J. Agr. Sci. Tech. (2021) Vol. 23(1): 209-220

Virulence and Mating Type Distribution of *Didymella rabiei* in Chickpea Growing Areas of Turkey

N. Nalcaci[1], A. Turan[1], S. Basbuga[1], F. N. Kafadar[1], D. Isler Ceyhan[1], A. Anay[2], D. Mart[3], E. Ogut[4], K. Sarpkaya[5], and C. Can[1*]

ABSTRACT

Chickpea (*Cicer arietinum* L.) is a traditional crop species in Turkey that is cultivated in almost every province providing prominent economic income. Turkey has an important resource for both *Cicer* spp diversity and their phytopathogens like ascohyta blight caused by *Didymella rabiei* (Kovachevski) von Arx wherein resistance/tolerance is broken every 4-5 years in cultivated chickpea cultivars. In order to breed resistant/tolerant varieties in chickpea against *D. rabiei*, detailed and up to date analyses on population characterization is needed. This study was undertaken to define current aggressiveness patterns, pathotype and mating type distribution of *D. rabiei* population in chickpea growing areas of Turkey. The *D. rabiei* isolates were assigned to 5 virulence groups in which existence of pathotype IV, a new and aggressive group, was defined for the first time from farmers' fields and research institutes exhibiting continuous arm race between plant and pathogen. The isolates in each pathotype group depicted statistically important difference (P≤ *0.05*) in virulence levels on chickpea genotypes. The mating type distribution of 971 *D. rabiei* isolates was 1:1 for *Mat 1.1* and *Mat 1.2* isolates (X^2= 0.87, P= 0.35) exhibiting random sexual reproduction. Overall, the data obtained revealed the unstable aggressiveness nature of *D. rabiei* population in Turkey, which, in turn, explains frequent resistance overcome in registered chickpea genotypes leading to epidemics.

Keywords: Ascochyta blight, Biotic stress, Chickpea genotypes, *Cicer arietinum* L., Pathotypes.

INTRODUCTION

Chickpea is widely grown in Turkey and covers a total of harvest area of 392,673 ha with production of 470,000 tons in 2017 (FAOSTAT, 2020) and the main chickpea producing areas in Turkey are the Mediterranean, Southeastern and Central Anatolia regions. However, chickpea production has been in falling trend for the last 20 years in Turkey and one of the reasons for this reduction is a/biotic stresses. Ascochyta blight caused by *Didymella rabiei* (Kovachevski) von Arx [Anamorph: *Ascochyta rabiei* (Passerini) Labrousse] is one of the major biotic stresses (Kaiser and Kusmenoglu, 1997). *D. rabiei* has been reported from every continent in the world where chickpea is produced like Europe, Canada, Australia, Asia, Mediterranean countries and the USA, and it causes reductio in seed yield of 50-70% in chickpea (Nene, 1981; Trapero-Casas and Kaiser, 1992). *D. rabiei* also infects wild annual *Cicer* spp creating a model system to study

[1] Biology Department, University of Gaziantep, Gaziantep, Turkey.
[2] Mersin Directorate of Agricultural Quarantine, Mersin, Turkey.
[3] Eastern Mediterranean Agricultural Research Institute, Adana, Turkey.
[4] Agricultural Quarantine Directorate, Antalya, Turkey.
[5] Karabuk University, Engineering Department, Karabuk, Turkey.
[*]Corresponding author; e-mail: can@gantep.edu.tr

plant pathogen specification and coevolution in the Levant where the first domestication of chickpea occurred (Ozkilinc and Can, 2019).

