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Mating type distribution, genetic diversity and population structure of *Ascochyta rabiei*, the cause of Ascochyta blight of chickpea in western Iran

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Summary. Ascochyta blight (caused by *Ascochyta rabiei*) is an important disease of chickpea. Mating type distribution, genetic diversity and population structure *A. rabiei* isolates from western Iran, using specific matting type primers, and ISSR and SSR molecular markers. Two mating types were identified, with the 57% of isolates belonging to MAT1-1. Ten ISSR markers produced 78 polymorphic bands with an average polymorphism information content (PIC) value of 0.33. Seven SSR markers showed high allelic variation (four to seven alleles) with the average PIC value of 0.61. The generated dendrogram using neighbor joining approach with ISSR and SSR marker data grouped isolates in three clusters. Combined dendrogram and model-based population structure analysis divided the isolates into two distinct populations. No significant correlation was found between geographical origins of isolates and their genetic diversity patterns, although the isolates from North Kermanshah and Kurdistan were closely grouped, and most of isolates from Lorestan and Kermanshah were clustered in a separate group. This relative spatial correlation between geographical locations and *A. rabiei* grouping indicated high genetic diversity within populations and no significant gene flow between distinctly geographical regions. This suggests the nece0ssity of continuous monitoring of *A. rabiei* populations in order to design effective chickpea breeding strategies to control the disease.

Keywords. ISSR, SSR, population structure, Ascochyta blight, sexual reproduction.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important food legume, which provides human feed as a stable, rich and cheap source of vegetar-

ian protein (Varshney *et al.*, 2013). Chickpea originated from Middle-East, North Africa and Central Asia (van der Maesen, 1987; Talebi *et al.*, 2008). Chickpea is produced in over 50 countries and India is the most important producer, with average yields of approx. 900 kg ha^{-1}. Iran ranks ninth in the world for chickpea production, with 2% of world production (Merga *et al.* 2019).

International chickpea seed yields are less than potential yield due to the narrow genetic base of most improved cultivars and uniform reaction to different abiotic and biotic stresses (Ghaffari *et al.,* 2014). Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Lab. (syn: *Didymella rabiei* Kov.), is one of the most important fungal diseases of chickpea. The disease may cause yield losses up to 100% under favourable cool and humid conditions (Ahmad *et al.,* 2014; Farahani *et al.,* 2019). In Western Iran (Kermanshah, Kurdistan and Ilam provinces) AB is a serious damaging disease of chickpea, and in spring seasons (April to May), the disease is often severe (Nourollahi *et al.* 2011; Azizpour and Rouhrazi, 2017). Integrated strategies have to be applied to reduce AB, including agronomic practices (crop rotation and adjusting sowing date), application of fungicides and use of durably resistant chickpea cultivars (Kimurto *et al.,* 2013; Vafaei *et al.,* 2016; Farahani *et al.,* 2019). Employment of resistant varieties has been considered the most effective, economic and environmentally-friendly strategy to manage the disease (Singh and Reddy, 1996; Varshney *et al.,* 2009).

Both asexual and sexual reproduction has been reported for *A. rabiei*. The asexual stage occurs during the host growing season and the sexual stage develops on infected seed and crop residues during the winter (Trapero-Casas and Kaiser, 1992; Nourollahi *et al.,* 2011). Sexual reproduction plays an important role in genetic diversity of fungal pathogens, which enable them to rapidly overcome host resistance genes as a response to selection pressure imposed by resistant cultivars (Chen and McDonald, 1996; Aghamiri *et al.,* 2015). This variability of the pathogen led to breakdown of resistance in chickpea germplasm. Knowledge of pathogen diversity and mating type systems is fundamental for effective diseases management and breeding programmes (Varshney *et al.,* 2009; Mehrabi *et al.,* 2015; Baite and Dubey, 2018). However, field assessment of the genetic structure in *A. rabiei* populations may be not reliable due to lack of powerful discriminating tools and variable environmental condition. Measuring pathogen genetic diversity based on molecular markers at the DNA level can provide unbiased estimates of genetic variation which is independent of culture conditions (Nourollahi *et al.,* 2011).

