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Distribution, Morphological Characterization and Pathogenicity of *Fusarium sacchari* Associated with Pokkah Boeng Disease of Sugarcane in Peninsular Malaysia

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ABSTRACT

Pokkah boeng disease on sugarcane has been recorded in almost all countries where sugarcane is grown commercially. The objectives of this study were to survey the distribution of *Fusarium sacchari* associated with pokkah boeng disease throughout Peninsular Malaysia, to isolate and identify the causal organisms by using morphological characteristics, and to ascertain the pathogenicity of *F. sacchari* based on Koch's postulates. A total of 58 strains of *F. sacchari* were obtained throughout sugarcane plantations, small holders and household compounds within seven states i.e. Kedah, Perlis, Penang, Kelantan, Terengganu, Pahang and Johor in Peninsular Malaysia. The highest number of *F. sacchari* strains was obtained from Kedah (48%), followed by Perlis (25%), Penang (3%), Pahang (3%), Kelantan (5%), Terengganu (8%) and Johor (8%). For identification of *F. sacchari*, carnation leaves agar (CLA) and potato dextrose agar (PDA) media were used with emphasis for characterizations of colony features, growth rates, shapes and sizes of macroconidia and microconidia, conidiogeneous cells and chlamydospores. In plant house pathogenicity tests, healthy seedlings of sugarcane cultivar PS-81-362 were inoculated by injection and soaking techniques with conidial suspension ($2x10^6$ conidia/ml) of selected strains of *F. sacchari, F. proliferatum and F. subglutinans*. All strains of F. sacchari tested were pathogenic to sugarcane plants with disease severity index (DSI) varying from 0.3 to 5.0 (0 for no visible symptoms and 5 for plants with symptoms of twisted, wrinkled and shortened leaves or death). There were no significant ($p>0.05$) difference in DSI caused by strains of *F. sacchari* on variety PS-81-362 for both inoculation techniques, although they were significantly different compared with the control. This knowledge would be invaluable in developing our understanding on the interaction between *F. sacchari* with the host plants.

Keywords: Sugarcane, pokkah boeng, *Fusarium sacchari*

INTRODUCTION

In Peninsular Malaysia, the largest sugarcane plantations are situated in Felda Chuping, Perlis and Gula Padang Terap, Kedah for local consumption. The increased importation of sugarcane and other sugar crops from 5,304.48 tonnes in 1990 to 10,491.73 tonnes in 2004 based on the Food and Agriculture Organization of the United Nations (FAO) statistics indicates the demand for sugar in Malaysia. One of the limiting factors in sugarcane production is diseases caused by fungi, bacteria, viruses and

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nematodes that affect the quantity and/or quality of harvested crops (Edgerton 1955; Martin *et al.*, 1961; Sharma, 2006). Moreover, one or more diseases can occur on virtually every sugarcane plant in the field (Barnes, 1974; Hideo, 1988). Pokkah boeng (a Javanese word) is one of the most important diseases of sugarcane in Southeast Asia (Benigno and Quebral, 1977; Giatgong, 1980; Salleh, 1994, 2007; Semangun, 1988; Singh, 1980) and the world over (Agrios, 2005). Provided the environment is conducive, the disease can cause significant quality reduction in varieties with high sugar yields (Dohare *et al.*, 2003; Duttamajumder *et al.*, 2004). Approximately 40.8 - 64.5% sugars can be reduced from sugarcanes infected with pokkah boeng disease, depending on the cultivars (Dohare *et al.*, 2003).

Fusarium sacchari in the Section Liseola is generally responsible for causing this disease (Egan *et al.*, 1997; Nirenbergh and O'Donnell, 1998; Leslie and Summerell, 2006). The pathogen is transmitted by air currents (Martin *et al.*, 1961; Raid and Lentini, 1991) and airborne spores will colonize the leaves, flowers and stems of the plant (Burgess, 1981). The curve structure of macroconidia of *Fusarium* species can easily be dispersed by rain splash (Deacon, 2006).

The objectives of this study were to survey the distribution of *F. sacchari* associated with pokkah boeng disease throughout Peninsular Malaysia, to isolate and identify the causal organisms based on morphological characteristics, and to ascertain the pathogenicity of *F. sacchari* based on Koch's postulates. Data from the distribution, morphological characteristics of causal organism, and the pathogenicity test on healthy sugarcane will provide integrated information in formulating feasible control measures against the disease in Malaysia.