Determining genetic differences within populations of phytopathogenic fungi is prerequisite to define effective control strategies, to select and test hybrid progenies in plant breeding programs (Taylor and Ford, 2007; Tahir *et al*., 2019). *D. rabiei* is a sexually propagating heterothallic fungus and has two forms in mating type loci, namely, *Mat 1.1* and *Mat 1.2.* Sexual stage occurs when two mating type groups of isolates exist in the area and this generates recombination leading to new generations comprising different virulence level (Wilson and Kaiser, 1995). Existence of genetic and aggressiveness difference has been reported in *D. rabiei* populations from major chickpea producing countries as well as wild annual *Cicer* spp exhibiting sympatric distribution with cultivated chickpea (Ozkilinc *et al*., 2011). Difference in virulence levels in *D. rabiei* was classified into pathogenic groups, virulence forms, pathotypes and races (Pande *et al*., 2005; Kanouni *et al*., 2011). Pathotype is defined as a group or sub groups within a population that incite a certain level of disease severity in a set of host genotypes, whereas a race is a qualitative measurement of virulence assigned by a certain resistance gene in a host genotype (Taylor and Ford, 2007). Several studies were conducted to disclose aggressiveness/virulence difference and assigning races/pathotypes in *D. rabiei* population from leading chickpea producing countries. Four pathotype groups from Syria, namely, I, II, III and IV were reported based on aggressiveness on five chickpea genotypes (ILC-1929, ILC-482, ILC-3279, ICC-12004, ICC-3996) and were used to classify virulence levels within the *D. rabiei* population (Udupa *et al*., 1998; Imtiaz *et al*., 2011). Pathogenic difference from India, Lebanon, Iran, Pakistan Canada and Spain revealed 3-14 different pathotypes/races in *D. rabiei* population by using 3-15 chickpea differentials (Vir and Grewal, 1974; Jamil *et al*., 2000). The incoherent results obtained through all the reports could be due to differences in test procedures used, disease ratings, chickpea differential genotypes, climatic conditions (temperature, light regime, humidity) that greatly affect first infection and symptom development of *D. rabiei* (Taylor and Ford, 2007).

In Turkey, *D. rabiei* exhibit pathogenic and genetic variation as well as mating type difference. Kaiser and Kusmenoglu (1997) determined teleomorph stage on overwintered chickpea debris in 15 provinces of Turkey and defined mating type distribution as 59 and 41% for *Mat 1.1* and *Mat 1.2*, respectively. Accordingly, Türkkan and Dolar (2009) used 7 differential chickpea genotypes (ILC-1929, ILC-482, F8, ICC-1903, ILC-249, ILC-3279, ICC-3996) to define pathotypes and physiological races of 64 *D. rabiei* isolates collected from 18 major chickpea cultivating provinces of Turkey. The isolates were classified into three pathotypes (I, II and III) and 6 races where pathotype I had the highest pathogenic variability and included four races.

Chickpea breeding studies against *D. rabiei* in chickpea is currently being conducted in 7 major research institutes and some universities in Turkey under collaboration with International Center for Agricultural Research in the Dry Areas (ICARDA) and The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Understanding the variability of the fungal population associated with infection could improve disease control strategies and, consequently, success in breeding efforts relies on critical investigation and chase of the pathogen population. Considering genetic and pathogenic variability of *D. rabiei* population in Turkey, this study aimed to: (i) Identify *D. rabiei* mating type distribution in Turkey, (ii) Define current aggressiveness patterns of *D. rabiei* isolates in major chickpea growing areas, and (iii) Collect the isolates to generate gene bank for further studies. The data obtained would provide

information to chickpea breeding studies against ascochyta blight in Turkey.

MATERIALS AND METHODS

Didymella rabiei Isolates

A total of 1257 isolates from 45 chickpea cultivating provinces belonging to 7 regions of Turkey were surveyed during 2014-2016 growing seasons and *D. rabiei* isolates was collected from 1251 fields covering 3206.6 ha area (Table 1, Figure 1). The chickpea plant materials exhibiting disease symptoms on stems, lateral branches, and pods were cultured on Potato Dextrose Agar (PDA: Merck, Darmstadt, Germany) following surface sterilization and incubated at 18-20^{o}C, 12/12 day/light regime for 5-8 days (Can *et al*., 2007). The isolates were then single-spored and maintained at -80^{o}C in glycerol stock and on Whatman filters at -20^{o}C for long time storage.

Pathotype Screening

Representative *D. rabiei* isolates for pathotypes I, II and III were provided by Dr. Weidong Chen (Washington State University, USA) (Chen *et al*., 2004). The isolates were grown on CSMDA (Chickpea Seed Meal Dextrose Agar) at 20^{o}C, 12/12 hour light/dark conditions for 8-12 days (Trapero-Casas and Kaiser, 1992). Conidia were collected into 5-10 mL of sdH_2O by scrabbling off the Petri dishes, filtered through cheese cloth to remove remaining

Table 1. Survey studies and *D. rabiei* isolate collection during 2014-2016 chickpea growing seasons.

