Genetic Diversity and Mating Type Distribution of *Ascochyta rabiei* Populations Affecting Chickpea

B. Attar[1*], S. Aahmed[2], M. Kayim[3], A. Hamwieh[4], E. Choueiri[5], H.A. Ghannam[6] and A. Alabdullah[7]
(1) School of Natural and Environmental Sciences, Newcastle University, United Kingdom; (2) International Center for Agricultural Research in The Dry Areas (ICARDA), Rabat, Morocco; (3) Department of Plant Protection, Faculty of Agriculture, University of Çukurova, Adana, Türkiye; (4) ICARDA, Cairo, Egypt; (5) Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, Lebanon; (6) Department of Plant Food Legumes Research, Agricultural Research Center, Cairo, Egypt; (7) The John Innes Centre, Norwich Research Park, Norwich, United Kingdom.
[*]Email address of the corresponding author: basematt@hotmail.com

Abstract

Attar, B., S. Aahmed, M. Kayim, A. Hamwieh, E. Choueiri, H.A. Ghannam and A. Alabdullah. 2025. Genetic Diversity and Mating Type Distribution of *Ascochyta rabiei* Populations Affecting Chickpea. Arab Journal of Plant Protection, 43(2): 185-193. https://doi.org/10.22268/AJPP-001313

Chickpea Ascochyta blight, caused by *Ascochyta rabiei,* is the most important disease of chickpea in many countries. Understanding the genetic diversity of the pathogen is very important to identify resistance sources to virulent populations in national and international chickpea breeding programs. Moreover, knowledge on the existence of mating types can help to fine tune Ascochyta blight management practices. Eight simple sequence repeats (SSR) markers were used to estimate the genetic diversity of 96 isolates collected from four countries (Uzbekistan, Syria, Lebanon, and Türkiye). A total of 29 bands were recorded, of which 26 were polymorphic. The study showed that the isolates were genetically diverse and isolates from Uzbekistan showed the highest diversity. Following phylogenetic analysis, the isolates were clustered into four groups and isolates from Uzbekistan were distributed in all groups. The two mating types were found in the four countries and no significant deviation from 1:1 ratio for Türkiye and Uzbekistan isolates.

Keywords: *Ascochyta rabiei*, SSR markers, diversity, mating type.

Introduction

Ascochyta blight (AB) of chickpea, *Ascochyta rabiei* is an important foliar pathogen negatively affecting yield and quality of chickpea crops in many countries (Pande *et al.*, 2005). Development and deployment of resistant chickpea cultivars are the most reliable and economical approach for disease management (Li *et al.*, 2015; Sharma *et al.*, 2016). However, many AB disease outbreaks have been reported associated with the emergence of aggressive pathogen populations (Bar *et al.*, 2021; Imtiaz *et al.*, 2011; Reddy & Kabbabeh, 1985). Sexual reproduction of *A. rabiei* can take place in the presence of the two mating types (MAT1-1 and MAT1-2), it has been reported in many countries (Atik *et al.,* 2011; Attar *et al.,* 2020; Bencheqroun *et al.,* 2021; Getaneh *et al.,* 2021; Navas-Cortés *et al.,* 1998b). Sexual reproduction plays a role as the primary source of inoculum and contributes in genetic diversity to the pathogen population.

Genetic diversity of *A. rabiei* populations was reported from different countries using differential chickpea genotypes and molecular markers. High genetic variation was reported in Italy (Fischer *et al.*, 1998), Spain (Navas-Cortés *et al.*, 1998a), South Asia, West Asia and north Africa (Bayraktar *et al.*, 2007; Bencheqroun *et al.*, 2021; Farhani *et al.*, 2021; Hussain & Malik, 1991; Jamil *et al.*, 2000; Nourollahi *et al.*, 2011; Morjane *et al.*, 1994; Santra *et al.*, 2001; Udupa *et al.*, 1998), America, Australia (Bar *et al.*, 2021; Phan *et al.*, 2003; Vail & Banniza, 2009) and East Africa (Getaneh *et al.*, 2021). The ICARDA chickpea breeding program is developing germplasm that can be used in many countries where AB is a major production threat.

Most of the studies describing genetic variation of *A. rabiei* were reported from individual countries. The objective of this study was to assess the genetic diversity and mating type distribution of pathogen isolates collected from four countries that represent different environmental conditions for chickpea production.

Materials and Methods

Isolates collections

Surveys were conducted in chickpea fields and research centers in Uzbekistan, Türkiye, Lebanon, and historical collection from Syria (Table 1). Infected stem samples from Lebanon, Syria and Türkiye were collected from winter planted crops in farmer and research fields, whereas samples from Uzbekistan were collected from spring planted crops from farmers' fields. In addition to field collected isolates, 10 reference isolates collected from Syria and used to screen breeding lines developed by ICARDA chickpea breeding program were included in the mating type and diversity analyses (Table 1).

Pathogen isolation

Infected chickpea stems were surface sterilized by soaking in 0.25% NaOCl (1/10 dilution of product containing 2.5 % NaOCl) for 5 min, washed in sterilized distilled water for 1 min and dried on paper towels before being transferred to chickpea seed meal dextrose agar (CSMDA: 4% chickpea

https://doi.org/10.22268/AJPP-001313

flour; 2% dextrose; 2% agar in 1 L. distilled water). For reference isolates and pathotypes, mycelial plugs were used to initiate new cultures on CSMDA. Petri dishes were incubated at 21-23°C with a 16 h/8 h light/dark photoperiod. Seven days after incubation, single spore isolates were prepared from each isolate by making three dilutions (1:10, 1:100, 1:1000) of spore suspension from the isolate in sterilised distilled water. The dilutions were then transferred to three Petri dishes containing water agar medium (each dilution in one Petri dish), and the Petri dishes were incubated for 24 h under the same temperature and light regime mentioned above. After 24 h, the Petri dish containing the lowest spore concentration was inspected under stereomicroscope, one germinated spore was transferred to a new Petri dish containing CSMDA by using sterilised needle, and this single spore isolate was allowed to grow for seven days. A small plug of actively growing single spore culture of each isolate was transferred to microcentrifuge tubes containing 1.5 ml of sterilized deionized water and kept at -20°C until further use. For genetic diversity and mating type analyses, 96 and 78 isolates were used, respectively.

