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FULL PAPER

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Population structure of *Magnaporthe oryzae* isolates from green foxtail in Japan examined by DNA fingerprint analysis

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Abstract The population structure of *Magnaporthe oryzae* from green foxtail (*Setaria viridis*) in Japan was examined by DNA fingerprint analyses using the transposable elements MGR586 and MAGGY as probes. Fifteen *M. oryzae* isolates from green foxtail were collected from 11 Japanese prefectures so that a macrogeographic population of this pathogen is represented. All the 15 isolates were sorted into distinct haplotypes by DNA fingerprint analyses with both probes. Furthermore, similarities between the DNA fingerprint profiles of the 15 isolates were exclusively low; i.e., if lineages are arbitrarily established based on greater than 70% similarities in isolates, the 15 isolates could be categorized into 13 distinct lineages by DNA fingerprinting with both probes. We also examined the MGR586 DNA fingerprint variations of this pathogen in 9 microgeographic populations each of which contained 20 to 24 isolates collected from a 1 m^2 or 50 m^2 area. In all the 9 populations, more than 2 haplotypes, which shared less than 70% similarities, were identified in the DNA fingerprint profiles. These results suggested that *M. oryzae* isolates from the green foxtail in Japan possessed a complex lineage structure, even at the microgeographic scale.

Key words DNA fingerprint analysis · Green foxtail · *Magnaporthe oryzae* · *Setaria viridis*

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Introduction

Magnaporthe oryzae B. Couch (anamorph *Pyricularia oryzae*) (Couch and Kohn 2002) is the causative agent of blast disease in many gramineous plants, including wild plant species. This fungal species is genetically diverse and includes several subgroups, each of which has a restricted range of host species. These subgroups have been designated as pathotypes (Kato et al. 2000); i.e., the *Oryza* pathotype is pathogenic to rice (*Oryza sativa* L.), the *Setaria* pathotype is pathogenic to foxtail millet [*Setaria italica* (L.) P. Beauv.], the *Panicum* pathotype is pathogenic to common millet (*Panicum miliaceum* L.), etc. Economically, the most important pathotype is the *Oryza* pathotype, i.e., the rice blast fungus. Knowledge of the genetic diversity and population structure of the rice blast fungus is indispensable for breeding strategies for resistant rice cultivars, and thus population genetic analysis of this pathogen continues to be an active area of research. In particular, DNA fingerprint analyses using dispersed repetitive DNA sequences have been applied to rice pathogen populations from many rice-growing countries or regions and provide valid information concerning the population structure of the rice pathogen (Levy et al. 1991, 1993; Chen et al. 1995; Roumen et al. 1997; Sone et al. 1997; Don et al. 1999a,b; Kumar et al. 1999; Park et al. 2003). Although rice blast populations from each country or region appear genetically diverse, isolates within the populations are ordinarily sorted into a limited number of groups based on genetic similarity inferred from the fingerprint data. Each group is considered to constitute a lineage comprising members related by clonal descent (Kumar et al. 1999). In contrast to the rice blast fungus, however, little is known about the population structure of *M. oryzae* isolates belonging to other pathotypes. The pathotypes mentioned above, in general, include isolates from wild plant species, but there is no report concerning the population structure of blast pathogens infecting wild grass.

In this report, we focused on the population structure of the blast fungus from green foxtail [*Setaria viridis* (L.) Beauv.], which is a close relative of foxtail millet (Dekker

2003) and a commonly distributed wild grass species in Japan. In addition to blast isolates from green foxtail, we previously taxonomically characterized those from another commonly distributed wild *Setaria* species, namely, giant foxtail (*S. faberii* Herrm.), by DNA analyses, mating tests, and pathogenicity assays (Yamagashira et al. 2008). We concluded that the blast isolates from the wild foxtails were closely related to those from foxtail millet and should be classified into the *Setaria* pathotype of *M. oryzae*. To analyze the population structure of *M. oryzae* from green foxtail, we performed DNA fingerprint analysis using the transposable elements MAGGY (Leong et al. 1994; Farman et al. 1996a) and MGR586 (Hamer et al. 1989) as probes; further, the haplotypic and lineage diversity of the pathogen populations from green foxtail were examined on macro- and microgeographic scales in Japan. MAGGY and MGR586 are a DNA-type transposon and a retrotransposon with long terminal repeats, respectively, and both transposons have been widely used for population genetic studies in the rice blast fungus.

