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Genetic characterization of *Pyrenophora graminea* isolates and the reactions of some barley cultivars to leaf stripe disease under greenhouse conditions

Harun BAYRAKTAR[1,*], Kadir AKAN[2]

[1]Department of Plant Protection, Faculty of Agriculture, Ankara University, 06110 Ankara - TURKEY

[2]The Central Research Institute for Field Crops, Ankara - TURKEY

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Abstract: The genetic diversity of *Pyrenophora graminea*, the causal agent of barley leaf stripe disease, was characterized by restriction fragment length polymorphism (RFLP) of the rDNA internal transcribed spacer region and intersimple sequence repeat (ISSR) analysis. A total of 45 *P. graminea* isolates recovered from different geographic origins and barley cultivars or genotypes produced identical RFLP banding patterns with *Hinf*I, *Msp*I, *Hin*6I and *Bsu*RI restriction enzymes. ISSR analysis indicated that there was little genetic variability among the isolates. A dendrogram based on Jaccard's coefficient separated the isolates into 4 clusters with 12% dissimilarity. In the dendrogram, the isolates collected from different geographic regions in Turkey were clustered separately from the Italian isolates. These results indicated that the Turkish isolates of *P. graminea* were genetically homogeneous and may be derived from a single gene pool. In addition, 13 isolates were tested for pathogenic variability on 48 barley cultivars. The cultivars Durusu, Balkan 96 (Igri), Çumra 2001 and Anadolu 98 were found to be resistant to all leaf stripe isolates.

Key words: Barley (*Hordeum* spp.), barley leaf stripe, genetic resistance, ISSR, PCR-RFLP

Pyrenophora graminea izolatlarının genetik karakterizasyonu ve bazı arpa çeşitlerinin çizgili yaprak lekesi hastalığına karşı sera şartlarında reaksiyonları

Özet: Arpa çizgili yaprak lekesi etmeni olan *Pyrenophora graminea* içerisindeki genetik farklılıklar rDNA internal transcribed spacer bölgesindeki restriksiyon parça uzunluk polimorfizmleri (RFLP) ve intersimple sequence repeat (ISSR) yöntemleri kullanılarak tanımlanmıştır. Farklı coğrafik bölge ve arpa çeşit veya genotiplerinden elde edilen 45 *P. graminea* izolatı *Hinf*I, *Msp*I, *Hin*6I ve *Bsu*RI restriksiyon enzimleri ile kesim sonucunda aynı RFLP bant profillerini oluşturmuşlardır. ISSR analizi ise izolatlar arasında ufak genetik farklılıkların bulunduğunu göstermiştir. Jaccard'ın katsayısı ile oluşturulan dendrogramda tüm izolatlar % 12 farklılık ile 4 gruba ayrılmıştır. Türkiye'nin farklı coğrafik bölgelerinden elde edilen izolatlar dendrogramda İtalyan izolatlarından ayrı olarak gruplanmıştır. Bu sonuçlar *P. graminea*'nın Türk izolatlarının genetik olarak homojen olduğunu ve muhtemelen tek bir gen havuzundan ortaya çıktığını göstermiştir. Ayrıca 13 izolat patojenik farklılıkları için 48 arpa çeşidi üzerinde test edilmiş ve Durusu, Balkan 96 (Igri), Çumra 2001 ve Anadolu 98 çeşitlerinin tüm izolatlara karşı dayanıklı olduğu görülmüştür.

Anahtar sözcükler: Arpa (*Hordeum* spp.), arpa çizgili yaprak lekesi, genetik dayanıklılık, ISSR, PCR-RFLP

* E-mail: bayrakta@agri.ankara.edu.tr

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crops in Turkey, with a cultivated area of 2.97 million ha and a production of 7.3 million t year^{-1} (FAOSTAT 2010). Leaf stripe disease in barley is caused by *Pyrenophora graminea* Ito & Kurib. [anamorph: *Drechslera graminea* (Rabenh. ex Schltdl.) Shoemaker]. The fungus is seed-borne and grows internally within the infected plant. The pathogen causes yellow and brown stripes to grow longitudinally along the leaf. Infected plants are stunted and produce sterile spikes (Zad et al. 2002). The disease reduces the yield and quality of barley and causes economically important yield losses in many countries (Porta-Puglia et al. 1986; Arabi et al. 2004). Yield losses from 3.3% to 15% have been reported in Turkey (Damgacı and Aktuna 1983; Aktaş 1984; Mamluk et al. 1997).

