Variability of *Fusarium* species Associated with Bakanae Disease of Rice based on their Virulence, Vegetative and Biological Compatibilities

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Bakanae is one of the most important diseases of rice in Southeast Asia. Variability of 212 strains of *Fusarium* associated with the disease in Malaysia and Indonesia were examined. Most of the strains (59.0%) were classified into five *Fusarium* species in section Liseola i.e. *F. fujikuroi* (the most frequent, 37.3%), followed by *F. verticillioides, F. proliferatum, F. sacchari* and *F. subglutinans*. Pathogenicity tests on a susceptible rice seedlings variety MR 211 confirmed that only isolates identified as *F. fujikuroi* were pathogenic and able to produce typical bakanae symptoms. Results on vegetative compatibility tests indicated that there was a substantial genetic diversity within the five species. Several isolates (2.4%) were classified as heterokaryon self-incompatible (HSI) based on their inability to form a heterokaryon. In crosses with seven standard testers of mating populations (MP-A to MP-G), 69.3% of the strains were assigned to five *Gibberella fujikuroi* species complex i.e. MP-A, MP-B, MP-C, MP-D and MP-E based on their ability to produce viable ascospores. The isolates of *F. fujikuroi* belong to MP-C. The present data provide baseline information for *Fusarium* species associated with bakanae disease of rice in Malaysia and Indonesia as well as for the genotypes involved in causing the disease on rice in this region.

Keywords: *Fusarium fujikuroi,* bakanae, vegetative compatibility group, virulence and mating population

Bakanae is a Japanese word that refers to an abnormal elongation frequently seen in the infected rice plants (Webster and Gunnell 1992). In Malaysia, the disease was first observed causing serious problems in 1985 during the second rice planting season in Kedah, Kelantan and Perak (Saad 1986). The disease is seedborne and caused by *F. fujikuroi* Nirenberg (Nirenberg 1976), the anamorph stage of *Gibberella fujikuroi* Sawada. Previous researchers e.g. Amoah *et al.* (1995, 1996), Nirenberg and O'Donnell (1998) and Desjardins *et al.* (2000) reported

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several species of *Fusarium* in the section Liseola were associated with the disease i.e. *F. verticillioides, F. fujikuroi* and *F. proliferatum* that belong to mating population (MP) A, C and D, respectively.

Morphological characteristics of *F. fujikuroi* strains are similar to *F. proliferatum* but they are distinguishable by the presence of pyriform microconidia of the latter species (Nirenberg and O'Donnell 1998). However, Leslie and Summerell (2006) reported the pyriform microconidia produced in both species. They can be distinguished by mating type and DNA sequences. Confusion on morphological characters occurred also in differentiating *F. subglutinans* and *F. sacchari*. The present study focuses also on mating type analyses, followed by a pathogenicity test and vegetative compatibility (VC).

VC also known as heterokaryon compatibility is another useful tool for identification and reveals the genetic diversity of several fungal genera, including *Fusarium* (Leslie 1993). VC was developed when hyphae of two strains formed anastomoses and fused to a stable heterokaryon. The strains are therefore classified in the same vegetative compatibility group (VCG; Leslie 1996), while those that cannot form heterokaryons are considered as vegetative incompatible and are therefore grouped in different VCGs. Strains of the same VCGs have similarity in genetic characteristics and share more traits than strains in different VCGs (Leslie 1996).

MP analysis clarified the taxonomy of *G. fujikuroi* species complex. In the last 25 years three different anamorphs of *G. fujikuroi* were reported by Nelson *et al.* (1983): *F. moniliforme, F. subglutinans* and *F. proliferatum*. *G. fujikuroi* consists of at least seven genetically distinct biological species or mating populations (Leslie 1995). MP-A and MP-F shared the *F. moniliforme* anamorph (Mansuestus *et al.* 1997). The concept of MPs had been up-dated and currently by Leslie and Summerell (2006): MP-A: *F. verticillioides* (*G. moniliformis*), MP-B: *F. sacchari* (*G. sacchari*), MP-C: *F. fujikuroi* (*G. fujikuroi*), MP-D: *F. proliferatum* (*G. intermedia*), MP-E: *F. subglutinans* (*G. subglutinans*).

In this paper we identified the strains of *Fusarium* isolated from rice plants showing typical bakanae symptoms in Peninsular Malaysia and Indonesia into species in the section Liseola based on morphological, vegetative and sexual characteristics.

Materials and Methods

Isolation of *Fusarium*

A series of surveys was conducted in 1987–1988 and 2004–2005 in 21 major rice production areas in Peninsular Malaysia and Indonesia. A total of 30 rice stem samples at the booting stage that showed typical bakanae symptoms were recovered for each production area. Isolations were conducted by surface-sterilization with 1% of sodium hy-

pochlorite (NaOCl) for 1 min, rinsed with several changes of sterile water and plated on peptone pentachloronitrobenzene agar (PPA) (Papavizas 1967). *Fusarium* cultures were purified by a single-spored isolation technique (Toussoun and Nelson 1968, Burgess *et al.* 1994) and incubated under standard growth conditions (Salleh and Sulaiman 1984). Isolations were made from a total of 630 stem pieces.

Identification using morphological characteristics

All single-spore *Fusarium* isolates were identified by observing the morphological characteristics grown on potato dextrose agar (PDA, Booth 1971), carnation leaf-pieces agar (CLA) (Fisher *et al.* 1982) and soil extract agar (SEA) (Klotz *et al.* 1988). The Methuen handbook of colour chart (Kornerup and Wancher 1978) was used to determine the pigmentation of the cultures on PDA. The morphological characteristics were observed following Burgess *et al.* (1994) and Leslie and Summerell (2006) under a light microscope (Olympus model BX-50F4) and photographed using a JVC camera model KY-F55BE with an image analyzer-SIS programme.

