

BAKANAЕ DISEASE OF RICE IN MALAYSIA AND INDONESIA: ETIOLOGY OF THE CAUSAL AGENT BASED ON MORPHOLOGICAL, PHYSIOLOGICAL AND PATHOGENICITY CHARACTERISTICS

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Abstract: Bakanae disease on rice has been recorded almost in all countries where paddy is grown commercially, especially in Asian countries, including Malaysia and Indonesia. Bakanae disease was widespread in Peninsular Malaysia and three provinces of Indonesia with the range of disease severity from scale 1 to 5 and disease incidence from 0.5 to 12.5% during 2004–2005 main growing seasons. A total of five *Fusarium* species belonging to section Liseola and their allied i.e. *Fusarium fujikuroi*, *F. proliferatum*, *F. sacchari*, *F. subglutinans* and *F. verticillioides* were isolated and identified on the basis of their morphological characteristics. Literature data showed that the bakanae disease of rice all over the world is caused by *F. fujikuroi* and probably some other *Fusarium* species from section Liseola or allied. However, from pathogenicity tests that have been carried out by using variety MR 211 of rice it was evident that *F. fujikuroi* was highly virulent and the only species involved in causing bakanae disease. Therefore, this species was the only one detected to be able to produce gibberellic acid – (GA_3) with R_f value 0.40 and 0.62, developed in solvent systems isopropanol:ammonia:water (10 : 1 : 1), v/v/v and chloroform:ethyl acetate:formic acid (5 : 4 : 1), v/v/v, respectively. This knowledge would be invaluable in developing our understanding on the interaction between *F. fujikuroi* and the host plants.

Key words: *Fusarium fujikuroi*, section Liseola, rice, bakanae disease, gibberellic acid

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INTRODUCTION

In nature, it is still not possible to discern between pathogenic and non-pathogenic *Fusarium* isolates, because some of the species although are not pathogenic but they are obviously associated with unhealthy plants and appear as saprophytes or endophytes (Nelson *et al.* 1983). This phenomenon also deals with an etiology of bakanae disease that is still under discussion. Most of the studies implicated that *F. fujikuroi* or other species in the section *Liseola* and their allied species are also involved in causing the disease. Initially, the pathogen responsible for the bakanae disease of rice was identified as *F. moniliforme* Sheldon (Snyder and Hansen 1945; Booth 1971; Nirenberg 1976) and later re-identified as *F. fujikuroi* Nirenberg (Nirenberg 1976), the anamorph of *Gibberella fujikuroi* Sawada. Some earlier phytopathologists, who only used morphological characters to distinguish the species, believed that *F. moniliforme* was the only species involved (Snyder and Hansen 1945; Nirenberg 1976; Nelson *et al.* 1983). Recent findings revealed conflicting results and suggested that other species of *Fusarium* in the section *Liseola* may be involved in infection of bakanae disease (Amoah *et al.* 1995, 1996; Desjardins *et al.* 1997, 2000).

Since the identity of bakanae pathogen seems to be still unclear, a pathogenicity test was conducted to verify whether *Fusarium* spp. from the section *Liseola* isolated from naturally infected rice plants in Malaysia and Indonesia could fulfill the Koch's postulate. The objectives of presented study were to survey the distribution of bakanae disease and to identify the causal agent of the disease basing on morphological and physiological characteristics and a pathogenicity test.

MATERIALS AND METHODS

Disease Incidence and Severity in the Field

A series of field samplings was conducted during 2004–2005 main growing seasons at 19 rice granary areas in six states throughout the Malaysian peninsula (Kedah, Perak, Selangor, Pahang, Terengganu and Kelantan) and three provinces in Indonesia (East Java, Samarinda and Padang). Disease symptoms and growth stages of rice at all sampling areas were observed at germination, seedling growth, tillering, booting, heading/anthesis, milky, dough, yellow-ripe and mature stages (MacLean *et al.* 2002). The disease incidence (%) was calculated as follows (Teng and James 2001), with slight modifications:

$$\text{Disease incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants in } 10 \text{ m}^2 \text{ plots}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Area of plants infected (m}^2\text{)}}{\text{Total area (m}^2\text{)}} \times 100$$

At least five plots (10 m²) were investigated in every sampling area. The disease incidence was later converted to disease severity scale (0–9) following IRRI Standards Evaluation System for rice, where 0 = no disease observed; 1 = less than 1%; 5 = 1–25% and 9 = 26–100% (Anon 1996).

