TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2021) 45: 1-12

doi:10.3906/tar-2003-78

Comparison of phenotypic and marker-assisted selection in Turkish cultivars and global genotypes of chickpea for resistance to pathotypes of *Ascochyta rabiei* (Pass.) Labr.

Hüseyin KABAKCI, Göksel ÖZER*

Department of Plant Protection, Faculty of Agriculture, Bolu Abant İzzet Baysal University, Bolu, Turkey

Received: 23.03.2020 • **Accepted/Published Online:** 26.08.2020 • **Final Version:** 10.02.2021

Abstract: The pathotypes of *Ascochyta rabiei*, which causes ascochyta blight, show a high pathogenic variation. Forty-four chickpeas, including 26 cultivars commonly cultivated in Turkey and 18 global genotypes, were characterized for resistance status to ascochyta blight following inoculation with four pathotypes of *A. rabiei*. The pathogenicity experiments were conducted using the whole-plant inoculation method and completely randomized designs with three replicates. The pathogenicity tests revealed that 32, 17, 3, and 1 chickpea showed resistance reactions to pathotypes I, II, III, and IV, respectively. These chickpeas were genotyped with STMS (GAA47, TA146, and TA194), SCAR ($SCK13_{603}$, $SCAE19_{336}$, and $SCY17_{590}$), and an allele-specific (CaETR) MAS markers closely linked to QTLs located on linkage groups 2 and 4 for ascochyta blight resistance. QTL_{AR1} predicted blight resistance to pathotype I with a rate of 65.9%. Strong correlations at $P < 0.01$ were found between resistance reactions of chickpeas to pathotype II and QTL_{AR2}, which made it possible to predict resistance in 79.55%–81.82% of studied chickpeas. A significant association was found between QTL_{AR3} and resistance to pathotypes III and IV, which are the most aggressive groups among *A. rabiei* isolates. Three chickpea genotypes (ICC 3996, ICC 12004, and ICC 4475) showed a high level of resistance to pathotypes I, II, and III. ICC 3996 was the only genotype with resistance to pathotype IV. This study is the most comprehensive phenotypic study yet for determining the resistance status of chickpeas against pathotype IV, and the first study showing a significant association between a MAS marker linked to QTL_{AR3} and blight resistance to pathotypes III and IV. Breeders should include the isolates in pathotype III and IV groups into pathogenicity tests due to the increase in their prevalence. The markers linked to QTLs determining the resistance to these pathotypes should be emphasized, and the efficiency of the use of these markers in breeding programmes should be increased.

Key words: Ascochyta blight, *Cicer arietinum*, MAS, pathotype, resistance

1. Introduction

Chickpea (*Cicer arietinum* L.) is a diploid (2n = 2x = 16), self-pollinated, annual species with a genome size of about 740 Mbp (Arumuganathan and Earle, 1991). It is the third most widely grown grain legume in the world, with an annual global production of about 17.22 million tons[1]. Yields and profitability in chickpea production are generally low and unstable in most chickpea-growing countries, mainly due to the adverse effects of multiple abiotic and biotic stresses. Fungal pathogens induce significant chickpea diseases worldwide, limiting production economically. Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labrousse, is a damaging disease of chickpea worldwide (Nene and Reddy, 1987). The disease affects all aboveground parts of plants and causes necrotic lesions on leaflets, stems, pods, and seeds, where pycnidia are usually observed. Ascochyta blight is capable of potentially causing complete yield losses under favourable conditions where cold and humid weather prevails during flowering to the podding stage (Pande et al., 2005).

The control of AB is dependent on fungicide applications; however, these applications alone are generally not economical due to the necessity of repeating application several times under long-term suitable environmental conditions for disease development (Chongo et al., 2004; Atik et al., 2011). An increase in the resistance of the pathogen to some fungicides, such as strobilurin, has emerged in chickpea growing-areas (Wise et al., 2009). An integrated approach for disease management must be eco-friendly, sustainable, and the most effective strategy for successful chickpea production. Integrated disease management aims to control AB with several components, primarily including the use of resistant or tolerant cultivars, cultural practices (rotation of

[1] FAO (2020). Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC (accessed 14 March 2020).

* Correspondence: gokozer@gmail.com

chickpea every 4 years, use of disease-free seeds, selection of fields with little or no AB history), and seed treatment and foliar spray with fungicides (Strange et al., 2004).

The use of cultivars resistant to AB is considered to be the best option for long-standing disease management. Several sources of partial resistance in chickpea and wild *Cicer* species to the disease have been identified and successfully incorporated in chickpea breeding programmes for developing new resistant cultivars worldwide (Collard et al., 2001; Chen et al., 2004; Tar'an et al., 2007; Sharma and Ghosh, 2016). Although the phenotypic selection of resistant cultivars is one of the most efficient tools used to improve breeding populations for quantitative traits, it has several bottlenecks, such as laborious, time-consuming screening procedures and instability in different environmental conditions. There have been different attempts to accelerate phenotypic selection, such as using real-time PCR for accurate monitoring of disease progression in plant materials (Bayraktar et al., 2016).

The pathogen populations show high pathogenic variability caused mostly by sexual recombination, which is realized at the teleomorphic stage [*Didymella rabiei* (Kovatsch.) Arx], and selection pressure on the pathogen has stimulated the adoption of improved resistant chickpea cultivars. The pathogenic variability within the pathogen populations is characterized by a set of differential host genotypes and designated as 'pathotype'. The pathotyping system, which was described by Udupa et al. (1998) and modified by Imtiaz et al. (2011), is reasonable and it is the most used system: pathotype I (least aggressive), pathotype II (aggressive), pathotype III (highly aggressive), and pathotype IV (most aggressive). The genes associated with aggressiveness have not been fully identified; further research is needed for that. The occurrence of a new pathotype with the increased aggressiveness of the current pathotypes has overcome resistance in several cultivars. As for the host, several quantitative trait loci (QTL) have been identified for AB resistance by genetic mapping. Different authors have characterized two major QTLs (QTL_{AR1} and QTL_{AR2}) located and validated on linkage group 4 (LG4) and QTL_{AR3} on LG2, respectively, associated with resistance to different pathotypes (Singh and Reddy, 1983; Santra et al., 2000; Tekeoğlu et al., 2002; Flandez-Galvez et al., 2003: Millan et al., 2003; Udupa and Baum, 2003; Cho et al., 2004; Iruela et al., 2006, 2007; Madrid et al., 2012). To track the QTLs associated with blight resistance successfully during the development of new chickpea cultivars, marker-assisted selection (MAS) provides more effective, cheaper, and faster breeding methods when compared to phenotypic screening.