Different molecular methods, including SSR (Geistlinger *et al.,* 2000; Phan *et al.,* 2003b), AFLP (Varshney *et al.,* 2009), RAPD (Santra *et al.,* 2001), and mating-type specific markers (Phan *et al.,* 2003a; Ozer *et al.,* 2012), have been developed and utilized for genetic diversity assessment in *A. rabiei* populations from different countries, including Turkey (Bayraktar *et al.,* 2007), Pakistan (Ali *et al.,* 2012), India (Varshney *et al.,* 2009) and other chickpea growing areas (reviewed by Pande *et al.,* 2005). High levels of genetic and pathogenic diversity have also been reported in Iranian *A. rabiei* populations (Nourollahi *et al.,* 2011; Azizpour and Rouhrazi, 2017). Biased mating type distribution has been reported in populations of this fungus collected from west and north-west Iran, that was dominated by the Mat1-1 mating type (Nourollahi *et al.,* 2011; Azizpour and Rouhrazi, 2017). In Turkey and Tunisia, Mat1-2 has been reported as the dominant mating type (Rhaiem *et al.,* 2007; Taylor and Ford, 2007). Previous studies on pathogenicity, genetic diversity using SSRs and Specific mating type DNA markers among Iranian isolates of *A. rabiei* have not been comprehensively described, and most previous Iranian studies were focused on a province or small geographical region (Younessi *et al.,* 2004; Vafaei *et al.,* 2016; Nourollahi *et al.,* 2011; Azizpour and Rouhrazi, 2017).

The present study was undertaken to: (i) assess pathogenicity *A. rabiei* isolates collected from the west of Iran; (ii) assess genetic diversity of these isolates using SSR and ISSR markers; and (iii) characterize mating type distribution of Iranian *A. rabiei* isolates from different provinces using MAT-specific primers.

MATERIALS AND METHODS

Ascochyta rabiei *sampling and isolation*

Seventy-five *A. rabiei* isolates were collected from infected chickpea fields of western Iran provinces (20 isolates from Kurdistan, 46 from Kermanshah and nine from Lorestan) during the 2017 and 2018 growing seasons (Table 1). These provinces are geographically juxtaposed, but due to substantial mountains between them, their climatic conditions are different. Kurdistan has cold winters with mild summers and greater annual precipitation than the other two provinces. Samples were collected from 15 locations with minimum distance between the locations of 10–15 km. At each location, samples were chosen every 20 m along a row from three to five parts of each infected field. Infected leaves were removed to a laboratory, were surface sterilized with sodium hypochlorite (0.5%) for 2 min, and washed twice

Table 1. Numbers and mating types of 75 *Ascochyta rabiei* isolates collected from western Iran.

Province	No. of isolates	Mat1-1	Mat1-2	X^{2*}	*P*
Kermanshah	46	30	16	4.26	0.039
Kurdistan	20	9	11	0.20	0.655
Lorestan	9	4	5	0.11	0.739
Total	75	43	32	1.61	0.204

*Chi-square values were calculated under the null hypothesis of a 1:1 ratio of equal proportions of *Mat1-1* and *Mat1-2.*

with sterilized distilled water. Samples were then plated onto CSMDA (40 g of chickpea seed meal, 20 g dextrose and 18 g agar in 1 L sterilized distilled water). Plates were incubated for 7 to 10 d at 20/22°C. Single pycnidium isolates were obtained from the isolation plates, and were stored on CSMDA for pathogenicity tests.

Pathogenicity tests

Pathogenicity test for all isolates was carried out on the two susceptible chickpea cultivars 'Bivanij' (Iranian landrace susceptible check) and ILC1929 (International susceptible check) (Farahani *et al.* 2019). Purified single pycnidium *A. rabiae* isolates were grown in potato dextrose broth (PDB). The inoculum of each isolate was prepared in a water solution containing 0.20% of Tween 20, and was adjusted to 6×10^5 conidia mL^{-1}.

Two-week-old chickpea seedlings of the susceptible cultivars were inoculated with each isolate, and inoculated plants were kept in the dark under plastic bags for 24 h. The plants were then transferred to a controlled greenhouse where environmental conditions were maintained at 23/18°C day/night, a 16 h photoperiod, and ≥85% relative humidity (Farahani *et al.,* 2019). After 2 weeks, disease reactions on both cultivars were assessed using a 0–9 scale (Pande *et al.,* 2011; Farahani et al. 2019). Plants that showed disease scores greater than 5 were considered susceptible to *A. rabiae* (Farahani *et al.,* 2019).