The development of resistant cultivars is the most practical and effective means of disease control. The knowledge of genetic and pathogenic variability within fungal isolates is important to improve the efficiency of breeding schemes and control strategies. Thus, much research has been conducted on the morphological, physiological, and pathogenic variability of *P. graminea* (Gatti et al. 1992; Bembelkacem et al. 2000; Arabi et al. 2002).

A number of different molecular approaches have been used for analyzing the genetic variability among plant pathogen populations. The internal transcribed spacer (ITS) region of the nuclear rDNA provides useful inter- and intraspecific polymorphisms in eukaryotic organisms (Cooke et al. 2000; Guillemaut et al. 2003). Allowing DNA amplification between microsatellite regions, the intersimple sequence repeat (ISSR) technique has been extensively used to estimate the genetic diversity in fungal pathogens (Jana et al. 2005; Bayraktar and Dolar 2009). Genetic diversity within populations of *P. graminea* has been characterized by the restriction fragment length polymorphism (RFLP) of ITS and intergenic spacer (IGS) regions on the ribosomal DNA and random amplified polymorphic DNA (RAPD) analysis (Pecchia et al. 1998; Arabi and Jawhar 2007; Bakonyi and Justesen 2007). However, there are no reports on the determination of genetic diversity among *P. graminea* isolates using microsatellite markers.

The objective of this study was to determine the extent of genetic diversity among Turkish isolates of *P. graminea* using polymerase chain reaction (PCR)-RFLP and ISSR techniques for the first time and to record the reactions of commonly grown barley cultivars to this pathogen under greenhouse conditions.

Materials and methods

Fungal material

The fungal isolates used in this study were recovered from infected leaves collected from different barley cultivars or genotypes and different geographic origins (Table 1). Barley leaf tissues showing necrosis and chlorosis were surface-sterilized in a 1% sodium hypochlorite solution for 3 min, rinsed thoroughly with sterile distilled water, air-dried, and placed in petri dishes containing potato dextrose agar (PDA) medium (Merck, Germany). The petri dishes were incubated for 7 days at 22 °C with a 12-h dark/light cycle. A total of 45 *P. graminea* isolates derived from hyphal tips were preserved on filter paper at 4 °C.

DNA extraction

Genomic DNA was extracted using a preparation method modified from that of Weiland et al. (1999). The mycelia of all isolates grown on PDA medium at 22 °C for 7 days were collected by scraping with a sterile spatula, and then they were ground in liquid nitrogen and suspended in 500 µL of extraction buffer (2% cetyltrimethylammonium bromide, 1.4 M sodium chloride, 20 mM EDTA, and 0.1 M Tris-HCl, pH 8.0). The mixture was incubated for 30 min at 65 °C and extracted twice with phenol-chloroform-isoamyl alcohol (24:1:1, v/v/v). After the addition of 25 µL of RNAse-A, the suspension was extracted again with chloroform-isoamyl alcohol. The DNA was precipitated with a 0.5 volume of 7.5 M ammonium acetate and a 1.5 volume of isopropanol. The pellet was rinsed with ethanol, suspended in TE buffer (pH 7.4), and stored at –20 °C.

PCR-RFLP analysis

The ITS region of the genomic rDNA was amplified with the primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC), as described

Table 1. The isolates of *Pyrenophora graminea* used in the study.