Effective use of MAS requires highly saturated molecular markers linked to the quantitative traits. Several molecular markers have been employed for the identification of QTLs associated with resistance to ascochyta blight. A sequence-tagged microsatellite sites (STMSs) marker, GAA47, was determined to be an indicative marker for QTL_{AR1} (Iruela et al., 2006), which coincided with a QTL on LG4A for resistance to pathotype II (Cho et al., 2004). An allele-specific and codominant marker (CaETR) tightly linked to QTL_{AR1} was produced based on the sequence polymorphism of an ethylene receptor-like gene to select genotypes containing QTL_{AR1} (Madrid et al., 2012). Two dominant sequences characterized by amplified region (SCAR) markers ($SCK13_{603}$ and $SCAE19_{336}$) and one codominant SCAR marker ($SCY17_{590}$) were found to be as tightly linked to QTL_{AR2} as the TA146 marker, which is an indicative STMS marker for this QTL (Iruela et al., 2006). Iruela et al. (2007) noted that QTL_{AR3} on the LG2 locus is strongly associated with STMS marker TA194. These markers have been claimed to discriminate susceptible and resistance phenotypes of chickpea to AB. Codominant markers have been used to detect heterozygous individuals in early generations with a high probability (Madrid et al., 2013). Understanding the genetic bases of both resistance in chickpea and virulence in *A. rabiei* is needed to overcome difficulties in the phenotyping of resistance to AB, which is crucial for developing cultivars with more stable resistance.

This study was designed to (i) evaluate the resistance level of Turkish cultivars and a global genotype collection of chickpea against four pathotypes of *A. rabiei* under controlled conditions, (ii) characterize the chickpea cultivars and genotypes with MAS markers, (iii) examine the relationship between GAA47, CaETR, $SCK13_{603}$, $SCAE19_{336}$, $SCY17_{590}$, TA146, and TA194 markers and host–pathotype interactions. Consequently, our primary goal was that the results of this study would provide useful information for breeding programmes in developing resistant varieties to AB.

2. Materials and methods

2.1. Fungal isolates and inoculum preparation

Fungal isolates were supplied as pure cultures from the collection of *A. rabiei* isolates by Prof. Canan Can (Department of Biology, Faculty of Science, Gaziantep University). Pathotype characterization was previously classified based on differentials determined by Udupa et al. (1998) and Imtiaz et al. (2011). The isolates representing four pathogenic groups of *A. rabiei* were grown on chickpea seed meal dextrose agar (CSMDA: chickpea seed meal 40 g, dextrose 20 g, and agar 20 g L^{-1}) at 22 ± 1 °C with a 14-h light photoperiod under cool-white light combined with near-UV light. After 2 weeks of incubation, conidia were harvested in sterile distilled water with a sterile glass rod from the agar plate. Conidial concentrations were

determined with a haemocytometer and adjusted to 2 × 10^5 pycnidiospores mL^{-1}.

2.2. Plant materials and pathogenicity assay

Twenty-six chickpea cultivars commonly grown in Turkey were supplied from commercial companies, Akdeniz University, and the Research Institutes of the Republic of Turkey Ministry of Agriculture and Forestry (Table 1). Eighteen global genotypes of chickpea, including differentials, were provided by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) National Center for Genetic Resources (NCGS) (Table 2).

The determination of the resistance status of chickpeas to the pathotypes was evaluated using the whole-plant inoculation method. Chickpea seeds were surface-sterilized with 0.5% sodium hypochlorite solution for 5 min, dipped in 70% ethanol for 1 min, and rinsed twice in sterile distilled water. Five seeds for each chickpea were sown at a depth of 2 cm in a 16-cm diameter plastic pot (14.5 cm depth) filled with about 2 L of a substrate containing a mixture of sterile vermiculite, sterile soil, and peat (KTS 1, Klasmann-Deilmann, Bösel, Germany) (1:1:1, v/v). Plants were transferred to a growth room and maintained for 2 weeks with 16 h of light at 22 ± 1 °C and 8 h of dark at 18 ± 1 °C, with a relative humidity varying from 50% to 70%. Aerial parts of 15-day-old plants were sprayed with the conidial suspension to run-off (approximate 2 mL per plant). Control plants were treated with sterile distilled

Table 1. The characterization of the cultivars of chickpea from Turkey.

Number	Cultivar	Origin	Seed	Pathotypes[c]				CaETR	GAA47	SCY17	SCAE19	SCK13	TA146	TA194
			Type[b]	I	II	III	IV							
1	GÜLÜMSER	Black Sea ARI[a]	K	R	R	S	S	+	-	+	+	+	+	-
2	ZUHAL		K	R	S	S	S	+	+	-	-	-	-	-
3	ÇAĞATAY		K	S	S	S	S	+	+	+	+	+	+	-
4	SEZENBEY		K	R	S	S	S	+	+	-	-	-	-	-
5	HASANBEY	East Mediterranean Transitional Zone ARI	K	R	R	S	S	+	+	+	+	+	+	-
6	YAZICI		K	R	R	S	S	+	-	+	+	+	+	-
7	AĞSAKALLI		K	S	S	S	S	+	-	-	-	-	-	-
8	SEÇKIN		K	R	R	S	S	+	+	+	+	+	+	-
9	İNCI		K	R	R	S	S	+	+	+	+	+	+	-
10	UZUNLU 99	Field Crops Central Research Institute	K	S	S	S	S	+	-	+	+	+	+	-
11	AKÇIN 91		K	S	S	S	S	-	-	+	+	+	+	-
12	GÖKÇE		K	S	S	S	S	-	+	+	+	+	+	-
13	ARAS	Olgunlar Company	K	R	S	S	S	+	+	-	-	-	-	-
14	GÖKSU		K	R	R	S	S	+	+	+	+	+	+	-
15	HISAR	Transitional Zone ARI	K	S	S	S	S	+	+	-	-	-	-	-
16	ÇAKIR		K	R	S	S	S	+	+	-	-	-	-	-
17	AKCA		K	R	R	S	S	+	+	+	+	+	+	-
18	YAŞA 05		K	R	S	S	S	+	-	+	+	+	+	-
19	AZKAN		K	R	R	S	S	+	-	+	+	+	+	-
20	CANITEZ 87		K	S	S	S	S	-	-	-	-	-	-	-
21	IŞIK 05		K	R	S	S	S	+	+	-	-	-	-	-
22	SARI 98	Aegean ARI	K	S	S	S	S	-	-	-	-	-	-	-
23	OMPAR 3	Akdeniz University	K	S	S	S	S	-	+	+	+	+	+	-
24	SAĞEL	Ankomer Company	K	R	R	S	S	-	-	+	+	+	+	-
25	DIYAR 95	GAP Int. ARI	K	R	R	S	S	+	+	+	+	+	+	-
26	ILGAZ	Mersin Com. Exchange	K	R	S	S	S	+	+	-	-	-	-	-

[a]ARI Agricultural Research Institute; [b]K: Kabuli type and D: Desi type, [c]Phenotypic response of pathotypes R: Resistant and S: Susceptible

Table 2. The characterization of the genotypes of chickpea from USDA-ARS-NPGS.