Vegetative compatibility group

Pure cultures were placed on a complete medium (CM) (Correll *et al.* 1987) for generation of actively growing colonies with a dense fungal growth. Plates of minimal medium (MM) and PDA containing 2.5% of $KClO_3$, hereafter called MMC and PDC respectively, were inoculated with 2 mm^2 mycelial discs taken from an actively growing colony. The colonies began to produce fan-like, chlorate resistant sectors (CRSs) after 7 days. The individual sectors from each colony were transferred to MM slants containing $NaNO_3$ as a sole nitrogen source. Sectors that produced thin expensive growth on MM were selected and considered to be *nit* mutants, and subsequently kept at 4° C for phenotyping. Those that grew densely as a wild type or reverted cultures were discarded.

The physiological phenotypes of the *nit* mutants were interpreted by their growth on modified-MM by replacing $NaNO_3$ with $NaNO_2$ (0.5 gL^{-1}), hypoxanthine (0.2 gL^{-1}) or ammonium tartrate (1.0 gL^{-1}). The plates were incubated in complete darkness and colony growth was scored after 4 days of incubation, and classified as *nit1, nit3* or NitM following Klittich and Leslie (1988).

Pairings of *nit* mutants were made on MM following a procedure described by Leslie (1993). The strains were classified as either heterokaryon self-compatible (HSC) that formed heterokaryon (robust growth) or heterokaryon self-incompatible (HSI) when the *nit* mutants were unable to form heterokaryon (only thin growth at intersection of the colonies) at the line of contact between the mutants. Only *nit* mutants of the HSC strains obtained from different parents were paired

with each other. Two complementing strains were termed vegetatively compatible and designed as members of the same VCG. Whereas, strains that did not complement each other were termed as vegetatively incompatible and assigned in different VCGs. VCGs of all *Fusarium* strains were identified by pairing the possible combinations of the *nit* mutants from each strain.

Pathogenicity test

The susceptible rice cultivar MR 211 seeds were provided by the Malaysian Agricultural Research and Development Institute (MARDI). The seeds were treated with benomyl-thiram, washed several times with sterile water, and soaked in 50 ml spore suspension (10^6 spores/ml) of 7 days-old cultures of *F. fujikuroi* (46 strains), *F. verticillioides* (9 strains), *F. proliferatum* (7 strains), *F. sacchari* (5 strains), and *F. subglutinans* (3 strains). Inoculated and control seeds (soaked in 50 ml sterile water) were sown on sterilized rice field soils (2 times at 15 psi for 20 min, 24 h intermittently) in plastic trays (33 × 23 × 10 cm). Fifteen seeds were planted in each tray with four replicates and arranged in a complete randomized design (CRD) in the plant house at the School of Biological Sciences, Universiti Sains Malaysia.

The seedlings were scored into disease scale based on: 0 = healthy and uninfected plants (no external symptoms); 1 = normal growth but leaves beginning to show yellowish-green; 2 = abnormal growth, elongated, thin and yellowish-green leaves; 3 = abnormal growth, elongated, chlorotic, thin and brownish leaves; and 4 = seedlings with fungal mass on the surface of infected plants or died. Disease severity index (DSI) was calculated at 40 day after inoculation (dai) following the procedure of Ooi (2002) with slight modifications for rice:

$$\text{DSI} = \frac{\sum (\text{number of plants in the specific scale x disease scale})}{\text{Total number of plants}}$$

$$= \frac{\sum (n \times 0) + (n \times 1) + (n \times 2) + (n \times 3) + (n \times 4)}{\text{Total number of plants}}$$

The tissues of all infected plants were plated onto PPA and the fungi were re-isolated and re-identified. The DSI were analysed by non-parametric technique (Friedman test) by using SPSS programme version 11.0.

Biological compatibility

Standard testers strains for each MP groups (MP-A to MP-G) were obtained from the Department of Plant Pathology, Kansas State University, U.S.A and used as one of the parent in entire crossing. Crosses were made on carrot agar (CA) following the standard protocols (Hsieh

et al., 1977; Klittich and Leslie, 1992). Strains expected as female parents were inoculated onto thick CA plates (90 × 15 mm) while strains serving as male parents were inoculated on CM slants (6 – 8 ml of CM in 16 × 150 mm test tubes) on the same day. After 7 days, conidia from the male parent strain were suspended in 3–5 ml of 2.5% Tween 60. Then, approximately one ml of the spore suspension was spread on the surface of the female parent on the CA plate, and worked into the mycelia of the female colony with a glass rod (hockey stick). The fertilized plates were incubated at 26 ± 1°C in an incubator (Vindon model S. No.15112) with cool-white and near-UV fluorescent tubes (approximately 1,900 lux). All strains were crossed with all seven standard mating-type testers of *G. fujikuroi* species complex. All crosses were inspected for formation of perithecia and viable ascospores during an incubation period of up to two months. Fertile strains were crossed and produce perithecia that exude a cirrhus of ascospores. Ascospores from representative crosses were plated out on water agar (WA) to demonstrate their viabilities. Results showed 82% of the ascospores are germinated and viable. Sterile strains will not be fertile in crosses with any of the standard mating-type testers in either the original or a replicated set of crosses, after being repeated at least twice.

Perithecia were observed *in situ* by using a compound microscope (FESEM Leo Supra model 50 VP Carl-Zeiss SMT). Asci and ascospores were observed through the mounted slides by using an Olympus light microscope model BX-50F4.

