Mycologia Iranica 6(2): 89 – 99, 2019 Original Article DOI: 10.22043/mi.2020.122233

Genetic diversity of *Ascochyta lentis* in Ilam and Kermanshah provinces using SSR Markers

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Abstract: *Ascochyta* blight caused by *Ascochyta lentis* is one of the most important diseases of lentil in the world. This disease has been reported in various areas in Iran causing serious damages every year. Currently, the use of resistant cultivars is considered the most effective and environmentally sustainable strategy for disease control. This study was carried out to determine the genetic diversity of *A. lentis* populations. For this purpose, samples were collected from eight different regions of Ilam and Kermanshah provinces including: Ilam, Ayvan, Malekshahi, Sirvan, Chardavol, Islamabad, Gilan-e Gharb, Sarpul-e Zahab. After isolation, purification and morphological identification, genetic variation of 94 *A. lentis* isolates were assessed using three pairs of SSR primers. Based on molecular analysis, 34 alleles were observed among the populations, and average number of alleles was 1.688. The amount of marker index (MI) in the ArA03T primers was the highest. The results of AMOVA analysis showed 92% variability within populations and 8% diversity among the populations. Total gene diversity (Ht) and gene diversities between populations (Hs) were estimated 0.315 and 0.269, respectively. Gene diversity attributable to differentiate among population (Gst) was 0.146, while gene flow (Nm) was 2.903. Cluster analysis based on UPGMA method showed the lowest genetic distance between Ayvan and Sarpol-e Zahab, afterward Ilam and the highest genetic distance between Islamabad and seven remaining populations. This is the first report and comprehensive survey of *A. lentis* pathogen genetic diversity in Iranian lentil fields. Results of this study can be useful in breeding programs with the aim of producing *A. lentis* resistant cultivars and developing effective methods for disease control.

Key words: Ascochyta blight, gene flow, lentil, microsatellite

INTRODUCTION

Lentils (*Lens culinaris* Medik.) are self-pollinating diploid (2n = 14) annual cool season legumes with important role in human diet as a cheap protein source. Legume crops as well as lentils can fix nitrogen through symbiotic bacteria in their root nodules and play important roles in crop rotation and sustainable agriculture (Gan et al. 2006, Hajibarat et al. 2015).

Lentil production is constrained by different diseases, among various diseases, Ascochyta bight is a major problem in many temperate regions in the world, that can significantly reduce crop production (Khazaei et al. 2016). It has been reported to cause up to 70% yield losses in Canada, 30–50% in the USA, and 50% in Australia (Sari et al. 2017). This disease is prevalent in Kermanshah and Ilam provinces which are among the most lentil production regions in Iran and causes severe losses every year. Symptoms of Ascochyta blight on lentil are described as grey to tan spots, lesions on leaflets, stems, flowers, and pods, with dark margins. Lesions often have tiny black fruiting bodies (pycnidia) in the center, usually associated with severe reduction in yield and quality, especially under favorable conditions (Kaiser 1997). *Ascochyta lentis* survives in plant debris, soil and infected seeds. *Ascochyta lentis* reproduces asexually, conidia in flask-shaped pycnidia, and ascospores from pseudothecia. Ascospores and conidia can spread the disease up to hundreds of meters by wind and rain (Rhaiem et al. 2007).

The control of Ascochyta blight is successful only through integrated approaches: Crop rotation, seeds free pathogen, seed treatment and foliar fungicide (Ford *et al*. 2000). However, the use of resistance lentil varieties is the most economic and safe environmentally method in controlling of this disease (Andrahennadi et al. 1993).

Knowledge of the amount and distribution of genetic diversity between and within pathogen

Submitted 3 March 2019, accepted for publication 6 Aug. 2019
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populations is a prerequisite for the development of resistant cultivars. Identification of diversity by morphological characters is highly variable in *A. lentis* isolates which are influenced by cultural conditions (Kiprop et al. 2002). The genetic structure of a population is the amount and distribution of genetic variation within and among populations of a species and reflects the evolutionary history of populations (McDonald & Linde 2002). The amount of genetic variation within a population indicates how rapidly a pathogen can develop and provides valuable information in predicting the effectiveness of control measures such as host resistance genes or chemical control (McDermott & McDonald 1993). The main evolutionary forces that contribute to the genetic structure within and between populations are gene flow, genetic drift, reproduction/mating systems, population size, and selection (Morjane et al. 1994, Peever et al. 2004, Phan et al. 2003, Santra et al. 2001, Udupa et al. 1998). Therefore, it is important to assess the amount and distribution of genetic variation within pathogen species which is essential for developing strategies to reduce disease risk (Nasir 1998, Ford et al. 2000).

