Natural Infection of Wild Grass Species with Rice Blast Fungus Suggested by DNA Fingerprinting*

Motoaki KUSABA**, Le Dinh DON**, Alfredo S. URASHIMA**, Yukiko ETO**,
Yukio TOSA**, Hitoshi NAKAYASHIKI**, Masaaki YAMAMOTO**
and Shigeyuki MAYAMA**

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Blast disease of rice (*Oryza sativa*) is a serious disease in many rice growing countries, caused by *Pyricularia oryzae* Cavara [= *P. grisea* (Cooke) Sacc¹²⁾ teleomorph: *Magnaporthe grisea* (Hebert) Barr]. The fungus has been isolated from several other gramineous plant species coexistent with rice in the agroecosystem⁶⁾. Whether these plants serve as collateral hosts for the rice pathogen is an important question for blast management.

Recent molecular genetic analyses have suggested that *Pyricularia* isolates from different hosts are genetically distinct¹³⁾ and that pathogen populations infecting hosts other than rice do not provide inocula against rice crops in the field³⁾. However, the results are contradictory in several pathogenicity studies of *Pyricularia* isolates^{2,5,9,10,17)}. Results from artificial inoculation tests showed that numerous gramineous species commonly found in rice-growing areas were susceptible to the rice blast fungus²⁾. Furthermore, several researchers have reported cross-pathogenicity of *Pyricularia* species isolated from rice and other hosts^{5,9,10,17)}. These observations suggest that some grass species could be reservoirs of inoculum for infection of rice. Kato and Yamaguchi⁵⁾ found in cross-inoculation tests that field isolates from Italian ryegrass (*Lolium multiflorum*), sweet vernalgrass (*Anthoxanthum odoratum*) and reed canarygrass (*Phalaris arundinacea*) were pathogenic on rice and vice versa. The isolates from these host species also parasitized several other grass species that were susceptible to rice isolates. Since the host range of these isolates was very similar to that of rice isolates, Kato and Yamaguchi⁵⁾ proposed that these weed isolates were intrinsically rice blast isolates. In addition to these isolates, isolates from tall fescue (*Festuca arundinacea*) were pathogenic on rice and also were suggested to be rice blast isolates¹⁷⁾.

MAGGY is an LTR-retrotransposon⁴⁾ present in a high copy number in isolates from rice, foxtail millet (*Setaria italica*) and green bristlegrass (*S. viridis*)¹⁵⁾. We also found multiple copies of MAGGY in isolates from Italian ryegrass, sweet vernalgrass, reed canarygrass

and tall fescue. Dispersed repeated DNA sequences such as MAGGY have been widely used for fingerprinting and phylogenetic analyses of *Pyricularia* isolates^{7,8,16)}. In these studies, diverse isolates population could be differentiated into lineages, or groups of isolates with similar DNA banding patterns, which were inferred to be related by their descent from a common ancestor. In the present study, genetic relationships between rice isolates and the isolates from the four weed grasses were examined by DNA fingerprinting analysis using MAGGY as a probe to obtain further evidence that grass weeds can be naturally infected with the rice blast fungus.

A total of 18 isolates, comprised of 11 isolates from rice (including one isolate from *O. longistaminata*) and seven Japanese isolates from five weed grass species were used in this study (Table 1). The pathogenicity of these isolates on rice is presented in Table 1. All isolates were pathogenic on rice, except for an isolate from buffel grass (*Cenchrus ciliaris*) (Kato *et al.*, in preparation). The buffel grass isolate, which also has multiple copies of MAGGY, was nonpathogenic on rice. These isolates were maintained on barley grains under dry conditions at 5°C for long-term storage. For DNA extraction, fungal isolates were grown in 50 ml potato dextrose broth in 100 ml Erlenmeyer flasks at 23°C for 4 days. Total DNA of each isolate was prepared from the resulting mycelia by the method of Adachi *et al.*¹⁾, digested with *Bam*HI (Toyobo), and fractionated on a 0.8% agarose gel (GP-36: Nacalai tesque) in 0.5×TBE buffer. The fractionated DNA was transferred to nylon membranes (MagnaGraph, MSI) and fixed by UV irradiation according to the manufacturer's instructions. The 0.56 kb *Sal*I-*Bam*HI fragment (SB) isolated from a MAGGY clone pMGY23¹⁵⁾ was used as a probe for detecting DNA fingerprints. The DNA probe was labeled with biotin using NEBlot-Photoprobe Kit (New England). Hybridization was carried out in 6×SSC containing 5×Denhardt's solution, 0.5% sodium dodecyl sulfate and 100 µg of sonicated salmon sperm DNA

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** Laboratory of Plant Pathology, Faculty of Agriculture, Kobe University, Rokkodai, Nada-ku, Kobe 657-8501, Japan
神戸大学農学部植物病理学研究室