Results

Morphological characteristics

A total of 212 *Fusarium* isolates were obtained from the bakanae-infected rice plants in 21 major rice growing areas in Peninsular Malaysia and Indonesia. The isolates were identified based on their morphological characters into five species in Liseola while the other six species were *F. semitectum, F. oxysporum, F. solani, F. equiseti, F. longipes* and *F. chlamydosporum* that belong to other sections. The five *Fusarium* species in the section Liseola i.e. *F. fujikuroi, F. verticillioides, F. proliferatum, F. sacchari* and *F. subglutinans,* were the most frequently (59.9%) recovered from bakanae-infected rice plants in Malaysia. However, only three species of *Fusarium* in the section Liseola namely *F. fujikuroi, F. sacchari* and *F. verticillioides* were recovered from Indonesia.

All strains of *Fusarium* species associated with species in section Liseola produced pigmentations of white to dark violet. *F. fujikuroi, F. verticillioides* and *F. proliferatum* produced microconidia in chains (short, medium or long), and false heads with varying shapes such as oval to long oval, clavate with or without flattened base, or pyriform. However, *F. sacchari* and *F. subglutinans* only produced microconidia

in false heads. The macroconidia of the five *Fusarium* species were pedicellate, thin-walled, and almost straight; microconidiophores were monophialides with or without polyphialides. Chlamydospores were not produced by all species, but some strains of *F. verticillioides* produced big spherical cells, termed as swollen cells (pseudochlamydospores). The morphology of the swollen cells was variable and transverse septa were observed in some of the swollen cells.

Vegetative compatibility group

Spontaneous CRSs were readily recovered from 127 wild type strains of *F. fujikuroi, F. verticillioides, F. proliferatum, F. sacchari* and *F. subglutinans* when cultured and incubated for 7 days on both chlorate media; MMC or PDC. Majority of the CRSs generated on chlorate media did not have ability to utilize the nitrate and identified as *nit* mutants. However, some *nit* mutants reverted to wild-type growth (dense growth) and these mutants were therefore classified as *crn* mutants. Majority of the *nit* mutants recovered were *nit1*.

Physiological complementation between *nit* mutants with different loci of mutations was indicated by the development of dense growth where mycelia of the *nit* mutant colonies came in contact and anastomosed to form heterokaryons. The complementation occurred more rapidly and subsequent growth of the resulting heterokaryons was more robust in the pairings between NitM and *nit1* or NitM and *nit3* mutants than those between *nit1* and *nit3* mutants, where usually they formed weak heterokaryons (Figure 1). When *nit1* and *nit3* mutants were paired, complementation usually occurred after 2 – 3 weeks. In some pairings between *nit1* and *nit3* mutants recovered from the same parental strain, no heterokaryon was observed. Some NitM mutants were able to complement with one another, but no complementation was observed between any of the *nit1*, as could *nit3*.

Majority (97.6%) of the *Fusarium* strains were classified as HSC, however three strains of *F. fujikuroi* were identified as HSI. The strains of *F. fujikuroi, F. verticillioides, F. proliferatum, F. sacchari* and *F. subglutinans* were grouped into 26, 8, 8, 7 and 4 VCGs, respectively. Based on the number of VCG, variations were also observed within strains obtained from Kuala Selangor; where C115 to C120 *(F. fujikuroi)* were classified in different groups even though the isolates from the same sampling area. However, several VCGs comprised of strains from the same locations, e.g. the members of: A203 from Seberang Perak, Perak; A204 from Pasir Puteh, Kelantan; A205 from Tulong Agung, East Java; B401 from Pendang, Kedah; B402, B403 B404 and C110 from Rompin, Pahang; C103 and C105 from Padang, Sumatra; C104, C109 and D302 from Sungai Besar, Selangor; C107 from Merlimau, Melaka; C108 and D301 from Sekinchan, Selangor; C111 from Seberang Perai, Penang; C112 from Jabi, Terengganu; D303 and D304 from Tumpat, Kelantan.

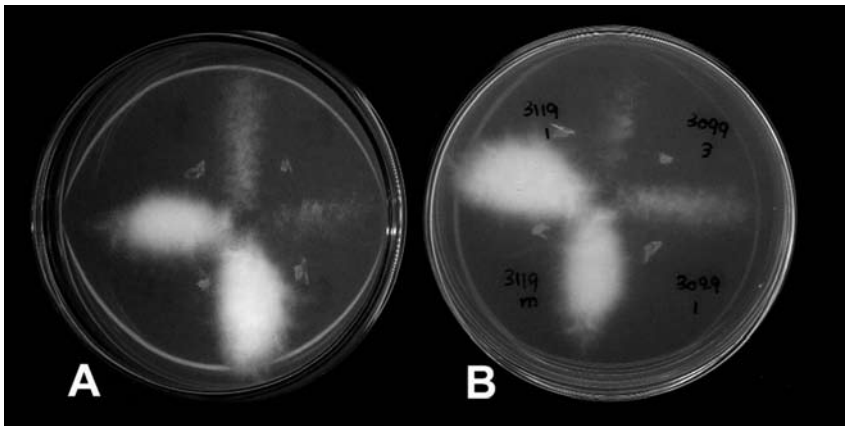

Fig. 1. Comparison between robust heterokaryon (*nit1* and NitM) and weak heterokaryon (*nit1* and *nit3*) in the same pairing (A = front, B = reverse)

Pathogenicity test

The first symptom on rice seedlings inoculated with *F. fujikuroi* was observed as early as 5 day after inoculation. The inoculated seedlings were several centimeters taller compared to healthy (control) seedlings, the leaves became thinner and yellowish followed by the whole leaf quickly turned brownish, then dried and died before mature. Several infected tillers produced wiry (stiff) adventitious roots at the first or second nodes. At the advanced stages of infection, the pathogenic fungi produced white mycelium and sometimes pinkish sporodochia on the stem just above water level, 10 – 40 day after inoculation. Through microscopic examination, the fungal mass consisted of a large number of conidia. However, some infected plants appeared normal until maturity but produced empty and discolored grains.

