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SHORT COMMUNICATION

Mating Populations of *Fusarium* Section Liseola from Rice, Sugarcane and Maize

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Abstrak: Kajian pengawanan telah dijalankan ke atas 78 pencilan spesies *Fusarium* seksyen Liseola yang telah dipencilkan daripada padi, tebu dan jagung. Berdasarkan ujian persilangan dengan strain penguji spesies kompleks *Gibberella fujikuroi*, 64.1% (50 daripada 78 pencilan) berjaya mengacuk-subur dengan strain penguji populasi kacukan A hingga E. Keputusan daripada kajian pengawanan ini menunjukkan daripada 50 pencilan yang mengawan, 19 termasuk dalam populasi kacukan A (*Gibberella moniliformis*), 18 dalam populasi kacukan B (*Gibberella sacchari*), 4 dalam populasi kacukan E (*Gibberella subglutinans*), 6 dalam populasi kacukan D (*Gibberella intermedia*) and 3 dalam populasi kacukan C (*G. fujikuroi*). Pengenalpastian beberapa populasi kacukan pada padi, tebu dan jagung boleh dianggap sebagai entiti biologi penting di lapangan.

Kata kunci: *Fusarium*, Liseola, Populasi Kacukan

Abstract: Mating studies were conducted on 78 isolates of *Fusarium* species section Liseola from rice, sugarcane and maize. From the crosses with tester strains of *Gibberella fujikuroi* species complex, 64.1% (50 out of 78 isolates) were cross-fertile with tester strains of mating populations A to E. The results of the mating studies showed that of the 50 isolates, 19 belonged to mating population A (*Gibberella moniliformis*), 18 to mating population B (*Gibberella sacchari*), 4 to mating population E (*Gibberella subglutinans*), 6 to mating population D (*Gibberella intermedia*) and 3 to mating population C (*G. fujikuroi*). Identification of several mating populations from rice, sugarcane and maize could be important biological entities under field conditions.

Keywords: *Fusarium*, Liseola, Mating Populations

Fusarium species section Liseola commonly has teleomorph in the *Gibberella fujikuroi* species complex. This complex can be divided into nine biological species designated as mating populations A to J (Leslie & Summerell 2006). Members of the section Liseola or *G. fujikuroi* species complex have a worldwide distribution and has caused diseases on a wide variety of economically important crops such as rice, maize and sugarcane (Leslie 1995).

Among the methods used to distinguish species in the section Liseola is by using mating study or crosses with standard tester strain (Leslie 1991) since each anamorph belongs to one or more sexually compatible mating populations.

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Classifying isolates of *Fusarium* by mating study would place the isolates in biological species.

In mating study of *G. fujikuroi* species complex, the female parent is the tester strain for the mating population and the male parent is the isolate to be identified. Members of *G. fujikuroi* species complex are generally heterothallic in which sexual interaction requires interaction between two morphologically indistinguishable spores but different in their mating types allele or *MAT* allele (Leslie & Summerell 2006). For crosses to occur, one isolate must carry *MAT*-1 allele and the other *MAT*-2 allele.

Being fertile or having the ability to mate among isolates of *Fusarium* in section Liseola could provide information on genetic diversity, population genetics and can be important for plant pathological purposes. Therefore, the objective of the present study was to identify the mating populations present in *Fusarium* species section Liseola isolated from rice, sugarcane and maize.

Isolates of *Fusarium* section Liseola used in this study were isolated from the field and obtained from the stock culture (Table 1). The isolates were identified using DNA sequencing of *α*-translation elongation factor gene (TEF-1*α*).

The tester strains to determine the mating population of *G. fujikuroi* species complex (mating populations A to G) were obtained from Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas, USA: A–00149 (*MATA–1*), A–00999 (*MATA–2*), B–03853 (*MATB–1*), B–03854 (*MATB–2*), C–01993 (*MATC–1*), C–01995 (*MATC–2*), D–04854 (*MATD–1*), D–04583 (*MATD–2*), E–00990 (*MATE–1*), E–02192 (*MATE–2*), F–4094 (*MATF–1*), F–4093 (*MATF–2*), G–05111 (*MATG–1*), G–05112 (*MATG–2*). The standard sexual crosses were carried out according to Klittich and Leslie (1992) and repeated twice. Tester female strains were inoculated on carrot agar (CA) plates and the *Fusarium* isolates (male strains) were inoculated on complete medium (CM) on the same day and incubated at 25°C for 7 days.