Materials and methods

Fungal materials

A total of 202 *M. oryzae* isolates were examined in this study; they were collected from 24 sites in Japan, and the locations of these 24 collection sites are shown in Fig. 1. The names of the collection sites shown in Fig. 1 have been designated by the abbreviated name of the prefectures where the collection sites are located (e.g., Sa), years of collection (e.g., 04), and identification numbers within the prefectures (e.g., –1). The abbreviated names Na, Sa, Ku, Hi, Hy, Si, Ky, Gi, Ka, Ch, Ib, Mi, and To correspond to Nagasaki, Saga, Kumamoto, Hiroshima, Hyogo, Siga, Kyoto, Gifu, Kanagawa, Chiba, Ibaraki, and Miyagi Prefectures and the Metropolis of Tokyo, respectively. The isolates were collected during 2004–2007. To analyze the population structure of *M. oryzae* from green foxtail on a macrogeographic scale in Japan, 15 isolates were collected from infected leaves on naturally growing green foxtail plants from the following 15 sites in 11 prefectures: Na05-1, Na05-2, Sa05-1, Sa05-2, Hi06-1, Hy05-1, Ky05-1, Si05-1, Gi06-1, Ka05-1, Ka05-2, Ch05-1, Ib05-1, Mi05-1, and Mi05-2. The range of distances between the sampling sites was 300 m (Mi05-1 and Mi05-2) to 1160 km (Na05-1 and the 2 sites in Miyagi). The 15 sampling sites, together, cover a wide geographic range of Japan, i.e., the Kyushu, Chugoku, Kinki, Chubu, Kanto, and Tohoku regions. To analyze the population structure on a microgeographic scale, samples were collected at the following 9 sites in 6 prefectures (as shown in Fig. 1): Sa04-3, Sa06-4, Sa06-5, Ku06-1, Gi06-2, Ka06-3, Ka06-4, To07-1, and Ib07-2. Each sampling site was approximately 5×10 m^2 (Sa04-3) or 1×1 m^2 (the remaining 8 sites), and 20–24 infected leaves were randomly collected from each site (1 leaf per plant). *M. oryzae* isolates were obtained from all the infected leaves at each site (1 isolate per leaf), and 20–24 isolates from each site were obtained and considered as a microgeographic population in this study. All the isolates used in this study were collected by single-spore isolation from blast lesions on the infected leaves sampled as previously described (Kusaba et al. 2006). After monoconidial isolation, these isolates were grown for 7 days on filter paper disks with potato sucrose agar (PSA) medium, and the filter paper disks with mycelia were thoroughly desiccated and stored at –25°C for long-term storage. For short-term storage, all the isolates used were grown on PSA slants and maintained at 22°C. The representative isolate of each collection site was deposited in the Microorganisms Section of the NIAS Genebank (MAFF), National Institute of Agrobiological Science, Tsukuba, Japan under accession numbers 240996 to 241039.

Hybridization

Extraction of total DNA from each isolate was performed as previously described (Luo et al. 2005). The total DNA was digested with *Eco*RI (for MGR586 DNA fingerpinting) or with *Bam*HI (for MAGGY DNA fingerprinting), and fractionated on a 0.8% agarose gel in 0.5× TBE buffer at 20 V for 30 h. The fractionated DNA was transferred to a MSI nylon membrane (Osmonics, Westborough, MA, USA) and fixed by UV irradiation following the manufacturer's instructions. pMGR-T1 containing an almost complete copy of MGR586 transposon (Farman et al. 1996b) and pMGY-SB containing *Sal*I-*Bam*HI fragment (SB) from a MAGGY clone pMGY23 (Eto et al. 2001) were used as hybridization probes for MGR586- and MAGGY DNA fingerprinting, respectively. These probes were labeled with biotin by using NEBlot Phototope Kit (New England Biolabs, Beverly, MA, USA). Hybridization with the biotin-labeled probes and detection of target DNA were performed as previously described (Luo et al. 2005).

Statistical analysis of fingerprint data

DNA fingerprint profiles of the isolates used were visually scored using a binary system, i.e., the presence of a given fragment was recorded as 1 and an absence as 0. Similarities between DNA fingerprint profiles were calculated by using the formula of Nei and Li (1979): $F = 2Nxy/(Nx + Ny)$, where Nxy is the number of fragments shared by two isolates, and Nx and Ny are the number of fragments in each isolate, x and y. Dendrograms were constructed based on the similarity coefficient using the unweighted pair-group method with arithmetic average (UPGMA) program in NTSYSpc (Rohlf 1998). The robustness of clusters in the UPGMA-based dendrograms was determined by bootstrap analysis with 1000 replications using the program WINBOOT (Yap and Nelson 1996).

