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Genetic Diversity of Iranian Isolates of Barley Scald Pathogen (*Rhynchosporium secalis*) Making Use of Molecular Markers

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ABSTRACT

Leaf blotch disease of barley, caused by *Rhynchosporium secalis* is a major disease of barley in Iran. Its worldwide occurrence and economic importance in barley production has motivated studies on the population genetic structure of this pathogen. Random Amplified Polymorphic DNA (RAPD) method was utilized to investigate the genetic diversity of populations of *R. secalis* isolated from barley leaves. A total of 94 isolates, representing five geographically distinct populations, were collected from diverse climatic regions in Iran. Genetic diversity was studied using eleven RAPD primers. Out of a total of 119 fragments generated by random decamer primers, 89 (75.1%) were polymorphic with an average of 8.1 polymorphic fragments per primer. Cluster analysis of RAPD data using UPGMA and simple matching coefficient method distinguished 33 main groups at 75% similarity level. The similarity between isolates ranged from 0.62 to 0.89. In total, 42 molecular phenotypes (haplotype) were distinguished among the 94 isolates by 11 RAPD primers. Haplotype one was found in all the five regions of Iran surveyed. Some haplotypes were specific to a single region while others found in several regions. There was little correlation observed between genetic *vs.* geographical distance suggesting that they were independent of each other.

Keywords: Barley, Genetic diversity, *Rhynchosporium secalis,* Scald.

INTRODUCTION

The fungus *Rhynchosporium secalis* (Oudem) J. J. Davis causes scald of barley, rye, triticale, and of various other members of the family *Poaceae* (Welty and Metzger, 1996). *R. secalis* can be a significant pathogen where barley is grown (Tekauz, 1991) and under its favorable conditions for growth can cause 10 to 40% yield reductions (Khan, 1986). The fungus is a serious pathogen in Australia, East Africa, Europe, Middle East and South Africa (Zaffarano *et al.,* 2006). The disease is currently a major constraint to barley production in Iran as a result of intensive crop cultivation and favorable environmental conditions that are conducive to its development. In Iran, yield losses of up to 35% are common on barley landraces and could reach 65% in epidemic years. It is thought that the primary inoculum of *R. secalis* consists of conidia dispersed by rain splash or mycelia found in infected seeds (Stedman, 1980). The teleomorph of *R. secalis* has not yet been identified.

The economic importance and worldwide occurrence of barley production motivated several studies on genetic diversity and population genetic structure of this pathogen. Such molecular markers as isozymes (Burdon *et al.*, 1994), Restriction Fragment Length Polymorphisms (RFLPs) (McDonald *et al.,* 1999) and random amplified polymorphic DNAs (RAPDs) (Goodwin *et al.,* 2002) have been utilized to assess the genetic variability of the fungus.

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RAPD markers have been widely made use of in assessing the genetic diversity, genome mapping and molecular diagnostics of many fungal species (Zaffarano *et al.,* 2006). The technique is simple and does not require any prior knowledge of discriminating markers (Williams *et al.,* 1990).

It has been reported that local *R. secalis* populations are extremely diverse for molecular markers. Zaffarano *et al.* (2006) found about 40% of the worldwide RFLP variation in *R. secalis* was found in a 1 m² sampling area and about 60% of the variation was found in a single barley field. Arabi *et al.* (2008) reported high genetic variation among *R. secalis*, collected from barley fields in Syria, through RAPD markers.

It seems that the genetic variation in *R. secalis* populations is generally high (Burdon *et al*., 1994). The mechanisms of genetic variability in *R. secalis* are still not clear, but hypotheses have included mutation, asexual recombination, migration and cryptic sexual stage (Goodwin *et al.,* 1994; Newman and Owen, 1985; Newton, 1989). Knowledge of the population biology of *R. secalis* would be useful to more effective use of resistance genes in plant-breeding programs, (Zhan *et al.,* 1998) and provides useful information on the epidemiology and evolutionary history of the pathogen (Linde *et al.,* 2009). To the best of our knowledge, however, there has been no report on the genetic variability and organization of *R. secalis* in Iran.