At present, the absolute information about genetic diversity of *A. lentis* is not available in Iran. Molecular techniques based on the polymerase chain reaction (PCR) have been used for genetic diversity assessment, molecular taxonomy, evolutionary studies, and diagnosis of fungal species (Williams et al. 1990, Clulow et al. 1991, Nasir & Hoppe 1991, Welsh et al. 1991, McDonald 1997). Different molecular markers are available for investigation of genetic variability in fungal plant pathogens. SSRs are molecular markers routinely used for this purpose, and they are abundant and highly polymorphic, distributed over the euchromatic (Active Chromatin Sequence = ACS) parts of the genome (Schlotterer & Ellegren 1998, Zane et al. 2002). Numerous studies including genetic diversity of fungal populations in different parts of the world (Arnau et al. 1994, Peltonen et al. 1996), virulence and pathogenic variation in different pathogens (Kolmer et al. 1995, Sicard et al. 1997) and differentiating of aggressive and nonaggressive fungal isolates (Ford et al. 2000), have been conducted on this topic. Significant genetic variation was shown in *A. lentis* populations in Italy (Fischer et al. 1995), and

Tunisia (Geistlinger et al. 2000, Morjane et al. 1994). In Australia (from Victoria, South Australia, Western Australia and New South Wales) and the Pacific northwest of California, only modest genetic diversity and a single mating type have been reported (Kaiser. 1997, Phan et al. 2003). Similar studies on other plant pathogenic fungi have emphasized the importance of molecular approaches to characterize genetic diversity within and between isolates including *Sclerotinia sclerotiorum* (Sirjusingh & Kohn 2001), *Ascochyta rabiei* (Nourollahi et al. 2011), *Rhizoctonia. solani* (Mwang Òmbe et al. 2007) and *Fusarium oxysporum* (Stewart et al. 2006), while, the genetic diversity of *A. lentis* isolates have not yet been surveyed in Iran.

The aim of this study was to determine the genetic diversity of *A. lentis* isolates collected from Ilam and Kermanshah provinces using three SSR sequences.

MATERIALS AND METHODS

Sampling, fungal isolation

Lentil plants with brown or black lesions on stem, leaves and pods were randomly collected in the sampling season in 2018, from eight different regions including: Ilam, Ayvan, Malekshahi, Sirvan, Chardavol, Islamabad, Gilan-e Gharb, Sarpul-e Zahab in the western of Iran. The regions have different altitude and climate, and separated by substantial mountain ranges from 50-300 km. Isolates of each region were considered as a population (Fig. 1). Symptomatic samples were cut into 2-5 mm pieces, surface sterilized by dipping into domestic bleach solution (1% NaOCl) for 1-2 min, washed three times with sterile distilled water, dried with sterile filter papers and placed on potato dextrose agar (PDA) or chickpea seed meal dextrose agar (CSDA).

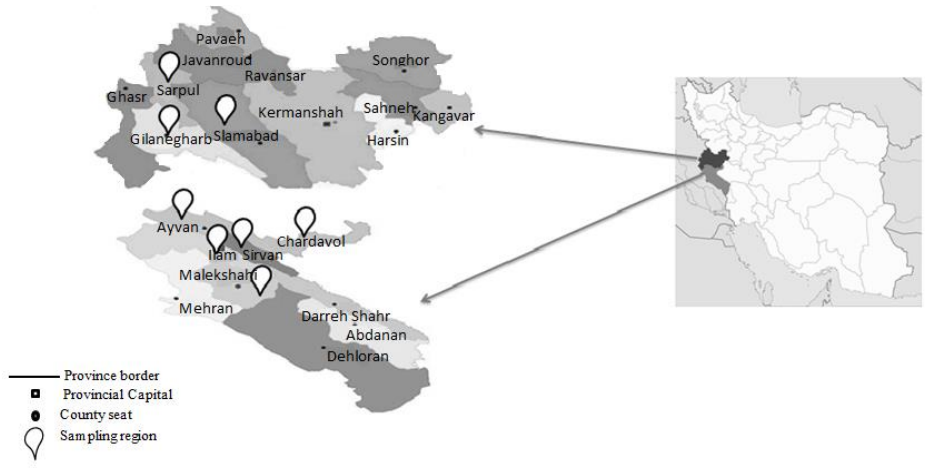


Fig.1. Geographical origins of *Ascochyta lentis* populations in Ilam and Kermanshah provinces

Samples were incubated at 25 °C for three days in an incubator with a 12-h photoperiod to induce production of conidia. Isolates were purified by single-spore method via harvesting conidia from a single pycnidium with a sterile needle and streaking them on a 2% water agar plate. Conidia were incubated as above and single germinated conidia were transferred onto PDA or CSDA plates. Simultaneously, a piece of 4cm sterile paper was placed on the culture medium and incubated with a 12-h photoperiod for 2-3 weeks. After the growth of mycelia, purified isolates were stored on sterile filter papers at -20 °C. Isolates were identified based on morphological characteristics such as pycnidia, pycnidiospore and colony growth traits (Barnett & Hunter. 1972), and then identification was confirmed based on molecular techniques with SSR primer pairs specific to *Ascochyta*.