Evaluation of mating ability

Evaluation of the mating ability of isolates tested by using tester isolates Y93-164a-1 (*MAT1-1*) and F1-63 (*MAT1-2*)

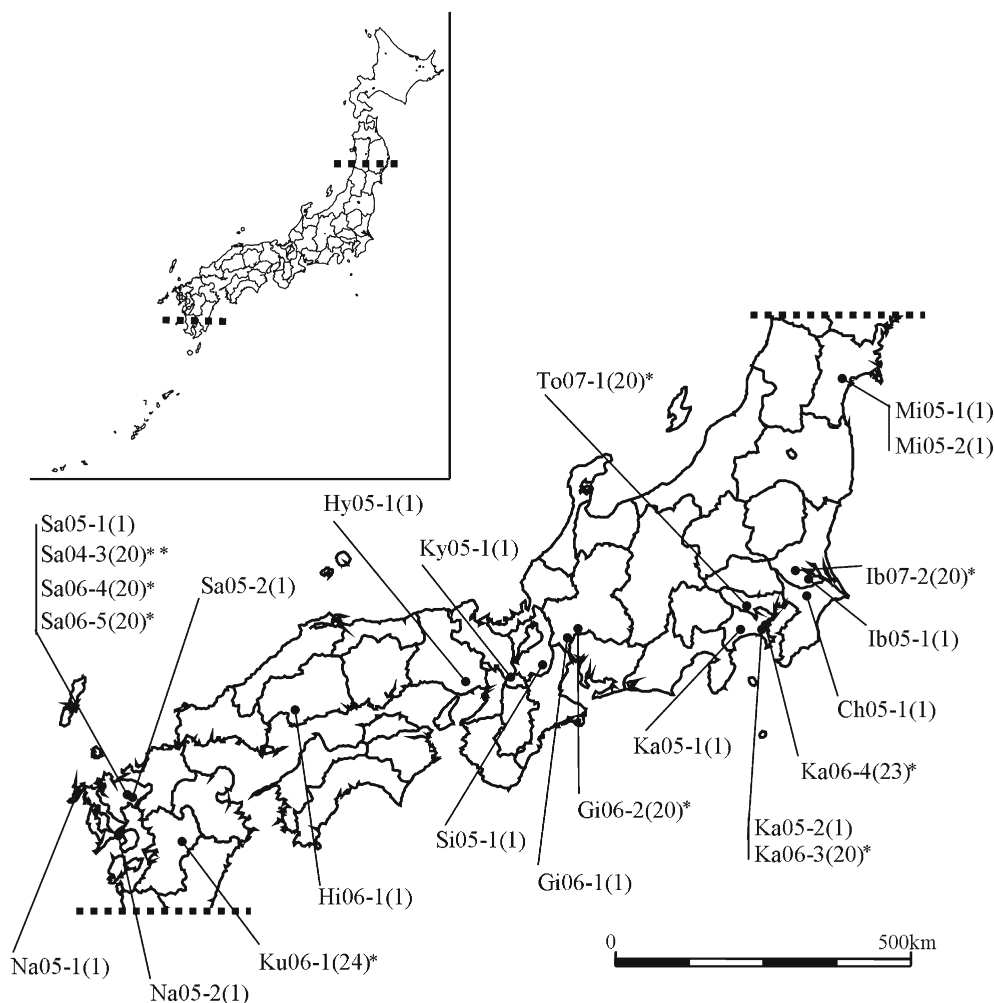


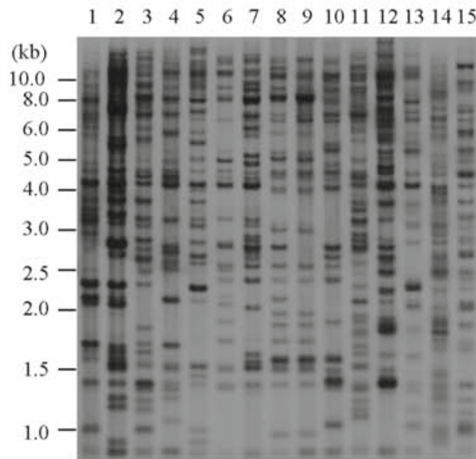
Fig. 1. Locations of collection sites of *Magnaporthe oryzae* isolates from green foxtail in Japan. Sites located within 5 km in distance are plotted by a *single dot*. Names of the collection sites are designated as abbreviated names of prefectures where the collection sites are located, years of collection, and identification numbers within the prefectures. The abbreviated names (*Na*, *Sa*, *Ku*, *Hi*, *Hy*, *Si*, *Ky*, *Gi*, *Ka*, *Ch*, *Ib*, *Mi*, and *To*) correspond to Nagasaki, Saga, Kumamoto, Hiroshima, Hyogo, Siga, Kyoto, Gifu, Kanagawa, Chiba, Ibaraki, and Miyagi Prefectures and the Metropolis of Tokyo, respectively. Numbers of isolates collected from each site are indicated in *parentheses*. The symbols* and ** represent areas of sites 1 m^2 and 50 m^2, respectively. These areas are shown for the sites where more than one isolate was collected. The representative isolate of each collection site was deposited in the Microorganisms Section of the NIAS Genebank (MAFF), National Institute of Agrobiological Science, Tsukuba, Japan. Accession numbers were: 240996, Na05-1; 240997, Na05-2; 240998, Sa05-1; 240999, Sa05-2; 241000 to 241010, Sa04-3; 241011 and 241012, Sa06-4; 241013, 241014 and 241015, Sa06-5; 241016 and 241017, Ku06-1; 241018, Hi06-1; 241019, Hy05-1; 241020, Ky05-1; 241021, Si05-1; 241022, Gi06-1; 241023 and 241024, Gi06-2; 241025, Ka05-1; 241026, Ka05-2; 241027 and 241028, Ka06-3; 241029 and 241030, Ka06-4, 241031, 241032 and 241033; To07-1; 241034, Ch05-1; 241035, Ib05-1; 241036 and 241037, Ib07-2; 241038, Mi05-1; 241039, Mi05-2