The highest DSI of 3.3 was shown by seedlings inoculated with strains R078 and followed by strains R042, R050 and R077 with DSI of 3.0. In contrast, a single strain (R048) that has been identified by morphological, VCG and MP characteristics as *F. fujikuroi* was non-pathogenic to rice with DSI of 0.1 and not significantly (P>0.05) different with the DSI of control seedlings.

Biological compatibility

In mating crosses, 69.3% of strains could be assigned to at least one of the seven MPs of *G. fujikuroi* species complex (MP-A to MP-E), where MP-C was the more frequent. Perithecia were produced within 2–3 weeks after spermatization (Figure 2). Out of 18 strains of *F. verticillioides,* 13 strains (72.2%) were crossed-fertile with MP-A tester and were assigned as *G. moniliformis*. In crosses with MP-B and MP-D

tester strains, 12 strains were crossed-fertile strains for each MP and belonged to *G. intermedia* and *G. sacchari*, respectively. Most of the strains were highly fertile, with dozens to hundreds of matured perithecia per plate. In contrast, only a single strain was crossed-fertile with MP-E tester that belonged to *G. subglutinans*.

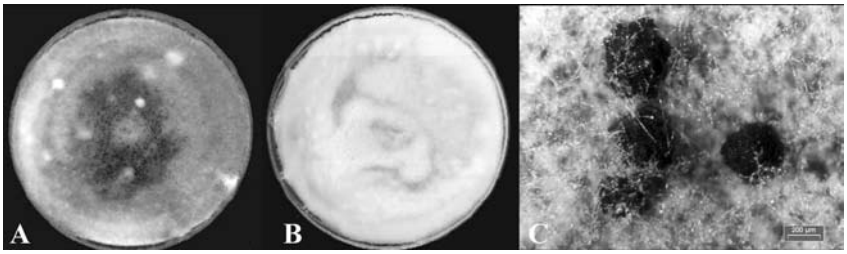

Fig. 2. Perithecia on CA plate, *in situ* observation. (A) positive-scored, perithecia were produced (black dots); (B) negative-scored, no perithecia were produced; (C) Perithecia of *G. intermedia*

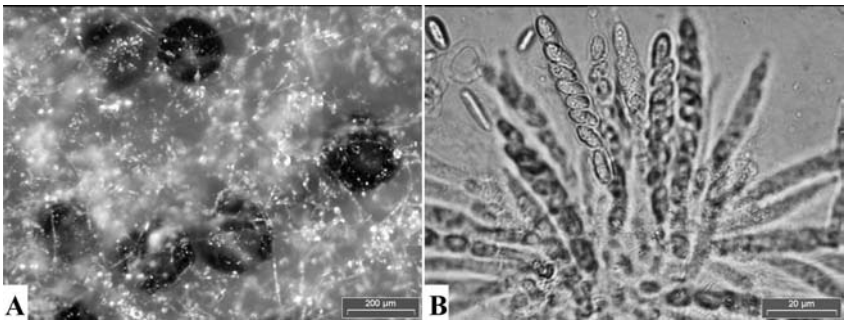

Fig. 3. Perithecia and ascospores of *G. fujikuroi* (MP-C). A) perithecia on CA, *in situ* observation; B) ascospores in asci produced by crossing strains R018 with C01996

Most of the MP-C showed low fertility with a few mature perithecia per plate. Perithecia were rare to abundant, usually with diameter 277.5 µm; ranging from 214.3 – 357.1 µm (Figure 3A), usually formed within 2 – 3 weeks after spermatization. Ascospores were oval-shaped with or without septate, usually 1-septate, 3.6 – 7.1 × 7.9 – 14.3 µm (Figure 3B). Most randomly selected ascopsores and plated on WA were viable.

Discussion

The results indicated that the species of *Fusarium* associated with rice plants were diverse. *F. fujikuroi* was the most frequent species isolated from rice showing typical bakanae symptoms in 21 major granary areas in Peninsular Malaysia and Indonesia. This species is prov-

Table 1. – Geographic locations, vegetative compatibility groups, disease severity index and mating populations of *Fusarium* strains isolated from rice with symptomatic bakanae disease