Pathogenicity test

All stages of the pathogenicity test on lentil were performed according to Chen et al. 2004. For this experiment, first, local lentil seeds were disinfected with 0.5% NaOCl for three minutes and washed three times with sterile distilled water. Then, lentil seeds were planted in vases with three replications separately for each isolate in greenhouse conditions. For inoculum production, based on the sampling areas, one isolate from each area was randomly selected as the group representative such as: AL1, AL23, AL37, AL45, AL62, ALK66, ALK7 and ALK84. Conidia were harvested from two weeks old colonies grown on PDA under alternating cycles of 12h of light/darkness at room temperature by adding sterile water and gently scraped off with a glass rod. Conidial suspensions were adjusted at a concentration of 3×10^5 spores/ml and sprayed to run off on two weeks old seedlings in the greenhouse at (25±3 °C, RH = 90%), while the control plants were sprayed with sterile water (Chen *et al.* 2004). Humidity (100 %) was maintained by covering plants with plastic bags for the first 48hr. The pots were irrigated in a timely manner and kept in greenhouse condition. Fifteen days after inoculation, inoculated plants were checked for possible symptoms and the results were recorded.

DNA extraction

To obtain the mycelial mass, liquid cultures were initiated by adding 2-4 mm^2 pieces of filter papers to 250- mL Erlenmeyer flasks containing 100 mL PDB medium (potato dextrose broth). Flasks were incubated at room temperature approximately 25 °C on a rotary shaker for 6–8 days. Mycelia were collected by filtration through sterile filter papers with a vacuum funnel. Mycelia were harvested, frozen and stored at −20 °C. DNA was extracted using a modified hexadecyl-trimethyl ammonium bromide (CTAB) procedure (Doyle & Doyle 1987). Genomic DNA was isolated from the single spore culture of each isolate (Murray & Thompson 1980). Mycelia were ground in liquid nitrogen and suspended in 2% CTAB extraction buffer (1.4 M NaCl, 0.1 M Tris-HCl, pH 8.0, 20 mM EDTA, 0.2% β-mercaptoethanol). Samples were treated with five units RNase at 37 °C for 30 min, and then extracted with chloroform-isoamyl alcohol 24:1 (v/v). DNA in the supernatant was precipitated with isopropanol, rinsed with ethanol, and adjusted to a final concentration of 20 ng/µl in TE (pH 7.4). The quality of the extracted DNA was visually checked on 0.8 % agarose gels.

SSR amplification and analysis

Three SSR primer pairs (Table 1) were selected on the basis of their high PIC as described by (Geistlinger *et al.* 2000). Primer aliquots for each marker were prepared by mixing 20 pmoles of appropriate forward and reverse primers in 1×TE (1 mM EDTA, 10 mM Tris–HCl, pH 8.0) and used for the amplification of individual microsatellite loci. PCR amplification was performed in a 25 µl reaction volume containing 2.5 µl of 10X PCR Buffer, 1.5 mM MgCl2, 0.2 mM of dNTPs mix (100 mM of each dNTPs), 1µl of each forward and reverse primer, 0.6 U of Taq polymerase with 25 ng of template DNA. Amplification was performed using Biometra (USA) thermal cycler, PCR conditions for SSR were as follows; one initial denaturation step at 94 °C for 4 min followed by 35 cycles of 94 °C for 1 min, annealing at 59 ℃ for 1 min (appropriate annealing temperature were used for each primers set between 52-60 °C) and 72 °C for 2 min. The thermal cycles were terminated by a final extension at 72 °C for 5 min. Amplified products were resolved in 2 % agarose gel at 60 V cm^{-1} using in Tris Boric Acid EDTA (1X TBE) buffer and stained with DNA Safe Stain at 0.5 mg ml^{-1} and photographed under UV Trans-laminator with Bio-Rad Gel Doc. (American). A 1 kb ladder (Gene Ruler TM, Fermantas) was used as a molecular size standard.

Table 1. SSR primer sequences used in this study (Geistlinger *et al.* 2000).

Primer	motif 3"→5"	Primer Sequences	Size(bp)	Allele	Tm (℃)	PIC	EMR	MI
ArA03T	$(GAA)_{31}$	TAGGTGGCTAAATCTGTAGG CAGCAATGGCAACGAGCACG	379	11	58	0.343	400	128
ArH05T	$(CTT)_{18}$	CATTGTGGCATCTGACATCAC TGGATGGGAGGTTTTTGGTA	197	14	56	0.307	300	90
ArH06T	$(CAA)_9(CAG)_7(CAA)_{21}$	CTGTCACAGTAACGACAACG ATTCCAGAGAGCCTTGATTG	167	9	56	0.300	300	90

