Indian Journal of Biochemistry & Biophysics
Vol. 60, March 2023, pp. 196-208
DOI: 10.56042/ijbb.v60i3.61501

STMS markers related to Ascochyta blight resistance in chickpea

Ilhan Dogan[1]*, Ibrahim Ilker Ozyigit[2], Mustafa Enes Genc[2], Fatih Tabanli[2], Durdane Mart[3], Ozcan Yorgancilar[4], Meltem Turkeri[3], Evren Atmaca[4] & Aysel Yorgancilar[4]

[1]Sakarya University of Applied Sciences, Vocational School of Health Services at Akyazi, Sakarya, Türkiye

[2]Marmara University, Faculty of Science, Department of Biology, Istanbul, Türkiye

[3]Republic of Türkiye Ministry of Agriculture and Forestry, Cukurova Agricultural Research Institute, Adana, Türkiye

[4]Republic of Türkiye Ministry of Agriculture and Forestry, Transitional Zone Agricultural Research Institute, Eskisehir, Türkiye

Received 16 March 2022; *revised 10 February 2023*

Chickpea (*Cicer arietinum* L.) is one of the important legume crops and is cultivated large-scale throughout Türkiye as well as the world. Ascochyta blight, caused by the fungal phytopathogen *Ascochyta rabiei*, is the leading reason for the highest yield losses among the diseases known for chickpea. The pathogen exhibits high genetic diversity in Türkiye. Therefore, resistancy using Sequence Tagged Microsatellite Site (STMS) markers related with the genes that provide resistance against Ascochyta blight was investigated for the 205 chickpea breeding lines grown in different parts of Türkiye. The analysis for Ascochyta blight resistance was performed using Ta2, Ta146 and Ts54. It was demonstrated that Ta2, Ts54 and Ta146 were the STMS markers having distinguishable features for the detection of Ascochyta blight resistance and were shown to be used in credible fashion for the selection of resistant chickpea breeding lines.

Keywords: *Ascochyta rabiei*, *Cicer arietinum*, *Didymella rabiei,* Molecular breeding, Molecular markers, Yield losses

As important pulse crop, chickpea (*Cicer arietinum* L.) is cultivated widely throughout the world, from the Mediterranean Region to Australia and Myanmar in the east, and to Mexico and Chile in the west, and even to the tropics[1,2]. Chickpea was grown on an area of 12.65 million ha with an annual production of 12.09 million tons in over fifty countries in 2016[3] and this was accounted for ~29% of the world pulse production[4]. The leading country for worldwide chickpea production is India and it is followed by Pakistan, Türkiye, Australia, Myanmar, Ethiopia, Iran, Mexico, Canada and the USA[5].

Chickpea is an economically important legume crop for the large-scale agricultural production in Türkiye, after haricot bean and sweet pea. According to data provided by TUIK[6], the area for chickpea cultivation is about 395 310 ha and the annual production is about 470 000 tons in Türkiye. It is noticed that the scale down rates for the chickpea production is observed, as well with the becoming less of total chickpea cultivation area in years in Türkiye[7].

Although the preferability features of chickpea such as being a rich nitrogen source, increasing soil fertility and being a valuable protein source are important criteria in chickpea cultivation, crop productivity is low due to its vulnerability to biotic and abiotic stresses[3,8-10]. Despite, many of chickpea breeding lines, were being developed in Türkiye, due to many reasons, one of which is being related with Ascochyta blight, they could not be efficiently used in large-scale agricultural production[7,11].

Ascochyta blight as a biotic stress, caused by pathogenic fungus *A. rabiei* that has capability of being alive, especially in ongoing cool, cloudy, and humid weather conditions between seasons, is the source for the varying degrees of yield losses. Spreading of contagiousness between fields occurs through infected seeds and/or infected crop debris. As a major cause, *A. rabiei* under certain conditions could wipe out entire yield in a field[12,13]. Climatic conditions (*e.g.* temperature, humidity and precipitation rates, and wind velocity) affect emerging and spreading of Ascochyta blight[14]. A proportional relationship exists between the volume of precipitation and the severity of disease in terms of emergencing of the infection and transporting of spores[15].

In terms of enhancing crop productivity, having resistant breeding lines against Ascochyta blight is an important issue to meet the farmers' needs. As an effort for enhancing crop productivity, identification

*Correspondence:
E-mails: ilhandogan@subu.edu.tr

of the resistant genotypes of chickpea breeding lines for Ascochyta blight using molecular breeding techniques associated with molecular markers is the main goal of this research. For this, by utilizing of STMS (PCR based sequence tagged microsatellite site marker-assisted selection), was used for the generation of locus-specific amplification products that exhibited considerable differences due to variations in sequence tagged microsatellite sites, the resistant genotypes of chickpea breeding lines for Ascochyta blight were determined.

Materials and Methods

Plant material (*C. arietinum* L.)

Genus *Cicer* belonging to the family Fabaceae (Leguminosae) comprises 43 species including 9 annuals and 35 perennials[16,17]. *C. arietinum* is an annual self-pollinated diploid pulse crop that has a total of 8 chromosomes and has a genome size of 750 M bp[18] and is believed to be originated from the area covering of south-eastern Türkiye and northern part of Syria. Chickpea is a nutritionally important supplementary source being rich in terms of proteins, carbohydrates, unsaturated fatty acids, sterols, vitamins, and certain minerals. It is cultivated in all over the world[19,20]. *C. arietinum*, an annual species in the Monocicer group from the Genus *Cicer*, is having of features: bushy appearance; 50 to 80 cm stem and 5 to 15 mm leaf lengths; shades of grey, black or green seeds; each inflated, elliptic, obovate or elongate-rhomboid, acuminate pod having 1 to 10 seeds; and flowers with 5-29 mm long white or red corolla. Chickpea shows wide-spreading in temperate climatic regions and a 90-100 day-period for maturation is required[21]. *C. arietinum* used for cultivation showed no resistance against diseases, especially for Ascochyta blight; therefore, the crossbreeding practices were conducted between *C. arietinum* and the ILC482, ILC3279, FLIP84-92C and FLIP84-79C chickpea varieties having resistance[22] (Table 1).

DNA markers used in this study

In this work, the marker-assisted selection was performed for the identification of resistant breeding lines against Ascochyta blight developed in Türkiye. The PCR-based technique including of using STMSs (1-6 bp long-short repetitive motifs distributed throughout a genome) is being highly convenient in evaluation of DNA polymorphism[23] and is commonly employed for characterization of genetic variability[24].

The results from the earlier studies showed that the genetic variation in the populations of *A. rabiei* in Türkiye was higher than that of other countries[23]. In our study, the susceptibility tests via employing of the band profiles of the chickpea breeding lines for Ascochyta blight were performed using Ta2, Ta146 and Ts54 STMS markers.

