Ozyigit II *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 1, Article number 13502
DOI:10.15835/nbha52113502
Research Article

Investigation of resistance using STMS markers against Ascochyta blight in the chickpea varieties

Ibrahim Ilker OZYIGIT[1*], Ilhan DOGAN[2*], Fatih TABANLI[1], Durdane MART[3], Ozcan YORGANCILAR[4], Meltem TURKERI[3], Evren ATMACA[4], Aysel YORGANCILAR[4]

[1]*Marmara University, Faculty of Science, Department of Biology, Istanbul, Türkiye; ilkozyigit@marmara.edu.tr (*corresponding author); fatih_969_1991@hotmail.com*
[2]*Sakarya University of Applied Sciences, Vocational School of Health Services at Akyazi, Sakarya, Türkiye; ilhandogan@subu.edu.tr (*corresponding author)*
[3]*Republic of Türkiye Ministry of Agriculture and Forestry, Cukurova Agricultural Research Institute, Adana, Türkiye; durdane.mart@tarimorman.gov.tr; meltemturkeri@tarimorman.gov.tr*
[4]*Republic of Türkiye Ministry of Agriculture and Forestry, Transitional Zone Agricultural Research Institute, Eskisehir, Türkiye; ozcan.yorgancilar@tarimorman.gov.tr; evren.atmaca@tarimorman.gov.tr; aysel.yorgancilar@gthb.gov.tr*

Abstract

Chickpea (*Cicer arietinum* L.), a prominent legume plant, is an important agricultural plant that is widely grown both in Türkiye and around the world. Ascochyta blight, caused by the fungal phytopathogen *Ascochyta rabiei*, is one of the major causative agents responsible for yield reductions across the spectrum of chickpea diseases. The impact of diseases varies depending on crops, countries, seasons and cropping systems, and yield loss data collected under well-defined conditions is limited. It is noteworthy that this pathogen shows significant genetic diversity in Türkiye's agricultural environment. In light of this, this study aimed to conduct a research to determine the resistant/tolerant and susceptible genotypes of 34 certificated chickpea varieties grown in different regions of Türkiye by using Sequence Tagged Microsatellite Site (STMS) markers that are related to the genes that provide resistance against Ascochyta blight. The results obtained in this study showed that the primers Ta2, Ta146 and Ts54 used as STMS markers have distinctive features in providing highly effective results in the detection of resistant/tolerant and susceptible varieties of Ascochyta blight.

Keywords: Ascochyta blight; *Ascochyta rabiei*; chickpea; *Didymella rabiei;* molecular breeding; molecular markers

Introduction

Regarding nutritional properties, cholesterol-free and low-fat legumes are preferable in comparison with other plant-derived foods for feeding the world population (Tang *et al.*, 2022; Ketnawa and Rawdkuen, 2023). Chickpea (*Cicer arietinum* L.) is one of the most important legumes and is widely consumed by humans, especially in tropical and subtropical regions of the world (e.g., the Mediterranean, Near East, Central Asia and

Received: 04 Nov 2023. Received in revised form: 18 Dec 2023. Accepted: 29 Jan 2024. Published online: 11 Mar 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

America) (Kafadar *et al*., 2019; Achari *et al*., 2023). Global chickpea cultivation area is about 13.81 (13.72) million ha with an annual production of 12.09 (14.25) million tons (FAOSTAT, 2019). The grains of chickpeas contain 59% carbohydrate, 29% protein, 5% oil, 3% fiber and 4% ash and are rich in vitamins, amino acids, calcium, phosphorus, iron, magnesium, and potassium (Yadav *et al.,* 2018; Varol *et al.,* 2020). Chickpeas are also known to have protective properties against cardiovascular diseases, cancer and diabetes (de Camargo *et al.,* 2019; Varol *et al.,* 2020).

Chickpea is one of the main food sources in human nutrition and an important legume for large-scale agricultural production in Türkiye (Pisuttu *et al*., 2023). Chickpea cultivation area in Türkiye is approximately 520 000 ha and Türkiye's annual production is approximately 630 000 tons (FAOSTAT, 2019; Tuik, 2023). According to the production and consumption rates, chickpea is the most cultivated species in the edible legumes in Türkiye (Sudupak *et al.,* 2004; Dogan *et al*., 2023). Being rich in minerals, chickpea contains 875 mg K, 331 mg P, 105 mg Ca, 24 mg S, 11.5 mg Mg, 8.2 mg Se, 6.2 mg Fe and 3.4 mg Zn per 100 g (Khetarpaul, 2018).

Ascochyta blight disease caused by *Didymella rabiei* (Kovachevski) von Arx [anamorph: *Ascochyta rabiei* (Passerini) Labrousse], adversely affects chickpea production rate (Zhang *et al.,* 2019). The host-specific fungal phytopathogenic *A. rabiei*, first identified in 1911, is classified in Ascomycota and is effective on all the above-ground parts of plant species in genus *Cicer* (Frenkel *et al.,* 2007; Ozkilinc *et al.,* 2011). Ascochyta blight is the disease that causes the highest yield loss among the diseases seen in chickpeas. The disease agent is recorded in approximately 35 countries producing chickpeas (Vandana *et al.,* 2020; Bell *et al.,* 2022). Environmental and climate conditions suitable for pathogen development can lead to 100% yield loss in agricultural areas (Kahraman and Ozkan, 2015; Mart *et al*., 2022). The pathogen shows high genetic diversity in Türkiye (Ozkilinc *et al*., 2011; Asrat *et al*., 2016). If the precipitation rate stays above 150 mm between October and April, the effects of the disease can be excessive (Javaid *et al*., 2014; Kahraman and Ozkan, 2015). The main symptoms of the disease are pale leaf tips, leaf lesions, stem lesions that cause fractures, and lesions on the seed coat that cause seed infection. Stem fractures and seed shell infections are the most dangerous of these symptoms (Owati *et al.,* 2017; Rodda *et al*., 2017). In addition, mobilization of infected seeds causes *A. rabiei* to spread over wide geographic areas. *A. rabiei* can survive more than 5 years in the seeds of diseased chickpeas (Acikgoz *et al*., 1994; Rubiales *et al.,* 2018). There is a negative correlation between the grain size of the plant and Ascochyta blight as is that an increase in the grain size of the plant causes a decrease in the resistance of the plant (Toker and Cagirgan, 2004). Therefore, in the development of coarse grained, blight-resistant Ascochyta chickpea varieties, it is primarily necessary to have advanced knowledge about the genotypic characteristics of the plant and the disease-causing agent. The loss of plant stability against the disease is the most important challenge in agricultural practices.

Genetic marker-assisted identification of traits related to common diseases is highly preferred in plant breeding applications as it provides accurate results and saves time for screening (Simsek *et al.,* 2020; Jha *et al.,* 2022). Genetic marker systems including Sequence Tagged Microsatellite Site (STMS), Sequence-Tagged Site (STS), Sequence Characterized Amplified Region (SCAR), Randomly Amplified Polymorphic DNA (RAPD), Arbitrary Primed-PCR (AP-PCR), Inter Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), Retrotransposon Microsatellite Amplified Polymorphism (RE-MAP) were developed based on PCR amplification and are commonly employed for a variety of applications such as genome mapping, gene tagging, genetic diversity, phylogenetic analysis and forensic investigations (Kalendar and Schulman, 2006; Filiz *et al.,* 2018; Jenfaoui *et al.,* 2021; Hocaoglu-Ozyigit *et al.,* 2022). Advances in the sequencing of genomes have led to the generation of SNP markers for a variety of applications such as genome-wide association studies (GWAS), marker developments, genomic selections, germplasm characterizations (Semagn *et al.,* 2014; Vishwakarma *et al.,* 2019).

