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Comparison of Different Screening Methods for Selection of Ascochyta Blight Disease on Chickpea (Cicer arietinum L.) Genotypes

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Research Article

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Abstract

Chickpea (*Cicer arietinum* L.) is the second most important and commonly grown edible food legume crop all over the world. However, chickpea cultivation and production are mainly affected by Ascochyta Blight (AB) disease that results in up to 100% loss in areas having high humidity and warm temperature conditions. Various screening methods are used in the selection of chickpea genotypes for resistance to AB disease. These methods are Natural Field Condition (NFC), Artificial Epidemic Field Condition (AEFC), Marker Assisted Selection (MAS), and Real-Time PCR (RT-PCR). The study was conducted between the 2014 to 2016 growing seasons with 88 chickpea test genotypes. The results of the screening were used to sort the genotypes into the three categories susceptible (S), moderately resistant (MR), and resistant (R). Using MAS screening, 13, 21, and 54 chickpea genotypes were identified as S, MR, and R, respectively. For RT-PCR screening, 39 was S, 31 was MR, and 18 genotypes were R. NFC screening revealed 7, 17, and 64 genotypes that were S, MR, and R while 74 and 6 genotypes were S and MR, 8 genotypes were R to AB disease in the AEFC method.

Introduction

Chickpea (Cicer arietinum L. 2n: 16) is the second most widely grown pulse crop and a major plant-derived protein source for human nutrition in the world after dry bean. It is produced in 153 countries in different parts of the world. It is grown over 15 million•ha^{-1} with a production about 15.9 million tons. Yield of chickpea in the World is 1058 kg/ha. Turkiye accounts for more than 475,000 tons of chickpea production (Faostat, 2021).

Chickpea cultivation in Turkiye is done in dry farming method without irrigation (Bayramoglu et al., 2010; Erman et al., 2012). The climate conditions of the chickpea growing areas are characterized by low annual total precipitation, low winter temperatures, hot and dry summers, and the majority of precipitation (65%) falling in winter and spring (Kusmenoglu et al.,1995).

In Turkiye, chickpea is grown in both the spring and the fall seasons. In high altitudes, it is cultivated in the spring, whereas at lower altitudes, it is sown in the fall (Aydogan et al., 2009).

Efficiency and profitability are low in chickpea production. The distinctive traits of legumes, as well as a variety of biotic and abiotic stress factors, are to blame for chickpeas' low productivity (Slinkard et al., 2000). Ascochyta rabiei-caused blight disease (AB) is the main disease limiting chickpea yield in Turkiye (Kusmenoglu et al., 1996). To prevent the illness known as Ascochyta chickpea blight, planting time is postponed in Turkiye. Blight disease, which causes significant economic losses (Haware et al., 1986), is seen in more than 40 countries (Bhardwaj et al., 2010; Sharma et al., 2016). When conditions are suitable for the epidemic, it can cause up to 100% product losses and it is also known that the producer has not obtained any products during this epidemic season season (Trapero-Casas et al.,1986; Nene et al., 1987; Pand et al., 2005; Singh et al., 2007). This indicates the global importance of the disease.

The severity and occurrence of AB in cultivated chickpea is highly weather-dependent with damaging effects at both vegetative and podding stages of the crop in regions with cool (15˚C – 25˚C) and humid weather conditions (> 150 mm precipitation) during the crop growing season. The pathogen causes severe blight epidemics and substantial yield losses, especially in susceptible cultivars and under favorable disease conditions (Sing et al., 1990; Shtienberg, 2010). Due to the strong genotype x environment (G × E) interaction, the disease status may vary significantly from year to year depending on the presence of the pathogen in the environment (McDonald et al., 2002).

Many studies have been conducted on the inheritance of the durability of As- cochyta blight resistance (Bhardwaj et al., 2009; Tekeoglu et al., 2000). The majority of research state that disease re sistance had a quantitative feature and was connected to many quantitative trait loci (QTL) (Tekeoglu et al., 2002; Anbessa et al., 2009). One of the methods from cultural, host plant resistance (use of resistant varieties), chemical control and biological control is used to combat the disease (Foresto et al., 2023). Using resistant cultivars while planting is the most efficient, cost-efficient, and ecologically beneficial technique out of these (Li et al., 2015; Chen et al., 2004; Jimenez-Diaz et al.,1993).

Hence, increasing host tolerance and developing resistant cultivars are the key goals of chickpea breeding studies, particularly in nations where the AB is severe and extensive. Varieties of techniques are used to screen breeding materials to develop resistant

varieties and lines.

In addition to field, greenhouse, and controlled climatic circumstances (Pande et al. 2011a; Kaur et al. 2014; Varshney et al. 2014), molecular techniques like Real-Time PCR and Marker Assisted Selection (MAS) is also utilized in breeding projects to screen genetic material for the presence of the AB (Kumar et al., 2016). Field conditions artificial inoculation test method used to screen breeding material for disease was developed by ICARDA and ICRISAT (Nene et al., 1981; Singh et al., 1984). In the method; in the observation garden to be established in the field, a sensitive control is placed in every two to four rows. Conidia suspensions obtained from diseased plants collected one year ago, are sprayed on the plants. Two evaluations are made, the first after the sensitive control dies, and the second during the pod-setting period. In a study was conducted by ICARDA and ICRISAT, 15,300 acceptance and desi materials were tested for AB in field and greenhouse conditions. 12 kabuli, 3 desi type chickpea AB was found to be resistant to Pathotype 1, 2, 3, 4 (Singh et al.1992). 112 materials of Kabuli and desi type were tested in 51 locations in different countries. In the trial, lines numbered ILC 72, 191, 3279 and 3856 were found to be resistant in 8 of 11 countries (Singh et al., 1984).

In natural conditions, no intervention is made on the spread and severity of the disease, except for the presence of sensitive varieties in the trial. Observations of disease are made twice under natural circumstances during bean tying and during harvest. Namely, the material is assessed for disease. Under natural and artificial field epidemic conditions, there is a strong genotype x environment (G × E) interaction, which causes the disease state to alter significantly from year to year depending on the presence of the pathogen in the environment (McDonald et al., 2002). Environmental conditions significantly affect the severity and prevalence of the disease in Natural Field Conditions (NFC) and Artificial Epidemic Field Conditions (AEFC).

Marker Assisted Selection (MAS) is a further technique for genotype-based AB screening of chickpeas. For qualities that are challenging to select, such as disease resistance and abiotic stressors, MAS is particularly beneficial. Because it is simpler than phenological screening, is unaffected by environmental influences, is safer, and enables early selection, MAS is frequently used to check breeding material for disease (Yorgancilar et al., 2015).

SSR, SCAR, ISSR and RAPD techniques are used in these scans (Ali et al., 2008). Resistant gene-based markers are developed and used in selection for different diseases in different plants. For example, SSR marker for Leaf rust (Puccinia recondita f.sp.tritici) in wheat (Suenaga et al., 2003), SA598 SCAR for Gall midge (Orseolia oryzae) in paddy (Sardesai et al., 2002), SCAR and CAPS for Sugarcane mosaic virus (SCMV) in maize (Dussle et al., 2002), CAPS (Graner et al., 2000) for Leaf rust (Puccinia hordei) in barley.

Resistant QTLs have also identified for Ascochyta blight of chickpea. Many markers are used for MAS (Iruela et al., 2006; Imtiaz et al., 2008; Castro et al., 2013; Sudheesh et al., 2021). It is stated that 4 SCAR (the Sequence Characterized Amplified Region) markers are used for QTLs (QTL_{AR1} and QTL_{AR2}), which are identified as being associated with the resistance of Ascochyta blight disease caused by Ascochyta rabiei in an Kabuli × Desi RIL population (Iruela et al., 2006). SCAR markors detect local varieties, advanced breeding lines, disease susceptibility and resistance of allele of cultivars in 90% (Madrid et al., 2013).

Another screening method used for Ascochyta Blight is Real-Time PCR (RT- PCR). Phan et al., (2002) developed a PCR-RFLP assay for the detection of the pathogen in infected leaves or seeds of the host (chickpea) using primers target- ing the conserved sequences of the internal transcribed spacer (ITS) regions of A. rabiei. The technique is a sensitive method for measuring pathogen DNA. With this technique, the severity of the disease can be determined and the disease can be monitored (Gachon et al., 2004; Schena et al., 2004; Pache et al., 2013). The method is widely used to detect and identify pathogens and its amount in infected seeds and plant tissues of the host, and to disease severity (Udapa,1997; Rigotti, 2002; Jime´nez-Ferna´ndez et al., 2011; Leiminger et al., 2015).The article is a output of the multidisciplinary project titled “Development of Germplasm Tolerant to Chickpea Blight (Ascochyta rabiei) by Combining Classical and Modern Breeding Techniques”.