MATERIALS AND METHODS

Morphological Characteristics and Identification

Samples of sugarcane plants with pokkah boeng symptoms were observed and collected from sugarcane plantations, small holders and household compounds in seven states Kedah, Perlis, Penang, Kelantan, Terengganu, Pahang and Johor in Peninsular Malaysia between November 2004 and March 2005 (Table 1).

Fifty-eight single-spored cultural strains of fungi grown on PDA (potato dextrose agar) and CLA (carnation leaves agar) plates were used for identification based on morphological characteristics. PDA was used to measure fungal growth rates and pigmentation, while CLA was used to determine the shapes and sizes of macroconidia and microconidia, conidiogenous cells and chlamydospores formation. Morphological characteristics on CLA were observed after 7 - 10 days of incubation under standard growth conditions (Salleh and Sulaiman, 1984). The identification of *F. sacchari* was done based on the taxonomic guidelines by Nirenberg and O'Donnell (1998) and Leslie and Summerell (2006).

Pathogenicity Tests

The experiment was conducted on PS-81-362 sugarcane seedlings variety (provided by Gula Padang Terap Plantation in Kedah) between September 2005 and March 2006 in the plant house at the School of Biological Sciences, Universiti Sains Malaysia (USM). Each strain of *F. sacchari, F. proliferatum and F. subglutinans* isolates were grown on PDA plates as described by Salleh and Sulaiman (1984). Conidial suspension of each strain was prepared by pouring sterile distilled water onto 7 day-old cultures, shaken thoroughly, pooled, and the concentration was adjusted to $2x10^6$ conidia/ml by using a haemocytometer. Apparently healthy stalks were cut into a single-bud with one node. The stalks were surface-disinfected by dipping in 0.26% NaOCl solution for 30 min before inoculation with the inoculum.

Inoculation was carried out first by injecting the suspension (5 ml/plant) into young spindle leaves with a sterile syringe needle. In the second technique, single-bud stalks (one-node) were soaked overnight at room temperature in 500 ml of the inoculum suspension in a plastic container. The single-bud stalk pieces were then planted in sterile garden soil (autoclaved for 20 min at 1.05 $kgcm^{-2}$). All control plants were inoculated with sterile water. Every treatment was replicated three times. After 15 days of inoculation (dai), the plants were checked twice a week for six months in the plant house. The day temperature was 30.3 - 35.1°C and the night temperature was 23.3 - 30.6°C. All tissues of sugarcane plants inoculated with *F. sacchari* strains that showed pokkah boeng symptoms and controls were re-isolated for *F. sacchari* and re-identified as described above.

TABLE 1
Source of selected strains of *F. sacchari* used in pathogenicity tests

Species	Strain no.	Locality
F. sacchari	K3257U	Gula Padang Terap, Kedah
	K3268U	Gula Padang Terap, Kedah
	K3271U	Felda Chuping, Perlis
	R3287U	Felda Chuping, Perlis
	K3305U	Gula Padang Terap, Kedah
	D3325U	Rantau Panjang, Kelantan
	K3352U	Baling, Kedah
	T3334U	Setiu, Terengganu
F. proliferatum	K3240U	Gula Padang Terap, Kedah
	K3241U	Gula Padang Terap, Kedah
	K3245U	Gula Padang Terap, Kedah
	C3336U	Kuantan, Pahang
	C3340U	Kuantan, Pahang
F. subglutinans	K3267U	Gula Padang Terap, Kedah
	K3270U	Gula Padang Terap, Kedah
	K3308U	Kupang, Kedah
	R3276U	Felda Chuping, Perlis
	R3281U	Felda Chuping, Perlis

Disease Assessment

The symptoms were scored based on disease scale from 0 to 5 by following the scoring system devised by Elmer (2002) (Table 2) with slight modifications for sugarcane at 15, 30, 60, 90, 120, 150 and 180 days after inoculation (dai).

Disease Severity Index (DSI) was calculated by using the disease scale as follows:

$$DSI = \frac{\sum(A \times n)}{\sum B} \times \frac{100}{6}$$

A = disease scale (0, 1, 2, 3, 4 or 5)
n = number of stalks for each disease scale
B = total number of stalks

Statistical Analysis

DSI was analysed using non-parametric statistics; Friedman test and Mann-Whitney tests ($P<0.05$) were used to compare the inoculation techniques (soaking and injection) using SPSS programme version 11.5.