This study aims to determine the genotypes of chickpea varieties that are resistant/tolerant and susceptible to Ascochyta blight, which is one of the most important problems in agricultural areas in Türkiye as well as in the world. For this purpose, the genotypes of certificated chickpea varieties grown in Türkiye were examined using STMS markers that have relationships with the genes providing resistance to Ascochyta blight (Tekeoglu *et al*., 2002) by determinations of the rates of resistance and sensitivity.

Materials and Methods

Study material (Cicer arietinum L.)

Genus *Cicer* comprises 43 species; 9 annuals, 33 perennials and 1 unclassified species (Sethy *et al*., 2006). *C. arietinum* belonging to the family Fabaceae (Leguminosae) (Jain *et al*., 2013) is a diploid plant having 8 chromosomes and a genome size of 750 mega base pairs (Mbp) (Sethy *et al.,* 2006; Singh *et al.,* 2015). *C. arietinum* shows a wide geographical distribution in Türkiye as well as in the world, from the Mediterranean Region to Australia, Myanmar, Mexico, and Chile and even to the tropics (Vasishtha *et al.,* 2014). The chickpea species can grow in a wide range of territory in terms of elevation, from sea level to more than 2500 m altitude (Damte and Ojiewo, 2016). *C. arietinum* used for agricultural production is not a species resistant to diseases, especially Ascochyta blight; therefore, crossbreeding practices are being carried out between *C. arietinum* and the resistant chickpea varieties ILC482, ILC3279, FLIP84-92C and FLIP84-79C (Tivoli and Banniza, 2007).

STMS marker assisted selection

STMS and similar molecular markers are commonly used to determine of genetic variations and population genetic structures of plant fungal pathogens (Milgroom and Peever, 2003; Ozkilinc *et al*., 2015). For this reason, these markers are preferred in our research for studying genetic variation because of knowledge about *A. rabiei* which shows higher genetic variation in the Southeastern Anatolia Region of Türkiye than in other countries (Ozkilinc *et al.,* 2015). Different microsatellite loci (total of 20) were determined in the genus *Cicer* for the identification of Ascochyta blight (Geistlinger *et al*., 2000; Leo *et al*., 2015).

Certificated chickpea varieties (Table 1) were planted (20 seeds per row) in the order of 2 rows, each with 2 m length and 0.45 m intervals in the sowing season in the experimental field in Eskisehir-Central Disease Observation Garden. In addition to certificated chickpea varieties to be tested, a chickpea variety, 'Canitez 87', was planted as susceptive control in an order of one row between 2 rows. Also, the same set of plantations was done in the greenhouse using pots. In this study, the certificated chickpea varieties taken from the greenhouse were tested by Ascochyta blight resistant STMS markers for resistance using the STMS technique while the chickpea varieties grown in the field were used for determining the rate of susceptibilities of chickpea varieties to Ascochyta blight. Seeds of chickpea varieties and the control, planted in pots, were grown in the greenhouse after necessary maintenance. When the plants reached a length of approximately 10-15 cm, the samples were taken from the young leaves of certified chickpea varieties and controlled for DNA isolation and stored at -86 °C.

Table 1. Certificated chickpea varieties, developed in Türkiye were used in this research

No	Certificated chickpea varieties	Certificate Issuer	Certification date
1	Ena 102-1	Eastern Anatolian Agricultural Research Institute	—
2	Ena 102-9	Eastern Anatolian Agricultural Research Institute	—
3	Aziziye 94	Eastern Anatolian Agricultural Research Institute	16.05.1994
4	Ena 87-3	Eastern Anatolian Agricultural Research Institute	—
5	Gokce	Field Crops Central Research Institute	09.05.1997
6	Dikbas	Field Crops Central Research Institute	12.04.2006
7	Uzunlu 99	Field Crops Central Research Institute	30.04.1999
8	Er 99	Field Crops Central Research Institute	30.04.1999
9	Kusmen 99	Field Crops Central Research Institute	30.04.1999
10	Akcin 91	Field Crops Central Research Institute	01.05.1991
11	Sezenbey	Black Sea Agricultural Research Institute	13.04.2012
12	Cagatay	Black Sea Agricultural Research Institute	27.04.2001
13	Zuhal	Black Sea Agricultural Research Institute	13.04.2012
14	Seckin (Adana)	Eastern Mediterranean Agricultural Research Institute	11.04.2011
15	Aksu (Maras)	East Mediterranean Transitional Zone Agricultural Research Institute/Kahramanmaras	06.04.2009
16	Inci (Adana)	Cukurova Agricultural Research Institute	30.04.2003
17	Aydin (İzmir)	Eastern Mediterranean Agricultural Research Institute	—
18	Hasanbey (Adana)	Eastern Mediterranean Agricultural Research Institute	11.04.2011
19	Menemen 92	Aegean Agricultural Research Institute	11.05.1992
20	Sari 98	Aegean Agricultural Research Institute	15.05.1998
21	Izmir 92	Aegean Agricultural Research Institute	11.05.1992
22	Aydin 92	Aegean Agricultural Research Institute	11.05.1992
23	Cevdetbey 98	Aegean Agricultural Research Institute	15.05.1998
24	Yasa 05	Anatolian Agricultural Research Institute	27.04.2005
25	Cakir	Transitional Zone Agricultural Research Institute	13.04.2012
26	Azkan	Transitional Zone Agricultural Research Institute	06.04.2009
27	Canitez-87	Anatolian Agricultural Research Institute	27.04.1987
28	Hisar	Transitional Zone Agricultural Research Institute	04.04.2008
29	Isik 05	Anatolian Agricultural Research Institute	27.04.2005
30	Ilgaz	Itas Exporters' Association Seeds and Research Industry and Trade Joint Stock Company	13.04.2012
31	ILC 482	Southeastern Anatolian Agricultural Research Institute	01.05.1991
32	Diyar 95	Southeastern Anatolian Agricultural Research Institute	20.04.1995
33	TAEK-Sagel	Turkish Atomic Energy Authority	12.04.2006
34	Arda	Southeastern Anatolian Agricultural Research Institute	08.04.2013

DNA isolation from the certificated chickpea varieties and control

DNA isolations were performed from young leaves of certificated chickpea varieties when they reached a length of approximately 10-15 cm. The following DNA isolation protocol was applied for each sample. 0.4 g leaf sample is thoroughly crushed in liquid nitrogen. Following the evaporation of the liquid nitrogen, the sample was thoroughly ground using a mortar after adding 3.5 mL of isolation buffer to the sample. The solution in the mortar was taken into 1.5 mL Eppendorf tubes each containing 0.5 ml. The samples were placed in a water bath at 60-65 °C and kept for 30 minutes, stirring occasionally. The samples taken from the water bath were mixed well and centrifuged at 12000 rpm for 5-10 min. after adding 500 μL chloroform:isomyl alcohol buffer solution into each tube. The supernatant was taken into a sterile Eppendorf tube using a pipette. Approximately 350-400 µL of cold isopropanol (2/3 volume to volume) was added for precipitation of nucleic acids. After DNA precipitation by centrifugation, isopropanol was carefully discarded. 500 µL of the wash buffer was added to DNA at the bottom of the tube and allowed to stand for 20 min. Following 5 min. centrifugation at 5000 rpm, the supernatant was discarded and the tubes were left open at room conditions to allow the DNA at the bottom of the tube to dry. After dissolving of DNA in 100 µL TE by mixing, 1 µL of RNase was added to the tube to remove the RNA from the DNA. The purity and quantification of the obtained DNA were determined using a Nano Volume Spectrophotometer (OptizenNanoQ). According to the DNA concentration obtained, DNA was diluted to a certain ratio and prepared for PCR.