Isolates	Geographic origin	Barley cultivar (cv.) or genotypes	Year
1001	Eskişehir	-	2007
1003	Ankara-Haymana	-	2007
1004	Burdur	-	2007
1005	Adana	-	2007
1006	Konya	-	2007
1007	Ankara-Polatlı	-	2007
1008	Konya	-	2007
1009	Ankara-Haymana	-	2007
1010	Afyon	-	2007
1011	Ankara-Haymana	-	2007
1012	Ankara-Yenimahalle	-	2007
1014	Ankara-Yenimahalle	cv. Bülbül 89	2007
1015	Ankara-Yenimahalle	cv. Yerçil-147	2007
1022	Italy	-	2004
1024	Ankara-Yenimahalle	cv. Yerçil-147	2005
1025	Italy	-	2004
1026	Konya	SAB-VD-11	2008
1027	Konya	KAB-VD-20	2008
1040	Ankara-Haymana	MA-VD-1-326	2008
1041	Ankara-Haymana	YA-BVD-1/135	2008
1042	Ankara-Yenimahalle	cv. Sur 93	2008
1043	Ankara-Haymana	cv. Karatay 94	2008
1044	Ankara-Haymana	MA-VD-3/557	2008
1046	Ankara-Haymana	MA-VD-3/525	2008
1047	Ankara-Haymana	PAT-PRO/123	2008
1048	Ankara-Haymana	MA-VD-2/464	2008
1049	Ankara-Haymana	MA-VD-1-325	2008
1050	Ankara-Haymana	YA-BVD-2/250	2008
1051	Ankara-Haymana	YA-BVD-2/258	2008
1052	Ankara-Haymana	YA-BVD-2/215	2008
1053	Ankara-Haymana	cv. Yesevi 93	2008
1054	Ankara-Haymana	PAT-PRO-120	2008
1055	Ankara-Haymana	YA-BVD-2-249	2008
1056	Ankara-Haymana	PAT-PRO/139	2008
1057	Ankara-Haymana	PAT-PRO/173	2008
1058	Ankara-Haymana	PAT-PRO/33	2008
1059	Ankara-Haymana	MA-VD-4-609	2008
1060	Ankara-Haymana	PAT-PRO/113	2008
1061	Ankara-Haymana	MA-VD-2/442	2008
1062	Ankara-Haymana	MA-VD-3/541	2008
1063	Ankara-Haymana	PAT-PRO-155	2008
1064	Ankara-Haymana	PAT-PRO-114	2008
1065	Ankara-Haymana	PAT-PRO-174	2008
1066	Ankara-Haymana	MA-VD-4/517	2008
1067	Ankara-Haymana	MA-VD-4/608	2008
1068	Ankara-Haymana	PAT-PRO-20	2008
1069	Ankara-Haymana	MA-317	2008

by White et al. (1990). Amplification was performed in a total volume of 50 µL containing 10× PCR buffer, 2 mM of $MgCl_2$, 0.5 µM of each primer, 0.2 mM of dNTPs, and 2 U of Taq DNA polymerase (MBI Fermentas, Lithuania). PCR amplification was carried out in a thermal cycler (Whatman-Biometra Model T1, Göttingen, Germany) with an initial denaturation step of 2 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 57 °C, and 2 min at 72 °C, and ending with 1 cycle of 10 min at 72 °C. The PCR products were visualized on 1% agarose gel in 1× TAE buffer by staining with ethidium bromide (Sambrook et al. 1989).

Without additional purification, 8 µL of the amplified products were digested with the enzymes *Hinf*I, *Msp*I, *Hin*6I, and *Bsu*RI according to the manufacturer's instructions (MBI Fermentas). Restriction fragments were separated electrophoretically at 100 V on 2% agarose gels in 1× TAE buffer and visualized under UV light by staining with ethidium bromide (Sambrook et al. 1989). The sizes of the bands were evaluated by comparison with their molecular weight relative to a DNA ladder using the Gene Tools bioimaging system from Syngene software (Cambridge, England).

ISSR analysis

PCR reaction was carried out in 25-µL reaction volumes containing 10× PCR buffer, 2.5 mM of $MgCl_2$, 0.24 µM of primer, 0.2 µM of dNTPs, and 1 U of Taq DNA polymerase (MBI Fermentas). PCR amplification was performed in a thermal cycler with 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature, and 2 min at 72 °C, with a final extension of 10 min at 72 °C (Table 2). The PCR products were visualized on 1.4% agarose gel as described above.

The reaction of some barley cultivars to leaf stripe disease

Thirteen isolates of *P. graminea*, representing different geographic locations and genetic groups, were evaluated for their virulence on 48 barley cultivars under greenhouse conditions (Table 3). The inoculum was prepared according to the sandwich method modified from Mohammad and Mahmood (1974). Isolates were grown on PDA medium at 22 °C for 10 days. Barley seeds were surface-sterilized as described above and placed on half of the fungal colony in each plate, and then the other half of the agar was inverted over the barley seeds under sterile conditions. Inoculated seeds were incubated for 72 h at 22 °C, and for an additional week at 4 °C. Ten

Table 2. The sequence and annealing temperatures of the ISSR primers used and the number and size of the total DNA fragments.