Species	Strain	Rice variety; geographic locations (town, state, country)	VCG	DSI	MP
F. fujikuroi	R005	Variety MR219; Tanjong Karang, Selangor, MY	C101	2.3[a]	–
	R006	Variety MR219; Tanjong Karang, Selangor, MY	C101	2.3[a]	–
	R007	Variety MR211; Sekinchan, Selangor, MY	C101	2.0[ab]	–
	R012	Variety MR211; Sekinchan, Selangor, MY	C101	1.8[b]	–
	R014	Variety MR211; Sekinchan, Selangor, MY	C101	NE	C+
	R017	Variety MR211; Sekinchan, Selangor, MY	C101	2.3[a]	–
	R019	Variety MR211; Sekinchan, Selangor, MY	C101	2.2[a]	–
	R026	Variety MR211; Sekinchan, Selangor, MY	C101	2.1[ab]	–
	R027	Variety MR211; Sekinchan, Selangor, MY	C101	NE	C+
	R028	Variety MR211; Sekinchan, Selangor, MY	C101	2.0[ab]	–
	R029	Variety MR211; Sekinchan, Selangor, MY	C101	2.0[ab]	–
	R031	Variety MR211; Sekinchan, Selangor, MY	C101	2.4[a]	–
	R032	Variety MR211; Sekinchan, Selangor, MY	C101	2.4[a]	C+
	R033	Variety MR211; Sekinchan, Selangor, MY	C101	NE	C+
	R034	Variety MR211; Sekinchan, Selangor, MY	C101	2.9[a]	C+
	R035	Variety MR211; Sekinchan, Selangor, MY	C101	2.8[a]	–
	R038	Variety MR211; Sungai Besar, Selangor, MY	C101	NE	C+, D+
	R040	Variety MR211; Sungai Besar, Selangor, MY	C101	NE	C+, D+
	R041	Variety MR211; Sungai Besar, Selangor, MY	C101	2.5[a]	–
	R042	Variety MR211; Sungai Besar, Selangor, MY	C101	3.0[a]	C+
	R044	Variety MR211; Sungai Besar, Selangor, MY	C101	NE	C+, D–
	R046	Variety MR211; Sungai Besar, Selangor, MY	C101	2.8[a]	–
	R050	Variety MR211; Sungai Besar, Selangor, MY	C101	3.0[a]	C+
	R004	Variety MR219; Seberang Perak, Perak, MY	C102	NE	C+
	R008	Variety MR211; Sekinchan, Selangor, MY	C102	NE	C+
	R009	Variety MR211; Sekinchan, Selangor, MY	C102	2.1[ab]	–
	R010	Variety MR211; Sekinchan, Selangor, MY	C102	1.7[b]	–
	R011	Variety MR211; Sekinchan, Selangor, MY	C102	NE	C–

Species	Strain	Rice variety; geographic locations (town, state, country)	VCG	DSI	MP
	R021	Variety MR211; Sekinchan, Selangor, MY	C102	NE	C+
	R022	Variety MR211; Sekinchan, Selangor, MY	C102	NE	C+
	R055	Variety MR219; Petaling, Kelantan, MY	C102	2.0[ab]	–
	R056	Unknown variety; Padang, Sumatra, ID	C103	NE	C+
	R059	Unknown variety; Padang, Sumatra, ID	C103	NE	C+
	R060	Unknown variety; Padang, Sumatra, ID	C103	NE	C+
	R061	Unknown variety; Padang, Sumatra, ID	C103	NE	C+
	R063	Unknown variety; Padang, Sumatra, ID	C103	NE	C+
	R045	Variety MR211; Sungai Besar, Selangor, MY	C104	NE	C+
	R047	Variety MR211; Sungai Besar, Selangor, MY	C104	NE	C+
	R048	Variety MR211; Sungai Besar, Selangor, MY	C104	0.1[c]	C+
	R049	Variety MR211; Sungai Besar, Selangor, MY	C104	2.0[ab]	–
	R057	Unknown variety; Padang, Sumatra, ID	C105	NE	C+
	R058	Unknown variety; Padang, Sumatra, ID	C105	2.8[a]	C+
	R062	Unknown variety; Padang, Sumatra, ID	C105	2.9[a]	C+
	R064	Unknown variety; Padang, Sumatra, ID	C105	NE	C+
	R066	Unknown variety; Kampung Paya, Kedah, MY	C106	1.8[b]	–
	R067	Unknown variety; Kampung Paya, Kedah, MY	C106	1.7[b]	–
	R069	Variety MR219; Pendang, Kedah, MY	C106	NE	C+ D–
	R070	Variety MR219; Pendang, Kedah, MY	C106	NE	C+ D–
	R071	Variety MR219; Merlimau, Melaka, MY	C107	2.0[ab]	C+
	R072	Variety MR219; Merlimau, Melaka, MY	C107	NE	C+
	R073	Variety MR219; Merlimau, Melaka, MY	C107	NE	C+
	R074	Variety MR219; Merlimau, Melaka, MY	C107	2.1[ab]	C+
	R015	Variety MR211; Sekinchan, Selangor, MY	C108	2.9[a]	C+
	R018	Variety MR211; Sekinchan, Selangor, MY	C108	2.5[a]	C+
	R036	Variety MR211; Sungai Besar, Selangor, MY	C109	2.9[a]	C+
	R037	Variety MR211; Sungai Besar, Selangor, MY	C109	NE	C+
	R051	Variety MR219; Rompin, Pahang, MY	C110	2.0[ab]	C+
	R052	Variety MR219; Rompin, Pahang, MY	C110	NE	–
	R075	Variety MR219; Seberang Perai, Penang, MY	C111	1.7[b]	–
	R076	Variety MR219; Seberang Perai, Penang, MY	C111	2.1[ab]	C+
	R078	Variety MR219; Jabi, Terengganu, MY	C112	3.0[a]	C+
	R079	Variety MR219; Jabi, Terengganu, MY	C112	3.3[a]	C+
	R001	Variety MR219; Seberang Perak, Perak, MY	C113	2.1[ab]	–
	R003	Variety MR219; Seberang Perak, Perak, MY	C114	2.2[a]	C–
	R013	Variety MR211; Sekinchan, Selangor, MY	C115	2.0[ab]	–