The objective followed in this study was to estimate the extent and origin of the potential genetic diversity of *R. secalis* populations in Iran.

MATERIALS AND METHODS

Field Sampling Method

Barley leaves infected with *R. secalis* were sampled from five provinces of Iran namely: Ilam, Kermanshah, Lorestan, Khuzestan, and Golestan during the years 2006-2008 (Table 1). Leaf samples were collected from naturally infected fields near the end of the growing season (February, March and April). The isolates were collected from different locations and from each location of 5,000 ha, one sample was selected. The geographic distances among these isolates varied from 5 to 1,700 km. Populations were defined as the pooled samples of isolates from different locations within a region.

Isolation of the Fungus

In total, 94 samples of *R. secalis*, used in this study, were isolated from infected leaves. Leaves were placed in paper envelopes and allowed to air-dry at room temperature for at least 72 hours. Dried leaves were soaked for 10 sec in 70% ethanol, surface sterilized for 60 seconds in a 0.5% sodium hypochlorite solution, and rinsed in sterile distilled water for 1 minute. Leaves were dried up in between paper towels and placed on the plastic screen that rested on rubber bands above damp filter paper in a Petri dish. Petri dishes were incubated in the dark at 15°C for 48 hours to induce fungal growth and sporulation. Purification of isolates was conducted using single spore method. Mycelial turfs were transferred to Perti dishes containing Potato Dextrose Agar (PDA, Merck). Following incubation at 17°C for 10- 14 days, colonies were spread across fresh PDA plates and incubated for an additional 21 days at 17°C (McDonald *et al.,* 1999).

Spores and mycelial fragments were harvested by adding 2 ml of sterile distilled water and scarping gently across the mycelium with a sterile glass microscope slide. The resulting slurry of spores and mycelia was used to inoculate 50-ml flasks containing potato dextrose broth amended with Kanamycin at 50 μg ml^{-1}. Inoculated flasks were incubated on a shaker at 17°C for 25 days (Salamati *et al.,* 2000).

DNA Extraction

Mycelium was harvested by filtration through filter paper in a Buchner funnel,

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Table 1. Isolate number, origin and date of sampling of *Rhynchosporium secalis* collected from five provinces of Iran during 2006-2008.