Identification and Morphological Characteristics

Samples of rice plants with bakanae symptoms were collected and isolated on pentachloronitrobenzene agar (PPA). Thirty-four single-spore isolates grown on PDA (potato dextrose agar) and CLA (carnation leaf agar) plates were used for identification based on morphological characteristics. PDA was used to measure growth rate and pigmentation of culture, while CLA was used to determine shape and size of macroconidia and microconidia, conidiogenous cells and chlamydospore formation. Morphological characteristics on CLA were determined after 7–10 days of incubation under standard growth condition. The identification of *Fusarium* spp. was done basing on the taxonomic guidelines by Nirenberg and O'Donnell (1998) and Leslie and Summerell (2006).

Gibberellic Acid (GA₃) Detection

The isolates were cultured in 50 ml of sterile Richard's Solution in triplicate and incubated under static condition for 10 days (Johnston and Booth 1983). To the control flasks were supplemented with distilled water treated in the same manner. After ten days of incubation the medium was filtered through the filter paper with 0.7 µm of the pore size (Whatman no. 1) and pH of the filtrate was adjusted to 2.5 by using 1N HCl and extracted following Hasan (2002) with slight modification. The suspended residue was spotted on a silica gel TLC plate alongside with gibberellic acid standard (GA₃; Sigma) and positioned in vertical direction by using two specific solvent systems i.e. isopropanol:ammonia:water (10 : 1 : 1), v/v/v and chloroform:ethyl acetate:formic acid (5 : 4 : 1), v/v/v (Chang and Jacobs 1973; Hasan 2002). Visualization process was completed according to Hasan (2002) and the R_f values of GA₃ was calculated (Fessenden *et al.* 2001).

Development of Symptoms and Disease Severity Index (DSI)

A rice variety MR 211 was provided by the Malaysian Agricultural Research and Development Institute (MARDI), Pulau Pinang, Malaysia. Selected isolates representing *F. fujikuroi*, *F. proliferatum*, *F. verticillioides*, *F. sacchari* and *F. subglutinans* were cultured on PDA plates for 7 days under the standard growth conditions. Afterwards the plates were flooded with sterile water and obtained conidial suspensions were pooled and adjusted to 1 × 10⁶ conidia/ml. Rice seeds were treated with benomyl-thiram and soaked in 50 ml spore suspension of each strain for 12 h. Control (non-inoculated) seeds were soaked in the same amount of sterile water.

Inoculated and control seeds were sown on sterilized rice field soils (autoclaved at 121°C, 15 psi, 2 times 24 h intermittently) in plastic trays (33 × 23 × 10 cm). Fifteen seeds were planted in each tray with triplicate and arranged in a complete randomized design (CRD) in the greenhouse at the School of Biological Sciences, University Sains Malaysia with day and night temperatures of 30.3–35.1°C and 23.3–30.6°C, respectively. Seedlings were fertilized every two weeks with 3g/tray of N:P:K = 21 : 21 : 21 and irrigated daily with tap water.

External disease symptoms were continuously observed and the seedlings were scored according to the disease scale from 0 to 4 (Table 1). The disease severity index (DSI) was calculated for each treatment following Ooi (2002) with slight modifications of rice within 5, 10, 20, 30 and 40 days after inoculation. Disease Severity Index, DSI was calculated using on the following formula:

$$\begin{aligned}
 \text{DSI} &= \frac{\sum (\text{number of plants in the specific scale} \times \text{disease scale})}{\text{Total of plants}} \\
 &= \frac{\sum (n \times 0) + (n \times 1) + (n \times 2) + (n \times 3) + (n \times 4)}{\text{Total of plants}}
 \end{aligned}$$

After scoring, the plants tissues were used for the fungal re-isolation and re-identification for accomplishment of the Koch's postulate. The data of DSI were analysed by non-parametric technique (Friedman test) using SPSS programme version 11.0.