was as previously described (Yamagashira et al. 2008). Y93-164a-1 and F1-63 are highly fertile, hermaphroditic *M. oryzae* isolates. When the test isolate was interfertile with one of the testers, perithecia formed along the borderline of their mycelial mats within 1 month. The perithecial formation was determined with a stereomicroscope, and asci and ascospores were observed using a microscope.

Results and discussion

To analyze the population structure of *M. oryzae* from green foxtail on a macrogeographic scale, 15 green foxtail isolates were collected from 15 distinct sites in 11 prefectures in Japan during 2005 to 2006 (Fig. 1). The 15 isolates were subjected to MGR586 DNA fingerprint analysis (Fig. 2A). On an average, 30 resolvable fragments could be counted for each profile, ranging from 1 to 10 kb, and DNA fingerprint similarities between the DNA fingerprint profiles were calculated based on the presence or absence of these resolvable fragments in all 105 possible pairwise combinations of the 15 isolates (Fig. 2C). The pairwise comparisons of the DNA fingerprint profiles revealed a high genetic diversity among the isolates (Fig. 2C). There was no pair combination with a 100% similarity (identical DNA fingerprint profile shared between 2 isolates in a combination) observed; in other words, each of the 15 isolates possessed its own DNA fingerprint haplotype. Furthermore, 103 of the 105 possible combinations showed less than 65% simi-

A



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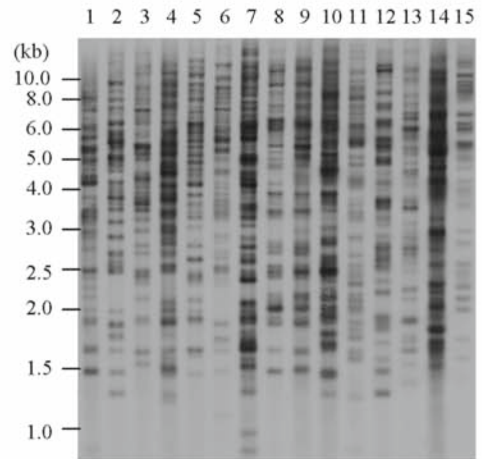

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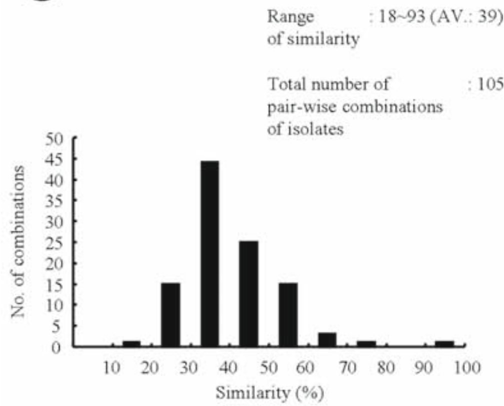

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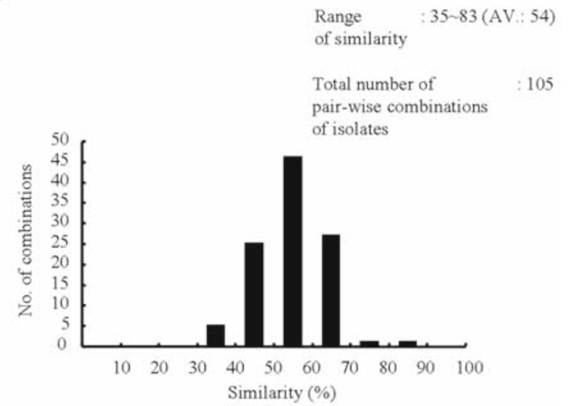

