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Population Genetic Analyses of *Gibberella fujikuroi* Isolates from Maize in Korea

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We analyzed 88 strains of *Gibberella fujikuroi* (Anamorph: *Fusarium* section *Liseola*) from maize in Korea for mating population, mating type, fumonisin production, vegetative compatibility, and random amplified polymorphic DNA (RAPD) patterns. We found 50 strains that were *MATA-2*, 22 that were *MATA-1*, 1 that was *MATD-1*, and 15 that were not reproducibly fertile with any of the mating type testers. Of the 50 *MATA-2*, 15 were female fertile, while 10 of the 22 *MATA-1* strains were female fertile. A total of 1,138 nitrate non-utilizing (*nit*) mutants were recovered from a total of 88 strains. These strains were grouped into 39 vegetative compatibility groups (VCGs) by demonstrating heterokaryosis between *nit* mutants. A single maize ear could be infected by more than one VCG of *F. moniliforme*. RAPD analysis measured genetic diversity among 63 strains of *F. moniliforme*. Several VCGs were distinguished by RAPD fingerprinting patterns. Most strains produced significant levels of fumonisins. However, 6 *MATA-2* strains from a single VCG produced higher levels of fumonisin B_3 than that of fumonisin B_1 or B_2. From these data, we concluded that most Korean strains of *F. moniliforme* associated with maize belonged to mating population A and produced significant levels of fumonisins. Furthermore, RAPD analysis could differentiate strains associated with different VCGs.

Keywords : *Fusarium*, fumonisin, *Gibberella fusikuroi*, RAPD, vegetative compatibility.

Gibberella fujikuroi (Sawada) Ito in Ito & K. Kimura, with anamorphs in section *Liseola* of the genus *Fusarium*, is a complex species containing at least eight genetically distinct mating populations (=biological species) (Britz et al., 1999; Klittich et al., 1997; Leslie, 1991). This fungus is widely distributed worldwide and is a pathogen of a variety of important crops including maize, rice, and sorghum (Desjardins et al., 1997; Klittich et al., 1997; Leslie et al., 1990). In addition to their capabilities as plant pathogens, strains from this group of fungi are capable of producing significant quantities of mycotoxins including fumonisins (Gelderblom et al., 1988), fusaric acid (Marasas et al., 1984), fusarin C (Wiebe et al., 1981), and moniliformin (Marasas et al., 1984). Fumonisins induce equine leukoencephalomalacia and pulmonary edema in swine (Harrison et al., 1990; Kellerman et al., 1990; Ross et al., 1990). The presence of fumonisins in feeds has also been correlated with cancer-promoting activity in rats (Gelderblom et al., 1991).

In maize, *Fusarium moniliforme* is associated with root, stalk, and ear rots, which cause substantial losses of the crop (Shurtleff, 1980). Previous extensive surveys of *F. moniliforme* strains isolated from maize, mainly in the USA and Africa, reported that most strains were genetically diverse members of mating population A and can produce significant quantities of fumonisins (Desjardins et al., 1994; Leslie et al., 1992a; Leslie et al., 1992b; Marasas et al., 1979; Nelson et al., 1991). There was also considerable variation in the amount of fumonisin produced by strains from different geographic sources. For example, many Nepalese strains collected from maize consistently produced no detectable fumonisins. Genetic analysis of these strains showed that changes at a single locus, designated *fum1*, could block fumonisin production (Desjardins et al., 1992; Plattner et al., 1996).

The application of the vegetative compatibility group (VCG) technique for identifying different *F. moniliforme* strains has provided a relatively simple way to distinguish between strains that are morphologically identical (Klittich and Leslie, 1989; Leslie, 1993). Strains that are vegetatively compatible can form a stable heterokaryon belong to the same VCG, and are identical at a set of at least 10 *vic* loci (Correll et al., 1987; Klittich and Leslie, 1988). Strains belonging to the same VCG may be clones of one another, but strains that are in different VCGs are certainly distinct. Thus, several investigators have used VCG analysis to determine the minimum amount of genetic variability present within a population or related set of strains in many

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plant pathogenic fungi (Correll et al., 1986; Correll et al., 1987; Puhalla et al., 1985). Leslie et al. (1992) reported that all field strains of *F. moniliforme* associated with maize was in distinctive VCGs. This suggested that these strains of *F. moniliforme* which produced fumonisin B_1 were not clones of a single widely distributed strain, but rather were genetically diverse. Furthermore, Kedera et al. (1994) reported that most maize plants in the field appeared to have multiple infections of *F. moniliforme* originating from 2 or more genetically distinct strains. However, the relatedness or genetic diversity among strains within a VCG is not well understood. It is prerequisite to have molecular and/or neutral markers to assess genetic diversity among clonal lineages in fungi.

Random amplified polymorphic DNA (RAPD) analysis has been extensively used as a diagnostic tool for distinguishing genetic differences that define races or populations of plant pathogenic fungi (Foster et al., 1993; Guthie et al., 1992; Nicholson et al., 1994). Recently, the RAPD technique was applied in *F. moniliforme* to distinguish mating populations (Amoah et al., 1996), but it was not developed well enough to be used as a diagnostic tool at the population level.

The objectives of this study were i) to determine the mating populations and mating types of *F. moniliforme* isolates from maize in Korea, ii) to detect the capability of these strains to produce fumonisins, and iii) to determine the genetic diversity of maize strains by VCG and RAPD analyses.

Materials and Methods

Media. *Fusarium* complete medium (CM) (Correll et al., 1987) was used to maintain fungal cultures throughout the experiments. Peptone/pentachloronitrobenzene (PCNB) medium (Nash et al., 1962), carnation leaf agar (CLA), and KCl medium (Fisher et al., 1982) were used to isolate and identify *F. moniliforme*. *Fusarium* minimal medium (MM) (Correll et al., 1987) was used to generate and characterize the phenotypes of *nit* mutants.

