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Mating Behavior, Mycotoxin Production, and Vegetative Compatibility of *Gibberella fujikuroi* Species Complex from Sorghum in Korea

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***Fusarium* isolates of *Gibberella fujikuroi* species complex were obtained from sorghum grown in five provinces of Korea in 1996 and 1997. These isolates were characterized based on their mating behavior, mycotoxin production, and vegetative compatibility. Only three mating populations (A, D, and F) were recovered from a total of 155 isolates examined. The relative frequency of the mating populations was significantly different: F was predominat (80%), while D and A were observed at low frequencies of 9% and 3%, respectively. Female fertile isolates were more common within F (44 out of 124) than D (2 out of 14), while none of the five A isolates were female fertile. The inbreeding effective population sizes (N_e) for mating type and male/hermaphrodite ratios in mating population F were 93% and 77% of the count, respectively. Isolates of mating populations A and D produced significant amounts of fumonisins, while F isolates produced none or only traces of fumonisin B_1. In contrast, F isolates produced higher amounts of moniliformin (average of 3,820 ppm) than A and D isolates (averages of 77 and 1,819 ppm, respectively). Fifty-one isolates were tested for vegetative compatibility using nitrogen non-utilization mutants of each isolate, and 44 vegetative compatibility groups (VCGs) were identified. A single VC type (VC1) was found in all of the five A isolates examined. Six of the D isolates examined consisted of three VC types: two for VC2, two for VC3, and the rest for VC4. All of the F isolates tested were incompatible in every combination and, thus, each constituted a unique VCG.**

Keywords **:** *Gibberella fujikuroi*, mating populations, fumonisins, sorghum, vegetative compatibility.

Gibberella fujikuroi is a complex of at least eight mating populations that represent different biological species (designated by the letters A-H) (Hsieh et al., 1977; Leslie, 1991). These mating populations are important pathogens of agricultural crops in many areas of the world (Correll et al., 1992; Leslie et al., 1990). In addition to yield loss caused by these populations, they have been associated with human and animal toxicoses due to the production of toxic secondary metabolites such as moniliformin (Marasas et al., 1984; Marasas and Nelson, 1987), fusarin C (Wiebe and Bjeldanes, 1981), fusaric acid (Marasas et al., 1984), and fumonisins (Gelderblom et al., 1988). Among these mycotoxins, fumonisins primarily produced by the mating populations A and D have been recently given much attention by many scientists because they have been shown to be potentially carcinogenic. Therefore, production of mycotoxins by *G. fujikuroi* is of particular concern because of the nearly universal distribution of these species in crops (Leslie et al., 1990; Munkvold and Desjardins, 1997) and the ability to be internally seedborne in symptomless, apparently healthy grain (Thomas and Buddenhagen, 1980).

Each mating population within the *G. fujikuroi* species complex has been distinguished mainly by sexual fertility to tester strains (Leslie, 1991). This is because morphological characters, which usually work for identification of other *Fusarium/Gibberella* species, are not sufficient in identifying different biological species in this complex. Genetic variations within each mating population have been investigated using several mycological characters such as distribution of mating type (Leslie, 1991), frequency of hermaphrodite (Leslie and Klein, 1996), and vegetative compatibility (VC) (Leslie, 1993). Along with mating tests, vegetative compatibility group (VCG) systems for identifying different *G. fujikuroi* complex have provided a relatively simple way to distinguish and analyze fungal populations (Leslie, 1993). In the fungal population, strains can be classified into different VCGs based on their ability to form heterokaryons with one another (Puhalla and Spieth, 1983).

Recently, several molecular markers (RAPD, AFLP, nucleotide sequence analysis, etc.) have been used to characterize the population of each biological species, as well as to identify biological species in this complex (O'Donnell and Cigelnik, 1997; Xu and Leslie, 1995).

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Previous studies have shown that six mating populations of the *G. fujikuroi* species were recovered from sorghum but two mating populations, A (anamorph *Fusarium moniliforme*, syn. *F. verticillioides*) and F (anamorph *F. thapsinum*) were dominant (Klittich and Leslie, 1992; Mansuetus et al., 1997). These two mating populations have been reported to be strikingly different in terms of production of mycotoxins (Leslie et al., 1996; Marasas et al., 1986). In general, members of the A mating populations produce significant quantities of fumonisins along with moniliformin, while strains of the F mating populations produce little or none of fumonisin but with significant quantities of moniliformin.

This study aimed to investigate systematic population studies of *G. fujikuroi* complex from sorghum in Korea. Specifically, this study sought to: a) identify mating populations of *G. fujikuroi* isolates from sorghum in Korea and determine their relative frequencies; b) investigated their ability to produce mycotoxins; c) determined how mating types and female fertility affect population structure, as estimated by the inbreeding effective population number (N_e); and d) determine the number of VC groups within mating population.

Materials and Methods

Sorghum samples. Twenty samples of sorghum were collected in 1996 and 1997 from farmers' stocks in five provinces in Korea namely Gangwon, Gyeongbuk, Chungnam, Chungbuk, and Jeonnam.