Number	Genotype	Seed	Chen et al. (2004)[b]		Hamwieh et al. (2013)[b]			Pathotypes[b]				CaETR	GAA47	SCY17	SCAE19	SCK13	TA146	TA194
		Types[a]	I	II	I	II	III	I	II	III	IV							
1	ILC 1272	K	-	-	-	-	-	S	S	S	S	-	-	-	-	-	-	-
2	**ICC 3996**	D	R	R	*R*	*R*	*R*	R	R	R	R	-	-	+	+	+	+	+
3	FLIP 84-92C	K	R	S	-	-	-	R	S	S	S	+	+	+	+	+	+	-
4	DWELLEY	K	R	S	-	-	-	R	S	S	S	+	+	-	-	-	-	-
5	**ICC 12004**	D	R	R	*R*	*R*	*R*	R	R	R	S	-	-	+	+	+	+	+
6	ILC 195	K	-	-	*S*	*S*	*S*	R	R	S	S	+	+	+	+	+	-	-
7	RPIP 12-069-103	D	S	S	-	-	-	S	S	S	S	-	-	-	-	-	-	-
8	ICC 3279	K	R	S	*R*	*R*	*S*	R	R	S	S	+	+	+	+	+	+	-
9	**ILC 1929**	K	S	S	-	-	-	S	S	S	S	-	-	-	-	-	-	-
10	ILC 249	K	R	S	-	-	-	R	S	S	S	-	-	-	-	-	-	-
11	ILC 72	K	R	S	*R*	*S*	*S*	R	S	S	S	+	+	+	+	+	+	-
12	ICC 4475	D	R	R	-	-	-	R	R	R	S	-	-	+	+	+	+	+
13	ILC 1903	D	R	S	-	-	-	R	S	S	S	-	-	+	-	-	+	-
14	**ILC 482**	K	R	S	*R*	*S*	*S*	R	S	S	S	-	-	-	-	-	-	-
15	ILC 200	K	R	R	*R*	*R*	*S*	R	R	S	S	-	-	+	+	+	+	-
16	ILC 4935	D	R	S	-	-	-	R	S	S	S	-	-	-	-	-	-	-
17	ICC 13416	K	-	-	-	-	-	R	S	S	S	-	-	-	-	-	-	-
18	ILC 247	K	-	-	-	-	-	R	R	S	S	-	-	+	+	+	+	-

[a]K: Kabuli type and D: Desi type; [b]Phenotypic response of pathotypes R: Resistant and S: Susceptible; ILC 1929, ILC 482, ILC 3279, and ICC 12004, which are written in bold within the table, are used as differential sets for pathotyping.

water. Plants were immediately covered with a transparent plastic bag to ensure sufficient moisture for successful infection during the first 48 h. After the plastic bag was removed, plants were maintained in the growth room adjusted as before preinoculation for 2 weeks. The severity of disease was scored based on a nonparametric 1–9 rating scale (1, healthy plant to 9, dead plant), which was described by Chen et al. (2004) as slightly modified from Reddy and Singh (1984). Chickpea cultivars and genotypes scored 1.0 to 5.0 were considered resistant while those scored 5.1 to 9.0 were considered susceptible, according to Türkkan and Dolar (2009). The scores were measured for each plant, and the average of the scores of the five plants in a pot represented one experimental unit. The experiments were conducted using completely randomized designs with three replicates, and all experiments were repeated twice at different times. Disease phenotyping results, which were previously reported by Chen et al. (2004) and Hamwieh et al. (2013) for genotypes used in this study, were included in this study to confirm our results (Tables 1 and 2).

2.3. DNA isolation and molecular characterization

A modified version of the CTAB-based method, as described in the DArT protocol[2], was used to extract genomic DNA from plants. About 100 mg of young leaf tissue of 2-week-old seedlings was harvested from each chickpea, ground using a mortar and pestle in liquid nitrogen, and immediately transferred into a 1.5-mL microfuge tube containing 750 µL of preheated (65 °C) extraction–lysis buffer (125 mM Tris-HCl pH 8.0, 25 mM EDTA pH 8.0, 2% CTAB, 2% PVP-40, 0.8 M NaCl, 0.5% sodium disulfite, and 1% sarcosyl). The sample was incubated at 65 °C for 60 min, and shaken gently every 15 min. Approximately 750 µL of chloroform/isoamyl alcohol (24:1 v/v) was added, mixed for 10 min, and then centrifuged at 12,000 *g* for 15 min. The supernatant was transferred to a clean centrifuge tube; 0.6 volume isopropanol at room temperature was added to the tube and mixed well to precipitate DNA. After centrifugation at 12,000 *g* for 5 min, the supernatant was discarded. The pellet was washed twice with 70% ethanol and then dried at

[2] Diversity Arrays Technology. Website http://www.diversityarrays.com [accessed 24 Jan 2021]

room temperature. The resultant DNA was dissolved with sterile ultrapure water and adjusted to 50 ng/μL using a DS-11 FX spectrophotometer (DeNovix Inc., Wilmington, DE, USA) for PCR assays.

The 26 cultivars and 18 genotypes of chickpea were characterized using STMS markers (GAA47, TA146, and TA194; Winter et al., 2000), an allele-specific marker (CaETR; Madrid et al., 2012), and SCAR markers ($SCK13_{603}$, $SCAE19_{336}$, and $SCY17_{590}$; Iruela et al., 2006) associated with QTL_{AR1}, QTL_{AR2}, and QTL_{AR3} (Table 3), respectively. PCR reaction was carried out in a total volume of 20 μL containing 50 ng of plant genomic DNA, 1 × DreamTaq buffer, 0.5 μM each of primers, 0.2 mM dNTPs, and 1-unite Ampliqon TEMPase Hot Start DNA polymerase (Berntsen, Rødovre, Denmark). Amplification reactions were performed using a T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) starting at 95 °C for 15 min, followed by 35 cycles of a denaturation step at 95 °C for 45 s, an annealing step (58 °C for 50 s, 60 °C for 30 s, and 50 °C for 30 s for STMS, CaETR, and SCAR markers, respectively), and an extension step at 72 °C for 45 s, with a final extension step at 72 °C for 5 min. Amplification products were separated on 2% agarose gels and stained with ethidium bromide. The Gene Ruler 100-bp plus DNA ladder (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used as a size standard. The DNA banding patterns were visualized and documented by a G:Box F3 gel documentation system (Syngene, Cambridge, UK).

