Genetics

Mating Behavior of Japanese Isolates of *Pyricularia oryzae*

S. Itoi, T. Mishima, S. Arase, and M. Nozu

Faculty of Agriculture, Shimane University, Matsue 690, Japan.
Accepted for publication 1 July 1982.

ABSTRACT

Itoi, S., Mishima, T., Arase, S., and Nozu, M. 1983. Mating behavior of Japanese isolates of *Pyricularia oryzae*. Phytopathology 73:155-158.

Fertile Japanese isolates of *Pyricularia oryzae* were mated with the most fertile hermaphroditic isolates from ragi, *Eleusine coracana*, by using the three-points culture method. The hermaphroditic tester lines (mating type *A* or *a*) produced perithecia with either white beaks or black beaks, when mated with an isolate of opposite mating type. Each monoconidial isolate from ragi or rice was crossed with the *A* and *a* tester lines by inoculating them at three points on a Misato-Hara medium. Matings between compatible hermaphroditic tester lines produced two bands of perithecia. Each isolate produced its own band of perithecia on its own side. Some additional isolates from ragi functioned as hermaphrodites, but most functioned only as males. In crossings between a compatible tester line and an isolate functioning as a male, one band of perithecia was produced on the side of the tester line. None of the crosses between two isolates from ragi functioning as males, even between *A* and *a*, was fertile. Crossing between every isolate from rice and a compatible tester line produced only one band of perithecia on the side of the tester line. Fertile isolates of *P. oryzae* collected from rice in Japan could only function as males.

In 1971, Hebert (1) found the perfect state of *Pyricularia grisea* (Cke.) Sacc. in culture and demonstrated heterothallism in this fungus. He designated the mating types or compatibility groups as + or −. Since then, efforts have been made to produce the perfect state of *Pyricularia oryzae* Cavara, the causal fungus of rice blast. In 1976, the perfect state of *P. oryzae* from rice was produced among matings of isolates from *Eleusine coracana* (L.) Gaertn. (ragi or finger millet) (3,6,10). Two kinds of mating types, *A* and *a*, were recognized in isolates from ragi in Japan. Only mating *A* was found in Japanese isolates of *P. oryzae* from rice (3,7). In 1977, Yaegashi (8) showed that both *A* and *a* mating types are present in rice isolates of *P. oryzae* from Japan. In 1979, Kato and Yamaguchi (4) obtained the perfect state of *P. oryzae* from rice in matings between a Japanese isolate and an Indonesian isolate. To date, however, no perithecia have been produced in crosses between Japanese *A* and *a* rice isolates of *P. oryzae*. The nature of this infertility between both mating-type groups of Japanese rice isolates has not been elucidated. According to Raper (5) many ascomycete fungi are hermaphroditic. In a previous paper (2) we described the hermaphrodites of *Pyricularia* isolated from the ragi.

The purpose of the studies reported here was to describe the mating behavior of isolates of *P. oryzae* collected in Japan when mated with the most fertile hermaphrodites from ragi. The results may provide valuable information for considering the possibility of perithecial production in the rice blast fungus.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Nineteen fertile monoconidial field isolates of *P. oryzae* from ragi were collected from several prefectures of Japan during 1979–1980. These isolates were from Tochigi Prefecture in 1979 (G10-1) and 1980 (G12-9, G16-5, G17-8, G18-1, G18-7, and G19-4), from Niigata Prefecture in 1980 (F13-3 and F17-4), from Kagawa Prefecture in 1979 (Z2-1, Z5-1, and Z7-1) and 1980 (Z13-3, Z17-1, Z38D, and Z47A) from Shimane Prefecture in 1979 (P2-6), and from Kumamoto Prefecture in 1980 (K20-1 and K22-1). Of 19 isolates listed, two of mating type *A* (G10-1, P2-6) and three of mating type *a* (Z2-1, Z5-1, and Z7-1) were fertile hermaphrodites (2). Perithecia with white beaks were produced in a band on the side of mating type *A* and perithecia with black beaks were produced in a band on the side of mating type *a* when crossed. The hermaphrodites of mating type *A* forming perithecia with white beaks were designated as *A*(♀♂) and the isolates of mating type *a* forming perithecia with black beaks as *a*(♀♂). These isolates were selected as tester lines for use in determining the mating characteristics of the Japanese isolates of *P. oryzae*.