Regions	# Provinces	# Districts	Area covered (ha)	# Fields	# Isolates
Southeastern Anatolia	6	116	1072.1	182	190
Eastern Anatolia	5	36	109.4	38	40
Blacksea	5	47	109.7	91	152
Central Anatolia	10	121	840.2	341	261
Mediterranean	7	103	431.6	275	272
Aegean	7	114	502.3	227	221
Bosporus	5	45	141.3	97	121
Total	45	582	3206.6	1251	1257

Figure 1. Chickpea producing provinces of Turkey surveyed for *D. rabiei* collection during 2014-2016.

mycelia, counted on Thoma chamber to final concentration of 5×10^5 conidia mL^{-1} (Chen *et al*., 2004). Selected 237 *D. rabiei* isolates were tested to define their aggressiveness patterns (Table 2) on ILC 1929 (susceptible to pathotypes I, II, III and IV), ILC 482 (resistant to pathotype I, susceptible to pathotypes II, III and IV), ILC 3279 (resistant to pathotypes I and II, susceptible to pathotypes III and IV), ICC 12004 (resistant to pathotypes I, II and III, susceptible to pathotype IV (Udupa *et al*., 1998; Imtiaz *et al*., 2011). The differential chickpea genotypes were originally obtained from ICARDA and were produced in the Eastern Mediterranean Research Institute (Adana-Turkey). The seeds of each genotype were surface sterilized with 2% commercial NaOCI solution before planting on sterile soil:perlite:peat (1:1:1) mixture in 10×12 cm pots. Experiments were conducted with three replicates and each replicate contained 5 germinated plants. Registered chickpea variety cv. Sarı was used as susceptible check in all the experiments.

The pots were incubated in growth chambers under the 12 hours light regime at 20±2°C with 85-90% humidity. Ten days after germination, plants were sprayed with conidia suspension of 5×10^5 conidia mL^{-1} until run off, whereas the control plants were applied with sdH_2O. The inoculated plants were covered with plastic bags for 24 hours to facilitate conidial infection. The plants were watered when necessary and disease ratings were recorded every three days until 21 days after inoculation using 1-9 scale (Singh *et al*., 1981; Reddy and Kabbabeh, 1985). Disease Severity Index (DSI) was calculated and the Area Under Disease Curve (AUDPC) and AUDPC% values were defined as the percentage of maximum possible area for three-week period according to Campbell and Madden (1990). Reactions of chickpea genotypes to *D. rabiei* isolates were defined as resistant or susceptible by DSI values of 21 days after inoculations (Benzohra *et al.*, 2011). Each disease assay was conducted independently as a completely randomized design with pots randomized within the growth chamber. Disease severity scores were recorded for each plant and the mean of scores represented one replicate. Histograms of disease scores were constructed using the mean of three replicates per isolate (each replicate consisted of disease scores of 5 plants). Tukey HSD test of variance was applied to AUDPC% values using SPSS v.25 (IBM Institute Inc.) with chickpea genotypes and pathoypes as main factors.

Mating Type Analyses

D. rabiei isolates were grown in Potato Dextrose Broth (PDB: Difco, Detroit, USA) media for 7-10 days in controlled incubators and fungal mycelia were used to isolate total genomic DNA using modified CTAB protocol (Peever *et al*., 2004). Mating type (MAT) groups were determined through multiplex PCR amplifications with SP21, COM1 and Tail 5 primers (Barve *et al*., 2003). PCR was conducted in 25 µL containing 10-20 ng μL^{-1} DNA template, 2 mM $MgCl_2$, 0.2 mM of each dNTP (Thermo Scientific), 1-unit Taq DNA polymerase (Thermo Scientific), 1X Taq DNA polymerase buffer, 10 nM of each primer. The PCR cycling parameters were denatured at 94°C for 3 minutes, followed by 45 cycles

Table 2. Pathotype scoring and identifications of *D. rabiei* isolates (Udupa *et al*., 1998; Imtiaz *et al*., 2001).

Pathotypes	Chickpea genotypes			
	ILC 1929	ILC 482	ILC 3279	ICC 12001
I	S	R	R	R
II	S	S	R	R
III	S	S	S	R
IV	S	S	S	S

of 30 seconds at at 94°C, 45 seconds at 60°C and 2.5 minutes at 60°C with final extension of 10 minutes at 72°C (Barve *et al*. 2003). The products were electrophoresed 1X TAE containing 1.5% agarose gels at 80V cm^{-1} for 2 hours along with 1 kb DNA ladder (Thermo Scientific), stained with ethidium bromide and visualized with gel documentation system. The *D. rabiei* isolates exhibiting ~700 bp and ~500 bp product were assigned as *Mat 1.1* and *Mat 1.2*, respectively. The probability of a greater Chi-square (χ^2) value under the null hypothesis of a 1:1 ratio of equal proportions of *Mat 1.1* and *Mat 1.2* was calculated.

