Comparative Genetic Analysis of *Magnaporthe oryzae* Isolates Causing Gray Leaf Spot of Perennial Ryegrass Turf in the United States and Japan

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ABSTRACT

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Gray leaf spot caused by *Magnaporthe oryzae* is a serious disease of perennial ryegrass (*Lolium perenne*) turf in golf course fairways in the United States and Japan. Genetic relationships among *M. oryzae* isolates from perennial ryegrass (prg) isolates within and between the two countries were examined using the repetitive DNA elements MGR586, Pot2, and MAGGY as DNA fingerprinting probes. In all, 82 isolates of *M. oryzae*, including 57 prg isolates from the United States collected from 1995 to 2001, 1 annual ryegrass (*Lolium multiflorum*) isolate from the United States collected in 1972, and 24 prg isolates from Japan collected from 1996 to 1999 were analyzed in this study. Hybridization with the MGR586 probe resulted in approximately 30 DNA fragments in 75 isolates (designated major MGR586 group) and less than 15 fragments in the remaining 7 isolates (designated minor MGR586 group). Both groups were represented among the 24 isolates from Japan. All isolates from the United States, with the exception of one isolate from Maryland, belonged to the major MGR586 group. Some isolates from Japan exhibited MGR586 fingerprints that were identical to several isolates collected in Pennsylvania. Similarly, fingerprinting analysis with the Pot2 probe also indicated the presence of two distinct groups: isolates in the major MGR586 group showed fingerprinting profiles comprising 20 to 25 bands, whereas the isolates in the minor MGR586 group had less than 10 fragments. When MAGGY was used as a probe, two distinct fingerprint types, one exhibiting more than 30 hybridizing bands (type I) and the other with only 2 to 4 bands (type II), were identified. Although isolates of both types were present in the major MGR586 group, only the type II isolates were identified in the minor MGR586 group. The parsimony tree obtained from combined MGR586 and Pot2 data showed that 71 of the 82 isolates belonged to a single lineage, 5 isolates formed four different lineages, and the remaining 6 (from Japan) formed a separate lineage. This study indicates that the predominant groups of *M. oryzae* associated with the recent outbreaks of gray leaf spot in Japan and the United States belong to the same genetic lineage.

Additional keywords: genetic diversity, *Pyricularia grisea*, *Pyricularia oryzae*, turfgrass

Gray leaf spot of perennial ryegrass (*Lolium perenne* L.) recently has emerged as a serious problem in the maintenance of perennial ryegrass (prg) for golf course fairways in the United States. Outbreaks of gray leaf spot on annual ryegrass (*L. multiflorum* Lam.) grown for forage in the southern United States first were reported in the early 1970s in Louisiana and Mississippi (1,2). In 1991, the disease first was reported on prg turf in golf course fairways in Pennsylvania (15). Severe outbreaks of the disease causing more than 98% loss of turf were reported in 1998 (27) and, since then, outbreaks of gray leaf spot have occurred every 2 to 3 years, resulting in seri-

ous loss of turf in the northeast and mid-Atlantic region (4,27). The disease also has been reported from the Midwest (9,18,33), New England states (21), and western United States (28) in recent years. Outbreaks of gray leaf spot also have occurred on prg turf in golf courses in Japan (24). The disease first was reported in western Japan (Yamaguchi prefecture) in 1996. Subsequently, the disease spread to eastern Japan (Tochigi prefecture) in 1997, northern Japan (Akita prefecture) in 1998, and to at least six prefectures by 1999 (23).

Gray leaf spot of prg is caused by *Magnaporthe oryzae* Couch (3), anamorph *Pyricularia oryzae* Cavara (14) (formerly designated as *Pyricularia grisea* (Cooke) Sacc. (19) collectively), which infects more than 50 gramineous hosts. The fungus is known to exhibit a high degree of phenotypic and genotypic variability, and has been studied extensively (17,34). Various molecular markers have been used to characterize the genetic structure of *M. oryzae* isolates (3,6,8,14,25,30,32). The

