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Mating Type Alleles of *Magnaporthe grisea* in Korea

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한국에서 분리한 도열병균의 교배형

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ABSTRACT : Twenty six isolates of *Magnaporthe grisea* originated from rice and other gramineous hosts in Korea were tested for mating type with MAT1-1 and MAT1-2 standard isolates. Ninety three and 64% of rice and grass isolates mated and produced perithecia with standard isolates, respectively. Both mating types were found from rice and non-rice isolates, but MAT1-1 allele of *M. grisea* was predominant in Korea.

***Key words* : Mating types, *Magnaporthe grisea*, sexual reproduction.**

Magnaporthe grisea (Hebert) Barr is a hermaphroditic ascomycete (1) and its anamorph, *Pyricularia grisea*, has a wide range of hosts including rice and several other gramineous plants. Isolates causing rice blast have been classified as *P. oryzae* separately from *P. grisea*. The facts that these two anamorphs have the same sexual stage and interfertility have led to the proposition of grouping both anamorphs into *P. grisea* (9). Rice blast is the most destructive disease in rice throughout the world (8). Controlling this disease has been very difficult, because resistant cultivars to this disease have been broken due to frequent appearances of new races. Epidemiological models have been formulated to understand frequent changes of races in the rice fields. However, little is known about the genetic basis of race shifting of this pathogen in nature.

Sexual reproduction between non-rice isolates of *P. grisea* was first reported by Hebert in 1971, followed by crossings of several weed isolates and rice isolates (4, 12, 13, 15, 16, 17). Mating of this fungus is controlled by two alleles at one locus, designated MAT1-1 and MAT1-2 (14). Previously two alleles were designated as A and a in many literatures, but recent genetic evidence of matings of this fungus clearly showed that either allele is not dominant to the other. Mating type alleles were segregated into 1:1 ratio in the progeny analysis of F_1 (5). Since isolates pathogenic to rice have low fertility and most of them are female-sterile, it was necessary to improve their fertility for genetic analyses. Recently isolates which are fertile enough to carry out genetic analyses of rice isolates and pathogenic to rice were obtained by the breeding of field isolates in the laboratory (6). In this study we report the existence of both mating types of *M. grisea* pathogenic to rice and other gramineous hosts in Korea and possibility of sexual recombination in the field.

Isolates of *M. grisea* used in this experiment were obtained from the Korean Research Institute of Chemical Technology or Agricultural Sciences Institute, Rural Development Administration. Standard isolates of mating type (70-6 and 70-15) were obtained from Dr. A. Ellingboe at University of Wisconsin, USA (Table 1). The detailed descriptions of these isolates were described in previous report (6). Unknown isolates were paired with standard isolates of both mating types on oatmeal medium (50 g oatmeal per liter) and incubated for 2~4 weeks under continuous fluorescent light at 22℃. After 2 weeks, lines formed in the junction between two isolates were taken and observed under microscope.

Total 26 isolates of *M. grisea* isolated from rice and other hosts were tested for their mating type. Fourteen from 15 rice isolates mated with the oppo-

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Table 1. Mating type allele and origin of *Magnaporthe grisea* isolates

Isolates	Mating type	Origin
MG01	MAT1-1	*Oryza sativa*
MG02	MAT1-1	*Oryza sativa*
MG03	MAT1-1	*Oryza sativa*
MG04	MAT1-1	*Oryza sativa*
MG05	MAT1-1	*Oryza sativa*
MG06	MAT1-1	*Oryza sativa*
MG07	MAT1-1	*Oryza sativa*
MG08	MAT1-1	*Oryza sativa*
MG14	MAT1-1	*Oryza sativa*
MG15	MAT1-1	*Oryza sativa*
MG16	MAT1-1	*Oryza sativa*
MG17	–	*Oryza sativa*
MG18	MAT1-1	*Oryza sativa*
MG19	MAT1-1	*Oryza sativa*
MG20	MAT1-2	*Oryza sativa*
MG10	MAT1-2	*Echinochloa crus-galli*
MG11	–	*Digitaria sanguinalis*
MG12	–	*Digitaria sanguinalis*
MG104	MAT1-1	*Digitaria sanguinalis*
MG108	–	*Digitaria sanguinalis*
MG101	MAT1-1	*Setaria italica*
MG105	MAT1-1	*Setaria italica*
MG102	MAT1-1	*Eleusine coracana*
MG103	MAT1-1	*Festuca elatior*
MG106	MAT1-2	*Phleum pratense*
MG107	–	*Panicum miliaceum*
70-15[a]	MAT1-1	*Oryza sativa*
70- 6[a]	MAT1-2	*Oryza sativa*

[a] 70-15 and 70-6 are the standard isolates of mating type.