RESULTS

D. rabiei isolations were successfully conducted from the symptom exhibiting chickpea plants collected during the survey studies in 2014-2016 (3 years) and a total of 1257 isolates were collected from 7 regions of Turkey (Table 1). The isolates were cultured on Whatman papers and placed at -20°C for long term storage to maintain *D. rabiei* germplasm for future studies. A total of 237 *D. rabiei* isolates that were collected from 106 chickpea growing districts belonging to 44 provinces of Turkey was included for pathotyping studies. The highest number of isolates were classified as low virulent (below 12% of DSI value at 21st day on cv. Sarı, ILC 482, ILC 1929, ILC 3279, ICC 12004) exhibiting single group statistically (Table 3). Aegean, Eastern, and Bosporus regions had the low virulence level isolates whereas Mediterranean, Southeastern, Black Sea, and Central Anatolia regions consisted of five aggressiveness groups. Additionally, the most aggressive isolates (pathotype IV) were defined within the *D. rabiei* population of Central, Blacksea, and Mediterranean regions (Tables 2 and 4). Furthermore, *D. rabiei* isolates obtained from 8 research institutes of Turkey and experimental fields of the Çukurova University (Adana-Turkey) contained high number of pathotype IV isolates (Table 4).

The chickpea cultivars (ILC 482, ILC 1929, ILC 3279, ICC 12004) used to define the pathotype groups of *D. rabiei* population in Turkey exhibited considerable variation among isolates (Figure 3). The AUDPC% values of differential genotypes of each pathotype group depicted statistically significant variation ($P< 0.0001$) amtaong *D. rabiei* isolates (Figures 2 and 3).

Similarly, virulence levels of *D. rabiei* isolates on chickpea differentials exhibited statistically significant variation with Tukey HSD test ($P\leq 0.05$) (Table 5). Mating type distribution was defined by assessing 971 *D. rabiei* isolates collected from 116 districts of chickpea growing fields of Turkey. Both mating types existed in 42 provinces of Turkey and exhibited 1:1 distribution for *Mat1.1* and *Mat 1.2* isolates ($X^2= 0.87$, $P= 0.35$) (Table 6, Figure 4).

DISCUSSION

Chickpea is mainly cultivated in the climatic and sub climatic zones of the world

Table 3. Distrubution of *D. rabiei* virulence groups in Turkey.

Regions	# Province	# District	# Low virulence	# P I	# P II	# P III	# P IV
Central Anatolia	10	29	5	14	2	11	11
Mediterranean	7	17	18	1	1	2	10
Aegean	7	20	39	-	1	-	1
Southeastern Anatolia	6	17	13	3	1	10	6
Blacksea	6	10	4	2	5	11	20
Bosporus	5	9	27	-	-	-	-
Eastern Anatolia	3	4	19	-	-	-	-
Total	44	106	125	20	10	34	48

Table 4. Virulence levels of *D. rabiei* isolates in research institutes of Turkey.

Research Institutes[a]	*Mat 1.1*	*Mat 1.2*	# Low virulence	# P I	# P II	# P III	# P IV
GAPTAEM	17	1	-	-	5	-	2
GAPUTAEM	10	14	1	-	3	4	6
KTAE	16	17	6	-	-	6	5
TARM	5	12	5	-	1	-	3
GKTAE	8	11	3	2	4	-	1
DATAE	16	4	1	-	1	-	6
DAGKTAE	17	5	4	-	1	1	4
CUTB	13	7	2	-	-	2	8
Total	102	71	22	2	15	13	35

[a]GAPTAEM: Southeastern Anatolia Agricultural Research Institute, Diyarbakır; GAPUTAEM: Southeastern Anatolia International Agricultural Research Institute, Sanliurfa; KTAE: Blacksea Agricultural Research Institute, Samsun; TARM: Field Crops Central Research Institute, Ankara; GKTAE: Transitional Zone Agricultural Research Institute, Eskisehir; DATAE: Eastern Mediterranean Research Institute, Adana; DAGKTAE: East Mediterranean Transitional Zone Agricultural Research Institute, K. Maras, CUTB: Çukurova University Field Crops Experimental Plots.