	R016	Variety MR211; Sekinchan, Selangor, MY	C116	2.5[a]	–
	R020	Variety MR211; Sekinchan, Selangor, MY	C117	NE	C+
	R024	Variety MR211; Sekinchan, Selangor, MY	C118	NE	C+
	R025	Variety MR211; Sekinchan, Selangor, MY	C119	2.0[ab]	–
	R030	Variety MR211; Sekinchan, Selangor, MY	C120	NE	C+
	R039	Variety MR211; Sungai Besar, Selangor, MY	C121	2.7[a]	–
	R043	Variety MR211; Sungai Besar, Selangor, MY	C122	2.6[a]	–
	R054	Variety MR219; Peringat, Kelantan, MY	C123	NE	C–
	R065	Unknown variety; Samarinda, Kalimantan, ID	C124	2.1[ab]	C+
	R068	Unknown variety; Kampung Paya, Kedah, MY	C125	NE	C+
	R077	Unknown variety; Cuping, Perlis, MY	C126	1.3[b]	C+
	R002	Variety MR219; Seberang Perak, Perak, MY	HSI	NE	C+
	R023	Variety MR211; Sekinchan, Selangor, MY	HSI	2.5[a]	–
	R053	Variety MR219; Peringat, Kelantan, MY	HSI	NE	C–
F. verticillioides	R084	Variety MR219; Seberang Perak, Perak, MY	A201	0.2[c]	A–
	R086	Variety MR219; Seberang Perak, Perak, MY	A201	0.1[c]	–
	R087	Variety MR219; Seberang Perak, Perak, MY	A201	0.2[c]	–
	R088	Variety MR219; Seberang Perak, Perak, MY	A201	NE	A–
	R089	Variety MR219; Seberang Perak, Perak, MY	A201	0[c]	–
	R093	Variety MR219; Pasir Puteh, Kelantan, MY	A201	NE	A–
	R090	Variety MR211; Tanjong Karang, Selangor, MY	A202	NE	A–
	R091	Variety MR212; Sekinchan, Selangor, MY	A202	NE	A–
	R092	Variety MR211; Sungai Besar, Selangor, MY	A202	NE	A–
	R081	Variety MR219; Seberang Perak, Perak, MY	A203	NE	A–
	R083	Variety MR219; Seberang Perak, Perak, MY	A203	0[c]	A–
	R094	Variety MR219; Pasir Puteh, Kelantan, MY	A204	0.2c	–
	R095	Variety MR219; Pasir Puteh, Kelantan, MY	A204	NE	A–
	R096	Unknown variety; Tulong Agung, East Java, ID	A205	0[c]	A+
	R097	Unknown variety; Tulong Agung, East Java, ID	A205	NE	A+
	R080	Variety MR219; Teluk Intan, Perak, MY	A206	0[c]	–
	R082	Variety MR219; Seberang Perak, Perak, MY	A207	0[c]	A–
	R085	Variety MR219; Seberang Perak, Perak, MY	A208	NE	A–
F. proliferatum	R099	Variety MR212; Sekinchan, Selangor, MY	D301	0[c]	D+
	R100	Variety MR212; Sekinchan, Selangor, MY	D301	NE	D+
	R101	Variety MR211; Sungai Besar, Selangor, MY	D302	NE	D+
	R102	Variety MR211; Sungai Besar, Selangor, MY	D302	NE	D+
	R106	Variety MR219; Tumpat, Kelantan, MY	D303	NE	D+

Species	Strain	Rice variety; geographic locations (town, state, country)	VCG	DSI	MP
	R107	Variety MR219; Tumpat, Kelantan, MY	D303	NE	D+
	R108	Variety MR219; Tumpat, Kelantan, MY	D304	0.2[c]	D+
	R109	Variety MR219; Tumpat, Kelantan, MY	D304	0.3[c]	D–
	R105	Variety MR219; Rantau Panjang, Kelantan, MY	D305	0.1[c]	–
	R110	Unknown variety; Jitra, Kedah, MY	D305	0[c]	D+
	R098	Variety MR219; Seberang Perak, Perak, MY	D306	0[c]	D–
	R103	Variety MR219; Rompin, Pahang, MY	D307	NE	D–
	R104	Variety MR219; Rompin, Pahang, MY	D308	0[c]	D+
F. sacchari	R121	Variety MR219; Pendang, Kedah, MY	B401	0[c]	B–
	R122	Variety MR219; Pendang, Kedah, MY	B401	NE	B–
	R123	Variety MR219; Pendang, Kedah, MY	B401	NE	B–
	R111	Variety MR219; Rompin, Pahang, MY	B402	NE	B–
	R114	Variety MR219; Rompin, Pahang, MY	B402	0[c]	B+
	R113	Variety MR219; Rompin, Pahang, MY	B403	0[c]	–
	R117	Variety MR219; Rompin, Pahang, MY	B403	NE	B+
	R115	Variety MR219; Rompin, Pahang, MY	B404	NE	B+
	R116	Variety MR219; Rompin, Pahang, MY	B404	0[c]	B+
	R119	Unknown variety; Samarinda, Kalimantan, ID	B405	0[c]	B+
	R120	Unknown variety; Samarinda, Kalimantan, ID	B405	NE	B+
	R112	Variety MR219; Rompin, Pahang, MY	B406	NE	B+
	R118	Variety MR219; Rompin, Pahang, MY	B407	NE	B–
F. subglutinans	R124	Variety MR219; Tanjong Karang, Selangor, MY	E501	0[c]	–
	R125	Variety MR219; Rompin, Pahang, MY	E502	NE	E+
	R126	Variety MR219; Rompin, Pahang, MY	E503	0.1[c]	–
	R127	Variety MR219; Tumpat, Kelantan, MY	E504	0[c]	–

DSI = Disease severity index; NE = Not examined; DSI with different alphabets donate significant different at $p<0.05$, the data were analysed within the strains at the same day after inoculation

MP = Mating population; Score of strains fertility, (–) indicated that the strains were infertile; A+ (*MATA-2*), A– (*MATA-1*), B+ (*MATB-2*), B– (*MATB-1*), C+ (*MATC-1*), C– (*MATC-2*), D+ (*MATD-2*), D– (*MATD-1*), E+ (*MATE-2*) and E– (*MATE-2*).

en to be pathogenic to rice seedlings in a plant house experiment. Abnormal elongation of infected plants indicates that the pathogen has the ability to produce gibberellic acid (GA_3). *F. fujikuroi* has been isolated not only from rice (Hsieh *et al.* 1977, Amoah *et al.* 1996, Kini *et al.* 2002) but also from maize (Hsieh *et al.* 1977, Leslie 1991, 1995), and sorghum (Correll *et al.* 1989, Mansuetus *et al.* 1997).