Isolation and identification of *G. fujikuroi* species. From each sorghum sample, 100 seeds were soaked in 1% NaOCl for 1min, rinsed with sterilized water, and transferred onto potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI, U.S.A.), and incubated at 25°C for 4-7 days. Morphological characterizations were made from cultures grown on carnation-leaf agar (CLA), KCl agar, and PDA. All isolates originated from single uninucleate microconidia and maintained for long-term storage in 15% glycerol at -70°C.

Crosses. Sexual crosses were made on carrot agar as previously described (Klittich and Leslie, 1988). Twelve standard strains (A-F) for sexual cross were supplied by Dr. Yong-Hwan Lee, School of Agricultural Biotechnology, Seoul National University, Korea. The mating plates were incubated for 3-5 weeks at 25°C on an alternating 12 h/dark and 12 h/light cycle using a fluorescence light, and were examined weekly for the presence of perithecia and mature perithecia with ascospore mass extruded from the ostioles. All of the crosses were made with the standard tester as the female, and the unknown field isolate as the male. Tests for female fertility of the unknown isolate were made by reversing the roles of the two strains in the cross. A cross was scored as infertile if mature perithecia did not form after two attempts. The inbreeding effective population number (N_e) was calculated by using the equations of Leslie and Klein (1996).

Analysis of mycotoxin production. Mycelial plugs prepared from isolates grown on complete medium (CM) for 5 days were used to inoculate onto moistened rice in Erlenmeyer flasks autoclaved at 121 for 1 h each for 2 consecutive days. Cultures were incubated in the dark at 25°C for 28 days. The mycelial mass and substrate were dispersed onto a flask bottom and allowed to air dry in a ventilated hood. When dried, rice cultures were ground to the consistency of flour and stored at -20°C until used. The production of fumonisins in rice culture was assessed by high-performance liquid chromatography (HPLC) as described by Shephard et al. (1990) with a slight modification. Clean-up and extraction of moniliformin were made by using the method of Scott and Lawrence (1987) with a slight modification, and analyzed by HPLC.

Vegetative compatibility tests. Fifty-one of the representative isolates used in VC tests were selected from a total of 155 isolates based on mating type, fumonisin production, location, and color produced on PDA. Nitrate non-utilizing mutants (*nit* mutants) were generated on minimal agar with chlorate by the method of Correll et al. (1987). The physiological phenotypes of *nit* mutants recovered from the representative isolates were interpreted on the basis of their growth on media containing different nitrogen sources. Complementation tests between phenotypically distinct *nit* mutants were made on minimal medium. *Nit* mutants were assigned to the same VC group based on the ability of the derived *nit1* mutants to form a heterokaryon with complementary NitM mutants.

Results

Distributions of mating population, mating type, and female fertility. A total of 155 isolates were obtained from sorghum samples and were tested for sexual fertility with the standard tester strains. As shown in Table 1, of the 155 isolates examined, 143 were cross-fertile with one of the standard mating type testers and could produce fertile perithecia. Based on the sexual fertility, they could be assigned to one of three mating populations of *G. fujikuroi*. However, only one mating population F was predominant with 80% frequency in this collection. Relative frequencies of the other two mating populations, A and D, were 3 and 9%, respectively. Two opposite mating types were recovered from the F and D mating populations, but the ratios were significantly different from equal segregation for both. Female fertile isolates were found in these two mating populations but in most crosses, they showed low female fertility compared with the number of perithecia produced in tester strain. To determine a population size relative to the size of randomly mating populations, all members of which are hermaphrodite, the effective population numbers for both mating type ($N_{e(mt)}$) and male/hermaphrodite ratio ($N_{e(r)}$) were estimated for each mating population. However, a reliable estimate for both parameters could be made using the F mating population only because the sample

Table 1. Mating, female fertility, and inbreeding effective numbers of *Gibberella fujikuroi* isolates from sorghum

MAT[a]	No. of sexually fertile isolates	Fertility[b]		*Ne*[c] (% of count)	
		Male	Herm.	MAT	Fert.
Mating Population A					
MATA-1	5	5	0	MD[e]	ND
MATA-2	0	0	0		
Mating Population D					
MATD-1	3	2	1	ND	ND
MATD-2	11	10	1		
Mating Population F					
MATF-1	46	16	30	93	77
MATF-2	78	64	14		
Unknown[d]	12				
Total	155	97	46		

[a] Mating type is based on terminology proposed by Kerényi et al. (1999).
[b] 'Male' refers to function as the male parent in crosses with the standard testers. 'Herm' refers to hermaphrodites that can function as either male or female parent in crosses.
[c] Inbreeding effective number for mating type (MAT) or male/hermaphrodite (fertility) ratios.
[d] Sterile isolates with testers; when they were crossed with the standard mating type testers, no sexual structures were formed.
[e] *Ne* was not calculated; a reliable estimate could not be calculated because the sample sizes of the mating population A and D were too small.