In the study, Natural Field Conditions, Artificial Epidemic Field Conditions, Marker Assisted Selection and Real-Time PCR methods were used for Ascochyta Blight selection of the project material. The aim of the study is to compare the screening methods of chickpea breeding materials for AB and to determine the most effective method.

Materials and Methods

2.1. Material

The material consisted of eighty-four advanced chickpea lines (genotypes) and four checks [Çağatay, Gökçe, Azkan (kabuli type), ICC3996 (desi type)]. 84 genotypes were lines that could be cultivar candidates. The 4 checks were cultivars that are widely cultivated in Turkey with different disease resistance levels. These were evaluated for their reaction to Ascochyta blight (AB). All lines were kabuli types. Çağatay and Gökçe were susceptible, while Azkan (Aydin, et al, 2016) and ICC3996 (Zhou et al, 2019) were resistance to AB. The 50% flowering days of the materials of the experiment varied between 76–87 days. Whereas, the 100 grain weight of the materials was between 23.6 and 42.7 g.

2.2. Methods

The genotypes in the study were screened and evaluated using four screening methods for identifying AB disease resistance. These methods were Natural Field Condition (NFC), Artificial Epidemic Field Condition (AEFC), Marker Assisted Selection (MAS), and Real-Time PCR (RT-PCR).

Natural Field Condition (NFC): Yield and preliminary yield trials at Hay mana, Ankara, Turkiye were conducted using three and two replications under field conditions respectively during 2014 growing season. The plot dimensions are 6 m^2 (5 m × 0.3 m × 4 rows) and the height of the cultivation area is 1050 m above sea level. 45 seeds were used per m^2. During the cultivation process, the total amount of precipitation was 218.2 mm and the highest precipitation was in June with 74.8 mm. The climate data of the experimental area are given in Fig. 1. The amount of precipitation was more than the average for many years.

The trials were planted on March 6, 2014. AB observations were taken three times (flowering, podding and harvest stage). Thereafter, mean AB observations for each line were calculated. Disease scoring was recorded on 1–9 (1: resistance, 9: susceptible) disease rating scale (Reddy et al., 1984). Then, disease scores were modified as Pande et al., (2011a).

Artificial Epidemic Field Conditions (AEFC): Genotypes of the yield and preliminary yield trials in the breeding program of 2014 were used as material in the experiment with 88 genotypes sown with two replications in 1 m rows under the field conditions as a disease nursery at Haymana, Ankara. Climate data of experimental area is given in Fig. 1. The genotypes were sown on March 25, 2014.

Table 1
Scoring and classifying for AB disease.

R	1 = No infection
	2 = Highly resistant (1–5% of plants showed blight)
	3 = Resistant (6–10% showed blight)
MR	4 = Moderately resistant (11–15% showed blight)
	5 = Intermediate (16–40% showed blight)
S	6 = Moderately susceptible (41–50% showed blight)
	7 = Susceptible (51–75% showed blight)
	8 = Highly susceptible (76–100% showed blight)
	9 = All plants died

The isolates of Pathotype 1 were used as an artificial inoculation source. After 57 days from sowing, the trials were inoculated by spraying aqueous spore suspensions having a concentration of 5×10^5 spores/mL. The nursery was inoculated with diseased debris and sprinkler irrigation was provided to create humid conditions (Udupa et al., 2003; Chen et al., 2005).

The disease observations were taken when susceptible check genotypes had completely succumbed to AB disease. The evaluation of chickpea genotypes for AB reaction was performed by using a rating scale based on the severity of infection on

leaves, stems, and pods as proposed by Reddy et al. 1984. Disease observations were taken three times during the experiment. Afterwards, the average of three observations was calculated. Then these scores were grouped as shown in Table 1.

Marker Assisted Selection (MAS): The Sequence Characterized Amplified Region (SCAR) Genomic DNA was isolated from the leaves of the 88 genotypes. For DNA isolation, Gene Matrix Plant Fungi DNA Purification Kit (Cat No: E3595) was used and done according to Kwasniak et al., 2013. DNA quality and quantity measurements were by made using 1% agarose gel and Nanodrop ND- 1000 spectrophotometer. PCR reaction of the three SCAR markers; 15 ng DNA, 5 pmol forward primer, 5 pmol reverse primer, 0.5 mM total dNTP, 0.5 unit Go Taq DNA Polymerase (Promega) (containing 1.5 mM $MgCl_2$), 3 µl buffer (5× Buffer) carried out at a total of 15 µl. SCAR-primer sequences are presented in Table 2.

The PCR program used for the PCR reaction (Touchdown):

1) 3 minutes at 94˚C

2) 1 min at 94˚C

3) From 66˚C to 57˚C for 1 min 45 sec

2 min at 4.72˚C

It was applied as a total of 21 cycles, 10 minutes at 5.72˚C.

After PCR, PCR products of loci were visualized on 2% agarose gel and band profiles were determined. Definition of band profiles was made according to Iruela et al., 2006 ; and Winter et al., 1999. The materials were evaluated at the $SCY17_{590}$ mark, band profiles showed resistance of genotypes at 590 bp, and moderately resistance at $SCY19_{336}$ mark with 336 bp and susceptible if there was no band on $SCY19_{336}$ mark. Both markers ($SCY19_{336}$, $SCK13_{603}$) showed similar efficacy and it was observed that the $SCY17_{590}$ marker determined the genotype with a greater number of resistance alleles. When the markers $SCY19_{336}$, $SCK13_{603}$, and $SCY17_{590}$ were scanned in agarose gel electrophoresis for comparison among themselves, it was seen that all of these markers showed the presence of resistance allele and moderately resistant genotypes were not found.

Table 2
Information on SCAR primers' sequences

Marker name	The primer sequences (5'-3')
SCAE19336	Forward: gacagtccctccattatctaaac
SCAE19336	Reverse: gacagtccctatgtgtgagaat
SCK13603	Forward:ggttgtaccccatcctcccg
SCK13603	Reverse: ggttgtacccttgtgccacta
SCY17590	Forward:gacgtggtgactatctagc
SCY17590	Reverse: gacgtggtgaaaatagatacc
Source: Iruela et al., 2006	

In addition, classifying for disease was made in the marker evaluations. In this grouping, they were evaluated as a resistance (R), if the genotype was resistance to three markers, the genotype had moderately resistance (MR), if it was resistant for one or two markers, it was susceptible, if genotype was susceptible (S) for three markers.

Real-Time PCR (RT-PCR): The pathogen isolate was grown in petri dishes containing Chickpea-Flour-Dextrose-Agar medium for 14 days in an incubation room at 22˚C ± 1˚C and 12 hours of light (near UV) period. Chickpea-Flour- Dextrose-Agar (CSMDA: 40 g chickpea flour, 20 g dextrose, 20 g agar and 1 l pure water) medium is the most suitable medium for sporulation.

The concentration of this prepared spore suspension was determined by counting with a thoma slide and diluted with sterile water to 1×10^5 spores/ml. Study materials were grown in pots. Three Petri dishes were used for each inoculation point, and each Petri dish contained ten leaflets. Detection of *Ascochyta rabiei* in plant tissue was made by real-time PCR method that was reported by by Udupa et al. 1997 and subsequently developed by Bayraktar et al., 2016. The samples were taken from all genotypes on the 8th day after inoculation. Also, disease reactions in chickpea leaflets were calculated after each inoculation period. Disease incidence (%) was expressed as the proportion of diseased leaflets. Percent disease severity was evaluated from the affected leaflet size based on 0–5 scale 0: no lesion, 1: 10%; 2: 25%; 3: 50%, 4: 75%, and 5: 100% affected leaflets (Dolar et al.,1994). According to the classification, percent disease rate was evaluated into three categories; 0: up to 10% as resistant (R), 2–3: 10% – 50% as moderately resistant (MR), and 4–5 over 50% as susceptible (S).

In the study, the regression coefficient between the amount of pathogen in the leaf and tissue and the disease severity and incidence was calculated.