Table 1. Symptoms of rice plants were scored basing on disease scale 0–4

Disease scale	Disease symptoms
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; seedlings also shorter or taller than normal
3	abnormal growth, elongated; chlorotic, thin and brownish leaves; seedlings also shorter or taller than normal
4	seedlings with fungal mass on the surface of infected plants or died

RESULTS

Disease Incidence and Severity in the Field

All infected rice plants in the sampling areas showed typical bakanae symptoms expressed by abnormal and excessive growth comparing to healthy plants. There was an indication; however, that some varieties were resistant to bakanae. The leaves of infected plants were pale, thin, yellowish and slowly turned brownish and finally dried. Infected plants also produced stiff or wiry adventitious roots at lower nodes. In a more favourable environment, the fungus produced white or pink mycelium and abundant conidia in sporodochia on dried-up stems. Bakanae disease of rice was widespread (94.7% of the sampling areas) during 2004–2005 main growing seasons throughout the sampling areas, except for Yan, Kedah (Table 2). The highest disease severity of scale 5, was recorded in Rompin (Pahang), Sekinchan and Sungai Leman (Selangor), East Java and Padang (Indonesia). Disease incidence in Rompin (Pahang), Sungai Leman and Sekinchan (Selangor), East Java and Padang (Indonesia) was 12.5%, 5.3%, 2.5%, 5.0% and 12.0%, respectively. The granary area in Yan, Kedah showed 0% disease incidence and Samarinda, Kalimantan disease incidence was 0.1%.

Morphological Characteristics and Gibberellic Acid (GA₃) Production

Obtained isolates were identified as *F. fujikuroi*, *F. proliferatum*, *F. verticillioides*, *F. sacchari* and *F. subglutinans* based on their morphological characteristics on PDA and CLA (Table 3). Only *F. fujikuroi* isolates were able to produce GA₃ (Table 4). This feature could be consider as the main physiological character to distinguish *F. fujikuroi* from the other four species of *Fusarium* isolated from bakanae-infected rice in Malaysia and Indonesia. This is the only species of *Fusarium* that produced this

plant growth hormone causing abnormal elongation when presence in higher levels in infected plants. The range of R_f values were 0.322–0.509 (mean = 0.40) and 0.53–0.68 (mean = 0.62) for solvent systems isopropanol:ammonia:water (10 : 1 : 1), v/v/v and chloroform:ethyl acetate:formic acid (5 : 4 : 1), v/v/v, respectively.

Table 2. Disease severity (scale 0–9) of bakanae disease at 19 sampling areas in six states throughout the Malaysian peninsula and three provinces in Indonesia

Locality of granary areas		Growth stage of rice plants	Disease severity [scale 0–9] ^a	Disease incidence [%]
Malaysia	Langgar, Kedah	milky and mature	1	0.5
	Pendang, Kedah	milky and mature	1	0.9
	Tikam Batu, Kedah	milky and mature	1	0.5
	Yan, Kedah	tillering	0	0
	Seberang Perak, Perak	milky and mature	1	0.9
	Sungai Manik, Teluk Intan, Perak	milky and mature	1	0.5
	Sekinchan, Selangor	booting and milky	5	2.5
	Sawah Sempadan, Selangor	mature	1	0.7
	Sungai Nibong, Selangor	booting	1	0.5
	Sungai Besar, Selangor	booting	1	0.5
	Sungai Lemau, Selangor	milky	5	5.3
	Rompin, Pahang	milky	5	12.5
	Jabi, Terengganu	booting	1	0.8
	Pasir Puteh, Kelantan	booting	1	0.8
	Ladang Ana 2, Tumpat, Kelantan	booting	1	0.9
	Paklekbang, Tumpat, Kelantan	booting	1	0.5
Indonesia	East Java, Indonesia	booting	5	5.0
	Padang, Sumatra, Indonesia	booting	5	12.0
	Samarinda, Kalimantan, Indonesia	booting	1	0.1

^a0 = no disease severity; 1 = less than 1%; 5 = 1–25% and 9 = 26–100%

Development of Symptoms and Disease Severity Index (DSI)