Morphological characteristics of *F. fujikuroi* strains such as pigmentations, growth rates and length of the microconidial chains were highly variable. Morphologically, *F. fujikuroi* is similar to *F. proliferatum* (Leslie and Summerell, 2006). These closely related species were distinguished by the formation of pyriform microconidia (Nirenberg and O'Donnell 1998). However, later the strains of both species also were able to produce pyriform microconidia, thus they usually can only be distinguished by mating types as well as DNA sequence (Leslie and Summerell 2006). Working with morphological characteristics of *Fusarium* spp. in the section Liseola, considerable errors can be made by many, even the experienced researchers, particularly in separating *F. fujikuroi* and *F. proliferatum*. This is not surprising due to the fact that *F. proliferatum* was also implicated in bakanae disease (Amaoh *et al.* 1995, 1996). Therefore, pathogenicity test, VCG and MP were used to separate both species as well as *F. verticillioides* from *F. fujikuroi*.

VCG can also be used in differentiating the species as the *nit* mutants from strains in different species did not pair with each other. Spontaneous CRSs were recovered from all wild-type strains of *F. fujikuroi* when cultured on MMC and PDC containing 2.5% chlorate as a toxic analogue of nitrate with thin expansive growth colonies. The number of CRSs obtained depends on the strains and the amount of chlorate in the medium (Lui and Sundheim 1996). *Nit* mutants that have been generated are usually unable to reduce chlorate to chloride because of a lesion at one or more loci that control reductase, thus rendering them as a chlorate-resistance (Correll *et al.* 1986). The classes of *nit* mutants presumably reflect mutations at a nitrate reductase structural locus (*nit1*); a nitrate assimilation pathway-specific regulatory locus (*nit3*), and the loci (at least five) that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM). Some chlorate-resistant mutants, however, reverted to wild-type (dense growth); these mutants were classified as *crn* mutants.

Majority of the strains were classified as HSC, however three strains of *F. fujikuroi* were identified as HSI. The lack of complementation observed might be due to a difference in inability to anastomose among the HSI strains. Observations on HSI have been conducted in several strains of *Fusarium* species such as *F. oxysporum* (Vakalounakis and Fragkiadakis 1999) and *F. graminearum* (McCallum *et al.* 2004). Correll *et al.* (1987) suggested that HSI might be due to anomaly of the *nit* mutants themselves, in the lack of anastomosis. In addition,

the lack of complementation has also been caused by a double mutation in some of the *nit* mutants (Papa 1986). On the other hand, Correll *et al.* (1989) reported the low frequency of hyphal fusions per mm^2 on MM after they were paired may also caused HSI. HSI strains have averaged 0.2 and 1.1 hyphal fusions per mm^2 compared to 6.9 and 8.1 fusions per mm^2 for HSC that formed heterokaryon normally. Correll *et al.* (1989) also reported that HSI strains branched less frequently, then they would be fewer anastomosed cell formed. The strains in the same VCG consist of the same allele at each incompatible locus. Different VCGs showed high genotypic diversity; member of the same VCG are usually assumed to be clonal, where VCGs provide a multilocus phenotype that is suitable for measuring genotypic diversity.

The *in vitro* fertility of some strains suggested that there is a potential for a high recombination to occur in the rice field. This is important because a high possibility of genetic recombination in the field could improve the genetic pool available for the pathogenic population of *Fusarium* species in the section Liseola. In mating crosses, MP-C was more dominant compared to the other MPs. MP-C of *G. fujikuroi* has been reported to occur on several hosts plants, predominantly rice, mostly from Southeast Asia (Hsieh *et al.* 1977, Kuhlman 1982) and it has also been reported from maize (Leslie 1991, 1995), date palm (Abdalla *et al.* 2000) and sorghum (Leslie 1991, 1995), where *F. fujikuroi* is considered the anamorph of MP-C. Five strains of *F. fujikuroi* could interbreed with MP-C and MP-D and completed the meiosis process to produce viable progenies. Leslie *et al.* (2004) reported that some strains of MP-C and MP-D (minority) were able to crossed-fertile (interbreed) and produced viable ascospores. The viability of ascospores from a few representative crosses or other indicators of low fertility and where strains were able to crosses with testers of more than one MP, the ascospores of the strains were plated out and showed the ascospores were viable.

Therefore, conidial ontogeny, VCG and DSI were used to identify the species of sterile strains. For example, R001, R013, R016, R023, R039 and R043 were identified as *F. fujikuroi* based on diagnostic elongation symptoms of bakanae disease that has been shown throughout the pathogenicity test in the plant house. The morphological characteristics combined with VCGs as well as sexual compatibility in these studies have provided important information on the characteristics and genetic diversity of *Fusarium* strains associated with bakanae disease of rice in Peninsular Malaysia and Indonesia.

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References

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

Amoah B.K., MacDonald M.V., Rezanoor H.N., Nicholson P. (1996) The use of random amplified polymorphic DNA technique to identify mating groups in the *Fusarium* Section Liseola. *Plant Pathology* 45: 115–125.