A total of 21 isolates of *P. oryzae* from rice were used in this

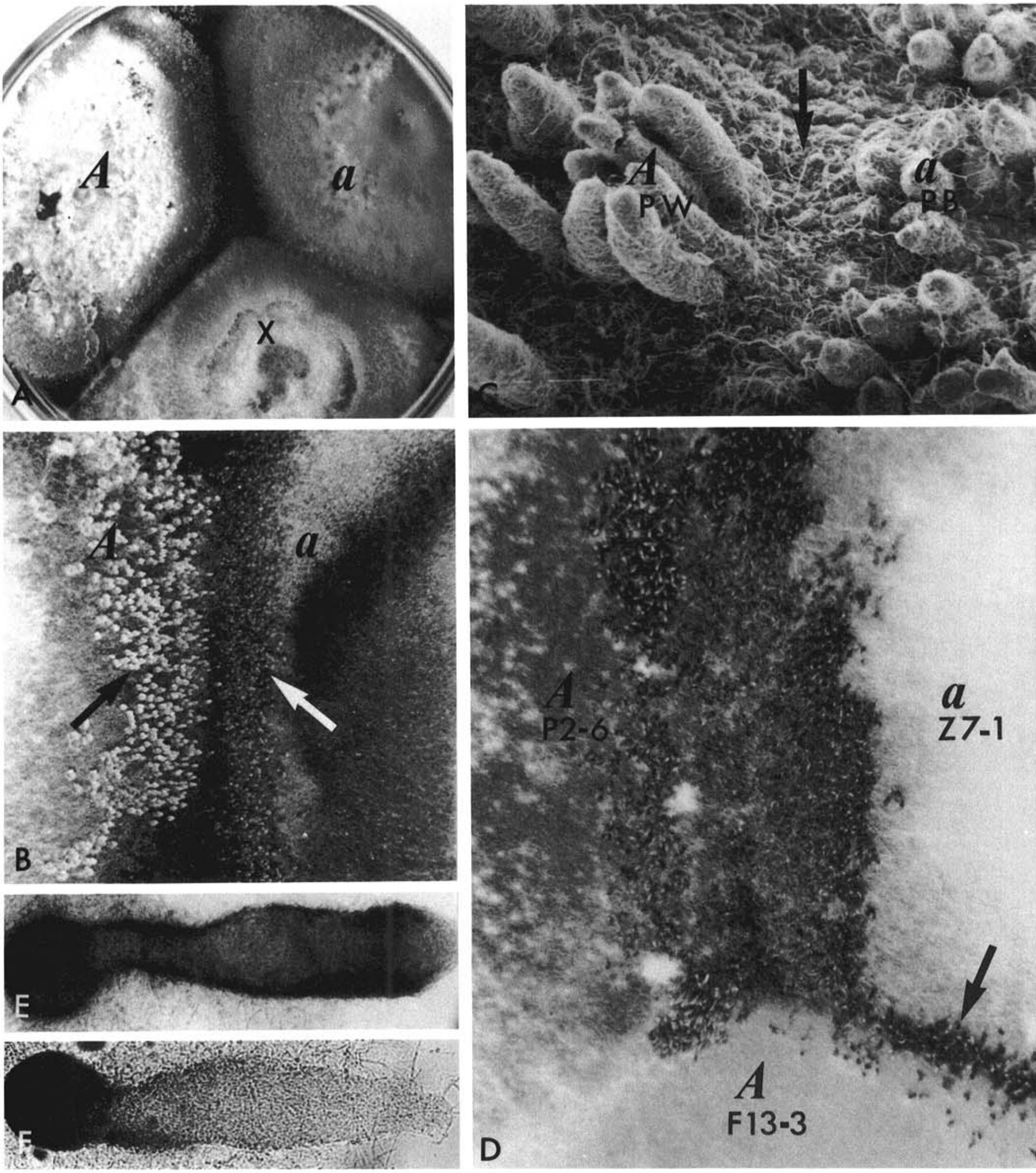


Fig. 1. Mating behavior of *Pyricularia oryzae* isolated from ragi in Japan. The three-points culture method, perithecial band formation, and two types of perithecia. **A,** The three-points culture method. The two tester lines (*A* and *a*) and an isolate of an unkown mating type (X) are crossed in one petri dish. **B,** Two bands of perithecia formed in matings between the two tester lines, *A*(♀♂) (G10-1) and *a*(♀♂) (Z2-1). Note one band of perithecia with white beaks on the side of mating type *A* (left arrow) and the other band of perithecia with black beaks on the side of mating type *a* (right arrow). **C,** Scanning electron micrograph of the two bands of perithecia shown in Fig. 1B (×1,000), showing clearly long white-beaked perithecia (PW) on one side of the junction of compatible colonies (arrow) and short black-beaked perithecia (PB) on the other side. **D,** Matings between two tester lines (P2-6 and Z7-1) and F13-3 by using the three-points culture method. Note two bands of perithecia (above) between the two tester lines (left, white-beaked perithecia; right, black-beaked perithecia) and a single band of black-beaked perithecia (below, arrow) between Z7-1 and F13-3. **E,** Black-beaked perithecium produced from a mating between a rice isolate (Oka8-1) and a compatible tester line (Z2-1). **F,** White-beaked perithecium produced from a mating between rice isolate (TY70-94) and a compatible tester line (G10-1).