Fungal strains. Sixty-three field strains of *F. moniliforme* (prefixed as Gf) were collected from 5 provinces in Korea including Kangwon, Kyonggi, Chungchong, Kyongsang, and Cheju provinces from September to October during 1996. Four maize ears were collected from Kangwon and a maize ear was collected each from Kyonggi, Chungchong, Kyongsang, and Cheju provinces, respectively. All maize samples were symptomless. The kernels of maize were surface-sterilized by dipping in 1% NaOCl for 1 min and rinsed with sterile distilled water for 30 sec. The kernels were then plated on peptone/PCNB medium and incubated for 3-5 days at 25°C. Uniform colonies grew out from all samples, and one colony was selected from each plate. One single spore (microconidium) from the colony was obtained by picking germinating conidia on 2% water agar, and was subcultured onto KCl medium or CLA. Identification of fungal strains was performed based on their morphological characteristic (Nelson et al., 1983). Twenty-five field strains of *F. moniliforme* (prefixed as M) were also obtained from symptomless kernels of maize samples collected from 5 provinces in Korea including Kyonggi, Chungchong, Kyongsang, Cholla, and Kangwon provinces during 1994. All strains used in this study were deposited in the Korean Collection for Type Culture (KCTC) at the Korean Research Institute of Bioscience and Biotechnology, Taejon, Korea. Mating type standard strains for *Fusarium* section *Liseola* were kindly supplied by Dr. John F. Leslie, Department of Plant Pathology, Kansas State University. A new mating type designation, the *MAT-1*/*MAT-2* system as proposed by Kerenyi et al. (1999), was employed in this study.

Crossing procedure. Crosses were made on carrot agar using the method of Klittich and Leslie (1988) with slight modifications. Initially, all field strains were crossed with the standard testers of mating population A, *MAT-1* (A00149) and *MAT-2* (A00999). Strains that were not fertile with these two testers were crossed with the standard testers of mating populations B, C, D, E, and F. An agar block of the maternal parent was transferred on carrot agar and incubated at 25°C with a 12 h/12 h dark and light cycle under 40 W cool-white fluorescent light. After 10-14 days, microconidial and mycelial suspensions from the paternal parent were poured on the lawn of maternal plates and spread with a sterile glass rod. After 10-14 days to mature dark blue perithecia were observed with oozing ascospores. Once mating population and type were identified, strains were tested for female fertility by reciprocal crosses in which the field strain functioned as the female parent and the standard tester as the male parent. All crosses were repeated at least twice.

Fumonisin production. Erlenmeyer flasks (300 ml) plugged with cotton were filled with 50 g of wheat grains and 15 ml distilled water and then autoclaved at 121°C for 1 h on each of two consecutive days. Each flask was inoculated with 10^7 conidia of a *F. moniliforme* strain and shaken once or twice daily for 3 days to distribute the inoculum evenly. The flask cultures were incubated in the dark at 25°C for 28 days. After that, the cultures were dried in a hood, ground to a fine meal in a blender, and stored at 20°C until analyzed. Fumonisin production was quantified by the procedure of Xie et al. (1997). For the analysis of fumonisins by high performance liquid chromatography (HPLC), the following equipment and conditions were used: instrument, tsp *Spectra* SYSTEM (Thermo Separation Products Inc., San Jose, CA, U.S.A.); Bondclone10 C_{18} column (4.9 mm by 300 mm; particle size, 10 μm; Phenomenex Co., Torrance, CA, U.S.A.); mobile phase, an acetonitrile gradient containing 1% acetic acid beginning at 20:80 of acetonitrile-water with a hold of 1 min and changing linearly to 80:20 of acetonitrile-water at 30 min with a final hold of 10 min; flow rate, 1 ml/min; FL3000 fluorescence detector, excitation at 336 nm and emission at 440 nm wavelength. The quantitation of fumonisins was performed by a PC1000 program that controlled the HPLC system.

Generation and characterization of *nit* mutants. Mycelial plugs of each strain from actively growing colony margins on CM were placed in the three sites of MM containing 3% chlorate

(MMC). The plates were incubated at 23°C under the dark and examined periodically for the appearance of fast-growing sectors from the initially restricted colonies. Fast-growing sectors were transferred to MM. Colonies that were resistant to chlorate and unable to utilize nitrate were considered *nit* mutants. The *nit* mutants were assigned to different phenotypic classes on the basis of their growth on media containing one of five different nitrogen sources: $NaNO_3$, $NaNO_2$, hypoxanthine, ammonium tartrate, or uric acid (Correll et al., 1987).

Assignment of strains to a VCG. Vegetatively compatible *nit* mutants at different loci may complement one another by forming a heterokaryon on MM, i.e., dense aerial growth develops where mycelia of *nit1* and NitM or *nit1* and *nit3* mutant colonies make contact (Puhalla, 1984). Pairings were made by placing mycelial blocks from each *nit* mutant 1.5 cm apart on MM, incubated under the conditions described above for 7-14 days, and then scored for complementation. *nit1* and NitM or *nit1* and *nit3* mutants representing all 88 strains were paired in all possible pairwise combinations on MM.