DNA isolations from the chickpea breeding lines and controls

The young leaves (each of them is about 10-15 cm long) from each of the chickpea breeding lines and controls were utilized for the DNA isolation practices performed according to the CTAB DNA isolation protocol[25]. Each of the leaf sample, taken about 0.05 g, was put into an Eppendorf tube and stored at −80°C for 1 h. The mechanical crush done by placing of iron balls into Eppendorf tube and following using of a shaker containing Eppendorf tube, each of the leaf tissue sample powdered was obtained. Each of the sample was suspended in 0.8 mL of pre-warmed (60°C) CTAB extraction buffer (containing 2% hexadecyltrimethylammonium bromide, 100 mM Tris HCl [pH 8], 20 mM EDTA, 1.4 M NaCl, 0.2% β-mercaptoethanol [added just before use], 0.1 mg/mL proteinase K [added just before use] and after adding 0.002 g of PVP, each sample was incubated at 65°C for 30 min. During the incubation period, each of the sample tube was gently mixed by inverting at every 5 min interval 0.8 mL of chloroform:octanol (24:1, *v/v*) was added to each of the sample and following centrifugation for 10 min. at 12000 rpm, the supernatant was transferred to a clean tube. After addition of 0.35-0.4 mL of 5 M NaCl (2/3 of the recovered volume) to each of the sample, incubation at −20°C for 1 hour was put into practice. For precipitation of DNA, two consecutive centrifugations were performed, first one at 10000 rpm for 1.5 min. and second one at 14000 rpm for 2 min. After supernatant removal, the pellet was washed twice with 1mL of 75% EtOH. Then, the supernatant was discarded and the DNA at the bottom of the tube was allowed to be dried by leaving the tube opened at room temperature. After dissolving the DNA in 0.1 mL of sterile TE by mixing, 1 µL of RNase was added to the tube for removing RNA from the DNA. At last, a Nano Volume Spectrophotometer (Optizen Nano Q) was employed for determining purity and quantification of the obtained DNA. Regarding to the DNA concentration obtained, the DNA was diluted in certain ratio and was prepared for PCR.

Table 1 — Chickpea breeding lines used in this research

No	Chickpea Breeding Lines	No	Chickpea Breeding Lines	No	Chickpea Breeding Lines
			At Adana Location		
1	No specific name for the population	32	EN 1822	63	FLIP 97-706 C
2	F3-03 (X201 TH165-8)	33	EN 1823	64	FLIP 07-216 C
3	EN 808	34	EN 1830	65	FLIP 03-42 C
4	EN 766	35	EN 1831	66	EN 1945
5	EN 952	36	EN 1799	67	EN 1949
6	C-100-2-2	37	FLIP 97-677 C	68	EN 1951
7	ENA 8-2	38	FLIP 06-111 C	69	EN 1964
8	FLIP 03-108 c	39	FLIP 06-59 C	70	Inci-1
9	FLIP 03-42 c	40	FLIP 06-133 C	71	EN 1967
10	FLIP 03-21 c	41	FLIP 06-97 C	72	EN 1974
11	F4-09 (X05 TH80-16105-31-2)	42	FLIP 06-33 C	73	UNHB 2011-11
12	F4-09 (X05 TH69-16124-8)	43	FLIP 06-39 C	74	UNHB 2011-79
13	F4-09 (X05 TH21-16139-12-3)	44	FLIP 07-211 C	75	UNHB 2011-42
14	F4-09 (X05 TH21-16189-12-4)	45	FLIP 06-104 C	76	ENA 192-7
15	ENA 144-10	46	FLIP 88-85 C	77	ENA 197-7
16	ENA 144-11	47	FLIP 06-105 C	78	EN 1898
17	ENA 144-16	48	FLIP 06-66 C	79	EN 1837
18	FLIP 05-150 C	49	FLIP 05-65 C	80	ENA 190-7
19	FLIP 05-170 C	50	FLIP 07-184 C	81	EN-1867
20	FLIP 01-24 C	51	FLIP 06-158 C	82	FLIP 03-14 C
21	EN 1683	52	FLIP 07-227 C	83	FLIP 03 8C
22	EN 1685	53	FLIP 09-18 C	84	FLIP 03 61C
23	EN 1750	54	FLIP 09-20 C	85	Seckin-1
24	EN 1751	55	Seckin-D	198	FLIP-01 54C H-1
25	EN 1685-1	56	FLIP 09-23 C	199	FLIP-01 39C H-2
26	UNHB-2010-52	57	FLIP 09-30 C	200	FLIP-00 34C H-3
27	UNHB-2010-95	58	FLIP 09-13 C	201	EN 2057 AD-1
28	UNHB-2010-96	59	FLIP 09-22 C	202	EN 2057 AD-2
29	UNHB-2010-97	60	FLIP 09-21 C	203	EN 1680 AD-3
30	EN 1788	61	KNGB 2012 12505	204	TATLAR AD-4
31	EN 1800	62	TB 2012/40	205	A-Z AD-5
			At Ankara Location		
86	AKN-2012-3 C-1	90	AKN-2012-3 C-5	94	AKN-2012-3 C-9
87	AKN-2012-3 C-2	91	AKN-2012-3 C-6	95	AKN-2012-3 C-10
88	AKN-2012-3 C-3	92	AKN-2012-3 C-7	96	AKN-2012-3 C-11
89	AKN-2012-3 C-4	93	AKN-2012-3 C-8	97	F3 1 POP-2012 C-12
			At Eskisehir Location		
98	ESN-16-13 NUD-14	107	ESN-24 13 NUBD-6	116	ESN-10 13 NUD-6
99	ESN-14-13 NUD-10	108	ESN-20 13 NUBD-2	117	ESN-2 13 NOUD-4
100	ESN-17 13 NUD-16	109	ESN-23 13 NBUD-5	118	ESN-1 13 NOUD-2
101	ESN-18 13 NUD-16	110	ESN-21 13 NBUD-3	119	ESN-3 13 NOUD-6
102	ESN-23 13 NBUD-7	111	ESN-22 13 NBUD-4	120	ESN-11 13 NUD-7
103	ESN-15 13 NUD 13	112	ESN-5 13 NOUD-13	121	ESN-4 13 NOUD-8
104	ESN-13 13 NUD-9	113	ESN-6 13 NOUD-14	122	ESN-8 13 NUD-5
105	ESN-12-23 NUD-8	114	ESN-7 13 NOUD-17		
106	ESN-18-13 NUBD-1	115	ESN-8 13 NUD-1		
			At Erzurum Location		
123	EN 1553	130	ENA 102-4	137	ENA 159-2
124	EN 1554	131	ENA 112-3	138	ENA 159-4
125	EN 1640	132	ENA 140-8	139	ENA 159-1
126	EN 2	133	ENA 129-6	140	ENA 87-3
127	ENA 55-2	134	UNGB-48	141	ENA 102-1
128	ENA 74-2	135	UNGB-41	142	ENA 101-9
129	ENA 81-1	136	UNGB-46		

(*Contd.*)

Table 1 — Chickpea breeding lines used in this research. (*Contd*.)

