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Genetic structure of Iranian *Pyricularia grisea* populations based on rep-PCR fingerprinting

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Abstract

The population structure of the rice blast fungus *Pyricularia grisea* was analyzed in two major rice-growing provinces of Iran using rep-PCR DNA fingerprinting. A total of 221 monoconidial isolates of the fungus was collected from 12 cultivars at ten regions during 1997–2000. Long-PCR conditions were used to amplify sequences lying between adjacent *Pot2* elements. The frequencies of *Pot2* lineages (isolates with ≥70% amplicon similarity) and haplotypes within lineages were determined. Phenetic analysis differentiated five *Pot2* fingerprint lineages, designated A, B, C, D and E. The most common fingerprint group, Lineage E, was recovered from all rice cultivars sampled and was distributed throughout the region. Haplotype E6, the most common haplotype within lineage E, was recovered from almost all regions. Lineage A, the second most common lineage, was found mainly in the western part of the sampled region. Haplotype A1 was found in most sites in the western province. Lineage A occurred at relatively high frequency on the susceptible local cultivar Binam, suggesting that lineage A is specifically adapted to Binam. To test this hypothesis, 193 additional isolates were recovered from four fields at two sites separated by approximately 100 km. This second, field-specific collection of isolates contained lineages A, C, D, and E. Approximately 64% and 29% of the isolates recovered from Binam (the shared cv. at two sites) grouped into lineages A and E, respectively. The other two susceptible cultivars at these sites were infected by lineage E at frequencies of 100% and 71%. Overall, these data indicated a low level of genetic diversity in the Iranian *P. grisea* population similar to that reported in other countries.

Introduction

Rice blast caused by the fungus *Pyricularia grisea* (perfect state: *Magnaporthe oryzae* (Rossman et al., 1990; Couch and Kohn, 2002) is a widespread and damaging disease in most rice-growing areas of the world (Ou, 1985). Populations of this pathogen are pathotypically diverse and there are numerous reports that this diversity may be due to continuous generation of novel pathogenic variation. Attention was focussed on pathotype analysis after the first cultivar bred to be resistant rapidly became susceptible in the field (Kiyosawa, 1972; Ou, 1979). Numerous pathotypes of the pathogen exist and usually are defined by their ability or inability to cause disease on a set of differential cultivars (Ling and Ou, 1969; Ou, 1985; Zeigler et al., 1994).

DNA fingerprinting is an important tool for analyzing population structure of *P. grisea*. Fingerprints provide information needed for rational deployment of resistance genes (Zeigler et al., 1994, 1995). Dispersed, repetitive transposable elements such as MGR586 have been used for analysis of *P. grisea* population structure in a number of DNA

fingerprinting studies (Hamer et al., 1989; Shull and Hamer, 1996). MGR586 RFLP fingerprinting showed that many populations around the world contain a limited number of isolate groups, called clonal lineages, which are composed of closely related individuals descended from asexual reproduction. Many of these clonal lineages appear to have a restricted virulence pattern (Levy et al., 1993; Xia et al., 1993; Correa-Victoria et al., 1994; Chen et al., 1995; Don et al., 1999).

Kachroo et al. (1994) reported an inverted-repeat transposable element, *Pot2*, which occurs with approximately 100 copies and is dispersed throughout the genome of all rice and non-rice pathogenic isolates of *P. grisea*. George et al. (1998) used rep-PCR genomic fingerprinting based on the *Pot2* element to examine population structure of *P. grisea*. They generated two outwardly directed primer sequences specific for *P. grisea* isolates from *Pot2*. They found that polymorphisms detected using the *Pot2* rep-PCR method closely corresponded with MGR586 RFLP fingerprints in a collection of *P. grisea* isolates from the Philippines and the Indian Himalayas. Rep-PCR fingerprinting was found to be reliable, reproducible, and highly discriminatory for assessing diversity in large collections of strains, especially when combined with computer-assisted data analysis (Rademaker and de Bruijn, 1997; Louws et al., 1999).

In Iran, rice blast was reported for the first time in Guilan province about 50 years ago (Sharif and Ershad, 1966). At present, blast is the most important disease of rice in two major rice production areas, the Guilan and Mazandaran provinces in northern Iran. Investigations on pathotype variation of *P. grisea* in Iran showed that the population structure was variable in different geographical areas (Fatemi and Rahimian, 1975; Izadyar, 1982; Bahrami and Izadyar, 1998; Javan-Nikkhah and Hedjaroude, 2000), but these studies were not designed to determine the genetic structure of Iranian *P. grisea* populations.