Seedlings inoculated with conidial suspensions of isolates of *F. fujikuroi* from Malaysia and Indonesia showed typical symptoms of bakanae, thus, the result indicated that *F. fujikuroi* is the casual agent of the disease. However, only 93.7% of the isolates of *F. fujikuroi* were pathogenic (Table 4). Control plants and plants inoculated with other *Fusarium* species remained healthy. The symptom developments on inoculated seedlings in the greenhouse were similar to those observed in the field. The first symptoms on rice seedlings inoculated with *F. fujikuroi* were observed after 5 days. Infected seedlings were several centimeters taller comparing to healthy

Table 3. Microscopic characteristics of *Fusarium* strains in the section of *Lesiola* isolated from bakanae-infected rice in Malaysia and Indonesia

Species	Colony features on PDA	Phialide	Chlamydo-spore	Microconidia			Macroconidia	
				chain	shape	size	shape	size
<i>F. fujikuroi</i>	white orange to pale orange or greyish violet to dark violet pigmentations	monophialide and simple polyphialides	absent	present	obovoid with flattened base, oval (0–1 septate), allantoid pyriform (rarely)	4.1–13.6 x 1.4–5.7 μm	falcate to almost straight, slender, 1–3 septate majority 3 septate	19.0–49.0 x 2.4–4.1 μm
<i>F. proliferatum</i>	white orange, greyish violet to dark violet turned to violet brown and almost black pigmentations	monophialide and polyphialides	absent	present	obovoid with flattened base, pyriform, allantoid	5.4–13.6 x 1.9–4.1 μm	falcate to almost straight, slender, 1–4 septate (majority 3 septate)	21.8–49.0 x 2.7–3.3 μm
<i>F. verticillioides</i>	no pigmentation or greyish violet to dark violet	simple and branches monophialide	absent	present	obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	4.1–12.2 x 1.6–2.9 μm	falcate to straight, slender, 3–5 septate (majority 3 septate)	19.4–49.0 x 2.2–4.1 μm
<i>F. sacchari</i>	orange white to greyish violet, some strains never produced pigmentation	monophialide and polyphialides	absent	absent	oval, allantoid, obovoid to elliptical, primary conidia	4.1–16.3 x 1.4–3.3 μm	long falcate and slender, slightly straight, almost 3 septate	16.3–58.3 x 2.2–5.3 μm
<i>F. subglutinans</i>	white orange pigmentation, sometimes colourless which turned to greyish violet	monophialide and polyphialides	absent	absent	oval (0–1 septate), primary conidia, allantoid, obovoid	4.1–19.0 x 1.4–3.3 μm	long falcate and slender (3–5 septate), slightly straight, almost 3 septate	16.3–59.8 x 2.7–3.5 μm

Table 4. Production of GA₃ and pathogenic isolates of *Fusarium* species

<i>Fusarium</i> spp.	Number of strains	^a GA ₃	Pathogenic strains [%]
<i>F. fujikuroi</i>	16	^{b+}	93.7
<i>F. proliferatum</i>	6	–	0
<i>F. verticillioides</i>	5	–	0
<i>F. sacchari</i>	5	–	0
<i>F. subglutinans</i>	2	–	0

^a + produced/presented in the cultures; – absent in the cultures; ^b out of 16 strains, only a single strain (B3141P) didn't produced GA₃

(control) plants (Fig. 1a, 1b). At first, infected leaves became thinner and yellowish. Then they quickly turned brownish, dried and died before maturing. Several infected tillers produced wiry (stiff) adventitious roots at the first or second nodes. At more advanced stages of infection, the pathogen produced white mycelium and sometimes pinkish sporodochia on the stem just above water level (Fig. 1c). Microscopic examination, revealed the fungal mass consisted of a large number of conidia. However, some infected plants showed normal growth until maturity, they but produced empty and discoloured grains.