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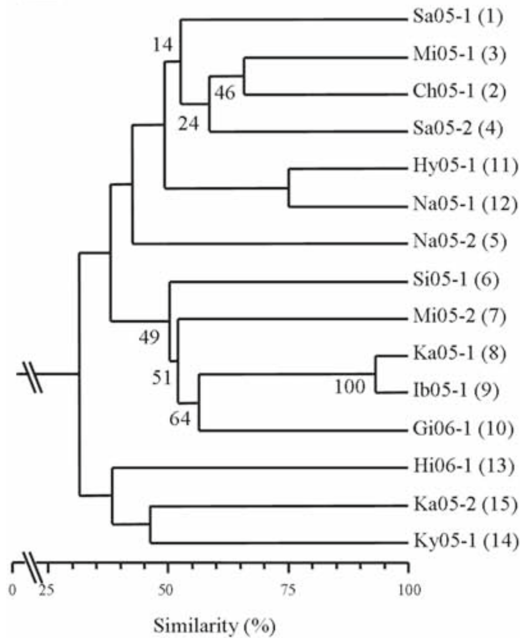

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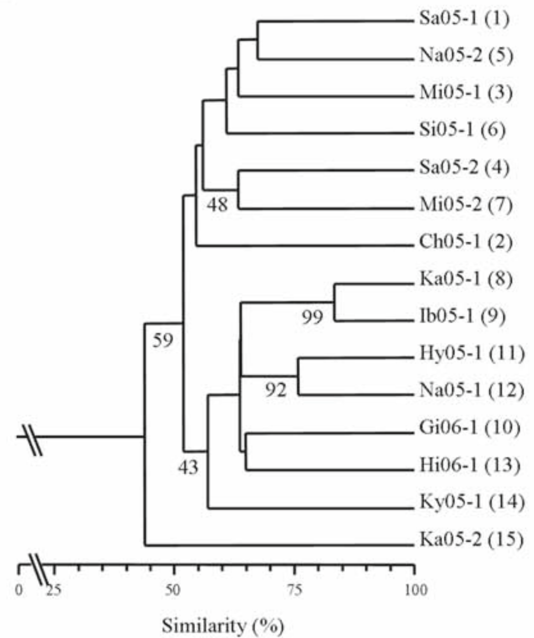

Fig. 2. DNA fingerprint analysis of *Magnaporthe oryzae* isolates from green foxtail at macrogeographic scale in Japan. Fifteen isolates used were collected from 15 sites in 11 prefectures: Na05-1 and -2 in Nagasaki, Sa05-1 and -2 in Saga, Hi06-1 in Hiroshima, Hy05-1 in Hyogo, Si05-1 in Siga, Ky05-1 in Kyoto, Gi06-1 in Gifu, Ka05-1 and -2 in Kanagawa, Ch05-1 in Chiba, Ib05-1 in Ibaraki, and Mi05-1 and -2 in Miyagi Prefectures. **A**, **B** DNA fingerprint profiles of the 15 isolates were generated by using pMGR-T1 (**A**) and pMGY-SB (**B**) as hybridization probes for MGR586 and MAGGY, respectively. Molecular sizes are shown on the *left*. **C**, **D** Histograms showing similarities between DNA fingerprint profiles in all 105 possible pairwise combinations of the 15 isolates. The similarities in **C** and **D** were calculated from the DNA fingerprint profiles shown in **A** and **B**, respectively, by using the formula of Nei and Li (1979). **E**, **F** UPGMA dendrograms were constructed from the DNA fingerprint similarity data shown in **C** (**E**) and **D** (**F**), respectively. The sites where isolates were collected are indicated as taxon labels. *Numbers in parentheses* with the taxon labels correspond to lane numbers of DNA fingerprint profiles shown in **A** and **B**, respectively. *Numbers at nodes* represent bootstrap support from 1000 iterations