After 7 days of incubation, mycelia from male strains were suspended in 1.5 ml 0.25% Tween 60 solution and spread on the surface of CA which was already colonised with tester strain's mycelium. A sterile spreader (hockey stick) was used to press the spore suspension into the mycelia of the female colony. The fertilised plates were incubated at 27°C and checked twice a week for 2 months to observe the production of perithecia. Perithecia were observed in situ while asci was crushed and mounted on microscopic slide to observe the ascospores under a light microscope (Olympus CX-41 Tokyo), and photographed using Xcam-*α* camera with an imaging software Cell A (Olympus, Tokyo).

From a series of crosses, 64.1% (50 out of 78 isolates) were cross-fertile with the tester strains of mating populations A to E (Table 2). None of the isolates were cross-fertile with tester strains of mating populations F and G. Of the 50 isolates, 19 belonged to mating population A (*Gibberella moniliformis*), 18 to mating population B (*Gibberella sacchari*), 4 to mating population E (*Gibberella subglutinans*), 6 to mating population D (*Gibberella intermedia*) and 3 to mating population C (*G. fujikuroi*).

Table 1: *Fusarium* species from rice, sugarcane and maize used in mating study.

Isolate	Location	*Fusarium* species
Isolates from rice		
0621	Cuping, Perlis	*F. fujikuroi*
0654	Kg. Paya, Seberang Perai, Penang	*F. verticillioides*
3053	Sungai Manik, Teluk Intan, Perak	*F. sacchari*
3054	FELCRA, Seberang Perak, Perak	*F. sacchari*
3055	FELCRA, Seberang Perak, Perak	*F. andiyazi*
3061	FELCRA, Seberang Perak, Perak	*F. andiyazi*
3063	FELCRA, Seberang Perak, Perak	*F. verticillioides*
3067	Kg. Apal, Jabi, Terengganu	*F. fujikuroi*
3068	Kg. Apal, Jabi, Terengganu	*F. verticillioides*
3073	Paklekbang, Tumpat, Kelantan	*F. andiyazi*
3074	Paklekbang, Tumpat, Kelantan	*F. proliferatum*
3075	Paklekbang, Tumpat, Kelantan	*F. proliferatum*
3078	Padang Sungai Laka, Rompin, Pahang	*F. sacchari*
3081	Padang Sungai Laka, Rompin, Pahang	*F. sacchari*
3082	Padang Sungai Laka, Rompin, Pahang	*F. sacchari*
3084	Padang Sungai Laka, Rompin, Pahang	*F. sacchari*
3086	Padang Sungai Laka, Rompin, Pahang	*F. andiyazi*
3087	Padang Sungai Laka, Rompin, Pahang	*F. sacchari*
3088	Padang Sungai Laka, Rompin, Pahang	*F. andiyazi*
3095	Sekinchan, Kuala Selangor, Selangor	*F. proliferatum*
3099	Sekinchan, Kuala Selangor, Selangor	*F. fujikuroi*
3101	Sekinchan, Kuala Selangor, Selangor	*F. fujikuroi*
3105	Sekinchan, Kuala Selangor, Selangor	*F. fujikuroi*
3122	Sekinchan, Kuala Selangor, Selangor	*F. fujikuroi*
3124	Tg. Karang, Kuala Selangor, Selangor	*F. verticillioides*
3132	Sungai Leman, Sungai Besar, Selangor	*F. fujikuroi*
3136	Sungai Leman, Sungai Besar, Selangor	*F. sacchari*
3137	Sungai Leman, Sungai Besar, Selangor	*F. andiyazi*
3151	Bukit Merah, Perak	*F. fujikuroi*
3170	Permatang Tok Jaya, Penang	*F. fujikuroi*
3208	Permatang Tok Jaya, Penang	*F. fujikuroi*
P1	Gurun, Kedah	*F. proliferatum*
P2	Gurun, Kedah	*F. proliferatum*
P3	Gurun, Kedah	*F. oxysporum*
P4	Gurun, Kedah	*F. proliferatum*