Analysis of PCR products

The susceptibility tests for Ascochyta blight were done by STMS analysis by exploiting of STMS markers on the DNA samples taken from the leaf parts of chickpea varieties. The markers for producing specific and stable band profiles were: Ta2 (F: 5'-AAATGGAAGAAGAATAAAAACGAAAC-3' and R: 5'-TTCCATTCTTTATTATCCATATCACTACA-3') yielding of 175 bp-long PCR product, Ta146 (F: 5'-CTAAGTTTAATATGTTAGTCCTTAAATTAT-3' and R: 5'-ACGAACGCAACATTAATTTTATATT-3') yielding of 161 bp-long PCR product and Ts54 (F: 5'-TACAAGTTAAAAATGAATAAATATTAATA-3' and R: 5'-GAAATTTAGAGAGTCAAGCTTTAC-3') yielding of 209 bp-long PCR product (Tekeoglu *et al.*, 2002; Kottapalli *et al.*, 2009). PCR mix (for each reaction) was contained 2µL PCR buffer (10X), 4µLdNTP mix (10-50 µM), 1.2 µL of $MgCl_2$ (1-4 mM), 2 µL of STMS primer I (0.1-1 µM), 2 µL of STMS primer II (0.1-1 µM) (Santagen), 5 µL (50 ng) of template DNA, and 0.2 µL (5 units) of Taq DNA polymerase, and at the end, the sterile de-ionized water was added to each mix (prepared as 20 µL). An AERIS-BG096 Gradient Thermal Cycler was used for the amplifications. The PCR program was as follows: initial denaturation at 9 6°C for 2 minutes; followed by 35 cycles of denaturation at 96 °C for 20 seconds, primer annealing at 55 °C for 50 seconds, elongation at 60 °C for 50 seconds and a final extension step at 60 °C for 5 minutes. Amplification products were run on a 4% agarose gel used (in 1X TBE) for separation at 96-100 V (70 mA) for 2-3 hours. Following ethidium bromide staining, the gel imaging and analysis system (Gel Logic 200 Imaging System) was employed for evaluation of PCR products. A molecular size marker (GeneRuler 100 bp Plus DNA Ladder, ready-to-use, Thermo Fischer Scientific) was used for analyzing and estimating of sizes of amplification products.

Results and Discussion

Having 7 different geographical regions, in which chickpea cultivation is being carried out through, Türkiye has different climatic conditions seen in these geographic regions. One of the most important problems encountered in chickpea cultivation is fungal diseases, especially emerging from following heavy rainy seasons. As a fungal disease, anthracnose caused by *A. rabiei* is the most seen one. This fungal species, which likes to live in humid environments, can easily be transported from one region to another through rainfall. This deeply affects the transmission rates and the severity of the disease. In the geographical regions of the country, in which our research was conducted in, different rates of disease severity were observed that occurred to depending on the climatic conditions. According to our data, the region where the disease severity was found to be highest the Marmara region with a rate of 40.35%. For the Marmara region, the data were collected from 65 agricultural fields throughout the region. The Aegean, Black Sea, Mediterranean, Central Anatolia, Southeastern Anatolia, and Eastern Anatolia regions with disease severity rates of 29.20%, 28.40%, 18.98%, 14.43%, 13.87%, and 1.77%, respectively, followed by the Marmara Region. To collect data, 141 agricultural fields in the Aegean Region, 58 agricultural fields in the Black Sea Region, 158 agricultural fields in the

Mediterranean Region, 219 agricultural fields in Central Anatolia, 125 agricultural fields in Southeastern Anatolia and 57 agricultural fields in Eastern Anatolia regions were screened, respectively.

Samples harvested from the fields in 2014 were used in disease severity analyzes and molecular breeding studies. The most important factors in the transmission of anthracnose disease are the high rainfall and humidity rates. When the precipitation rates of the regions in 2014 were examined, it was seen that the Marmara Region received 886 mm of precipitation with an increase of 33% compared to the average, and ranked first among the regions (Table 2). In addition, the Marmara region, which had the highest disease severity rate, ranked first with 40.35%. The fact that the highest humidity rate among the regions is in the Marmara Region may be one of the reasons for the widespread occurrence of the disease (Figure 1). The Black Sea Region is located in the north of our country and is the region that receives the highest annual precipitation. In 2014, this region received 880 mm of precipitation and the disease severity rate in the region was found to be 28.40%., the precipitation rates and the disease severity rates seen in 2014 in the other regions were respectively found to as 816 mm and 18.98% for the Mediterranean region; 756 mm (higher than average by 20%) and 29.90% for the Aegean Region (the increased rate of precipitation may have caused the high disease severity of the region in that period); 536 mm and 1.77% for the Eastern Anatolia Region; 464 mm and 13.87% for the Southeastern Anatolia Region (having smallest land area compared to other regions); and 456 mm and 14.43% for Central Anatolia Region (having a continental and drier climate) (Table 2 and Figure 1). Although the rainfall is relatively high, the Eastern Anatolia Region, where at least the severity of anthracnose disease is seen, may be due to the low humidity rate characteristic of the region.

Table 2. The annual precipitation rates (in mm) of the regions in the period of the experiment

Regions	2013 (in mm)	2014 (in mm)	2015 (in mm)
Marmara	607	886	638
Aegean	702	756	652
Mediterranean	649	816	647
Central Anatolia	290	456	442
Black Sea	797	880	727
Eastern Anatolia	491	536	533
Southeastern Anatolia	489	464	532

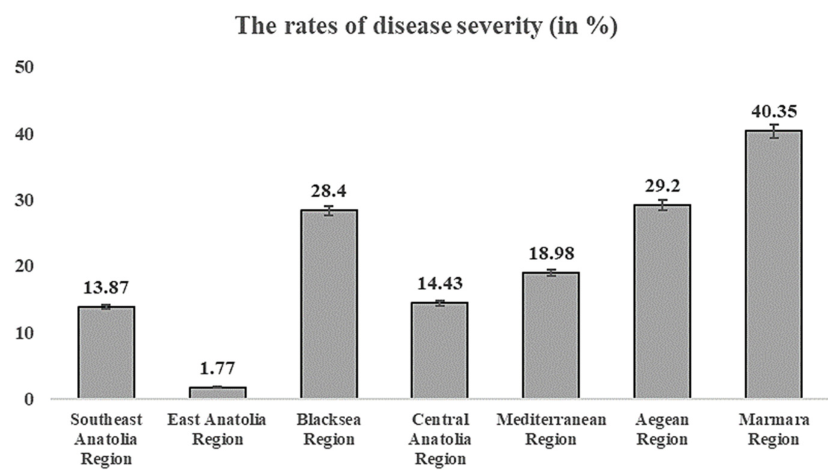
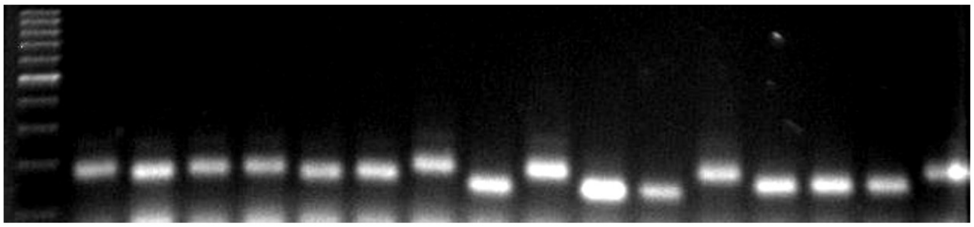