DNA extraction

Mycelial disks (4 × 5 mm diameter) were added to 250 ml glass flasks containing 50 ml potato dextrose broth. The isolates were incubated for six days using a rotary shaker (50 rpm) at 21-23°C with a 16h:8h light/dark photoperiod. The mycelium was harvested by using sterilized cheese cloth together with vacuum filtration before being freeze-dried for five days and stored at -30°C.

Freeze-dried mycelium (50 mg) was ground to powder in ceramic mortars containing liquid nitrogen (Chongo *et al.*, 2004). The powder was resuspended in 1200 µl CTAB buffer (Atik *et al.*, 2011), transferred to 2 ml Eppendorf tubes and incubated at 65°C for 60 min. Chloroform:isoamyl alcohol (600 µl of 24:1 v/v) was added and the samples shaken by hand gently for 15 min before centrifugation at 13,000 rpm for 20 min. The supernatant was recovered and mixed with 700 µl of isopropanol (4°C), with gentle shaking for 2 min, followed by incubation at -20°C for 10 min. The samples were centrifuged at 13000rpm for 15 min and the supernatant was discarded. The resulting pellet was washed twice by adding 1000 µl of 70% molecular grade ethanol and air dried for three hours. Finally, the pellet was dissolved in 100µl of TE buffer. The quantity and quality of the extracted DNA were assessed following separation using agarose gel electrophoresis, and staining using ethidium bromide.

Microsatellite analysis

The genetic diversity of *A. rabiei* isolates was investigated using eight microsatellite primers pairs taken from (Geistlinger *et al.,* 2000; Nourollahi *et al.,* 2011; Rhaiem *et al.,* 2008). (Table 2). PCR reactions were carried out in a total volume of 25 µl containing 2 µl of DNA template (15 ng/µl), 2.5µl of each primer (10mM), 1µl of 2mM dNTP mix, 2.5µl of buffer (15mM $MgCl_2$ final) and 1µl of Taq DNA polymerase (Promega). Cycling conditions consisted of an initial denaturation at 95°C for 2 min followed by 35 cycles of 94°C for 20 s, 57°C for 25 s, 67°C for 23 s, and a final extension at 72°C for 5 min. Amplicons were separated in 8% polyacrylamide gels, stained with ethidium bromide and size of fragments were estimated by comparison to 100 bp DNA ladder.

SSR data analysis

The binary format "0" or "1" allelic data matrix was used to create an allele-size coded matrix for the eight markers or loci, used to calculate and plot number of alleles, number of private alleles, allele frequencies and allelic patterns across populations using GenAlEx 6.5 (Peakall & Smouse, 2006). To measure the polymorphism of SSR markers, the polymorphism information content (PIC) values were calculated using the formula of Liu *et al.* (2011):

$$PIC = 1 - \sum_{J=1}^{n} P_{ij}^2$$

A letter coded matrix for the eight markers was created based on the allelic data matrix of "0" or "1" to perform the population genetic analysis using POPGENE version 1.32 (Yeh *et al.*, 1999), including number of observed alleles (Na), and number of effective alleles (Ne). Nei's genetic diversity (h) and Shannon's information index (I) were computed based on the allele frequencies obtained. Sample homozygosity (F) was calculated using the overall Ewens-Watterson Test for Neutrality, using 1000 simulated samples (Manly, 1985). GenAlEx 6.5 was used to estimate the Analysis of Molecular Variance (AMOVA) (Liu *et al.*, 2011). The binary format "A" or "C" allelic data matrix was used to perform phylogenetic analysis. Phylogenetic trees were constructed (Kumar *et al.*, 2018) using the UPGMA statistical method (Sneath & Sokal, 1973) within MEGA 7 and evolutionary distances were computed using the p-distance method (Nei & Kumar, 2000). The robustness of the nodes of the phylogenetic tree were assessed from 1000 bootstrap replicates. The genetic identity matrix was calculated using BioEdit Sequence Alignment Editor Ver.7.1.946v (Hall, 1999). Principal component analysis (PCA) was performed using the prcomp (R) function using the transformed allelic data matrix with allele sizes of eight markers across isolates.

Table 1. Summary of isolates information used for the study.

Country	Locations	Year of collection	No. of isolates
Lebanon	Bekaa-Taanayel	2013	1
	Baalbek-Kfar Dan	2013	3
	Zahleh-Terbol	2013	9
Syria	Latakia-Jableh	2012	5
	Aleppo-Tel Hedya	2012	10
	NA	1982	6
	NA	1980	2
	NA	1995	1
	Kaljebrine	2004	1
Türkiye	Adana-Balcali	2013	14
	Adana-Sofulu	2013	9
	Sanliurfa-Adiyaman	2013	2
Uzbekistan	Tashkent-Parkent	2012	4
	Samarqand-Payarik	2012	12
	Jizzakh-Gallaorol	2012	5
	Jizzakh-Bakhmal	2012	12
Total			**96**

Table 2. Microsatellite primers used to test the diversity of *A. rabiei* populations collected from four countries.