Results

Natural Field Condition (NFC): In screening method of NFC about 62 (73%), 17 (19%), and 5 (8%) genotypes were as R, MR, and S, respectively. While Azkan and ICC 3996 were resistant to AB, Çağatay and Gökçe were found to be susceptible in checks. In this growing season rainfall, relative humidity, and temperature were not suitable for occurrence and spread of AB in Haymana. The classifications of the AB disease observation among the genotypes in yield trials of 2014 under the natural field condition are given in Fig. 2.

The seasonal rainfall and relative humidity were appropriate for spore production and mycel development but the temperature was not favorable. Stages of flowering and pod filling period were on June in Haymana. At these stages, chickpea is the most sensitive period for AB spread. June had good conditions for AB spread with 74.8 mm rainfall and 65.6% relative humidity, while there were not suitable temperatures (17.9˚C). Therefore, the disease did not exist and spread in this season. As a general rule, if rainfall, relative humidity, and temperature are missing or insufficient, the crop is either less affected or not damaged by the AB disease. Therefore, NFC had the highest number of resistant material.

Artificial Epidemic Field Condition (AEFC): Artificial inoculation conditions and climatic conditions during the growing season had a positive effect on the development and severity of the disease. 72.6 and 6 genotypes of a total number of 84 advance lines in AEFC were susceptible (score: 6, 7, 8, 9), moderately resistance (score: 4, 5) and resistance (score: 1, 2, 3), respectively (Fig. 3). Çağatay and Gökçe were identified as a susceptible (score: 8, 9) while Azkan and ICC3996 were detected as a resistant in the method of AEFC. Under AEFC, 9% of the genotypes were resistant (R), 7% were moderately resistance (MR), while 84% were susceptible (S). Resistant genotypes of out of checks were line Tüb 18, 19, 70, 71, 72, and 82.

Marker Assisted Selection (MAS): PCR reactions of 3 SCAR ($SCY19_{336}$, $SCK13_{603}$, and $SCY17_{590}$) markers were studied in 88 chickpea genotypes for MAS against AB in the study, using both agarose gel electrophoresis and capillary electrophoresis conditions. The separation of the genotypes carrying the resistance allele was determined according to the band profiles.

The results showed that 84 genotypes and four standards scanned with the help of three markers. About 21 sensitive genotypes and they were classified as a moderately resistant (MR), and in 13 genotypes, were considered susceptible because the resistance alleles could not be determined (S) (Table 3).

Table 3
The AB resistant and susceptible genotypes using 3 SCAR markers for the MAS of chickpea germplasm.

Genotype	Molecular Screening				Genotype	Molecular Screening			
	SCAE19 336	SCK 13 603	SCY17 590	Response *		SCAE19 336	SCK 13 603	SCY17 590	Response *
Tüb-01	+	+	+	R	Tüb-50	+	+	+	R
Tüb-02	+	+	+	R	Tüb-51	+	+	+	R
Tüb-03	+	-	-	MR	Tüb-52	+	-	-	MR
Tüb-04	+	-	-	MR	Tüb-53	+	+	+	R
Tüb-05	+	+	+	R	Tüb-54	-	-	-	S
Tüb-06	+	+	+	R	Tüb-55	-	-	-	S
Tüb-07	+	+	+	R	Tüb-56	+	-	-	MR
Tüb-08	+	+	+	R	Tüb-57	+	-	-	MR
Tüb-09	+	+	-	MR	Tüb-58	+	+	+	R
Tüb-10	+	+	+	R	Tüb-59	+	-	-	MR
Tüb-11	+	+	+	R	Tüb-60	-	-	-	S
Tüb-12	+	+	+	R	Tüb-61	+	+	+	R
Tüb-13	+	+	+	R	Tüb-62	-	-	-	S
Tüb-14	+	+	+	R	Tüb-63	+	+	+	R
Tüb-16	+	+	+	R	Tüb-64	-	-	-	S
Tüb-18	+	+	+	R	Tüb-65	+	+	+	R
Tüb-19	+	+	+	R	Tüb-66	+	+	+	R
Tüb-20	+	+	+	R	Tüb-67	+	+	+	R
Tüb-21	-	-	-	S	Tüb-68	+	+	+	R
Tüb-22	+	+	+	R	Tüb-69	+	+	+	R
Tüb-23	+	-	-	MR	Tüb-70	+	-	-	MR
Tüb-25	+	-	-	MR	Tüb-71	+	-	-	MR
Tüb-26	+	+	-	MR	Tüb-72	+	+	+	R
Tüb-27	+	-	-	MR	Tüb-74	+	+	+	R
Tüb-28	+	+	-	MR	Tüb-75	+	+	+	R
Tüb-29	+	+	-	MR	Tüb-76	+	+	+	R
Tüb-30	+	+	-	MR	Tüb-78	+	+	+	R
Tüb-31	-	-	-	S	Tüb-79	+	+	+	R
Tüb-33	+	+	-	MR	Tüb-82	+	+	+	R
Çağatay	+	+	+	R	Tüb-84	-	-	-	S
Gökçe	+	+	+	R	Tüb-86	+	+	+	R

Genotype	Molecular Screening				Genotype	Molecular Screening			
	SCAE19 336	SCK 13 603	SCY17 590	Response *		SCAE19 336	SCK 13 603	SCY17 590	Response *
Tüb-37	+	-	+	MR	Tüb-87	+	+	+	R
Tüb-38	+	+	+	R	Tüb-93	+	+	+	R
Tüb-39	+	+	+	R	Tüb-96	-	-	-	S
Tüb-40	+	+	+	R	Tüb-97	+	-	+	MR
Tüb-41	+	+	+	R	Tüb-100	+	-	-	MR
Tüb-42	+	+	+	R	Tüb-105	+	+	+	R
Tüb-43	+	+	+	R	Tüb-108	+	+	+	R
Tüb-44	-	-	-	S	Tüb-114	+	+	+	R
Tüb-45	+	+	+	R	Tüb-119	+	+	+	R
Tüb-46	-	-	-	S	Tüb-121	+	+	+	R
Tüb-47	+	+	-	MR	Tüb-124	+	+	+	R
Tüb-48	-	-	-	S	**Azkan**	+	+	+	R
Tüb-49	-	-	-	S	**ICC 3996**	+	+	+	R

In the study conducted with three MAS markers, 61%, 24%, and 15% of the genotypes were evaluated as a resistant, moderately resistant and susceptible, respectively. All checks in this method were identified as resistant genotypes.

Real-Time PCR (RT-PCR): The disease incidence in 8 of the chickpea genotypes tested in the study was 100%. Furthermore 15 genotypes which showed a disease incidence of 0% – 10%, 30 genotypes with 11% – 40% resistance and 43 genotypes with 40% – 100% were resistant (R), moderately resistant (MR) and susceptible (S) respectively.

The susceptible checks, Çağatay and Gökçe had disease severity and incidence level of 2%, 6.67%, 5.33% and 5.33%, 23.33% respectively. In this method, Çağatay was resistant, while Gökçe was determined as moderate resistant. Among the resistance standards, Azkan was evaluated as the resistant (R) group with 1.93% disease severity or 10% disease incidence, and ICC3996 had no dis- ease severity and disease incidence (Table 4). It was determined that the genotypes evaluated with the RT-PCR method in the study were 21% resistant (R), 35% moderately resistant (MR) and 44% susceptible.

Positive relationship was determined between the amount of DNA (ng) of the pathogen in leaf with the percent disease severity and the percent disease incidence. The relationship between the amount of pathogen in leaf and the disease severity (r^2: 0.53) and between the disease severity and disease incidence (r^2: 0.73) was significant. However, the relationship between the pathogen amount in the leaf (ng) with the disease incidence % (r^2: 0.29) was not significant. The presence of the pathogen in the plant or increase in the amount of DNA of the pathogen did not mean that the plant will be more severely diseased. While Tüb-14: 0.08 ng and Tüb-41: 0.22 had very little pathogenic DNA, the percentage of disease rate can be as high as 40% and 70%, respectively. On the contrary, the amount of disease in Tüb-38 (6.71 ng) and Tüb-49 (4.55 ng), which have high DNA content in the leaf, remained at a low level of disease incidence of 20% and 30%, respectively.

Table 4
DNA amount (ng), disease severity %, disease incidence %, and disease classification in plants inoculated with the de detached leaf inoculation method.