study. Six (Ken54-04, Ken60-19, TY70-94, P2b, Ina72, and Naga87) are long-term stock cultures obtained from other investigators. Seven (R15-1, R21, R24, R25, R26, R27, and R28) are new isolates collected at different localities of Shimane Prefecture during 1978–1979. Nine other isolates were collected from eight prefectures during 1980: these isolates were from Hyogo Prefecture (Hyo4-1), Tottori Prefecture (Tot3-2 and Tot10-1), Okayama Prefecture (Oka8-1), Kochi Prefecture (Ko1-4), Tokushima Prefecture (Tok1-1), Fukuoka Prefecture (Fu1-2), and Nagasaki Prefecture (Na6-1).

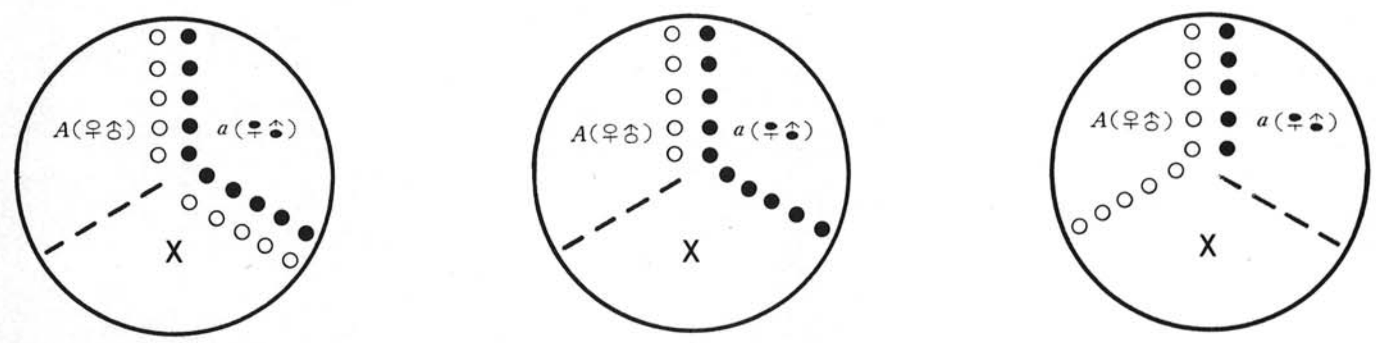
In our cross experiments, the three-points culture method was employed. On the medium, the two tester lines of mating type *A* and *a* and each monoconidial isolate (X) to be crossed were inoculated separately at three points (Fig. 1A). The plate was incubated at 28 C for 10 days and then kept at 20–25 C under continuous near-ultraviolet illumination (Black Light fluorescent lamp, National, FL20S BL-B). After 3–4 wk, the cultures were inspected for perithecial band formation under a dissecting microscope and perithecial types were determined under a light microscope. All the mating experiments were made on a Misato-Hara agar with and without sterilized rice straw in petri dishes. This medium was composed of yeast extract, 2 g; soluble starch, 10 g; agar, 10–15 g; and distilled water, 1 L. All crosses were carried out at least three times.

Two perithecial bands produced in matings between both tester lines were also examined under a scanning electron microscope. Specimens were fixed with glutaraldehyde and osmic acid, followed by critical-point drying and coating with gold. The specimens were examined with a MSM 30 scanning electron microscope operating at 15 kV.

