The Changes in Susceptibility Status of the Old and of the Newly Registered Chickpea (*Cicer arietinum* L.) Cultivars with Respect to the Blight Disease Caused by the Pathotypes of *Ascochyta rabiei* (Pass.) Labr.

Promjene osjetljivosti starih i novoregistriranih kultivara slanutka (*Cicer arietinum* L.) na snijet prouzročenu patotipovima *Ascochyta rabiei* (Pass.) Labr.

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THE CHANGES IN SUSCEPTIBILITY STATUS OF THE OLD AND OF THE NEWLY REGISTERED CHICKPEA (Cicer arietinum L.) CULTIVARS WITH RESPECT TO THE BLIGHT DISEASE CAUSED BY THE PATHOTYPES OF Ascochyta rabiei (Pass.) Labr.

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SUMMARY

Blight disease, caused by Ascochyta rabiei (Pass.) Labrousse (teleomorph = Didymella rabiei), is one of the most important biotic stress factors affecting chickpea production worldwide. The high variation in disease severity among different chickpea cultivars and the decrease in the resistance of the cultivars over time make it necessary to test the cultivars regularly. The aim in this research was to determine and evaluate the changes in the susceptibility of chickpea cultivars, which were developed in different years and widely cultivated, against A. rabiei pathotypes in Turkey. A three-replication pot experiment was conducted in a randomized plot design in the climate chamber in 2021. Fifteen registered chickpea cultivars (including one susceptible and one susceptible control cultivar) and four pathotypes of chickpea blight disease agent A. rabiei were used in the study. While Pathotype-IV was determined as the most aggressive, it was followed by the Pathotype-III, Pathotype-II, and Pathotype-I, respectively. The Azkan cultivar, included as a Tolerant (T) control in the experiment, had the Mid-Susceptible/Susceptible (MS/S) values, which can be explained by the decrease in resistance over time. However, it is opined that the main reason for the better resistance values of Akçin-91, registered in 1991, and Gökçe, registered in 1997, was provoked by the genetic basis of these cultivars, when compared to the recently registered cultivars.

Keywords: Ascochyta rabiei, pathotype, disease severity, resistance level, chickpea
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cultivars in Turkey, which, in turn, explains a frequent

A. rabiei population in Turkey disclose the adaptation

knowledge about pathogen aggressiveness and identifi-
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frequent resistance overcome in registered chickpea
cultivars investigated in Turkey (Naççäç et al., 2021). In

registered chickpea cultivars investigated in Turkey (Naççäç et al., 2021). In the studies carried out in Turkey in recent years, it is reported that four pathotypes of A. rabiei exist (Mart et al., 2016). A diversity of aggressiveness patterns and rapid evolution of highly virulent isolates within the A. rabiei population in Turkey disclose the adaptation

Plasticity of this destructive and prolific fungal pathogen (Naççäç et al., 2021). Knowledge about pathogen aggressiveness and identification of sources of resistance to different pathotypes is very useful for the proper decisions in plant breeding programs (Farahani et al., 2019). The aim in this study was to determine the susceptibility of chickpea cultivars developed and widely grown in Turkey to pathotypes of A. rabiei and the changes that occurred over the years.

MATERIALS AND METHODS

Plant material

In the study, registered chickpea cultivars, widely cultivated in Central Anatolia, Southeastern Anatolia, Mediterranean, Black Sea, and the Aegean Regions and developed by institutions, were used as plant material. Tolerant Azkan (Aydin et al., 2016) and susceptible Izmir-92 (Mart et al., 2016) cultivars were used as control, total of 15 chickpea cultivars (Hisar, İşik-05, Yaşa-05, Çakır, Çağataş, Arda, Azkan, İzmir-92, Sarı-98, Akçın-91, Üzunlu-92, Gökçe, Aksu, Inci and Hasanbey) were tested.

Ascochyta rabiei isolates

Ascochyta rabiei isolates belonging to pathotype I (26 ESK 403/13), pathotype II (26 SGZ 04/14), pathotype III (18MRK 08/14), and pathotype IV (06 ENS 11/14), which were isolated from chickpea and known to be virulent, were used in susceptibility tests of chickpea cultivars to A. rabiei (Kabakçı & Özer, 2021). The isolates were provided by Prof. Dr. Canan CAN’s collection (Faculty Member of Gaziantep University, Faculty of Arts and Sciences, Department of Biology). All the pathotypes were obtained in 2021, and the trials were conducted in the same year.

Cultivation of Ascochyta rabiei and preparation of spore suspension

A. rabiei isolates belonging to pathotypes I, II, III, and IV were grown in plastic Petri dishes (10 cm in diameter) by transferring them to the Potato Dextrose Agar (PDA-Difco) medium in a refrigerated incubator for fourteen days at 22±1°C and 12 hours of light. The 10 ml of sterile distilled water was added on the developed A. rabiei cultures. The fungus was scraped from the surface of the agar medium with a sterile spatula. The resulting suspension was filtered through sterile filter paper and the mycelium and agar pieces were removed from the suspension. The concentration of the prepared spore suspension was adjusted to 2x10⁵ spores/ml by counting with a Thoma slide (Hemocytometer) (Singh et al., 1981) and was used in the inoculation of chickpea plants.