identification of several repetitive DNA sequences in the *M. oryzae* genome (7,8,11) has facilitated population genetic analyses of isolates from rice and as well as other grasses. *M. oryzae* isolates from prg have an rDNA internal transcribed spacer sequence identical to that of isolates from wheat (*Triticum aestivum* L.), belong to a single mating type (MAT1-2) (25,31), and consist of three closely related lineages (32). Additionally, Farman (6) has documented that prg isolates collected in the United States were very similar in fingerprint patterns to the MGR586, MAGGY, RETRO5, Pot2, and MGR583 probes, and were distinct from isolates from crabgrass, rice, and *Setaria* spp. Although the prg isolates are genetically similar to those from wheat, they are clearly distinct groups, suggesting that the isolates causing gray leaf spot of prg are host specific (6,32). Recently, Tosa et al. (25) reported that the MGR586 fingerprints of Japanese prg isolates showed close similarity to the U.S. isolates. The prg isolates from Japan consisted of two distinct populations: one is highly virulent on and specific to prg, whereas the other is less virulent on prg but has some virulence on wheat.

Although genetic diversity among the isolates of *M. oryzae* causing gray leaf spot of prg in the United States and Japan has been investigated independently, the genetic relationships among the isolates from the two countries have not been extensively documented. Therefore, this study was undertaken to determine the genetic relationship between the prg isolates of *M. oryzae* from the United States and Japan.

MATERIALS AND METHODS

Collection and maintenance of isolates. Prg samples with gray leaf spot symptoms were collected from golf course fairways and roughs in Pennsylvania from 1995 to 2001. Symptomatic prg leaves were selected randomly from the samples, washed in tap water, placed on moist filter paper in petri dishes, and incubated for 24 to 48 h at 22°C to induce sporulation. Conidia were dislodged from individual sporulating lesions onto potato-dextrose-agar (PDA) plates. Monoconidial isolates of *M. oryzae* were transferred to fresh PDA plates. For long-term storage, the isolates from the United States were preserved as previously described (29). Additionally, *M.*

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oryzae isolates from other states in the United States were obtained from Dr. Larry Stowell (Pace Turfgrass Research Institute, San Diego, CA), Dr. Andrew Hamblin (University of Illinois), Dr. Paul Vincelli (University of Kentucky), Dr. Gail Schumann (University of Massachusetts), Dr. Joseph Vargas (Michigan State University), Dr. Bruce Clarke (Rutgers, the State University of New Jersey), and Dr. Peter Dernoeden (University of Maryland). Isolates obtained from prg samples submitted to the turfgrass disease research laboratory at the Pennsylvania State University by Van Cortlandt Golf Course (Bronx, NY), Meadow Farms Golf Club (Locust Grove, VA), and Highland Springs Golf Course (Wellsburg, WV) also were included (Table 1). The isolates of *M. oryzae* from the United States also included an isolate from annual ryegrass collected by Dr. Larry Trevathan in 1972 (The American Type Culture Collection, accession number 64557). Isolates collected in Japan were obtained from Dr. A. Tanaka and Dr. T. Tani (Institute for Green Science, Japan) and were maintained at 4°C on barley seed. In this procedure, barley seeds

with husks were soaked in 1% sucrose solution in test tubes (13 by 75 mm) at room temperature for 24 h. The excess sucrose solution was removed and the test tubes were capped with cotton plugs and sterilized for 20 min. A 3-by-3-mm PDA block containing actively growing mycelia was placed in each test tube and incubated at room temperature for 7 to 10 days. When the seeds were colonized, they were transferred to a container with silica gel and stored at 4°C (10). In total, 58 monoconidial isolates from the United States and 24 from Japan were included in this study (Table 1).

DNA extraction. Individual isolates of *M. oryzae* were retrieved from storage, placed on full-strength PDA plates, and incubated at 28°C under 12-h (day and night) cycles of fluorescent light (76 $\mu\text{E}^{-2} \text{s}^{-1}$) for 7 to 10 days. Mycelial plugs were transferred to 50 ml of 2YEG broth (2 g of yeast extract and 10 g of glucose per liter), and incubated at 26°C for 3 days. The mycelial mats were harvested, freeze dried, and ground to a powder in liquid nitrogen using a mortar and pestle. The ground powder then was transferred to a micro-

centrifuge tube containing 1 ml of DNA isolation buffer (100 mM LiCl, 100 mM EDTA, 10 mM Tris-HCl, pH7.5, and 0.5% sodium dodecyl sulfate [SDS]) and mixed thoroughly by vortexing. After incubation at 55°C for 30 min, the mycelial debris was precipitated by centrifugation at 10,000 $\times g$ for 10 min. The supernatant was transferred to another tube and extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and then once with an equal volume of chloroform:isoamyl alcohol (24:1). Genomic DNA was precipitated from the aqueous phase with isopropanol and recovered by centrifugation at 10,000 $\times g$ for 15 min. The DNA was washed with 1 ml of 70% ethanol, dried in vacuum, and dissolved in 50 μl of Tris-EDTA containing RNase (50 $\mu\text{g}/\text{ml}$) by incubating at room temperature overnight. The dissolved DNA sample was incubated at 37°C for 1 h and used for Southern analysis.