Genotypes	The amount of pathogen DNA (ng)	Disease severity %	Disease incidence %	Response	Genotypes	The amount of pathogen DNA (ng)	Disease severirty %	Disease incidence %	Responce
Tüb-01	3,23	23,33	73,33	S	Tüb-50	3,76	15,33	46,67	S
Tüb-02	20,55	56,19	90,48	S	Tüb-51	0,393	1,33	6,67	R
Tüb-03	4,66	16,67	70	S	Tüb-52	1,782	21,9	66,67	S
Tüb-04	4,86	24,67	76,67	S	Tüb-53	2,578	5,33	20	MR
Tüb-05	1,95	8	36,67	MR	Tüb-54	9,007	45,24	80	S
Tüb-06	0,28	8,67	36,67	MR	Tüb-55	1,145	2,67	13,33	MR
Tüb-07	1,26	8,67	40	MR	Tüb-56	0,215	2,67	13,33	MR
Tüb-08	4,47	10	33,33	MR	Tüb-57	0,509	6,67	23,33	MR
Tüb-09	0,28	11,33	30	MR	Tüb-58	17,7	28	63,33	S
Tüb-10	0,63	8,67	30	MR	Tüb-59	10,54	22	56,67	S
Tüb-11	0,41	2	10	R	Tüb-60	5,411	16,67	43,33	S
Tüb-12	0,37	2	10	R	Tüb-61	2,331	9,33	36,67	MR
Tüb-13	3,48	6	23,33	MR	Tüb-62	43,05	59,05	100	S
Tüb-14	0,08	8	40	MR	Tüb-63	12,94	26,67	76,19	S
Tüb-16	0,09	4	20	MR	Tüb-64	8,515	22,86	76,19	S
Tüb-18	3,88	62	90	S	Tüb-65	0,043	2	10	R
Tüb-19	0,12	4,67	23,33	MR	Tüb-66	50,6	96,19	100	S
Tüb-20	1,99	10,67	43,33	S	Tüb-67	65,4	72,38	90,48	S
Tüb-21	0,81	2	6,67	R	Tüb-68	8,964	37,14	76,92	S
Tüb-22	0,52	1,33	6,67	R	Tüb-69	0,1	0	0	R
Tüb-23	0,11	2,67	10	R	Tüb-70	0,37	2,67	13,33	MR
Tüb-25	0,15	2	10	R	Tüb-71	4,879	14	30	MR
Tüb-26	0,01	0,67	3,33	R	Tüb-72	80,96	72,81	96,67	S
Tüb-27	2,08	16,67	73,33	S	Tüb-74	11,32	100	100	S
Tüb-28	1,74	9,33	40	MR	Tüb-75	134,9	78,1	100	S
Tüb-29	1,22	5,33	23,33	MR	Tüb-76	9,479	21,9	61,9	S
Tüb-30	0,84	6	26,67	MR	Tüb-78	31,77	34,29	52,38	S
Tüb-31	5,53	12	43,33	S	Tüb-79	1,346	10	46,67	S
Tüb-33	1,28	14	56,67	S	Tüb-82	0,024	1,43	7,14	R
Çağatay	0,004	2	6,67	R	Tüb-84	12,43	44,05	100	S

Genotypes	The amount of pathogen DNA (ng)	Disease severity %	Disease incidence %	Response	Genotypes	The amount of pathogen DNA (ng)	Disease severirty %	Disease incidence %	Responce
Gökçe	1,15	5,33	23,33	MR	Tüb-86	3,479	39,17	91,67	S
Tüb-37	0,5	3,33	13,33	MR	Tüb-87	18,39	58,57	85,71	S
Tüb-38	6,71	6,67	20	MR	Tüb-93	11,32	37,14	100	S
Tüb-39	0,22	4,67	23,33	MR	Tüb-96	0,785	4	16,67	MR
Tüb-40	15,36	51,7	93,1	S	Tüb-97	16,86	24	40	MR
Tüb-41	0,22	14,67	70	S	Tüb-100	3,375	11,33	40	MR
Tüb-42	2,43	8,67	36,67	MR	Tüb-105	83,37	65,71	100	S
Tüb-43	0,16	2	10	R	Tüb-108	51,53	95,24	100	S
Tüb-44	0,57	0,67	3,33	R	Tüb-114	0,786	4	20	MR
Tüb-45	0,66	4	10	R	Tüb-119	2,245	29,52	100	S
Tüb-46	0,94	4,67	20	MR	Tüb-121	0,585	23,33	93,33	S
Tüb-47	0,01	0	0	R	Tüb-124	3,004	46	100	S
Tüb-48	2,15	14,67	60	S	**Azkan**	0,64	1,93	10	R
Tüb-49	4,55	10,67	30	MR	**ICC 3996**	0	0	0	R

Discussion

The number of resistant genotypes was higher in the disease observation per- formed in NFC. In Haymana, the relative humidity (> 60%) reached the most favorable values for the formation, development, and spread of the disease during the flowering and pod filling period (June), when plant is the most sensitive to AB. Pande et al., (2011a, b) also stated that the disease is more widespread in environments with high humidity (> 60%). However, moisture is not a sufficient condition for the spread and occurrence of the disease. Temperature is also an important factor for the AB. In June, which is the flowering and pod filling period in Haymana, the average temperature was 17.9˚C. This temperature value is below 20˚C, which is stated to be positively correlated with the occurrence of the disease (Trapero-Casas et al., 1992). In addition, temperature and humidity values, which can be considered appropriate under natural epidemic not noted in the early stage of growth, but during later period, it increased resistance. Singh et al. [64] also had similar observations and found susceptibility at later stages of the plant development in the early part of crop growth, when relative humidity was high (> 60%), cool temperatures (minimum < 5˚C, and maximum < 15˚C) were found to limit blight epidemics. Among the AB disease screening methods examined, the most resistant material was found in NFC with 73%. The reason for that there is no suitable conditions (humidity, temperature, and precipitation) in the location when the plant is the most sensitive stage.

In AEFC, relative humidity and spring irrigation good for disease development was carried out after inoculation (Chen et al., 2005). In this study, Pathotype 1 was used for the inoculation. Different genotypes were defined as a resistance, moderately resistance, and susceptible like many other studies (Chen et al., 2004; Singh et al., 1993; Gayacharan et al., 2020; Benzohra et al., 2013).

Among the examined disease screening methods, the most susceptible number of genotype was found in AEFC method with 84%. The results of the study are compatible with Gayacharan et al., 2020, who found that 10.6% genotypes were susceptible and 87.4% were highly susceptible in their study in 1970.

The dilution or concentration of the inoculation source (spore suspension), the infection of different spores from the environment, the inoculated isolate, the type of pathotype, spores its prevalence, aggressiveness, climatic conditions, time, and number of application of the inoculant influence the effectiveness of the method. Also, a positive correlation between field condition and controlled environment screening technique for AB was reported by Pande et al., (2011a).

In MAS experiment, 2 QTL (QTLAR1, QTLAR2) have relationship with AB disease were reported that in a study including three SCAR marker ($SCY19_{336}$, $SCK13_{603,}$ and $SCY17_{590}$) of QTLAR2 especially the $SCK13_{603}$ marker has been shown to be closely linked to the associated gene as cM, and it is recommended that this marker can be used primarily in susceptible/sensitive discrimination Iruela et al., (2006). In the study, both markers showed similar efficacy, $SCY17_{590}$ marker determined genotype with a higher number of resistance alleles differently. When the markers $SCY19_{336}$, $SCK13_{603}$, and $SCY17_{590}$ scanned in agarose gel electrophoresis were compared among themselves, it was seen that all of these markers showed the presence of resistance allele. Resistance could not be determined in at least one of the three SCAR markers in materials with moderately resistance. In the study with 3 markers, genotypes showing resistance allele in all markers reached 61% of the total genotype. This amount is close to the durability (73%) in natural conditions. The number of susceptible genotypes in MAS was lower than AEFC that suspicion increased about the efficiency of the marker. However, Ali et al., (2008) used 3 SCR (SCY603, 590, and SCADA SCY19) markers for the screening of 21 local chickpea genotypes and noted that one STMS marker (TA 146) and three SCAR markers ($SCAE19_{336}$, $SCK13_{603}$, $SCY17_{590}$) covering the distance of 0.5 cM on this linkage group were linked with resistance in genotypes. The genome walking method used in a study was useful to sequence flanking regions of the marker $SCK13_{603}$ tightly linked to QTLAR2 for AB resistance Iruela et al., (2009). There are studies that are compatible with our MAS findings but contradict our AEFC findings. One allele specific marker (CaETR) and one codominant $SCAR17_{590}$ marker and reported that these two markers contributed efficiently to the selection of new chickpea varieties with better combinations of alleles to ensure durable resistance to the AB (Bouhadida et al., 2013).