(continued on next page)

Table 1: *(continued)*

Isolate	Location	*Fusarium* species
P5	Gurun, Kedah	*F. sacchari*
Isolates from sugarcane		
3238	Padang Terap, Kedah	*F. proliferatum*
3246	Padang Terap, Kedah	*F. sacchari*
3257	Padang Terap, Kedah	*F. verticillioides*
3262	Padang Terap, Kedah	*F. sacchari*
3277	Chuping, Perlis	*F. verticillioides*
3281	Chuping, Perlis	*F. sacchari*
3295	Chuping, Perlis	*F. sacchari*
3308	Kupang, Kedah	*F. sacchari*
3310	Kupang, Kedah	*F. sacchari*
3324	Rantau Panjang, Kelantan	*F. proliferatum*
3327	Rantau Panjang, Kelantan	*F. sacchari*
3336	Kuantan, Pahang	*F. sacchari*
3343	Kg Awah, Pahang	*F. sacchari*
3349	Kuantan, Pahang	*F. sacchari*
3350	Kuantan, Pahang	*F. sacchari*
3354	Alor Setar, Kedah	*F. proliferatum*
3358	Bagan Serai, Perak	*F. sacchari*
T1	Chuping, Perlis	*F. verticillioides*
T2	Chuping, Perlis	*F. verticillioides*
T3	Chuping, Perlis	*F. sacchari*
T4	Chuping, Perlis	*F. sacchari*
T5	Chuping, Perlis	*F. verticillioides*
T6	Chuping, Perlis	*F. sacchari*
T7	Chuping, Perlis	*F. sacchari*
T8	Chuping, Perlis	*F. sacchari*
T9	Chuping, Perlis	*F. sacchari*
Isolates from maize		
JB1	Gurun, Kedah	*F. verticillioides*
JB2	Gurun, Kedah	*F. verticillioides*
JB3	Gurun, Kedah	*F. verticillioides*
JB4	Gurun, Kedah	*F. verticillioides*
JD4	Gurun, Kedah	*F. verticillioides*
JF1	Gurun, Kedah	*F. verticillioides*
JF5	Gurun, Kedah	*F. fujikuroi*
B1	Gurun, Kedah	*F. verticillioides*

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Table 1: *(continued)*

Isolate	Location	*Fusarium* species
B2	Gurun, Kedah	*F. verticillioides*
B3	Gurun, Kedah	*F. verticillioides*
B4	Gurun, Kedah	*F. verticillioides*
B5	Gurun, Kedah	*F. verticillioides*
B6	Gurun, Kedah	*F. verticillioides*
B9	Gurun, Kedah	*F. verticillioides*
B10	Gurun, Kedah	*F. sacchari*
F1	Gurun, Kedah	*F. verticillioides*
F2	Gurun, Kedah	*F. sacchari*

Table 2: Mating populations of 78 isolates of *Fusarium* species section Liseola from rice, sugarcane and maize.

Isolates	*Fusarium* species	*Gibberella* species	Mating population
0654 (R)	*F. verticillioides*	–	–
3063 (R)	*F. verticillioides*	*G. moniliformis*	A
3068 (R)	*F. verticillioides*	–	–
3124 (R)	*F. verticillioides*	–	–
3257 (SC)	*F. verticillioides*	–	–
T1 (SC)	*F. verticillioides*	*G. moniliformis*	A
T2 (SC)	*F. verticillioides*	*G. moniliformis*	A
3277 (SC)	*F. verticillioides*	–	–
T5 (SC)	*F. verticillioides*	*G. moniliformis*	A
JB1 (M)	*F. verticillioides*	–	–
JB2 (M	*F. verticillioides*	*G. moniliformis*	A
JB3 (M)	*F. verticillioides*	–	–
JB4 (M)	*F. verticillioides*	*G. moniliformis*	A
JD4 (M)	*F. verticillioides*	–	–
JF1 (M)	*F. verticillioides*	*G. moniliformis*	A
B1 (M)	*F. verticillioides*	*G. moniliformis*	A
B2 (M)	*F. verticillioides*	*G. moniliformis*	A
B3 (M)	*F. verticillioides*	*G. moniliformis*	A
B4 (M)	*F. verticillioides*	*G. moniliformis*	A
B5 (M)	*F. verticillioides*	*G. moniliformis*	A
B6 (M)	*F. verticillioides*	*G. moniliformis*	A
B9 (M)	*F. verticillioides*	*G. moniliformis*	A