Table 1. *Pyricularia* isolates used in this study

Host	Isolate ^{a)}	Locality ^{b)}		Collector	Pathogenicity ^{c)} on rice
<i>Oryza sativa</i> L. (rice)	0903-4[003]	Tochigi	Japan	M. Yamada	+
	2012-1[007]	Ishikawa	Japan	M. Yamada	+
	88A[433]	Ibaraki	Japan	M. Yamada	+
	IN77-32-1-1	Mysore	India	H. Kato	+
	UN-05-1	Yunnan	China	M. Iwano	+
	ISH-5-1	Yunnan	China	M. Iwano	+
	CHNOS59-6-11	Yunnan	China	N. Hayashi	+
	CHNOS60-8-1	Yunnan	China	N. Hayashi	+
	Br11	Paulo Frontin	Brazil	S. Igarashi	+
	CD276	—	Cote d'Ivoire	J. M. Bidaux	+
<i>Oryza longistaminata</i> A. Chev. et Roehr.	CD262	—	Cote d'Ivoire	J. M. Bidaux	+
<i>Phalaris arundinacea</i> L. (reed canarygrass)	NI901	Tochigi	Japan	N. Nishihara	+
	NI921	Tochigi	Japan	N. Nishihara	+
<i>Anthoxanthum odoratum</i> L. (sweet vernalgrass)	NI904	Tochigi	Japan	N. Nishihara	+
<i>Festuca arundinacea</i> Schreb. (tall fescue)	NI899	Tochigi	Japan	N. Nishihara	+
<i>Lolium multiflorum</i> Lam. (Italian ryegrass)	GFIT1-1	Gifu	Japan	H. Kato	+
	GFIT6-1	Gifu	Japan	H. Kato	+
<i>Cenchrus ciliaris</i> L. (buffel grass)	NI981	Kumamoto	Japan	N. Nishihara	—

a) Race numbers are shown in square brackets.

b) —, unknown.

c) +, pathogenic ; —, nonpathogenic.

Fig. 1. DNA fingerprints of 18 *Pyricularia* isolates from rice and five grass weed species. The lanes contained DNA from : 1, 88A ; 2, 0903-4 ; 3, 2012-1 ; 4, UN-05-1 ; 5, ISH-5-1 ; 6, CHNOS59-6-11 ; 7, CHNOS60-8-1 ; 8, Br11 ; 9, CD262 ; 10, CD276 ; 11, IN77-32-1-1 ; 12, NI981 ; 13, NI901 ; 14, NI921 ; 15, NI904 ; 16, NI899 ; 17, GFIT1-1 ; 18, GFIT6-1. The sizes of DNA (in kilobases) are indicated on the left.

per ml at 68°C. Hybridized membranes were washed at 68°C, with the final wash in 0.1×SSC containing 0.1% sodium dodecyl sulfate. Detection of DNA blots was

performed by using the Phototope-Star Detection Subkit (New England).

The resulting DNA fingerprints are shown in Fig. 1.

Fig. 2. Dendrogram showing the level of genetic relatedness of 18 *Pyricularia* isolates from rice and five grass weed species. Hosts and localities of isolates are shown in parentheses.

Hybridization fragments larger than 5 kb in size were excluded from the evaluation for polymorphisms of the fingerprints. Consequently, the hybridization produced 154 different resolvable fragments (smaller than 5 kb in size) in the isolates tested. The 18 isolates were classified into distinctive fingerprint patterns. A cluster analysis of the fingerprint data was used to calculate the similarity coefficients⁽¹¹⁾ between isolates, and to construct a dendrogram using UPGMA⁽¹⁴⁾ (Fig. 2).

In the dendrogram, all rice isolates were grouped into a single cluster, but with a very low similarity level (14%). Japanese rice isolates 88A and 0903-4 were relatively close to each other with 69% similarity. However, the other Japanese rice isolate 2012-1 and eight isolates from the other four countries were classified into groups different from the two Japanese isolates with lower than 17% similarities. Such low levels of similarity suggested genetic diversification within the rice isolates populations. One isolate from buffel grass, which is not pathogenic to rice, was most distant from all the other isolates with only a 5% similarity in the dendrogram. On the other hands, six Japanese isolates from the four weed grass species were closely related to Japanese rice isolates with higher than 80% similarity levels. These weed isolates were divided into three groups, each of which was close to one of the three Japanese rice isolates.

Kato *et al.* (in preparation) have already confirmed that the same isolates from the four weeds used in this study were pathogenic not only on rice but also on other host plants which were susceptible to the rice blast fungus. Thus, these pathogenicity tests suggest, as did previous field studies^(2,5,9,10,17), that the isolates from these four weed species are the rice pathogen with the ability to infect these grass weeds.

Such weeds may act as collateral hosts of the rice blast fungus in the agroecosystem. On the other hand,

Borromeo *et al.*⁽³⁾ reported that populations of weed isolates do not provide inoculum to rice in the field in the Philippines. They examined genetic relationships among isolates from rice and 16 weed grass species in the Philippines based on RFLP analyses using 10 single-copy DNA probes. The dendrogram constructed from the RFLP data showed that the rice isolates were distinct from those on other host species. However, their isolates from weed grass hosts did not include any from *Lolium*, *Anthoxanthum*, *Phalaris* or *Festuca*. Thus, some weed species may serve as collateral hosts for the rice pathogen, whereas others may not.

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和 文 摘 要

草場基章・Le Dinh DON・Alfredo S. URASHIMA・衛藤由希子・土佐幸雄・中屋敷均・山本正晃・眞山滋志：DNAフィンガープリンティング分析により示唆されたイネいもち病菌の野生イネ科植物への自然感染

MAGGYをプローブとしたDNAフィンガープリント分析により、世界各地から採集したイネいもち病菌と日本産野生イネ科植物いもち病菌との遺伝的類縁関係を調査した。供試菌から得られたDNAフィンガープリントに基づきクラスター分析を行ったところ、日本産と外国産イネ菌の類縁性は、27%以下であった。一方、日本産ネズミムギ、ハルガヤ、クサヨシ、オニノウシノケグサ各菌は日本産イネ菌に対して80%以上の高い類縁性を示した。以上の結果から、これら日本産の野生イネ科植物の菌はイネ菌がこれら植物に感染したものであると推定される。

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