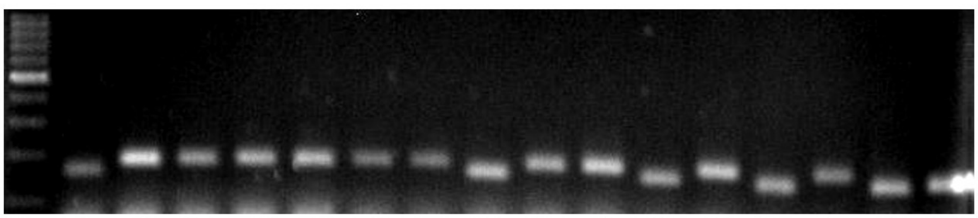
Figure 1. The rates of disease severity (in %) in the regions of Türkiye in the year of 2014

Sequence Tagged Microsatellite Sites, short repeat motifs consisting of 1-6 bases in the genome, are used for producing high-level polymorphisms and are highly sensitive for identification of species and are co-dominant and sensitive neutral markers frequently preferred in research for characterization of population genetic structures (Dutech *et al.*, 2007; Ozkilinc *et al.*, 2015). By STMS technique, primers can be used for amplifying a region in the genomic DNA. The results obtained by using STMS Ta2, Ta146 and Ts54 primers were used for determining of susceptibility of chickpea varieties against Ascochyta blight in a way of generating approximately 175, 161 and 209 bp-long DNA bands as PCR products that were visualized for STMS analyses (given in Figures 2-10, respectively). The data obtained from the identifications of susceptibility of 34 certificated chickpea varieties developed in Türkiye and the field data from the disease evaluations are shown in Table 3. The resistance and susceptibility of certificated 34 chickpea varieties were determined as follows: 17 resistant and 17 susceptible against the disease via Ta2 primer; 23 resistant and 11 susceptible against the disease via Ta146; and 24 resistant and 8 susceptible against the disease via Ts54 primer.



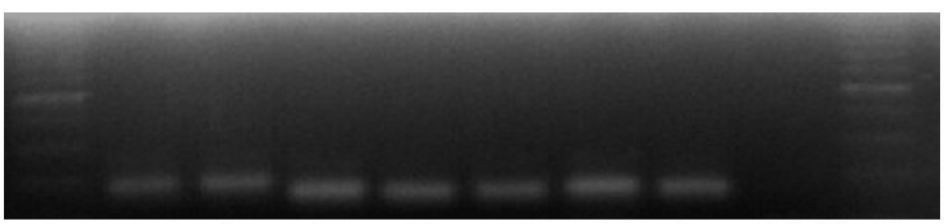
L F R 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 2. Susceptibility parameters of certificated chickpea varieties through 1-14 using STMS Ta2 primer [(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), R: *Cicer reticulatum* (susceptible chickpea variety for Ascochyta blight as negative control), 1: Ena 102-1, 2: Ena 102-9, 3: Aziziye 94, 4: Ena 87-3, 5: Gokce, 6: Dikbas, 7: Uzunlu 99, 8: Er 99, 9: Kusmen 99, 10: Akcin 91, 11: Sezenbey, 12: Cagatay, 13: Zuhal, 14: Seckin (Adana)].



L F R 15 16 17 18 19 20 21 22 23 24 25 26 27 28

Figure 3. Susceptibility parameters of certificated chickpea varieties through 15-28 using STMS Ta2 primer
[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), R: *Cicer reticulatum* (susceptible chickpea variety for Ascochyta blight as negative control), 15: Aksu (Maras), 16: Inci (Adana), 17: Aydin (Izmir), 18: Hasanbey (Adana), 19: Menemen 92, 20: Sari 98, 21: Izmir 92, 22: Aydin 92, 23: Cevdetbey 98, 24: Yasa 05, 25: Cakir, 26: Azkan, 27: Canitez 87, 28: Hisar].



L F 29 30 31 32 33 34 H_2O (C) L

Figure 4. Susceptibility parameters of certificated chickpea varieties through 29-34 using STMS Ta2 primer
[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), 29: Isik 05, 30: Ilgaz, 31: ILC-482, 32: Diyar-95, 33: TAEK-Sagel, 34: Arda, H_2O (as control)].

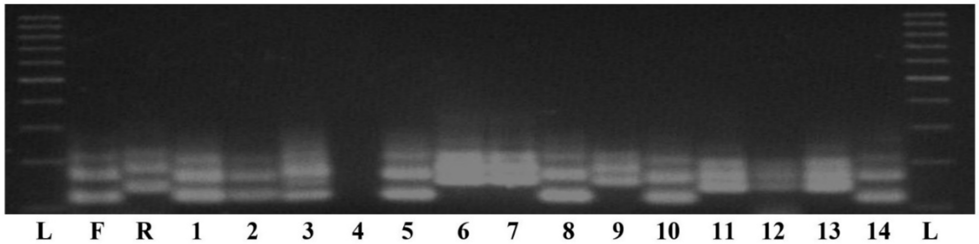


Figure 5. Susceptibility parameters of certificated chickpea varieties through 1-14 using STMS Ta146 primer

[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), R: *Cicer reticulatum* (susceptible chickpea variety for Ascochyta blight as negative control), 1: Ena 102-1, 2: Ena 102-9, 3: Aziziye 94, 4: —, 5: Gokce, 6: Dikbas, 7: Uzunlu 99, 8: Er 99, 9: Kusmen 99, 10: Akcin 91, 11: Sezenbey, 12: Cagatay, 13: Zuhal, 14: Seckin (Adana)].

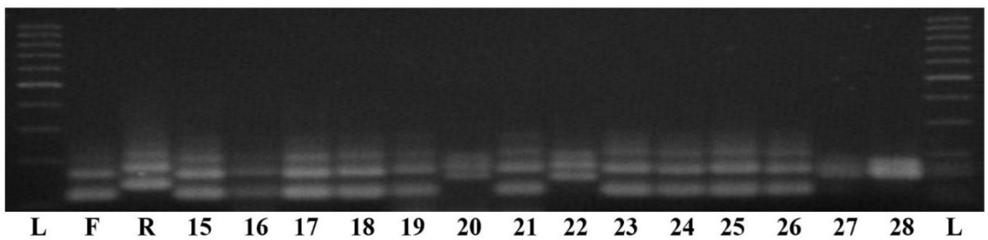


Figure 6. Susceptibility parameters of certificated chickpea varieties through 15-28 using STMS Ta146 primer

[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), R: *Cicer reticulatum* (susceptible chickpea variety for Ascochyta blight as negative control), 15: Aksu (Maras), 16: Inci (Adana), 17: Aydin (Izmir), 18: Hasanbey (Adana), 19: Menemen 92, 20: Sari 98, 21: Izmir 92, 22: Aydin 92, 23: Cevdetbey 98, 24: Yasa 05, 25: Cakir, 26: Azkan, 27: Canitez 87, 28: Hisar].