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Table 2: *(continued)*

Isolates	*Fusarium* species	*Gibberella* species	Mating population
F1 (M)	*F. verticillioides*	*G. moniliformis*	A
3055 (R)	*F. andiyazi*	*G. moniliformis*	A
3061 (R)	*F. andiyazi*	*G. moniliformis*	A
3073 (R)	*F. andiyazi*	–	–
3086 (R)	*F. andiyazi*	*G. moniliformis*	A
3088 (R)	*F. andiyazi*	–	–
3137 (R)	*F. andiyazi*	*G. moniliformis*	A
3053 (R)	*F. sacchari*	–	–
3054 (R)	*F. sacchari*	–	–
3078 (R)	*F. sacchari*	*G. sacchari*	B
3081 (R)	*F. sacchari*	*G. sacchari*	B
3082 (R)	*F. sacchari*	*G. sacchari*	B
3084 (R)	*F. sacchari*	*G. sacchari*	B
3087 (R)	*F. sacchari*	*G. sacchari*	B
3136 (R)	*F. sacchari*	–	–
P5 (R)	*F. sacchari*	*G. sacchari*	B
3246 (SC)	*F. sacchari*	–	–
3262 (SC)	*F. sacchari*	*G. sacchari*	B
3281 (SC)	*F. sacchari*	*G. sacchari*	B
3295 (SC)	*F. sacchari*	*G. sacchari*	B
3308 (SC)	*F. sacchari*	*G. subglutinans*	E
3310 (SC)	*F. sacchari*	*G. subglutinans*	E
3327 (SC)	*F. sacchari*	*G. sacchari*	B
3336 (SC)	*F. sacchari*	–	–
3343 (SC)	*F. sacchari*	–	–
3349 (SC)	*F. sacchari*	*G. subglutinans*	E
3350 (SC)	*F. sacchari*	*G. sacchari*	B
T3 (SC)	*F. sacchari*	*G. sacchari*	B
T4 (SC)	*F. sacchari*	*G. sacchari*	B
T6 (SC)	*F. sacchari*	*G. sacchari*	B
T7 (SC)	*F. sacchari*	–	–
T8 (SC)	*F. sacchari*	*G. subglutinans*	E
T9 (SC)	*F. sacchari*	*G. sacchari*	B
3358 (SC)	*F. sacchari*	*G. sacchari*	B
B10 (M)	*F. sacchari*	*G. sacchari*	B

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Table 2: *(continued)*

Isolates	*Fusarium* species	*Gibberella* species	Mating Population
F2 (M)	*F. sacchari*	*(G. sacchari*	B
0621 (R)	*F. fujikuroi*	–	–
3067 (R)	*F. fujikuroi*	–	–
3099 (R)	*F. fujikuroi*	*G. intermedia*	D
3101 (R)	*F. fujikuroi*	–	–
3105 (R)	*F. fujikuroi*	–	–
3122 (R)	*F. fujikuroi*	*G. fujikuroi*	C
3132 (R)	*F. fujikuroi*	*G. intermedia*	D
3151 (R)	*F. fujikuroi*	–	–
3170 (R)	*F. fujikuroi*	*G. intermedia*	D
3208 (R)	*F. fujikuroi*	–	–
JF5 (M)	*F. fujikuroi*	–	–
3074 (R)	*F. proliferatum*	*G. intermedia*	D
3075 (R)	*F. proliferatum*	–	–
3095 (R)	*F. proliferatum*	*G. fujikuroi*	C
P1 (R)	*F. proliferatum*	–	–
P2 (R)	*F. proliferatum*	–	–
P4 (R)	*F. proliferatum*	*G. intermedia*	–
3238 (SC)	*F. proliferatum*	*G. fujikuroi*	C
3324 (SC)	*F. proliferatum*	*G. intermedia*	D
3354 (SC)	*F. proliferatum*	–	–

Note: R: rice; SC: sugarcane; M: maize.