No	Chickpea Breeding Lines	No	Chickpea Breeding Lines	No	Chickpea Breeding Lines
			At Adana Location		
			At Samsun Location		
143	X00 TH 119 C-1	150	KNM-10-103	157	KNM 10 146
144	NBUD-012-104 C-2	151	KNM-10-105	158	KNM 10 162
145	NBUD-012-103 C-3	152	KNM-10 114	159	KNM 10 171 SAM
146	C-300-16 M C-4	153	KNM 10 117	160	KNM 10 196 SAM
147	NBUD-12-101 C-5	154	KNM 10 122	161	KNM 10 235 SAM
148	C-100-67 M C-6	155	KNM 11 125	162	KNM 10 274 SAM
149	UBUT-12-102 C-7	156	KNM 11 127	163	KNM 10 283 SAM
			At Urfa Location		
164	URFA C-1	171	URFA C-8	178	URFA C-15
165	URFA C-2	172	URFA C-9	179	URFA C-16
166	URFA C-3	173	URFA C-10	180	URFA C-17
167	URFA C-4	174	URFA C-11	181	URFA C-18
168	URFA C-5	175	URFA C-12	182	URFA C-19
169	URFA C-6	176	URFA C-13	183	URFA C-20
170	URFA C-7	177	URFA C-14		
			At Maras Location		
184	EN-2052	186	EN-1554		
185	EN-957 (C100-60M)	187	EN-1630 (X98 AK7)		
			At Diyarbakir Location		
188	DIYARBAKIR C-1	192	DIYARBAKIR C-5	196	DIYARBAKIR C-9
189	DIYARBAKIR C-2	193	DIYARBAKIR C-6	197	DIYARBAKIR C-10
190	DIYARBAKIR C-3	194	DIYARBAKIR C-7		
191	DIYARBAKIR C-4	195	DIYARBAKIR C-8		

Screening the chickpea breeding line genotypes for Ascochyta blight

The chickpea breeding lines grown in our country were obtained from Adana, Urfa, Maras, Ankara, Eskisehir, Erzurum, Samsun and Diyarbakir Regions. The individuals belonging to the breeding lines were germinated from the seeds in pots under greenhouse conditions. After reaching adequate lengths, the young leaf parts of the individuals (about 10-15 cm long) were used for DNA isolation. In addition to the chickpea breeding lines to be tested, Flip8492(C3) (resistant) and *C. reticulatum* (susceptive) were planted regarding with using as positive and negative controls. The samples from the breeding lines and controls were tested using STMS molecular markers specific to Ascochyta blight for determining susceptibilities.

STMS analyzes

The STMS markers (as following: Ta2, F: 5'-AAATGGAAGAAGAATAAAAACGAAAC-3' and R: 5'-TTCCATTCTTTATTATCCATATCACTACA-3', yields 175 bp-long PCR product; Ta146, F: 5'-CTAAGTTTAATATGTTAGTCCTTAAATTAT-3' and R: 5'-ACGAACGCAACATTAATTTTATATT-3', yields 161 bp-long PCR product; and Ts54, F: 5'-TACAAGTTAAAAATGAATAAATATTAATA-3' and R: 5'-GAAATTTAGAGAGTCAAGCTTTAC-3', yields 209 bp-long PCR product) were exploited for generating specific and stable band profiles for the DNAs from the chickpea breeding lines in terms of determining whether one of which is susceptible or resistant against Ascochyta blight[26,27]. The PCR mix set-up for each reaction was prepared as: 20 µL final volume containing 2 µL PCR buffer (10X), 4 µL dNTP mix (10-50 µM), 1.2 µL of $MgCl_2$ (1-4 mM), 2 µL of STMS primer I (0.1-1 µM) and 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 3.6 µL of sterile de-ionized H_2O. Each PCR run was set up as: an initial 2 min. denaturation at 96°C; followed by 35 cycles of:a 20 sec of denaturation at 96°C, a 50 sec of primer annealing at 55°C, a 50 sec of elongation at 60°C; and a 5 min. of final extension step at 60°C. An AERIS-BG096 Gradient Thermal Cycler was employed for the amplifications and separation of amplification products in PCR runs were done using 4% agarose gel (in 1X TBE). 96-100 V (70 mA) for 2-3 h was applied for separation. After application of ethidium bromide staining, the gel imaging and analysis system (Gel Logic 200 Imaging System) was used for evaluation of PCR products. For the estimation of the sizes

of amplification products, GeneRuler 100 bp Plus DNA Ladder (ready-to-use, Thermo Fischer Scientific) was utilized.

Results and Discussion

Türkiye is divided into 7 different geographical regions where different climatic conditions are seen, suitable for chickpea farming. One of the most important problems encountered with chickpea breeding practices is to generate resistant lines against fungal diseases, especially for Ascochyta blight caused by *Ascochyta rabiei* that lives in humid environments and can easily be transported from one region to another through precipitation. Humidity and high rates of precipitation deeply affect the rates of transmission and the severity of the disease observed in fields used for chickpea cultivation. According to our results, different severity levels for the disease were observed in the geographical regions where the research was conducted depending on the climatic conditions (Table 2). Accordingly, the region that the highest severity of the disease observed was the Marmara Region, a 40.35% rate of which was found. The total number of farm fields that research was carried out in the Marmara Region was 65. Regarding with the disease severity observed, the Marmara Region was followed by the Aegean Region, a 29.20% observation rate, of which was found by carrying it out in a total of 141 farm fields. For the Black Sea Region, located in the north part of the country, a 28.40% disease severity rate was monitored and the data with regarding to the disease severity came from 58 farm fields where the research was conducted throughout the Black Sea Region. The disease severity rate recorded in the Mediterranean region, located in the south part of the country, and having a moderate climate, was 18.98%. The data was collected from a total of 158 farm fields throughout the Mediterranean Region. The Mediterranean Region was followed by the Central Anatolia Region with a disease severity rate of 14.43%, and the related data was obtained from a total of 219 farm fields. The disease severity rate recorded in the Southeastern Anatolia Region, of which the research conducted in a total of 125 farm fields was found to be 13.87%. The lowest disease severity rate found to be in the East Anatolia Region, located in the east part of the country, and the rate observed was 1.77%. The data was collected from a total of 57 farm fields in that region.

Table 2 — Depending on the climatic conditions, the different rates of disease severity observed in the geographical regions of Türkiye where the research was conducted.

Regions	The rates of disease severity (in %)
Marmara	40.35
Aegean	29.2
Mediterranean	18.98
Central Anatolia	14.43
Black Sea	28.4
East Anatolia	1.77
Southeast Anatolia	13.87

The samples used in disease severity analyzes as well as molecular breeding experiments were obtained from the agricultural fields harvested in 2014. As stated above, the most important factors in the transmission of Ascochyta blight are the high rates of precipitation and humidity. The annual precipitation rates (in mm) of the regions of Türkiye seen in the year of 2014 were given in (Fig. 1). When the observed precipitation rates belonging to 2014 were examined, the Marmara Region received an 886 mm of precipitation with a level of 33% surplus compared to the average precipitation rate of the country and sat on the first rank in this regard. In terms of disease severity rate, the Marmara Region came first with 40.35%. And the fact that because of having the highest humidity rate of the country, this could be the reason for the large level of disease spreading seen in the Marmara Region. The Black Sea Region, located in the north part of the country receives the highest annual precipitation in general. In 2014, this region received an 880 mm of the precipitation rate and the disease severity rate in the region was observed as 28.40%. The Mediterranean Region received an 816 mm of the precipitation rate and the disease severity percentage was seen as 18.98% in 2014. In the Aegean Region, the precipitation percentage in 2014 was found to be as 20% that was higher than the average and as being 756 mm. This went-up, observed as 20.20%, was