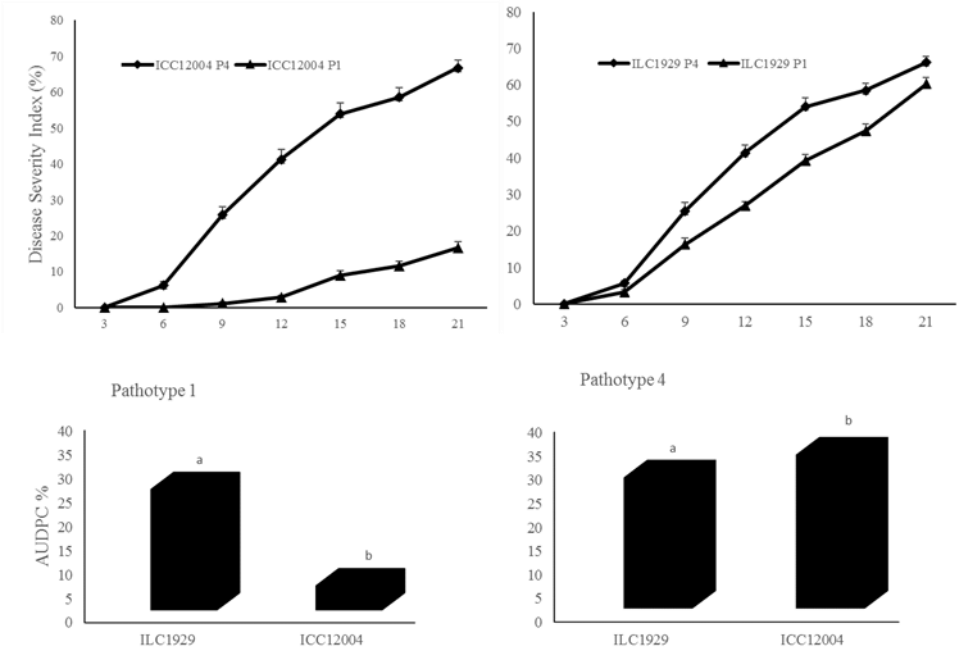

Figure 2. Disease Severity Index (DSI) and Area Under Disease Curve (AUDPC, %) values of pathotypes IV (P IV) and I (P I) following inoculation of ICC 12004 (*D. rabiei* resistant genotype) and ILC 1929 (*D. rabiei* susceptible genotype). Means±SE in the same column with the same letters are not significantly different with Tukey HSD test (P≤ 0.05).

and *D. rabiei* is considered as the major biotic stress factor negatively effecting seed quality and quantity where cool and humid conditions are prevalent during vegetative growth stage (Kanouni *et al*., 2011). Turkey is one of the main chickpeas producing countries in the world ranking fifth after India, Australia, Myanmar and Pakistan (FAOSTAT 2020). Chickpea breeding studies are being conducted in 7 major research institutes in Turkey (Table 4) and the resistance breeding against *D. rabiei* is

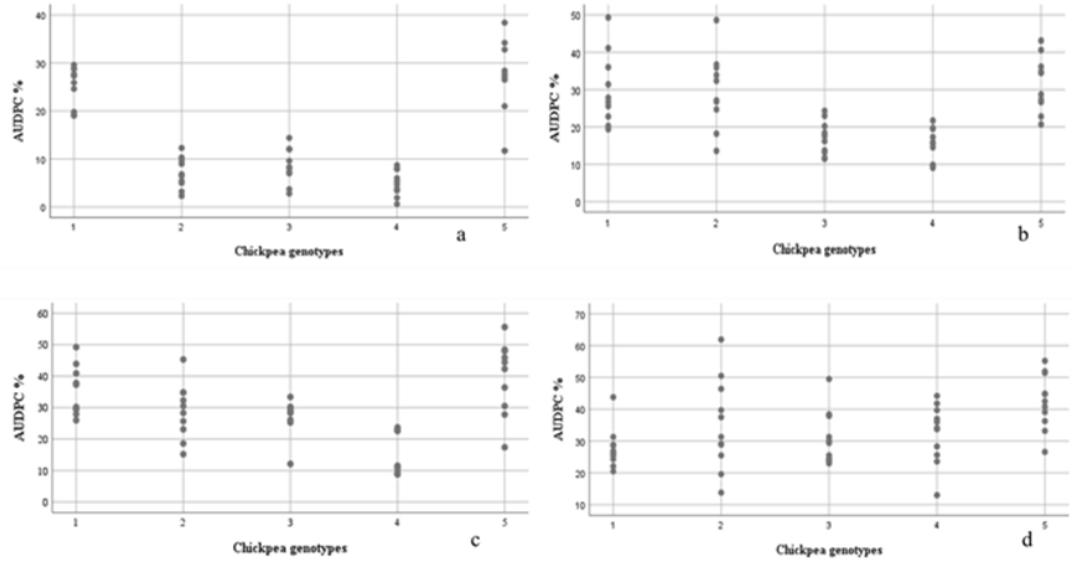

Figure 3. Distribution of Area Under Disease Curve (AUDPC, %) values of Pathotypes I (a), II (b), III (c), and IV (d) isolates on chickpea genotypes. The numbers in X-axis indicate cv. Sarı (1), ILC 1929 (2), ILC 482 (3), ILC 3279 (4), and ICC 12004 (5).