– : No perithecia were observed.

site standard isolates. Thirteen isolates were identified as MAT1-1 and one isolate was MAT1-2 (Table 1). This is very high frequency of mating in the crossings of *M. grisea* rice field isolates compared to other reports (10, 15, 18). This result also indicates the importance of using high fertile standard isolates for the crosses of rice isolates. The standard isolates used in this experiment were enhanced for their fertility by breeding in the laboratory. Seven out of 11 non-rice isolates mated with standard isolates. Five and two isolates were identified as MAT 1-1 and MAT1-2, respectively. MAT1-2 isolates were isolated from barnyard grass (*Echinochloa crus-galli*) and timothy (*Phleum pratense*). These results clearly showed that both mating type alleles were found from rice and non-rice isolates in Korea, although only 26 isolates were screened. Perithecia formation was initiated after 2 weeks when paired with opposite standard isolate on oatmeal agar medium under continuous fluorescent light. Fully matured perithecia were observed around after 3~4 weeks. Several crosses produced only perithecia without asci and ascospores, whereas most of crosses formed perithecia with asci and ascospores. Perithecia, singly or in groups, were partially embedded in oatmeal agar predominantly 2 mm in depth (Fig. 1C). The mature perithecia formed with 70-6 standard isolates were brown to dark brown and globose, measuring 70~220 μm (mean 140 μm) in diameter. The ostiolar beaks ranged from 40 μm to 140 μm in width (mean 60 μm) and 150 μm to 590 μmm in length (mean 415 μmm). However, perithecia formed with 70-15 standard isolate were slightly bigger in the diameter of perithecial base (mean 160 μm) and longer in the length of ostiolar beak (470 μmm) than those formed with 70-6 standard isolate. The size of ascospore formed from the cross with 70-15 standard (8.0 μm×22 μm) was slightly bigger than those from the cross with 70-6 (6.5 μm×18 μm). The size of asci was virtually the same in both crosses (mean 10 μm×90 μm in width and length). The morphology of sexual organs observed in this experiment was similar to previous other reports (1, 2). No perithecia was formed when two opposite mating types of rice isolates were paired. This indicated that all rice field isolates of *M. grisea* were female-sterile and act as only male. This female-sterility was evident when paired with hermaphroditic standard isolates; only one line of perithecia was formed on the side of hermaphroditic standard isolate instead of two lines (Fig. 1A and 1B).

Sexual recombination in nature was not observed, although both mating types were found in the field. This phenomenon is believed that most of isolates in the field are female-sterile. The frequency of MAT1-1 or MAT1-2 in the fields was variable depending on the geographic origin. Recently Notteghem and Silue (7) reported the distribution of mating type alleles of rice isolates from world-wide collection. Fifty-two percentage of isolates tested were formed perithecia when paired with the standard isolates. Both mating types, MAT1-1 and MAT1-2, were nearly equal frequencies in West Africa, Philippines, and North South America, whereas MAT 1-1 was predominant in Northern Asia. However,