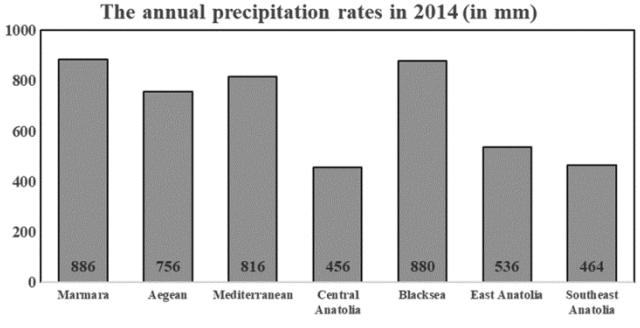

Fig. 1 — The annual precipitation rates (in mm) of the regions of Türkiye seen in the year of 2014

thought to be a periodic increase in the severity rate of the disease in the region. The East Anatolia Region received a 536 mm of the precipitation rate in 2014 but, despite this, it was the region where the severity rate of the disease was found to be lowest, as being 1.77%. Despite of rainfall intensity, the reason for the low incidence of the disease might be due to the relative low humidity seen in the region. The Southeast Anatolia Region, being the smallest one in terms of surface area in Türkiye, received a 464 mm of the precipitation rate and the disease severity rate was determined as 13.87%. Finally, the Central Anatolia Region is located in the central part of the country and has a continental and more arid climate. This region received a 456 mm of the precipitation rate throughout the year of 2014, with having a disease severity rate of 14.43%.

The reports show that one major problem for the chickpea producers is Ascochyta blight that causes yield losses. Identifying of the resistant chickpea breeding lines for this devastating disease that *A. rabiei* is the cause (Fig. 2) will provide advantages to the farmers for alleviation of field losses.

In our study, STMSs were used for the determination of the breeding lines that were resistant to Ascochyta blight. STMSs used for producing high-level polymorphisms are highly sensitive for identification of species in terms of characterization of population genetic structures[23]. In this work, STMSs as markers were utilized in amplifications using specific primers and the generated PCR products were visualized for STMS analyzes. The results obtained using STMS Ts54 primer via an approximate 209 bp-long DNA band generation regarding with determination of susceptibility of the chickpea breeding lines against Ascochyta blight were given below (Figs 3 & 4).

The results obtained using STMS Ta146 and STMS Ta2 primers that are being used to generate approximate 161 and 175 bp-long DNA bands for determination of the susceptibility of chickpea breeding lines against Ascochyta blight were given below (Figs 5-8).

The data taken from resistancy/susceptibility evaluations of the 205 chickpea breeding lines using STMS primers was given in (Table 3).

Fig. 2 — Disease lesions of Ascochyta blight in chickpeas observed during the field trails (Photos were taken by General Directorate of Agricultural Research and Policies[28]

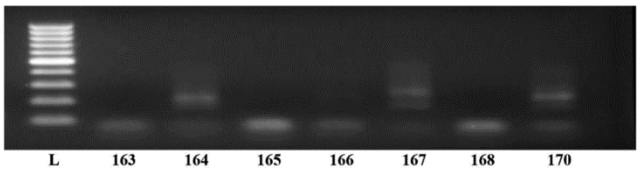

Fig. 3 — Susceptibility analysis of chickpea breeding lines through 163-168, and 170 using STMS Ts54 primer. Band formation in the 209 bp region indicates that the plant is resistant to Ascochyta blight disease. As a result of the analysis done using a 0-1000 bp ladder, bands showing resistance in the 209 bp region were detected in individuals numbered 164, 166, 167 and 170, in which polymorphic band formation was observed. L: 100 bp DNA Ladder Plus, 163: KNM 10 283 SAM, 164: URFA C-1, 165: URFA C-2, 166: URFA C-3, 167: URFA C-4, 168: URFA C-5, and 170: URFA C-7

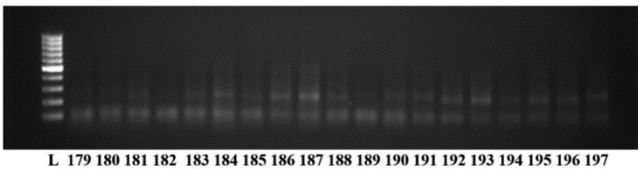

Fig. 4 — Susceptibility analysis of chickpea breeding lines through 179-197using STMS Ts54 primer. As a result of the analysis done using a 0-1000 bp ladder, the band in the 209 bp region indicates the resistance in individuals having polymorphic band formation. As shown in Table 3, individuals numbered 179, 181, 182, 183, 184, 185, 186, 188, 189, 190, 191, 192, 193, 195, 196 and 197 were observed being as resistant. L: 100 bp DNA Ladder Plus, 179: URFA C-16, 180: URFA C-17, 181: URFA C-18, 182: URFA C-19, 183: URFA C-20, 184: EN-2052 Maras, 185: EN-957 (C100-60M Maras), 186: EN-1554 Maras, 187: EN-1630 (X98 AK7) Maras, 188: DIYARBAKIR C-1, 189: DIYARBAKIR C-2, 190: DIYARBAKIR C-3, 191: DIYARBAKIR C-4, 192: DIYARBAKIR C-5, 193: DIYARBAKIR C-6, 194: DIYARBAKIR C-7, 195: DIYARBAKIR C-8, 196: DIYARBAKIR C-9, and 197: DIYARBAKIR C-10

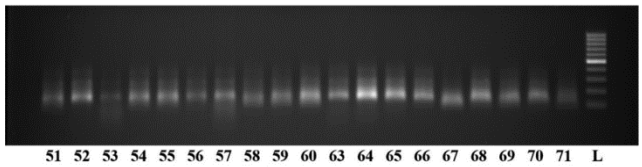

Fig. 5 — Susceptibility analysis of chickpea breeding lines through 51-60, and 63-71 using STMS Ta146 primer. As a result of the analysis done using a 0-1000 bp ladder, the band in the 161 bp region indicates the resistance in individuals having polymorphic band formation. As shown in Table 3, individuals numbered 52, 53, 54, 55, 56, 57, 63, 64, 65, 66, 68 and 70 were observed being as resistant. L: 100 bp DNA Ladder Plus, 51: FLIP 06-158 C, 52: FLIP 07-227 C, 53: FLIP 09-18C, 54: FLIP 09-20-C, 55: Seckin-D, 56: FLIP 09-23 C, 57: FLIP 09-30 C, 58: FLIP 09-13 C, 59: FLIP 09-22 C, 60: FLIP 09-21 C, 63: FLIP 97-706 C, 64: FLIP 07-216 C, 65: FLIP 03-42 C, 66: EN 1945, 67: EN 1949, 68: EN 1951, 69: EN 1964, 70: Inci-1, and 71: EN 1967

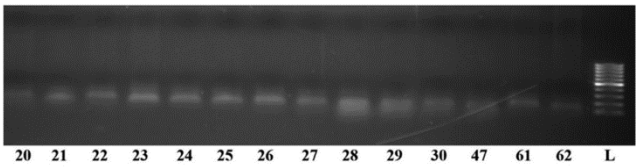

Fig. 6 — Susceptibility analysis of chickpea breeding lines through 20-30, 47, and 61-62 using STMS Ta146 primer. As a result of the analysis done using a 0-1000 bp ladder, the band in the 161 bp region indicates the resistance in individuals having polymorphic band formation. When the bands appearing in the gel were examined, band formation was observed in the 146 bp region in some individuals, as in individuals 21 and 23. Individuals numbered 21, 23, 24, 25, 26, 27, 28, 29, 30, 47 and 62 were observed being as resistant. L: 100 bp DNA Ladder Plus, 20:FLIP 01-24 C, 21:EN 1683, 22: EN 1685, 23: EN 1751, 24: EN 1751, 25: EN 1685-1, 26: UNHB-2010-52, 27: UNHB-2010-95, 28: UNHB-2010-96, 29: UNHB-2010-97, 30: EN 1788, 47: FLIP 88-85 C, 61: KNGB 2012 12505, and 62: TB 2012/40