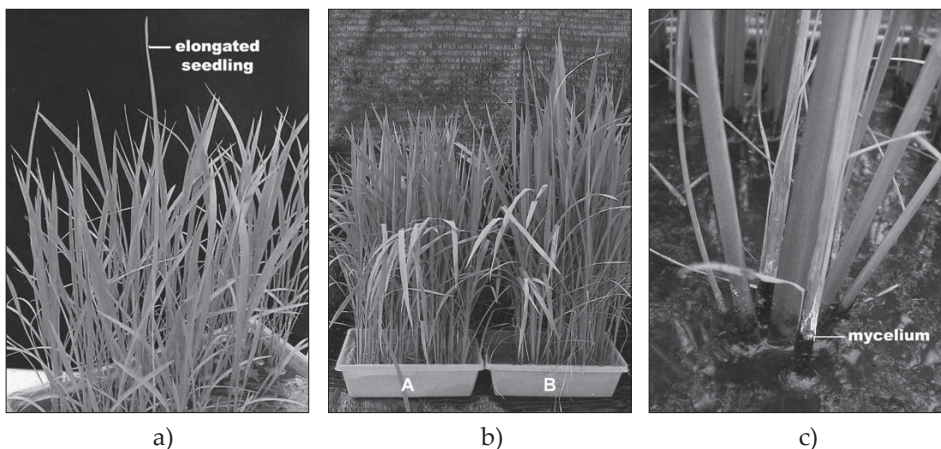


Fig. 1. Typical bakanae symptoms: (a) an elongated seedling, 20 days after inoculation; (b) healthy (control) seedlings (A) shorter than infected seedlings (B), 70 days after inoculation; (c) Pinkish fungal mass (mycelium) above water level on dried-up seedlings

The DSI for all pathogenic isolates of *F. fujikuroi* were dramatically increased after 10 to 30 days as illustrated in table 5. The highest DSI of 3.4 was recorded for seedlings inoculated with isolates T3068P after 70 days and this was followed by isolates A3067P, B3102P, B3105P, B3122P, B3127P, B3133P and B3143P with DSI ranges between 2.9 to 3.1. The value of DSI for seedlings inoculated with other isolates of *F. fujikuroi* was less than 2.8. In contrast, a single isolate (B3141P) that has been identified on the basis of morphological characteristics as *F. fujikuroi* was also non-pathogenic with DSI of 0.1 and was not significantly ($p \leq 0.05$) different from the DSI of control seedlings.

Table 5. Disease Severity Index (DSI) of inoculated and control rice seedlings at different days after inoculation (sowing) with isolates of *Fusarium* spp.