Two of the four SCARs showed significant alignment with genes or proteins related to disease resistance in other species and one of them ($SCK13_{603}$) was sited in the highly saturated region linked (Iruela et al., 2006). It is determined that it is resistant to ICC3996 pathotypes 1 and 2 (Chen et al., 2004) are compatible to the findings of this study, while susceptible checks (Gökçe and Çağatay) have resistance allele in three SCAR markers, they are identified as susceptible in AEF and NFC. In a study with 23 Tunisian chickpea genotypes found that V10 line showed resistance allele in CaETR and heterozygous of $SCAR17_{590}$, it is moderately resistant under natural conditions and controlled conditions (Bayraktar et al., 2016). The results are comparable to these findings. None of various AB resistance quantitative trait loci (QTLs) have been reported to be used in MAS.

A number of screening techniques under field and controlled environments have been reported for AB (Pande et al., 2005). Resistant cultivars are difficult to obtain due to the continuous evolution of the fungus and appearance of new pathotypes that overcome the resistance of existing cultivars. In addition, disease resistance is considered a quantitative trait and numerous QTLs have been identified on the chickpea genetic map [69]. Breeders are attempting to combine genes in a new cultivars to improve the level and durability of resistance but this process is further complicated when different QTLs or genes control the same phenotype. SCAR markers have some advantages such as highly reproducible, quick and simple, locus specific, despite SCAR markers have the disadvantages such as need gene sequence to design markers and sometimes radioactive isotopes is required (Dar et al., 2019).

Traditional methods of isolation and identification of A. rabiei are time-consuming. Polymerase Chain Reaction (PCR) techniques offer advantages over traditional plant disease diagnosis because organisms do not need to be cultured prior detection by PCR. Real-time PCR has been referred as a rapid, sensitive, and specific method for pathogen detection, the evaluation of host resistance, epidemiological studies, and disease management (Schena et al., 2004; Schaad et al.,, 2002).

Although there is a relationship between the amount of DNA of the pathogen in the leaf and the rate of disease, it has been observed that this is not very important because genotypes have both active and passive defence responses to stop initial pathogenic attacks and to prevent successful invasion and spread to neighboring cells (Coram et al., 2006). Passive defence mechanisms include preformed structural and chemical barriers such as glandular trichomes, which secrete antifungal isoflavones antifungal isoflavones (Armstrong-Cho et al., 2005). Active defence systems in plants may employ R genes to

recognise pathogen-specific effectors encoded by the Avrgenes (McDonald et al., 2002), leading to effector-triggered immunity (ETI) and possible programmed cell death (PCD) via a hypersensitive response (HR) (Jones et al.,2006). Real-time PCR compared to other screening method AEFC, while the number of resistance materials in the real-time PCR method is 18, this amount is only 8 in AEFC. Also, only 3 materials (Azkan, Tüb-18, and ICC3996) were found resistance in the real-time PCR technique in the AEFC screening method.

The PCR-based method developed can simplify both plant disease diagnosis, and pathogen monitoring in an early phase, as well as aid in effective management practices that avoid the disease advance and minimize losses (Valetti et al., 2021). Real time PCR has many advantages over conventional PCR. These advantages are; 1) it does not require the use of post PCR processing, 2) avoiding the risk of cross contamination, 3) reduction of the assay labor and material costs, and 4) in- crease the sensitivity and specificity and allows the accurate quantification the target pathogen (Kumar et al., 2016).

On the other hand, real-time PCR technique has some disadvantages such as contamination of the plant tissues by spores of the pathogen is possible. This is because genotypes have both active and passive defense responses to stop initial pathogenic attacks, prevent successful invasion, and spread to neighboring cells (Coram et al., 2006). This could have occurred during the sampling of tissues for the analysis, or naturally by spores transported on the surface of the trunk (Chandelier et. al., 2018). Furthermore, conventional lab-based PCR technology requires expensive laboratory equipment and skilled personnel, which is a major disadvantage in adopting this technology as a detection method for on-site purposes (DeShields et al., 2018).

In this study, 4 different screening methods, namely NFC, AEFC, MAS, and RT-PCR are used in the selection of AB disease resistance for chickpea genotypes. In the study, Azkan, ICC3996, and Tüb-82 were determined as a resistant within all methods for Pathotype 1. Among the examined screening methods, significant differences occurred in the level of resistance and number of genotypes in expression for AB disease. It was determined that the most effective method among the screening methods was AEFC. Resistance for AB of the genotype is one of the most important selection criteria for chickpea breeding strategy. The method of material selection for the disease should be effective, accurate, fast, and economic.

The occurrence of the disease in the NFC method depends on the environ- mental conditions, and there is an uncertainty of the inoculation source. In the AEFC method, it is partially dependent on environmental conditions, and that there may be isolates other than the given inoculant. In the MAS method, there is the lack of an effective marker for environment and genotypes cannot be de- fined precisely with markers for the disease. These facts make it useful for the early detection of infected tissues in the RT-PCR method. Considering all these unfavorable conditions, it was concluded that using fully controlled environ mental conditions and the artificial inoculation is the most effective method for screening chickpea genotypes in the AB disease evaluation.

Declarations

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

1. Ali, H., Iqbal, N., Haq, M.A., Shah, T.M., Atta, B. and Hameed, A. (2008). Detection of Qtls For Blight Resistance in Chickpea Genotypes with DNA Based Markers. *Pak. J. Bot., 40*(4): 1728, 2008.
2. Anbessa, Y., Taran, B., Warkentin, T.D., Tullu, A. and Vandenberg, A. (2009) Genetic Analyses and Conservation of QTL for Ascochyta Blight Resistance in Chick- pea (Cicer arietinum L.). *Theoretical and Applied Genetics*, 119, 757-765. https://doi.org/10.1007/s00122-009-1086-2