Statistical analysis

Sampling regions in different geographical situation were defined as different populations. The bands generated by SSR primers that were repeatable and clearly visible with a high intensity were scored manually for the presence (1) or absence (0) in each isolate. The pairwise distance among the isolates was calculated from the binary matrix using the simple matching (SM) coefficient (Sneath & Sokal 1973) that is recommended for haploid fungi (Kosman & Leonard 2005). Genetic similarity between pairs was estimated using Jaccard's similarity coefficient. The dendrogram was constructed from the Jaccard similarity coefficients matrix with UPGMA method (Rolhf 1990). For each primer pair, the polymorphic information content (PIC) and, marker index (MI) were calculated. The polymorphic information content (PIC) was calculated using $PIC_i = 2f_i (1-f_i)$, where i is the information of marker I, f_i is the frequency of the amplified allele (presence of fragments) and $(1 – f_i)$ is the frequency of the null alleles (Roldan-Ruiz *et al*. 2000). The genetic variation was computed by averaging PIC estimates over all loci (Nei & Li. 1979). The marker index (MI) was calculated by MI = PIC x EMR, where EMR is the "effective multiplex relationship" given by the product of the total number of fragments per primer (n) and the fraction of polymorphic fragments (β) (Varshney et al. 2007). Genotypic diversity (H) among isolates was estimated from allelic frequencies using the equation $H=1 - \Sigma xi^2$, where, xi is the frequency of the i^{th} allele (Nei. 1973). The coefficient of population subdivision (GST) was computed as (Ht – Hs)/Ht, where, Ht is the total genetic diversity and Hs is the average gene diversity over all subgroups (Nei. 1973). the allele frequencies at polymorphic loci, the Nm values (effective migration rate), and the genetic identity among populations for characterize genetic variation, observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (He) and Shannon's information index (I) were calculated for each locus and population. Mean values of gene diversity in total populations (H_t), gene diversity between populations (H_s), proportion of gene diversity attributable to differentiateamong populations (Gst) and estimate of gene flow from G_{st} (N_m) were estimated across loci (McDermot & McDonald 1993). All above calculations were performed using POPGENE ver. 1.31 (Yeh et al. 1999) and Gen Alex ver. 6.501 (Peakall & Smouse 2012).

RESULTS AND DISCUSSION

Fungal isolation

Ninety-four isolates of *A. lentis* were isolated from lentil plants with symptom of brown or black lesions disease. According to the pathogenicity test results all *A. lentis* isolates were pathogenic on lentil (Fig. 2). Inoculated plants showed brown to black lesions on leaves and all isolates caused discoloration symptoms of blight while such symptoms were not observed in control treatments. Disease severity increased after four to seven days of inoculation. The symptoms developed into circular to oval elongated spots with irregular boundaries. Black pycnidia were seen in the lesions. The lesions on the petioles and stems were generally elongated and often girdled these tissues. After three weeks, broken stems and dead plants were observed. Finally, pathogenicity test was confirmed based on Koch's principles by re-isolating the fungus from the infected plants.

Pathogenicity test

Isolates were identified according to the morphological and microscopic characteristics (Barnett & Hunter 1972). The colony's features were highly variable (Fig. 3). Colonies on PDA were flat, submerged, and white at the beginning and gradually became grey to dark. Mycelia were thin, and pycnidia were abundantly formed at the center of the colony. Pycnidia were spherical, globular form, pale to dark brown color and size with 90-160 µm in diameter. Conidia were cylinder or ellipsoid form with one or two cells with rounded ends, straight, and the size of 8-15× 4-6 µm in diameters.

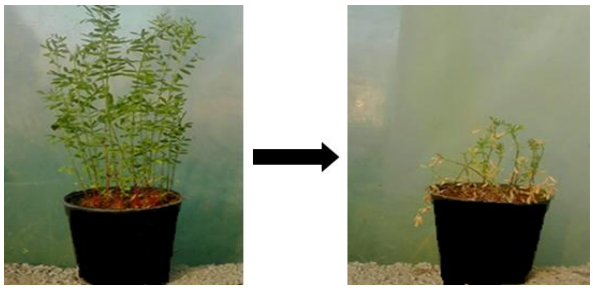


Fig. 2. The pathogenicity test of *Ascochyta lentis* isolates on lentil seedlings three weeks after inoculation.

SSR analysis

Three pairs of SSR primers were used for amplification of loci in 94 *A. lentis* isolates (Fig. 4). A summary of characteristics of three microsatellite loci is given in Table 2. Number of alleles, percentage of polymorphism and polymorphic information content (PIC) of SSR primers pairs were evaluated, the mean number of PIC values was 0.316, which reflects the informative content of the primers. The total number of alleles and the percentage of polymorphic alleles were 34 and 100 %, respectively. The total alleles originated from ArH05T, ArA03T and ArH06T were 14, 11 and 9 alleles per locus, respectively. The highest number of alleles was observed in ArA05T while ArH06T showed the lowest number.

Various population genetic parameters in microsatellite loci for all populations such as: Observed allele number, effective numbers, are given in table 2. Observed allele number (Na) and effective numbers (Ne), the average number of alleles per locus was the highest in Malekshahi and Sirvan populations