2.4. Statistical analysis

Levene's test was performed to assess the homogeneity of variance before the analysis of variance (ANOVA). ANOVA was applied to the score data from the pathogenicity tests for each pathotype. The means of statistically different treatments were compared using Fisher's least significant difference test (LSD) with Statistical Analysis System (SAS Version 9.0; SAS Institute Inc., Cary, NC, USA). Pearson's coefficient of correlation between experiments, as well as between the disease scores caused by *A. rabiei* pathotypes I, II, III, and IV and the markers, was also calculated using JMP®, Version 13.2.0 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Pathogenic variation

Pathogenicity tests were conducted independently twice using isolates representing pathotypes I, II, III, and IV of *A. rabiei*. The isolates caused high levels of disease severity in both the first and second experiments. The disease scores for the chickpea cultivars and genotypes were evaluated using a 1–9 scale. Simple analysis between two pathogenicity experiments indicated that the disease scores for the same chickpea were highly correlated ($r = 0.94$), so further statistical analyses were performed by combining the data from both experiments. Significant differences were observed in pathogenicity among chickpeas, while no significant differences among replications were observed when inoculated with each of the pathotypes I, II, III, and IV (Table 4).

Table 3. Characteristics of primer pairs linked to quantitative trait loci, used in this study.

Marker type	Primer		Primer sequence (5'-3')	Linkage group - QTLs	Reference
Allele specific	CaETR	Fw	CAGGAAGTTCAATGGCCCTA	LG4 QTL_{AR1}	Madrid et al., 2013
		Rev1	TAAGTTGTGACAAAAGACTCAATCG		
		Rev2	TGTGGCACAGTGGACCCCATCT		
SCAR	SCK13	Fw	GGTTGTACCCCATCCTCCCG	LG4 QTL_{AR2}	Iruela et al., 2006
		Rev	GGTTGTACCCTTGTGCCACTA		
	SCY17	Fw	GACGTGGTGACTATCTAGC		
		Rev	GACGTGGTGAAATAGATACC		
	SCAE19	Fw	GACAGTCCCTCCATTATCTAAAC		
		Rev	GACAGTCCCTATGTGTGAGAAT		
STMS	GAA47	Fw	CACTCCTCATGCCAACTCCT	LG4 QTL_{AR1}	Winter et al., 1999
		Rev	AAAATGGAATAGTCGTATGGGG		
	TA146	Fw	CTAAGTTTAATATGTTAGTCCTTAAATTAT	LG4 QTL_{AR2}	
		Rev	ACGAACGCAACATTAATTTTATATT		
	TA194	Fw	TTTTTGGCTTATTAGACTGACTT	LG2 QTL_{AR3}	
		Rev	TTGCCATAAAATACAAAATCC		

Table 4. Analysis of variance for the disease scores observed on 44 chickpea cultivars and genotypes against *Ascochyta rabiei* pathotypes.

Pathotype	Source	*df*[a]	Mean square	*F* Ratio	*P* > *F*	$LSD_{0.01}$
I	Chickpeas	44	27.00	185.90	<.0001[b]	0.57
	Replicates	5	0.17	1.17		
	Error	220	0.15			
	C. Total	269				
II	Chickpeas	44	27.36	197.77	<.0001[b]	0.56
	Replicates	5	0.25	1.81		
	Error	220	0.14			
	C. Total	269				
III	Chickpeas	44	14.13	87.98	<.0001[b]	0.60
	Replicates	5	0.47	2.90		
	Error	220	0.16			
	C. Total	269				
IV	Chickpeas	44	11.04	74.92	<.0001[b]	0.58
	Replicates	5	0.38	2.55		
	Error	220	0.15			
	C. Total	269				

[a]*df*: degrees of freedom
[b]Significant P < 0.0001

Against pathotype I, which is the least aggressive group, 32 out of 44 chickpea cultivars and genotypes were found to be resistant (Tables 1 and 2). Disease scores ranged from 1.40 to 7.93, with a mean of 4.21. ICC 12004 genotype was the most resistant, while cv. UZUNLU 99 was the most sensitive to pathotype I. Pathotype II showed more aggressiveness with a disease severity of >5 on 27 chickpeas; 17 of the tested chickpeas were resistant. The disease severity ranged from 1.93 (ICC 3996) to 8.6 (ÇAĞATAY), with a mean of 6.12. Chickpea cultivars and genotypes mostly exhibited sensitive reactions to pathotypes III and IV, which are the most aggressive groups of *A. rabiei* isolates. No Turkish cultivar was resistant to pathotypes III and IV, while three genotypes (ICC 3996, ICC 12004, and ICC 4475) had disease scores of lower than 5.0 against pathotype III. ICC 3996 was found to be the only genotype resistant to pathotype IV. Disease scores observed for chickpea cultivars and genotypes ranged from 3.00 to 8.80 (mean, 7.27), and from 3.27 to 8.67 (7.85) for pathotypes III and IV, respectively (Figure 1).

3.2. Molecular characterization

CaETR locus, a codominant molecular marker linked to QTL_{AR1}, was amplified with CaETR-Fw and CaETR-Rev1 primers. The amplified products were the expected sizes for the resistant (1034 bp) and susceptible (304 bp) chickpeas (Figure 2). CaETR-Rev2 primer was used for a multiplex PCR together with the primers to discriminate resistance and susceptible genotypes of chickpea. The multiplex PCR characterized 25 chickpeas as resistant and the remaining 19 chickpeas as susceptible, and defined all cultivars and genotypes of chickpea as homozygotes (Tables 1 and 2). Similarly, QTL_{AR1} was also detected in 21 chickpeas with GAA47 STMS marker, which was considered to be closely linked to this QTL. A high correlation ($r = 0.65$ at $P < 0.01$) occurred between results of CaETR and GAA47 markers.

$SCK13_{603}$, $SCAE19_{336}$, $SCY17_{590}$, and TA146 markers associated with QTL_{AR2} were employed to characterize chickpeas for the existence of QTL. The markers determined that the quantitative trait locus in most chickpeas with high correlations ranged from 0.91 to 1. The codominant marker $SCY17_{590}$ also showed that all chickpeas were homozygous for QTL_{AR2} polymorphism.

The frequency of QTL_{AR3} in the screened chickpeas was estimated using an indicative STMS marker TA194. Three genotypes (ICC 3996, ICC 12004, and ICC 4475) were detected to have this QTL located in LG2.