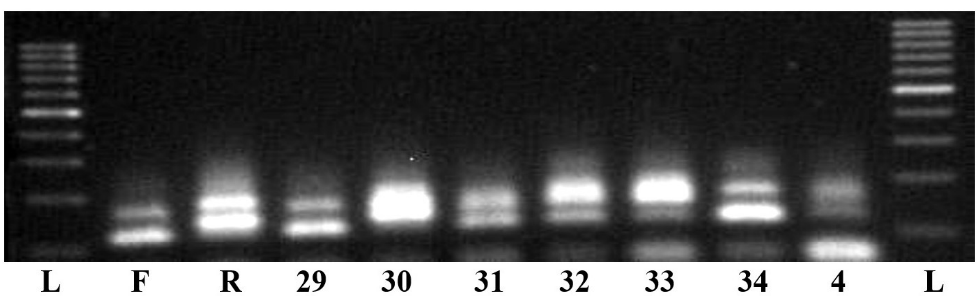


Figure 7. Susceptibility parameters of certificated chickpea varieties through 29-34 and 4 using STMS Ta146 primer

[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), 29: Isik 05, 30: Ilgaz, 31: ILC-482, 32: Diyar-95, 33: TAEK-Sagel, 34: Arda, 4: Ena 87-3].

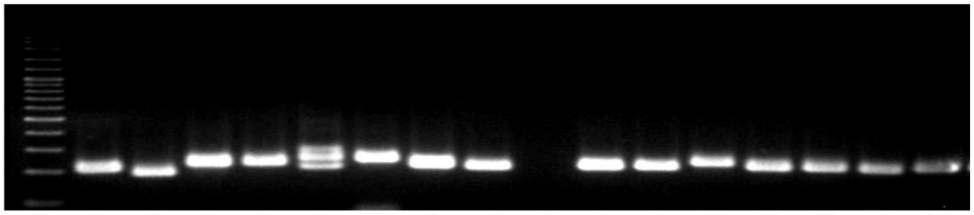


Figure 8. Susceptibility parameters of certificated chickpea varieties through 1-14 using STMS Ts54 primer

[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), R: *Cicer reticulatum* (susceptible chickpea variety for Ascochyta blight as negative control), 1: Ena 102-1, 2: Ena 102-9, 3: —, 4: Ena 87-3, 5: Gokce, 6: Dikbas, 7: —, 8: Er 99, 9: Kusmen 99, 10: Akcin 91, 11: Sezenbey, 12: Cagatay, 13: Zuhal, 14: Seckin (Adana)].

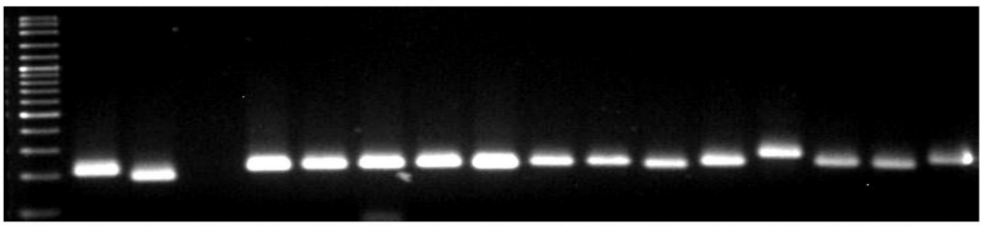


Figure 9. Susceptibility parameters of certificated chickpea varieties through 15-28 using STMS Ts54 primer

[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), R: *Cicer reticulatum* (susceptible chickpea variety for Ascochyta blight as negative control), 15: —, 16: Inci (Adana), 17: Aydin (Izmir), 18: Hasanbey (Adana), 19: Menemen 92, 20: Sari 98, 21: Izmir 92, 22: Aydin 92, 23: Cevdetbey 98, 24: Yasa 05, 25: Cakir, 26: Azkan, 27: Canitez 87, 28: Hisar].

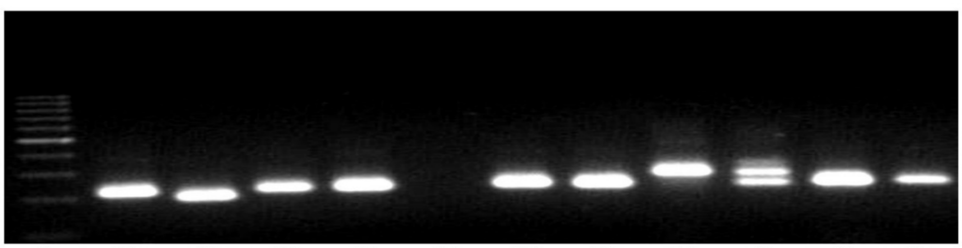


Figure 10. Susceptibility parameters of certificated chickpea varieties through 29-34 and 4 using STMS Ts54 primer

[(L: 100 bp DNA Ladder Plus, F: Flip 8492(C3) (resistant chickpea variety for Ascochyta blight as positive control), 29: Isik 05, 30: Ilgaz, 31: ILC-482, 32: Diyar-95, 33: TAEK-Sagel, 34: Arda, 3: Aziziye 94, 7: Uzunlu 99, 15: Aksu (Maras)].

Table 3. The data obtained from identifications of susceptibility of 34 certificated chickpea varieties developed in Türkiye using STMS primers and the field data for the disease evaluations

No	Certificated Chickpea Varieties	R/S using Ta2 producing 175 bp-long band		R/S using Ta146 producing 161 bp-long band		R/S using Ts54 producing 209 bp-long band		The field data for the disease evaluations
F	Flip8492(C3)	R		R		R		Resistant against the disease
C	*C. reticulatum*		S		S		S	Susceptible against the disease
1	Ena 102-1	R		R		R		—
2	Ena 102-9	R		R		R		—
3	Aziziye 94		S		S			Tolerant against the disease
4	Ena 87-3		S	R		R		—
5	Gokce	R		R			S	Tolerant against the disease
6	Dikbas		S		S		S	Moderate tolerant against the disease
7	Uzunlu 99	R			S	R		Moderate tolerant against the disease
8	Er 99		S	R			S	Tolerant against the disease
9	Kusmen 99		S		S		S	Moderate tolerant against the disease
10	Akcin 91	R		R		R		Moderate tolerant against the disease
11	Sezenbey		S		S		S	Tolerant against the disease
12	Cagatay		S		S		S	Tolerant against the disease
13	Zuhal		S		S		S	Tolerant against the disease
14	Seckin (Adana)	R		R			S	The disease was not detected during the experimental period
15	Aksu (Maras)	R		R		R		Resistant against the disease
16	Inci (Adana)	R		R		R		Tolerant against the disease
17	Aydin (İzmir)	R		R		R		Resistant against the disease
18	Hasanbey (Adana)	R		R		R		The disease was not detected during the experimental period
19	Menemen 92	R		R		R		Resistant against the disease
20	Sari 98		S		S	R		Resistant against the disease
21	Izmir 92	R		R		R		Resistant against the disease
22	Aydin 92	R			S	R		Resistant against the disease
23	Cevdetbey 98		S	R		R		Resistant against the disease
24	Yasa 05	R		R		R		Resistant against the disease
25	Cakir		S	R		R		Moderate tolerant against the disease
26	Azkan	R		R		R		Resistant against the disease
27	Canitez-87		S		S	R		Susceptible against the disease
28	Hisar	R			S	R		Resistant against the disease
29	Isik 05	R		R		R		Tolerant against the disease
30	Ilgaz		S	R		R		—
31	ILC 482		S	R				The disease was not detected during the experimental period
32	Diyar 95		S	R		R		The disease was not detected during the experimental period
33	TAEK-Sagel		S	R		R		—
34	Arda		S	R		R		Tolerant against the disease
F: as positive control-resistant, C: as negative control-susceptible, R: Resistant, and S: Susceptible								