DNA extraction. Fungal cultures were grown in *Fusarium* complete broth for 7 days at 25°C with a 12 h dark/12 h light cycle. The mycelia were harvested, washed with sterile distilled water, and freeze-dried. Freeze-dried mycelia (1 g) were ground to a fine powder in liquid nitrogen. The mycelial powder was transferred to a 1.5 ml-microcentrifuge tube and lysis buffer (50 mM Tris-HCl, 50 mM EDTA, 3% SDS, and 1%-mercaptoethanol) was added. DNA was extracted twice with phenol: chloroform (1:1, v/v). Finally, total genomic DNA was precipitated by ethanol, and the DNA was dissolved in TE buffer (10 mM Tris-HCl, pH 7.5; 0.1 mM EDTA). The quantity of DNA was measured using a TKO 100 Flurometer (Hoefer, San Francisco, CA, U.S.A.).

RAPD analysis. Eight 10-mer primers, OPB-04, OPD-06, OPD-16, OPE-11, OPE-15, OPE-16, OPF-09, and OPF-16 (Operon Technologies, Alameda, CA, U.S.A.) were used for RAPD analysis. The amplification reactions were performed in 50 ml containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.25 mM dNTP mixture (Takara Shuwo Co., Ltd, Japan), 10 pmol of 10-mer random primer, 10 ng of genomic DNA, and 2 unit of *Taq* polymerase (Takara Shuzo Co., Ltd, Japan). Amplification was performed in a Perkin-Elmer/Cetus GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT, U.S.A.) for 40 cycles of 1 min at 94°C, 1 min at 40°C, and 2 min at 72°C using the fastest available transitions between each temperature. Polymerase chain reaction (PCR) products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide.

Statistical analysis. RAPD profiles corresponding to the strains were scored visually for the presence or absence of DNA bands. Only the major amplification products were scored, and this was based on the assumption that products of the same size and electrophoretic mobility in different strains were identical. Data obtained from the eight primers were pooled together. Each DNA band for isolates was used to construct a matrix of similarities between all pairs of isolates based on Dice's coefficient [F=2Nxy/(Nx + Ny)], where Nxy is the number of bands either present or absent in both isolates x and y and Nx + Ny is the total number of bands observed for that pair of isolates. Based on this similarity matrix, the average linkage method of hierarchical agglomerative clustering (unweighed pair group method arithmetic mean analysis in the SAHN program of the NTSYS-pc package (Rohlf, 1992) was used to produce a cluster dendrogram of isolates.

Results

Identification of fungal strains. The identification of the field strains collected from maize samples was performed based on the morphological characteristics reported by Nelson et al. (1983). Aerial mycelium was white to pale orange or violet in almost half or the entire colony. No sporodochia were produced. Sclerotia developed and were usually blue or dark blue if present. Macroconidia were observed on CLA, were slightly curved and septated, and their length ranged between 23-69 (average 46) μm. Microconidia formed in chains (3 to 58 spores) on monophialides on KCl medium. Based on these mycological characteristics, fungal strains were identified as *Fusarium* Section *Liseola*.

Mating population, type, and female fertility. Since the mating population A is the most popular in maize, we first tested all 88 strains for fertility with the A mating population testers; *MATA-1* (A00149), and *MATA-2* (A00999). Mature dark blue perithecia oozing ascospores were observed in the majority of plates about 14 days after incubation. In 25 strains (M1-M25) that were collected from 5 provinces of Korea in 1994, only A mating population (19 out of 25 strains) was found while the other 6 strains were not fertile. Both *MATA-1* and *MATA-2* mating types existed in all regions sampled. Among 63 strains collected from 5 provinces in Korea during 1996, 40 were identified as *MATA-2*, 13 were *MATA-1*, one was *MATD-1*, and 9 could not be determined. Overall, 50 strains were *MATA-2*, 22 were *MATA-1*, 1 was *MATD-1*, and 15 were not reproducibly fertile with any other mating type testers. Of the 50 *MATA-2* strains, 15 were female fertile, while 10 of the 22 *MATA-1* strains were female fertile (Table 1).

Production of fumonisins. Most strains produced significant quantities of fumonisins in the wheat culture. Strains of M1-M25 averaged 353 μg/g of fumonisin B_1 (FB_1), 131 μg/g of fumonisin B_2 (FB_2) and 18 μg/g of fumonisin B_3 (FB_3). Strains of Gf1-Gf63 averaged 212 μg/g of FB_1, 74 μg/g of FB_2 and 65 μg/g of FB_3 (Table 1). In general, mating population A strains produced more FB_1 than FB_2 or FB_3. However, 6 strains (Gf33, Gf35, Gf36, Gf37, Gf38 and Gf40) produced higher level of FB_3 compared to FB_1 or FB_2. These 6 strains averaged 42 μg/g of FB_1, 62 μg/g of FB_2, and 350 μg/g of FB_3. They were all mating type *MATA-2* and in the same VCG (see below). Among the 88 strains, 8 did not produce detectable levels of fumonisins.

Vegetative compatibility group. To find the optimum conditions for the generation of *nit* mutants, MM supplemented

Table 1. Mating population, mating type, geographic origin, vegetative compatibility group, and fumonisin production by *Fusarium moniliforme* strains used in this study