Table 5. Area Under Disease Curve (AUDPC, %) values of *D. rabiei* aggressiveness groups on chickpea differentials.

Genotypes	Virulence levels of *Didymella rabiei* isolates				
	Low virulence	P I	P II	P III	P IV
Sarı	11.92±2.01 a	25.95±2.81 b	31.52±2.35 b	39.67±3.65 c	42.41±2.58 c
ILC 1929	9.45±1.77 a	25.07±1.33 b	30.04±3.03 b	35.23±2.45 bc	27.66±1.86 a
ILC 482	7.96±0.82 a	7.05±1.02 a	29.79±3.16 b	28.86±2.77 b	34.93±4.24 ab
ILC 3279	7.71±1.61 a	8.53±3.66 a	16.98±1.43 a	27.22±1.82 b	30.69±2.49 a
ICC 12004	5.65±1.40 a	5.02±0.85 a	15.30±1.42 a	15.23±2.13 a	32.45±2.76 ab

a-c Means±SE in the same column with the same letters are not significantly different with Tukey HSD test (P≤0.05).

Table 6. Mating type distribution of *D. rabiei* isolates in Turkey.

Regions	# District	Mat 1.1	Mat 1.2	Total	X^{2a}	P
Southeastern Anatolia	18	71	32	88	6.55	0.01
Eastern Anatolia	3	17	30	47	3,6	0.06
Blacksea	7	56	58	114	0.04	0.85
Central Anatolia	29	68	121	189	14.86	0.000 1
Mediterranean	22	158	104	262	11.13	0.000 8
Aegean	23	12.	94	216	3.63	0.057
Bosporus	14	23	32	55	1.47	0.22
Total	116	500	471	971	0.87	0.35

a Chi-square value was calculated under the null hypothesis of a 1: 1 ratio of equal proportions of *Mat 1.1* and *Mat 1.2.*

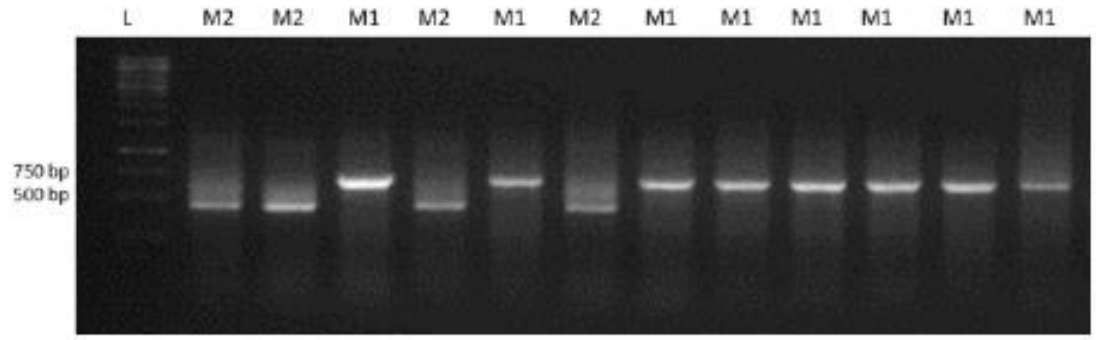

Figure 4. PCR products of *Mat1.1* and *Mat1.2* isolates of *D. rabiei*. L: 1 kb DNA ladder; M1: *Mat1.1*; M2*: Mat1.2*.

one of the major issues. *D. rabiei* population in chickpea growing areas in Turkey exhibit variable pathogenic and genetic structure (Türkkan and Dolar, 2009; Ozkilinc and Can, 2019) hence chickpea resistance to this prolific plant pathogen is easily broken causing major yield loses when the climatic factors are favorable (Sharma and Ghosh, 2016). This study was conducted to define population structure of *D. rabiei* through aggressiveness patterns and mating type distribution in 45 provinces of the 7 regions in Turkey. The results obtained through this study contain the current pathogenic variability of *D. rabiei* population in chickpea growing areas of Turkey.

Survey studies were conducted for 3 consecutive years during 2014-2016 chickpea growing seasons, covering 3,206.6 ha and 1251 fields were inspected (Table 1). These studies recovered a total of 1257 *D. rabiei* isolates, which were single-spored and maintained in long term storage conditions as germplasm for further studies comprising the largest collection of *D. rabiei* in Turkey.