3. Armstrong-Cho, C. and Gossen, B.D. (2005) Impact of Glandular Hair Exudates on Infection of Chickpea by Ascochyta rabiei. *Canadian Journal of Botany*, 83, 22-27. https://doi.org/10.1111/j.1467-7652.2006.00208.x
4. Aydoğan, A., Gürbüz, A., Karagül, V. and Aydin, N. (2009) Yüksek Alanlarda Kişlik Nohu (Cicer arietinum L.) Yetiştirme İmkanlarinin Araştirilmasi. *Tarla Bitkileri Merkez Araştirma Enstitüsü Dergisi*, 18, 11-16.
5. Aydin, M.H., Oğuz, A., Erdemci, I. and Krademir, Ç. (2016) Control of Ascochyta Blight (Ascochytha rabiei) in Chickpea in Winter Sowing in Southeastern Anatolia. *The Journal of Turkish Phytopathology*, 45, 87-96.
6. Bayramoğlu, Z. and Gündoğmuş, E. (2010) Kurak İklim Bölgelerinde Organik Tarim Ve
7. Geleceği: Konya İli Örneği. Proceedings of International Conference on Organic Agriculture in Scope of Environmental Problems, Famagusta, 3-7 February 2010, 254-263.
8. Bayraktar, H., Özer, G., Aydoğan, A. and Palacioğlu, G. (2016) Determination of Ascochyta Blight Disease in Chickpea Using Real-Time PCR. *Journal of Plant Diseases and Protection*, 123, 109-117. https://doi.org/10.1007/s41348-016-0017-0
9. Benzohra, I.E., Bendahmane, B.S., Labdi, M. and Benkada, M.Y. (2013) Sources of Resistance in Chickpea Germplasm to Three Pathotypes of Ascochyta rabiei (Pass.)
10. Labr. In Algeria. *World Applied Sciences Journal,* 21, 873-878.
11. Bhardwaj, R., Sandhu, J.S., Kaur, L., Gupta, S.K., Gaur, P.M. and Varshney, R. Genetics of Ascochyta Blight Resistance in Chickpea. *Euphytica, 171*, 343. https://doi.org/10.1007/s10681-009-0020-7
12. Bouhadida, M., Benjannet, R., Madrid, E., Amri, M. and Kharrat, M. (2013) Efficiency of Marker-Assisted Selection in Detection of Ascochyta Blight Resistance in Tunisian Chickpea Breeding Lines. *Phytopathologia Mediterranea,* 52, 202-211.
13. Castro, P., Rubio, J., Madrid, E., Fernández-Romero, M., Millán, T. and Gil, J. Efficiency of Marker-Assisted Selection for Ascochyta Blight in Chickpea. *The Journal of Agricultural Science*, 153, 56-67. https://doi.org/10.1017/S0021859613000865
14. Chandelier, A., Massot, M., Fabreguettes, O., Gischer, F., Teng, F. and Robin, C. Early Detection of Cryphonectria parasitica by Real-Time PCR. *European Journal of Plant Pathology*, 153, 29-46. https://doi.org/10.1007/s10658-018-1538-0
15. Chen, W., Coyne, C.J., Peever, T.L. and Muehlbauer, F.J. (2004) Characterization of
16. Chickpea Differentials for Pathogenicity Assay of Ascochyta Blight and Identification of Chickpea Accessions Resistant to Didymella rabiei. *Plant Pathology*, 53, 759-769. https://doi.org/10.1111/j.1365-3059.2004.01103.x
17. Chen, W., Mcphee, K.E. and Muehlbauer, F.J. (2005) Use of a Mini-Dome Bioassay and Grafting to Study Resistance of Chickpea to Ascochyta Blight. *Journal of Phytopathology*, 153, 579-587. https://doi.org/10.1111/j.1439-0434.2005.01022.x
18. Cho, S., Chen, W. and Muehlbauer, F.J. (2004) Pathotype-Specific Genetic Factors in
19. Chickpea (Cicer arietinum L.) for Quantitative Resistance to Ascochyta Blight. *Theoretical and Applied Genetics*, 109, 733-739. https://doi.org/10.1007/s00122-004-1693-x
20. Cobos, M.J., Rubio, J., Strange, R.N., Moreno, M.T., Gil, J. and Millan, T. (2006) A New QTL for Ascochyta Blight Resistance in an RIL Population Derived from an Interspecific cross in Chickpea. *Euphytica*, 149, 105-111. https://doi.org/10.1007/s10681-005-9058-3
21. Coram, T.E. and Pang, E.C. (2006) Expression Profiling of Chickpea Genes Differentially Regulated during a Resistance Response to Ascochyta rabiei. *Plant Biotechnology Journal*, , 647-666. https://doi.org/10.1111/j.1467-7652.2006.00208.x
22. Dar, A.A., Mahajan, R. and Sharma, S. (2019) Molecular Markers for Characterization and Conservation of Plant Genetic Resources. *Indian Journal of Agricultural Sciences*, 89, 1789 https://doi.org/10.56093/ijas.v89i11.95286
23. De Shields, J.B., Bomberger, R.A., Woodhall, J.W., Wheeler, D.L., Moroz, N., Johnson, D.A. and Tanaka, K. (2018) On-Site Molecular Detection of Soil-Borne Phyto- pathogens Using a Portable Real-Time PCR System. *Journal of Visualized Experiments*, 132, e56891. https://doi.org/10.3791/56891-v
24. Dolar, F.S., Tenuta, A. and Higgins, V.J. (1994) Detached Leaf Assay for Screening Chickpea for Resistance to Ascochyta Blight. *Canadian Journal of Plant Pathology,* 16, 215-220. https://doi.org/10.1080/07060669409500756

25. Dussle, C.M., Quint, M., Xu, M.L., Melchinger, A.E. and Lübberstedt, T. (2002) Conversion of AFLP Fragments Tightly Linked to SCMV Resistance genes Scmv1
and Scmv2 into Simple PCR-Based Markers. *Theoretical and Applied Genetics*, 105, 1190-1195. https://doi.org/10.1007/s00122-002-0964-7
26. Erman, M., Çiği, F. and Çelik, M. (2012) Potasyum Uygulamasının Farklı Nohut Çeşitlerinde Verim, Verim Öğeleri ve Nodülasyona Etkileri. Tarim Bilimleri Araştirma Dergisi, 5, 124-127. http://www.nobel.gen.tr FAOSTAT (2021) http://www.fao.org/faostat/en/#home
27. Foresto, E., Carezzano, M.E., Giordano, W. and Bogino, P. (2023) Ascochyta Blight in Chickpea: An Update. *Journal of Fungi*, 9, Article 203.
https://doi.org/10.3390/jof9020203
28. Gachon, C., Mingam, A. and Charrier, B. (2004) Real-Time PCR: What Relevance to Plant Studies? *Journal of Experimental Botany*, 55, 1445-1454.
https://doi.org/10.1093/jxb/erh181
29. Gayacharan, Rani, U., Singh, S., Basandrai, A.K., Rathee, V.K., Tripathi, K., Singh, N., Dixit, G.P., Rana, J.C., Pandey, S., Kumar, A. and Singh, K. (2020) Identification of Novel Resistant Sources for Ascochyta Blight (Ascochyta rabiei) in Chickpea. *PLOS ONE*, 15, e0240589. https://doi.org/10.1371/journal.pone.0240589
30. Graner, A., Streng, S., Drescher, A., Jin, Y., Borovkova, I. and Steffenson, B. (2000)
31. Molecular Mapping of the Leaf Rust Resistance Gene Rph7 in Barley. *Plant Breeding*, 119, 389-392.
https://doi.org/10.1046/j.1439-0523.2000.00528.x
32. Haware, M.P., Nene, Y.L. and Mathur, S.B. (1986) Seed-Borne Diseases of Chickpea.
Technical Bulletin 1, Danish Goverment Institute of Seed Pathology for Developing Countries, Copenhagen, 9-15.
33. Imtiaz, M., Materne, M., Hobson, K., van Ginkel, M. and Malhotra, R.S. (2008) Molecular Genetic Diversity and Linked Resistance to Ascochyta Blight in Australian
34. Chickpea Breeding Materials and Their Wild Relatives. *Australian Journal of Agricultural Research*, 59, 554-560.
https://doi.org/10.1071/AR07386
35. Iruela, M., Pistón, F., Cubero, I.C., Millán, T., Barro, F. and Gil, J. (2009) The Marker $SCK13_{603}$ Associated with Resistance to Ascochyta Blight in Chickpea is Located in a Region of a Putative Retrotransposon. *Plant Cell Reports*, 28, 53-60.
https://doi.org/10.1007/s00299-008-0609-7
36. Iruela, M., Rubio, J., Barro, F., Cubero, J.I., Millán, T. and Gil, J. (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, 278-287. https://doi.org/10.1007/s00122-005-0126-9
37. Jimenez-Diaz, R.M., Crino, O., Halila, M.H., Mosconi, C. and Trapero-Casas, A.T. Screening for Resistance to Fusarium Wild and Ascochyta Blight in Chick- pea. In: Singh, K.B. and Saxena, M.C., Eds., Breeding for Stress Tolerance in Cool Season Food Legumes, John Wiley and Sons Ltd, Chichester, 77-95.
38. Jiménez-Fernández, D., Montes-Borrego, M., Jiménez-Díaz, R.M., Navas-Cortés,
39. J.A. and Landa, B.B. (2011) In Planta and Soil Quantification of Fusarium oxysporum f. sp. ciceris and Evaluation of Fusarium Wilt Resistance in Chickpea with a Newly Developed Quantitative Polymerase Chain Reaction Assay. *Phytopathology*, 250-262. https://doi.org/10.1094/PHYTO-07-10-0190
40. Jones, J.D. and Dangl, J.L. (2006) The Plant Immune System. *Nature*, 4, 323-329.
https://doi.org/10.1038/nature05286
41. Kaur, S., Kimber, R.B., Cogan, N.O., Materne, M., Forster, J.W. and Paull, J.G. SNP Discovery and High-Density Genetic Mapping in Faba Bean (Vicia faba L.) Permits Identification of QTLs for Ascochyta Blight Resistance*. Plant Science,* 218, 47-55.
https://doi.org/10.1016/j.plantsci.2013.11.014