The objective in this study was to use *Pot2* rep-PCR fingerprinting to determine the genetic structure of populations of *P. grisea* existing on commonly grown rice cultivars from the major rice-producing areas in northern Iran. The goal was to determine how genetic diversity was partitioned spatially and according to cultivars to determine if there was an association between particular clonal lineages and cultivars or regions.

Material and methods

Research site

The study region was situated in the two most important rice-growing provinces, Guilan and Mazandaran, in northern Iran near the Caspian Sea (Figure 1). This is the largest rice-producing region in Iran, with over 450,000 ha of rice grown each year, representing approximately 75% of the Iranian rice crop. In these provinces, traditional non-improved indica cultivars of rice that are normally susceptible to leaf and neck blast predominate in an irrigated, transplanted cropping system. Modern, blast-resistant cultivars giving higher yields were introduced only a few years ago and have not yet been widely adopted. Northern Iran has a climate conducive to sporadic blast epidemics in years with high humidity, moderate temperatures, and high rainfall, but the potential number of cycles of pathogen reproduction is limited by the short growing season (3 months).

Collections of isolates

Two hundred and twenty-one monoconidial isolates were collected during 1997–2000 from leaf and panicle neck lesions on 12 rice cultivars. This collection of isolates originated from 10 regions distributed across the two provinces (Figure 1). Samples were collected approximately 7–10 days after symptoms first appeared on leaves and panicle necks. In each year, leaf blast samples were collected between 5 and 21 July and neck-panicle blast was collected between 6 and 20 August. Six to ten infected leaves and panicles were collected randomly in each field and 13–40 fields were sampled in each region (Figure 1). For each leaf or panicle a single conidium was isolated from a single lesion and maintained on desiccated filter paper as described previously (Leung and Taga, 1988). One isolate was analyzed from each field.

An additional 193 isolates were collected on 15 August 2000 from three traditional cultivars (susceptible to leaf and neck blast) growing in four fields at two sites separated by approximately 100 km. Fields were less than 1000 m apart in each site. Cultivar Binam (89 isolates total) was sampled in both sites and cultivars Taroom and Hasansaraii (52 isolates each) were sampled in sites 1 and 2, respectively. All three are traditional

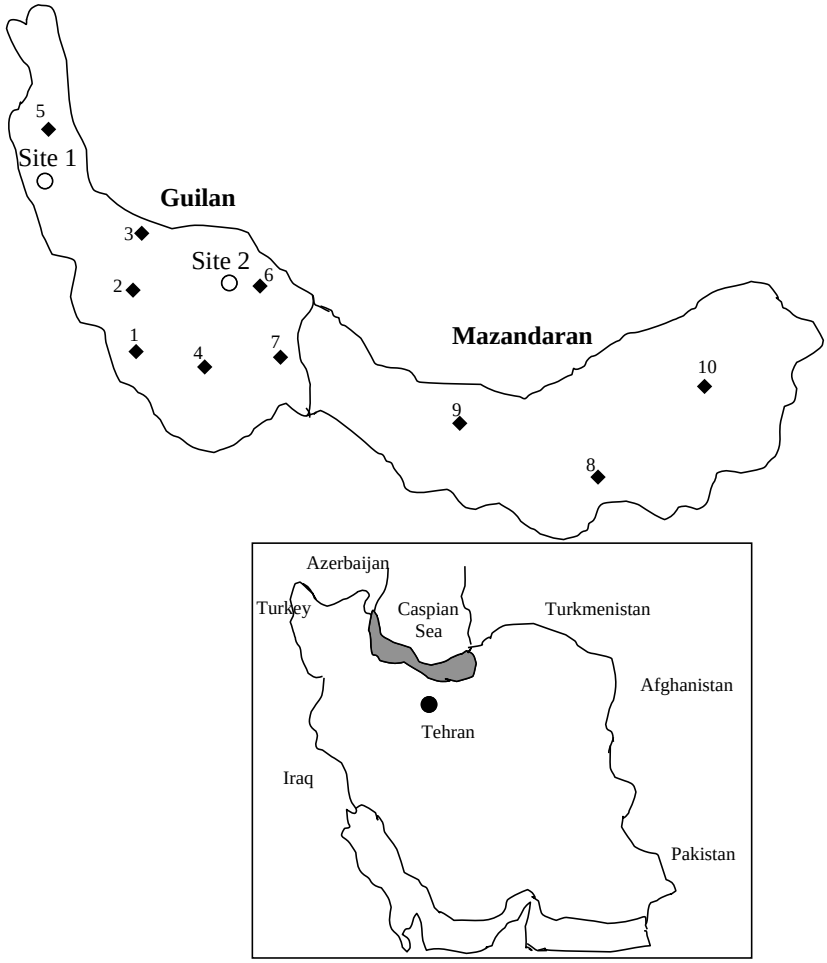

Figure 1. Location of *Pyricularia grisea* collection sites in the Iranian provinces of Gilan and Mazandaran. Names of the regions correspond to the numbers given in Table 1. The shaded area on the map represents the location of Guilan and Mazandaran within Iran. Open circles on the map show sites where two fields were sampled intensively.