No QTL evaluated in the present study was determined in CANITEZ 87, SARI 98, ILC 1272, RPIP 12-069-103, ILC 1929, ILC 249, ILC 482, ILC 4935, or ICC 13416. Four of these chickpea genotypes (ILC249, ILC482, ILC4935,

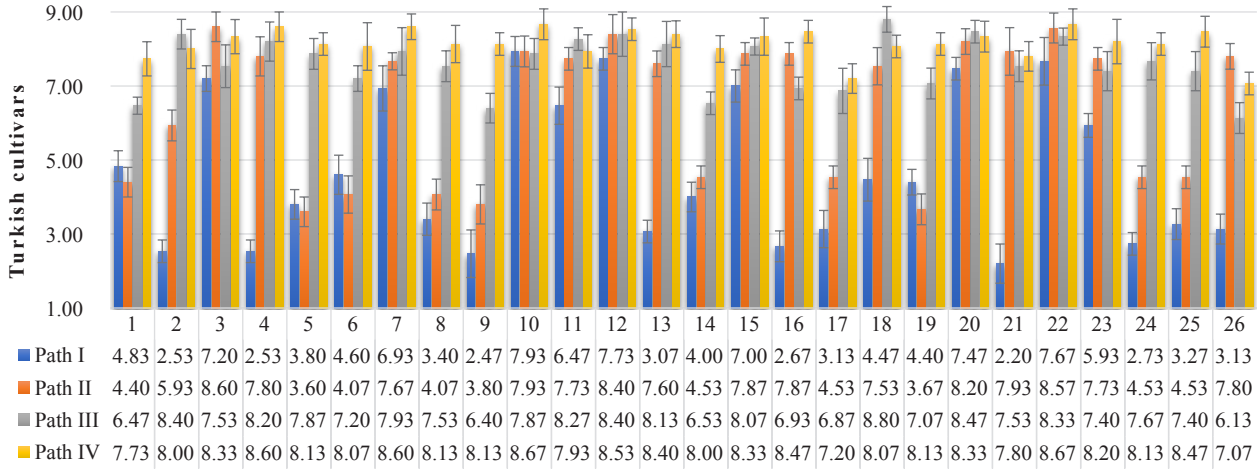

Figure 1. Mean disease severity of Turkish cultivars and global genotypes of chickpea (n = 6) inoculated with *A. rabiei* pathotypes based on the 1–9 rating scale. $LSD_{0.01}$: 0.57, 0.56, 0.60, and 0.58 for pathotypes I, II, III, and IV, respectively. The ordering information is retrieved from Tables 1 and 2.

and ICC13416) were phenotypically resistant only to pathotype I, while the other five were susceptible to all pathotypes.

3.3. Correlation analysis

Correlation between phenotype and genotype relationship in Turkish cultivars and global genotypes of chickpea for resistance to pathotypes of *A. rabiei* was calculated with Pearson's correlation coefficient; *r* values are given in Table 5. No correlation was detected between the response of chickpea cultivars and genotypes to pathotype I and the 7 markers tested. The association was highly significant between the SCAR markers and TA146 marker. These markers were also correlated with resistance to pathotype II. The perfect correlation ($r = 1$) was observed between TA194 and resistance to pathotype III. TA194 was also significantly correlated with resistance to pathotype IV.

4. Discussion

Ascochyta blight caused by *A. rabiei* is the most significant disease for chickpea production worldwide, particularly under favourable environmental conditions. Breeding to improve host resistance is a very efficient way to control the disease, but requires the continuous screening of genotypes and determination of the genetic and pathogenic variation in the pathogen populations (Sharma and Ghosh, 2016). The presence of a teleomorphic stage of *A. rabiei* causes a high level of genetic variability within the pathogen population (Bayraktar et al., 2007; Vail and Banniza, 2009; Özer et al., 2012, Atik et al., 2013). The genetic variability reorganizes the genes related to virulence and generates pathotypic variations (Pande et al., 2005). Pathogenic variability among *A. rabiei* isolates has been noted from several countries, including India (Ambarder and Singh, 1996), Syria (Atik et al., 2013), the USA (Chen et al., 2004; Peever et al., 2012), Pakistan (Iqbal et al., 2004), Spain (Navas-Cortes et al., 1998), Canada (Vail and Banniza, 2008), and Turkey (Türkkan and Dolar, 2009). Udupa et al. (1998) grouped the pathogenic variations of *A. rabiei* isolates into three pathotypes, for which the most valid and widely pathotyping system is

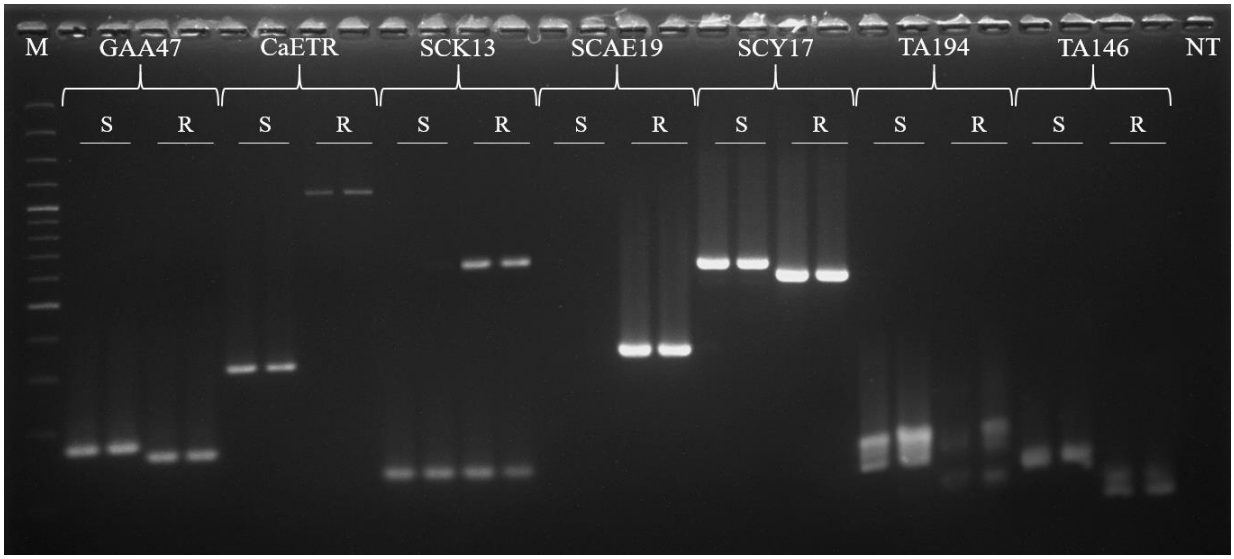

Figure 2. Fragment profiles provided MAS markers used in this study for resistant (R) and susceptible (S) chickpeas, NT = non template DNA. M (DNA marker) - GeneRuler 100 bp plus DNA ladders (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Table 5. Correlation matrix for study variables.

	CaETR	GAA47	SCY17	SCAE19	SCK13	TA146	TA194
Path. I	0.29	0.18	0.22	0.19	0.19	0.19	0.17
Path. II	0.13	0.01	0.66*	0.69*	0.69*	0.60*	0.34
Path. III	0.31	0.26	0.23	0.24	0.24	0.24	1.00*
Path. IV	0.18	0.15	0.13	0.13	0.13	0.13	0.56*
CaETR		0.65*	0.12	0.17	0.17	0.07	0.31
GAA47			0.06	0.10	0.10	0.01	0.26
SCY17				0.95*	0.95*	0.95*	0.23
SCAE19					1.00*	0.91*	0.24
SCK13						0.91*	0.24
TA146							0.24

*indicates significance of the correlation at $P < 0.01$.

used in the present study. Pathotype IV has been recently added to this system as the most aggressive group (Imtiaz et al., 2011). In this study, the resistance level of Turkish cultivars and global genotypes of chickpea was evaluated phenotypically against four pathotypes of *A. rabiei* with a seedling screening technique.