Strain number	KCTC number[a]	Mating population/ Mating type (Female fertility[b])	Fumonisin (μg/g)			Geographic origin	Vegetative compativility group
			B_1	B_2	B_3		
M01	KTCT-16247	*MATA-2* (+)	692	284	27	Kyonggi	01
M02	KTCT-16248	NF[c]	787	356	12	Kyonggi	02
M03	KTCT-16249	*MATA-1* (–)[c]	336	96	20	Kyonggi	03
M04	KTCT-16250	NF	335	365	10	Kyonggi	04
M05	KTCT-16251	*MATA-2* (–)	264	72	9	Chungchong	05
M06	KTCT-16252	*MATA-1* (–)	356	76	13	Chungchong	06
M07	KTCT-16253	*MATA-2* (–)	573	194	22	Chungchong	07
M08	KTCT-16254	*MATA-1* (–)	435	196	13	Chungchong	08
M09	KTCT-16255	*MATA-2* (+)	5	2	3	Chungchong	09
M10	KTCT-16256	NF	170	29	10	Chungchong	10
M11	KTCT-16257	*MATA-2* (+)	272	116	7	Chungchong	11
M12	KTCT-16258	NF	7	3	0.6	Kyungsang	12
M13	KTCT-16259	*MATA-1* (+)	298	89	12	Kyungsang	13
M14	KTCT-16260	NF	106	75	16	Kyungsang	14
M15	KTCT-16261	*MATA-2* (–)	–	–	–	Kyungsang	15
M16	KTCT-16262	*MATA-2* (–)	283	82	34	Chonlla	16
M17	KTCT-16263	*MATA-2* (–)	154	47	8	Chonlla	17
M18	KTCT-16264	*MATA-1* (+)	798	302	30	Chonlla	18
M19	KTCT-16265	*MATA-1* (+)	164	73	6	Chonlla	19
M20	KTCT-16266	NF	6	16	–	Chonlla	20
M21	KTCT-16267	*MATA-2* (+)	563	140	57	Chonlla	21
M22	KTCT-16268	*MATA-2* (+)	226	52	22	Chonlla	22
M23	KTCT-16269	*MATA-1* (+)	219	103	6	Chonlla	23
M24	KTCT-16270	*MATA-1* (+)	471	163	29	Chonlla	24
M25	KTCT-16271	*MATA-1* (–)	948	202	49	Kangwon	25
Gf01	KCTC-16183	*MATA-2* (–)	318	82	34	Kangwon	26
Gf02	KCTC-16184	*MATA-2* (+)	179	56	23	Kangwon	26
Gf03	KTCT-16185	*MATA-2* (–)	670	275	42	Kangwon	26
Gf04	KTCT-16186	*MATA-2* (+)	165	6	26	Kangwon	26
Gf05	KTCT-16187	*MATA-2* (–)	201	70	21	Kangwon	26
Gf06	KTCT-16188	*MATA-2* (–)	550	216	40	Kangwon	26
Gf07	KTCT-16189	*MATA-2* (–)	442	185	39	Kangwon	26
Gf08	KTCT-16190	*MATA-2* (–)	431	121	34	Kangwon	26
Gf09	KTCT-16191	*MATA-2* (+)	314	47	23	Kangwon	26
Gf10	KTCT-16192	*MATA-2* (+)	349	65	25	Kangwon	26
Gf11	KTCT-16193	*MATA-2* (–)	288	0.3	1	Kangwon	27
Gf12	KTCT-16194	*MATA-2* (–)	466	–	–	Kangwon	27
Gf13	KTCT-16195	*MATA-2* (–)	438	50	58	Kangwon	28
Gf14	KTCT-16196	*MATA-2* (–)	230	69	14	Kangwon	28
Gf15	KTCT-16197	*MATA-2* (–)	9	3	0.2	Kangwon	27
Gf16	KTCT-16198	*MATA-2* (–)	51	7	0.2	Kangwon	27
Gf17	KTCT-16199	*MATA-2* (–)	523	132	54	Kangwon	28
Gf18	KTCT-16200	*MATA-2* (–)	122	28	10	Kangwon	28
Gf19	KTCT-16201	*MATA-2* (–)	2	1	0.1	Kangwon	27
Gf20	KTCT-16202	*MATA-2* (–)	731	200	65	Kangwon	28
Gf21	KTCT-16203	*MATA-2* (+)	–	–	–	Kangwon	26
Gf22	KTCT-16204	*MATA-2* (+)	632	252	42	Kangwon	26

Table 1. Continued

Strain number	KCTC number[a]	Mating population/ Mating type (Female fertility[b])	Fumonisin (μg/g)			Geographic origin	Vegetative compativility group
			B_1	B_2	B_3		
Gf24	KTCT-16206	*MATA-2* (−)	471	253	79	Kangwon	26
Gf25	KTCT-16207	*MATA-2* (−)	265	87	21	Kangwon	26
Gf26	KTCT-16208	*MATA-2* (−)	4	2	1	Kangwon	26
Gf27	KTCT-16209	*MATA-2* (−)	7	4	1	Kangwon	29
Gf28	KTCT-16210	*MATA-2* (−)	78	13	6	Kangwon	29
Gf29	KTCT-16211	*MATA-2* (−)	6	1	0.4	Kangwon	29
Gf30	KTCT-16212	*MATA-2* (−)	25	7	0.5	Kangwon	29
Gf31	KTCT-16213	*MATA-2* (+)	485	166	34	Kangwon	26
Gf32	KTCT-16214	*MATA-2* (−)	523	374	306	Kangwon	30
Gf33	KTCT-16215	*MATA-2* (−)	197	98	556	Kangwon	30
Gf34	KTCT-16216	*MATA-2* (−)	305	98	30	Kangwon	26
Gf35	KTCT-16217	*MATA-2* (−)	20	56	308	Kangwon	30
Gf36	KTCT-16218	*MATA-2* (+)	12	43	223	Kangwon	30
Gf37	KTCT-16219	*MATA-2* (−)	6	76	399	Kangwon	30
Gf38	KTCT-16220	*MATA-2* (+)	1.4	46	269	Kangwon	30
Gf39	KTCT-16221	*MATA-2* (−)	41	138	36	Kangwon	26
Gf40	KTCT-16222	*MATA-2* (+)	5	52	345	Kangwon	30
Gf41	KTCT-16223	*MATA-1* (−)	11	2	12	Kyonggi	31
Gf42	KTCT-16224	*MATA-1* (−)	37	19	8	Kyonggi	31
Gf43	KTCT-16225	*MATA-1* (−)	567	169	52	Kyonggi	31
Gf44	KTCT-16226	*MATA-1* (−)	320	106	20	Kyonggi	31
Gf45	KTCT-16227	*MATA-1* (−)	597	104	111	Kyonggi	32
Gf46	KTCT-16228	NF	0.2	–	–	Kyungsang	33
Gf47	KTCT-16229	*MATD-2* (−)	438	53	62	Kyungsang	34
Gf48	KTCT-16230	NF	4	1	1	Kyungsang	35
Gf49	KTCT-16231	NF	0.3	0.1	0.3	Kyungsang	36
Gf50	KTCT-16232	NF	8	1.2	2	Kyungsang	36
Gf51	KTCT-16233	NF	1	0.3	0.4	Kyungsang	36
Gf52	KTCT-16234	*MATA-1* (+)	29	25	3	Cheju	37
Gf53	KTCT-16235	*MATA-1* (+)	–	–	–	Cheju	37
Gf54	KTCT-16236	*MATA-1* (+)	–	–	–	Cheju	37
Gf55	KTCT-16237	*MATA-1* (−)	–	–	–	Cheju	37
Gf56	KTCT-16238	*MATA-1* (−)	1	0.2	–	Cheju	37
Gf57	KTCT-16239	*MATA-1* (+)	6	5	0.1	Cheju	37
Gf58	KTCT-16240	*MATA-1* (−)	1	0.3	1	Cheju	37
Gf59	KTCT-16241	*MATA-1* (−)	6	4	1	Cheju	37
Gf60	KTCT-16242	NF	51	14	19	Chungchong	38
Gf61	KTCT-16243	NF	–	–	–	Chungchong	38
Gf62	KTCT-16244	NF	–	–	–	Chungchong	38
Gf63	KTCT-16245	NF	136	80	22	Chungchong	39