cultivars that are highly susceptible to blast. Transect sampling was used in each field. One infected neck panicle was collected every 10 m along eight transects separated by 5 m in each field.

DNA preparation and Pot2 *fingerprinting*

Isolates were grown in Frie's medium (Van Etten and Daly, 1980) at room temperature for 5–7 days with constant shaking on a rotary shaker. Mycelia were harvested, then frozen at −80 °C and lyophilized prior to use. Lyophilized mycelia were ground in liquid nitrogen into a fine powder with a mortar and pestle. Fungal genomic DNA was extracted using DNeasy Plant Mini Kits (QIAGEN) following the manufacturer's instructions or using a phenol/chloroform method (Lee et al., 1988). About 40 mg of powdered mycelium was suspended in 400 µl of extraction buffer (50 mM, Tris–HCl; 50 mM EDTA and 3% SDS) and 4 µl of RNase in a 2 ml microcentrifuge tube. With the phenol/chloroform method, the suspension was incubated at 65°C for 30 min and centrifuged for 15 min at 14,000*g* in a microcentrifuge. The supernatant was extracted with 700 µl of phenol:chloroform:isoamyl alcohol (25:24:1). Nucleic acids were precipitated by adding a one-tenth volume of sodium acetate and 600 µl of cold 95% EtOH, then centrifuged for 5 min at 14,000*g*. The nucleic acid pellet was dissolved in 600 µl of cold

70% EtOH and centrifuged for 5 min at 14,000*g*. The nucleic acid pellet was dried in a vacuum centrifuge and then dissolved in 100 µl of 10 mM Tris, 1 mM EDTA (TE). DNA quality and concentration were determined by electrophoresis on 0.7% agarose gels.

Sequences of the two primers (*Pot2*-1: 5′ CGGAAGCCCTAAAGCTGTTT 3′ and *Pot2*-2: 5′ CCCTCATTCGTCACGTTC 3′) were as described by George et al. (1998). These sequences were based on the ends of the repetitive element *Pot2*, an inverted repeat found in *P. grisea* (Kachroo et al., 1994).

Amplification was performed in 20-µl volumes in a 0.2 ml 96 well PCR-microtiter plate containing 3 µl of genomic DNA (representing a total of 1–10 ng), 2 µM each of four dNTPs, 1 µM each of the two primers and 2.25 units of Taq DNA polymerase (Amersham Pharamacia) in PCR buffer no. 9 (10 mM Tris, pH 9.2; 25 mM KCl; 1.5 mM $MgCl_2$; 15 mM $(NH_4)_2SO_4$). Buffer no. 9 was supplied by Stratagene. PCR amplifications were conducted in a Biometra DNA Thermal Cycler T1. PCR conditions were (1) 2 min denaturation at 96 °C; (2) four cycles of 1 min denaturation at 96 °C, 1 min annealing at 62 °C, and 10 min extension for 10 min at 65 °C; (3) 26 cycles of 30 s denaturation at 96 °C, 1 min annealing at 62 °C, and 10 min extension for 10 min at 65 °C; and a final extension for 15 min at 65 °C. Amplified DNA fragments were separated by electrophoresis in a 1.1% agarose gel in 0.5X TBE (89 mM Tris, pH 7.8, 89 mM boric acid, and 2mM EDTA). To visualize the DNA fingerprints, 10 µl of PCR product was loaded into each well and gels were run for 14 h at 50 V. DNA bands were stained with ethidium bromide, visualized under 280 nm UV light and then photographed with a GelDoc 2000 system (BIO-RAD). PCR analysis was done at least two or three times for each DNA sample to ensure only reproducible bands were scored. Only bands that amplified consistently were scored and used for the analysis.