RESULTS

Matings between ragi isolates and the tester lines. In matings between the two *A* and *a* tester lines, two bands of perithecia developed. Many perithecia with white beaks were produced in a band on the side of mating type *A* (G10-1). Likewise, many perithecia with black beaks were formed in a band on the side of mating type *a* (Z2-1) (Fig. 1B). Three other tester lines behaved similarly. In a scanning electron micrograph of the two perithecial bands (Fig. 1C), one group of perithecia with long white beaks (PW) can be seen on the side of mating type *A* (left) and another group of perithecia with short black beaks (PB) can be seen on the side of mating type *a* (right). Three ragi isolates (Z13-3, Z38D, and Z47A) also developed two bands of perithecia when mated with the compatible tester line (Fig. 2, group I). A band of perithecia with white beaks was produced on the side of each of these isolates and a band of perithecia with black beaks was produced on the side of the tester line, *a*(♀♂). The results indicate that ragi isolates Z13-3, Z38D, and Z47A were mating type *A* and behaved as hermaphrodites. The crosses between the *a*(♀♂) tester lines Z2-1, Z5-1, and Z7-1 and ragi isolates K20-1, K22-1, F13-3, F17-4, and Z17-1 were also fertile, but only perithecia with black beaks were produced in a band on the side of the tester line (Fig. 2, group II). An example of such a cross is shown in Fig. 1D. There are two bands of perithecia between P2-6 and Z7-1 and a single band between F13-3 and Z7-1. The five mating type *A* ragi isolates in the group II had apparently degenerated sexually and could function only as males. The remaining six ragi isolates (G12-9, G16-5, G17-8, G18-1, G18-7, and G19-4) reacted with the *A*(♀♂) tester line to produce only perithecia with white beaks in a band on the side of the tester line (Fig. 2, group III). These six mating type *a* ragi isolates also had degenerated sexually and functioned only as males. None of the crosses between these sexually degenerate isolates, even between *A* (group II) and *a* (group III), was fertile.

Matings between rice isolates and the tester lines. Each monoconidial isolate of *P. oryzae* from rice was crossed with the *A*(♀♂) and *a*(♀♂) tester lines. Two bands of perithecia developed between tester lines. Crossings between every rice isolate and a compatible tester line produced only one band of perithecia on the side of the tester line. Eighteen rice isolates had the same sexual behavior as the ragi isolates in group II and produced only one



Group I A(♀♂)	Group II A(♂)	Group III a(♂)
	Ragi isolates	
Z13-3, Z38D, Z474*	K20-1, K22-1, F13-3 F17-4, Z17-1	G12-9, G16-5, G17-8 G18-1, G18-7, G19-4
	Rice isolates	
None	Ken54-04, Ina72, Naga87 P2b, R15-1, R21, R24 R25, R26, R27, R28 Hyo4-1, Tot3-2, Oka8-1 Ko1-4, Tok1-1, Fu1-2 Na6-1	Ken60-19, TY70-94 Tot10-1

Fig. 2. Results of crosses between *Pyricularia oryzae* isolates from ragi or rice and the two tester line groups. *A*(♀♂) designates the *A* tester line group (G10-1, P2-6), which form white-beaked perithecia in compatible crosses. *a*(♀♂) designates the *a* tester line group (Z2-1, Z5-1, Z7-1), which form black-beaked perithecia in compatible crosses. X represents monoconidial field isolates from ragi or rice to be crossed. After being crossed with tester lines, the field isolates were divided into three groups. Group I: Forming two bands of perithecia in matings with the *a*(♀♂) tester line group. Group I isolates were hermaphroditic and were designated as *A*(♀♂). Group II: Forming a single band of black-beaked perithecia on the side of the *a*(♀♂) tester line group. Group II isolates functioned as a male and were designated as *A*(♂). Group III: Forming a single band of white-beaked perithecia on the side of the *A*(♀♂) tester line group. Group III isolates functioned as a male and were designated as *a*(♂). *Z47A was used as the tester line in some matings.

band of perithecia with black beaks (Fig. 1E) on the side of the tester line (Fig. 2, group II). Three rice isolates (Ken60-19, TY70-94, and Tot10-1) reacted with the *A*(♀♂) tester line and perithecia with white beaks (Fig. 1F) developed only in one band on the side of the tester line (Fig. 2, group III). The rice isolates in group II (Fig. 2) belong to mating type *A* and the rice isolates in group III (Fig. 2) belong to mating type *a*. All seem to have sexually degenerated and could function only as males.

DISCUSSION

In 1980, hermaphrodites were found in *Pyricularia oryzae* from ragi, *Eleusine coracana* (2). This was apparently the first report of hermaphroditism in *Pyricularia*. There are also two compatibility groups, *A* and *a*, in *P. oryzae*. The most fertile hermaphrodites (*A*♀♂): G10-1, P2-6; *a*(♀♂): Z2-1, Z5-1, Z7-1) were used as the tester lines in the present study. In matings between *A* and *a* tester lines, sexual reproduction occurred in two bands. This means that both tester lines behaved as hermaphrodites. The three new isolates (Z13-3, Z38D, and Z47A) were found to be hermaphroditic and were designated as *A*(♀♂). Yaegashi (8) stated that *Pyricularia* isolates from ragi and goosegrass (*Eleusine indica*) were most fruitful compared with the fertile isolates from other hosts. It is difficult to find a valid reason for this. The fact, however, that hermaphrodites are commonly found among isolates from ragi may supply a reason for their high fertility.