DNA fingerprinting. Southern hybridization was performed using the ECL Direct Nucleic Acid Labeling and Detection System (Amersham Biosciences, Buckinghamshire, UK). Probes used in this study

Table 1. Isolates of *Magnaporthe oryzae* from *Lolium perenne* and *L. multiflorum* from the United States and Japan used in the study

<i>M. oryzae</i> isolate	Abbreviation	Host of origin	Location	Year of collection	Collector
ATCC-64557	U49	<i>L. multiflorum</i>	Mississippi, United States	1972	L. Trevathan
MD2-1	U60	<i>L. perenne</i>	Maryland, United States	1995	P. Dernoeden
NJ1-2	U66	<i>L. perenne</i>	New Jersey, United States	1995	B. Clarke
MD1-1	U68	<i>L. perenne</i>	Maryland, United States	1995	P. Dernoeden
PgZen96-227-1	U50	<i>L. perenne</i>	Michigan, United States	1996	K. Gard
PgZen96-227-2	U51	<i>L. perenne</i>	Michigan, United States	1996	K. Gard
PgZen96-227-4	U52	<i>L. perenne</i>	Michigan, United States	1996	K. Gard
WK3-1	WK3-1	<i>L. perenne</i>	Yamaguchi, Japan	1996	A. Tanaka and T. Tani
WK3-2	WK3-2	<i>L. perenne</i>	Yamaguchi, Japan	1996	A. Tanaka and T. Tani
WK4-1	WK4-1	<i>L. perenne</i>	Yamaguchi, Japan	1996	A. Tanaka and T. Tani
WK4-2	WK4-2	<i>L. perenne</i>	Yamaguchi, Japan	1996	A. Tanaka and T. Tani
WK4-3	WK4-3	<i>L. perenne</i>	Yamaguchi, Japan	1996	A. Tanaka and T. Tani
WK4-4	WK4-4	<i>L. perenne</i>	Yamaguchi, Japan	1996	A. Tanaka and T. Tani
TP-1	TP-1	<i>L. perenne</i>	Tochigii, Japan	1997	A. Tanaka and T. Tani
TP-2	TP-2	<i>L. perenne</i>	Tochigii, Japan	1997	A. Tanaka and T. Tani
TP-3	TP-3	<i>L. perenne</i>	Tochigii, Japan	1997	A. Tanaka and T. Tani
TP-4	TP-4	<i>L. perenne</i>	Tochigii, Japan	1997	A. Tanaka and T. Tani
TP-5	TP-5	<i>L. perenne</i>	Tochigii, Japan	1997	A. Tanaka and T. Tani
TP-6	TP-6	<i>L. perenne</i>	Tochigii, Japan	1997	A. Tanaka and T. Tani
PgVA13M-06	U61	<i>L. perenne</i>	Virginia, United States	1998	W. Uddin
PgWV17I-05	U63	<i>L. perenne</i>	West Virginia, United States	1998	W. Uddin
PgPA05T-01	U64	<i>L. perenne</i>	Pennsylvania, United States	1998	W. Uddin
PgPA18C-04	U65	<i>L. perenne</i>	Pennsylvania, United States	1998	W. Uddin
PgNJ15C-03	U67	<i>L. perenne</i>	New Jersey, United States	1998	B. Clarke
AK-1	AK-1	<i>L. perenne</i>	Akitai, Japan	1998	A. Tanaka and T. Tani
AK-2	AK-2	<i>L. perenne</i>	Akitai, Japan	1998	A. Tanaka and T. Tani
AK-3	AK-3	<i>L. perenne</i>	Akitai, Japan	1998	A. Tanaka and T. Tani
AK-4	AK-4	<i>L. perenne</i>	Akitai, Japan	1998	A. Tanaka and T. Tani
AK-5	AK-5	<i>L. perenne</i>	Akitai, Japan	1998	A. Tanaka and T. Tani
FI-2	FI-2	<i>L. perenne</i>	Chiba, Japan	1998	A. Tanaka and T. Tani
FI-3	FI-3	<i>L. perenne</i>	Chiba, Japan	1998	A. Tanaka and T. Tani
FI-4	FI-4	<i>L. perenne</i>	Chiba, Japan	1998	A. Tanaka and T. Tani
FI-5	FI-5	<i>L. perenne</i>	Chiba, Japan	1998	A. Tanaka and T. Tani
PA02V18.1	U1	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PA02V22.1	U2	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PA02V26.2	U3	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PgPA32H01.1	U4	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PgPA32H03.1	U5	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PgPA32H07.1	U6	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PgPA33B.01	U7	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PgPA33B.03	U8	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin

