983; Burgess *et al.*, 1994). Corn (*Zea mays*) belongs to dicotyledonous angiosperm plant and it is a member of the grass family Poaceae.

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Fusarium species can cause plant diseases, such as wilt, rot, abnormal growth and decay, on vegetables, wood, herbs, and ornamental plants. Besides being plant pathogens, *Fusarium* species may also produce secondary metabolites, such as mycotoxins (i.e. beauvericin, fumonisins, and moniliformin), as well as phytotoxins (fusaric acid and gibberallic acid) problems (Booth, 1971; Summerell *et al*., 2003). Fumonisins and moniliformin can cause serious mycotoxicoses on humans and animals (Summerell *et al*., 2003), whereas, fusaric acid and gibberellic acids can respectively lead to stunting and abnormal elongation on the growth of plants.

Meanwhile, the *Fusarium* species in the section Liseola and their allies have been reported as potential pathogens on corn and caused Fusarium ear rot disease. However, the identity and aetiology of this pathogen is still highly debated. Therefore, this study was conducted to isolate and identify the *Fusarium* from the samples showing typical symptoms of the disease. Recently, *Fusarium* species such as *F. proliferatum*, *F. oxysporum*, *F. nygamai*, *F. semitectum*, *F. solani*, *and F. verticillioides* were successfully isolated from corn showing typical symptoms of ear rot disease in four states of Malaysia, namely, Perlis, Pulau Pinang, Sabah and Sarawak (Darnetty *et al*., 2008). However, no report is available on the distribution and diversity of *Fusarium* isolates obtained from the west coast (Selangor), east coast (Pahang) and Southeast areas (Johor) of Peninsular Malaysia.

MATERIALS AND METHODS

Corn Samples

A total of 657 corn samples were obtained from 12 main corn growing areas throughout Malaysia. All the samples were surface sterilized with 10% Clorox® and rinsed in several changes of sterile water.

The Isolation of Fusarium *Isolates and Monospore Culture*

The samples were placed on semi-selective medium for *Fusarium*, peptone pentachloronitrobenzene agar (PPA) as described by Nash & Snyder (1962), and incubated for 7 days under standard growth conditions (Salleh & Sulaiman, 1984). The cultures were single-spored following a standard protocol by Burgess *et al.* (1994). After 7 days of incubation, the cultures were transferred onto potato dextrose agar (PDA) and carnation leaf agar (CLA; Fisher *et al*. 1982) for species identification.

Morphological Characteristics and Species Identification

The cultures on PDA were used for observing the macroscopic characteristics such as colony features, growth rate and pigmentation. For microscopic characterization, pure cultures were transferred onto CLA and soil extract agar (SEA; Klotz *et al*., 1988). The microscopic characteristics such as conidia ontogeny, as well as the presence of chlamydospores and types of conidiophores were examined following the procedure by Burgess *et al*. (1994) and Leslie & Summerell (2006). The observations were done using a light microscope (Olympus model BX-50F4) and photographed using a JVC camera model KY-F55BE, with an image analyzer-SIS programme. The *Fusarium* isolates were identified based on the morphological characteristics into species level following Nelson *et al.* (1983), Burgess *et al*. (1994) and the Fusarium Laboratory Manual (Leslie & Summerell, 2006). The pure cultures were maintained on water agar (WA) as short-term working cultures (Burgess *et al*., 1994).

Diversity of the Fusarium *Species*

The diversity of the *Fusarium* species isolated within Peninsular Malaysia was calculated based

on Shannon-Weiner Index (Spellerberg, 2008), as follows:

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

where: H' = value of Shanon-Weiner Index

$\sum$ 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 220 isolates of *Fusarium* were obtained from the corn plant samples showing typical symptoms of *Fusarium* ear rot disease in 12 locations throughout the main corn growing areas in Malaysia. The typical symptoms of *Fusarium* ear rots are a white to pink- or salmon-coloured mold (fungal mass), beginning anywhere on the ear or scattered throughout. Some infected ears show brown necrotic lesions at the end of the cob (*Figs. 1A-C*). Often, the decay begins with insect-damaged kernels, by corn borer or bird feeding as a first infection and is later infected by fungi as a secondary infection. Normally, the disease does not involve the whole ear or kernels, but a portion of the corn ear. The infected kernels are frequently appeared as tan or brown, or have white streaks of the fungal mycelia.