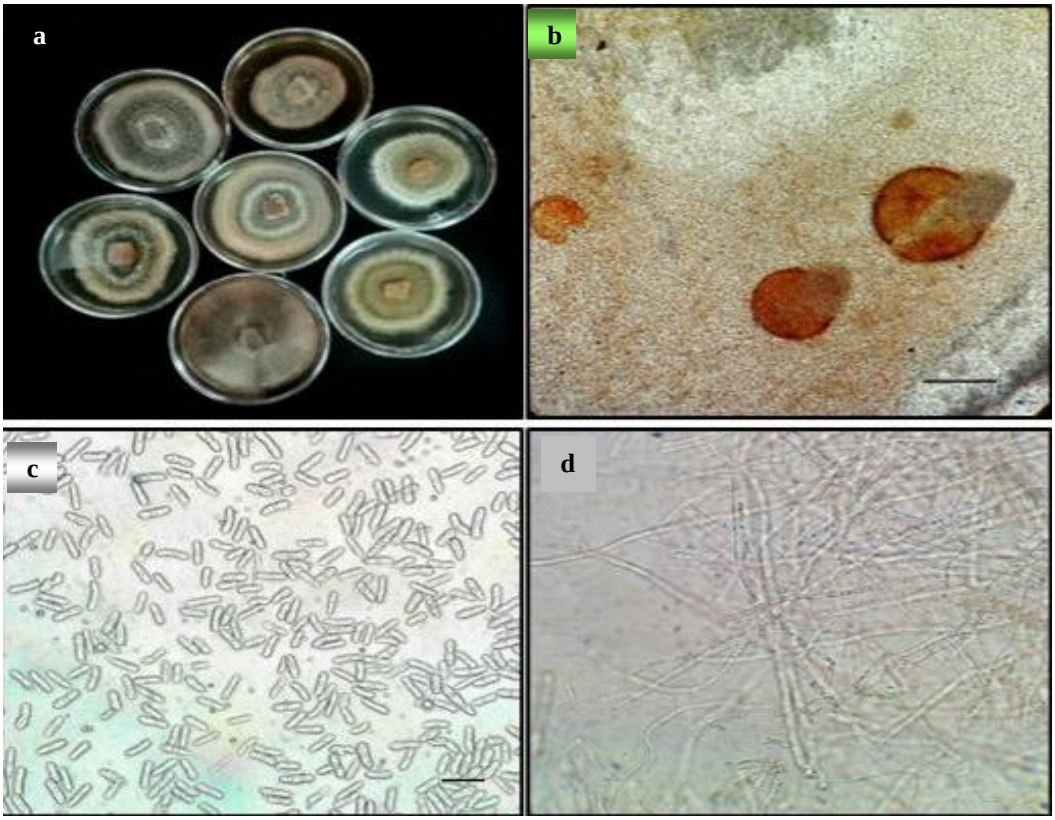


Fig. 3. Morphological features of *Ascochyta lentis* on (PDA) (a), Pycnidia (b), Conidia (c), and Mycelia with 400x magnification (d).

(2 alleles) while the lowest was observed in the Islamabad population (1.2 alleles). The value of gene diversity (He) and (I) were also high in Malekshahi (H = 0.349; I = 0.526) and low in Ilam (He = 0.213; I = 0.333).

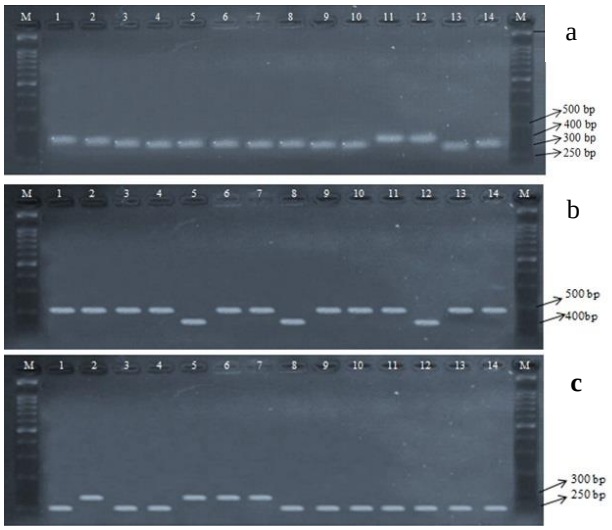


Fig. 4. Amplification profile of *A. lentis* isolates using ArA03T (a), ArH05T (b), ArH06T (c) SSR primer pairs. Numbers indicate the isolates (1 - 14), M indicates DNA size marker (Ladder 1 kb).

The average genetic distance was calculated among the eight populations. Nei's pairwise genetic distances between the populations varied from 0.008 to 0.169. The lowest genetic distance was found between Ayvan and Sarpul-e Zahab, while the highest genetic distance was revealed between Chardavol and Islamabad (Table 3).

The total gene diversity (Ht) and gene diversities between subpopulations (Hs) were estimated to be 0.315 and 0.269, respectively. Furthermore, gene diversity attributable to differentiate among populations (Gst) was 0.146, while gene flow (Nm) was 2.903.

Cluster analysis of isolates showed five groups that each group contains of isolates from different geographical origins (Fig. 5). Group 1 was the largest group and included isolates from all populations. Dendrogram of population showed a distinction between the Islamabad population and the seven remaining populations (Fig. 6).

Table 2. Genetic diversity estimates in *Ascochyta lentis* populations based on microsatellite loci

Population	N	Na	Ne	I	He
Ilam	13	1.600	1.304	0.333	0.213
Ayvan	14	1.800	1.393	0.411	0.259
Malekshahi	14	2.000	1.582	0.526	0.349
Sirvan	11	2.000	1.503	0.480	0.311
Chardavol	12	1.600	1.380	0.368	0.236
Slamabad	10	1.200	1.379	0.345	0.224
Gilanegharb	10	1.800	1.547	0.491	0.328
Sarpolezahab	10	1.500	1.383	0.357	0.234
Average	11	1.688	1.434	0.414	0.269

I: Shannon's Information index, He: Nei's (1973) gene diversity, Ne: Effective number of alleles, Na: Observed number of alleles, N: Number of isolates

The AMOVA of genetic variation in *A. lentis* populations revealed that 8 % of the variance

occurred among populations and 92 % within populations (Fig. 7).