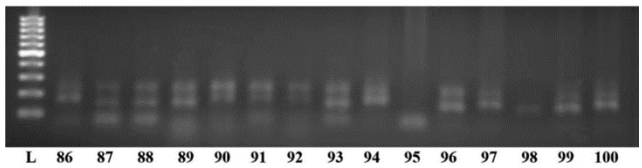

Fig. 7 — Susceptibility analysis of chickpea breeding lines through 86-100 using STMS Ta2 primer. As a result of the analysis done using a 0-1000 bp ladder, the band in the 175 bp region indicates the resistance in individuals having polymorphic band formation. Polymorphic band formation in individuals in the gel ran was observed in the 175 bp region and these individuals were shown in table 3 as resistant certificated lines. Individuals numbered 86, 91, 98 and 100 were observed being as resistant. L: 100 bp DNA Ladder Plus, 86: AKN-2012-3 C-1, 87: AKN-2012-3 C-2, 88: AKN-2012-3 C-3, 89: AKN-2012-3 C-4, 90: AKN-2012-3 C-5, 91: AKN-2012-3 C-6, 92: AKN-2012-3 C-7, 93: AKN-2012-3 C-8, 94: AKN-2012-3 C-9, 95: AKN-2012-3 C-10, 96: AKN-2012-3 C-11, 97: F3 1 POP-2012 C-12, 98: ESN-16-13 NUD-14, 99: ESN-14-13 NUD-10, and 100: ESN-17-13 NUD-16

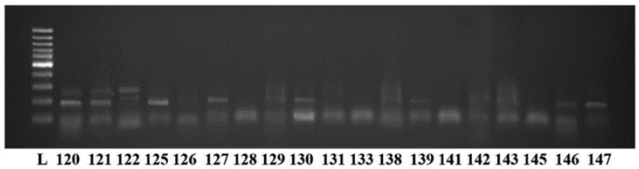

Fig. 8 — Susceptibility analysis of chickpea breeding lines through 120-122, 125-131,133, 138-139, 141-143, 145-147using STMS Ta2 primer. As a result of the analysis done using a 0-1000 bp ladder, the band in the 175 bp region indicates the resistance in individuals having polymorphic band formation. Polymorphic band formation in individuals in the gel ran was observed in the 175 bp region and these individuals were shown in table 3 as resistant certificated lines. Individuals numbered 120, 121, 125, 127, 129, 130, 138, 139, 142, 143, 146 and 149 were observed being as resistant. L: 100 bp DNA Ladder Plus, 120: ESN-11 13 NUD-7, 121: ESN-4 13 NOUD-8, 122: ESN-8 13 NUD-5, 125: EN 1640, 126: EN 2, 127: ENA 55-2, 128: ENA 74-2, 129: ENA 81-1, 130: ENA 102-4, 131: ENA 112-3, 133: ENA 129-6, 138: ENA 159-4, 139: ENA 159-1, 141: ENA 102-1, 142: ENA 101-9, 143: X00 TH 119 C-1, 145: NBUD-012-103 C-3, 146: C-300-16 M C-4, and 147: NBUD-12-101 C-5

In this research, high polymorphism ratios were detected and used for identification of the resistant/susceptible chickpea breeding lines against Ascochyta blight by employing STMS primers. Similar to our research, a number of studies were carried out previously using molecular markers for similar purposes. For example, RAPD technique was used for identification of the resistant bean breeding varieties against bean anthracnose (*Colletotrichum lindemuthianum*) disease. The strains that are the cause of that disease are having very variable genetic structures. Molecular markers related with the disease resistant genes were used for the detection[29]. Similarly, 3 RAPD and 2 ISSR markers were used for identification and mapping of the QTLs conferring resistance to Ascochyta blight in chickpea[30,31]. Also, 6 STMS markers were found to be related to Ascochyta blight resistance[26,32]. In a study performed by Cingilli *et al.*[33], 49 chickpea varieties grown in Türkiye were examined using STMS, ISP and RAPD markers for the identification of Ascochyta blight. Resistant genotypes were observed by using a RAPD UBC 733 primer and a STMS Ta2 primer. Also, STMS primers, Ta2, Ts54 and Ta146, were utilized in a study for determination resistance in the chickpea breeding varieties for Ascochyta blight[27]. In a similar study, the devastating disease, Ascochyta blight was identified by 82% accuracy using Ta2 primer in the chickpea breeding lines[34]. In another study performed by Upaydhyaya *et al.*[35], 48 SSR markers were used for characterization of chickpea in purpose of crop

Table 3 — The data obtained from identifications of susceptibility of 205 chickpea breeding lines using STMS primers for the disease evaluations

No	Chickpea Breeding Lines	R/S using Ts54 producing 209 bp-long band	R/S using Ta146 producing 161 bp-long band	R/S using Ta2 producing 175 bp-long band
F	Flip8492(C3)	R	R	R
C	*C. reticulatum*	S	S	S
1	No specific name for the population	R	R	R
2	F3-03 (X201 TH165-8)	R	S	S
3	EN 808	S	S	S
4	EN 766	S	R	R
5	EN 952	S	R	R
6	C-100-2-2	R	R	R
7	ENA 8-2	S	S	S
8	FLIP 03-108 c	S	R	S
9	FLIP 03-42 c	R	R	R
10	FLIP 03-21 c	R	R	R
11	F4-09 (X05 TH80-16105-31-2)	S	R	S
12	F4-09 (X05 TH69-16124-8)	R	S	R
13	F4-09 (X05 TH21-16139-12-3)	S	S	R
14	F4-09 (X05 TH21-16189-12-4)	R	R	S
15	ENA 144-10	R	R	
16	ENA 144-11	S	R	R
17	ENA 144-16	S	R	S
18	FLIP 05-150 C	R	R	R
19	FLIP 05-170 C	S	R	R
20	FLIP 01-24 C	S	S	S
21	EN 1683	S	R	R
22	EN 1685	S	S	S
23	EN 1750	S	R	R
24	EN 1751	S	R	S
25	EN 1685-1	S	R	R
26	UNHB-2010-52	S	R	S
27	UNHB-2010-95	S	R	R
28	UNHB-2010-96	S	R	S
29	UNHB-2010-97	S	R	S
30	EN 1788		R	S
31	EN 1800	R	S	S
32	EN 1822	S	R	R
33	EN 1823	S	S	S
34	EN 1830	S	R	R
35	EN 1831	S	R	R
36	EN 1799	S	S	S
37	FLIP 97-677 C	S	S	R
38	FLIP 06-111 C	S	R	S
39	FLIP 06-59 C	S	S	S
40	FLIP 06-133 C	S	S	R
41	FLIP 06-97 C	S	S	S
42	FLIP 06-33 C	S	R	R
43	FLIP 06-39 C	S	R	R
44	FLIP 07-211 C	R	S	S
45	FLIP 06-104 C	R	R	S
46	FLIP 88-85 C	S	R	S
47	FLIP 06-105 C		R	R
48	FLIP 06-66 C	R	R	S
49	FLIP 05-65 C	S	R	S
50	FLIP 07-184 C	S	R	S
51	FLIP 06-158 C	S	S	S
52	FLIP 07-227 C	R	R	R
53	FLIP 09-18 C	S	R	R