The high level of variation in the aggressiveness of different pathotypes has been determined by studies (Türkkan and Dolar, 2009; Ali et al., 2013). The disease severity caused by isolates increased from pathotype I (least virulent) to IV (most virulent). Some of the 18 global genotypes of chickpeas used in this study were previously evaluated by Chen et al. (2004) to determine resistance to pathotypes I and II. Hamwieh et al. (2013) also employed pathotype III in their experiments, which allowed us to compare the consistency of our phenotypic results.

Pathotype I, which is considered to have the lowest aggressiveness (Udupa et al., 1998; Imtiaz et al., 2011) caused the lowest disease severity values, with susceptible reactions for 12 out of 44 chickpeas in this study. Of the chickpeas in the growth chamber experiments, 63.7% were resistant to pathotype I, which is similar to the 62.8% observed by Hamwieh et al. (2013), who evaluated the reaction of 43 chickpea genotypes to *A. rabiei*. Chen et al. (2004) found 37 out of 40 genotypes to be resistant to pathotype I. All genotypes used jointly with these studies

gave the same reaction against pathotype I, except for the ILC 195 genotype in Hamwieh et al. (2013) (Table 2).

The result of the reaction test of chickpeas to pathotype II were 17 resistant and 27 susceptible reactions. ILC 200, ICC 12004, and ICC 3996 genotypes showed a resistant reaction in all studies evaluating the aggressiveness of pathotype II. In addition, ICC 3279 was resistant in the study carried out by Hamwieh et al. (2013), which is in accordance with our results, whereas Chen et al. (2004) reported the opposite. Aydın et al. (2016) showed that although İNCI and HASANBEY were susceptible to *A. rabiei* in field conditions contrary to the findings of our study, 9 chickpea cultivars reacted similarly to those observed in our study.

No Turkish cultivars showed a resistant response to pathotype III, which is the most aggressive group according to the triple pathotype system described by Udupa et al. (1998); however, three Desi-type genotypes (ICC 12004, ICC 4475, and ICC 3996) were highly resistant to this pathotype. This finding was particularly consistent with those reported by Gaur (2015) and Hamwieh et al. (2013). These genotypes were also reported to be resistant against some isolates obtained from the northwestern United States by Chen et al. (2004). This pathotype was highly aggressive to all thirteen chickpea germplasms studied by Benzohra et al. (2013). None of the eighteen selected chickpea genotypes, including ICC3996 and 6 Moroccan varieties, was resistant to pathotype III, which was present in the majority of the surveyed regions of Morocco (Bencheqroun et al., 2018). Türkkan and Dolar (2009) noted that most of the pathotype III isolates obtained from chickpea-growing areas in Turkey caused disease severity <5 for ICC 3996, which was considered resistant. The resistance of ICC 12004 to pathotypes I, II, and III has been confirmed in several studies (Atik et al., 2012; Şahin, 2015; Attar, 2016).

Bayaa et al. (2004) reported an extremely aggressive pathotype in Syria that can overcome the high resistance of ICC 12004 and ICC 3996 chickpea lines. Imtiaz et al. (2011) declared a new pathotype (IV) that can affect chickpea genotypes such as ICC 12004 known for high resistance to pathotypes I, II, and III. The susceptible reaction to ICC 12004 is the characterization phenomenon for identifying pathotype IV. In our study, this most aggressive pathotype group was included in the aggressiveness test and caused catastrophic disease in almost all chickpeas except for ICC 3996. Similarly, Attar (2016) could not determine a highly resistant genotype to pathotype IV among 200 genotypes studied. Kemal et al. (2017) found all genotypes to be susceptible to this pathotype.

The presence of QTL_{AR1}, QTL_{AR2}, and QTL_{AR3} located on LG4 and LG2 were examined in Turkish cultivars and global genotypes of chickpea, and the relationship of the QTLs with resistance to the disease was evaluated with phenotypic and molecular analysis in this study. The markers were commonly used to screen the QTLs for AB resistance in recombination breeding lines derived from an interspecific cross in chickpea. However, we tested the effectiveness of these markers in populations where the distorted segregation was not present to determine the resistance of chickpea to different pathotypes of *A. rabiei*.

GAA47 and CaETR markers have been successfully employed to characterize QTL_{AR1} in chickpea genotypes solely or together (Iruela et al., 2006; Bouhadida et al., 2013; Gil et al., 2017). Nevertheless, some conflicting results were obtained from the evaluation of genotypes for blight resistance with the CaETR locus. Madrid et al. (2013) correctly predicted the phenotypes of 27 of 40 accessions (74.6%) using this marker. Our results demonstrated that CaETR was more effective than GAA47 in predicting phenotypic resistance to pathotypes. The highest level of expectation with the CaETR marker was realized in predicting blight resistance against pathotype I, with a rate of 65.9%. A strong correlation was statistically ($P < 0.01$) estimated between the markers. However, no correlation was found between QTL_{AR1} and resistance reactions to any pathotype in this study. Contrary to our result, Castro et al. (2015) and Gil et al. (2017), who selected the parental lines in the crossing programmes, found a significant association between the markers linked to QTL_{AR1} ($P < 0.05$) and the phenotypic reaction. No heterozygote chickpea was detected with the codominant CaETR marker in this study. This observation was also consistent with the very low frequency of heterozygotes in the previous study conducted by Bouhadida et al. (2013), who detected only one heterozygote in 23 chickpea genotypes.

TA146 STMS and SCAR ($SCK13_{603}$, $SCAE19_{336}$, and $SCY17_{590}$) markers produced specific products determining the existence of QTL_{AR2} in most of the chickpeas used in this study. Significant associations ($r < 0.9$) were observed among the markers. The use of markers made it possible to correctly predict resistance in 79.55%–81.82% of collected chickpeas. A strong correlation was found between QTL_{AR2} and blight resistance to pathotype II as estimated, which is consistent with the results of Cho et al. (2004), Iruela et al. (2006), and Castro et al. (2015). The codominant $SCY17_{590}$ simultaneously determined all chickpeas as homozygotes for the resistant or susceptible state.

The microsatellite marker TA194 was employed to detect QTL_{AR3} located on LG2. We found that TA194 showed linkage to blight resistance against pathotype III at 100% probability. This QTL was also noted to be closely linked to blight resistance by Iruela et al. (2007) and Castro et al. (2015). A significant correlation was observed between this QTL and the resistance of genotypes ICC 3996, ICC 12004, and ICC 4475 to pathotype III. The presence of this QTL guarantees resistance against pathotypes I, II, and III.

QTL_{AR3} was also significantly correlated with resistance to pathotype IV.