PCA (Principal Component Analysis) showed the genetic differences among isolates within populations (Fig. 8). The first and second principal coordinates was 26.22 % and 22.80 % of the variation, respectively. The results suggest that geographical separation plays a key role in the formation of populations. PCA allows for visualizing the patterns of genetic relationship without altering the data itself and finds patterns within a multidimensional data set (Nourollahi & Madahjalali 2017).

Table 3. Information about genetic distance between pairs of *A. lentis* populations.

Population	Ilam	Ayvan	Malekshahi	Sirvan	Chardavol	Slamabad	Gilanegharb	Sarpolezahab
Ilam	****							
Ayvan	0.017	****						
Malekshahi	0.031	0.038	****					
Sirvan	0.077	0.066	0.035	****				
Chardavol	0.031	0.023	0.038	0.053	****			
Slamabad	0.157	0.132	0.111	0.067	0.169	****		
Gilanegharb	0.101	0.090	0.060	0.105	0.144	0.102	****	
Sarpolezahab	0.026	0.008	0.059	0.083	0.044	0.131	0.083	****

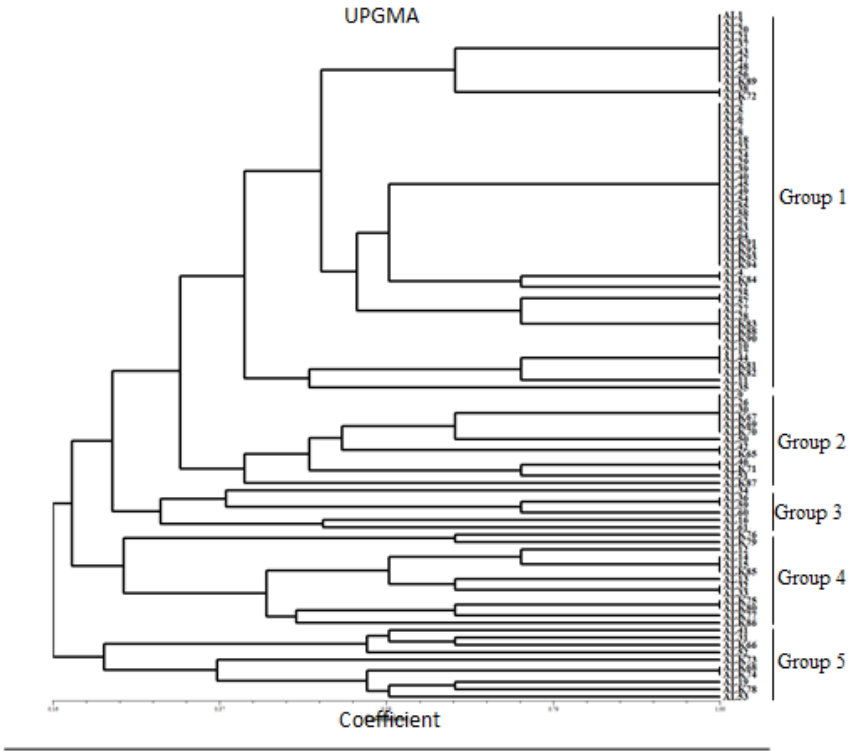
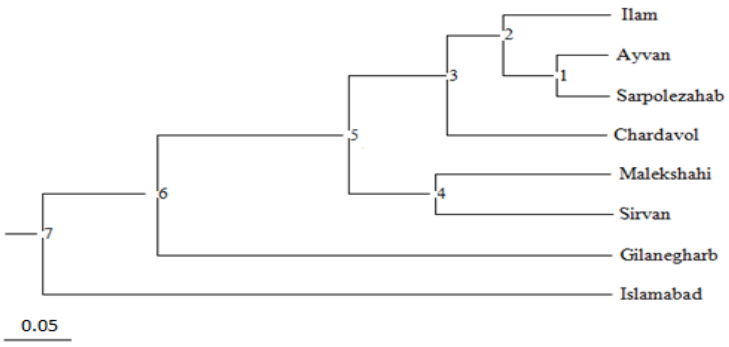


Fig.5. Dendrogram of *Ascochyta lentis* isolates using Jaccard similarity coefficient based on UPGMA method

Fig.6. Dendrogram of genetic relationships between eight populations of *Ascochyta lentis* as reconstructed by UPGMA using Nei's genetic distance



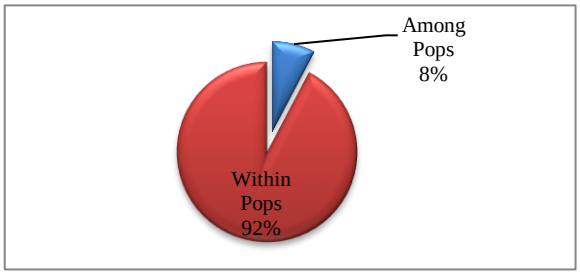


Fig.7. Percentage of molecular variance analysis (AMOVA) within and between populations