Amoah B.K., Rezanoor H.N., Nicholson P., MacDonald M.V. (1995) Variation in the *Fusarium* Section Liseola: pathogenicity and genetic studies of isolates of *Fusarium moniliforme* Sheldon from different hosts in Ghana. *Plant Pathology* 44: 63–572.

Booth C. (1971) The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey.

Burgess L.W., Summerell B.A., Bullock S., Gott K.P., Backhouse D. (1994) *Laboratory Manual for Fusarium Research*. University of Sydney.

Correll J.C., Klittich C.J.R., Leslie J.F. (1987) Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77: 1640–1646.

Correll J.C., Klittich C.J.R., Leslie J.F. (1989) Heterokayon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). *Mycological Research* 93*:* 21–27.

Correll J.C., Puhalla J.E., Schneider R.W. (1986) Vegetative compatibility groups among nonpathogenic root colonizing strains of *Fusarium oxysporum*. *Canadian Journal Botany* 64: 2358–2361.

Desjardins A.E., Manandhar H.K., Plattner R.D., Manandhar G.G., Poling S.M., Maragos C. M. (2000) *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. *Applied and Environmental Microbiology* 66: 1020–1025.

Fisher N.L., Burgess L.W., Toussoun T.A., Nelson P.E. (1982) Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151–153.

Hsieh, W.H. Smith S.N., Snyder W.C. (1977) Mating groups in *Fusarium moniliforme*. *Phytopathology* 67: 1041–1043.

Kini K.R., Leth V., Mathur S.B. (2002) Genetic variation in *Fusarium moniliforme* isolated from seeds of different host species from Burkina Faso based on Random amplified polymorphic DNA analysis. *Journal of Phytopathology* 150: 209–212.

Klittich C.J.R., Leslie J.F. (1988) Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* 118: 417–423.

Klittich C.J.R., Leslie J.F. (1992) Identification of a second mating population within the *Fusarium moniliforme* anamorph of *Gibberella fujikuroi*. *Mycologia* 84: 541–547.

Klotz L.V., Nelson P.E., Toussoun, T.A. (1988) A medium for enhancement of chlamydospore formation in *Fusarium* species. *Mycologia* 80: 108–109.

Kornerup A., Wancher J.H. (1978) *Methuen handbook of colour*. Eyre Methuen Ltd.

Kuhlman E.G. (1982) Varieties of *Gibberella fujikuroi* with anamorph in *Fusarium* Section *Liseola*. *Mycologia* 74: 759–768.

Leslie J.F. (1991) Mating populations in *Gibberella fujikuroi* (*Fusarium* Section Liseola). *Phytopathology* 81: 1058–1060.

Leslie J.F. (1993) Fungal vegetative compatibility. *Annual Review Phytopathology*, 31: 127–150.

Leslie J.F. (1995) *Gibberella fujikuroi* available populations and variable traits. *Canadian Journal of Botany* 73: 282–291.

Leslie J.F. (1996) Fungal vegetative compatibility – promises and prospects. *Phytoparasitica* 24: 3–6.
Leslie J.F. Summerell B.A. (2006) *The Fusarium Laboratory Manual*. Blackwell Publishing Ltd, UK.
Leslie J.F., Zeller K.A., Wohler M., Summerell B.A. (2004) Interfertility of two mating population in the *Gibberella fujikuroi* species complex. *European Journal of Plant Pathology* 110: 611–618.
Liu W., Sundheim L. (1996) Nitrate nonutilizing mutants and vegetative compatibility groups in *Fusarium poae*. *Fungal Genetic and Biology* 20: 12–17.
Mansuetus A.S.B., Odvody G.N., Frederiksen R.A., Leslie J.F. (1997) Biological species in the *Gibberella fujikuroi* species complex (*Fusarium* Section Liseola) recovered from sorghum in Tanzania. *Mycological Research* 101: 815–820.
McCallum B.D., Tekauz A., Gilbert J. (2004) Barrage zone formation between vegetatively incompatible *Fusarium graminearum* (*Gibberella zeae*) isolates. *Phytopathology* 94: 432–437.
Nelson P.E., Toussoun T.A., Marasas W.F.O. (1983) Fusarium *species: An Ilustrated Manual for Identification*. The Pennsylvania State University Press.
Nirenberg H.I. (1976) Untersuchungen über die Morphologische and Biologische Differenzierung in *Fusarium* – Sektion Liseola. *Mitt. Biol. Bundesansi. Land-Forstwirtsch. Berlin – Dahlem* 169: 1–117.
Nirenberg H.I., O'Donnell K. (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* 90: 434–458.
Ooi K.H. (2002) Characterization and chemical control of *Fusarium oxysporum*, a causal agent of vascular wilt disease of Roselle. *PhD. thesis*. Universiti Sains Malaysia, Malaysia.
Papa K.E. (1986) Heterokaryon incompatibility in *Aspergillus flavus*. *Mycologia* 78: 98–101.
Papavizas G.C. (1967) Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. *Phytopathology* 57: 848–852.
Saad A. (1986) Penyakit bakanae di Malaysia. *Berita Penyelidikan MARDI* 8: 15.
Salleh B., Sulaiman B. (1984) Fusaria associated with naturally diseased plants in Penang. *Journal of Plant Protection in the Tropics* 1: 47–53.
Toussoun T.A., Nelson P.E. (1968) *A pictorial guide to the identification of Fusarium species according to the taxonomic system of Snyder and Hansen*. The Pennsylvania State University Press, University Park and London.
Vakalounakis D.J., Fragkiadakis G.A. (1999) Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. *Phytopathology* 89: 161–168.
Webster R.K., Gunnell P.S. (1992) *Compendium of rice diseases*. The American Phytopathological Society Press, St. Paul, Minn.

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