Isolate number	Province	Specific location	Date of sampling	Isolate number	Province	Specific location	Date of sampling
Rs4	KH[a]	Dezful	2007	Rs78	Kh	Izee	2007
Rs5	G[b]	Gonbad kavoos	2007	Rs79	Kh	Lali	2007
Rs6	Kh	Shoosh	2007	Rs84	Kh	Bibigole	2007
Rs7	Kh	Shoosh	2007	Rs85	L	Romeshkan	2008
Rs9	G	Gonbad kavoos	2007	Rs86	L	Masoor	2008
Rs10	Kh	Shooshtar	2007	Rs90	L	Koohdasht	2008
Rs11	Kh	Bagh malek	2008	Rs91	L	Koohdasht	2008
Rs13	Kh	Lali	2008	Rs93	I	Ivan	2008
Rs14	G	Ashoora deh	2007	Rs95	G	Gorgan	2007
Rs15	G	Bandare-torkaman	2007	Rs96	G	Gonbad kavoos	2007
Rs16	G	Bandare-torkaman	2007	Rs100	L	Nargese	2008
Rs17	G	Gorgan	2007	Rs101	K	Gahvareh	2008
Rs19	Kh	Dezful	2007	Rs103	K	Mahidasht	2008
Rs20	L[c]	Koohdasht	2008	Rs104	K	Kozaran	2008
Rs22	L	Koohdasht	2008	Rs105	K	Siah gel	2008
Rs24	L	Koohdasht	2008	Rs107	K	Kerned	2008
Rs25	I	Saleh abad	2007	Rs111	K	Sohrabi	2008
Rs26	L	Pole dokhtar	2008	Rs112	K	Eslam abad	2008
Rs27	L	Veisian	2008	Rs113	K	Homail	2008
Rs28	I	Ivan	2008	Rs115	K	Homail	2008
Rs29	I	Ilam	2008	Rs116	K	Darbabdam	2008
Rs31	K[d]	Gilan gharb	2008	Rs117	K	Mersad	2008
Rs32	I[e]	Sarableh	2006	Rs118	K	Ravansar	2008
Rs34	I	Shabab	2006	Rs121	K	Ravansar	2008
Rs35	K	Eslam abad	2008	Rs122	K	Mahidasht	2008
Rs38	I	Mehran	2007	Rs123	K	Kerned	2008
Rs39	K	Sarpole zahab	2008	Rs128	K	Siah gel	2008
Rs40	I	Chavar	2006	Rs129	G	Gonbad kavoos	2008
Rs41	G	Kordkooi	2007	Rs131	I	Lomar	2007
Rs42	I	Abdanan	2006	Rs137	K	Gilan gharb	2008
Rs45	G	Agh ghola	2007	Rs138	K	Gilan gharb	2008
Rs46	I	Darehshahr	2006	Rs139	K	Ravansar	2008
Rs47	I	Darehshahr	2006	Rs140	K	Kermanshah	2008
Rs48	L	Mamolan	2007	Rs142	K	Kermanshah	2008
Rs49	L	Khorram abad	2007	Rs145	K	Kozaran	2008
Rs50	L	Khorram abad	2007	Rs147	K	Kozaran	2008
Rs53	L	Koohdasht	2008	Rs149	I	Darehshahr	2006
Rs55	L	Koohdasht	2008	Rs150	I	Malekshahi	2007
Rs57	L	Koohdasht	2008	Rs151	K	Homail	2008
Rs58	Kh	Andimeshk	2007	Rs152	K	Darebadam	2008
Rs61	Kh	Shooshtar	2007	Rs156	K	Eslam abad	2008
Rs65	L	Azad bakht	2008	Rs158	L	Koohdasht	2008
Rs68	Kh	Shoosh	2007	Rs159	L	Koohdasht	2008
Rs69	Kh	Dezful	2007	Rs160	L	Visian	2008
Rs72	Kh	Izee	2007	Rs161	L	Mamolan	2008
Rs73	I	Sarableh	2007	Rs162	L	Navehkesh	2008
Rs74	I	Abdanan	2006	Rs163	L	Pole dokhtar	2008

[a] Khuzestan; [b] Golestan; [c] Lorestan; [d] Kermanshah, [e] Ilam.

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frozen in liquid nitrogen and ground into a fine powder using mortar and pestle. DNA from each fungal isolate was extracted through modified Hexadecyl-Trimethylammonium Bromide (CTAB) extraction procedure (von Korf *et al.,* 2004). Extracted DNA was resuspended in TE buffer (1 mM EDTA; 10 mM Tris-Hcl, pH 8.0), transferred to 1.5 ml microfuge tubes and stored at -20°C. The DNA was quantified using spectrophotometric analysis and diluted to a final concentration of 7 ng μl^{-1} and used for Polymerase Chain Reactions (PCR) (Bouajila *et al.,* 2007).

RAPD-PCR Assay

RAPD amplification was conducted using 41 decamer primers obtained from Operon Technologies, Inc. Almeda, CA, USA. Amplification was performed in a 25 µl reaction volume containing, 1x PCR Buffer, 0.3 mM dNTPs (Fermentas), 1 U *Taq* polymerase (Fermentas), 0.35 mM MgCl2, 0.4 µM primer and 7 ng of genomic DNA per µl of reaction mixture. Amplification was performed using Eppendorf Master cycler (Eppendorf Netheler-Hinz GMBH, Hamburg, Germany), programmed for initial denaturation at 96°C for 2 minutes and 40 cycles of 94°C for 1 minute, 34°C for 1 minute and 72°C for 2 minutes. Amplification was completed withthin a 7 minutes final extension at 72°C. Amplified products were electrophoresed in 1.5% agarose gel at 80 V, using 1x TBE buffer. The gels were stained with ethidium bromide (0.5 µg ml^{-1}) and photographed under UV light.