DNA extraction from isolates

Single pycnidium *A. rabiae* isolates grown on CSMDA were each used to inoculate 50 mL PDB and then incubated at 20/22°C on an orbital shaker (100 rpm) for 5 d. Fungal biomass was harvested from each culture by filtration through Miracloth, was rinsed with distilled water, and then finely ground in liquid nitrogen. These preparations were then subjected to DNA extraction using the CTAB method (Weising *et al.,* 1991).

Mating type assay

Mating types of all 75 *A. rabiei* isolates was determined using the multiplex MAT-specific PCR assay (Barve et al., 2003). Primer combinations of SP21, Tail5 and Com1 were used in a single PCR reaction carried out in 20 µL reaction volume, containing 1× PCR buffer, 25 ng sample DNA, 2 µM primer, 200 µM of each dNTPs, 2.5 mM $MgCl_2$ and 1.5 units of Taq DNA polymerase (Cinnagene). PCR amplifications were carried out in an Eppendorf thermocycler (Ali *et al.,* 2012) as follows: initial denaturation at 95°C for 3 min followed by 35 cycles of 94°C for 20 s, 58°C for 20 s, 72°C for 40 s, and a final extension at 72°C for 10 min. PCR products were separated on 1.5% agarose gels, stained with ethidium bromide and visualized under UV light using a gel documentation system (Bio-Rad).

ISSR analyses

A set of 10 primers (UBC set, University of British Columbia, Canada) were used to determine genetic diversity of the *A. rabiei* isolates (Table 2). PCR reactions were each performed in 20 µL reaction volume containing 1× PCR buffer, 30 ng sample DNA, 2.5 µM primer, 200 µM of each dNTPs, 2.5 mM $MgCl_2$ and 1.5 units of Taq DNA polymerase (Cinnagene). PCR amplifications were carried out in an Eppendorf thermocycler (Germany), as follows: initial denaturation at 95°C for 2 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at optimum *Ta* for 60s, and extension at 72°C for 90 s. A final extension cycle at 72°C for 10 min followed. PCR products were separated on 1.5% agarose gels, stained with ethidium bromide and visualized under UV light using a gel documentation system (Bio-Rad).

SSR analysis

A set of seven SSR markers (Supplementary Table S1), previously described by Geistlinger *et al.* (2000) and Hayden *et al.* (2008), was used to determine the genetic diversity of the 75 *A. rabiei* isolates. PCR reactions were each performed in 20 µL reaction volume containing 1× PCR buffer, 15 ng sample DNA, 2 µM primer, 200 µM of each dNTP, 2.5 mM $MgCl_2$ and 1.5 units of Taq DNA polymerase (Cinnagene). PCR amplifications were carried out using an Eppendorf thermo cycler (Varshney

et al., 2009) as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of 94°C for 20 s, 53°C for 30 s, 67°C for 30 s. PCR products were separated on 3% metaphor agarose gels, stained with ethidium bromide and visualized under UV light using a gel documentation system (Bio-Rad).

Data analyses

DNA bands obtained with ISSR primers were scored visually for the presence (1) or absence (0) of bands for all the *A. rabiei* isolates. Frequencies of incidence of all polymorphic alleles for each SSR marker were calculated and used for determining statistical parameters. Each band identified as an allele and scored as 'a', 'b', etc., from largest to smallest sized band. Number of alleles (Na), effective number of alleles (Ne), heterozygosity (μe), Shannon-index (I) and polymorphism information content (PIC) were calculated for ISSR and SSR markers using GENALEX 6.1 software (Peakall and Smouse 2006). Marker indices (MI) were obtained by multiplying the average PIC with the effective multiplex ratio. Cluster analysis was conducted on the basis of a neighbor joining (NJ) tree using dissimilarity matrix using DARwin 5.0.128 (Perrier *et al.,* 2003). For the analysis of population structure, a Bayesian model-based analysis was performed using STRUCTURE 2.1 software (Pritchard *et al.,* 2000). A Monte Carlo Markov chain method was used to estimate allele frequencies in each of the K populations and the degree of admixture for each individual plant. The number of clusters was inferred using five independent simultaneous runs with 10,000 replications, using the admixture model and correlated allele frequencies with the K value ranging from 1 to 10.