In the present study, the isolates of *Fusarium verticillioides* were isolated from rice, sugarcane and maize. 65% of the isolates from the 3 hosts were cross-fertile with tester strains of mating population A (*G. moniliformis*). Similar results were obtained by Leslie (1995) in which isolates of *G. moniliformis* isolated from maize, banana, rice and pine from various geographic origins were inter-fertile. Successful crosses in mating population A (*G. moniliformis*) which were observed in this study can be considered as high. The potential of sexual reproduction can be increased when both mating types of *G. moniliformis* were widely distributed and the frequency of female fertility was high (Leslie & Klein 1996). None of the isolates of *F. verticillioides* were cross-fertile with tester strains from mating population F (*Gibberella thapsina*) although *F. verticillioides* has been reported to share similar morphological characteristics with *Fusarium thapsinum*.

Four isolates identified as *Fusarium andiyazi* (3055, 3061, 3086, 337) from rice were cross-fertile with tester strains of mating population A which contrasted with a study by Marasas *et al*. (2001). In the study, none of the *F. andiyazi* isolates from sorghum were inter-fertile with tester strains of mating populations A to H. However, until more isolates and larger population of *F. andiyazi* are examined, the inter-fertility between *F. andiyazi* and mating population A remain inconclusive. Moreover, female-fertile strains have not been

identified and mating-type tester strains for *F. andiyazi* have not been synthesised.

Among the isolates of *Fusarium fujikuroi*, one isolate (3122) was cross-fertile with tester strain of mating population C (*G. fujikuroi*) and three isolates (3099, 3132 and 3170) were cross-fertile with tester strain of mating population D (*G. intermedia*). As for isolates of *Fusarium proliferatum*, only three isolates (3074, P4 and 3324) were cross-fertile with tester strain of mating population D (*G. intermedia*). Two isolates of *F. proliferatum* (3095 and 3238) were cross-fertile with mating population C (*G. fujikuroi*).

Although mating populations C and D can interbreed and produce viable progeny (Leslie *et al*. 2004), the present study with limited number of isolates did not show any interbreeding of isolates from both mating populations. According to Leslie *et al.* (2004), the separation between *F. fujikuroi* and *F. proliferatum* based on mating test is not complete and naturally occurring hybrids between the two species have been recovered. Both *F. fujikuroi and F. proliferatum* are regarded as sibling species and shared many similarities morphologically and by mycotoxin production (Desjardins *et al*. 1997). Leslie (1995) reported that based on isozyme patterns, isolates from mating populations C and D were difficult to resolve.

Eighteen isolates of *Fusarium sacchari* from rice and sugarcane were cross-fertile with mating population B (*G. sacchari*) and four isolates from sugarcane were cross-fertile with mating population E (*G. subglutinans*). The results indicated that both *F. sacchari* and *F. subglutinans* were closely related. Several studies have shown that both species are closely related and their morphological characteristics are very similar. Leslie (1995) reported that *F. subglutinans* isolated from mango showed similar isozyme patterns with isolates of *F. sacchari* of mating population B. O'Donnell *et al*. (1998) also reported that *F. subglutinans* associated with mango malformation were closely related to isolates in mating population B.

Although the application of biological species concept or mating studies are one of the methods to define species in *G. fujikuroi* species complex, the method has limitation and disadvantages which could explain why only 64.1% of the isolates were able to cross-fertile with the tester strains. The limitations and disadvantages of biological species concept or mating studies were described in details by Desjardins (1993) and Kvas *et al*. (2009). However, despite the disadvantages, mating studies provide information on the variations associated with morphology and DNA sequences within a well-defined species, thus allowing a better understanding between species especially species not known to reproduce sexually (Kvas *et al*. 2009).

In conclusion, there are complex of *G. fujikuroi* species complex mating populations occurred in rice, sugarcane and maize which suggested that mating populations could be important biological entities under field conditions.

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