[a] KCTC = Korean Collection for Type Culture.
[b] + and – indicate female fertility and female sterile, respectively.
[c] NF indicates not fertile with any tester strain of mating populations A, B, C, D, E, and F.

with varying amount of chlorate concentrations was initially tested. The frequency of chlorate-resistant sectors was dependent upon the strains and the amount of chlorate in the medium. The MM with 3% chlorate was found to be the most efficient to induce the mutations in this experiment. When the chlorate-resistant sectors were transferred to MM, two mutant classes were observed; *nit* and chlorate-resistant, nitrate utilizing (CRUN). The *nit* mutants grew as

thin and wispy colonies while CRUN mutants grew similar to the wild type. Of the 1,138 chlorate-resistant sectors recovered from 88 strains of *F. moniliforme*, 1,104 (97%) were *nit* mutants and 34 (3%) were CRUN. The phenotypes of the *nit* mutants were determined by their colony morphology on media containing five different nitrogen sources. Of the 1,104 *nit* mutants, 840 (73.8%) were identified as *nit1*, and 177 (15%) and 87 (7%) were NitM and *nit3*, respectively. The frequencies of *nit* mutants were similar among different sources of strains. Physiological complementation among different *nit* mutants was indicated by the development of dense aerial growth where the mycelia of the *nit* mutant colonies came in contact and anastomosed to form heterokaryons. When all pairwise combinations, mainly *nit1* and NitM, from all 88 strains were made on MM (7,593 combinations), 39 nonoverlapping VCGs were identified. Thirty strains were incompatible with all others, and thus each strain constituted a unique VCG. Fifty-eight strains were compatible with other isolates and thus constituted nine VCGs. Twenty-five strains of *F. moniliforme* (prefixed as M) collected during 1994 were classified into 25 VCGs, therefore indicating that each strain constitutes a unique VCG. Fourteen nonoverlapping VCGs were identified among 63 strains of *F. moniliforme* collected during 1996. One to 4 VCGs were identified from the strains obtained from a single maize ear (Table 2).

RAPD analysis. Eight random primers were used to analyze polymorphisms by RAPD analysis among the 63 strains (Gf series) of *F. moniliforme* collected from maize during 1996. Two to 8 DNA fragments were amplified from each strain by each primer. The sizes of amplified DNAs ranged from 200 bp to 1.5 Kbp. Among the 8 primers used for amplification, 3 (OPF-09, OPE-16 and OPD-16) exhibited the distinct features of amplified products. Especially, primers OPE-09 could differentiate those strains belonging to different VCGs. In VCG 26, 29, and 30, strains belonging to the same VCG had unique RAPD patterns, but were different from those associated with other VCGs (Fig. 1). Strains belonging to VCG30 produced higher levels of FB_3 than FB_1 or FB_2. These strains also exhibited a unique RAPD band pattern by primer OPF-09 (Fig. 1). When all bands amplified with 8 primers were scored and a dendrogram was constructed, 8 major groups were clustered. This grouping matches to a VCG in this fungus as shown in the dendrogram in Fig. 2.

Table 2. Vegetative compatibility groups of *Fusarium monilifome* strains used in this study

Geographic origin	Strains collected in 1994		Strains collected in 1996[a]	
	No. of strains	No. of VCG	No. of strains	No. of VCG
Kangwon	1	1	40	5
Kyonggi	4	4	5	2
Chungchong	7	7	4	2
Kyongsang	4	4	6	4
Cholla	9	9	–	–
Cheju	–	–	8	1
Total	25	1	63	14

[a] All strains were isolated from single maize ear except Kangwon where each 10 strains were isolated from 4 maize ears.