Primer	Annealing temp. (°C)	Total band number	Size (bp) min-max
$DDB(CCA)_5$	64	7	360-1720
$(GA)_8T$	50	3	490-2037
$(AC)_8YA$	56	6	400-1480
$(AG)_8T$	56	5	490-1530
$(GA)_8C$	52	4	755-1450
$(ATG)_6$	50	2	990-1180
$DVD(AAG)_5$	45	6	450-2870
$BDB(ACA)_5$	45	6	630-2880

Y = Pyrimidine; D = G, A, or T; B = C, G, or T; V = A, C, or G

Table 3. The reaction of 48 barley cultivars to 13 *Pyrenophora graminea* isolates under greenhouse conditions.

Cultivars	Isolates of *Pyrenophora graminea*													No. of isolates evaluated as immune or resistant on individual cultivars
	1001	1003	1004	1005	1006	1007	1010	1011	1042	1047	1051	1055	1057	
	Percentage of leaf stripe infection[a]													
Tokak 157/37	0	50	14	0	36	18	0	11	0	0	0	0	0	11
Zafer 160	56	33	30	40	36	0	0	0	29	0	0	0	0	7
Obruk 86	0	33	0	13	19	11	0	0	0	0	0	0	9	12
Anadolu 86	6	77	14	0	13	0	0	20	14	6	10	0	0	12
Bülbül 89	0	70	17	0	13	0	0	0	8	13	0	0	0	12
Tarm-92	29	89	33	33	29	0	0	0	13	10	0	18	0	8
Yesevi 93	0	45	10	33	11	0	0	10	0	0	0	0	0	11
Orza 96	0	30	11	0	11	0	0	0	9	7	0	7	0	12
Çetin 2000	0	17	14	25	78	13	10	33	0	0	0	0	0	10
Aydanhanım	0	55	0	14	0	20	0	25	80	33	0	33	0	8
Avcı-2002	75	20	0	63	33	38	0	100	25	5	0	0	0	7
Zeynel Ağa	20	0	0	0	22	0	0	22	0	13	0	0	0	11
Cumhuriyet 50	9	38	11	0	10	0	0	0	10	0	10	0	17	12
Yerçil-147	0	0	20	10	38	0	0	20	0	0	0	0	0	12
Erginel 90	64	44	0	38	38	10	0	10	0	14	0	0	0	9
Bilgi-91	29	10	0	100	13	0	0	17	13	0	20	0	0	11
Kalaycı-97	18	25	0	11	14	0	0	0	0	8	0	0	0	12
Çıldır 02	25	46	25	0	23	0	0	0	0	8	0	8	0	9
İnce-04	10	92	13	38	25	0	0	38	0	17	20	0	18	9
Karatay 94	0	9	33	29	9	11	0	25	0	6	0	0	15	10
Kıral-97	50	17	8	0	67	0	10	0	0	0	0	0	0	11
Beyşehir	20	22	57	0	33	36	0	25	0	10	0	13	22	7
Konevi	45	100	38	22	38	0	0	30	17	20	0	0	14	7
Larende	88	48	75	14	30	0	0	50	67	29	6	0	44	5
Yeşilköy 387	33	40	20	25	50	50	0	88	38	0	0	0	0	6
Balkan 96 (Igri)	0	20	0	0	0	0	0	13	10	0	0	0	0	13
Sladoran	20	13	0	0	27	0	0	11	25	0	0	0	0	11
Bolayır	0	25	50	31	40	0	0	20	38	14	10	0	0	8
Şahin-91	38	33	70	25	10	0	0	17	10	18	0	25	29	7
Sur-93	33	22	17	11	17	25	0	27	29	25	25	10	11	6
Efes-3	10	15	30	10	17	0	0	0	0	14	0	0	0	12
Anadolu 98	20	0	8	20	13	0	0	9	20	7	0	0	0	13
Efes 98	9	21	29	25	0	27	0	18	50	0	10	25	11	7
Angora	14	38	0	40	0	0	14	20	0	0	0	0	0	11
Çumra 2001	13	0	0	11	0	0	0	0	13	10	10	0	0	13
Çatalhüyük2001	0	31	11	10	8	0	0	0	13	0	0	0	0	12
Başgül	11	69	0	27	11	0	0	7	13	20	8	0	10	11
Atılır	80	33	67	67	57	0	0	57	29	0	60	20	56	4
Fırat	13	22	30	14	73	0	0	0	14	0	17	0	0	10
Meriç	13	60	0	9	0	17	0	71	50	9	0	0	0	10
Yıldız	29	22	0	0	0	0	0	18	0	29	13	0	0	10
Durusu	12	0	15	0	0	0	0	0	0	11	8	0	0	13
Erciyes	67	60	0	63	29	14	0	67	33	20	14	11	33	6
Bornova 92	0	45	0	0	33	0	0	25	0	9	0	0	0	10
Şerifehanım98	29	9	27	18	33	17	0	0	57	0	0	0	24	8
Vamıkhoca98	75	100	50	24	0	0	0	80	40	9	0	25	14	6
Akhisar98	22	50	17	11	9	13	0	0	33	38	0	0	0	9
Quantum	8	33	0	20	27	36	0	0	58	0	0	0	0	9
Mean[b]	22.72[b]	36.14[a]	17.99[b]	19.65[b]	22.72[b]	7.39[de]	0.71[f]	20.48[b]	17.81[bc]	8.98[cd]	5.02[def]	4.05[ef]	6.84[de]	