RESULTS

Pathogenicity tests and mating type distribution

Initially, the pathogenicity of 75 *A. rabiei* isolates was confirmed on two susceptible genotypes. Prominent morphological differences were not seen between isolates. Both chickpea genotypes showed high susceptibility to all the isolates. Symptoms of Ascochyta blight appeared on the Iranian susceptible landrace, Bivanij 2–4 d earlier than on ILC1929 (Supplementary Table S2).

The multiplex PCR using the mating type primers amplified two amplicons (490 bp for Mat1-2 and 700 bp for Mat1-1), across the 75 *A. rabiei* isolates collected from three major chickpea growing provinces (Table 1; Supplementary Figure 1). Both mating types were found in isolates collected from different provinces. Isolates from Kurdistan and Lorestan showed equal distribution for both mating types, while isolates from Kermanshah had different proportions ($P = 0.039$) of the two mating types. This may have resulted from the low number of collected isolates from the other provinces or the geographical differences of the collection sites. Overall, 57% of the isolates were the MAT1-1 mating type and 43% were Mat1-2, but the mating type ratio was not significantly different ($P = 0.204$) from 50:50 (Table 1).

ISSR markers diversity analysis

The ISSR markers revealed clear and scorable bands per primer for all the studied isolates (Supplementary Figure 1). Ten ISSR markers amplified 78 polymorphic bands with an average of 7.8 bands per primer (Table 2). The maximum number of polymorphic bands was obtained using UBC807 (ten bands) and the minimum

Table 2. Numbers of polymorphic bands (NPB), heterozygosity (μe), Shannon indices, polymorphism information content (PIC), and marker indices (MI) of ISSR markers used for defining genetic diversity of 75 *Ascochyta rabiei* isolates.

Primer	Sequence	NPB	μe	Shannon Index (I)	PIC	MI
UBC807	AGAGAGAGAGAGAGAGT	10	0.48	0.58	0.42	4.2
UBC815	CTCTCTCTCTCTCTCTG	8	0.38	0.47	0.38	3.04
UBC818	CACACACACACACACAG	8	0.36	0.46	0.37	2.96
UBC857	ACACACACACACACACYG	7	0.33	0.43	0.34	2.72
UBC822	TCTCTCTCTCTCTCTCA	9	0.42	0.51	0.32	2.88
UBC860	TGTGTGTGTGTGTGTGRA	8	0.31	0.49	0.31	2.48
UBC864	ATG ATG ATG ATG ATG ATG	6	0.29	0.37	0.29	1.74
UBC880	GGAGAGGAGAGGAGA	7	0.30	0.39	0.29	2.03
UBC895	AGAGTTGGTAGCTCT TGA TC	8	0.28	0.42	0.32	2.56
UBC899	CAT GGT GTT GGT CAT TGT TCC A	7	0.28	0.34	0.28	1.96
Mean		7.8	0.34	0.44	0.33	2.65

number using UBC864 (six bands). The PIC values for the ISSR primers ranged from 0.28 to 0.42, with an average of 0.33 per primer. The marker index (MI) of the primers ranged from 1.74 (UBC864) to 4.2 (UBC807) (Table 2). At the population level the average of Shannon-index was 0.44 and heterozygosity (μe) was 0.34. Cluster analysis using ISSR markers grouped the *A. rabiei* isolates into three distinct clusters (Supplementary Figure 2). Cluster I consisted of nine isolates, all collected from Kermanshah. Cluster II comprised 30 isolates that divided into two sub-clusters, and all isolates from Lorestan province grouped closely along with a few isolates from Kermanshah grouped in first sub-cluster. Cluster III contained 36 isolates and divided into two sub-clusters. All isolates in Cluster III originated from Kermanshah and Kurdistan provinces, while all isolates from Kurdistan grouped in a second sub-cluster (Supplementary Figure 2).