Pathotype groups of isolates collected from farmers' fields and research institutes' experimental plots were defined as cv. Sari, ILC 482, ILC 1929, ILC 3279, and ICC 12004, wherein selected 237 *D. rabiei* isolates were screened. The aggressiveness of *D. rabiei* isolates were classified into 5 groups based on DSI values at 21st day after inoculations (Tables 3 and 4). Türkkan and Dolar (2009) tested the virulence levels of 67 *D. rabiei* isolates collected from 18 provinces of Turkey and reported 3 pathotype groups (pathotypes I, II and III) in 2009. Our isolates collection was conducted during 2014-2016. This result may indicate that during this short period of time, *D. rabiei* population of chickpea in Turkey underwent change in aggressiveness patterns through recombination, since teleomorph stage had previously been reported (Kaiser and Kusmenoglu, 1997). These findings were also supported by distribution of virulence groups of isolates from farmers' fields and research institute experimental plots. The isolates collected from farmers' fields exhibited high number of low virulence isolates (52.74%), whereas the isolates from the research institutes, where continuous breeding studies against ascochyta blight are being conducted, had 40.21% pathotype IV isolates (Tables 3 and 4). The chickpea breeding materials from universities, private sectors and research institutes in Turkey are screened for resistance/tolerance to *D. rabiei* in 7 main research institutes' experimental plots (Table 4) before registration. These plots are artificially or naturally contaminated with *D. rabiei* and contain well-established resident *D. rabiei* population. Therefore, *D. rabiei* isolates present in these areas may try to overcome the resistance/tolerance of chickpea genotypes and, through the interaction between plant and pathogen,

more aggressive isolates may be generated through sexual recombination (Afshari, 2008; Peever *et al*., 2004; Imtiaz *et al*., 2011). The aggressiveness patterns calculated with AUDPC% values of each pathotype group statistically differed on chickpea differential genotypes (Table 5). The isolates with low virulence were placed into a single group, whereas there was statistical difference in aggressiveness among pathotypes I, II, III, and IV isolates. These differences could also be explained by instability of *D. rabiei* virulence factors and horizontal gene transfer (Hamza *et al*., 2000; Verma *et al*., 2016).

The Mating type of *D. rabiei* isolates exhibited 1:1 (Mat 1.1/Mat1.2) distribution in Turkey (X^2= 0.87; P= 0.35; Table 6), however, there were differences among regions wherein both mating types existed in close proximity. These results suggested random sexual propagation of *D. rabiei* in Turkey as reported from nearby chickpea producing countries such as Syria, Lebanon, and Iran (Reddy and Sing, 1990; Atik *et al*., 2011). This result also may explain the diverse aggressiveness patterns of *D. rabiei* from chickpea producing areas of Turkey.

There are 7 regions in Turkey (Table 1) and the mean precipitation for the last 30 years varies among regions: the Central Anatolia had the least (406.5 mm) and the Black sea region had the highest (696.5 mm) precipitations according to Ministry of Agriculture and Forestry, Meteorology Directory of Turkey. Additionally, the mean temperatures change annually among regions but Aegean, Mediterranean and Southeastern Anatolia regions usually have higher temperatures in spring and summer than those of the other regions. The fluctuations among regions in terms of mean rainfall and temperatures may incite the occurrence and distribution of teleomorph stage of *D. rabiei* in Turkey (Kaiser and Kusmenoglu, 1997).

Disease severity index (%) and AUDPC (%) values of ILC 1929 (susceptible) and ICC 12004 (resistant) genotypes following inoculation with pathotypes I and IV isolates are given in Figure 3. First symptoms of *D. rabiei* pathotype I isolates appeared on 3 days after inoculation (dai) in both of the genotypes, but the symptoms were apparent in ILC 12004 on 9th day with pathotype I isolates. The percent of AUDPC values were statistically different for pathotypes I and IV isolates in both of the genotypes ($P \leq 0.05$). These results outlined high aggressiveness of pathotype IV isolates and this is the important step to show the existence of new aggressive isolate in Turkey. Türkkan and Dolar (2009) reported occurrence of pathotypes I, II and III from chickpea growing areas of Turkey and we conclude that *D. rabiei* undergo extensive recombination leading to the occurrence of more virulent genotypes that, in turn, overcome the resistance of chickpea genotypes.