larities (the similarities ranged from 18% to 93%, and the average was 39%). Two exceptional combinations with relatively high similarities were isolates from Ka05-1 and Ib05-1 and from Hy05-1 and Na05-1; they showed 93% and 75% similarities, respectively (also see Fig. 2E). An UPGMA dendrogram was constructed from the similarity data of the MGR586 DNA fingerprint profiles (Fig. 2E). In the dendrogram, except for 2 clusters supporting groups of the 2 pairs of isolates mentioned above, no isolate grouping was observed until a 65% similarity level, i.e., the remaining 11 isolates separated into distinct branches at this similarity level. A majority of clusters in the dendrogram were formed at less than 65% similarity levels and supported by low bootstrap values. MAGGY DNA fingerprint analysis was also performed to confirm the genetic diversity among the 15 isolates (Fig. 2B). For MAGGY DNA fingerprint profiles, on an average, 45 resolvable fragments ranging from 1 to 10 kb could be counted for each isolate. Similar to the MGR586 DNA fingerprint profiles, MAGGY DNA fingerprint profiles were highly divergent among the 15 isolates. As shown in Fig. 2D, no pair combination with a 100% similarity was observed in the 105 possible pair combinations of the isolates, and 103 of the 105 possible combinations showed less than 68% similarities (the similarities ranged from 35% to 83%; the average was 54%). The 2 exceptional combinations with high MGR586-DNA fingerprint similarities, i.e., isolates from Ka05-1 and Ib05-1 and from Hy05-1 and Na05-1, also showed relatively high MAGGY DNA fingerprint similarities, 83% and 75%, respectively (also see Fig. 2F). Similar to the dendrogram shown in Fig. 2E, a majority of the isolates clustered with weak bootstrap support at a less than 68% similarity level in the UPGMA dendrogram constructed with the MAGGY-DNA fingerprint data (Fig. 2F). Precedent studies concerning DNA fingerprint analysis of rice blast fungal populations from several countries or regions have identified clear lineage structures in the pathogen populations (Levy et al. 1991, 1993; Chen et al. 1995; Roumen et al. 1997; Sone et al. 1997; Don et al. 1999a,b). In these studies, isolate groupings (clusters) were formed discontinuously along an axis of similarity levels in a UPGMA dendrogram constructed from the DNA fingerprint data. Statistically robust clusters, which were considered as lineages, were initially formed at high similarity levels (more than 80% similarities in most cases) in the dendrograms. Sequentially, superclusters, usually with weak statistical support, were formed at lower similarity levels (less than 70% similarities in most cases) and separated from the clusters corresponding to the lineages by relatively long interval branches. On the other hand, such a clear lineage structure could not be observed in either of the dendrograms in our study (Fig. 2E,F). As an exceptional case, the lack of lineage structure was reported for the rice blast population from Korea (Park et al. 2003). Park et al. (2003) analyzed the lineage structure of the Korean rice blast population containing 328 isolates collected from 1981 to 2000 based on the MGR586- and MAGGY DNA fingerprints. In their study, 13 clusters that tended to correlate with collection year of the isolates were observed in the dendrogram constructed from the combined data of MGR586- and MAGGY DNA fingerprints. However, almost all the clusters were distinguished from each other by 70%–85% similarities; i.e., relatively short interval branches separated these clusters. Furthermore, none of the clusters was supported by bootstrap values greater than 10%. From the results, Park et al. (2003) concluded that the Korean rice blast population was composed of a single clonal lineage. However, from our study we considered the lineage structure of the green foxtail blast population to be dissimilar to that of the Korean rice blast population; i.e., almost all haplotypes detected in this study represent distinct lineages. Fifteen isolates used could be sorted into 13 lineages, i.e., 2 pairs of isolates, namely, isolates from Ka05-1 and Ib05-1 and from Hy05-1 and Na05-1, respectively, corresponded to 2 distinct lineages and the other 11 isolates corresponded to the remaining 11 lineages, respectively. The number of the putative lineages in the green foxtail pathogen population was higher than those in the rice pathogen populations from several countries or regions, such as the Philippines (Chen et al. 1995), Japan (Sone et al. 1997; Don et al. 1999a), Columbia (Levy et al. 1993), the United States (Levy et al. 1991), Vietnam (Don et al. 1999b), and the European countries (Roumen et al. 1997). Isolates within each of the rice blast populations were considered to be sorted into limited number of lineages, 2 (present-day Japan) to 10 (the Philippines).

Another interesting feature of the dendrograms shown in Fig. 2E,F is that the relationships among the isolates tended not to correlate with the geographic origins of the isolates. Among the 15 isolates, 3 pairs of isolates were collected from the same prefectures, i.e., isolates from Sa05-1 and Sa05-2 in Saga, from Na05-1 and Na05-2 in Nagasaki, and from Mi05-1 and Mi05-2 in Miyagi Prefecture, respectively (see Fig. 1). However, none of the 3 pairs formed a single cluster in both dendrograms. Mi05-1 and Mi05-2 were located closest to each other among the 15 sampling sites; the distance between the 2 sites was only 300 m. In spite of

Table 1. MGR586 DNA fingerprint variation in microgeographic populations of *Magnaporthe oryzae* isolates from green foxtail

Collection site[a]	No. of isolates	No. of haplotypes[b]	Similarity[c] (%)	No. of putative lineages[d]	
				70%	50%
Sa04-3	20	11 (55)	31–88 (51)	8	4
Sa06-4	20	2 (1)	26	2	2
Sa06-5	20	3 (3)	48–62 (54)	3	2
Ku06-1	24	2 (1)	34	2	2
Gi06-2	20	2 (1)	25	2	2
Ka06-3	20	2 (1)	33	2	2
Ka06-4	23	2 (1)	56	2	1
To07-1	20	3 (3)	20–60 (38)	3	2
Ib07-2	20	2 (1)	20	2	2