SSR markers diversity analysis

Seven SSR loci were used for genetic diversity analysis in the 75 *A. rabiei* isolates, and these showed clear and scorable amplicons (Supplementary Figure 1). The SSR loci analyzed produced 38 alleles with an average of 5.42 alleles per marker. The numbers of alleles ranged from 4 to 7, where the maximum number of alleles was observed in ArH05T and ArH06T (Table 3). The numbers of effective alleles ranged from 4.28 (ArH06T) to 1.97 (ArR01T) with an average value of 3. PIC ranged from 0.48 (ArR01T) to 0.76 (ArH06T) with an average of 0.61. The Shannon's information index (I) ranged from 1.06 (ArA06D) to 2.17 (ArH06T) (Table 3). Gene diversity (μe) ranged from 0.56 to 0.84 with a mean of 0.69. Genetic relationships between the *A. rabiei* isolates based on polymorphic bands from seven SSR markers grouped the isolates into three major clusters (Supplementary Figure 3). Cluster I contained seven isolates that all originated from Kermanshah province. Cluster II comprised 39 isolates divided into two sub-clusters. One sub-cluster contained seven isolates from Kurdistan and the other contained isolates from different regions. Cluster III comprised 29 isolates in two sub-clusters. In this cluster, isolates from Kurdistan and Kermanshah showed distinct patterns from isolates collected from Lorestan province.

Table 3. Numbers of alleles (Na) and effective alleles (Ne), heterozygosity (μe), Shannon indices, polymorphism information content (PIC), and marker indices (MI) of SSR markers used for determining genetic diversity of 75 *Ascochyta rabiei* isolates.

SSR locus	Na	Ne	μe	Shannon index (I)	PIC	MI
ArH02T	6	3.07	0.72	1.68	0.63	3.78
ArH05T	7	4.17	0.81	2.11	0.73	5.11
ArH06T	7	4.28	0.84	2.17	0.76	5.32
ArR12D	5	2.79	0.69	1.47	0.59	2.95
ArA03T	5	2.68	0.63	1.39	0.56	2.80
ArR01T	4	1.97	0.56	1.11	0.48	1.92
ArA06D	4	2.10	0.59	1.06	0.52	2.08
Mean	5.43	3.01	0.69	1.57	0.61	3.42

Genetic structure of Ascochyta rabiei *isolates*

The general dendrogram (Figure 1) that was constructed using the combined data of all molecular markers (ISSRs and SSRs) grouped the *A. rabiei* isolates in two major clusters. Cluster I comprised isolates mostly collected from Kurdistan, and few isolates from north Kermanshah and Lorestan. Cluster II divided into two sub-clusters, one with all isolates originating in Kermanshah, and the other with isolates from Kermanshah, Lorestan and three from Kurdistan. The NJ-cluster analysis showed no relationships with geographical origin, but most isolates in cluster I were from closely associated regions (Kurdistan and North Kermanshah), except for two isolates from Lorestan.

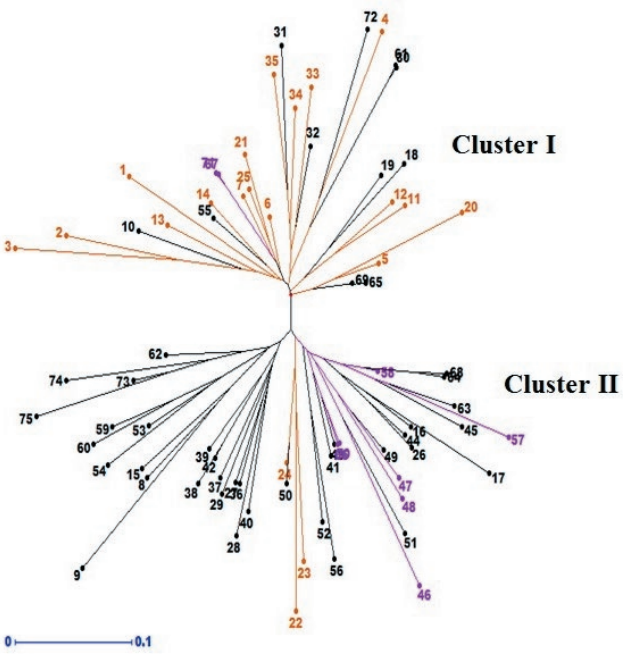

Figure 1. Neighbor joining (NJ) phylogenetic tree from pooled ISSR and SSR molecular data for 75 *Ascochyta rabiei* isolates collected from the western Iran provinces of Kermanshah (black), Kurdistan (red) and Lorestan (blue).