(continued on next page)

included MGR586 in pCB586 (8), Pot2 (11) in pPO-H (5), and MAGGY (7) in pMGY23 (26). These plasmids were maintained in *Escherichia coli* strain DH5 α , extracted with QIAprep Spin Miniprep Kit (Qiagen, Hilden), and labeled following the manufacturer's instructions. Total DNA (500 ng) from each of the isolates were digested with *Eco*RI and fractionated on a 0.8% agarose gel in 1 $\times$ Tris-acetate-EDTA buffer. The fractionated DNA was transferred to a nylon membrane (Hybond-N⁺; Amersham Biosciences) with 20 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and fixed by UV irradiation. The membrane was hybridized overnight with a labeled probe in Gold hybridization buffer (Amersham Biosciences) containing 0.5 M NaCl at 42°C. After hybridization, the membrane was washed twice in primary wash buffer (6 M urea, 0.4% SDS, 0.5 $\times$ SSC) for 20 min at 42°C, followed by two washes in 2 $\times$ SSC for 5 min at room temperature. Detection of target DNA was performed according to the manufacturer's instructions.

Construction of dendrograms. Bands were scored manually and represented as binary data with "1" for the presence of a

band and "0" for absence. MGR586 bands were scored from the bottom of the gel up to 10 kb, whereas Pot2 bands were scored from the bottom of the gel up to 12 kb. Genetic similarities were calculated using an neighbor-joining (NJ) program (20) and a maximum parsimony program in PAUP 4.0 (22). NJ trees were constructed from similarities calculated using Nei and Li's index of genetic similarity for restriction fragment length polymorphism comparisons (16). Maximum parsimony trees were constructed using the heuristic search option.

RESULTS

DNA fingerprinting with the MGR586 probe resulted in approximately 30 DNA fragments in 75 of the 82 isolates (Fig. 1A, represented by 13 isolates shown from the second to fourteenth lanes). All 75 isolates (designated major MGR586 group) exhibited highly similar fingerprint profiles. The remaining seven isolates (designated minor MGR586 group) had less than 15 bands (Fig. 1A, represented by two isolates shown at the first and last lanes). Among the 24 prg isolates from Japan, both MGR586 groups were present. With the

exception of an isolate (U68) collected in Maryland in 1995, all the U.S. isolates belonged to the major MGR586 group and exhibited high similarity to those in the major MGR586 group from Japan. The annual ryegrass isolate, U49, collected in Mississippi in 1972, also belonged to the major MGR586 group. To determine the genetic similarity among these isolates, the presence or absence of individual bands was converted into a binary (0 or 1) matrix. Based on the resulting matrix, an unrooted tree was constructed using the NJ method, which then was rooted using the minor MGR586 group in Japan as an out-group (Fig. 2A). The major MGR586 group in Japan was further divided into three subgroups (Fig. 2A). Isolates TP1, TP6, LW1, LW2, LW3, FI2, FI3, FI4, and FI5 collected from Tochigi, Yamanashi, and Chiba were identical, forming a subgroup (J1). Although TP5, an isolate from Tochigi, was slightly different from these isolates, it was close enough to be grouped with J1. The remaining isolates from Tochigi, TP2, TP3, and TP4, formed the second subgroup (J2), while all the isolates from Akita (AK1, AK2, AK3, AK4, and AK5) clustered together, forming the third

Table 1. (continued from preceding page)