In our experiments, many ragi isolates did not behave hermaphroditically, but behaved like males. These isolates reacted with one of the tester lines and produced perithecia in one band on the side of tester line. These isolates were considered to have only the ability to function as males and could be designated as *A*(♂) or *a*(♂). Many ascomycete fungi are hermaphroditic (5). These ragi isolates may originally have been hermaphroditic but lost the female function or may have degenerated sexually. In consequence, matings of these degenerate isolates between themselves, even between *A* and *a*, were all sterile. Matings between these degenerate isolates and nondegenerate (hermaphroditic) compatible tester line produced one band of perithecia on the side of the nondegenerate line. These results suggest that the female function may be an important factor conditioning the fertility of isolates.

None of the *P. oryzae* isolates from rice collected in Japan behaved as hermaphrodites. All fertile *P. oryzae* isolates from rice behaved only as males. This means that *P. oryzae* from rice may have been hermaphroditic, and then lost the female character due to degeneration of sex from hermaphroditism to unisexuality (maleness). However, the male function of rice isolates has been inherited up to the present time (for more than 20 yr) in long-term stock cultures on artificial media. This seems to be the nature of sexual characteristics in *P. oryzae*. Furthermore, the conversion of sexual potential among naturally occurring ragi isolates may explain the nature of variability in *P. oryzae*.

It is generally considered that hybridization is an important means of pathogenic variation in fungi having a sexual state as part of their life cycle. In *Pyricularia,* however, the perfect state has not yet been found in nature. According to Yaegashi (9), pathogenicity and mating type in *Pyricularia* were inherited independently, which made it possible to cross various types of isolates. He (8) also stated that both mating types of the rice isolates were present in some areas at the same time in Japan. Therefore, if hybridization occurred in nature, it could play an important role in developing new types of isolates having different pathogenicity. However, as for the *P. oryzae* from rice in Japan, there are no opportunities for perithecial formation in nature due to degeneration of sex.

LITERATURE CITED

1. Hebert, T. T. 1971. The perfect stage of *Pyricularia grisea*. Phytopathology 61:83-87.
2. Itoi, S., Yamamoto, J., Karino, S., Arase, S., and Kato, H. 1980. Hermaphroditic isolates of *Pyricularia* isolated from ragi, *Eleusine coracana* (L.) Gaertn. Ann. Phytopathol. Soc. Jpn. 46:549-552.
3. Kato, H., Yamaguchi, T., and Nishihara, N. 1976. The perfect state of *Pyricularia oryzae* Cav. in culture. Ann. Phytopathol. Soc. Jpn. 42:507-510.
4. Kato, H., and Yamaguchi, T. 1979. Reproductive phase of *Pyricularia oryzae* (6). On the mating of *Pyricularia oryzae* Cav. with each other. (Abstr.) Ann. Phytopathol. Soc. Jpn. 45:121.
5. Raper, J. R. 1966. Life cycle, basic patterns of sexuality, and sexual mechanisms. Pages 473–511 in: The Fungi. G. C. Ainsworth and A. S. Sussman, eds. Academic Press, New York.
6. Ueyama, A., and Tsuda, M. 1975. Formation of the perfect state in culture of *Pyricularia* sp. from some graminaceous plants. (Preliminary report). Trans. Mycol. Soc. Jpn. 16:420-422.
7. Ueyama, A., Tsuda, M., and Nakagawa, H. 1977. Mating types for perfect state of *Pyricularia oryzae*. Trans. Mycol. Soc. Jpn. 18:312-317.
8. Yaegashi, H. 1977. On the sexuality of blast fungi, *Pyricularia* spp. Ann. Phytopathol. Soc. Jpn. 43:432-439.
9. Yaegashi, H. 1978. Inheritance of pathogenicity in crosses of *Pyricularia* isolates from weeping lovegrass and finger millet. Ann. Phytopathol. Soc. Jpn. 44:626-632.
10. Yaegashi, H., and Nishihara, N. 1976. Production of the perfect stage in *Pyricularia* from cereals and grasses. Ann. Phytopathol. Soc. Jpn. 42:511-515.