The *Fusarium* isolates were single-spored and tentatively identified into 10 species (namely, *F. equiseti*, *F. longipes*, *F. nygamai*, *F. oxysporum*, *F. pseudograminearum*, *F. proliferatum*, *F. semitectum*, *F. solani*, *F. subglutinans*, and *F. verticillioides*) as shown in Table 1. Among the 10 species, *F. proliferatum* (Section Liseola) was the most prevalent species and widely distributed (it was found to be present in seven locations examined with 58 isolates). On the contrary, three species, namely, *F. equiseti*, *F. longipes* and *F. nygamai*, were noted as the least frequent species with a single isolate each. The *Fusarium* species in the Section Gibbosum (*F. equiseti* and *F. longipes*) and Section Arthrosporiella (*F. semitectum*) were also isolated and have previously been reported to be frequently present as saprophytes on various plants including maize (Nelson *et al*., 1983, Summerell *et al*., 2003; Leslie & Summerell, 2006). However, *F. proliferatum*, *F. verticillioides*, *F. solani* and *F. oxysporum* commonly cause plant diseases on a variety of crops (Summerell *et al*., 2003; Leslie & Summerell, 2006).

The *Fusarium* species associated with the corn samples, showing the typical symptoms of Fusarium ear rot disease in Malaysia, are very

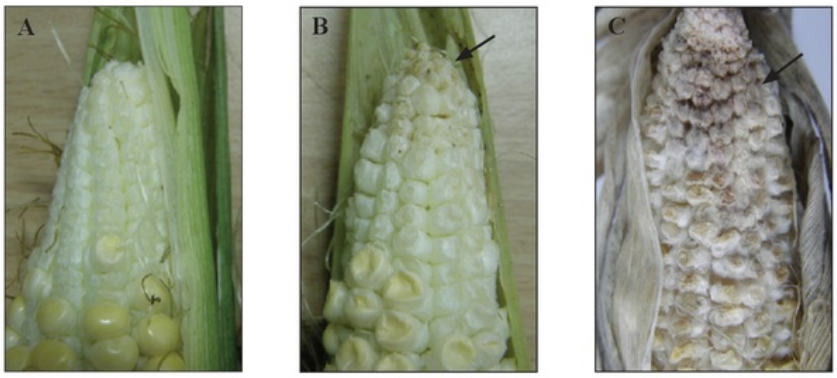

Fig. 1: Samples of the corn obtained from Cameron Highland, Pahang; A) Healthy corn; and B-C) infected cobs showing brown necrotic lesion on the ears (arrows). Scale bar = 0.7cm

diverse, based on Shanon-Weiner Index. The *Fusarium* species isolated from the samples obtained from Semenyih was the highest in term of its diversity, with H'= 1.72, and this was followed by Banting (1.46), TPU UPM and MARDI Pulau Pinang (1.41), Cameron Highland (1.25), Jerantut (0.69), Lanchang (0.51), and Seri Medan (0.46). The lowest index (0) was obtained by the samples from Jengka, Kuala Berang and Kota Kinabalu (Table 1).

The cultures were identified as the *Fusarium* species based on several diagnostic characteristics, such as the presence of chlamydospores, microconidia, macroconidia and the type of conidiophores. The number of macroconidia septation is variable, depending on the species. Meanwhile, *F. equiseti*, *F. longipes*, and *F. pseudograminearum* only produced macroconidia and without microconidia. Most of the species in Section Liseola and their allied (which were tentatively identified as *F. nygamai*, *F. proliferatum*, and *F. verticillioides*) produced microconidia in chains and/or false heads. Another archetypal characteristic of this particular section is that the majority of the species were unable to produce chlamydospores.

The most dominant species was morphologically identified as *F. proliferatum* that belongs to the section Liseola and their allied. This species has a worldwide distribution and is frequently isolated on an economically important plant including maize (Nelson *et al.*, 1983; Marasas *et al.*, 1984). Oláh *et al.* (2006) state that *F. proliferatum* is a weak pathogen of maize and it enters into host tissues during germination.