Species	Strains number	Locality	***Disease Severity Index (DSI) at different days							
			5	10	20	30	40	50	60	70
<i>F. fujikuroi</i>	*R621P	Perlis, MY	0.5c	0.9c	1.3b	1.3b	1.3b	1.3b	1.3b	1.3b
	*P655P	Penang, MY	0.7cd	0.9c	1.2b	1.7b	2.1c	2.1c	2.2c	2.2c
	T3067P	Terengganu, MY	1.3e	1.4d	2.1d	2.8cd	3.0d	3.0d	3.0d	3.0d
	T3068P	Terengganu, MY	0.9d	1.3d	2.2d	3.1d	3.3e	3.3e	3.4e	3.4e
	B3102P	Selangor, MY	1.2e	1.3d	2.0d	2.9cd	2.9d	3.0d	3.0d	3.1d
	B3105P	Selangor, MY	0a	0.6b	1.5bc	2.0bc	2.5cd	2.8d	3.0d	3.0d
	B3120P	Selangor, MY	0.7cd	0.8bc	1.3b	2.0bc	2.4cd	2.9d	2.9d	2.9d
	B3122P	Selangor, MY	0.5c	0.8bc	1.3b	2.1bc	2.9d	2.9d	2.9d	2.9d
	B3127P	Selangor, MY	0.4c	0.8bc	1.6c	2.3bc	2.9d	3.0d	3.0d	3.0d
	B3133P	Selangor, MY	0.5c	0.8bc	1.8c	2.9cd	3.0d	3.0d	3.0d	3.0d
	B3141P	Selangor, MY	0a	0a	0a	0.1a	0.1a	0.1a	0.1a	0.1a
	B3143P	Selangor, MY	0.4c	0.6b	1.2b	2.1bc	3.0d	3.1 d	3.1de	3.1de
	I3208P	Sumatra, Indonesia	0.5c	0.9c	2.7e	2.7cd	2.8d	2.8 d	2.8d	2.8d
	I3214P	Sumatra, Indonesia	0.6c	1.3d	2.9e	2.9cd	2.9d	2.9 d	2.9d	2.9d
**I3422P	Samarinda, Indonesia	0a	0.1a	1.3b	2.1bc	2.1c	2.1c	2.2c	2.2c	
M3237P	Melaka, MY	0.6	0.8bc	1.1b	1.6b	2.1c	2.1c	2.1c	2.1c	
<i>F. proliferatum</i>	*K664P	Kedah, MY	0a	0a	0a	0a	0a	0a	0a	0a
	A3054P	Perak, MY	0a	0a	0a	0a	0a	0a	0a	0a
	D3074P	Kelantan, MY	0.1ab	0.2a	0.2a	0.2a	0.2a	0.2a	0.2a	0.2a
	D3075P	Kelantan, MY	0.2ab	0.2a	0.3a	0.3a	0.3a	0.3a	0.3a	0.3a
	C3089P	Pahang, MY	0a	0a	0a	0a	0a	0a	0a	0a
	B3095P	Selangor, MY	0a	0a	0a	0a	0a	0a	0a	0a
<i>F. verticillioides</i>	A3056P	Perak, MY	0a	0a	0a	0a	0a	0a	0a	0a
	A3057P	Perak, MY	0a	0a	0a	0a	0a	0a	0a	0a
	A3058P	Perak, MY	0a	0a	0.2a	0.2a	0.2a	0.2a	0.2a	0.2a
	D3070P	Kelantan, MY	0.1ab	0.1a	0.2a	0.2a	0.2a	0.2a	0.2a	0.2a
	I3410P	East Java, Indonesia	0a	0a	0a	0a	0a	0a	0a	0a
<i>F. sacchari</i>	C3080P	Pahang, MY	0a	0a	0a	0a	0a	0a	0a	0a
	C3081P	Pahang, MY	0a	0a	0a	0a	0a	0a	0a	0a
	C3084P	Pahang, MY	0a	0a	0a	0a	0a	0a	0a	0a
	I3420P	Samarinda, Indonesia	0a	0a	0a	0a	0a	0a	0a	0a
	K3222P	Kedah, MY	0a	0a	0a	0a	0a	0a	0a	0a
<i>F. subglutinans</i>	D3077P	Kelantan, MY	0a	0a	0a	0a	0a	0a	0a	0a
	B3124P	Selangor, MY	0a	0a	0a	0a	0a	0a	0a	0a
Control (sterile distilled water)			0a	0a	0a	0a	0a	0a	0a	0a

*Isolates were obtained from series of survey conducted during 1987–1988; **Isolates were isolated from upland rice; ***DSI in each column with different letters are significantly different at $p \leq 0.05$

Some isolates of *F. proliferatum* (D3074P and D3075P) and *F. verticillioides* (A3058P and D3070P) caused stunting symptom (disease scale 2). The DSI values ranged from 0.2 to 0.3 after 70 days. However, the strains were considered as non-pathogenic based on the DSI values which were not significantly ($p \leq 0.05$) different from those of the control seedlings. The DSI of treated seedlings significantly ($p \leq 0.05$) increased from 5 to 40 days but not later.

DISCUSSION

Bakanae disease of rice was widespread in all sampling areas throughout Malaysia and three provinces in Indonesia in 2004–2005 growing seasons, except for Yan, Kedah. In some sampling areas such as Pahang and Selangor the disease was very severe, and was the highest disease severity (scale 5). On the basis of morphological, physiological and pathogenicity tests, we identified the causal agent of bakanae disease of rice as *F. fujikuroi*. The abnormal elongation of seedlings was probably caused by large quantities of GA₃ produced by the pathogens (Sun and Snyder 1981). *F. fujikuroi* usually produced large quantities of gibberellic acid (GA₃) in cultures and induced bakanae symptoms in experiments with artificial inoculation. GA₃ is a simple growth hormone promoting the elongation of plant cells (Johnson and Coolbaugh 1990). The role of GA₃ in pathogenicity was attributed to the fact that it caused abnormal elongation when concentration of GA₃ was higher than normal.

Although the culture filtrates of the fungus were shown to be able to induce bakanae symptoms in rice seedlings, this phenomenon was not common among isolates of bakanae pathogens (Ou 1985), indicating variations in GA₃ production. Sunder and Satyavir (1998) reported that the isolates of *F. moniliforme* varied greatly in producing GA₃ in liquid culture. Not all isolates of *F. fujikuroi* caused typical elongation of bakanae symptoms. Some infected seedlings were stunted with yellowish leaves after 5 to 10 days, for example, those inoculated with I3208P and I3214P and later showed abnormal elongation symptom. In certain cases some infected plants appeared normal until maturity but later produced empty and discoloured grains. The same observations were also reported by Sun and Snyder (1981), Ou (1985) and Schnitzler (1989).