Statistical Analysis

The DNA banding patterns were scored as binary digit code of 0 and 1-character states for either the absence or presence of band, respectively, each of which was treated as an independent character regardless of its intensity. Only clear and reproducible bands were scored. All the amplifications were repeated twice while only reproducible bands were considered for analysis. Similarity of the isolates was estimated using UPGMA program of NTSYS-pc (version 2.02) software. The simple matching coefficient was employed for cluster analysis and for construction of similarity dendrogram (Rohlf, 1990).

The haplotypes were identified as based on the method suggested by kolmer (Kolmer *et al.,* 1995).

Pairwise genetic distance among populations was measured using Nei's (Nei, 1972) genetic distance using the MVSP 3.131 (Kovach computing services Wales, UK) program.

Analysis of Genetic Diversity and Structure of Population

Allelic diversities were estimated using Nei diversity parameters. Gene diversity in the total population (H_T) was divided into the gene diversities within (H_S) and between (D_{ST}) subpopulations, $H_T = H_S+D_{ST}$ (Nei, 1978).

$H_T= 1- \Sigma X^2_i +H_{S/}n_s$

$H_S= n(1- \Sigma X^2_i)/n-1$

Where, x_i is the frequency of the *i*th allele at a locus (the frequency of the marker) while *n* the harmonic mean of the sample size (n_i) from each of *S* subpopulations. Both polymorphic and monomorphic markers were included, as recommended by Nei (1978).

Genetic differentiation relative to the total population was calculated through the coefficient of gene differentiation (Nei, 1978): $G_{ST}=D_{ST}+H_T.G_{ST}$ can take values between 0.0 (no differentiation between subpopulations) and 1.0 (complete differentiation between subpopulations).

Gene flow (N_m) between populations was estimated by use of the following formula (Boeger *et al*., 1993).

Nm= 0.5[(1/GST)-1]

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Where, N is the effective population size and m the fraction of immigrant individuals in a population.

If $Nm < 1$, then local populations tend to differentiate; if $Nm \geq 1$, then there will be little differentiation among populations and migration is more pronounced than the genetic drift (Wright, 1951).

RESULTS AND DISCUSSION

Some eleven of 41 primers utilized in this study revealed DNA polymorphism among the 94 isolates of *R. secalis* tested, while the other primers either did not yield consistent results, or produced non-distinguishable bands under the conditions employed. The number of amplified products ranged form seven (OPL1) to fourteen (OP508) per each primer. The average number of amplified products obtained per primer was 10.8. The sizes of the amplified products ranged from 0.2 kb to 3.4 kb (Figure 1). Overall, the RAPD patterns revealed a high level of polymorphism. Out of a total of 119 fragments generated by random decamer primers, 89 (75.1%) were polymorphic with an average of 8.1 polymorphic products per primer (Table 2). In total, 33 genotypic groups were detected among the 94 isolates using UPGMA method as well as simple matching coefficient (Figure 2).

The similarity between isolates ranged from 0.62 to 0.89, indicating that no two isolates were 100% similar. Analysis with Dice and Jaccard's coefficients indicated the same results.

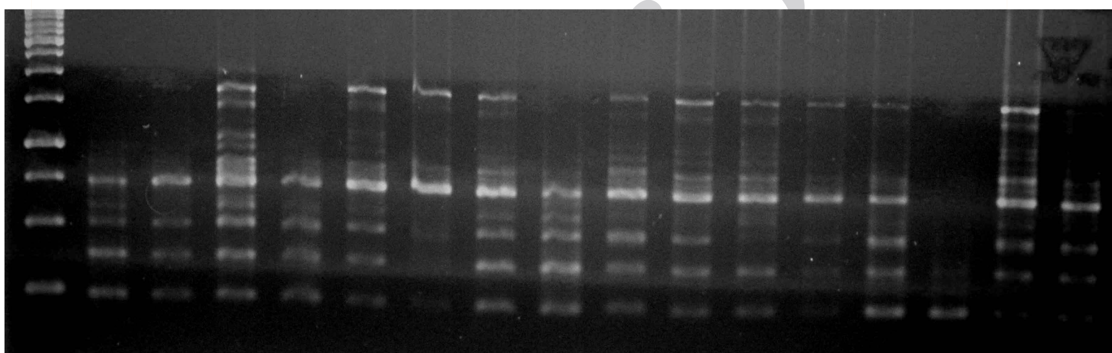

Figure 1. RAPD profiles of 16 isolates of *R. secalis* using OPA2 primer (left to right ladder 1kb standard, isolates Rs4,9,14,20,24,34,50,55,64,72,90,95,104,131,144 153.