42. Kumar, R.R., Kumar, M., Nimmy, M.S., Kumar, V., Sinha, S., Shamin, M.D. and Dharamsheela, T. (2016) Diagnosis of Pulse Disease and Biotechnological Approaches for Their Management. In: Biswas, S.K., Kumar, S. and Chand, G., Eds., Diseases of Pulse Crops and Their Sustainable Management, Biotech Book, New Delhi, 519-542
43. Küsmenoğlu, İ. and Aydin, N. (1995) The Current Status of Lentil Germplasm Exploitation for Adaptation to Winter Sowing in the Anatolian Highlands. In: Keating, J.D.H. and Küsmenoğlu, I., Eds., *Autumn-Sowing of Lentil in the Highlands of West Asia and North Africa*, CRIFC, Ankara, 64-71.
44. Küsmenoğlu, İ. and Meyveci, K. (1996) Chickpea in Turkey. In: Saxena, N.P., Saxena, M.C.,
45. Johansen, C., Virman, S.M. and Harris, H., Eds., Adaptation of Chickpea in the West Asia and North Africa Region, ICRISAT-ICARDA, 67.
46. Kwasniak, M., Majewski, P., Skibior, R., Adamowicz, A., Czarna, M., Sliwinska, E. and Janska, H. (2013) Silencing of the Nuclear RPS10 Gene Encoding Mitochondrial Ribosomal Protein Alters Translation in Arabidopsis Mitochondria. *The Plant Cell*, 25, 1855-1867. https://doi.org/10.1105/tpc.113.111294
47. Leiminger, J., Bäßler, E., Knappe, C., Bahnweg, G. and Hausladen, H. (2015) Quantification of Disease Progression of Alternaria spp. On Potato Using Real-Time PCR. *European Journal of Plant Pathology*, 141, 295-309. https://doi.org/10.1007/s10658-014-0542-2
48. Li, H., Rodda, M., Gnanasambandam, A., Aftab, M., Redden, R., Hobson, K., Rosewarne, G.,
49. Materne, M., Kaur, S. and Slater, A.T. (2015) Breeding for Biotic Stress Resistance in Chickpea: Progress and Prospects. *Euphytica*, 204, 257-288. https://doi.org/10.1007/s10681-015-1462-8
50. Madrid, E., Chen, W., Rajesh, P.N., Castro, P., Millán, T. and Gil, J. (2013) Allele-Specific Amplification for the Detection of Ascochyta Blight Resistance in Chickpea. *Euphytica*, 189, 183-190. https://doi.org/10.1007/s10681-012-0753-6
51. McDonald, B.A. and Linde, C. (2002) Pathogen Population Genetics, Evolutionary Potential and Durable Resistance. *Annual Review of Phytopathology*, 40, 349-379. https://doi.org/10.1146/annurev.phyto.40.120501.101443
52. Millàn, T., Winter, P., Jüngling, R., Gil, J., Rubio, J., Cho, S., Cobos, M.J., Iruela, M., Rajesh, P.N., Tekeoglu, M., Kahl, G. and Muehlbauer, F.J. (2010) A Consensus Genetic Map of Chickpea (Cicer arietinum L.) Based on 10 Mapping Populations. *Euphytica*, 175, 175-189. https://doi.org/10.1007/s10681-010-0157-4
53. Nene, Y.L., Haware, M.P. and Reddy, M.V. (1981) Chickpea Deaseses. Resistance Screening Techniques. ICRISAT Information Bulten 10, ICRISAT.
54. Nene, Y.L. and Reddy, M.V. (1987) Chickpea Diseases and Their Control. In: Saxena, M.C. and Saxena, K.B., Eds., The Chickpea, CAB International, Wallingford, 233-270.
55. Pande, S., Siddique, K.H.M., Kishore, G.K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T.W. and Crouch, J.H. (2005) Ascochyta Blight of Chickpea (Cicer arietinum L.): A Review of Biology, Pathogenicity and Disease Management. *Australian Journal Agricultural Research*, 317-332. https://doi.org/10.1071/AR04143
56. Pande, S., Sharma, M., Gaur, P., Tripathi, S., Kaur, L., Basandrai, A. and Siddique, K.H.M. (2011a) Development of Screening Techniques and Identifcation of New Sources of Resistance to Ascochyta Blight Disease of Chickpea. *Australasian Plant Pathology*, 40, 149 https://doi.org/10.1007/s13313-010-0024-8
57. Pande, S., Sharma, M., Mangla, U.N., Ghosh, R. and Sundaresan, G. (2011b) Phytophthora Blight of Pigeonpea [Cajanus cajan (L.) Millsp.]: An Updating Review of Biology, Pathogenicity and Disease Management. *Crop Protection*, 30, 951-957. https://doi.org/10.1016/j.cropro.2011.03.031
58. Pasche, J.S., Mallik, I., Anderson, N.R. and Gudmestad, N.C. (2013) Development and Validation of a Real-Time PCR Assay for the Quantification of Verticillium dahliae in Potato. *Plant Disease*, 97, 608-618. https://doi.org/10.1007/s10658-004-4842-9
59. Phan, H.T.T., Ford, R., Bretag, T. and Taylor, P.W.J. (2002) A Rapid and Sensitive Polymerase Chain Reaction (PCR) Assay for Detection of Ascochyta rabiei, the Cause of Ascochyta Blight of Chickpea. *Australasian Plant Pathology*, 31, 31-39. https://doi.org/10.1071/AP01056

60. Reddy, M.V. and Singh, K.B. (1984) Evaluation of a World Collection of Chickpea Germ Plasm Accessions for Resistance to Ascochyta Blight. *Plant Disease*, 68, 901.
61. Rigotti, S., Gindro, K., Richter, H. and Viret, O. (2002) Characterization of Molecular Markers for Specific and Sensitive Detection of Botrytis Cinerea Pers.: Fr. in Strawberry (Fragariaxananassa Duch.) Using PCR. *FEMS Microbiology Letters*, 169-174.
62. Sardesai, N., Kumar, A., Rajyashri, K.R., Nair, S. and Mohan, M. (2002) Identification of an AFLP Marker Linked to Gm7, a Gall Midge Resistance Gene and Its Conversion to a SCAR Marker for Its Utility in Marker Aided Selection in Rice. *Theoretical and Applied Genetics*, 105, 691-698. https://doi.org/10.1007/s00122-002-1035-9
63. Schaad, N.W. and Frederick, R.D. (2002) Real-Time PCR and Its Application for Rapid Plant Disease Diagnostics. *Canadian Journal of Plant Pathology*, 24, 250-258. https://doi.org/10.1080/07060660209507006
64. Schena, L., Nigro, F., Ippolito, A. and Gallitelli, D. (2004) Real-Time Quantitative PCR: A New Technology to Detect and Study Phytopathogenic and Antagonistic Fungi. *European Journal of Plant Pathology*, 110, 893-908. https://doi.org/10.1007/s10658-004-4842-9
65. Sharma, M. and Ghosh, R. (2016) An Update on Genetic Resistance of Chickpea to Ascochyta Blight. *Agronomy*, 6, Article 18. https://doi.org/10.3390/agronomy6010018
66. Shtienberg, D. (2010) Applications of Epidemiology in the Management of Ascochyta Blight in Chickpea and Lentil. In: Chew, W., Muehlbauer, F.J. and Sharma, H.C., Eds., *Compendium of Chickpea and Lentil Diseases and Pests, APS Press*, *St Paul*, p. 22.
67. Singh, K.B., Reddy, M.V. and Nene, Y.L. (1984) International Testing of Chickpeas for Resistance to Ascochyta Blight. *Plant Disease*, 68, 782-784. https://doi.org/10.1094/PD-69-782
68. Singh, K.B. and Reddy, M.V. (1990) Patterns of Resistance and Susceptibility to Races of Ascochyta rabiei among Germ Plasm Accessions and Breeding Lines of Chickpea*. Plant Disease, 74*, 127-129. https://doi.org/10.1094/PD-74-0127
69. Singh, K.B., Reddy, M.V. and Hawara, M.P. (1992) A Review of the Kabuli Chickpea Disease-Resistant Breeding Research at ICARDA. In: Singh, K.B. and Saxena M.C.,Eds., Disease Resistance Breeding in Chickpea, ICARDA, 23-54.
70. Singh, K.B. and Reddy, M.V. (1993) Resistance to Six Races of Ascochyta rabiei in the World Germplasm Collection of Chickpea. *Crop Science*, 33, 186-189. https://doi.org/10.2135/cropsci1993.0011183X003300010033x
71. Singh, G., Chen, W., Rubiales, D., Moore, K., Sharma, Y.R. and Gan, Y. (2007) Diseases and Their Management. In: Yadav, S.S., Redden, R.J., Chen, W. and Chen, W., Eds., *Chickpea Breeding and Management*, CAB International, Wallingford,497-519. https://doi.org/10.1079/9781845932138.024
72. Slinkard, E.A., Solh, M.B. and Vandenberg, A. (2000) Breeding for Yield: The Direct Approach. In: Knight, R., Ed., *Linking Research and Marketing Opportunities for Pulses in the 21st Century, Current Plant Science and Biotechnology in Agriculture*, Vol. 34, Springer, Dordrecht, 183-190. https://doi.org/10.1007/978-94-011-4385-1_16
73. Suenaga, K., Singh, R.P., Huerta-Espino, J. and William, H.M. (2003) Microsatellite Markers for Genes Lr34/Yr18 and Other Quantitative Loci for Leaf Rust and Stripe Rust Resistance in Bread Wheat. *Phytopathology*, 93, 881-890. https://doi.org/10.1094/PHYTO.2003.93.7.881
74. Sudheesh, S., Vahrood, H.V., Braich, S., Dron, N., Hobson, K., Cogan, O.I. and Kaur, S. (2021) Application of Genomics Approaches for the Improvement in Ascochyta Blight Resistance in Chickpea. *Agronomy*, 11, Article 1937. https://doi.org/10.3390/agronomy11101937
75. Tekeoglu, M., SantrA, D.K., Kaiser, W.J. and MuehlbaueR, F.J. (2000) Ascochyta Blight Resistance Inheritance in Three Chickpea Recombinant Inbred Line Populations. *Crop Science*, 40, 1251-1256. https://doi.org/10.2135/cropsci2000.4051251x
76. Tekeoglu, M., Rajesh, P.N. and Muehlbauer, F. (2002) Integration of Sequence Tagged Microsatellite Sites to the Chickpea Genetic Map. *Theoretical and Applied Genetics*, 105, 847-854. https://doi.org/10.1007/s00122-002-0993-2
77. Trapero-Casas, A. and Jiménez-Díaz, R.M. (1986) Inflance of Sowing Date on Fusarium Wilt and Ascochyta Blight of Chickpea in South Spain. Proceedings of the International Food Legume Research Conference on Pea, Lentil, Faba Bean and