Agarwal *et al.* (1989) reported that diseased plants at advanced stages of infection showed collar infection followed by death within 14 to 42 days. However, in this experiment, most of the infected seedlings died after 5 to 40 days, while other plants survived until maturity but produced sterile, empty, and discoloured seeds. The first 72 h (3 days) during germination of the seeds appeared to be the most critical period for the infection, mainly because of exudation of amino acids and sugars, acting as rich energy substrates for the actively growing pathogen (Rajagopalan and Bhuvanewari 1975). Therefore, the infected plants were taller than controls after 5 to 40 days. In addition, heavily infected plants died within 10 to 40 days and statistical analysis showed that the DSI was significantly ($p \leq 0.05$) different between days and isolates.

Period of soaking the seeds in inoculum (conidial suspension) also assists the germinating pathogen to penetrate into the seeds. Twelve hours exposure of seeds to 1.0×10^6 conidia/ml inoculum was selected following Rosmayati (1988) with slight modifications. Ahmed *et al.* (1986) soaked the seeds for 24 h, but in less concentrated suspension. Concentrations of the inoculums could also affect the disease severity.

Normally, the disease severity becomes higher when higher concentrations of inoculum are applied (Ahmed *et al.* 1986). Disease suppression was found to be less or more dependent on the virulence of each isolate; different isolates showed different levels of virulence. Ahmed *et al.* (1986) also reported that shoot elongation was significantly higher at 3.1×10^4 conidia/ml or more for 48 h soaking and maximum elongation of seedlings was attained when the seedlings were inoculated with 1.25×10^5 conidia/ml. Abnormal elongation was not observed if the inoculum levels were lower than those concentrations.

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POLISH SUMMARY

CHOROBA BAKANAE RYŻU W MALEZJI I INDONEZJI: ETIOLOGIA SPRAWCY NA PODSTAWIE CECH MORFOLOGICZNYCH, FIZJOLOGICZNYCH I PATOGENICZNYCH

Choroba bakanae występuje niemal we wszystkich rejonach uprawy ryżu, a zwłaszcza w krajach azjatyckich, takich jak Malezja czy Indonezja. W czasie głównych sezonów wegetacyjnych 2004–2005 choroba rozprzestrzeniła się na półwyspie Malezyjskim oraz w trzech prowincjach Indonezji, a jej występowanie utrzymywało się na poziomie 0,5–12,5%. Nasilenie określono w skali 1–5. Ze zgromadzonego materiału roślinnego na podstawie cech morfologicznych patogenów wyodrębniono ogółem pięć gatunków *Fusarium* należących do sekcji *Liseola*: *F. fujikuroi*, *F. proliferatum*, *F. sacchari*, *F. subglutinans*, *F. verticillioides*. Z badań prowadzonych na świecie wynika, że choroba jest wywoływana przez *F. fujikuroi* oraz prawdopodobnie inne gatunki *Fusarium* z sekcji *Liseola*. Wyniki prezentowanych badań nad patogennością wyodrębnionych izolatów patogenu na odmianie ryżu MR 211 wykazały, że gatunek *F. fujikuroi* charakteryzował się silną wirulencją i był jedynym głównym sprawcą choroby bakanae ryżu. Spośród wszystkich zidentyfikowanych gatunków *F. fujikuroi* jako jedyny wytwarzał kwas giberelinowy – (GA₃) z parametrem R_t wynoszącym 0,40 i 0,62 w warunkach doświadczeń laboratoryjnych przy zastosowaniu roztworów – izopropanol: amonika: woda (10 : 1 : 1), v/v/v oraz chloroform: octan etylu: kwasmrówkowy (5 : 4 : 1), v/v/v. Prezentowane wyniki badań stanowią cenne wzbogacenie wiedzy w zakresie interakcji pomiędzy patogenem *F. fujikuroi* a rośliną gospodarzem.