(*Contd.*)

Table 3 — The data obtained from identifications of susceptibility of 205 chickpea breeding lines using STMS primers for the disease evaluations (*Contd.*)

No	Chickpea Breeding Lines	R/S using Ts54 producing 209 bp-long band	R/S using Ta146 producing 161 bp-long band	R/S using Ta2 producing 175 bp-long band
F	Flip8492(C3)	R	R	R
C	*C. reticulatum*	S	S	S
54	FLIP 09-20 C	S	R	R
55	Seckin-D	S	R	S
56	FLIP 09-23 C	S	R	S
57	FLIP 09-30 C	S	R	S
58	FLIP 09-13 C	S	S	S
59	FLIP 09-22 C	S	S	S
60	FLIP 09-21 C	S	S	S
61	KNGB 2012 12505	S	S	S
62	TB 2012/40	S	R	S
63	FLIP 97-706 C	S	R	R
64	FLIP 07-216 C	S	R	R
65	FLIP 03-42 C	R	R	R
66	EN 1945	S	R	S
67	EN 1949	S	S	R
68	EN 1951	S	R	S
69	EN 1964	S	S	S
70	Inci-1	S	R	R
71	EN 1967	S	S	R
72	EN 1974	S	S	R
73	UNHB 2011-11	S	S	S
74	UNHB 2011-79	S	R	S
75	UNHB 2011-42	S	S	S
76	ENA 192-7	S	S	S
77	ENA 197-7	S	S	S
78	EN 1898	S	R	S
79	EN 1837	S	S	S
80	ENA 190-7	S	R	R
81	EN-1867	S	S	R
82	FLIP 03-14 C	S	S	R
83	FLIP 03 8C	S	S	S
84	FLIP 03 61C	S	S	R
85	Seckin-1	S		R
86	AKN-2012-3 C-1	S	S	R
87	AKN-2012-3 C-2	S	S	S
88	AKN-2012-3 C-3	S	R	S
89	AKN-2012-3 C-4	S	S	S
90	AKN-2012-3 C-5	S	R	S
91	AKN-2012-3 C-6	S	R	R
92	AKN-2012-3 C-7	S	S	S
93	AKN-2012-3 C-8	S	R	S
94	AKN-2012-3 C-9	S	S	S
95	AKN-2012-3 C-10	S	R	S
96	AKN-2012-3 C-11	S	R	S
97	F3 1 POP-2012 C-12	S	S	S
98	ESN-16-13 NUD-14	S	S	R
99	ESN-14-13 NUD-10	S	R	S
100	ESN-17 13 NUD-16	S	R	R
101	ESN-18 13 NUD-16	R	R	R
102	ESN-23 13 NBUD-7	R	S	R
103	ESN-15 13 NUD 13	S	S	S
104	ESN-13 13 NUD-9	S	R	R
105	ESN-12-23 NUD-8	R	S	R
106	ESN-18-13 NUBD-1	R	R	R
107	ESN-24 13 NUBD-6	R	S	S

(Contd.)

Table 3 — The data obtained from identifications of susceptibility of 205 chickpea breeding lines using STMS primers for the disease evaluations (*Contd.*)

No	Chickpea Breeding Lines	R/S using Ts54 producing 209 bp-long band	R/S using Ta146 producing 161 bp-long band	R/S using Ta2 producing 175 bp-long band
F	Flip8492(C3)	R	R	R
C	*C. reticulatum*	S	S	S
108	ESN-20 13 NUBD-2	S	S	S
109	ESN-23 13 NBUD-5	R	R	R
110	ESN-21 13 NBUD-3	R	S	S
111	ESN-22 13 NBUD-4	R	R	S
112	ESN-5 13 NOUD-13	S	S	R
113	ESN-6 13 NOUD-14	S	S	R
114	ESN-7 13 NOUD-17	S	S	S
115	ESN-8 13 NUD-1	S	S	S
116	ESN-10 13 NUD-6	S	S	R
117	ESN-2 13 NOUD-4	S	S	R
118	ESN-1 13 NOUD-2	S	S	R
119	ESN-3 13 NOUD-6	S	S	R
120	ESN-11 13 NUD-7	S	R	R
121	ESN-4 13 NOUD-8	R	R	R
122	ESN-8 13 NUD-5	S	S	S
123	EN 1553	R	S	R
124	EN 1554	R	S	S
125	EN 1640	R	S	R
126	EN 2	R	S	S
127	ENA 55-2	R	S	R
128	ENA 74-2	R	R	S
129	ENA 81-1	S	S	R
130	ENA 102-4	R	R	R
131	ENA 112-3	R	S	S
132	ENA 140-8	S	S	R
133	ENA 129-6	R	R	S
134	UNGB-48	S	S	R
135	UNGB-41	R	S	R
136	UNGB-46	R	S	R
137	ENA 159-2	S	S	S
138	ENA 159-4	S	S	R
139	ENA 159-1	R	S	R
140	ENA 87-3	S	S	R
141	ENA 102-1	S	R	S
142	ENA 101-9	R	R	R
143	X00 TH 119 C-1	S	S	R
144	NBUD-012-104 C-2	S	S	R
145	NBUD-012-103 C-3	R	R	S
146	C-300-16 M C-4	R	S	R
147	NBUD-12-101 C-5	R	S	R
148	C-100-67 M C-6	R	R	R
149	UBUT-12-102 C-7	S	S	S
150	KNM-10-103	R	S	R
151	KNM-10-105		S	R
152	KNM-10 114	R	S	S
153	KNM 10 117	S	S	R
154	KNM 10 122	S	S	S
155	KNM 11 125	R	S	S
156	KNM 11 127	S	S	S
157	KNM 10 146	R	S	S
158	KNM 10 162	S	S	R
159	KNM 10 171 SAM	R	S	R
160	KNM 10 196 SAM	R	S	R
161	KNM 10 235 SAM	R	S	S

(Contd.)