<i>M. oryzae</i> isolate	Abbreviation	Host of origin	Location	Year of collection	Collector
PgPA33B.06	U9	<i>L. perenne</i>	Pennsylvania, United States	1999	W. Uddin
PgIL9825-1	U53	<i>L. perenne</i>	Illinois, United States	1999	A. Hamblin
PgIL9825-3	U54	<i>L. perenne</i>	Illinois, United States	1999	A. Hamblin
PgIL9825-4	U55	<i>L. perenne</i>	Illinois, United States	1999	A. Hamblin
LW-1	LW-1	<i>L. perenne</i>	Yamanashi, Japan	1999	A. Tanaka and T. Tani
LW-2	LW-2	<i>L. perenne</i>	Yamanashi, Japan	1999	A. Tanaka and T. Tani
LW-3	LW-3	<i>L. perenne</i>	Yamanashi, Japan	1999	A. Tanaka and T. Tani
PgPA34M1.1	U10	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA34M1.2	U11	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA34M1.3	U12	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA35H1.1	U13	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA35H2.1	U14	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA35H4.1	U15	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgNJ36B.1.2	U17	<i>L. perenne</i>	New Jersey, United States	2000	B. Clarke
PgPA08N7.1	U19	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA08N7.2	U20	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA08N7.3	U21	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA02V28.1	U22	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA02V28.2	U23	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgMA37G1.1	U25	<i>L. perenne</i>	Massachusetts, United States	2000	G. Schumann
PgMA37G2.1	U26	<i>L. perenne</i>	Massachusetts, United States	2000	G. Schumann
PgMA37G2.3	U27	<i>L. perenne</i>	Massachusetts, United States	2000	G. Schumann
PgPA39C1.3	U28	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA39C2.1	U29	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA39C2.3	U30	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA09Lu6.1	U31	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA09Lu6.2	U32	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgPA09Lu6.3	U33	<i>L. perenne</i>	Pennsylvania, United States	2000	W. Uddin
PgKY40V1.1	U34	<i>L. perenne</i>	Kentucky, United States	2000	P. Vincelli
PgKY40V2.1	U35	<i>L. perenne</i>	Kentucky, United States	2000	P. Vincelli
PgKY40V2.3	U36	<i>L. perenne</i>	Kentucky, United States	2000	P. Vincelli
PgILLin	U56	<i>L. perenne</i>	Illinois, United States	2000	A. Hamblin
PgCA1.1.1	U37	<i>L. perenne</i>	California, United States	2001	L. Stowell
PgCA1.2.3	U38	<i>L. perenne</i>	California, United States	2001	L. Stowell
PgCA1.3.4	U39	<i>L. perenne</i>	California, United States	2001	L. Stowell
PgPAWH1.1.1	U40	<i>L. perenne</i>	Pennsylvania, United States	2001	W. Uddin
PgPAWH1.2.1	U41	<i>L. perenne</i>	Pennsylvania, United States	2001	W. Uddin
PgPAWH2.2.2	U42	<i>L. perenne</i>	Pennsylvania, United States	2001	W. Uddin
PgPALu1.1	U43	<i>L. perenne</i>	Pennsylvania, United States	2001	W. Uddin
PgNYVC1.3.1	U47	<i>L. perenne</i>	New York, United States	2001	W. Uddin
PgNYVC1.4.2	U48	<i>L. perenne</i>	New York, United States	2001	W. Uddin

subgroup (J3). Some of the isolates from the United States also belonged to these subgroups. For example, four U.S. isolates, U10, U11, U12, and U65, that were collected from Pennsylvania in 1998 and 2000, exhibited identical MGR586 fingerprints to most of the J1 isolates (Fig. 2A). An NJ dendrogram constructed using U68 (sole member of the minor MGR586 group in the United States) as an outgroup also showed a similar topology (*data not shown*).

Fingerprinting analysis of the *M. oryzae* isolates with the Pot2 probe produced similar groupings. All the isolates in the major MGR586 group showed very similar Pot2 profiles, comprising 20 to 25 bands, within the group (Fig. 1B). In contrast, the isolates in the minor MGR586 group showed unique patterns with only a few bands. An NJ dendrogram, constructed from the Pot2 data matrix using the minor MGR586 group in Japan as an outgroup, was very similar to that generated using the MGR586 data matrix (Fig. 2B). As with MGR586, the four isolates from the United States (U10, U11, U12, and U65) showed a Pot2 fingerprint identical to that of the J1 isolates from Japan (Fig. 2B). The NJ dendrogram constructed using the isolate U68 as an outgroup also showed a similar topology (*data not shown*).