Primer	Sequence 5'-3'	Expected size (bp)
ArA03T-F	TAGGTGGCTAAATCTGTAGG	379
ArA03T-R	CAGCAATGGCAACGAGCACG	
ArA06T-F	CTCGAAACACATTCCTGTGC	162
ArA06T-R	GGTAGAAACGACGACGAATAGGG	
ArR08T-F	GTGAGCTACTTAGCACCTCTGT	365
ArR08T-R	GCTGTGTCGGGTTGAGTAAC	
ArH02T-F	CTGTATAGCGTTACTGTGTG	365
ArH02T-R	TCCATCCGTCTTGACATCCG	
ArH05T-F	CATTGTGGCATCTGACATCAC	197
ArH05T-R	TGGATGGGAGGTTTTTGGTA	
ArH06T-F	CTGTCACAGTAACGACCAACG	167
ArH06T-R	ATTCCAGAGAGCCTTGATTG	
ArR04D-F	GCTTAGTTGGGCTTGTTACTT	160
ArR04D-R	CACACCTCTCTACCAATGAGAC	
ArH08D-F	ACTTTGACTTCGACTTCGACT	162
ArH08D-R	GTGGAAGAGAAGTGGATTGAC	

Mating type analysis

Mating type was determined using multiplex MAT-specific PCR with three primers. MAT1-1 specific primer Sp21 (ACAGTGAGCCTGCACAGTTC), MAT1-2 specific primer Tail 5 (CGCTATTTTATCCAAGACACACC) and flanking region-specific primer Com1 (GCATGCCATATCGCCAGT) were combined in equal concentrations in a single PCR (Barve *et al.*, 2003). Multiplex PCR reactions contained 2µl of DNA template (15ng/µl), 1µl of 10x PCR buffer (containing 15mM $MgCl_2$), 1µl of 2mM NTPs mix, 1µl of Taq Polymerase (Invitrogen Life Technologies), 2µl of each primer (10mM), and the final volume of multiplex PCR was completed to 25µl by using ultra-pure molecular grade water. Cycling was performed using a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA) with an initial denaturation step of 95°C for 5 min, followed by 44 cycles of 95°C, for 30 s, 60°C for 30 s each and 72°C for 1 min, with a final extension of 72°C for 5 min. Amplified products were separated by electrophoresis on a 1.5% agarose gel stained by ethidium bromide and photographed under UV illumination.

Results

Pathogen genetic diversity

Five SSR markers (ArA03T, ArA06T, ArR08T, ArH06T and ArR04D) were found to be polymorphic, with allele numbers ranging from 2 for ArA06T to 12 for ArA03T with an average of 5.20 per locus (Table 3). A total of 29 alleles were amplified from the DNA of 96 *A. rabiei* isolates used in this study. The effective number of alleles for polymorphic loci ranged from 1.44 (ArA06T) to 5.99 (ArA03T). The PIC values varied widely from 0.00 (ArH02T, ArH05T and ArH08D) to 0.83 (ArA03T), with an average of 0.37 indicating the existence of genetic diversity among pathogen isolates. The average estimated gene flow (Nm) for the 5 polymorphic loci among pathogen populations was 1.06 ($1 < Nm < 4$). The allele frequency ranged from 0.01 in the ArA03T allele with band size of 690 bp (ArA03T-690) to 0.81 in the allele ArA06T-600 (Figure 1b). Eight private alleles were found in isolates collected from Uzbekistan (7 private alleles) and one from reference isolate (Figure 1-A). Four loci (ArA06T, ArR08T, ArH06T and ArR04D) were common with at least one allele in all populations (Figure 1-A). The highest average number of observed alleles and effective alleles (3.13±0.97 and 2.15±0.49, respectively) were found within the *A. rabiei* isolates collected from Uzbekistan (Table 4). Shannon's information index (I) and Nei's gene diversity (h) of the different populations ranged from 0.23 to 0.69 (average 0.43) and from 0.12 to 0.37 (average = 0.25), respectively, indicating significant genetic diversity among the studied populations. Isolates from Uzbekistan showed the highest values for Shannon's information index (0.69±0.24) and Nei's gene diversity (0.37±0.12) compared with other isolates. The lowest values of the two diversity parameters (I= 0.23±0.10 and h: 0.12±0.06) were observed from isolates collected from Türkiye (Table 4).

Nei's genetic identity ranged from 0.73 (between Uzbekistan and Türkiye isolates) to 0.90 (between Syria and Lebanon and between Syria and Türkiye pathogen isolates). The highest genetic distance was observed between isolates from Türkiye and Uzbekistan (0.31), Lebanon and Uzbekistan (0.23), whereas the minimum genetic distance (0.10) was between isolates collected from Syria and Lebanon as well as isolates from Türkiye and Syria (Table 5). According to the identity matrix from the 8 SSR markers, our study showed 138 cases of 100% genetic similarity among the isolates studied. Although most of these cases were between isolates within a country, we reported 100% genetic similarity between samples from Syria and Türkiye, Syria, and Lebanon and between Lebanon and Türkiye.

The analysis of Molecular Variance (AMOVA) showed that variation among populations was low (29%), and very high (66%) within populations (Table 6).

Table 3. Summary of genetic variation statistics for the eight SSR loci.

Locus	Sample size	Na	Ne	PIC	Nm	F±se
ArA03T	96	12	5.99	0.83	0.91	0.22±0.01
ArA06T	96	2	1.44	0.30	1.97	0.80±0.03
ArR08T	96	3	2.64	0.62	0.39	0.68±0.03
ArH02T	96	1	1.00	0.00	-	-
ArH05T	96	1	1.00	0.00	-	-
ArH06T	96	4	2.02	0.50	1.54	0.57±0.03
ArR04D	96	5	3.63	0.72	2.08	0.49±0.03
ArH08D	96	1	1.00	0.00	-	-
Mean± SE		3.63±1.31	2.34±0.62	0.37±0.12	1.06±0.32	40.80±0.03

Na = Observed number of alleles; Ne = Effective number of alleles; PIC = Polymorphism information content; Nm = Estimates gene flow; F=Sample homozygosity.

Table 4. Genetic diversity of *A. rabiei* isolates using eight microsatellite loci[1,2].