Table 2. Sequence of primers, number of amplified fragments and percentage of polymorphic bands among 94 isolates of *Rhynchosporium secalis* in Iran.

Primer	Primer sequence(5'→3')	Total no. of bands	NPB[a]	Percentage of polymorphism PP%
Op515	GGG GGC CTC A	11	9	81.8
Op542	CCC ATG GCC C	12	11	91.6
Op519	ACC GGA CAC T	12	8	66.7
Opa2	TGC CGA GCT G	11	11	100
Op508	CGG GGC GGA A	14	12	85.7
Op540	CGG ACC GCG T	9	4	44.4
Op548	GTA CAT GGG C	9	8	88.9
Opl2	TGG GCG TCA A	10	9	90
Opl1	GGC ATG ACC T	7	4	67.1
Op516	AGC GCC GAC G	13	6	46.2
Opl5	ACG CAG GCA C	11	7	63.6
	Total	119	89	75.1%

[a] Number of polymorphic bands.

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In total 42 molecular phenotypes were distinguished among the 94 isolates by 11 primers using the method of Kolmer in the RAPD analysis (Kolmer *et al.*, 1995) (Table 3).

The haplotype one (11111111111), stood at a high frequency in all the regions.

The highest haplotype frequency was observed for Ilam Province (0.75) while the lowest found for Kermanshah Province (0.52) (Tables 3 and 4).

The analysis of population structure

Tabel 3. Molecular phenotypes (haplotypes) of *Rhynchosporium secalis* isolates collected from five provinces based on 11 random RAPD primers.

Haplotype group	Molecular phenotype	Numbers within population	Number in location
HP1	11111111111	32	K[a](12), L[b](7), KH[c](6), G[d](4), I[e](3[f])
HP2	11111112111	3	G(1), KH(2)
HP3	11111411111	1	KH(1)
HP4	21116111111	1	KH(1)
HP5	11111311211	1	KH(1)
HP6	11111116531	1	G(1)
HP 7	11113111111	3	G(1), K(1), L(1)
HP8	14111111111	4	L(2), K(2)
HP9	11111131311	2	L(1),I(1)
HP 10	11112311211	1	L(1)
HP11	21111112111	4	I(1), L(2), KH(1)
HP12	11111111131	2	KH(1), L(1)
HP 13	14111312132	1	I(1)
HP14	11111318218	1	KH(1)
HP15	11111312121	1	I(1)
HP 16	14112715111	1	G(1)
HP17	11111112112	2	I(1), L(1)
HP18	14111114331	1	I(1)
HP 19	11111111121	1	L(1)
HP20	11131111121	1	L(1)
HP21	11111312212	1	I(1)
HP 22	14111311182	1	L(1)
HP23	11111171232	1	K(1)
HP24	11111117131	1	I(1)
HP 25	12111112111	3	K(3)
HP26	12311111111	2	K(1), KH(1)
HP27	11111111212	3	K(2), L(1)
HP28	14111211111	2	G(1), K(1)
HP 29	11711111231	1	L(1)
HP30	21311112111	1	G(1)
HP31	11111511212	1	K(1)
HP 32	11211112111	2	K(1), L(1)
HP33	11111316211	1	K(1)
HP34	11112112111	2	K(1), L(1)
HP 35	11311111131	1	K(1)
HP36	11111212131	1	K(1)
HP37	11111211671	1	I(1)
HP 38	11114371132	1	K(1)
HP39	21221212111	1	I(1)
HP40	11111314132	1	L(1)
HP 41	14111111661	1	L(1)
HP42	61111112111	1	L(1)
Total	-	94	94

[a] Kermanshah; [b] Lorestan; [c] Khuzestan; [d] Golestan; [e] Ilam, [f] Number in location.