Santra *et al.* (2000) identified and mapped two QTL regions for Ascochyta blight. Mapping the quantitative trait loci responsible for resistance to Ascochyta blight in molecular breeding is considered an important input for growing chickpeas. Sharma and Gosh (2016) identified various markers conferring resistance to Ascochyta blight through QTL mapping method. Among these markers, STMS markers showing parallelism to the markers used in our study take attention. In another study, 83 RAPD, STMS, ISSR and RGA markers were found for seedling and body resistance, and they were successfully used in the detection of Ascochyta blight in the *Cicer* genome (Kukreja *et al*., 2018). Similarly, 6 different STMS markers were suggested to use for the productions of breeding chickpea genotypes resistant to Fusarium blight, a fungal disease (Sahu *et al*., 2020). Ascochyta blight, also known as anthracnose disease caused by the fungus *A. rabiei* leads to major yield losses in the agricultural production of chickpeas (Barilli *et al*., 2016). Knowing the susceptibility statutes of the certificated chickpea varieties against the disease can provide great advantages to farmers. STMS markers are commonly used in the determination of genetic similarities.

In this study, STMS markers were used for the determination of Ascochyta blight-resistance and sensitivity of certificated chickpea varieties in terms of comparisons done between the banding regions that specific primers specifically amplify. In a previous study, 6 STMS markers were found to be associated with anthracnose disease (Tekeoglu *et al*., 2002; Daba *et al*., 2016). In another study conducted in 2003, 49 different chickpea varieties grown in Türkiye were subjected to susceptibility tests against Ascochyta blight using 10 different RAPD, ISSR and STMS markers and the STMS Ta2 marker was successfully used for determining resistant chickpea varieties (Cingilli *et al.,* 2003). In a previous study performed in 2009, it was found that the STMS Ta2, Ta146 and Ts54 markers could be effectively used for the selection of the Ascochyta blight-resistant varieties (Kottapalli *et al*., 2009). In another study, it was determined that the STMS Ta2 marker was found to be able to differentiate the resistant chickpea varieties against Ascochyta blight in 82% (Yorgancilar *et al*., 2009). As our conclusion reported in our study, Yildirim *et al*., (2018) also stated that RAPD and STMS (Ta2, Ts54 and Ta146) markers are demonstrated to show the usability of them in molecular breeding programs. Additionally, STMS markers used in the identification of Ascochyta blight in chickpeas can be used as a reference point (Madrid *et al*., 2013). Similar to these previous studies, in this study, STMS primers produced a high polymorphism ratio for the identification of resistant/susceptible chickpea varieties against Ascochyta blight. Among them, Ta146 primer, which yields 161 bp-long bands used for the determination of resistance, produced a higher polymorphism ratio and showed better distinguishing features than Ta2 and Ts54 primers which yield 175 and 209 bp-long bands used for the determination of resistance, respectively. Analogies were found between the data obtained from the identifications of resistance of certificated chickpea varieties and the field data for the disease evaluations. The analogy rates between the data from PCR using Ta146 primer and the field data were found to be 85% and the analogy rates between the data from PCR using Ta2 and Ts54 primers and the field data were found to as ~76%, respectively. Finally, in this study, certified chickpea varieties developed in Türkiye were determined for resistance/tolerance to Ascochyta blight disease using STMS primers. This would be an important step in breeding practices that will provide significant advantages to breeders.

Conclusions

This study explored into the crucial issue of Ascochyta blight, a significant threat to chickpea cultivation in Türkiye and worldwide. The research aimed to identify resistant/tolerant and susceptible genotypes among 34 certified chickpea varieties using Sequence Tagged Microsatellite Site (STMS) markers associated with genes providing resistance against Ascochyta blight. The findings revealed that the STMS primers Ta2, Ta146, and Ts54, particularly the Ta146 primer, exhibited distinct features in effectively detecting resistant/tolerant and susceptible varieties. The high polymorphism ratio observed with these primers underscores their efficacy in identifying chickpea varieties' resistance levels. Notably, the Ta146 primer displayed superior distinguishing features compared to Ta2 and Ts54, displaying its potential for accurate resistance determination.

Furthermore, the study established analogies between the PCR data using Ta146 primer and field evaluations, with an impressive 85% correlation. Analogous rates for Ta2 and Ts54 primers were approximately

76%. This alignment between laboratory results and real-world disease evaluations underscores the reliability of the STMS markers in predicting resistance/tolerance in certified chickpea varieties.

Fundamentally, the identification of Ascochyta blight resistance in certified chickpea varieties through STMS primers represents a pivotal advancement in breeding practices. This research provides valuable insights for breeders, offering a strategic advantage in developing resilient chickpea varieties and mitigating the impact of Ascochyta blight on agricultural yields in Türkiye.

Authors' Contributions

Conceptualization: IIO, ID and DM; Data curation: IIO, ID and OY; Formal analysis: OY, MT, EA and AY; Funding acquisition: IIO and DM; Investigation: FT, OY, AY, EA and MT ; Methodology: IIO, ID and OY; Project administration: IIO and DM; Resources: IIO and DM; Software: FT, OY and EA; Supervision: IIO, ID and DM; Validation: IIO, ID, OY and DM; Visualization: FT; Writing - original draft: IIO and ID; Writing-review and editing: IIO and ID. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This investigation was funded by TUBITAK, 1003-Priority Areas, Research and Development Project Support Program under grant 113O070 and supported in part by Marmara University, Scientific Research Project under grant FEN-C-YLP-070617- 0365.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Achari SR, Mann RC, Sharma M, Edwards J (2023). Diagnosis of *Fusarium oxysporum* f. sp. *ciceris* causing Fusarium wilt of chickpea using loop-mediated isothermal amplification (LAMP) and conventional end-point PCR. Scientific Reports 13(1):2640. *https://doi.org/10.1038/s41598-023-29730-6*

Acikgoz N, Karaca M, Er C, Meyveci K (1994). Expanding the production and use of cool season food legumes. In: Muehlbauer FJ, Kaiser WJ (Eds). Current Plant Science and Biotechnology in Agriculture, Springer Netherlands, pp 388-398.

Asrat A, Tana T, Fikre A (2016). Response of chickpea (*Cicer arietinum* L.) to rates of nitrogen and phosphorus fertilizer at Debre Zeit, Central Ethiopia. In: Korbu L, Damte T, Fikre A (Eds). Harnessing chickpea value chain for nutrition security and commercialization of smallholder agriculture in Africa, pp 169.