Table 2. Mycotoxin production by *Gibberella fujikuroi* isolates from sorghum in Korea

Mycotoxin	No. (%) of positive samples	Mean level (range) (μg/g) in positive samples
Mating population A (5 isolates)		
Fumonisin B_1	5 (100)	586 (105-746)
Moniliformin	5 (100)	77 (4-223)
Mating population D (14 isolates)		
Fumonisin B_1	14 (100)	873 (14-3,274)
Moniliformin	14 (100)	1,819 (17-8,849)
Mating population F (124 isolates)		
Fumonisin B_1	0	–[a]
Moniliformin	121(97.5)	3,820 (60-14,437)

[a] Several isolates produced only traces (lower than 10 ppm) of fumonisin B_1.

sizes of the other two populations were too small. In the F mating population from sorghum in Korea, $N_{e(mt)}$ and $N_{e(f)}$ were 93% and 77% of the count, respectively.

Mycotoxin production. As shown in Table 2, 19 isolates belonged to either mating population A or D. Mating population A produced significant amounts of the fumonisin B_1 and mating population D showed large variability in fumonisin B_1 production. Meanwhile, all of the F mating population isolates produced none or only traces of fumonisin B_1 (lower than 10 ppm). In contrast, moniliformin was produced by all of the isolates from the three mating populations. Interestingly, moniliformin production based on average level was very much different among the mating populations. Isolates of the F mating population produced much more moniliformin than the other two mating populations, with the A mating population isolates producing the lowest level of moniliformin.

VC tests. All of the 51 representative isolates spontaneously formed chlorate-resistant sectors on chlorate medium. Of the 527 chlorate-resistant sectors recovered, 499 (94.7%) were *nit* mutants and 28 (5.3%) were chlorate-resistant nitrate utilizing mutants. The majority of *nit* mutants were *nit1* (76.2%), followed by NitM (21.4%), and *nit3* (2.4%). The two major mutants, *nit1* and NitM, were obtained from all the 51 isolates, but *nit3* mutants were identified only from 8 isolates. Representative mutants of *nit1* and NitM from the 51 isolates were paired with each other in 2,601 combinations on minimal medium. Forty-four vegetative compatibility groups, which were confirmed by heterokaryon formation, were identified. A single VC type (VC group 1) was found in all of the A mating population isolates. Three VC groups (VC group 2, 3, and 4) were identified among the mating population D isolates. All the F mating population isolates tested were incompatible in every combination, thus, each constituted a unique VC group. Self-incompatible isolates were also detected in mating populations D and F.

Discussion

This study showed that only mating population F is predominant (80%), and the other two mating populations A and D are present at much less frequencies (3 and 9%, respectively) on sorghum in Korea. This result is similar to that obtained from sorghum samples in the USA, where mating population F (74%) and D (13%) were predominant. The relative frequency of mating population F in this study is, however, much higher than that from Tanzanian sorghum samples (49%). The outnumbered female sterile isolates among the mating population F collection caused reduction in the effective population number relative to the population size in the ideal situation (23% reduction of the count). However, the value of the effective size number from this study is much higher than those from the USA and Tanzania (Leslie and Klein, 1996; Munsuetus et al., 1997). Based on the results, sexual reproduction within the F mating population in Korea is more frequent than in the USA and Tanzania. Leslie (Leslie and Klein, 1996) suggested that low sexual reproduction could cause local pop-

ulations to lose female fertility, and that such populations might diverge to form new species or sub-specific types as a result of their genetic isolation. When production of fumonisin and moniliformin was investigated in the 155 isolates, those belonging to either mating population A or D produced significant amounts of fumonisin and relatively small amount of moniliformin. Meanwhile, most isolates of the mating population F produced relatively higher amounts of moniliformin but none or only traces of fumonisin. This production pattern of both mycotoxins by these two mating populations are similar to those obtained in other studies (Nelson et al., 1991; Leslie et al., 1992), which may suggest that there is an obvious correlation between mating population and mycotoxin production. Moniliformin has been reported to be acutely toxic to laboratory animals (Leslie et al., 1996), and the effects of this toxin on human and animal health should be investigated systematically in the mating population F.

In most heterothallic fungi, the formation of heterokaryon between two genetically different haploid strains is an essential part of the life cycle, and vegetative compatibility systems generally act to restrict the transfer of nuclear and cytoplasmic elements during growth (Leslie, 1993). *Nit* mutants have been recovered from several species of *Fusarium* such as *F. moniliforme* (Klittich and Leslie, 1988; Puhalla and Spieth, 1983), *F. graminearum* (Bowden and Leslie, 1992), *F. solani* (Correll, 1986), and *F. oxysporum* (Correll et al., 1987; Puhalla, 1985). In this study, complementation between different *nit* mutants of several Korean *G. fujikuroi* revealed that 5 isolates of mating population A from different locations consisted of a single VCG, and that 40 isolates of mating population F were composed of distinct VCG. This result is in contrast with previous reports which showed that mating population A usually had relatively high levels of genotype variation, while mating population F appeared to be relatively clonal (Campbell et al., 1992; Desjardins et al., 1994; Klittich and Leslie, 1988). A total of 44 distinct VCGs identified in this study suggest that the exchange of genetic information within *G. fujikuroi* in Korea may be significantly limited.

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