Chickpea, Spokane, 6-11 July 1986, 233-270.

78. Trapero-Casas, A. and Kaiser, W.J. (1992) Development of Didymella rabiei, the Teleomorph of Ascochyta rabiei, on Chickpea Straw. *Phytopathology*, 82, 1261-1266. https://doi.org/10.1094/Phyto-82-1261
79. Udupa, S. and Weigand, F. (1997) DNA Markers and Breeding for Resistance to Ascochyta Blight in Chickpea. *Proceedings of the Symposium on Application of DNA Fingerprinting for Crop Improvement: Marker-Assisted Selection of Chickpea for Sustainable Agriculture, Aleppo*, 11-12 April 1994, 222.
80. Udupa, S.M. and 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. https://doi.org/10.1007/s00122-002-1168-x
81. Valetti, L., Cazon, L.I. and Crociara, C. (2021) Early Detection of Ascochyta Blight (Ascochyta rabiei) of Chickpea by Traditional PCR. *Crop Protection*, 143, Article ID: 105463. https://doi.org/10.1016/j.cropro.2020.105463 Varshney, R.K., Mohan, S.M., Gaur, P.M., Chamarthi, S.K., Singh, V.K., Srinivasan, S., Swapna, N., Sharma, M., Pande, S., Singh, S. and Kaur, L. (2014) Marker-Assisted Backcrossing to Introgress Resistance to Fusarium Wilt Race 1 and Ascochyta Blight in C 214, an Elite Cultivar of Chickpea. *The Plant Genome*, 7, 1-11. https://doi.org/10.3835/plantgenome2013.10.0035
82. Winter, P, Pfaff, T, Udupa, SM, Hüttel, B., Sharma, P.C., Sahim, S., Arreguin-Espinoza, R., Weigand F., Muehlbauer, F.J. and Kahl, G. (1999) Characterization and Mapping of Sequence-Tagged Microsatellite Sites in the Chickpea (Cicer arietinum L.) Genome. *Molecular and General Genetics (MGG),* 262, 90-101. https://doi.org/10.1007/s004380051063
83. Yorgancilar, M., Yakişir, E. and Erkoyuncu, M.T. (2015) Moleküler markörlerin bitki ıslahında kullanımı. Bahri Dağdaş Bitkisel Araştırma Dergisi. *Journal of Bahri Dagdas Crop Research*, 4, 1-12.
84. Zhou, Z., Bar, I., Sambasivam, P.T. and Ford, R. (2019) Determination of the Key Resistance wiseGene Analogs Involved in Ascochyta rabiei Recognition in Chick-
85. pea. Sec. *Plant Breeding*, 10, Article 644. https://doi.org/10.3389/fpls.2019.00644

Figures

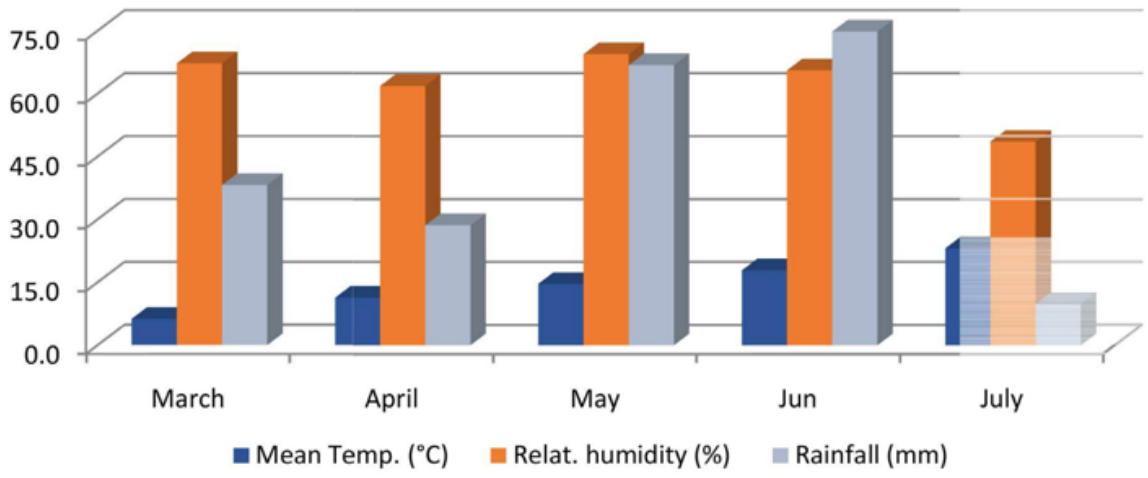

Figure 1

Mean temperatures, relative humidty, and rainfall during 2014 at Haymana, Ankara.

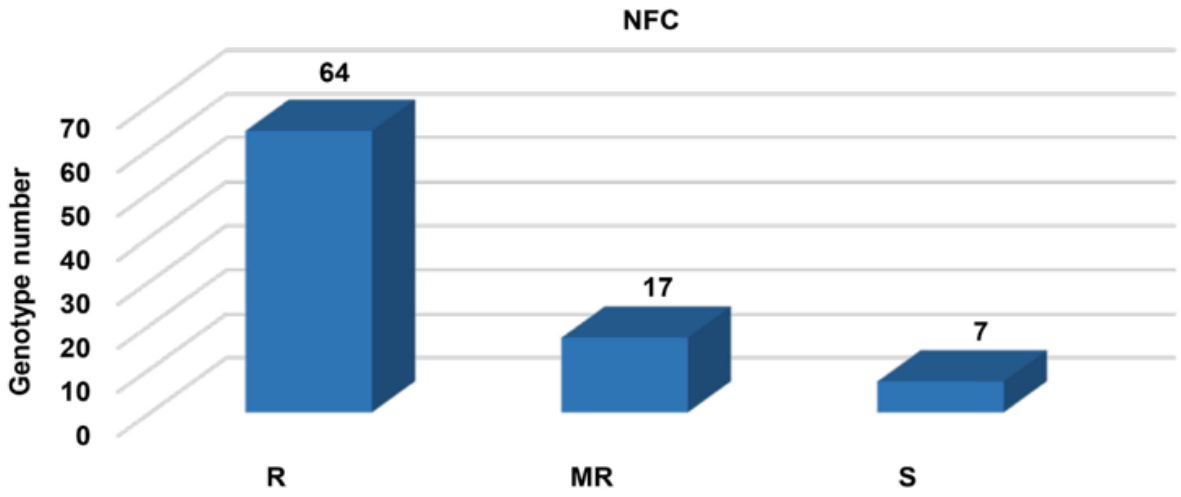

Figure 2

The classification of the disease observation for 88 chickpea genotypes under the Natural Field Conditions (NFC).

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Figure 3

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