Analysis of lineage structure

The fingerprints were first sorted visually into groups based on obvious similarities between amplified DNA profiles. Next, these preliminary groups were analyzed together on the same gel to confirm overall fingerprint identity or similarity and to quantify the number of shared amplicons among isolates. Lineages were assigned according to the similarity of *Pot2* amplification profiles. Forty-one isolates with different amplification patterns (haplotypes) were identified among five major fingerprint groups (hereafter called lineages). DNA from groups of isolates representing a particular lineage was electrophoresed together with representatives of other putative lineages to allow scoring of individual amplicon positions. For each resolved amplicon position between 550 bp and 5 kb, presence or absence of each amplicon was scored manually using a binary system (1 for presence and 0 for absence of each amplicon) for each isolate. Similarities between *Pot2* fingerprints among the fungal isolates were calculated using Nei and Li's index (Nei and Li, 1979) as $S_{xy} = 2n_{xy}/(n_x + n_y)$, in which n_{xy} is the number of amplicons shared by a given pair of isolates, n_x and n_y are the number of amplicons in isolates x and y. The binary data were used to calculate genetic distances between all pairs of isolates based on the mean character difference using the distance option in NTSYS (NTSYSpc, Numerical Taxonomy System, Exeter Software). Based on these genetic distances, cluster analysis was conducted and a dendrogram was constructed by using UPGMA (unweighted pair group method with arithmetic average). A cophenetic value was calculated using NTSYS. To evaluate the robustness of the resulting dendrogram, bootstrap analysis was performed using the program PAUP beta 4.0 (Swofford, 2000). The dendrogram was reconstructed 10,000 times by repeated sampling with replacement, and the frequency with which a particular grouping was formed was considered to reflect the robustness (bootstrap-support) of the group. Based on visual assessment, similarity coefficients, and the stability of each group in the dendrogram, a similarity level of ≥70% was used to define lineage groups. χ^2 tests were used to determine the significance of differences in the number of isolates found in different fingerprint groups, regions, or cultivars throughout the sampling area, and between cultivars at the two intensively sampled sites. Genotypic diversity in the fungal populations was measured using the formula $h = 1-\Sigma x_j^2$, where x_j was the lineage frequency within each population (Nei, 1975).

Table 1. Distribution of rep-PCR based fingerprint lineages of *Pyricularia grisea* in northern Iran

Region[a]	Year(s) sampled	No. of cultivars	No. of isolates[b]	No. of haplo-types	Isolates in each clonal lineage[c]				
					A	B	C	D	E
1. Phoman	1997–1999	6	27	11	8	2*	2	1	14
2. Somaesara	1997–1999	9	29	12	12	–	1	1	15
3. Anzali	1997–1999	8	18	9	3	1	–	1	13
4. Rasht	1997–1999	10	40	13	15	1	1	4*	19*
5. Talesh	1998–1999	5	13	4	4	–	–	–	9
6. Lahijan	1997–1999	4	19	4	4	–	–	1	14
7. Roudsar	1997–1999	3	18	8	4	–	–	–	14
8. Amol	2000	5	22	10	7	–	–	–	15
9. Noor	2000	4	14	7	2	–	–	–	12
10. Ghaemshahr	2000	4	15	6	1*	–	3*	–	11

* χ^2 test significant at the 5% level.
[a] Regions in northern Iran where isolates were collected. In each region, the isolates were collected at random from different fields within a 10-km radius.
[b] Each isolate was recovered from a different field in each region. The number of isolates for each region represents the number of sampled fields.
[c] Letters indicate rep-PCR fingerprint lineages.

Table 2. Frequencies of *Pyricularia grisea* rep-PCR lineages on different Iranian rice cultivars

Cultivar[a]	No. of isolates	No. of haplo-types	Predominant haplotypes[b]	Isolates in each clonal lineage (no.)[c]				
				A	B	C	D	E
Alikazemi	9	6	A1, E2, E6	4	–	–	–	5
Binam	62	23	A1, B2, C1, D1, E2, E6	24	1	3	3	31*
Domghermez	3	2	A1, E2	2	–	–	–	1
Domsiah	11	4	A1, E6	5	–	–	–	6
Domzard	6	6	D1, E6	-	–	–	2*	4
Hasani	8	7	E2, E6	2	–	–	–	6
Hasansaraii	5	5	A1, B2, E6	2	1*	–	–	2
Hashemi	16	8	A1, B2, E2, E6	4	1	–	1	10*
Khazar	7	5	E2, E6	1	–	–	–	6
Taroom	59	19	A1, C1, E2, E6	13	–	2	1	43*

* χ^2 test significant at 5% level.
[a] All cultivars except Khazar are traditional cultivars. One isolate was recovered from each of cultivars Neda (an Iranian improved cv.) and Tetep. The host cultivar was unknown for 34 isolates.
[b] Most common haplotypes within each lineage found on each cultivar as shown in Figure 3.
[c] Letters indicate rep-PCR fingerprint lineage.