RESULTS AND DISCUSSION

The general observable symptoms of pokkah boeng in the field are yellowing or chlorosis of young leaves with some showing red specks at early stages of infection, followed by crumpled and twisted leaves. These symptoms were mostly found in Padang Terap, Kedah and Chuping, Perlis, the two biggest sugarcane plantations in Peninsular Malaysia. Throughout the samplings, the sugarcane leaves were the main parts that showed noticeable symptoms of pokkah boeng. This occurred due to the pathogen involved being reported to be an air borne fungi (Martin *et al.*, 1961; Raid and Lentini, 1991). The highest numbers of strains (48%) were obtained from Kedah, followed by Perlis (25%), Terengganu (8%), Johor (8%), Kelantan (5%), Penang (3%) and Pahang (3%) from 58 strains (See *Fig. 1*).

TABLE 2
Disease severity index (DSI) used in disease assessment following Elmer (2002) with slight modifications for sugarcane

Disease scale	Symptom
0	no visible symptoms
1	slight chlorosis on young leaves
2	<10% of the leave areas showing chlorosis and/or 10% of the plant with twisted leaves symptoms
3	11% to 25% of the plant with twisted leaves symptoms
4	26 to 50% of the plant with twisted leaves symptoms and/or reddish specks develop within chlorotic parts
5	51 to 100% of the plant with twisted, wrinkled and shortened leaves symptoms or plant death

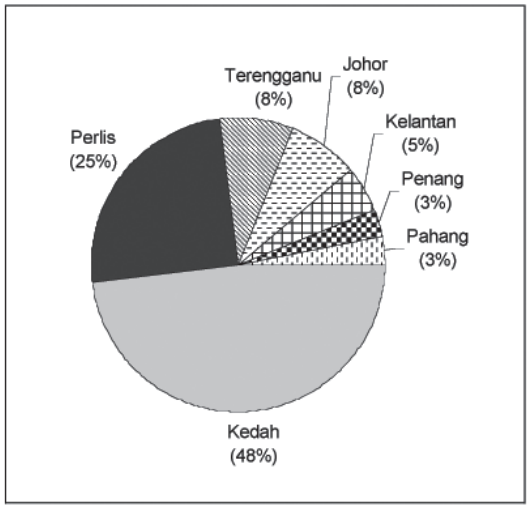

Fig. 1: Distribution of F. sacchari *according to states surveyed in Peninsular Malaysia*

All strains were identified as *F. sacchari* based on their morphological characteristics (*Fig. 2*). On PDA, the growth was rapid with abundance of mycelia that were colorless to pale violet and grayish violet. The pigmentations were colorless to grayish orange becoming purple with age. Colonies reached 2.3-3.7 cm in diameter at 25°C and 2.1-3.4 cm at 30°C after 3 days. Conidia produced on PDA were not uniform and not suitable for identification purposes. On CLA plates, *F. sacchari* were characterized by formation of microconidia that were produced abundantly in false-heads (*Fig. 2A*) from monophialides (*Fig. 2B*) and also polyphialides (*Fig. 2C*). The conidiophores were mostly branched at one level (*Fig. 2D*). Macroconidia (*Fig. 2E*) produced on CLA were sparse, slender, slightly falcate and thin-walled, 3 - 4 septate and the size ranges from 19.0 - 46.2 x 2.7 - 3.3 µm. The microconidia (*Fig. 2F*) were oval and slender 0 - 1 septate with sizes ranging from 5.4 - 15.0 x 1.9 - 4.1 µm. CLA is a natural substrate medium (Fisher *et al.*, 1982) that promotes sporulation rather than mycelial growth and at the same time false-heads can be seen directly in situ. On CLA plates the conidia were more abundant and uniform. The chlamydospores and microconidial chains were absent and these criteria are important in differentiating other Fusarium species in the Section Liseola (Nelson *et al.*, 1983).

Within six months of observation, the symptoms of pokkah boeng on young sugarcane plants variety PS-81-362 inoculated with different strains of *F. sacchari* varied. On day 15 until day 60 after inoculation, most of the inoculated sugarcane plants showed typical symptoms of pokkah boeng with chlorosis on the young leaves and some with twisted leaves. On day 90 until day 180 after inoculation, more twisted leaves appeared and reddish specks developed on the chlorotic parts. Some plants died and mycelium can be seen clearly at the nodes. Other young sugarcane plants which were inoculated with *F. proliferatum, F. subglutinans* and control plants remained healthy. There were no visible symptoms of pokkah boeng on sugarcane inoculated with *F. subglutinans* and *F. proliferatum* for both injection and soaking techniques. *F. sacchari* that were re-isolated from inoculated plants with pokkah boeng symptoms were identical to the original organisms isolated.