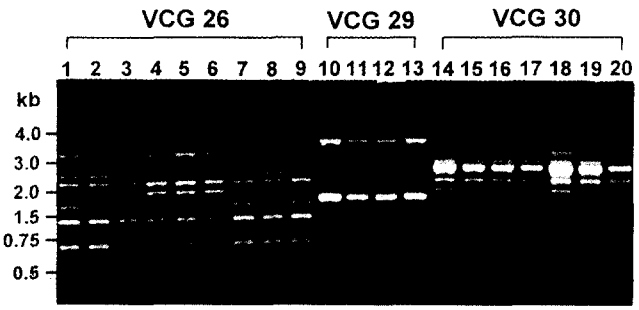

Fig. 1. Amplified DNA band patterns of genomic DNAs from *Fusarium moniliforme* strains by PCR with primer OPE-09. Lanes 1 to 20 are strains Gf1, Gf2, Gf3, Gf4, Gf22, Gf23, Gf24, Gf25, Gf26, Gf27, Gf28, Gf29, Gf30, Gf32, Gf33, Gf35, Gf36, Gf37, Gf38 and Gf40. Lanes 15 to 20 are strains which produce higher levels of FB_3 than FB_1 or FB_2.

Discussion

We have determined mating populations, mating types, and female fertilities of *F. moniliforme* strains collected from maize samples in Korea. Among the 88 strains tested, the A mating population was predominant; 72 out of fertile 73 strains. These results agree with previous reports that mating population A is predominant in maize, whereas mating population F is the major in sorghum (Leslie, J. F. 1991; Leslie, J. F. 1995; Leslie et al., 1996). Both mating type *MATA-1* and *MATA-2* were detected from all regions where maize samples were collected in Korea. Previously, it has been strongly suggested that sexual recombination might occur in the field, because both mating type strains exist in the same field or seed lots (Leslie, J. F. 1995). Desjardins et al. (1994) further reported that strains representing both mating types of *F. moniliforme* were recovered from the same ear of maize in Northeast Mexico. However, in this study strains obtained from a single ear of maize possess the same mating type. In other words, both mating type *MATA-1* and *MATA-2* strains exist in the same region or field, but only one mating type exists in a single ear of maize. However, our sample size was relatively small to support this conclusion. A more extensive survey should be conducted to determine the possible existence of both mating types in the same ear of maize in Korea and to under-

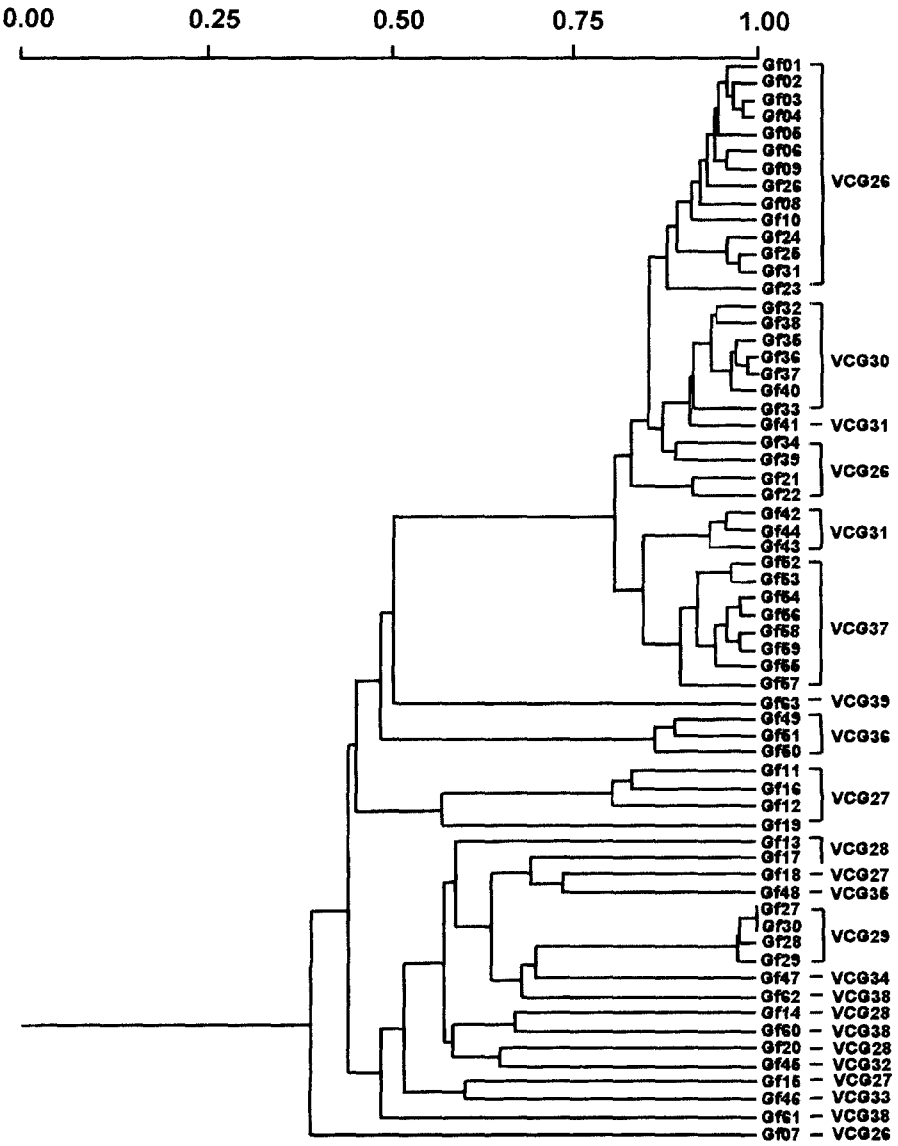

Fig. 2. Dendrogram of 63 *Fusarium moniliforme* strains collected from maize based on RAPD polymorphism. Data from 8 different 10-mer primers were pooled and used to construct a dendrogram. VCG numbers were indicated on the right for individual strains.

stand the possible frequency of sexual recombination in nature.