In conclusion, the pathotypes of *A. rabiei* have the potential to cause disease regardless of the level of resistance of chickpeas. The intensive efforts of chickpea breeding programmes have resulted in many varieties that are mostly resistant to the formerly common pathotypes I and II. Interestingly, no breeding studies have included pathotypes III and IV. The prevalence of pathotypes III and IV has been reported to have increased among *A. rabiei* populations derived from chickpea production areas of important chickpea-producing countries such as Turkey, Syria, and Algeria (Ali et al., 2013; Şahin, 2015; Attar, 2016; Benzohra et al., 2018). Therefore, resistance screening studies should include pathotypes III and IV for breeding programmes. Molecular tools are being integrated to speed up the process in conventional breeding programmes of introgressing genes into the chickpea, providing resistance to aggressive pathotypes. To the best of our knowledge, the present study is the first study showing a significant association between an MAS marker linked to QTL_{AR3} and AB resistance to pathotypes III or IV. Three chickpea genotypes (ICC 3996, ICC 12004, and ICC 4475) were identified as resistant to pathotypes I, II, and III, while the ICC 3996 genotype was resistant to all. These genotypes may provide useful resistance sources to be employed in chickpea breeding programmes. Further studies should be carried out to understand the relationships between the QTLs and the phenotypic resistance states to pathotypes.

Acknowledgements

We would like to thank Prof. Canan Can, USDA-ARS Germplasm Resources Information Network (GRIN), commercial companies, Akdeniz University, and Research Institutes of Republic of Turkey Ministry of Agriculture and Forestry for providing pathotypes of *Ascochyta rabiei*, the global genotypes, and cultivars of chickpea. The authors are grateful to Bolu Abant İzzet Baysal University Scientific Research Projects Coordination Unit (2016.10.06.1072). Thanks to Prof. Harun Bayraktar, who read and offered feedback on the manuscript. This paper contains part of the results of the master thesis by Hüseyin Kabakcı.

References

Ali H, Alam SS, Iqbal N (2013). Genetic and pathogenic variability of *Ascochyta rabiei* isolates from Pakistan and Syria as detected by universal rice primers. Plant Pathology and Microbiology 4: 212. doi: 10.4172/2157-7471.1000212

Ambardar VK, Singh SK (1996). Identification and elucidation of *Ascochyta rabiei* isolates of chickpea in Jammu. Indian Journal of Plant Pathology 26: 4-8.

Arumuganathan K, Earle ED (1991). Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter 9: 208-218. doi: 10.1007/BF02672069

Atik O, Baum M, El-Ahmed A, Ahmed S, Abang MM et al. (2011). Chickpea Ascochyta blight: disease status and pathogen mating type distribution in Syria. Journal of Phytopathology 159(6): 443-449. doi: 10.1111/j.1439-0434.2011.01788.x

Atik O, El-Ahmed A, Baum M, Ahmed S, Abang MM et al. (2012). Effects of mixed pathotypes of *Didymella rabiei* on the development of Ascochyta blight on chickpea. Arab Journal of Plant Protection 30 (2): 266-273.

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

Attar B (2016). Genetics of virulence and pathogenic diversity for Ascochyta blight affecting chickpea. MSc, Çukurova University, Adana, Turkey.

Aydın H, Oğuz A, Erdemci İ, Karademir Ç (2016). Control of Ascochyta blight (*Ascochyta rabiei*) in chickpea in winter sowing in Southeastern Anatolia. The Journal of Turkish Phytopathology 45: 87-98.

Bayaa B, Udupa SM, Baum M, Malhotra RS, Kabbabeh S (2004). Pathogenic variability in Syrian isolates of *Ascochyta rabiei*. In Proceedings of the 5th European Conference on Grain Legumes; Dijon, France. p. 306.

Bayraktar H, Dolar FS, Tör M (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.

Bayraktar H, Özer G, Aydoğan A, Palacıoğlu G (2016). Determination of Ascochyta blight disease in chickpea using real-time PCR. Journal of Plant Diseases and Protection 123 (3): 109-117. doi: 10.1007/s41348-016-0017-0

Bencheqroun SK, Udupa SM, Hamwieh A, Kemal SA (2017). Virulence and molecular diversity within *Ascochyta rabiei* in Moroccan population and evaluation of genotypic stability on chickpea. Conference on ICP2016; Marrakesh, Morocco. p. 59.

Benzohra IE, Bendahmane BS, Labdi M, Benkada MY (2013). Sources of resistance in chickpea germplasm to three pathotypes of *Ascochyta rabiei* (Pass.) Labr. in Algeria. World Applied Sciences Journal 21 (6): 873-878. doi: 10.5829/idosi.wasj.2013.21.6.2874

Benzohra IE, Bendahmane BS, Youcef M (2018). Pathotyping study of Algerian *Ascochyta rabiei* isolates using screening test of ascochyta blight severity assessment. International Journal of Biological Sciences and Research 1 (4): 222-231.

Bouhadida M, Benjannet R, Madrid E, Amri M, Kharrat M (2013). Efficiency of marker-assisted selection in detection of ascochyta blight resistance in Tunisian chickpea breeding lines. Phytopathologia Mediterranea 52 (1): 202-211. doi: 10.1111/10.14601/Phytopathol_Mediterr-12134

Castro P, Rubio J, Madrid E, Fernández-Romero MD, Millán T et al. (2015). Efficiency of marker-assisted selection for ascochyta blight in chickpea. The Journal of Agricultural Science 153 (1): 56-67. doi: 10.1017/S0021859613000865

Chen W, Coyne CJ, Peever TL, Muehlbauer FJ (2004). Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Pathology 53: 759-769. doi: 10.1111/j.1365-3059.2004.01103.x

Cho S, Chen W, Muehlbauer FJ (2004). Pathotype specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theoretical and Applied Genetics 109: 733-739. doi: 10.1007/s00122-004-1693-x

Chongo G, Gossen BD, Buchwaldt L, Adhikari T, Rimmer SR (2004). Genetic diversity of *Ascochyta rabiei* in Canada. Plant Disease 88: 4-10. doi: 10.1094/PDIS.2004.88.1.4

Collard BCY, Ades PK, Pang ECK, Brouwer JB, Taylor PWJ (2001). Prospecting for sources of resistance to ascochyta blight in wild *Cicer* species. Australasian Plant Pathology 30: 271-276. doi: 10.1071/AP01036

Flandez-Galvez H, Ford R, Pang ECK, Taylor PWJ (2003). An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged microsatellite site and resistance gene analog markers. Theoretical and Applied Genetics 106 (8): 1447-1456. doi: 10.1007/s00122-003-1371-4

Gaur PM (2015). Chickpea. In: Singh CB, Khare D (editors). Genetic Improvement of Field Crops. Jadalpur, India: Scientific Publishers, pp. 87-108.