The distribution of AUDPC values among isolates within each pathotype group is presented in Figure 3. The isolates assigned to pathotypes I, II, III, and IV exhibited statistically significant difference in aggressiveness on cv Sarı, ILC 1929, ILC 482, ILC 3279, and ICC 12004 ($P \leq 0.05$). Similarly, (Peever *et al*., 2012) tested F_1 progeny of AR20 (pathotype I)×AR628 (pathotype II) and concluded that virulence is quantitative in *D. rabiei*-chickpea pathosystem since resistance/susceptibility did not fit bimodal distribution. The data obtained through this study is in agreement with previous studies indicating complex nature of *D. rabiei* aggressiveness in chickpea.

D. rabiei-chickpea interaction create a good model system to study plant pathogen interactions since the pathogen infects wild annual and perennial *Cicer* spp like *C. isauricum*, *C. pinnatifidum* and *C. judaicum* (Can *et al*., 2007; Frenkel *et al*., 2009; Tekin *et al*., 2018). In natural ecosystems, *D. rabiei* never kills its host *C. pinnatifidum* and *C. isauricum* and, consequently, pathogenic diversity is low, whereas in agricultural ecosystems the pathogen exhibits more genotypic, phenotypic and virulence diversity (Pande *et al*., 2005;

Ozkilinc *et al*., 2010). Southeastern region of Turkey is placed within the Fertile Crescent, where first domestication of chickpea is assumed to have occurred during Neolithic revolution and that considering plant and pathogen coevolution, high genetic and pathogenic diversity of *D. rabiei* population is expected (Ozkilinc and Can, 2019). Diversity of aggressiveness patterns and rapid evolution of high virulent isolates within the *D. rabiei* population in Turkey disclose adaptation plasticity of this destructive and prolific pathogen. Therefore, continuous breeding efforts against *D. rabiei* in chickpea and systematic sampling must be done to define aggressiveness of newly forming isolates in order to diminish yield losses of chickpea in Turkey.

ACKNOWLEDGEMENTS

This study was fully supported by the Scientific and Technical Research Council of Turkey (TUBITAK) with project number 113O071.

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قدرت تهاجم و توزیع تیپ آمیزشی *Didymella rabiei* در مناطق کشت نخود در ترکیه

ن. نالکاسی، ا. توران، س. باسبوگا، ف. ن. کافادار، د. ایسلر سیهان، ا. آنای، د. مارت، ا. اوگوت، ک. سارپگایا، و س. کان

چکیده

نخود (*Cicer arietinum* L.) یکی از گونه های گیاهی سنتی ترکیه است که تقریبا در همه استانها کشت می شود و درآمد قابل توجهی به دست می دهد. ترکیه منابع مهمی برای تنوع هر دو گونه *Cicer* و عوامل بیماریزای گیاهی آن مانند سوختگی اسکوهایتا (*ascohyta blight*) (که عامل آن *Didymella rabiei (Kovachevski) von Arx* است دارد و در نتیجه هر ۴-۵ سال مقاومت کولتیوارهای کشت شده نخود شکسته می شود. به منظور بهنژادی برای ایجاد مقاومت/ تحمل در رقم های نخود بر علیه *D.rabiei* نیاز به تحلیل به روز از جزییات در مورد ویژگی های جمعیتی این عامل بیماری وجود دارد. هدف از اجرای این پژوهش تعیین و تعریف الگوی چیرگی (*aggressiveness patterns*)، تیپ آلوده کنندگی(*patotype*) و توزیع تیپ آمیزشی جمعیت *D. rabiei* درمناطق کشت نخود در ترکیه بود. جدایه های *D. rabiei* به ۵ گروه تهاجمی دسته بندی شد که در آن برای اولین بار وجود تیپ آلودکننده IV (که یک گروه تهاجی جدید است) در مزارع کشاورزان و موسسات تحقیقاتی تعریف شد و نشان از رقابت پیوسته بین گیاه و عامل بیماری داشت. جدایه ها در هر گروه از تیپ آلودکننده تفاوت های معنادار *($P \leq 0.05$)*مهمی نشان دادند. توزیع تیپ آمیزشی جدایه *971 D. rabiei* برای جدایه های *Mat1.1* و *Mat 1.2* برابر 1:1بود *($X^2=0,87$, $P=0,35$)*که حاکی از تولید مثل جنسی تصادفی بود. به طور کلی، داده های به دست آمده آشکار ساخت که طبیعت تهاجمی جمعیت *D. rabiei* در ترکیه نا پایدار است و این امر به نوبه خود شکست های مکرر مقاومت در ژنوتیپ های نخود های ثبت شده را که منجر به همه گیری و اپیدمی می شود توضیح می دهد.