The second highest isolate is *F. semitectum* which is regularly found as a secondary invader diseased tissues (Summerell *et al.*, 2003), soils (Burgess *et al.*, 1994), as well as from diverse aerial parts of several plants, including maize (Andrés Ares *et al.*, 2004), asparagus

TABLE 1
The occurrence and frequency of *Fusarium* species isolated from corns in Malaysia

Fusarium species	Total (number of isolates)	Main corn growing areas											
		Jerantut, Pahang	Jengka, Pahang	Lanchang, Pahang	Cameron Highland, Pahang	Banting, Selangor	Semenyih, Selangor	TPU, UPM, Selangor	Seri Medan, Johor	Senggarang, Johor	Kuala Berang, Terengganu	MARDI, Pulau Pinang	Kota Kinabalu, Sabah
F. equiseti	1	-	-	-	1	-	-	-	-	-	-	-	-
F. longipes	1	-	1	-	-	-	-	-	-	-	-	-	-
F. nygamai	1	-	-	-	-	-	1	-	-	-	-	-	-
F. oxysporum	20	-	-	-	10	3	3	2	-	-	-	2	-
F. pseudograminearum	19	-	-	-	19	-	-	-	-	-	-	-	-
F. proliferatum	58	-	-	3	-	2	6	21	5	3	-	18	-
F. semitectum	47	-	-	-	4	1	9	18	-	1	-	6	8
F. solani	15	1	-	-	-	3	9	-	-	-	-	2	-
F. subglutinans	34	1	-	11	1	6	5	8	-	-	1	1	-
F. verticillioides	24	-	-	-	1	-	1	11	1	3	-	7	-
Percentage (%)	-	0.9	0.4	6.4	16.4	6.8	15.5	27.3	2.7	3.2	0.4	16.4	3.6
Total (n)	220	2	1	14	36	15	34	60	6	7	1	36	8
Shannon-Weiner Index	-	0.69	0.0	0.51	1.25	1.46	1.72	1.41	0.46	1.0	0.0	1.41	0.0

(Al-Amodi, 2006), kangaroo paw (Satou *et al.*, 2001), beans (Dhingra & Muchovej, 1979), sorghum (Gopinath *et al.*, 1985), millet (Mathur *et al.*, 1973; 1975) and potatoes (Kim *et al.*, 1995). Meanwhile, *F. verticillioides* has been reported as a pathogen on maize which causes epidemics of maize ear rot. The species can be found worldwide, or wherever maize is cultivated (Leslie & Summerell, 2006).

Three species (namely, *F. equiseti*, *F. nygamai*, and *F. longipes*) were also isolated from the maize samples showing typical symptoms of Fusarium ear rot disease which were previously recovered, particularly from diverse hosts. These species are cosmopolitan soil inhabitants that have been recovered from many parts of the world, primarily as saprophytes or endophytes (Nelson *et al.*, 1983; Summerell *et al.*, 2003; Leslie & Summerell, 2006) and have also been isolated from maize (Logrieco *et al.*, 1998). Similarly, Logrieco *et al.* (1998) have reported that those species produce beauvericin and may be one of the contaminants of maize.

A total of 14 isolates of *F. pseudograminearum* were obtained from Cameron Highlands in Pahang, whereby the day and night temperature range is 18-25°C. Based on the colony growth requirement, this species is categorized as a low temperature tolerates fungus, and therefore, the species is usually found in the temperate region. Moreover, *F. pseudograminearum* is morphologically and culturally indistinguishable from *F. graminearum,* whereby both species can only be differentiated by observing the formation of perithecia on media such as CLA. *F. graminearum* is homothallic and able to abundantly produce perithecia on the media, although *F. pseudograminearum* is not capable of producing perithecia (Leslie & Summerell, 2006). *F. pseudograminearum* and *F. graminearum* are important plant pathogens worldwide, including the maize disease. Diseases of cereals, including maize, caused by these species are responsible for large economic losses due to the reduction in seed quality and contamination of grain with their secondary metabolites that are known as mycotoxin (Russell *et al.*, 2005).

In conclusion, ten species of *Fusarium* were isolated from 12 locations in Malaysia and they were morphologically identified as *F. equiseti*, *F. longipes*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. pseudograminearum*, *F. semitectum*, *F. solani*, *F. subglutinans*, and *F. verticillioides*. This is a rather comprehensive report on the diversity of the *Fusarium* species associated with corn in Malaysia and the role of these fungi in causing plant diseases; nonetheless, their biological species and toxigenicity still require further investigation.

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