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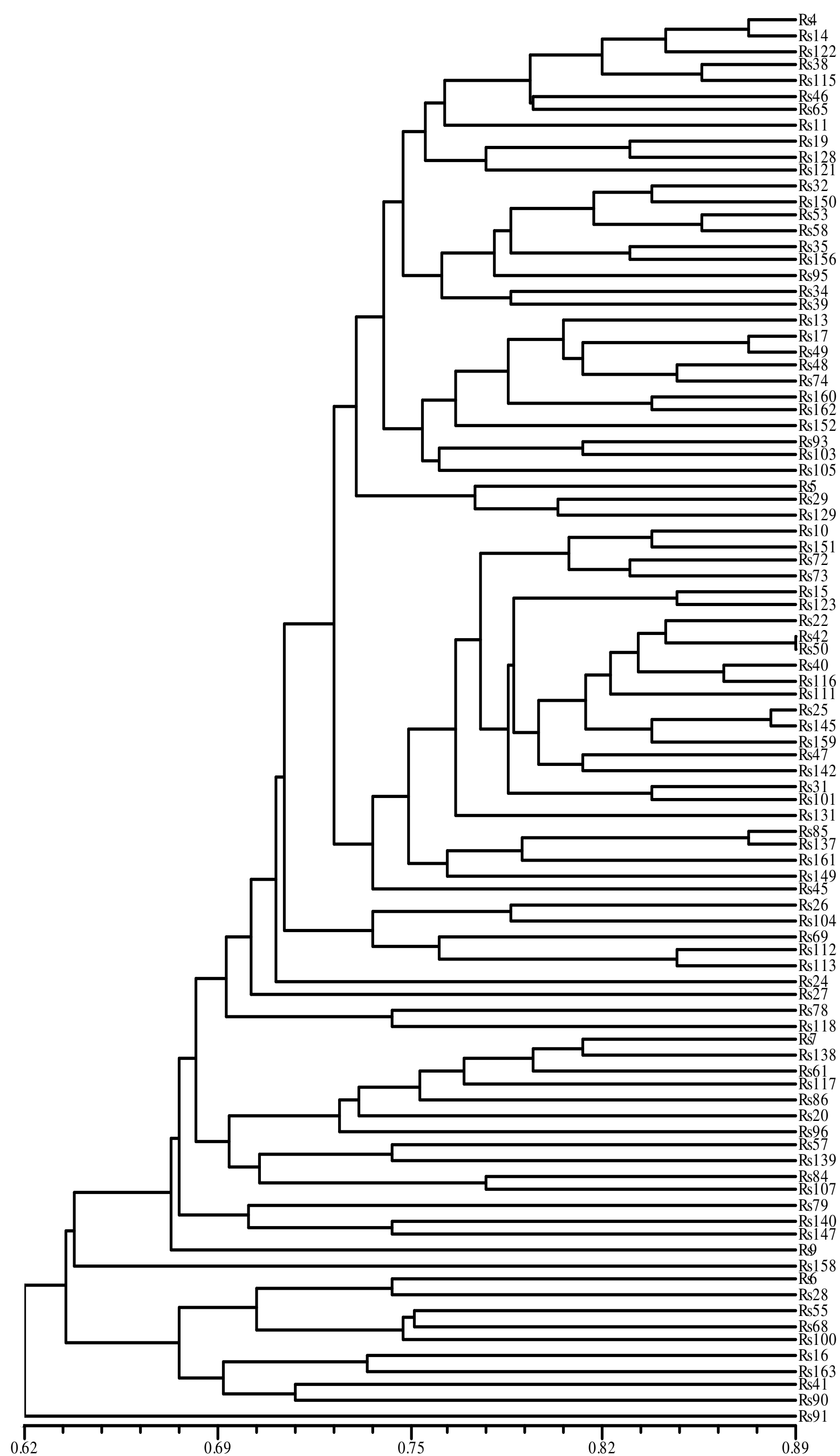

Figure 2. Similarity of 94 isolates of *Rhynchosporium secalis* based on RAPD markers using UPGMA clustering method and simple matching coefficient.