Population	N	Na	Ne	I	h	uh	P%
Uzbekistan	33	3.13±0.97	2.15±0.49	0.69±0.24	0.37±0.12	0.38±0.12	62.5
Lebanon	13	1.63±0.32	1.38±0.20	0.30±0.15	0.18±0.09	0.20±0.10	37.5
Syria	15	1.88±0.30	1.62±0.26	0.44±0.16	0.28±0.10	0.30±0.10	62.5
Türkiye	25	1.88±0.40	1.19±0.10	0.23±0.10	0.12±0.06	0.13±0.06	50.0
Reference isolates	10	2.13±0.40	1.71±0.28	0.51±0.17	0.30±0.10	0.34±0.11	62.5
Mean±SE	19.2±1.37	2.13±0.24	1.61±0.14	0.43±0.08	0.25±0.04	0.27±0.05	55.0±5.00

[1]N = Sample size; Na = Observed number of alleles; Ne = Effective number of alleles; I = Shannon's Information Index; h = Nei's gene diversity; uh = Unbiased gene diversity; P% = Polymorphic Loci. SE= standard error.
[2]Referece isolates: Six races and four pathotypes.

Table 5. Nei's genetic identity and genetic distance among pathogen populations using SSR markers[1,2].

Population	Uzbekistan	Lebanon	Syria	Türkiye
Uzbekistan	ID	0.79	0.85	0.73
Lebanon	0.23	ID	0.90	0.83
Syria	0.16	0.10	ID	0.90
Türkiye	0.31	0.19	0.11	ID

[1]Nei's genetic identity is above diagonal and Nei's genetic distance is below diagonal. [2]ID = refers to identical populations.

Table 6. Analysis of Molecular Variance (AMOVA) for variation between and within populations of pathogen isolates.

Source	DF	SS	MS	Est. Var.	%
Among regions	1	18.33	18.33	0.08	5
Among populations	3	25.51	8.50	0.49	29
Within populations	91	99.63	1.10	1.10	66
Total	95	143.46		1.67	100

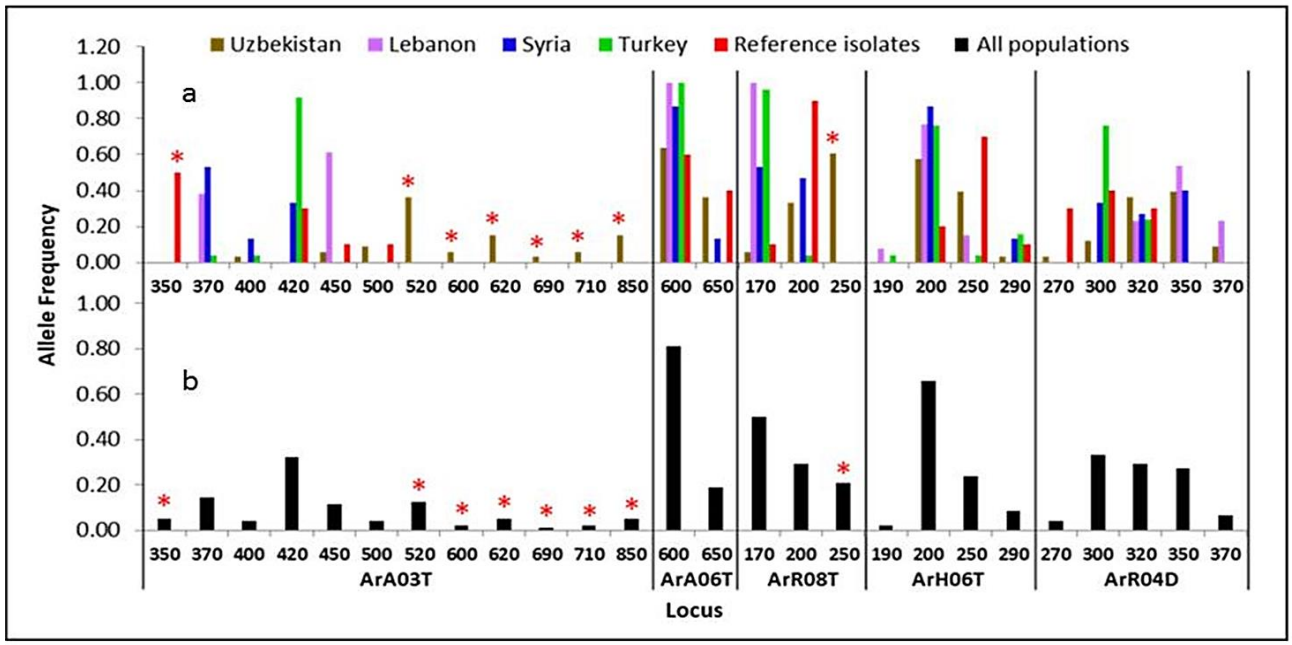

Figure 1. Allele frequency and private alleles across the polymorphic loci in *A. rabiei* populations. Allele frequency is shown for each of the *A. rabiei* populations (A) and as average of all populations (B). Red asterisk refers to private alleles.

Phylogenetic analysis

A phylogenetic tree was constructed using MEGA 7 based on the binary format "A" or "C" allelic data matrix of the 96 *A. rabiei* isolates. The relationships among different isolates were calculated using the UPGMA statistical method with evolutionary distances computed using the p-distance method. The phylogenetic tree consisted of two main clusters with each subdivided into two distinct groups (Figure 2). The first cluster, branched into group A and group B, contained 58 isolates that are mainly (86%; 50 isolates) from the Middle East countries. The second cluster, branched into group C and group D, contained more than 75% of the isolates from Uzbekistan. Group A was divided in two sub-groups (sub-group-a1 and sub-group-a2) and contained most of the isolates from Türkiye and Syria (24 and 10 isolates, respectively). Moreover, Group A mainly sub-group-a2 (Figure 2) consisted of isolates from Lebanon (5 isolates) and Uzbekistan (7 isolates). Group B contained mainly isolates from Lebanon clustered with one isolate from Uzbekistan collected from Payarik in Samarqand province and the reference pathotype 3. Most of the isolates from Uzbekistan (25 isolates) were clustered in groups C and D. Interestingly, most of the reference *A. rabiei* races (Race 2, Race 3, Race 4 and Race 6), originated from Syria and Lebanon, were grouped together in D with five isolates from Uzbekistan. Moreover, 20 isolates from Uzbekistan, five from Syria and one isolate from Türkiye were assigned to group C.