Barilli E, Cobos Mj, Rubiales D (2016). Clarification on host range of *Didymella pinodes* the causal agent of pea Ascochyta blight. Frontiers in Plant Science 7(27):592. *https://doi.org/10.3389/fpls.2016.00592*

Bell JC, Bound SA, Buntain M (2022). Biostimulants in agricultural and horticultural production. Horticultural Reviews 49:35-95. *https://doi.org/10.1002/9781119851981.ch2*

Cingilli H, Altinkut A, Akcin A (2003). Screening of Turkish chickpea (*Cicer arietinum* L.) genotypes for Ascochyta blight resistance using molecular markers. Biotechnology & Biotechnological Equipment 17(1):65-73. *https://doi.org/10.1080/13102818.2003.10819197*

Daba K, Deokar A, Banniza S, Warkentin T, Taran B (2016).QTL mapping of early flowering and resistance to Ascochyta blight in chickpea (*Cicer arietinum* L.). Genome 59(6):413-425. *https://doi.org/10.1139/gen-2016-0036*

Damte T, Ojiewo CO (2016). Current status of wilt/root rot diseases in major chickpea growing areas of Ethiopia. Archives of Phytopathology and Plant Protection 49(9-10):222-238. *https://doi.org/10.1080/03235408.2016.1180925*

De Camargo AC, Favero BT, Morzelle MC, Franchin M, Alvarez-Parrilla E, De La Rosa LA, Geraldi MV, Junior MRM, Shahidi F, Schwember AR (2019). Is chickpea a potential substitute for soybean? Phenolic bioactives and potential health benefits. International Journal of Molecular Sciences 20(11):2644. *https://doi.org/10.3390/ijms20112644*

Dogan I, Ozyigit II, Genc E, Tabanli F, Mart D, Yorgancilar O, Yorgancilar A (2023). STMS markers related to Ascochyta blight resistance in chickpea. Indian Journal of Biochemistry and Biophysics (IJBB) 60(3):196-208. *https://doi.org/10.56042/ijbb.v60i3.61501*

Dutech C, Enjalbert J, Fournier E, Delmotte F, Barres B, Carlier J, Tharreau D, Giraud T (2007). Challenges of microsatellite isolation in fungi. Fungal Genetical Biology 44(10):933-949. *https://doi.org/10.1016/j.fgb.2007.05.003*

FAOSTAT (2019). Food and agriculture organization of the United Nations Database, Food and Agriculture Organization Corporate Statistical Database, Türkiye.

Filiz E, Uras ME, Ozyigit II, Sen U, Gungor H (2018). Genetic diversity and phylogenetic analyses of Turkish rice varieties revealed by ISSR markers and chloroplast *trnl-F* region. Fresenius Environmental Bulletin 27(12):8351-8358.

Frenkel O, Shtienberg D, Abbo S, Sherman A (2007). The sympatric Ascochyta complex of wild *Cicer judaicum* and domesticated chickpea. Plant Patholology 56(3):464-471. *https://doi.org/10.1111/j.1365-3059.2006.01552.x*

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

Hocaoglu-Ozyigit A, Ucar B, Altay V, Ozyigit II (2022). Genetic diversity and phylogenetic analyses of Turkish cotton (*Gossypium hirsutum* L.) lines using ISSR markers and chloroplast trnL-F regions. Journal of Natural Fibers 19(5):1837-1850. *https://doi.org/10.1080/15440478.2020.1788493*

Jain M, Misra G, Patel RK, Priya P, Jhanwar S, Khan AW, … Chattopadhyay D (2013). A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). The Plant Journal 74(5):715-729. *https://doi.org/10.1111/tpj.12173*

Javaid A, Amin M, Athar MM (2014). Antifungal activity of *Melia azedarach* L. fruit extract against *Ascochyta rabiei* (Pass.) Lab. Pakistan Journal of Phytopathology 26(2):151-155.

Jenfaoui H, Uras ME, Bahri BA, Ozyigit II, Souissi T (2021). Morphological variation, genetic diversity and phylogenetic relationships of *Hypericum triquetrifolium* Turra populations from Tunisia. Biotechnology & Biotechnological Equipment 35(1):1505-1519. *https://doi.org/10.1080/13102818.2021.1977180*

Jha UC, Sharma KD, Nayyar H, Parida SK, Siddique KH (2022). Breeding and genomics interventions for developing Ascochyta blight resistant grain legumes. International Journal of Molecular Sciences 23(4):2217. *https://doi.org/10.3390/ijms23042217*

Kafadar FN, Ozkan A, Can C, Kar Y, Mart D, Ceyhan E (2019). Genetic and biochemical properties of *Cicer* spp reveal distinction between wild and cultivated chickpea genotypes. Legume Research 42(1):1-9. *https://doi.org/10.18805/LR-395*

Kahraman A, Ozkan Z (2015). Ascochyta blight of chickpea. Selcuk Journal of Agriculture and Food Sciences 29(2):62-66.

Kalendar R, Schulman AH (2006). IRAP and REMAP for retrotransposon-based genotyping and fingerprinting. Nature Protocols 1:2478-2484. *https://doi.org/10.1038/nprot.2006.377*

Ketnawa S, Rawdkuen S (2023). Properties of Texturized vegetable proteins from edible mushrooms by using single-screw extruder. Foods 12(6):1269. *https://doi.org/10.3390/foods12061269*

Khetarpaul N (2018). Total mineral content present (calcium, magnesium, potassium, phosphorus, iron, zinc, manganese, copper, and boron and nickel) in leaves of Desi and Kabuli chickpea varieties (on dry matter basis). The International Journal of Communication Systems 6(2):633- 635.

Kottapalli P, Gaur PM, Katiyar SK, Crouch JH, Buhariwalla HK, Pande S, Gali KK (2009). Mapping and validation of QTLs for resistance to an Indian isolate of Ascochyta blight pathogen in chickpea. Euphytica 165(1):79-88. *https://doi.org/10.1007/s10681-008-9762-x*

Kukreja S, Salaria N, Thakur K, Goutam U (2018). Fungal disease management in chickpea: current status and future prospects. In: Gehlot P, Singh J (Eds). Fungi and their Role in Sustainable Development: Current Perspectives. Springer, Singapore, pp 293-309. *https://doi.org/10.1007/978-981-13-0393-7_17*

Leo AE, Ford R, Linde CC (2015). Genetic homogeneity of a recently introduced pathogen of chickpea, *Ascochyta rabiei*, to Australia. Biological Invasions 17(2):609-623. *https://doi.org/10.1007/s10530-014-0752-8*

Madrid E, Chen W, Rajesh PN, Castro P, Millan T, 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*

Mart D, Can C, Ozyigit II, Turkeri M, Mart S, Yucel D (2022). The tolerance of some registered chickpea (*Cicer arietinum* L.) varieties against Ascochyta blight (*Ascochyta rabiei*) in the Eastern-Mediterranean region. Journal of Nutritional Health and Food Engineering 12(2):55-59. *https://doi.org/10.15406/jnhfe.2022.12.00356*

MGM (2023). Turkish State Meteorological Service, Türkiye. *https://www.mgm.gov.tr/*.