Table 3 — The data obtained from identifications of susceptibility of 205 chickpea breeding lines using STMS primers for the disease evaluations (*Contd.*)

No	Chickpea Breeding Lines	R/S using Ts54 producing 209 bp-long band		R/S using Ta146 producing 161 bp-long band		R/S using Ta2 producing 175 bp-long band	
F	Flip8492(C3)	R		R		R	
C	*C. reticulatum*		S		S		S
162	KNM 10 274 SAM		S	R		R	
163	KNM 10 283 SAM		S		S		S
164	URFA C-1	R		R			S
165	URFA C-2		S		S	R	
166	URFA C-3	R			S		S
167	URFA C-4	R		R			S
168	URFA C-5		S		S	R	
169	URFA C-6	R			S	R	
170	URFA C-7	R			S	R	
171	URFA C-8	R			S		S
172	URFA C-9		S		S	R	
173	URFA C-10	R			S	R	
174	URFA C-11	R			S		S
175	URFA C-12	R			S	R	
176	URFA C-13	R		R			S
177	URFA C-14		S		S		S
178	URFA C-15		S		S	R	
179	URFA C-16	R		R			S
180	URFA C-17		S	R			S
181	URFA C-18	R			S	R	
182	URFA C-19	R		R			S
183	URFA C-20	R		R			S
184	EN-2052 Maras	R		R			S
185	EN-957 (C100-60M Maras	R		R			S
186	EN-1554 Maras	R			S		S
187	EN-1630 (X98 AK7) Maras		S	R			S
188	DIYARBAKIR C-1	R		R		R	
189	DIYARBAKIR C-2	R		R		R	
190	DIYARBAKIR C-3	R			S		S
191	DIYARBAKIR C-4	R		R		R	
192	DIYARBAKIR C-5	R		R		R	
193	DIYARBAKIR C-6	R		R		R	
194	DIYARBAKIR C-7		S	R			S
195	DIYARBAKIR C-8	R			S	R	
196	DIYARBAKIR C-9	R		R			S
197	DIYARBAKIR C-10	R		R			S
198	FLIP-01 54C H-1	R			S		S
199	FLIP-01 39C H-2	R		R		R	
200	FLIP-00 34C H-3	R			S		S
201	EN 2057 AD-1	R		R			S
202	EN 2057 AD-2	R			S	R	
203	EN 1680 AD-3	R			S	R	
204	TATLAR AD-4	R			S		S
205	A-Z AD-5	R			S	R	

F: as positive control-resistant, C: as negative control-susceptible, R: Resistant, and S: Susceptible

improvement. A marker-assisted selection study was successfully carried out by Bouhadida*et al*.[36] in an effort of revealing resistance in the Tunisian chickpea breeding lines. A study, for defining of the pathotype-specific genetic factors in chickpea for quantitative resistance against Ascochyta blight were conducted by Cho *et al*.[37] using STMS primers. Population structure of *Ascochyta rabiei* in Australia was studied based on STMS fingerprints[38]. Validation of the 42 chickpea-STMS markers in lentil (*Lens culinaris* subsp. *culinaris*) cultivars of India was performed and all of them gave amplified products in lentil[39]. Moreover, new resistant markers against Ascochyta blight were determined by QTL mapping[40]. In another study, 83 RAPD, STMS, ISSR and RGA markers were obtained for seedling and body resistance and

they were successfully used in detection of Ascochyta blight in *Cicer* genom[41].

Development of resistant breeding lines against Ascochyta blight was the objective of this work and STMS markers were evaluated for potential identification of them. As mentioned above, Ascochyta blight is a major challenge regarding with having high chickpea productivity for farmers. The STMS markers in chickpea breeding programs as marker-assisted characterization are being used for improving productivity via identification of resistant cultivars to diseases. For the varieties developed through hybridization, in selections of resistant lines, precision and efficiency are provided by using STMS markers.

Screening of chickpeas through using of a number of STMS markers associated with resistance against Ascochyta blight was being undertaken; therefore, STMS markers were turned out to be the marker type of choice in chickpea breeding due to usability of them in producing high-level polymorphisms, which are highly sensitive for identification of population genetic structures. In our study, the results of resistancy and susceptibility tests using STMS Ta2 marker primer giving a resistance band of 175 bp in the chickpea genome revealed that among 205 breeding lines, 100 resistant and 104 sensitive chickpea genotypes were identified. With the use of STMS Ta146 marker primer, which gives a 161 bp resistance band, 94 resistant and 110 sensitive chickpea genotypes were determined. As well, with the use of the STMS Ts54 marker primer, which gives a 209 bp resistance band, 82 resistant and 123 sensitive chickpea genotypes were found. STMS markers showed high polymorphism in identifying resistant/susceptible genotypes of chickpea breeding lines against anthracnose disease that is caused by the pathogen *A. rabiei*. Among 205 breeding lines, the chickpea breeding lines numbered as 1-2,6,9-10,12, 14-15, 18, 31, 44-45,48, 52, 65, 101-102, 105-107, 109-111, 121, 123-128, 130-131, 133, 135-136, 139, 142, 145-148, 150, 152, 155, 157, 159-161, 164, 166-167, 169-171, 173-176, 179, 181-186, 188-193, 195-205 were found to be resistant against Ascochyta blight using STMS Ts54marker primer (Table 3). The chickpea breeding lines numbered as 1, 4-6, 8-11, 14-19, 21, 23-30, 32, 34-35, 38, 42-43, 45-50, 52-57, 62-66, 68, 70, 74, 78, 80, 88, 90-91, 93, 95-96, 99-101, 104, 106, 109, 111, 120-121, 128, 130, 133, 140-141, 145, 148, 162, 164, 167, 176, 179-180, 182-185, 187-189, 191-194, 196-197, 199 and 201 were found to be resistant against Ascochyta blight using STMS Ts54 marker primer (Table 3). And, the chickpea breeding lines numbered as 1, 4-6, 9-10, 12-13, 16, 18-19, 21, 23, 25, 27, 32, 34-35, 37, 40, 42-43, 47, 52-54, 63-65, 67, 70-72, 80-82, 84-86, 91, 98, 100-102, 104-106, 109, 112-113, 116-121, 123, 125, 127, 129-130, 132, 134-136, 138-140, 142-144, 146-148, 150-151, 153, 158-160, 162, 166, 168-170, 172-173, 175, 178, 181, 188-189, 191-193, 195, 199, 202-203 and 205 were found to be resistant against Ascochyta blight using STMS Ta2 marker primer. Finally, by this work, the resistant breeding lines were identified that will provide important benefits to breeders in fight against Ascochyta blight.

Acknowledgement

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 interest

All authors declare no conflict of interests.

References

1 García MO, Rocafull YR, Molina LZ, Aguilera JL, Garibay RO & García MCN, Identificación de rizobiospromotores del crecimiento vegetal asociados a garbanzo (*Cicer arietinum* L.). *Agron Mesoam*, (2023) 50929.

2 Pandey AK & Basandrai AK, Will *Macrophomina phaseolina* spread in legumes due to climate change? A critical review of current knowledge. *J Plant Dis Prot*, 128 (2021) 9.

3 Kumar M, Yusuf MA, Nigam M & Kumar M, An update on genetic modification of chickpea for increased yield and stress tolerance. *Mol Biotechnol*, 60 (2018) 651.

4 Kanouni H, Taleei A & Okhovat M, Ascochyta blight (*Ascochyta rabiei* (Pass.) Lab.) of chickpea (*Cicer arietinum* L.): breeding strategies for resistance. *Int J Plant Breed Genet*, 5 (2011) 1.

5 Zahra T, Ghramh HA, Batool M, Abid AD, Shahzad S, Shahbaz M & Hashim S, Temperature mediated influence of mycotoxigenic fungi on the life cycle attributes of *Callosobruchus maculatus* F. in stored chickpea. *J King Saud Univ-Sci*, 35 (2023) 102357.

6 TUIK. Statistical Data. http://www.tuik.gov.tr/yillik/Ist_gostergeler.pdf (2017).

7 Sezgin AC & Onur M, Traditional Turkish cuisine prepared with legumes. *JOGHAT (Online)*, 5 (2022) 170.

8 Kudapa H, Barmukh R, Garg V, Chitikineni A, Samineni S, Agarwal G & Varshney RK, Comprehensive Transcriptome Profiling Uncovers Molecular Mechanisms and Potential Candidate Genes Associated with Heat Stress Response in Chickpea. *Int J Mol Sci*, 24 (2023) 1369.