[a] Sa-3 was 50 m^2 in area, and other sites were 1 m^2 in area
[b] Numbers of all possible pairwise combinations of haplotypes in each population are shown in parentheses
[c] Range of similarities between DNA fingerprint profiles in all possible pairwise combinations of haplotypes in each population were calculated by using the formula proposed by Nei and Li (1979); averages of the similarities are shown in parentheses
[d] Putative lineages were arbitrarily established as isolates with greater than 70% and 50% similarity in each population, respectively

their closely related geographic origins, both the isolates from Mi05-1 and Mi05-2 clustered with isolates from remote locations in both dendrograms. For example, the isolate from Mi05-1 clustered with the isolate from Ch05-1 in Chiba Prefecture (Fig. 2E) and with those from Sa05-1 in Saga and Na05-2 in Nagasaki Prefecture (Fig. 2F). From this phenomenon, we speculated that high genetic variation existed in the green foxtail pathogen population on a microgeographic scale. To test our hypothesis, 9 populations comprising 20–24 *M. oryzae* isolates were obtained from green foxtail plants growing at 9 sites in 6 prefectures in Japan (Fig. 1, Table 1), and genetic variation within each of the 9 populations was analyzed by MGR586 DNA fingerprinting. Except for Sa04-3 in Saga Prefecture, each of the collection sites was approximately 1 m^2 in area; Sa04-3 was approximately 50 m^2 in area. DNA fingerprint profiles of the 20 isolates from Sa04-3 (Fig. 3A) are examples of DNA fingerprint variation in microgeographic populations. The number of haplotypes detected by the DNA fingerprint analysis within each population ranged from 2 to 11 (Table 1). As shown in Fig. 3A, isolates with identical DNA fingerprint profiles (haplotype) were observed in all the microgeographic populations, and such isolates were considered to be clonally propagated from a single isolate within each of the populations. In contrast to the homogeneity of the clonally propagated isolates, DNA fingerprint profiles were highly divergent among the isolates bearing distinct haplotypes. Except for the population from Sa04-3, similarities between DNA fingerprint profiles in all possible pairwise combinations of the haplotypes within each population were lower than 62% (Table 1). Therefore, if lineages were distinguished based on a 70% similarity (lineages are established as isolates with greater than 70% similarities), almost all haplotypes detected within each of the 9 microgeographic populations were discerned as distinct lineages (Table 1). In the population from Sa04-3, 11 haplotypes were detected. As shown in Fig. 3B, the 11 haplotypes in the population from Sa04-3 were separated into 5 branches and 3 clusters at a 70% similarity level in a UPGMA dendrogram constructed from the DNA fingerprint similarities. If a lower similarity, i.e., 50%, was employed for lineage discernment, lineage divergence was still observed in 8 of the 9 populations (see Table 1, Fig. 3B). These results support the high genetic variation at a microgeographic scale in the case of the green foxtail pathogen; i.e., the pathogen populations are considered to harbor more than 2 lineages even at the restricted areas.

For cultivated plant pathogens, artificial selection pressure, introduction of a new resistant cultivar, fungicide treatment, etc., could play an important role in the population dynamics of the pathogens. Don et al. (1999a) reported that the number of lineages in the rice blast population decreased in the agricultural environment in Japan especially during 1960–1976. They found that the Japanese rice blast population collected during 1972–1993 comprised only 2 lineages, which corresponded to 2 of the 5 lineages detected in rice blast isolates collected before 1960 in Japan. The other 3 lineages that existed before 1960 in Japan were considered to become extinct after this period. In Japan, some blast-resistant rice cultivars were widely introduced for commercial production in the early 1960s, but new populations that were virulent for the rice cultivars successively emerged and caused a breakdown of resistance in the rice cultivars within the few years after commercial production. Sone et al. (1997) suggested that the reduction in lineage diversity might be related to the breakdown, i.e., the introduction of resistant cultivars led to the lineage extinction in the rice blast population, and a small number of survivors, which acquired virulence for the resistant cultivars, restored the Japanese rice blast population after the breakdown. DNA fingerprint analyses in this study provided a complex lineage structure of green foxtail blast population in Japan. In contrast to the rice blast pathogen, it is rational to assume that the green foxtail pathogen has not faced any such artificial selection pressure in nature. As a result, the ancestral lineage diversity in the contemporary green foxtail pathogen population may have been retained.