When the MAGGY element was used as a probe, two distinct fingerprint types, type I (isolates exhibiting more than 30 bands) and type II (isolates with only 2 to 4 bands), were identified in the major MGR586 group (Fig. 1C). Both types were observed among the *M. oryzae* isolates from both countries. All the isolates belonging to the minor MGR586 group exhibited type II fingerprints. The functional copy of the MAGGY retrotransposon has

five *Eco*RI sites and produces four internal *Eco*RI fragments of 0.6, 0.7, 1.0, and 1.2 kb (26). Isolates that showed the type I fingerprints had all four internal *Eco*RI fragments with strong hybridization signal (Fig. 1C), indicating that the five *Eco*RI sites are conserved in most of the MAGGY copies in these isolates. These type I isolates were closely associated with each other on the NJ dendrograms constructed from the MGR586 and Pot2 data (Fig. 2), irrespective of their geographical origin.

The MGR586 and Pot2 data were combined to produce integrated NJ and parsimony trees (Fig. 3A and 3B). The integrated NJ tree (Fig. 3A) was similar to the NJ tree based on the Pot2 data (Fig. 2B). Based on the topology of the parsimony tree, the isolates were divided into six lineages, A, B, C, D, E, and F (Fig. 3B). The major MGR586 group recognized in the NJ dendrograms (Figs. 2A and B and 3A) included lineages A, B, C, and D. Lineage A, the largest group, was further divided into 10 subgroups, A1 through A10 (Fig. 3B). All the isolates that exhibited the MAGGY type I fingerprints belonged to the A9 subgroup regardless of their geographical origin. All Japanese isolates in the MGR586 major group belonged to either the A7 or A9 subgroup.

Lineage B, comprising two isolates from New York collected in 2001, was very closely related to lineage A (Fig. 3A). Lineage C consisted of a single isolate (U60) collected in Maryland in 1995. A single isolate, U49 (an annual ryegrass isolate), belonged to lineage D. Although lineage E consisted of the sole U.S. isolate in the MGR586 minor group, U68, lineage F included the six Japanese isolates in the MGR586 minor group.

The geographic origins of the *M. oryzae* isolates analyzed in this study are shown in a chronological order with their lineage or subgroup names (Fig. 4). In 1995, the first year of the major gray leaf spot outbreak in the United States, isolates U68 (lineage E), U60 (lineage C), and U66 (subgroup A9 with the MAGGY type I) were collected. The subgroup A1, which currently is predominant in the United States, also was identified in the early years of the outbreak. Various subgroups in lineage A were observed in the United States in 1998. More diverse subgroups were identified in lineage A between 1999 and 2001, in which subgroup A1 was predominant. In Japan, a serious outbreak of gray leaf spot was caused by isolates that belonged to lineage F in 1996. Subsequently, in 1997 and 1998, subgroups A7 and A9 (both MAGGY type I and MAGGY type II) were identified (Fig. 4). All the isolates collected from 1999 to 2001 also belonged to the subgroup A7. These subgroups of the *M. oryzae* isolates also were found in the United States.

DISCUSSION

This is the first study that has documented the genetic relationships among the isolates of *M. oryzae* associated with gray leaf spot epidemics in the United States and Japan. In Japan, two distinct populations, differentiated by their MGR586 and Pot2 fingerprint patterns, exist among the prg isolates of *M. oryzae* (25). In the present study, we found that the major group prevailing in Japan is highly similar to the isolates that are prevailing in the United States. Interestingly, the isolates from Japan that belonged to the A7 subgroup exhibited MGR586 or Pot2 fingerprints that were identical to certain

Fig. 1. Southern blot analysis of representative *Magnaporthe oryzae* isolates from the United States and Japan. Genomic DNA was digested with *Eco*RI, electrophoresed, and hybridized with **A**, MGR586, **B**, Pot2, and **C**, MAGGY. The arrows in C indicate internal fragments of the MAGGY retrotransposon.

A. MGR586

B. Pot2

Fig. 2. Neighbor-joining dendrograms of *Magnaporthe oryzae* isolates from the United States and Japan constructed from restriction fragment length polymorphism data using **A**, MGR586 and **B**, Pot2 probes. The Japanese isolates are enclosed in rectangles. The star symbols indicate isolates carrying approximately 30 copies of MAGGY (MAGGY type I). Numbers at nodes represent bootstrap support >50% from 1,000 replications.