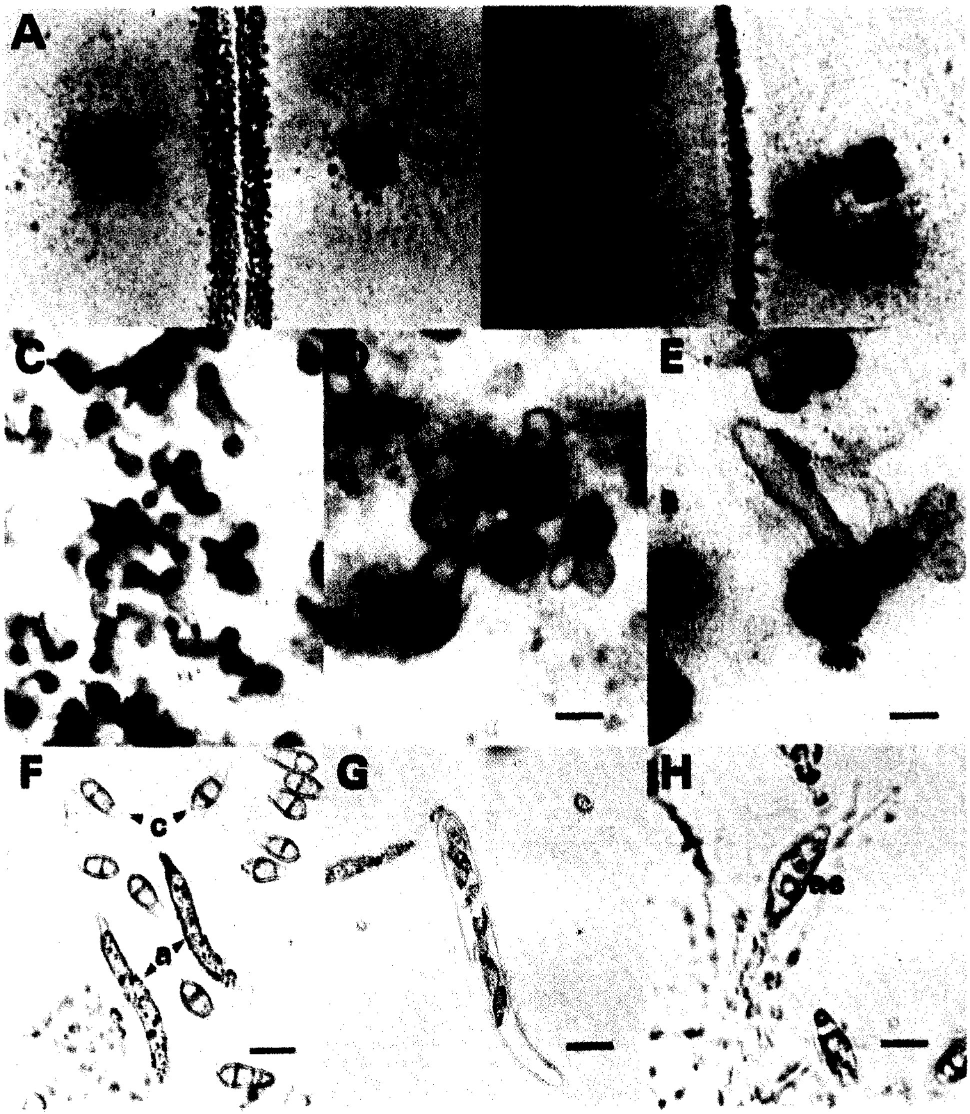

Fig. 1. Mating patterns and development of perfect stage in *Magnaporthe grisea*. A : Hermaphroditic mating pattern in the crossing between standard isolates (70-6 & 70-15). Two bands of perithecia were produced in the intersection of two fertile isolates, B : Mating pattern of a female-sterile isolate MG01 in the crossing to standard isolate 70-6. There is a single band of perithecia on the side of 70-6, C : Embedded perithecial bases and beaks protruded from oatmeal agar, D : Early stage of perithecial development in the crossing between isolates 70-6 and MG01, E : Matured perithecium and asci releasing by crushing a perithecium, F : Conidia (c) and asci (a) in pre-differentiation stage, G : Ascus with eight ascospores, H : Ascospores with three septae. Scale bars indicate 125 μm in D and E, 25 μm in F and G, and 13 μm in H.

only MAT1-1 was found in Europe and Central Africa. Yaegashi (15) reported that both mating types were found in Japan, but Kato and Yamaguchi (3) could not find any MAT1-2 type among 451 isolates tested. No information is available on the mating type distribution of *M. grisea* isolated from rice in Korea. Ryu *et al.* (10) reported the matings of *M. grisea* isolated from several cereals and grasses, but they did not indicate mating types of rice isolates. Our results indicate that Mat1-1 is predominant (93%) in Korea, although both mating type alleles were found.

In nature, only anamorph of *M. grisea*, *Pyricularia grisea*, exists. However, perithecia were formed on rice when inoculated with two compatible isolates in the laboratory condition (11). Most of rice isolates are female-sterile and have low fertility, but a few field isolates have hermaphroditic fertility (6, 7). These observations suggested that we can not completely exclude the possibility of sexual recombination in nature. Previous report (7) suggested that isolates pathogenic to rice seemed to have lost their ability for sexual reproduction. This process has not completed yet, because a few isolates maintain their fertility. Many pathogenic traits including host specificity and cultivar specificity were not directly correlated with the mating type alleles (5, 6). In Indonesia, only MAT1-2 isolates were found, but several races were identified (18).

Genetic analyses of fertile rice isolates would help to elucidate the pathogenic variations and other physiological and morphological traits important in pathogenesis. Although we examined relatively small number of isolates in this experiment for mating types of *M. grisea*, both mating type alleles were found from rice field isolates and MAT1-1 allele was predominant in Korea. The accurate distribution of mating type alleles of *M. grisea* in Korea awaits further investigation.

요 약

벼와 다른 여러 화본과 식물로부터 분리한 도열병균 (*Magnaporthe grisea*) 26균주를 표준균주와 대치배양해 각각의 교배형을 알아보았다. 벼에서 분리한 균주의 93%가, 그리고 다른 기주에서 분리한 균주의 64%가 각각 표준균주와 교배해 자낭각을 형성하였다. 벼와 다른 기주에서 MAT1-1과 MAT1-2교배형 모두 발견되었으며 이들 균주간에는 교배가 이루어지지 않아 모두 자성불임(female-sterile)임이 밝혀졌다. MAT1-1교배형이 벼와 다른 화본과 기주에서 분리한 균주군에서 각각 93, 71%로 나타나 한국에서는 MAT 1-1교배형이 우점함을 알 수 있었다.

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