[a] The evaluations were performed according to the disease scale modified from Tekauz (1983): immune (i): 0% leaf stripe; resistant (r): 1% to 5% leaf stripe; moderately resistant (mr): 6% to 20% leaf stripe; moderately susceptible (ms): 21% to 30% leaf stripe; susceptible (s): 31% to 70% leaf stripe; very susceptible (vs): 71% to 100% leaf stripe.

[b] Means followed by different letters differ significantly at P = 0.05 according to the least significant differences test (LSD: 6.4).

seeds of each cultivar were sown into plastic pots, 10 cm in diameter, and grown at 25 °C for 45 days under greenhouse conditions. For each isolate/cultivar combination, 3 pots were used and each assay was replicated 3 times. Plants were evaluated using a disease scale modified from that of Tekauz (1983; percentage infection = the number of infected plants / total number of plants × 100) and grouped into 6 categories as follows: immune (i): 0% leaf stripe; resistant (r): 1% to 5% leaf stripe; moderately resistant (mr): 6% to 20% leaf stripe; moderately susceptible (ms): 21% to 30% leaf stripe; susceptible (s): 31% to 70% leaf stripe; very susceptible (vs): 71 to 100% leaf stripe.

Data analysis

The mean values of *P. graminea* isolates tested on barley cultivars were compared by the least significant difference test (LSD, P = 0.05) using the MSTAT program package (Michigan State University, East Lansing, MI, USA).

Bands observed in each lane were scored as either 1 (present) or 0 (absent). The similarity coefficient was calculated for each pair of isolates using Jaccard's coefficient, and the resulting similarity matrix was further analyzed with the unweighted pair group method using arithmetic average (UPGMA). All data analysis was performed using the NTSYS-pc numerical taxonomy package, version 2.0 (Rohlf 1998).

Results

PCR-RFLP analysis

PCR amplification with primers ITS1 and ITS4 produced a single DNA fragment of approximately 600 bp from the *P. graminea* isolates. The PCR products were digested with each of the restriction enzymes *Hinf*I, *Hin*6I, *Msp*I, and *Bsu*RI, and all isolates showed a similar RFLP pattern. The restriction patterns of rDNA ITS region with *Hinf*I showed 2 fragments of 450 and 150 bp. After *Hin*6I digestion, all of the isolates showed 3 bands (250, 130, and 40 bp). The digestion of PCR products with *Msp*I gave 2 major bands of 450 and 150 bp. The enzyme *Bsu*RI produced 3 bands (310, 180, and 110 bp) in all of the isolates tested. Representative banding patterns from *Bsu*RI digestion are shown in Figure 1.

ISSR analysis

A total of 8 ISSR markers [DDB$(CCA)_5$, $(GA)_8$T, AC_8(YA), $(AG)_8$T, $(GA)_8$C, $(ATG)_6$, DVD$(AAG)_5$, and BDB$(ACA)_5$] were used to assess the genetic diversity among *P. graminea* isolates. The number of ISSR fragments produced per primer varied from 2 to 7 and ranged in size from 0.36 to 2.88 kb. The most informative primer was DDB$(CCA)_5$, producing 7 polymorphic bands, while primer $(ATG)_6$ was the least informative primer, with 2 fragments. In total, 8 primers produced 39 reliable ISSR bands, 18 of which (46.15%) were polymorphic (Table 2). Figure 2 shows the ISSR profiles of *P. graminea* isolates using the primer AC_8(YA).