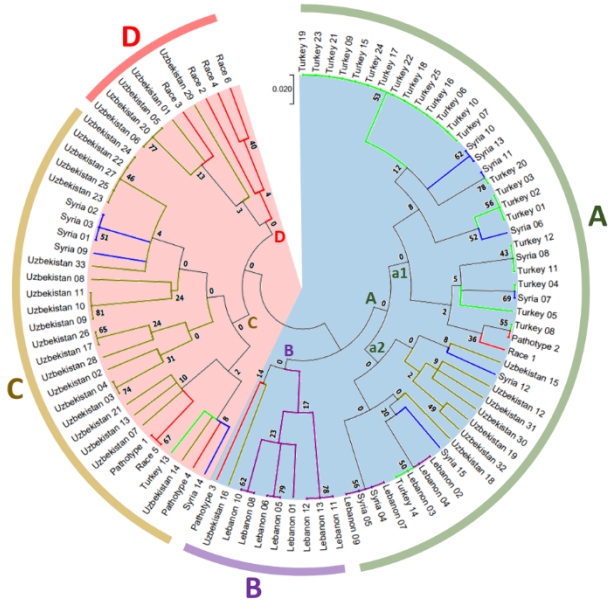

Figure 2. Phylogenetic tree showing the relationship among pathogen isolates collected from four different countries. Numbers on nodes refer to the bootstrap values.

Principal component analysis

PCA classified the isolates studied into two main groups that fit with the region of origin to some extent (Figure 3). The amount of variance accounted for the two-dimensional plot is 85.04 % of PC1 and 5.9% of PC2. Moreover, PCA clustering showed a high level of variation within the Uzbekistan pathogen population (Figure 3).

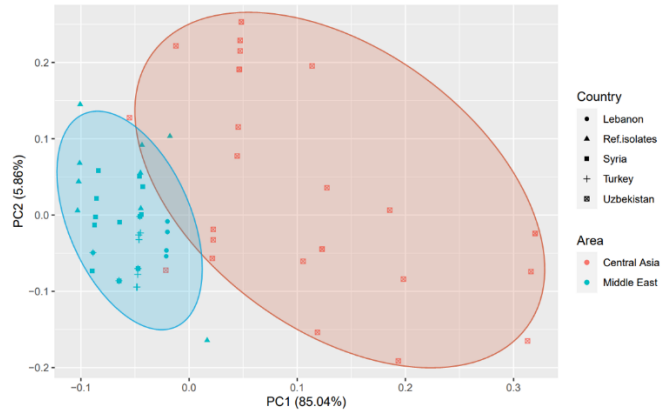

Figure 3. Relationships among *A. rabiei* isolates as revealed by principal component analysis.

Mating type distribution

Using the mating type markers, both mating types were observed amongst the isolates collected from each country (Table 7). Chi-squared analysis for MAT frequencies was not significantly different from 1:1 for isolates collected from Uzbekistan and Türkiye but was significant for isolates collected from Syria and Lebanon.

Table 7. Distribution of mating types for *A. rabiei* isolates collected from four countries.

Population	No. of isolates	MAT1-1	MAT1-2	X2	P
Uzbekistan	33	17	16	0.150	0.69
Türkiye	21	10	11	0.048	0.80
Syria	14	11	3	4.560	0.03
Lebanon	10	1	9	6.400	0.01
Total	**78**	**39**	**39**	**0.000**	**1.00**

Discussion

Chickpea Ascochyta blight is a threat to many countries where chickpea is traditionally produced by small holder and commercial farmers. In addition, there are reports that the disease is becoming increasingly important in Argentina and China where chickpea production is increasing (Chen *et al.*, 2017; Viotti *et al.*, 2012). Monitoring pathogen diversity is very important for developing resistant chickpea germplasm and *A. rabiei* has been reported infecting widely used chickpea cultivars signaling an apparent change in pathogenicity in some countries.

Simple sequence repeat (SSR) markers (microsatellites) have been used to better understand the genetic diversity of *A. rabiei* populations in many countries. Most of the SSR markers used in our study were polymorphic with the number of alleles ranging from 2-12, similar to isolates reported in other studies (Atik *et al.*, 2013; Bencheqroun *et al.*, 2021). In contrast, ArH02T, and ArH05T, markers were polymorphic in other *A. rabiei* populations (Atik *et al.*, 2013; Farhani *et al.*, 2021; Getaneh *et al.*, 2021). Based on genetic parameters like the Shannon

index and Nei's gene diversity the isolates studied showed high genetic diversity. Interestingly, this study showed that Pathotype 1 and Race 5 were identical. Moreover, one isolate collected from a farmer field in Balcali in Türkiye was identical to Pathotype 2. One of the notable cases was the full similarity among 14 Turkish isolates collected from different areas.

The average estimated gene flow (Nm) for the 5 polymorphic loci in the *A. rabiei* populations was 1.06 ($1 < Nm < 4$) indicating that there is no extensive gene flow among the populations, however it is enough to prevent populations from diverging because of genetic drift (Wright, 1931). There is a high possibility of seed exchange among Syria, Lebanon and Türkiye mainly for winter planted chickpea which could explain the exchange of isolates through infected seeds (Kaiser, 1997).

Using UPGMA, the isolates were grouped into four populations where those collected from Uzbekistan were distributed in all groups. Isolates collected from Syria and Türkiye were in groups C and A whereas most of the reference races were in group D. The reference pathotypes were in group A (pathotype-2), B (pathotype 3) and C (pathotypes 1 and 4). However, the reference pathotypes 3 and 4 are virulent on chickpea differentials and pathotypes 1&2 are low in their levels of virulence (Imtiaz *et al.*, 2011). The PCA grouped the isolates into two where all isolates collected from Syria, Lebanon, Türkiye and the reference isolates were grouped together, and the isolates from Uzbekistan formed a separate group. A recent publication showed that isolates collected from Morocco and Syria were grouped in a separate cluster using structural analysis (Bencheqroun *et al.*, 2021).