Results

Lineage structure among isolates from different cultivars across northern Iran

The 221 isolates were divided into five lineages designated A to E (Tables 1 and 2). These lineages were obtained by scoring 5-17 resolvable DNA amplicons ranging from 550 bp to 5 kb in size (Figure 2). DNA similarity among isolates within a putative lineage ranged from 70% to 100%. Cluster analysis was conducted using the 41 haplotypes identified among the five lineages. Bootstrap analysis showed that the clusters defining the five clonal lineages were robust (Figure 3). The low cophenetic value (0.18) was in agreement with the supported bootstrap groups.

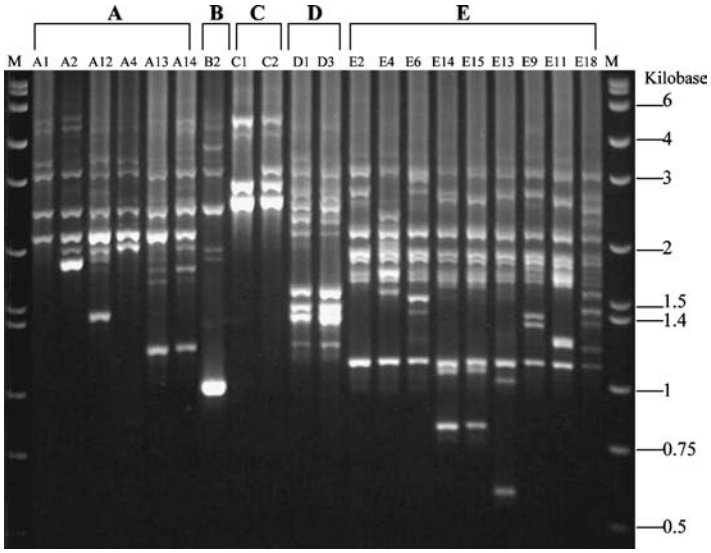

Figure 2. Agarose gel showing representative amplification patterns of five Iranian lineages of *Pyricularia grisea* generated by rep-PCR with *Pot2* primers. Capital letters represent each lineage. Numbers to the right indicate molecular weight in kilobase pairs of size markers in the first and last lanes. Individual haplotypes are indicated above each lane.

The overall frequency of lineages A, B, C, D and E across regions was 0.28, 0.02, 0.03, 0.04 and 0.63, respectively. χ^2 tests for heterogeneity of the fungal population in different regions and cultivars revealed that lineage frequencies were significantly different at $P < 0.05$ among regions and cultivars (Tables 1 and 2).

Lineage E was found in all sampled sites (Table 1) and isolates belonging to this lineage were sampled from all cultivars (Table 2). Most isolates of the second large fingerprint group, lineage A, were found in the western part of the sampled area (regions numbered 1–5 in Table 1). Though all five lineages were recovered from cv. Binam, lineage A occurred at a relatively high frequency on this cultivar, while on cv. Taroom the frequency of lineage E isolates was significantly higher. Lineage D was found at a low frequency and only in Guilan province. Like lineage D, lineage C was present at a low frequency but it was found in both Guilan and Mazandaran. Lineage B was found at a very low frequency in both provinces (Table 1).

Diversity of haplotypes within DNA fingerprint lineages

Some isolates having a large number of amplicons differed by only one weak amplicon. Though the weak amplicon was reproducible under our experimental conditions, we treated isolates with more than 95% similarity in DNA fingerprints as the same haplotype because we were not confident that this weak amplicon would be reproducible under all laboratory conditions. Using this 95% criterion for identity, the number of haplotypes detected was 14, 2, 4, 3 and 18 within lineages A, B, C, D, and E, respectively (Table 3, Figure 3). One haplotype predominated in each lineage with the exception of lineage E, which had two dominant haplotypes (Table 3). Approximately 57% of the isolates belonged to one of the seven most common haplotypes found among the five lineages (Table 3). The majority of the other haplotypes was represented by 1–3 isolates. Three haplotypes, named A1, E2, and E6, were widely distributed across the region. Haplotypes A1 and E2 were found mainly in the western-most region, particularly in Guilan province. Haplotype E6 was evenly distributed across sites in both provinces (Table 4). Haplotype D1 was found only in Guilan province.