The dendrogram based on Jaccard's coefficient confirmed a high level of genetic similarity among the isolates (Figure 3). Cluster analysis of the 45 isolates revealed a very homogeneous genetic structure in *P. graminea*. In the corresponding dendrogram, 4 major clusters of isolates were observed with 12% dissimilarity. Cluster I formed the largest group, with 25 isolates from different provinces, while cluster II included 6 isolates from different locations in Ankara Province. Cluster III consisted of the 12 isolates that were obtained from Ankara-Haymana, as well as

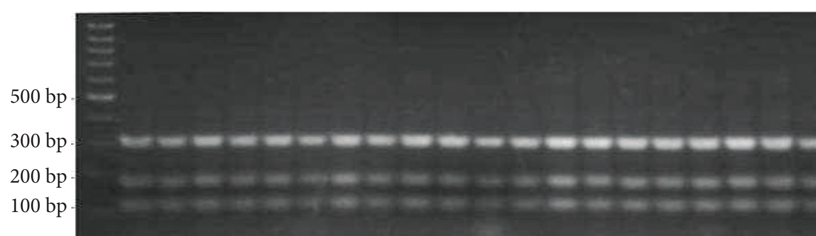

Figure 1. Restriction band patterns showing ITS region digestion of *Pyrenophora graminea* isolates with the enzyme *Bsu*RI. Lane 1: GeneRuler 100 bp DNA Ladder (MBI Fermentas).

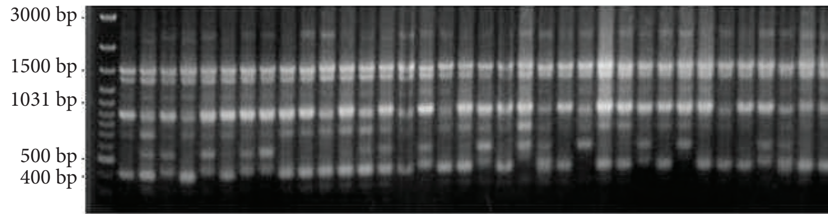

Figure 2. Electrophoretic banding patterns generated from *Pyrenophora graminea* isolates with the primer AC_8(YA). Lane 1: GeneRuler 100 bp DNA Ladder Plus (MBI Fermentas).