9 Hiremath PJ, Farmer A, Cannon SB, Woodward J, Kudapa H, Tuteja R & Varshney RK, Large-scale transcriptome analysis in chickpea (*Cicer arietinum* L.), an

orphan legume crop of the semi-arid tropics of Asia and Africa. *Plant Biotechnol J*, 9 (2011) 922.
10 Vargas-Blandino D, Cardenas-Travieso RM & San José de las Lajas M, Chickpea cultivation, a possible solution to climate change. *Cultivos Tropicales*, 42 (2021) 1.
11 Hamid Z, Lakmes A, Gumus H, Unsal NE & Kahraman A, Tolerance to Imazamox Herbicide Found after Screening of Advanced Generation Lentil Mutant Genotypes. *YYU J Agr Sci*, 31 (2021) 678.
12 Duzdemir O, Selvi B, Yanar Y & Yildirimi A, Sources of resistance in chickpea (*Cicer arietinum* L.) land races against Ascochyta rabiei causal agent of ascochyta blight disease. *Pak J Bot*, 46 (2014) 1479.
13 Kahraman A & Ozkan Z, Ascochyta blight of chickpea. *Selcuk J Agric Food Sci*, 29 (2015) 62.
14 Byamukama E, Pérez-Hernández O & Yabwalo DN, Plant disease threats to food security in the Northern Great Plains of North America. *Agron J*, 113 (2021) 4473.
15 Khaliq I, Spatiotemporal development of Ascochyta blight in chickpea from primary infection foci: insights from plant, pathogen and the environment interactions to inform an epidemic risk. *Eur J Plant Pathol*, 161 (2021) 331.
16 Mondal B, Chaturvedi SK, Das A, Kumar Y, Yadav A, Sewak S & Singh NP, Embryo rescue and chromosomal manipulations. In *Chickpea: Crop Wild Relatives for Enhancing Genetic Gains*. (Academic Press) 2020, 95.
17 Han S, Sebastin R, Lee KJ, Wang X, Shin MJ, Kim SH & Chung JW, Interspecific variation of seed morphological and micro-morphological traits in the genus *Vicia* (Fabaceae). *MRT*, 84 (2021) 337.
18 Singh M, Kumar K, Bisht IS, Dutta M, Rana MK, Rana JC & Sarker A, Exploitation of wild annual Cicer species for widening the gene pool of chickpea cultivars. *Plant Breed*, 134 (2015) 186.
19 Arora A, Panwar RK & Verma SK, In vitro screening of chickpea genotypes for drought related traits using PEG 6000. *J Pharmacogn Phytochem*, 9 (2020) 3438.
20 Yegrem L, Nutritional composition, antinutritional factors, and utilization trends of Ethiopian chickpea (*Cicer arietinum* L.). *Int J Food Sci,* 2021 (2021) 1.
21 Khan HA, Siddique KH, Munir R & Colmer TD, Salt sensitivity in chickpea: Growth, photosynthesis, seed yield components and tissue ion regulation in contrasting genotypes. *J Plant Physiol*, 182 (2015) 1.
22 Tivoli B, Baranger A, Avila CM, Banniza S, Barbetti M, Chen W & Muehlbauer FJ, Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. *Euphytica*, 147 (2006) 223.
23 Ozkilinc H, Thomas K, Abang M & Peever TL, Population structure and reproductive mode of *Didymella fabae* in Syria. *Plant Pathol*, 64 (2015) 1110.
24 Rai SK & Pandey-Rai S, Marker-assisted breeding for abiotic stress tolerance in horticultural crops. In *Stress Tolerance in Horticultural Crops*. (Woodhead Publishing) 2021, 63.
25 Ramchander L, Sadhukhan R, Das A, Banerjee J & Pramanik K, Screening for Phosphorus (P) Acquisition Efficient Genotypes and Identification of Sequence Variations among P-Efficient and Inefficient Genotypes in Chickpea (*Cicer arietinum* L.). *Legume Res-An Int J*, 44 (2021) 1405.
26 Tekeoglu M, Rajesh P & Muehlbauer F, Integration of sequence tagged microsatellite sites to the chickpea genetic map. *Theor Appl Genet*, 105 (2002) 847.
27 Kottapalli P, Gaur PM, Katiyar SK, Crouch JH, Buhariwalla HK, Pande S & Gali KK, Mapping and validation of QTLs for resistance to an Indian isolate of Ascochyta blight pathogen in chickpea. *Euphytica*, 165 (2009) 79.
28 TAGEM. https://www.tarimorman.gov.tr/TAGEM (2021).
29 Madakbas SY, & Ellialtioglu S, Studies on the Inheritance of Resistance to Bean Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lambs. Scrib.). *Bio Sci Res J*, *5*(1), (2012) 99-109.
30 Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ & Muehlbauer FJ, Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. *Crop Sci,* 40 (2000) 1606.
31 Madrid E, Chen W, Rajesh PN, Castro P, Millán T & Gil J, Allele-specific amplification for the detection of ascochyta blight resistance in chickpea. *Euphytica*, *189*,(2013)183-190.
32 Daba K, Deokar A, Banniza S, Warkentin TD & Tar'an B, QTL mapping of early flowering and resistance to ascochyta blight in chickpea. *Genome*, 59 (2016) 413.
33 Cingilli H, Altınkut A & Akcin A, Screening of Turkish Chickpea (*Cicer arietinum* L.) Genotypes for Ascochyta Blight Resistance using Molecular Markers. *Biotechnology & Biotechnological Equipment*, 17 (2003) 65.
34 Yorgancilar O, Bilir S& Cakir E, Testing the resistancy of chickpea lines and varieties grown in Anatolian agricultural research institute against ascochyta blight using molecular marker methods, Turkey VIII. Field Crops Congress, (October, Hatay, Turkey) 2009, 19.
35 Upadhyaya HD, Dwivedi SL, Baum M, Varshney RK, Udupa SM, Gowda CL & Singh S, Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum*L.). *BMC Plant Biology*, *8*(1), (2008) 1-12.
36 Bouhadida M, Benjannet R, Madrid E, Amri M & Kharrat M, Efficiency of marker-assisted selection in detection of ascochyta blight resistance in Tunisian chickpea breeding lines. *Phytopathologia Mediterranea*, (2013) 202.
37 Cho S, Chen W & Muehlbauer FJ, Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. *Theor Appl Genet*, 109 (2004) 733.
38 Phan HTT, Ford R & Taylor PWJ, Population structure of *Ascochyta rabiei* in Australia based on STMS fingerprints. *Fungal Divers*, 13 (2003) 111.
39 Agrawal PK & Katiyar AK, Validation of chickpea-STMS markers and DNA fingerprinting in lentil (*Lens culinaris* subsp. culinaris) cultivars of India. *Indian J Genet Plant Breed*, 68 (2008) 149.
40 Sharma M & Ghosh R, An update on genetic resistance of chickpea to ascochyta blight. *Agronomy*, 6 (2016) 1.
41 Kukreja S, Salaria N, Thakur K & Goutam U, Fungal disease management in chickpea: current status and future prospects. *Fungi and their role in sustainable development: current perspectives*, (2018) 293.