Host-specific collections of isolates from two sites

Lineages A, C, D and E were found among the 193 isolates collected from four fields in two sites

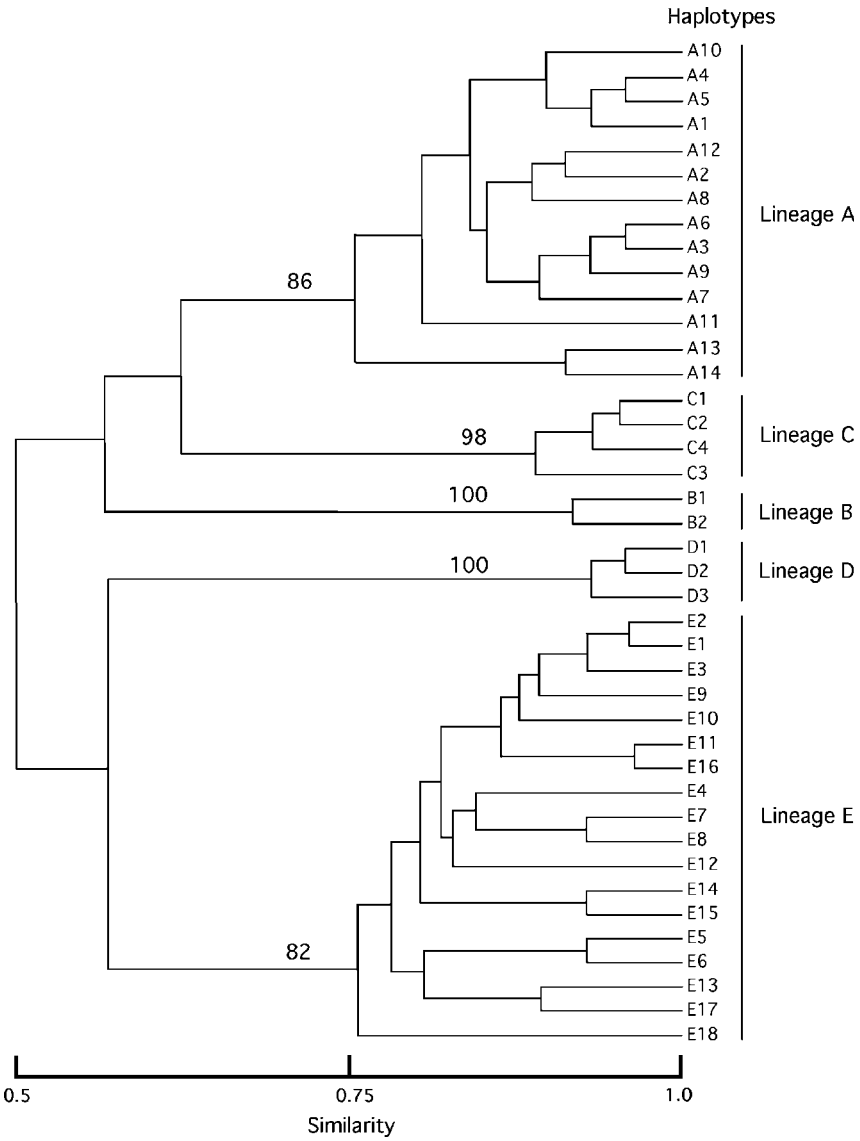

Figure 3. Dendrogram constructed using unweighted pair group method with arithmetic average (UPGMA) based on *Pot2* rep-PCR fingerprint data of *Pyricularia grisea*. The numbers on the main branches represent the bootstrap value based on 10,000 iterations. Each cluster formed at $\geqslant 70\%$ amplicon similarity was designated as a lineage.

(Table 5). The frequencies of the *Pot2* lineages and Nei's measurement of genetic diversity from the two sites are summarized in Table 5. Lineage A predominated on cv. Binam at both sites. χ^2 tests showed that lineage frequencies differed significantly ($P < 0.05$) among cultivars at both sites ($x^2 = 65.80$ at site 1 and 23.05 at site 2). The lineage frequencies were not significantly different for cv. Binam at the two sites.