Sexual reproduction is one of the drivers of population shifts in pathogens that can affect the performance of chickpea AB resistance cultivars and commonly used fungicides to control the disease (Owati *et al.*, 2017; Wise *et al.*, 2008). The two mating types were identified from all populations tested in this study and for Türkiye and Uzbekistan a 1:1 ratio was observed indicating a high probability of sexual reproduction if weather conditions are favorable (Navas-Cortés *et al.*, 1998b). Although many countries reported the presence of both mating types, this is the first report for the presence of both in Uzbekistan. The presence of the two mating types is reported from many countries and studies showed that ascospores germinate under a wide range of temperature and water potential and causes high disease severity over asexual spores (Trapero-Casas & Kaiser, 2007). A small number of studies showed that sexual reproduction can create isolates with high aggressiveness in affecting chickpea cultivars (Attar *et al.*, 2020; Peever *et al.*, 2012) Releases of ascospores from infected overwintering straw can initiate early infection; affect crop rotation; fungicide seed treatment and adoption of conservation agriculture where chickpea is included in the rotation (Salotti & Rossi, 2021).

It can be concluded from this study that sustainable production of chickpea by farmers is usually threatened by AB in many countries. The most effective means of managing the disease is through the development of resistant cultivars. However, resistance breaking *A. rabiei* populations are reported in many countries. In this study, high within population genetic diversity was observed using polymorphic SSR markers. The pathogen population from the Middle East was grouped separately from that of isolates from Uzbekistan. The two mating types were identified from all countries and this result will be useful to understand how sexual reproduction affects disease management practices. The genetic diversity reported in this study needs to be supported with field phenotyping using AB differential genotypes through network testing using spring and winter chickpea planting.

Acknowledgments

This study was partly supported by International Center for Agricultural Research in the Dry Areas (ICARDA) project on “Enhancing Food Security in Arab Countries” jointly funded by the Arab Fund for Economic and Social (AFESD-Kuwait: Grant # 200124), and Organization of the Petroleum Exporting Countries (OPEC) Fund for International Development (OFID-Vienna-Austria: Grant # 200153).
The authors acknowledge the support provided by Dr. Michel Afram, Director General of Lebanese Agricultural Research Institute (LARI). The assistance of Fouad Jreijiri, at LARI is also acknowledged. We also thank the support provided by Ali Endes, Faculty of Agriculture, Çukurova University.

الملخص

عطار، باسم، سعيد أحمد، مقدس كايم، علاء الدين حموية، إيليا الشويري، هند غنام وعبد القادر العبد الله. 2025. التنوع الوراثي وتوزع النمط التزاوجي لمجتمعات الفطر *Ascochyta rabiei* المسبب لمرض اللفحة على الحمص. مجلة وقاية النبات العربية، 43(2): 185−193.
https://doi.org/10.22268/AJPP-001313

يعدّ مرض لفحة الأسكوكيتا من أهم الأمراض التي تصيب محصول الحمص في العديد من البلدان. تعدّ دراسة التنوع الوراثي للفطر المسبب لهذا المرض أمراً مهماً للغاية لتحديد مصادر المقاومة للسلالات ذات الشراسة المرتفعة في برامج التأصيل الوطنية والدولية للحمص. علاوةً على ذلك، إن المعرفة بوجود الأنماط التزاوجية المختلفة للفطر قد يساعد في تصميم برامج الإدارة المتكاملة للمرض. تمّ استخدام ثمانية واسمات جزيئية من نوع التكرارات البسيطة المتسلسلة (SSR) لدراسة التنوع الوراثي لـ 96 عزلة من الفطر، متحصل عليها من أربع دول (أوزباكستان، سورية، لبنان وتركيا). تمّ تسجيل إجمالي 29 نطاقاً، منها 26 نطاقاً متعدد الأشكال. أظهرت الدراسة أن العزلات كانت متنوعة وراثياً، وكانت عزلات أوزباكستان أكثرها تنوعاً وتبايناً. بعد التحليل الوراثي، تمّ تجميع العزلات في أربع مجموعات، وتوزعت عزلات

أوزبكستان في جميع المجموعات. تمّ العثور على نوعي الأنماط التزاوجية في الدول الأربع، ولم يكن هناك ثمّة اختلافات معنوية عن النسبة 1:1 في عزلات تركيا وأوزباكستان.

كلمات مفتاحية: *Ascochyta rabiei*، SSR markers، التنوع، النمط التزاوجي.

عناوين الباحثين: باسم عطار[1*]، سعيد أحمد[2]، مقدس كايم[3]، علاء الدين حموية[4]، إيليا الشويري[5]، هند غنام[6] وعبد القادر العبد الله[7]. (1) معهد العلوم الطبيعية والبيئية، جامعة نيوكاسل، المملكة المتحدة؛ (2) المركز الدولي للبحوث الزراعية في المناطق الجافة (إيكاردا)، الرباط، المغرب؛ (3) قسم وقاية النبات، كلية الزراعة، جامعة شوكوروفا، أضنة، تركيا؛ (4) إيكاردا، القاهرة، مصر؛ (5) قسم وقاية النبات، محطة الأبحاث العلمية الزراعية، تل عمارة، لبنان؛ (6) قسم بحوث المحاصيل البقولية الغذائية، مركز البحوث الزراعية، القاهرة، مصر؛ (7) مركز جون إنز، نورويتش، المملكة المتحدة. *البريد الإلكتروني للباحث المراسل: basematt@hotmail.com

References

Atik, O., S. Ahmed, M.M. Abang, M. Imtiaz, A. Hamwieh and M.M. Yabrak. 2013. Pathogenic and genetic diversity of *Didymella rabiei* affecting chickpea in Syria. Crop Protection, 46:70-79. https://doi.org/10.1016/j.cropro.2012.12.012