Milgroom MG, Peever TL (2003). Population biology of plant pathogens: the synthesis of plant disease epidemiology and population genetics. Plant Disease 87(6):608-617. *https://doi.org/10.1094/PDIS.2003.87.6.608*

Owati AS, Agindotan B, Pasche JS, Burrows M (2017). The detection and characterization of QoI-resistant *Didymella rabiei* causing Ascochyta blight of chickpea in Montana. Frontiers in Plant Science 8:1165. *https://doi.org/10.3389/fpls.2017.01165*

Ozkilinc H, Frenkel O, Abbo S, Shtienberg D, Sherman A, Kahraman A, Can C (2011). Aggressiveness of eight *Didiymella rabiei* isolates from domesticated and wild chickpea native to Turkey and Israel, a case study. European Journal of Plant Pathology 131:529-537. *https://doi.org/10.1007/s10658-011-9828-9*

Ozkilinc H, Thomas K, Abang M, Peever TL (2015). Population structure and reproductive mode of *Didymella fabae* in Syria. Plant Pathology 64(5):1110-1119. *https://doi.org/10.1111/ppa.12359*

Pisuttu C, Risoli S, Moncini L, Nali C, Pellegrini E, Sarrocco S (2023). Sustainable strategies to counteract mycotoxins contamination and cowpea weevil in chickpea seeds during post-harvest. Toxins 15(1):61. *https://doi.org/10.3390/toxins15010061*

Rodda MS, Davidson J, Javid M, Sudheesh S, Blake S, Forster JW, Kaur S (2017). Molecular breeding for Ascochyta blight resistance in lentil: Current progress and future directions. Frontiers in Plant Science 8:1136. *https://doi.org/10.3389/fpls.2017.01136*

Rubiales D, Fondevilla S, Chen W, Davidson J (2018). Advances in Ascochyta research. Frontiers in Plant Science 9:22. *https://doi.org/10.3389/fpls.2018.00022*

Sahu VK, Tiwari S, Tripathi MK, Gupta N, Tomar RS, Ahuja A, Yasin M (2020). Molecular marker validation and identification of Fusarium wilt resistant chickpea genotypes. Indian Journal of Genetics and Plant Breeding 80(2):163-172. *https://doi.org/10.31742/IJGPB.80.2.6*

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

Semagn K, Babu R, Hearne S, Olsen M (2014). Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Molecular Breeding 33:1-14. *https://doi.org/10.1007/s11032-013-9917-x*

Sethy NK, Choudhary S, Shokeen B, Bhatia S (2006). Identification of microsatellite markers from *Cicer reticulatum*: molecular variation and phylogenetic analysis. Theoretical and Applied Genetics 112(2):347-357. *https://doi.org/10.1007/s00122-005-0135-8*

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

Simsek D, Samur D, Mutlu N (2020). Marker assisted backcross breeding for fusarium wilt (*Fusarium oxysporum* Schlecht. F. sp. *melongenae*) in eggplant. International Journal of Agricultural and Natural Sciences 13(2):91-100.

Singh M, Kumar K, Bisht Is, Dutta M, Rana MK, Rana JC, Bansal KC, Sarker A (2015). Exploitation of wild annual *Cicer* species for widening the gene pool of chickpea cultivars. Plant Breeding 134(2):186-192. *https://doi.org/10.1111/pbr.12254*

Sudupak MA, Akkaya MS, Kence A (2004). Genetic relationships among perennial and annual *Cicer* species growing in Turkey assessed by AFLP fingerprinting. Theoretical and Applied Genetics 108(5):937-944. *https://doi.org/10.1007/s00122-003-1505-8*

Tang X, Zheng Y, Liu TC, Liu J, Wang J, Lu Y, Zhou P (2022). Fragrant rapeseed oil consumption prevents blood cholesterol accumulation via promoting fecal bile excretion and reducing oxidative stress in high cholesterol diet fed rats. Journal of Functional Foods 88:104893. *https://doi.org/10.1016/j.jff.2021.104893*

Tekeoglu M, Rajesh P, Muehlbauer F (2002). Integration of sequence tagged microsatellite sites to the chickpea genetic map. Theoretical and Applied Genetics 105(6-7):847-854. *https://doi.org/10.1007/s00122-002-0993-2*

Tivoli B, Banniza S (2007). Comparison of the epidemiology of Ascochyta blights on grain legumes. European Journal for Plant Pathology 119(1):59-76. *https://doi.org/10.1007/s10658-007-9117-9*

Toker C, Cagirgan MI (2004). The use of phenotypic correlations and factor analysis in determining characters for grain yield selection in chickpea (*Cicer arietinum* L.). Hereditas 140(3):226-228. *https://doi.org/10.1111/j.1601-5223.2004.01781.x*

TUIK (2023). Statistical Data. *http://www.tuik.gov.tr/yillik/Ist_gostergeler.pdf* (in Turkish).

Vandana UK, Barlaskar NH, Kalita R, Laskar IH, Mazumder PB (2020). The vital foliar diseases of *Cicer arietinum* L. (chickpea): science, epidemiology, and management. In: Singh B, Singh G, Kumar K, Nayak S, Srinivasa N (Eds). Management of Fungal Pathogens in Pulses. Fungal Biology. Springer, Cham. pp 169-190. *https://doi.org/10.1007/978-3-030-35947-8_10*

Varol IS, Kardes YM, Irik HA, Kirnak H, Kaplan M (2020). Supplementary irrigations at different physiological growth stages of chickpea (*Cicer arietinum* L.) change grain nutritional composition. Food Chemistry 303:125402. *https://doi.org/10.1016/j.foodchem.2019.125402*

Vasishtha H, Srivastava RP, Verma P (2014). Effect of dehusking and cooking on protein and dietary fibre of different genotypes of desi, kabuli and green type chickpeas (*Cicer arietinum*). Journal of Food Science and Technology 51(12):4090-4095. *https://doi.org/10.1007/s13197-012-0909-3*

Vishwakarma G, Sanyal RP, Saini A, Sahu PK, Patel RRS, Sharma D, Tiwari R, Das BK (2019). GLADS: A gel-less approach for detection of STMS markers in wheat and rice. PloS One 14(11). *https://doi.org/10.1371/journal.pone.0224572*

Yadav P, Chandra R, Pareek N, Raverkar KP (2018). Screening of multi-trait mesorhizobium isolates for plant growth promotion and nitrogen fixation in chickpea (*Cicer arietinum* L.). International Journal of Current Microbiology and Applied 7(8):2592-2599. *https://doi.org/10.20546/ijcmas.2018.708.266*

Yildirim A, Kahraman A, Muehlbauer Fj, Tanyolaci B (2018). Identification of new QTL conferring resistance to anthracnose (*Ascochyta rabiei*) in a ril population of chickpea. Turkish Journal of Field Crops 23(2):93-99. *https://doi.org/10.17557/tjfc.467465*

Yorgancilar O, Bilir O, Cakir S, Atmaca E (2019). Testing the resistance of chickpea lines and varieties grown in Anatolian Agricultural Research Institute against Ascochyta blight using molecular marker methods. Türkiye VIII. Field Crops Congress, 19-22 October, Hatay, Türkiye. (in Turkish).

Zhang C, Chen W, Sankaran S (2019). High-throughput field phenotyping of Ascochyta blight disease severity in chickpea. Crop Protection 125:104885. *https://doi.org/10.1016/j.cropro.2019.104885*