Discussion

Over the last three decades, blast became the most important pathogen in most rice-producing areas in Iran. Pathotype diversity of *P. grisea* was the focus of two previous investigations in different rice-growing regions of Iran (Izadyar, 1982; Bahrami and Izadyar, 1998). In these earlier investigations using eight international standard

Table 3. Number and frequencies of haplotypes of *Pyricularia grisea* found within each of the five clonal lineages in northern Iran

Haplotypes	Isolates in each clonal lineage (%)[a]				
	A	B	C	D	E
1	39(60)	1 (25)	4 (58)	5 (63)	2 (1.5)
2	4 (6.1)	3 (75)	1 (14)	2 (25)	30 (22)
3	1 (1.5)	–	1 (14)	1 (12.5)	15 (11)
4	4 (6.1)	–	1 (14)	–	9 (6.6)
5	2 (3.1)	–	–	–	4 (3)
6	2 (3.1)	–	–	–	42 (31)
7	1 (1.5)	–	–	–	2 (1.5)
8	4 (6.1)	–	–	–	7 (5)
9	1 (1.5)	–	–	–	7 (5)
10	1 (1.5)	–	–	–	2 (1.5)
11	1 (1.5)	–	–	–	3 (2.2)
12	1 (1.5)	–	–	–	3 (2.2)
13	3 (4.6)	–	–	–	3 (2.2)
14	1 (1.5)	–	–	–	1 (0.7)
15	–	–	–	–	3 (2.2)
16	–	–	–	–	1 (0.7)
17	–	–	–	–	1 (0.7)
18	–	–	–	–	2 (1.5)

[a] Letters indicate rep-PCR fingerprint lineage and numbers indicate haplotypes within each lineage, shown in Figure 3.

Table 4. Distribution of the most common haplotypes within each clonal lineage of *Pyricularia grisea* in different sites of northern Iran

No. region	Haplotype[a]					
	A1	B2	C1	D1	E2	E6
1. Phoman	5	1	1	1	4	4
2. Somaesara	8	–	–	–	3	6
3. Anzali	3	1	–	1	2	5
4. Rasht	11	1	1	2	8	4
5. Talesh	–	–	–	–	1	2
6. Lahijan	2	–	–	–	4	4
7. Rodsar	4	–	–	–	2	4
8. Amol	1	–	–	–	1	6
9. Noor	3	–	–	–	1	1
10. Ghaemshahr	–	–	2	–	1	7

[a] Most common haplotypes found within the five fingerprint lineages shown in Figure 3.

differential cultivars (ISDC) of rice, twelve pathotypes were identified, with pathotype IA-89 comprising ~65% of the tested isolates. The work reported here represents the first attempt to characterize *P. grisea* populations in Iran using molecular genetic markers. *Pot2* rep-PCR fingerprinting differentiated five fingerprint lineages, designated A, B, C, D, and E, among 414 field isolates collected using two sampling strategies. Our results suggest that the population of *P. grisea* in Iran is largely clonal, with two lineages (E and A) dominating in the largest rice growing area. Lineage E was the most widely distributed and contained the largest number of haplotypes, suggesting this lineage is the most broadly adapted and possibly the most ancient lineage in northern Iran.

The frequency and distribution of lineage A, the second most common lineage, differed from lineage E. Most of the lineage A isolates were recovered in the central and western part of Guilan province (Table 1). In this case, it appears that

Table 5. Distribution of different rep-PCR fingerprint lineages of *Pyricularia grisea* on three cultivars at two sites in Guilan province, Iran

Sites[a]	No. of isolates[b]	Frequency of lineages					h[c]
		A	B	C	D	E	
Site 1							
Binam	39	0.72	0.00	0.05	0.03	0.18	0.45
Taroom	52	0.00	0.00	0.00	0.00	1.00	0.00
Average (%)		36	0	2.5	1.5	59	
Site 2							
Binam	50	0.58	0.00	0.00	0.04	0.38	0.52
Hasansaraii	52	0.25	0.00	0.00	0.04	0.71	0.43
Average (%)		41.5	0	0	4	54.5	
Total (%)		36	0	1	3	60	

[a] In Site 1, two cultivars Binam and Taroom were cultivated in two fields separated by approximately 1000 m. In Site 2, Binam and Hasansaraii were cultivated in adjacent fields.
[b] All isolates were recovered from neck blast samples collected 23–28 August 2000.
[c] Nei's measure of genetic diversity for each site based on frequencies of fingerprint lineages.

the distribution of the lineage is correlated mainly with the distribution of the host cultivar Binam, which was the predominant cultivar grown in this region. The correlation between lineage A and Binam was even more evident in the second collection of isolates made from three cultivars at two sites (Table 5). Among the 70 isolates belonging to lineage A in the second collection, ~81% were recovered from Binam. Thus, it appears that lineage A may exhibit host specialization towards Binam.