Recently, several analogues of fumonisin have been chemically characterized. Most naturally occurring strains of *G. fujikuroi* mating population A produce predominately FB_1 (70-80%), and lower levels of FB_2 (10-20%), FB_3 (10-20%), and FB_4 (5-10%), when grown on maize substrate. However, FB_1 deficient mutants have been identified and genetically characterized (Desjardins et al., 1996a; Desjardins et al., 1996b; Desjardins et al., 1992). Among these FB_1 deficient mutants, one mutant class produces high levels of FB_3 and FB_4 with no detectable FB_1 or FB_2. The mutation is designated as *fum3* and this genotype lacks the ability to hydroxylate position C-5. In our study, however, 6 strains produced FB_1, FB_2, and FB_3, but the production level of FB_3 was 6 to 8 times higher than that of FB_1 or FB_2. This production pattern of fumonisins by *F. moniliforme* has not been previously reported. Furthermore, these 6 strains composed a distinct unique VCG and clustered into the same group in RAPD analyses. The combination of these data suggests that these 6 strains are genetically distinct from other strains of *F. moniliforme*. Further molecular analysis of these strains may provide greater insights into the biosynthetic pathways of fumonisins.

Twenty-five strains of *F. moniliforme* (prefixed as M) were classed into 25 VCGs. It is not uncommon that each field strain of *F. moniliforme* represents a unique VCG as reported in previous studies (Kedera et al., 1994; Leslie et

al., 1992a). Through complementation tests of the *nit* mutants derived from 63 strains (Gf1-Gf63), 14 nonoverlapping VCGs were identified. We also showed that most maize plants are infected by one to four strains belonging to different VCGs. Kedera et al. (1994) reported that most maize plants appeared to have multiple infections of *F. moniliforme*; therefore, many plants are likely to contain genetically different strains. Our data also support this hypothesis, as none of the strains isolated from different ears in the same field of Kangwon province were grouped into the same VCG. On the other hand, it has been suggested that infection within an ear is relatively uniform and that only a few fungal strains are usually present per ear of maize (Kedera et al., 1994). However, 4 different VCGs were identified among 6 strains isolated from a single ear of maize in this study, which indicates that genetic diversity (number of VCGs/number of strains) within a single ear is as high as 0.67.

Although much research has been conducted on VCG analysis of *F. moniliforme* populations, little information is available on the relatedness or genetic diversity among strains within a VCG. It was required to use of molecular and/or neutral makers to access genetic diversity within a VCG. We showed that RAPD analyses could differentiate strains of *F. moniliforme* belonging to different VCGs. In other words, strains belonging to the same VCG exhibited similar DNA amplification patterns in RAPD analyses. Both techniques, VCG and RAPD analyses, have been extensively applied in the areas of research for differentiation of race and formae specialis in many plant pathogenic fungi (Guthie et al., 1992; Leslie et al., 1992a; Nicholson et al., 1994). However, our result presented here is one of the clearest relationships to date between VCG and RAPD analyses.

In conclusion, Korean strains of *F. moniliforme* associated with maize are affiliated with mating population A and produce significant levels of fumonisins. These strains of *F. moniliforme* are not clones of a single widely distributed strain, but are instead genetically diverse. Furthermore, RAPD analysis could differentiate strains belonging to different VCGs in this fungus.

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Literature Cited

Amoah, B. K., Macdonald, M. V., Rezanoor, N. and Nicholson, P. 1996. The use of random amplified polymorphic DNA technique to identify mating groups in the *Fusarium* section *Liseola*. *Plant Pathol.* 45:115-125.

Britz, H., Coutinho, T. A., Wingfield, M. J., Marasas, W. F. O., Gordon, T. R. and Leslie, J. F. 1999. *Fusarium subglutinans* f. sp. *pini* represents a distinct mating population in the *Gibberella fujikuroi* species complex. *Appl. Environ. Microbiol.* 65:1198-1201.

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

Correll, J. C., Puhalla, J. E. and Schneider, R. W. 1986. Vegetative compatibility groups among nonpathogenic root colonizing strains of *Fusarium oxysporum*. *Can. J. Bot.* 64:2358-2361.

Desjardins, A. E., Plattner, R. D. and Nelson, P. E. 1994. Fumonisin production and other traits of *Fusarium moniliforme* strains from maize in Northeast Mexico. *Appl. Environ. Microbiol.* 60:1695-1697.

Desjardins, A. E., Plattner, R. D. and Nelson, P. E. 1997. Production of Fumonisin B_1 and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. *Appl. Environ. Microbiol.* 63:1838-1842.

Desjardins, A. E., Plattner, R. D. and Proctor, R. H. 1996a. Genetic and biochemical aspects of fumonisin production. In: *Fumonisins in Foods*, ed. by L. S. Jackson, W. De Varies and L. B. Bullerman, pp. 165-173. Plenum Publication Co., New York, U.S.A.

Desjardins, A. E., Plattner, R. D. and Proctor, R. H. 1996b. Linkage among genes responsible for fumonisin biosynthesis in *Gibberella fujikuroi* mating population A. *Appl. Environ. Microbiol.* 62:2571-2576.

Desjardins, A. E., Plattner, R. D., Shackelford, D. D., Leslie, J. F. and Nelson, P. E. 1992. Heritability of fumonisin B_1 production in *Gibberella fujikuroi* mating population A. *Appl. Environ. Microbiol.* 58:2799-2805

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

Foster, L. M., Kozak, K. R., Loftus, M. G., Stevens, J. J. and Ross, I. K. 1993. The polymerase chain reaction and its application to filamentous fungi. *Mycol. Res.* 97:769-781.

Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R. and Kriek, N. P. J. 1988. Fumonisins: novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* 54:1806-1811.

Gelderblom, W. C. A., Kriek, N. P. J. , Marasas, W. F. O. and Thiel, P. G. 1991. Toxicity and carcinogenicity of *Fusarium moniliforme* metabolite; fumonisin B_1, in rats. *Carcinogenesis* 12:1247-1251.

Guthie, P. A. I., Magill, C. W., Fredericksen, R. A. and Odvody, G. N. 1992. Random amplified polymorphic DNA marker: a system for identifying and differentiating isolates of *Colletotrichum graminicola*. *Phytopathology* 82:832-835.

Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E. and Cole, J. R. 1990. Pulmonary edema and hydrothorax in swine

produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* 2:217-221.

Kedera, C. J., Leslie, J. F. and Claffin, L. E. 1994. Genetic diversity of *Fusarium* section *Liseola* (*Gibberella fujikuroi*) in individual maize stalks. *Phytopathology* 84:603-607.

Kellerman, T. S., Marasas, W. F. O., Thiel, P. G., Gelderblom, W. C. A., Cawood, M. and Coetzer, J. A. W. 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. *Onderstepoort J. Vet. Res.* 57:269-275.

Kerenyi, Z., Zeller, K., Hornok, L. and Leslie, J. F. 1999. Molecular standardization of mating type terminology in the *Gibberella fujikuroi* species complex. *Appl. Environ. Microbiol.* 65: 4071-4076.

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

Klittich, C. J. R. and Leslie, J. F. 1989. Chlorate-resistant, nitrate-utilizing mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *J. Gen. Microbiol.* 135:721-727.

Klittich, C. J. R., Leslie, J. F., Nelson, O. E. and Marasas, W. F. O. 1997. *Fusarium thapsinum* (*Gibberella thapsinum*): A new species in section *Liseola* from sorghum. *Mycologia* 89:643-652.

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

Leslie, J. F. 1993. Fungal vegetative compatibility. *Annu. Rev. Phytopathol.* 31:127-150.

Leslie, J. F. 1995. *Gibberella fujikuroi*: available populations and variable traits. *Can. J. Bot.* 73:S282-S291.

Leslie, J. F., Doe, F. J., Plattner, R. D., Shackelford, D. D. and Jonz, J. 1992a. Fumonisin B_1 production and vegetative compatibility of strains from *Gibberella fujikuroi* mating population "A" (*Fusarium moniliforme*). *Mycopathologia* 117:37-45.

Leslie, J. F., Marasas, W. F. O., Shephard, G. S., Sydenham, E. W., Stockenstrom, S. and Thiel, P. G. 1996. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and F mating populations of *Gibberella fujikuroi* (*Fusarium moniliforme*). *Appl. Environ. Microbiol.* 62:1182-1187.

Leslie, J. F., Pearson, C. A. S., Nelson, P. E. and Toussoun, T. A. 1990. *Fusarium* species from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathology* 80:343-350.

Leslie, J. F., Plattner, R. D., Desjardins, A. E. and Klittich, C. J. R. 1992b. Fumonisin B1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Phytopathology* 82:341-345.

Marasas, W. F. O., Kriek, N. P. J., Wiggens, V. M., Steyn, P. S., Towers, D. K. and Hastie, T. J. 1979. Incidence, geographic distribution, and toxigenicity of *Fusarium* species in South African corn. *Phytopathology* 69:1181-1185.

Marasas, W. F. O., Nelson, P. E. and Toussoun, T. A. 1984. *Toxigenic Fusarium Species: Identity and Mycotoxicology*. Pennsylvania State University Press, University Park.

Nash, S. M. and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. *Phytopathology* 52:567-572.

Nelson, P. E., Plattner, R. D., Shackelford, D. D. and Desjardins, A. E. 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. *Appl. Environ. Microbiol.* 57:2410-2412.

Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park, PA. 193pp.

Nicholson, P. and Rezanoor, H. N. 1994. The use of random amplified polymorphic DNA to identify pathotype and detect variation in *Pseudocercosporella herpotrichoides*. *Mycol. Res.* 98:13-21.

Plattner, R. D., Desjardins, A. E., Leslie, J. F. and Nelson, P. E. 1996. Identification and characterization of strains of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*) with rare fumonisin production phenotypes. *Mycologia* 88: 416-424.

Puhalla, J. E. 1984. A visual indicator of heterokaryon in *Fusarium oxysporum* from celery. *Can. J. Bot.* 62:540-545.

Puhalla, J. E. and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). *Exp. Mycol.* 9:39-47.

Rohlf, F. J. 1992. NTSYS-pc., version 1.7. *Numerical Taxonomy and Multivariate Analysis System*. Exeter Publishing, Setauket, New York.

Ross, P. F., Nelson, P. E., Richard, J. L., Osweiler, G. D., Rice, L. G., Plattner, R. D. and Wilson, T. M. 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. *Appl. Environ. Microbiol.* 56:3226-3226.

Shurtleff, M. C., ed. 1980. *Compendium of Corn Diseases*, 2nd ed. The American Phytopathological Society, St. Paul, MN, USA. 105 pp.

Wiebe, L. A. and Bjeldanes, L. F. 1981. Fusarin C, a mutagen from *Fusarium moniliforme* grown on corn. *J. Food. Sci.* 46: 1424-1426.

Xie, W., Mirocha, C. J. and Chen, J. 1997. Detection of two naturally occurring structural isomers of partially hydrolyzed fumonisin B_1 in corn by on-line capillary liquid chromatography-fast atom bombardment mass spectrometry. *J. Agric. Food Chem.* 45:1251-1255.