Present study clearly indicated the genetic variation among 94 *A. lentis* isolates collected from different regions. The present work was carried out to explore the possible application of SSR technique for identifying genetic structure in *A. lentis* isolates. Characteristics including high specificity, high polymorphism, good reproducibility and unambiguous measurability are advantages of microsatellite markers (Tenzer et al. 1999, Sahran & Naef 2008). SSR markers provide a powerful tool for taxonomic and population genetic studies (Britz et al. 2002). By SSR markers, alleles of different sizes were recorded in *A. lentis* genome. In this research, although sample sizes were not comparable, ArH05T primer identified the highest number of alleles at any locus. Geographically, the SSR data indicated that all eight populations had high levels of gene diversity, among these population, Chardavol and Islamabad showing the highest diversity. This genetic difference may be justified by the large geographic distance between the two populations rather than the other populations. These results are in accordance with results from the previous study on *A. lentis*, isolates from Tunisia, Pakistan, Turkey and Syria using microsatellite markers demonstrating that the population of this fungus is genotypically highly diverse (Geistlinger *et al*. 2000). Different research have been carried out on international populations of *A. lentis* using random amplified polymorphic DNA (RAPD) and a different level of genetic diversity was detected within and between populations (Ford et al. 2000). High genetic variability among *A. lentis* isolates from different lines has been reported by RFLPs of ribosomal DNA analysis in Australia (Taylor et al. 2007). The factor that has an important role on the genetic diversity of *A. lentis* populations is the mating system. With this assumption, if the sexual reproduction of *A. lentis* occurs in nature, ascospores may play a major role in population biology. The advantage of sexual reproduction is the adaptation to a new environment through recombination of genetic materials between the two mating strains. It also helps overcoming the selection pressure imposed by the introduction of resistant host genotypes and fungicides (Cherif et al. 2006). Both *A. lentis* mating types have been reported in at least 15 countries such as Tunisia, Turkey, USA, Canada and Spain (Rhaiem et al. 2007).

In a research of genetic variation of *A. lentis* isolates in India and southern Australia, both types of mat1-1, mat 2-2 considerably had high effect on genetic diversity (Varshney et al. 2009). These data suggest that *A. lentis* populations may be genetically diverse in countries where the host and the pathogen have been sympatric for a long period (Taylor & Ford 2007). The ratio between mating types may be tilted to differ from 1:1. It is also possible the lack of linkage disequilibrium may be due to historical sexual recombination. It has been reported that for the *A. lentis* pathosystem, the sign of past recombination cannot be eroded in field population even if asexual reproduction was enforced for 40 generations (Udupa et al. 1998, Imtiaz et al. 2011, Jamil et al. 2000). It is not clear in Iran whether sexual reproduction and the existence of virulent pathotypes are correlated or not. In this study, 92 % of the genetic diversity was distributed on a local level within populations, however, there was a high degree of genetic similarity between the populations which separated by low geographical distances like Ayvan and Sarpol-e Zahab. The low proportion of gene diversity attributable to differentiate among populations (Gst) was detected among all eight populations. Low Gst value (0.146) indicated little genetic differentiation among the eight populations and showed little evidence for geographical subdivisions among populations (Bayraktar 2010).

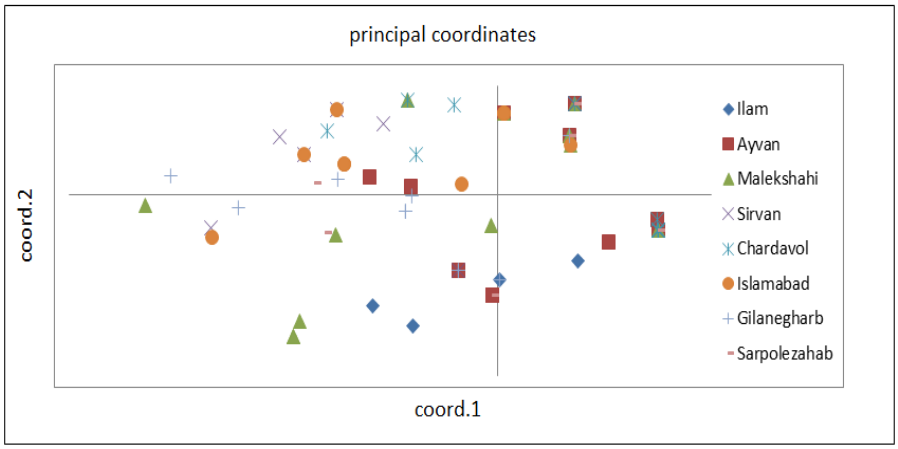


Fig. 8. Principal Component Analysis (PCA) based on SSR data for 94 individual *Ascochyta lentis* isolates belonged to eight populations.