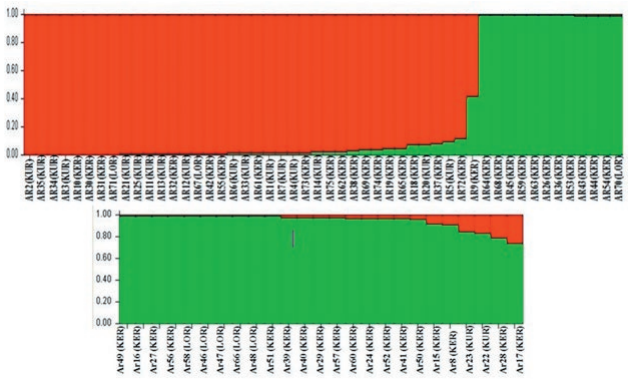

Figure 2. Membership coefficients estimated for *Ascochyta rabiei* isolates, determined from the greatest *a posteriori* likelihood using analysis with STRUCTURE and based on SSR and ISSR markers. The different isolates are represented by individual vertical bars divided into two colored segments (red and green) corresponding to the fraction of membership determined in Populations 1 and 2. The isolates are classified by province; Kermanshah (KER), Kurdistan (KUR) or Lorestan (LOR).

The genetic structure of the 75 *A. rabiei* isolates was further explored using the Bayesian clustering model implemented with the STRUCTURE software. This showed the greatest K value of 2, indicating the presence of two major clusters (Figure 2). The first population (Figure 2, indicated in red) comprised isolates from Kermanshah and Kurdistan, and two isolates (Ar67 and Ar71) from Lorestan. The second population (Figure 2, in green) included isolates from Kermanshah and Lorestan, and some isolates from Kurdistan. These results match those obtained from the NJ analysis (above), without significant correlation of isolate origin and population grouping.

DISCUSSION

Chickpea as cool season crops is mainly cultivated in many semi-arid areas, and seed yields and quality are reduced by major abiotic and biotic stresses (Kanouni *et al.,* 2011; Karami *et al.,* 2015). Ascochyta blight, caused by *A. rabiei*, is one of the most important fungal diseases of chickpea causing yield losses up to 100% in favourable cool and humid climates (Ahmad *et al.,* 2014; Farahani *et al.,* 2019). Variable pathogenicity and genetic structure in Iranian *A. rabiei* populations has been reported previously (Nourollahi *et al.,* 2011; Azizpour and Rouhrazi, 2017; Farahani *et al.,* 2019). Different factors, including sexual recombination, mutation, gene flow, migration, and selection pressure play important roles in population diversity of fungal pathogens (McDonald, 1997; Aghamiri *et al.,* 2015). This can lead to breakdown of host resistance in commercial cultivars (McDonald and Linde, 2002; Rhaiem *et al.,* 2007; Peever *et al.,* 2004; Ali *et al.,* 2012). Characterization of genetic diversity and population structure of fungal populations in disease epidemic areas is fundamentally important for effective disease management, farming practices and design of crop breeding strategies (Azizpour and Rouhrazi, 2017).

The present study was conducted to the define population structure of Iranian *A. rabiei* isolates, through outline of aggressiveness patterns, mating types and molecular marker diversity in isolates of this pathogen collected from three major chickpea growing areas of western Iran. All the assessed isolates showed high aggressiveness in pathogenicity tests. Two identified mating types had similar distributions, except for isolates those collected from Kermanshah province, where Mat1-1 was dominant. Nourollahi *et al.* (2011) showed that majorities (64%) of Iranian isolates collected from two provinces in western Iran were of Mat1-1, but within populations the proportions of each mating type were close to 50%.

Previous studies have showed biased distribution for *A. rabiei* mating types Mat1-1 in Tunisia and MAT1-2 in Turkey (Taylor and Ford, 2007; Rhaiem *et al.,* 2007). Fungi can reproduce in different ways through sexual, asexual or mixed mating systems (McDonald and Linde, 2002). Mixed mating types provide greater capability for overcoming resistance genes in crop resources (McDonald and Linde, 2002; Rhaiem *et al.,* 2007). In fungi, different mating types may be related to pathogenicity and fitness (Zhan *et al.,* 2002; Phan *et al.,* 2003a). However this is remains a question for Iranian populations of the pathogen, as the present study did not show relationship between aggressiveness and mating types in the *A. rabiei* population examined. These results indicate random sexual propagation of *A. rabiei* populations in Iran, has been reported in neighboring countries such as Turkey and Syria (Turkkan and Dolar, 2009; Atik *et al.,* 2011).