Atik, O., M. Baum, A. El-Ahmed, S.Ahmed, M.M. Abang and A. Hamwieh. 2011. Chickpea Ascochyta Blight: Disease Status and Pathogen Mating Type Distribution in Syria. Journal of Phytopathology, 159(6):443-449. https://doi.org/10.1111/j.1439-0434.2011.01788.x

Attar, B., S. Ahmad, M. Kayim, E. Choueiri, H.A. Ghannam and A. Hamwieh. 2020. Role of sexual reproduction in the aggressiveness of *Didymella rabiei* affecting chickpea. Arab Journal for Plant Protection, 38(1):17–24. https://doi.org/10.22268/AJPP-038.1.017024

Bar, I., P.T. Sambasivam, J. Davidson, L.M. Farfan-Caceres, R.C. Lee and R. Ford. 2021. Current population structure and pathogenicity patterns of *Ascochyta rabiei* in Australia. Microbial Genomics, 7:000627. https://doi.org/10.1099/mgen.0.000627

Barve, M.P., T. Arie, S.S. Salimath, F.J. Muehlbauer and T.L. Peever. 2003. Cloning and characterization of the mating type (MAT) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a MAT phylogeny of legume-associated Ascochyta spp. Fungal Genetics and Biology, 39(2):151-167. https://doi.org/10.1016/S1087-1845(03)00015-X

Bayraktar, H., F.S. Dolar and M. Tör. 2007. Determination of genetic diversity within *Ascochyta rabiei* (Pass.) Labr., the cause of Ascochyta blight of Chickpea in Turkey. Journal of Plant Pathology, 89(3):341-347.

Bencheqroun, S.K., S. Ahmed, M. Imtiaz, A. Hamwieh, S.M. Udupa and Z. Kehel. 2021. Pathogen diversity and mating types of *Didymella rabiei* isolates collected from Morocco. Current Plant Biology, 29:100231. https://doi.org/10.1016/j.cpb.2021.100231

Chen, X., J. Ge, D. Ma, L. Ma, W. Liu and S.Qiang. 2017. Characterization and Identification of an epidemic strain of *Ascochyta rabiei* on Chickpeas in Northwest China. Journal of Phytopathology, 165(6):355-360. https://doi.org/10.1111/jph.12568

Chongo, G., B.D. Gossen, L. Buchwaldt, T. Adhikari and S.R. Rimmer. 2004. Genetic diversity of *Ascochyta rabiei* in Canada. Plant Disease, 88(1):4-10. https://doi.org/10.1094/PDIS.2004.88.1.4

Farhani, S., R. Talebi, M. Maleki, R. Mehrabi and H. Kanouni. 2021. Mating type distribution, genetic diversity and population structure of *Ascochyta rabiei*, the cause of Ascochyta blight of chickpea in western Iran. Phytopathologia Mediterranea, 60(1):3-11. https://doi.org/10.36253/phyto-11616

Fischer, C., A. Porta-Puglia and W. Barz. 1998. RAPD Analysis of pathogenic variability in *Ascochyta rabiei*. Journal of Phytopathology, 143(1):601-607. https://doi.org/10.1111/j.1439-0434.1995.tb00208.x

Geistlinger, J., K. Weising, P. Winter and G. Kahl. 2000. Locus-specific microsatellite markers for the fungal chickpea pathogen *Didymella rabiei* (anarmoph) *Ascochyta rabiei*. Molecular Ecology, 9(11):1939-1941. https://doi.org/10.1046/j.1365-294x.2000.01092-13.x

Getaneh, G., T. Tefera, E. Lemessa, S. Ahmed and D. Villinger. 2021a. Distribution and mating type characterization of chickpea blight (*Didymella rabiei* (Kov.) v. Arx) in Ethiopia. Current Biology, 28:100220. https://doi.org/10.1016/j.cpb.2021.100220

Getaneh, G., T. Tefera, F. Lemessa, S. Ahmed, T. Fite and J. Villinger. 2021b. Genetic Diversity and Population Structure of *Didymella rabiei* Affecting Chickpea in Ethiopia. Journal of Fungi, 7(10):820. https://doi.org/10.3390/jof7100820

Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium, 41:95–98.

Hussain, S. and B.A. Malik. 1991. Pathogenic variability in *Ascochyta rabiei* in Pakistan. International Chickpea Newsletter, 24:36–37.

Imtiaz, M., M.M. Abang, R.S. Malhotra, S. Ahmed, B. Bayaa and M. Baum. 2011. Pathotype IV, a new and highly virulent pathotype of *Didymella rabiei*, causing Ascochyta blight in chickpea in Syria. Plant Disease, 95(9):1192-1192. https://doi.org/10.1094/PDIS-04-11-0333

Jamil, F.F., N. Sarwar, M. Sarwar, J.A. Khan, J. Geistlinger and G. Kahl. 2000. Genetic and pathogenic diversity within *Ascochyta rabiei* (Pass.) Lab. populations in Pakistan causing blight of chickpea (*Cicer arietinum* L.). Physiological and Molecular Plant Pathology, 57(6):243-254. https://doi.org/10.1006/pmpp.2000.0303

Kaiser, W.J. 1997. Inter- and intranational spread of ascochyta pathogens of chickpea, faba bean, and lentil. Canadian Journal of Plant Pathology, 19(2):215-224. https://doi.org/10.1080/07060669709500556

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6):1547-1549. https://doi.org/10.1093/molbev/msy096

Li, H., M. Rodda, A. Gnanasambandam, M. Aftab, R. Redden and A.T. Slater. 2015. Breeding for biotic stress resistance in chickpea: progress and prospects. Euphytica, 204:257-288. https://doi.org/10.1007/s10681-015-1462-8

Liu, P., Y. Que and Y.B. Pan. 2011. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech, 13:129-136. https://doi.org/10.1007/s12355-011-0077-1

Manly, B.F.J. 1985. The Statistics of Natural Selection. Chapman and Hall, London, UK. 484 pp. https://doi.org/10.1007/978-94-009-4840-2