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revealed that 94% of the total genetic diversity was distributed within subpopulations, while only 3% of total genetic diversity occurred between subpopulations. The genetic differentiation between subpopulations was estimated as 0.05. The number of migrants per generation was estimated as 9.5. The lowest genotypic diversity values were observed in samples belonging to Khuzestan Province (Table 5). Genetic distance among different isolates ranged from 0.331 to 0 .728.

The genetic structure of Iranian isolates of *R. secalis* collected from different agro-ecological zones and from different barley host populations was investigated. The agro-ecological zones of Iran represent unique physical environments and it was intendedto determine whether *R. secalis* isolates in different zones were genetically different. A high level of genetic diversity was found to exist among *R. secalis* isolates in Iran. High genetic variability of *R. secalis* has also been reported from Australia (McDonald *et al.,* 1999), New Zealand (Cromey *et al.,* 1987), USA, Norway and South Africa (Zaffarano *et al.,* 2006).

The observed genetic structure of the *R. secalis* populations in this survey is not consistent with an asexual pathogen. The primary infective inoculum source of *R. secalis* is thought to be conidia that are dispersed by rain splash (Fitt *et al.,* 1989). It was expected that *R. secalis* populations would display a low level of genotypic diversity within local populations and large degree of population differentiation among populations because of the limited potential for long-distance dispersal of conidia distributed by rain splash. Salamati *et al.* (2000) suggested two alternative hypotheses to explain the high level of genetic diversity of *R. secalis* including, regular cycles of parasexual recombination. The regular sexual recombination may be occurring in these *R. secalis* populations and that ascospores from the unknown teleomorph are disseminated over distances of up to hundreds of kilometers. Salamati *et al.* (2000) speculated that *R. secalis* is the bearer of a telemorph that has not yet been recognized and this telemorph plays a significant role in the epidemiology and population biology of the fungus.

No evidence was found for geographic differentiation in pairwise comparison

Table 4. Number of random amplified polymorphic DNA (RAPD) haplotypes of *Rhynchosporium secalis* in five provinces of Iran.

Region	Sample size	No. of RAPD haplotypes	Haplotype frequency
Ilam	16	12	0.75
Golestan	11	8	0.73
Kermanshah	29	15	0.52
Lorestan	23	17	0.74
Khuzestan	15	9	0.60
Total	94	42	

Table 5. Genetic diversity among subpopulations of *Rhynchosporium secalis* estimated through RAPD markers.

Population	No of samples	No of haplotype	H_T [a]	H_S [b]	G_{ST} [c]	N_m [d]
Golestan	11	8	0.97	0.90	0.077	5.99
Ilam	16	12	0.98	0.93	0.053	8.93
Kermanshah	29	15	0.96	0.94	0.021	23.3
Lorestan	23	17	0.94	0.91	0.032	15.1
Khuzestan	15	9	0.92	0.87	0.057	8.27
Total	94	42	0.94	0.91	0.050	9.5

[a] Total gene diversity; [b] Gene diversity within subpopulations; [c] Gene differentiation between subpopulation, [d] Estimated of number of migrants per generation.