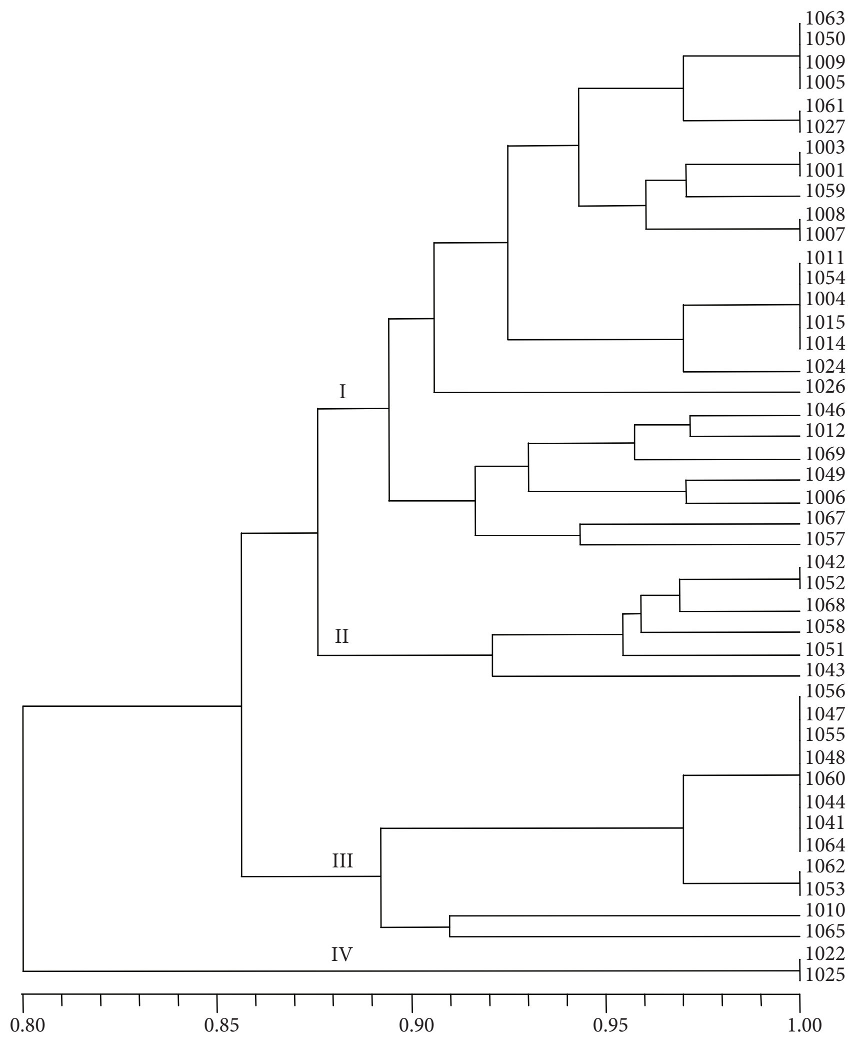

Figure 3. Dendrogram generated by UPGMA of Jaccard's coefficients based on the data amplified from *Pyrenophora graminea* isolates using 8 ISSR primers.

isolate 1010 from Afyon Province. Italian isolates 1022 and 1025 were grouped into cluster IV, separated from the Turkish isolates in the dendrogram by 20% dissimilarity. However, the clusters in the dendrogram did not show any direct relationship with geographical distribution or the pathogenicity of the Turkish isolates.

The reaction of barley cultivars to *P. graminea* isolates

Significant differences were observed in the reactions of the 48 barley cultivars to the 13 isolates of *P. graminea* (Table 3). The cultivars Durusu, Balkan 96 (Igri), Çumra 2001, and Anadolu 98 were found to be resistant to all leaf stripe isolates with a disease reaction of <20%. Out of all of the tested barley cultivars, 9 were resistant to 12 isolates of the pathogens while 8 and 6 cultivars showed resistant reactions to 11 and 10 isolates, respectively. However, the cultivar Atılır was the most susceptible cultivar and was only found to be resistant to 4 isolates; this was followed by the cultivar Larende, which was resistant to 5 isolates. The other cultivars showed resistant reactions to different numbers of isolates. Statistically significant levels of pathogenic variability were observed among the isolates. Detected leaf stripe infections ranged from 0.71% to 36.14%. Isolate 1003 was the most virulent, while isolate 1010 was found to be the least virulent.

Discussion

The genetic characterization of plant pathogens is important for the development of appropriate strategies in disease management programs. The present study is the first to attempt the genetic characterization of Turkish isolates of *P. graminea*. Both PCR-RFLP and ISSR markers revealed high levels of genetic similarity among *P. graminea* isolates from different geographical origins and different barley cultivars or genotypes. No polymorphisms were observed within the ITS region of *P. graminea* isolates digested with the 4 restriction enzymes tested. Using sequence variation in the ITS region of nrDNA, Stevens et al. (1998) detected small variations within the ITS1 and ITS2 regions of *P. graminea* isolates obtained from different countries that are consistent with our own results. They also reported that the one-base difference in the ITS1 region separated *P. graminea* isolates from *P. teres* and *P. hordei*, and that the ITS2 region was substantially identical among the *Pyrenophora* species pathogenic to barley. The low degree of genetic diversity within the ITS region was also observed in plant pathogens such as *Macrophomina phaseoli*, *Pyrenochaeta lycopersici*, and *Ascochyta rabiei* (Almeida et al. 2003; Infantino et al. 2003; Bayraktar et al. 2007). However, using ITS-RFLP, Arabi and Jawhar (2007) detected high levels of intraspecific variation within the *P. graminea* population in Syria and classified 56 isolates of *P. graminea* into 2 distinct clades. No relationship was observed between the ITS-RFLP patterns and geographic origins of Syrian isolates. Zein et al. (2010) classified Syrian isolates of *P. graminea* into 5 groups with a similarity index of 0.46 in a dendrogram based on ITS-RFLP analysis.

ISSR analysis is a PCR-based method that allows for the amplification of the DNA segments between inversely oriented microsatellite repeat regions and combines most of the advantages of RAPD and microsatellite analyses (Ratnaparkhe et al. 1998; Peever et al. 2002; Reddy et al. 2002). ISSR markers have been successfully used to estimate the extent of genetic diversity at inter- and intraspecific levels in a wide range of fungi (Rodrigues et al. 2004; Chadha and Gopalakrishna 2007; Gurjar et al. 2009). In this study, the ISSR technique was used for the first time to assess genetic diversity within this pathogen. ISSR analysis confirmed the high level of genetic similarity among the isolates revealed by PCR-RFLP analysis. The use of microsatellite markers enabled us to divide the *P. graminea* isolates into different groups at the molecular level. Turkish and Italian isolates of *P. graminea* were classified into genetically distinct groups in the dendrogram. However, cluster analysis did not reveal any spatial clustering of the Turkish isolates collected from different geographic origins. Isolates from the same area fell into different genotype groups, while a low level of genetic diversity was observed between the isolates of *P. graminea* from different barley-growing areas. For example, isolates 1005 and 1063, from Ankara and Adana provinces, were classified into the same genotype. Thus, these isolates may be derived from the same genetic population of *P. graminea* and could have expanded from one area to another with the transportation of infected seeds, spore dispersal, and human activities.