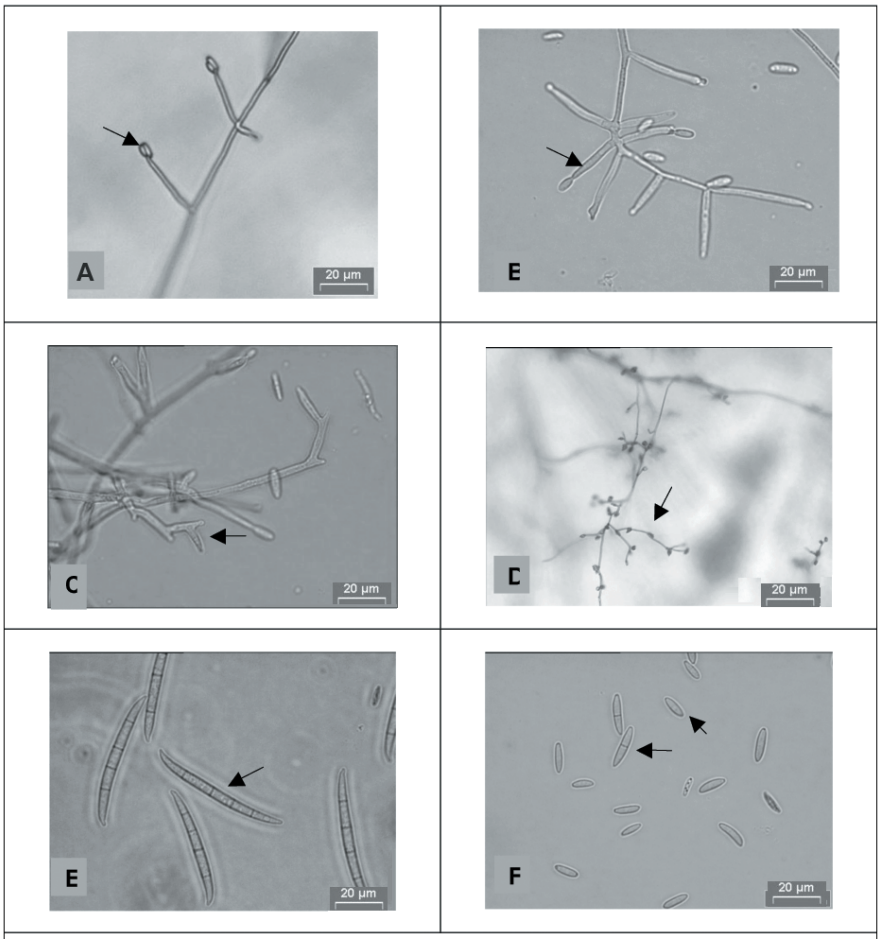

Fig. 2: Morphological characteristics of F. sacchari *from CLA; (A) Microconidia in false heads, (B) Simple monophialidic conidiophores, (C) Simple polyphialidic conidiophores, (D) Aerial mycelium with simple and prostrate conidiophores, (E) macroconidia with 4 septate, (F) 0 - 1 septate microconidia*

The DSI on variety PS-81-362 that was inoculated with strains of *F. sacchari* using injection (*Fig. 3*) and soaking (*Fig. 4)* techniques showed a steady increased in disease severity from 15 to 180 dai. Isolate R3287U showed the highest DSI for both techniques i.e. 4.3 for injection (*Fig. 3*) and 5.0 for soaking (*Fig. 4*) techniques. All strains of *F. sacchari* tested were pathogenic to sugarcane plants, as indicated by a mean DSI that was significantly different ($p<0.05$) from 15 to 180 dai, compared to that of control plants. There was no significant ($p>0.05$) difference in DSI between inoculation techniques by soaking and injection.

The results of inoculation tests in the plant house confirmed that pokkah boeng disease of sugarcane was caused by *F. sacchari*. The symptoms on inoculated plants were similar to those observed in the fields. The development of disease symptoms in the plant house was completed in six months when the syndromes of the typical pokkah boeng disease were observed. Sugarcane plants at actively growing stages are more susceptible to infection than the older canes (Martin *et al.*, 1961; Raid and Lentini, 1991). Therefore, susceptible sugarcane plants are easily infected with pokkah boeng disease during the early growth stages. The DSI for all sugarcane plants inoculated with different strains *F. sacchari* by both techniques steadily increased from 90-180 dai.