A. NJ

99

U68 (95 MD)

95

84

50

73

61

94

84

95

U1 (99 PA)
U2 (99 PA)
U3 (99 PA)
U4 (99 PA)
U5 (99 PA)
U13 (99 PA)
U14 (99 PA)
U15 (99 PA)
U17 (99 NJ)
U26 (99 MA)
U28 (99 PA)
U29 (99 PA)
U30 (99 PA)
U31 (99 PA)
U32 (99 PA)
U33 (99 PA)
U22 (99 PA)
U23 (99 PA)
U25 (99 MA)
U27 (99 MA)
U40 (99 PA)
U41 (99 PA)
U47 (99 NY)
U48 (99 NY)
U3 (99 PA)
U10 (99 PA)
U11 (99 PA)
U12 (99 PA)
U65 (99 PA)
TP1 (97 TG)
TP6 (97 TG)
LW1 (99 YN)
LW2 (99 YN)
LW3 (99 YN)
FQ (98 CB)
FQ (98 CB)
F4 (98 CB)
F6 (98 CB)
TP2 (97 TG)
U34 (99 KY)
U35 (99 KY)
U36 (99 KY)
U64 (98 PA)
U53 (99 L)
U54 (99 L)
U55 (99 L)
U63 (98 W)
U7 (99 PA)
U5 (99 PA)
U8 (99 PA)
U67 (98 NJ)
TP3 (97 TG)
TP4 (97 TG)
AK1 (98 AT)
AK2 (98 AT)
AK3 (98 AT)
AK4 (98 AT)
AK5 (98 AT)
WK3-1 (96 YG)
WK3-2 (96 YG)
WK4-1 (96 YG)
WK4-2 (96 YG)
WK4-3 (96 YG)
WK4-4 (96 YG)
U49 (72)
U60 (95 MD)

- 0.001 changes

B. Parsimony

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MAGGY is known to be the most active among the transposable elements that have been detected in *M. oryzae* thus far (5). A previous study (6) has shown that MAGGY fingerprints revealed the presence of three groups among prg isolates. The present study shows that the major group of prg isolates from the United States and Japan belong to two types (Fig. 1C). All the type I isolates belonged to subgroup A9 (Fig. 3B). If the A9 subgroup is derived from an ancestor with multiple copies of MAGGY (i.e., type I isolate), all isolates in the A9 subgroup should carry multiple copies of MAGGY. However, the A9 subgroup included both type I and type II isolates of *M. oryzae* (Fig. 3B), suggesting that the ancestor of the A9 subgroup

A new group (lineage B) was detected among *M. oryzae* isolates collected during the epidemics from 1999 to 2001 in the United States. Further, increased diversity with six additional subgroups (A2, A3, A4, A5, A6, and A8) was observed in lineage A during these years. It is noteworthy that one of the subgroups (A1) in the United States was found as far east as Massachusetts and as far west as California (Fig. 4). This subgroup appears to be predominant in the United States at present.

None of the isolates collected in the United States from 1995 to 2001, except one, showed fingerprint patterns similar to the Japanese isolates that belonged to the MGR586 minor group. Only one U.S.

The results of this study suggest that isolates in the same lineage (lineage A) could have been involved in the simultaneous outbreaks of gray leaf spot in Japan and the United States. It is possible that strains of the pathogen were imported to different regions of the United States and Japan independently from an unidentified source. Alternatively, considering that Japanese companies have imported paddy seed from the United States, they may have moved from the United States to Japan via seed trades. The widespread presence of a single lineage supports the possibility of seedborne transmission of the fungus. Studies have shown that the isolates of *M. oryzae* from rice are seed transmissible (12,13). Further, the lack of genetic diversity among the host population also could be an important factor influencing population diversity of the pathogen. Studies on the genetic back-

Fig. 4. Distribution patterns of *Magnaporthe oryzae* isolates associated with gray leaf spot epidemics on perennial ryegrass in the United States and Japan. Each isolate is plotted with its lineage or subgroup. A9 and A7, the two major subgroups prevailing in Japan, are marked with an oval and a black oval, respectively. The star symbols indicate isolates that belong to MAGGY type I.

ground of prg cultivars grown in the United States and Japan and the role of seedborne transmission of the fungus are warranted.

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