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involving populations from different regions (G_{ST}<0.08). Goodwin *et al.* (1993) estimated G_{ST} based on isozyme loci 0.16. Isolates were grouped in this study as based on geographic separation, but the results of population genetic analysis suggests that these geographic populations are not undergoing independent evolution and hence may be considered part of the same population. This finding is in agreement with those of McDonald *et al.* (1999) and Bouajila *et al.* (2007) who found low population differentiation among regional populations of *R. secalis* based on RFLP, AFLP and as well on microsatellite analysis. McDonald *et al.* (1999) obtained G_{ST} values ranging from 0.03 to 0.11, with an average of 0.07. Gene flow is a possible explanation for the high genotypic diversity, high genetic similarity, and a lack of geographic differentiation among *R. secalis* populations in Iran. RAPD marker used in this study revealed a high degree of genetic diversity in *R. secalis* populations from Iran. However, in a previous study of genetic variation in *R. secalis* from Africa and West Asia, von Korff *et al.* (2004) analyzed two field populations from Kef and found a high level of genetic differentiation between the tow populations. Similar results have been reported by Arabi *et al.* (2008) from Syria and Bouajila *et al.* (2007) in Tunisia. The present results suggest that *R. secalis* propagules are effectively disseminated over spatial scales of at least hundreds of kilometers, leading to only minor differences in the distribution of genetic diversity among populations on a regional scale. Conidia dispersed by rainsplash are not expected to travel long distances, so it is unlikely that these populations are linked by movement of conidia. It is more likely that gene flow among these populations occurs through infected seeds, fungal spores (possibly ascospores), or infected plant materials in hay, which can be disseminated over longer distances. Pathogens with propagules that disperse over relatively long distances can spread quickly into new geographic areas (Linde *et al.,* 2009). Several mechanisms could facilitate gene flow between populations of *R. secalis* in Iran, infected barley seed, stubble, debris or air dispersal of putative ascospores that are potentially, dispersible by wind, as important sources of inoculum of the scald disease. Exchange of infected breeding seeds among Iran provinces could have played a significant role in creating these relationships when barley and probably *R. secalis* on infected barley seeds were introduced into new areas. No sexual stage has been identified for *R. secalis*. It is possible that the sexual stage of *R. secalis* occurs on stubble between growing seasons playing some role in the establishment of a founder population (McDonald *et al.,* 1999). The observation of high levels of genetic variation in asexually reproducing fungi is not atypical (Gargouri *et al.,* 2003), however significant variation for a wide range of markers (pathogenicity, isozymes, ribosomal DNA and colony color) has been reported for populations of the imperfect fungus *R. secalis* (McDermott *et al.,* 1989; Goodwin *et al.,* 1994).

There existed a weak correlation between genetic and geographical distance suggesting that they are independent from each other. These isolates could have been derived from the same source population being disseminated from one area to another in association with their hosts. This is in consistence with the results reported by Bouajila *et al.* (2007) who found that cluster analysis did not reveal a close correlation between *R. secalis* and AFLP groups.

There is a huge trade in barley stubble used for animal feed among different barley growing regions in Iran. The susceptibility of most Iranian barley varieties to scald means that stubble obtained from these varieties and traded across the country is frequently infected and there is little or no restriction to gene flow among geographic populations of the pathogen throughout the country.

A high level of genetic diversity was revealed among a diverse set of 94 isolates, and through 11 RAPD primers utilized in

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this work, suggesting that there has been pervious dispersal of *R. secalis* isolates across Iran. This finding indicates the need to monitor the Iranian isolates of *R. secalis* for a detection of infection sources, predicting the spread of disease across locations and as well to study its local extension and recolonization.

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تنوع ژنتیکی جدایه های ایرانی پاتوژن عامل بیماری اسکالد جو (*Rhynchosporium secalis*) با استفاده از نشانگر مولکولی

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چکیده

سوختگی برگی که توسط قارچ *Rhynchosporium secalis* ایجاد می شود یکی از مهمترین بیماریهای جو در کشور می باشد. اهمیت اقتصادی و انتشار جهانی محصول جو باعث بوجود آمدن

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مطالعات زیادی در مورد ساختار ژنتیکی جمعیت در قارچ *R. secalis* شده است. تنوع ژنتیکی جمعیت های قارچ با استفاده از ۱۱ آغازگر RAPD مطالعه گردید. ۱۱۹ قطعه DNAتوسط آغازگرها تکثیر شد که ۸۹ قطعه بصورت چند شکلی بودند. تجزیه خوشه ای با استفاده از ضریب تشابه ساده و روش UPGMA نشان داد که جدایه ها در سطح تشابه ۷۵ درصد در ۳۳ گروه قرار گرفتند. تشابه بین جدایه ها بین ۶۲/ تا ۸۹/ برآورد شد. تعداد ۴۲ هاپلوتیپ در بین ۹۴ جدایه از قارچ شناسایی شد. هاپلوتیپ ۱ در همه نقاط مورد بررسی پراکنش داشت. تعدادی از هاپلوتیپها در یک منطقه خاص و تعدادی در چند منطقه دیده شدند. ارتباط کمی بین موقعیت جغرافیایی و تنوع ژنتیکی در بین جدایه ها وجود داشت.

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