This study is comparable with 123 Tunisian *A. lentis* isolates representatives of five regions were analyzed to estimate genetic diversity in Tunisia and provided a high level of genetic differentiation among subpopulations (Rhaiem et al. 2007).

In contrast, on a smaller scale in Tunisia, Morjane et al. (1994) examined the genetic variation among 50 isolates sampled from one single lentils field. The genetic structure of Australian *A. lentis* isolates were carried out where the genetic diversity among the populations was low. Gene Flow (Nm) is one of the evolutionary forces that can have a significant force on the genetic diversity of a population. In the absence of gene flow, genetic drift causes different allele frequencies at neutral loci, leading to differentiation in isolated populations (Keller et al. 1997). The high genetic similarity among populations of *A. lentis* suggests that gene flow has occurred across long distances. In this study, Gen flow average was 2.903 in all loci and populations, suggesting a level of gene flow that was greater than required to prevent populations from diverging by genetic drift (Keller et al. 1997). The distance between populations was very small, therefore, the genetic structure of *A. lentis* could be explained either by dispersal of pycnidiospores from one region to another through infected seeds, or plant debris as gene flow. The exchange of lentils seeds is possibly the mutual cause, because farmers in these regions often purchase seeds from other farmers. The low genetic similarity among *A. lentis* populations suggests that gene flow occurred across long distances. Infected seed can lead to persistence of genotypes if we consider that infected seed can explain the distribution and diversity of genotypes found at the end of the growing season in natural populations. Genetic drift and selection would limit the number of genotypes present in field populations (Shah et al. 1995). In this study, genetic characterization of *A. lentis* isolates was essential for the efficient management of *Ascochyta* blight through applying resistant cultivars in lentils grow in areas. Understanding of occurrence, distribution, and genetic relatedness of such pathogenic variants are essential for developing effective and efficient integrated disease management. Quarantine regulations will be needed to prevent the introduction of more diverse isolates into these populations and prevent transmission of any isolates from these areas to other regions of the country (Shah et al. 1995). Understanding the genetic structure of pathogen populations in the present study may provide insights into the epidemiology and evolutionary potential of pathogens and could lead to developing integrated strategies for disease management and breeding programs.

ACKNOWLEDGEMENTS

The financial assistance provided by Ilam University, is gratefully acknowledged.

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تنوع ژنتیکی *Ascochyta lentis* در استان های ایلام و کرمانشاه با استفاده از نشانگر های مولکولی SSR

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چکیده: بیماری برق‌زدگی عدس با عامل *Ascochyta lentis* یکی از مهم‌ترین بیماری‌های این گیاه در جهان به شمار می‌رود. این بیماری در مناطق مختلف عدس کاری ایران گزارش شده است و همه ساله باعث خسارت فراوانی می‌شود. در حال حاضر استفاده از ارقام مقاوم به لحاظ سازگاری با محیط زیست مؤثرترین راه کنترل بیماری است. این پژوهش به منظور بررسی و تعیین تنوع ژنتیکی قارچ عامل بیماری برق‌زدگی عدس انجام گرفت. به این منظور از مزارع آلوده عدس در استان های ایلام و کرمانشاه شامل هشت شهرستان از جمله: ایلام، ایوان، ملکشاهی، سیروان، چرداول، اسلام‌آباد، گیلانغرب و سرپل ذهاب نمونه‌برداری صورت گرفت. بعد از جداسازی، خالص‌سازی و شناسایی مورفولوژیکی، بررسی تنوع ژنتیکی ۹۴ جدایه *A. lentis* با استفاده از سه جفت نشانگر ریزماهواره (SSR) انجام گرفت. نهایتاً در مجموع ۳۴ آلل در بین جمعیت‌ها مشاهده شد. میانگین تعداد آلل‌های مشاهده شده (Na) در بین جمعیت ها ۱/۶۸۸ می باشد. نتایج تجزیه واریانس مولکولی نشان داد که ۹۲٪ از تنوع ژنتیکی در درون جمعیت‌ها و ۸٪ تنوع در بین جمعیت‌ها وجود دارد. تنوع ژنتیکی کل (Ht) و تنوع ژنی بین جمعیت ها (Hs) ۰/۳۱۵ و ۰/۲۶۹ به ترتیب تخمین زده شد. تنوع ژنتیکی مؤثر در تمایز بین جمعیت ها (Gst) ۰/۱۴۶ و میزان میزان جریان ژنی (Nm) ۲/۹۰۳ بود. تجزیه خوشه‌ای براساس روش UPGMA نشان داد که کمترین فاصله ژنتیکی بین ایوان و سرپل ذهاب، و بیشترین فاصله بین اسلام آباد با دیگر جمعیتها وجود دارد. این نخستین بررسی جامع از وضعیت تنوع ژنتیکی *A. lentis* در مزارع کشت عدس ایران است. این نتایج در برنامه اصلاح ارقام مقاوم برق زدگی عدس و گسترش روش های کنترل مفید خواهد بود.

کلمات کلیدی: برق‌زدگی، جریان ژنی، عدس، ریزماهواره

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تاریخ دریافت: ۱۳۹۷/۱۲/۱۳ تاریخ پذیرش: ۱۳۹۸/۰۵/۱۶