Both inoculation techniques were successful in inducing pokkah boeng symptoms on variety PS-81-362. Therefore, inocula at the concentration

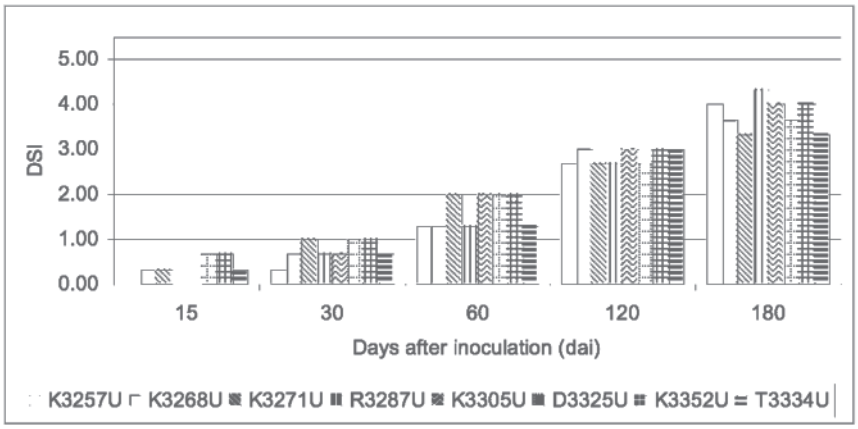

Fig. 3: Disease severity index (DSI) of young sugarcane plants var. PS-81-362 at different days after inoculation using injection technique with selected strains of F. sacchari

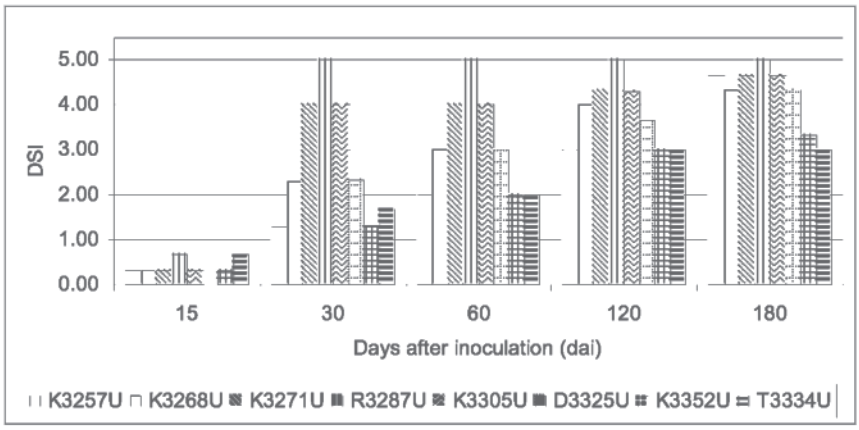

Fig. 4: Disease severity index (DSI) of young sugarcane plants var. PS-81-362 at different days after inoculation using soaking technique with selected strains of F. sacchari

of 2 x 10^6 conidia/ml injected into healthy plants appeared to be successfully transferred to the tender and fragile spindle leaves that facilitate pathogen penetration. Natural openings such as stomata and hydathodes on the leaves could also facilitate spore germination and penetration host of the tissues (Dickinson and Lucas, 1982; Agrios, 2005). For the soaking technique, dipping the plant into the inoculum suspension overnight provided direct exposure of the pathogen to the host making the penetration process easier. The duration of stalk immersion in the inoculum suspension influenced disease severity with longer immersion resulting in more severe symptoms (Aragaki, 1975).

Previous studies indicated that pokkah boeng disease on sugarcane was caused by *F. moniliforme var. subglutinans* in Peninsular Malaysia (Geh, 1973) but recent research reported that *F. sacchari* is the pathogen (Egan *et al.*, 1997; Nirenbergh and O'Donnell, 1998; Leslie and Summerell, 2006). *F. sacchar*i was successfully re-isolated from all symptomatic leaves that were inoculated in the plant house.

CONCLUSIONS

The study showed that pokkah boeng disease was widely distributed in growing sugarcane areas of in seven states i.e. Kedah, Perlis, Penang, Kelantan, Terengganu, Pahang and Johor, in

Peninsular Malaysia. Pathogenicity test results confirmed that the disease was caused by *F. sacchari*. The morphological characteristics that were described and derived from this research will assist in correct and quick identification of *F. sacchari* and other closely related *Fusarium* species in Section Liseola. The data from the distribution, morphological characteristics and the pathogenicity test on healthy sugarcane will provide integrated information in formulating feasible control measures against this disease in Malaysia.

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