Gil J, Castro P, Millan T, Madrid E, Rubio J (2017). Development of new kabuli large-seeded chickpea materials with resistance to Ascochyta blight. Crop and Pasture Science 68 (11): 967-972. doi: 10.1071/CP17055

Hamwieh A, Imtiaz M, Hobson K, Kemal SA (2013). Genetic diversity of microsatellite alleles located at quantitative resistance loci for Ascochyta blight resistance in a global collection of chickpea germplasm. Phytopathologia Mediterranea 52 (1): 183-191. doi: 10.14601/Phytopathol_Mediterr-12163

Imtiaz M, Abang MM, Malhotra RS, Ahmed S, Bayaa B et al. (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. doi: 10.1094/PDIS-04-11-0333

Iqbal SM, Ghafoor A, Ayub N, Ahmad Z (2004). Pathogenic diversity in *Ascochyta rabiei* isolates collected from Pakistan. Pakistan Journal of Botany 36 (2): 429-437.

Iruela M, Rubio J, Barro F, Cubero JI, Millán T et al. (2006). Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance. Theoretical and Applied Genetics 112 (2): 278-287. doi: 10.1007/s00122-005-0126-9

Iruela M, Castro P, Rubio J, Cubero JI, Jacinto C et al. (2007). Validation of a QTL for resistance to ascochyta blight linked to resistance to fusarium wilt race 5 in chickpea (*Cicer arietinum* L.). In: Tivoli B, Baranger A, Muehlbauer FJ, Cooke BM (editors). Ascochyta blights of grain legumes. Dordrecht, The Netherlands: Springer, pp. 29-37.

Kemal SA, Bencheqroun SK, Hamwieh A, Imtiaz M (2017). Effects of temperature stresses on the resistance of chickpea genotypes and aggressiveness of *Didymella rabiei* isolates. Frontiers in Plant Science 8: 1607. doi: 10.3389/fpls.2017.01607

Madrid E, Rajesh PN, Rubio J, Gil J, Millán T et al. (2012). Characterization and genetic analysis of an EIN4-like sequence (CaETR-1) located in QTL AR1 implicated in ascochyta blight resistance in chickpea. Plant Cell Reports 31 (6): 1033-1042. doi: 10.1007/s00299-011-1221-9

Madrid E, Chen W, Rajesh PN, Castro P, Millán T et al. (2013). Allele-specific amplification for the detection of ascochyta blight resistance in chickpea. Euphytica 189: 183-190. doi: 10.1007/s10681-012-0753-6

Millán T, Rubio J, Iruela M, Daly K, Cubero JI et al. (2003). Markers associated with Ascochyta blight resistance in chickpea and their potential in marker-assisted selection. Field Crops Research 84: 373–384. doi: 10.1016/S0378-4290(03)00103-5

Navas-Cortés JA, Trapero-Casas A, Jimenez-Diaz RM (1998). Phenology *of Didymella rabiei* development on chickpea debris under field conditions in Spain. Phytopathology 88 (9): 983-991. doi: 10.1094/PHYTO.1998.88.9.983

Nene YL, Reddy MV (1987). Chickpea diseases and their control. In: Saxena MC, Singh KB, editors. The Chickpea; CABI, UK: Oxon, pp. 233-270.

Özer G, Bayraktar H, Dolar FS (2012). Genetic diversity and mating-type distribution within populations of *Ascochyta rabiei* in Turkey. Indian Journal of Agricultural Sciences 82: 3-7.

Pande S, Siddique KHM, Kishore GK, Bayaa B, Gaur PM et al. (2005). Ascochyta blight of chickpea (*Cicer arietinum* L.): A review of biology, pathogenicity and disease management. Australian Journal of Agricultural Research 56: 317-332. doi: 10.1071/AR04143

Peever TL, Chen W, Abdo Z, Kaiser WJ (2012). Genetics of virulence in *Ascochyta rabiei*. Plant Pathology 61: 754-760. doi: 10.1111/j.1365-3059.2011.02566.x

Reddy MV, Singh KB (1984). Evaluation of a world collection of chickpea germplasm accessions for resistance to Ascochyta blight. Plant Disease 68: 900-901. doi: 10.1094/PD-68-900

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ, Muehlbauer FJ (2000). Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. Crop Science 40: 1606-1612. doi: 10.2135/cropsci2000.4061606x

Sharma M, Ghosh R (2016). An update on genetic resistance of chickpea to Ascochyta blight. Agronomy 6 (1): 18. doi: 10.3390/agronomy6010018

Singh KB, Reddy MV (1983). Inheritance of resistance to ascochyta blight in chickpea. Crop Science 23: 9-10. doi: 10.2135/cropsci1983.0011183X002300010003x

Strange RN, Gewiss E, Gil J, Millan T, Rubio J et al. (2004). Integrated control of blight of chickpea, *Cicer arietinum*, caused by the fungus *Ascochyta rabiei*: An overview. In: Proceedings of the 5th European Grain Legume Conference; Dijon, France. pp. 71-76.

Şahin N (2015). Pathogenic characterization and mating type analysis of *Didymella rabiei* causing Ascochyta blight of chickpea in the Central Anatolia Region. MSc, Gaziantep University, Gaziantep, Turkey.

Tar'an B, Warkentin TD, Tullu A, Vandenberg A (2007). Genetic mapping of ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using a simple sequence repeat linkage map. Genome 50 (1): 26-34. doi: 10.1139/g06-137

Tekeoglu M, Rajesh PN, Muehlbauer FJ (2002). Integration of sequence tagged microsatellite sites to the chickpea genetic map. Theoretical and Applied Genetics 105: 847-854. doi: 10.1007/s00122-002-0993-2

Türkkan M, Dolar F (2009). Determination of pathogenic variability of *Didymella rabiei*, the agent of ascochyta blight of chickpea in Turkey. Turkish Journal of Agriculture and Forestry 33 (6): 585-591. doi: 10.3906/tar-0901-34

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

Udupa SM, Baum M (2003). Genetic dissection of pathotype-specific resistance to ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theoretical and Applied Genetics 106: 1196-1202. doi: 10.1007/s00122-002-1168-x

Vail S, Banniza S (2008). Structure and pathogenic variability in *Ascochyta rabiei* populations on chickpea in the Canadian prairies. Plant Pathology 57: 665-673. doi: 10.1111/j.1365-3059.2008.01837.x

Vail S, Banniza S (2009). Molecular variability and mating-type frequency of *Ascochyta rabiei* of chickpea from Saskatchewan, Canada. Australasian Plant Pathology 38: 392-398. doi: 10.1071/AP09014

Winter P, Pfaff T, Udupa SM, Huttel B, Sharma PC et al. (1999) Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*C. arietinum* L.). Molecular Genetics and Genomics 262: 90-101. doi: 10.1007/s004380051063

Wise KA, Henson RA, Bradley CA (2009). Fungicide seed treatment effects on seed borne *Ascochyta rabiei* in chickpea. HortTechnology 19 (3): 533-537. doi: 10.21273/HORTSCI.19.3.533