The other three lineages existed at low frequencies. All isolates in lineage D were recovered from the central part of Guilan. The low frequency of lineage D (8 out of 221 isolates) suggests that it persists in the population at a low frequency or that it has been recently introduced to this region. In spite of the small total number of isolates present in lineage C, it was found on three cultivars in both Guilan and Mazandaran provinces. As with lineage D, it appears that lineage C persists in this region at a low frequency though it is more widely distributed.

Javan-Nikkhah et al. (2003) examined pathotype diversity in 50 isolates selected to represent the most frequent haplotypes among the five *Pot2* lineages (5 haplotypes from lineage A, 1 each from B, C, and D, and 12 from E) using the same ISDC cultivars as Izadyar (1982). Only six pathotypes were found. IC-25 was the most common pathotype (66%) and IA-89 was the second most common (22%). Pathotypes IA-8, IC-26, IC-29 and IF-1 made up the remaining 12%. No correlation was found between *Pot2* lineages and pathotypes. All 50 isolates were able to infect nine of the Iranian cultivars listed in Table 2, but were unable to infect cultivar Khazar. Khazar is resistant to leaf blast but is susceptible to neck blast in Iran.

The collection of isolates from four intensively sampled fields showed that the frequencies of lineages within individual fields were very similar to the frequencies calculated from across the region (Table 5). This suggests that the distribution of genetic diversity within a small number of representative fields may be a fair reflection of the diversity present across the entire sampled region. If this is confirmed with additional collections, future surveys of genetic diversity in this region could be conducted more efficiently by sampling a larger number of isolates from a limited number of fields planted to the major rice cultivars instead of sampling a small number of isolates from a large number of fields distributed across a wide geographical area. The finding also suggests that inoculum is efficiently disseminated across this region and that the sampled region consists of a single genetic neighbourhood. Though all 52 isolates from cv Taroom in site 1 were lineage E, five haplotypes were found, in proportions similar to those found in the entire Iranian sample (i.e., E2 = 23%, E6 = 60%, E4 = 6%, E13 = 8% and E18 = 4%).

MGR586 fingerprint analysis of *P. grisea* populations in other countries in Asia, Europe and the United States indicated that most populations are composed of a small number (typically 4–8) of clonal lineages (Levy et al., 1991; Levy et al., 1993; Chen et al., 1995; Roumen et al., 1997; Xia et al., 2000). Kumar et al. (1999) demonstrated that the Himalayan population of India is more diverse and concluded that the structure of some populations may be affected to some extent by sexual recombination. Despite its geographical proximity and historical connection to India through the Silk Road trade route, the population structure of *P. grisea* in northern Iran is comparable to other countries that have low genotype diversity. Among the five lineages detected, lineages A and E represented ~90% of all isolates. Within the A and E lineages, 32 haplotypes were detected. We cannot determine from these data what processes (e.g., sexual recombination, parasexual recombination, or mutation) created the different rep-PCR fingerprints within each clonal lineage, but *Pot2* transposition events have the potential to create the observed haplotype diversity.

The low level of genotype diversity detected with *Pot2* rep-PCR fingerprinting and the widespread distribution of four of the five lineages across the region can be due to several factors. One possibility is that relatively few genotypes of *P. grisea* have been introduced into northern Iran, and genotype flow from outside this area has not been frequent enough to introduce new genotypes. A short growing season and environmental conditions that favour sporadic blast epidemics make genetic drift more likely, which would maintain a relatively low level of diversity in the fungal population. The widespread distribution of common haplotypes is consistent with significant gene flow regionally. Evolution in these populations is likely characterized by selection among different mutants that occur within the existing clonal lineages followed by movement of selected genotypes regionally. The low level of pathotype diversity (Javan-Nikkhah et al., 2003) and lack of correlation between DNA fingerprint and pathotypes suggest that selection has favoured the same spectrum of virulence in each clonal lineage. There was no evidence for sexual recombination in this population, consistent with the dominant asexual reproduction observed in most rice-growing areas of the world. But it remains possible that further characterization of *P. grisea* isolates in Iran with mating-type or multiallelic, codominant, neutral genetic markers could provide evidence that sexual or parasexual recombination play a role in maintaining haplotype diversity of this fungus in Iran.

The results of this study can form the basis for a breeding program for new improved cultivars that are resistant to all clonal lineages. Though pathotype diversity appears to be limited, it is distributed across five different genetic backgrounds (clonal lineages), which may have an impact on the expression of virulence in the field. We can now choose a set of pathogen strains representing the entire known genetic diversity in the Iranian *P. grisea* population as inoculum while making selections for blast-resistant, improved rice cultivars in Iran.

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