The genetic homogeneity may also be associated with the geographic isolation of *P. graminea* populations in Turkey and the degree of sexual recombination under field conditions. Bakonyi and Justesen (2007) assessed genetic relationships within *P. graminea* collected from different countries using RAPD analysis and detected genetic similarity ranging from 86.8% to 97.6% among the isolates. A higher level of genetic similarity was observed between *P. graminea* and *P. teres* f. *maculata* than *P. teres* f. *teres*. Similarly, a low level of interspecific variation was detected within the other *Pyrenophora* and fungi species using different molecular markers (Infantino et al. 2003; Rau et al. 2003; Balmas et al. 2005).

The pathogenicity assays revealed a high level of variability in the reactions of seedling barley cultivars to *P. graminea* isolates. Of the barley cultivars tested, 4 cultivars (Durusu, Balkan 96 (Igri), Çumra 2001, and Anadolu 98) showed resistant reactions to all of the leaf stripe isolates. A further 9 cultivars were found to be resistant to 12 of the isolates. Similar variations in the seedling reaction of barley cultivars were observed in previous studies. Determining the reactions of some barley cultivars, Çetin et al. (1995) observed leaf stripe infection ranging from 15.4% to 96.3%. The cultivars Obruk, Anadolu, and Yesevi were found to be resistant, with a disease reaction of <20%. In this study, the cultivar Anadolu 98 was resistant to all isolates, Obruk 86 to 12 isolates, and Yesevi 93 to 11 isolates. Konak and Scharen (1994) classified the cultivars Tokak and Erbet into the resistant group, cultivar Cumhuriyet 50 into the moderately resistant group, cultivar Zafer into the moderately susceptible group, and cultivar Yerçil into the susceptible group with regards to isolate Mt 6. Similarly, our results showed cultivars Tokak 157/37, Cumhuriyet 50, and Zafer 160 to be resistant to 11, 12, and 7 isolates, respectively. The cultivar Yerçil 147 was found to be resistant to 12 isolates. Determining the reaction of 53 barley genotypes to 2 isolates, Tunalı (1995) grouped 3 entries as immune and 2 entries as resistant, with a leaf stripe occurrence of <5%. The cultivar Yerçil showed a disease reaction of 0% and 11.7% to the 2 isolates tested. Ulus and Karakaya (2007) determined cultivars Çumra 2001 and Yerçil 147 to be resistant to all isolates of *P. graminea*, a finding that is also consistent with our results. However, the cultivars Erginel 90, Orza 96, Çetin 2000, and Aydanhanım showed susceptible reactions to 3 isolates of the fungus. Our results showed these cultivars to be resistant to 9, 12, 10, and 8 isolates, respectively. These results indicated that the resistance reaction among barley cultivars is highly variable depending on the *P. graminea* isolates tested. Significant pathogenic differences were also detected among *P. graminea* isolates in this study. Variation in the aggressiveness of *P. graminea* isolates was reported by several authors (Zriba and Harabi 1995; Bembelkacem et al. 2000). However, no relationship was observed between the clustering in the dendrogram and the pathogenic variability of the 13 *P. graminea* isolates tested on different barley cultivars. The high- and low-pathogenic isolates (i.e., isolates 1003 and 1007) were included in the same cluster in the dendrogram.

In conclusion, the present study provides insight into the genetic variations among the Turkish isolates of *P. graminea* for the first time. These results indicate that the populations of *P. graminea* in Turkey are very closely related and probably emerged from the same gene pool. Further investigations using molecular techniques would be useful in order to obtain more insights into the population structure of *P. graminea* in Turkey. Furthermore, the use of the barley genotypes discussed in this study may be useful in decreasing the negative effects that result from chemical treatments and in developing resistant cultivars as candidate genitor plants.

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