Genetic diversity and population structure of 75 *A. rabiei* isolates were analyzed using ten ISSR and seven SSR markers. Both marker types showed high genetic diversity in the *A. rabiei* isolates. The polymorphism of SSR markers showed 4 to 7 alleles with an average 5.42 alleles per locus. In contrast, Nourollahi *et al.* (2011) and Barve *et al.* (2004) reported greater allelic variation in *A. rabiei* populations, which was likely due to the greater number and geographic diversity of isolates used in their studies. The average PIC values in the present study for ISSR markers was 0.33, and for SSR markers was 0.61. The high Shannon diversity index (I) and heterozygo-

sity (gene diversity) of SSR markers also indicated the diverse nature of the collected *A. rabiei* isolates.

NJ-cluster analysis using ISSR and SSR markers grouped *A. rabiei* isolates into three distinct clusters. The molecular data of ISSRs and SSRs were combined, to give the best interpretation of genetic diversity of the isolates examined, which grouped the isolates in two distinct clusters. The Kurdistan climate is Mediterranean, with cold winters, where most chickpea crops are sown in spring (February to March). These contrasts with south Kermanshah and Lorestan, which have a relatively warm climate with moderate winters, and where chickpea crops are mostly sown in autumn (November to December). These differences in chickpea growth conditions may influence the nature of *A. rabiei* isolates and their evolutionary processes. All *A. rabiei* isolates in this study showed high levels of pathogenicity and no significant relationships were detected between pathogenicity of isolates and collection sites.

Knowledge about pathogen genetic diversity and pathogenicity in different geographical areas can help plant breeders, to assist characterization of susceptible/resistant host germplasm against important diseases in chickpea, and for Ascochyta blight in particular (Farahani *et al.,* 2019; Montakhabi *et al.,* 2020). Results from the present study showed no correlation between *A. rabiei* isolate origin and genetic diversity pattern, although most of isolates collected from north Kermanshah and Kurdistan closely grouped in one cluster, and those collected from Lorestan and south Kermanshah grouped in another. This spatial relationship between geographical diversity and *A. rabiei* grouping indicated high genetic diversity within populations and no significant gene flow between distinctly geographical regions. Previous reports have indicated that some *A. rabiei* isolates from distinct continents grouped together, reflecting possible intercontinental migration by movement of pathogens through infected plants carried by seed exchange or agricultural vehicles (Kaiser, 1997; Nourollahi *et al.,* 2011). Iran, Turkey and India are the main centres of origins for chickpea domestication and their fungal pathogens (van der Maesen, 1987; Talebi *et al.,* 2008). The high genetic diversity of *A. rabiei* isolates detected in the present study supports the hypothesis that during long domestication and evolution of chickpea and *A. rabiei* in this area, genetic drift probably occurred. Distinct *A. rabiei* populations from Iran, Turkey and India indicate that pathogen migration rarely occurred, from closely-associated regions (Varsheny *et al.,* 2009; Nourollahi *et al.,* 2011). Abundance of asexual over sexual reproduction occurs in local populations (Morjane *et al.,* 1994; Keller *et al.,* 1997; Aghamiri *et al.,* 2015). Although, limited numbers of *A. rabiei* isolates were analyzed in this study, the present results showed high genetic diversity in isolates collected from different provinces. This study has given a basis for future strategies for breeding programmes and farming practices. Genetic variability and sexual recombination within populations may both increase the risks of increasingly diverse isolates that may overcome resistance in Iranian chickpea germplasm, and also enhance fungicide resistance in *A. rabiei* populations (McDonald and Linde, 2002; Varshney *et al.,* 2009).

In conclusion, the present results have shown interesting aspects of *A. rabiei* populations co-evolved with chickpea in their domestication origins or more probably linked to climate condition associated with differences in cropping seasons. In addition, the necessity of designing appropriate breeding strategies for chickpea improvement in each region is emphasized, due to the host and pathogen genetic differences within specific populations.

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