On the other hand, it may be assumed that the haplotypes or lineages observed in the DNA fingerprint profile in this study were rapidly generated by mutation during

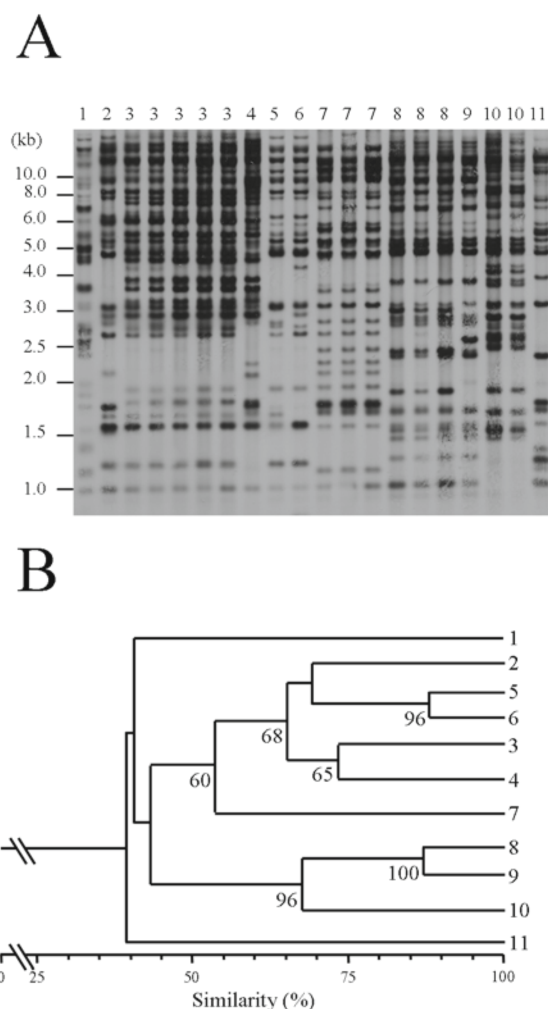

Fig. 3. DNA fingerprinting profiles and a dendrogram of *Magnaporthe oryzae* isolates. **A** MGR586 DNA fingerprinting profiles of 20 *M. oryzae* isolates from green foxtail in Sa04-3 in Saga Prefecture. The DNA fingerprint profiles were generated by using pMGR-T1 as a hybridization probe. Lane design, *1* to *11*, shows haplotypes detected in the DNA fingerprint profiles. Molecular sizes are shown on the *left*. **B** An UPGMA dendrogram constructed from DNA fingerprint similarities among the 11 haplotypes in the DNA fingerprint profiles. *Numbers at nodes* represent bootstrap support from 1000 iterations. Taxon labels correspond to numbers of haplotypes indicated *above the lanes* in **A**

asexual propagation, or by sexual and/or parasexual recombination. In particular, the haplotypic variations observed in the microgeographic populations appear to support the possibility of rapid generation of haplotypes or lineages. However, we previously observed a low level of fertility in the green foxtail pathogen (Yamagashira et al. 2008). In the previous study, the mating ability of 13 green foxtail isolates (all of which were used in this study as part of the macrogeographic population) were evaluated by crossing them with the highly fertile hermaphroditic tester isolates Y93-164a-1 (*MAT1-1*) and F1-63 (*MAT1-2*). Although 2 mating types were distributed in the 13 isolates, only 2 of them produced ascospores with 1 of 2 testers, and the remaining 11 produced no perithecium or barren perithecia in the cross experiments. In addition to the 15 isolates, in this study, we further evaluated the mating ability of the 20 isolates in the micropopulation from Sa04-3 and confirmed the low fertility level in these isolates, i.e., no perithecium was formed by crossing them with the tester isolates Y93-164a-1 and F1-63 (data not shown). Therefore, we assume that sexual recombination, if any, contributes little to the DNA fingerprint variations in the green foxtail pathogen. For the rice blast pathogen, mutations in the MGR586-DNA fingerprint profiles during asexual cell division and those induced by parasexual recombination were examined by several researchers (Wu and Magill 1993; Xia and Correll 1995; Zeigler et al. 1997). The mutations observed in these studies only slightly modified the DNA fingerprint profiles compared to those of the original haplotypes, but these mutations did not lead to the generation of new lineages. Although further study is required to clarify whether somatic mutations and parasexual recombination contributed to the divergence in DNA fingerprint profiles observed in this study, we consider the lineage diversity observed in the green foxtail blast populations in this study to be stable and reflect the genetic diversity in the pathogen populations.

In the previous study, we found differential pathogenicity reactions in landraces and a commercial cultivar of foxtail millet among *M. oryzae* isolates from green foxtail, indicating that physiological races on foxtail millet exist in the green foxtail pathogen population (Yamagashira et al. 2008). The population of green foxtail might harbor high heterogeneity in loci of resistance genes to the pathogen, and this may cause the race diversification and bring high genetic variation in the pathogen population on macro- and microgeographic scales. We are now attempting to perform an inoculation test of *M. oryzae* isolates from green foxtail to clarify the race diversification on green foxtail and to estimate the genetic heterogeneity of the blast resistance in green foxtail populations.

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