Morjane, H., J. Geistlinger, M. Harrabi, K. Weising and G. Kahl. 1994. Oligonucleotide fingerprinting detects genetic diversity among *Ascochyta rabiei* isolates from a single chickpea field in Tunisia. Current Genetics, 26:191-197. https://doi.org/10.1007/BF00309547

Navas-Cortés, J.A., E. Pérez-Artés, R.M. Jiménez-Diaz, A. Llobell, B.W. Bainbridge and J.B. Heale. 1998a. Mating type, pathotype and RAPDs analysis in *Didymella rabiei*, the agent of ascochyta blight of chickpea. Phytoparasitica, 26:199-212. https://doi.org/10.1007/BF02981435

Navas-Cortés, J.A., A. Trapero-Casas and R.M. Jiménez-Díaz. 1998b. Influence of relative humidity and temperature on development of *Didymella rabiei* on chickpea debris. Plant Pathology, 47(1):57-66. https://doi.org/10.1046/j.1365-3059.1998.00208.x

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York, USA. 348 pp.

Nourollahi, K., M. Javannikkhah, M.R. Naghavi, J. Lichtenzveig, S.M. Okhovat and S.R. Ellwood. 2011. Genetic diversity and population structure of *Ascochyta rabiei* from the western Iranian Ilam and Kermanshah provinces using MAT and SSR markers. Mycological Progress, 10:1-7. https://doi.org/10.1007/s11557-010-0668-3

Owati, A.S., B. Agindotan, J.S. Pasche and M. Burrows. 2017. The Detection and characterization of QoI-Resistant *Didymella rabiei* Causing Ascochyta blight of Chickpea in Montana. Frontiers in Plant Science, 8:1165. https://doi.org/10.3389/fpls.2017.01165

Pande, S., K.H.M. Siddique, G.K. Kishore, B. Bayaa, P.M. Gaur and J.H. Crouch. 2005. Ascochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity, and disease management. Australian Journal of Agricultural Research, 56(4):317-332. https://doi.org/10.1071/AR04143

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6(1):288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peever, T.L., W. Chen, Z. Abdo and W.J. Kaiser. 2012. Genetics of virulence in *Ascochyta rabiei*. Plant Pathology, 61(4):754-760. https://doi.org/10.1111/j.1365-3059.2011.02566.x

Phan, H.T.T., R. Ford and P.W.J. Taylor. 2003. Population structure of *Ascochyta rabiei* in Australia based on STMS fingerprints. Fungal Diversity, 13:111–129.

Reddy, A.M.V. and S. Kabbabeh. 1985. Pathogenic variability in *Ascochyta rabiei* (Pass.) Labr. in Syria and Lebanon. Phytopathologia Mediterranea, 24:265-266.

Rhaiem, A., M. Chérif, T.L. Peever and P.S. Dyer. 2008. Population structure and mating system of *Ascochyta rabiei* in Tunisia: evidence for the recent introduction of mating type 2. Plant Pathology, 57(3):540-551. https://doi.org/10.1111/j.1365-3059.2007.01779.x

Salotti, I. and V. Rossi. 2021. A mechanistic weather-driven model for *Ascochyta rabiei* infection and disease development in Chickpea. Plants, 10(3):464. https://doi.org/10.3390/plants10030464

Santra, D.K., G. Singh, W.J. Kaiser, V.S. Gupta, P.K. Ranjekar and F.J. Muehlbauer. 2001. Molecular analysis of *Ascochyta rabiei* (Pass.) Labr., the pathogen of ascochyta blight in chickpea. Theoretical and Applied Genetics, 102:676-682. https://doi.org/10.1007/s001220051696

Sharma, M., R. Ghosh, D. Rubiales, M. Carlota Vaz Patto and E. Prats. 2016. An update on genetic resistance of Chickpea to Ascochyta blight. Agronomy, 6(1):18. https://doi.org/10.3390/agronomy6010018

Sneath, H.A. and R.R. Sokal. 1973. Numerical Taxonomy: The principles and Practice of Numerical Classification. Freeman and Company, San Francisco, USA. 573 pp.

Trapero-Casas, A. and W.J. Kaiser. 2007. Differences between ascospores and conidia of *Didymella rabiei* in spore germination and infection of Chickpea. Phytopathology, 97(12):1600-1607. https://doi.org/10.1094/PHYTO-97-12-1600

Udupa, S.M., F. Weigand, M.C. Saxena and G. Kahl. 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. Theoretical and Applied Genetics, 97:299-307. https://doi.org/10.1007/s001220050899

Vail, S. and S. Banniza. 2009. Molecular variability and mating-type frequency of *Ascochyta rabiei* of chickpea from Saskatchewan, Canada. Australasian Plant Pathology, 38:392. https://doi.org/10.1071/AP09014

Viotti, G., M.A. Carmona, M. Scandiani, A.N. Formento and A. Luque. 2012. First report of *Ascochyta rabiei* causing Ascochyta blight of Chickpea in Argentina. Plant Disease, 96(9):1375-1375. https://doi.org/10.1094/PDIS-02-12-0153-PDN

Wise, K.A., C.A. Bradley, J.S. Pasche, N.C. Gudmestad, F.M. Dugan and W. Chen. 2008. Baseline sensitivity of *Ascochyta rabiei* to azoxystrobin, pyraclostrobin, and boscalid. Plant Disease, 92(2):295-300. https://doi.org/10.1094/PDIS-92-2-0295

Wright, S. 1931. Evolution in Mendelian populations. Genetics, 16(2):97-159. https://doi.org/10.1093/genetics/16.2.97

Yeh, F.C., R.C. Yang and T. Boyle. 1999. POPGENE: Microsoft Windows-based freeware for population genetic analysis, Release 1.31. University of Alberta, Edmonton, Alberta, Canada.

Received: December 14, 2023; Accepted: March 18, 2024

تاريخ الاستلام: 2023/12/14؛ تاريخ الموافقة على النشر: 2024/3/18