Pot trial

Cultivation of chickpea plants

The chickpea seed cultivars included in the experiment were kept in the 1% sodium hypochlorite (NaOCl) for three minutes before sowing, passed through distilled water three times, and its surface was sterilized and left to dry. Then the mixture containing soil, sand, and peat (1:1:1, v/v/v) was sterilized twice in autoclave at 121°C for 45 minutes, the soil mixture was filled into plastic pots (15 cm diameter), each of the eight chickpea seeds were sown in the pot and then, according to the plant emergence, the plants were provided to grow as five chickpea plants in each pot. The pot experiment was carried out in a randomized plot design with three replications in the growth chamber of Pamukkale University, Faculty of Applied Sciences, Department of Organic Agriculture Business Management (22±1°C, 12 hours light / 12 hours dark conditions). During the cultivation, planting-maintenance procedures were carried out according to the fertilization and water requirements.

Plant inoculation and disease evaluation

Spore suspensions (2x10⁵ spores/ml) were prepared from pathotypes I, II, III, and IV isolates, and 15-20 cm long chickpea plants grown in plastic pots were inoculated using a pressurized hand spray so that they were completely wet. Only sterile distilled water was sprayed on the plants used as control. Following the inoculation, the plants were covered with moist polyethylene bags to provide high humidity and kept in a humid environment for 48 hours before the bags were removed (Ilyas & Khan, 1986). In the meantime, water was sprayed on the plants with a pressurized hand spray four times a day at intervals of two hours to prevent them from being affected by the sudden drop in humidity. The pot experiment was carried out in the growth chamber (22±1°C, 95% humidity, 12 hours light / 12 hours dark conditions) in a randomized
plot design with three replications. After inoculation, weekly observations were made to follow the disease development in the plants and three weeks after the inoculation, the plants were evaluated according to the following formula:

\[ DSI = \frac{\sum (a \times 1) + (b \times 2) + (c \times 3) + (d \times 4) + (e \times 5) + (f \times 6) + (g \times 7) + (h \times 8) + (i \times 9)}{M} \]

where, a, b, c, d, e, f, g, h and i refer to the number of plants with degree 1, 2, 3, 4, 5, 6, 7, 8 and 9 respectively. M refers to the total number of plants.

Statistical analysis

Disease rates were calculated, and the obtained data were subjected to arcsin for transformation (Karman, 1971). The analysis of variance (ANOVA) was performed using JMP 13.2.1; 2017 (SAS Institute Inc., Cary, NC, USA) statistical software and the means were grouped by means of the LSD (0.01) test.

RESULTS AND DISCUSSION

The reactions of the registered chickpea cultivars to the four pathotypes of the Ascochyta blight agent A. rabiei were evaluated and found to be statistically significant at the p < 0.01 level (Table 1).

### Table 1. Analysis of Variance for the Disease Severity Index values and the mean of the sum of squares.

<table>
<thead>
<tr>
<th>Source / Izvor</th>
<th>DF</th>
<th>Pathotype-I / Patotip I</th>
<th>Pathotype-II / Patotip II</th>
<th>Pathotype-III / Patotip III</th>
<th>Pathotype-IV / Patotip IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication / Ponavljanje</td>
<td>2</td>
<td>Sum of Squares</td>
<td>Prob &gt; F</td>
<td>Sum of Squares</td>
<td>Prob &gt; F</td>
</tr>
<tr>
<td>Cultivar / Kultivar</td>
<td>14</td>
<td>0.01944</td>
<td>0.01944</td>
<td>0.26944</td>
<td>0.26944</td>
</tr>
<tr>
<td>Error / Greška</td>
<td>28</td>
<td>194.57778</td>
<td>&lt;.0001</td>
<td>121.1611</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total / Ukupno</td>
<td>44</td>
<td>199.03611</td>
<td>0.01944</td>
<td>125.2444</td>
<td>0.26944</td>
</tr>
</tbody>
</table>


In relation to the cultivars inoculated by Pathotype I, the DSI values of the cultivars were between 2.00-8.92. Four of the cultivars in the experiment were found to be R (Resistant) (İnci, Aksu Çağatay and Çakır), and four of them were found to be T (Tolerant) (Arda, Açıkgöz-91, Gökçe and Sari-98), and have a high level of resistance. The DSI values of cultivars against the Pathotype-II were determined to be between 2.25 and 8.25. Aksu R, İnci, Çakır, and Arda MR (Mid-Resistant) were detected, and Çağatay, Açıkgöz-91, and Gökçe were found to have the T resistance levels, respectively. The DSI values of Uzunlu-99 (8.25), Yaga-05 (7.67), and Hisar (7.58) cultivars were higher than the susceptible control İzmir-92 (7.58) cultivars. The lowest value was determined in Aksu (4.58), while the highest value was determined in Uzunlu-99 (8.33) against the Pathotype-III. It was determined that Aksu had the MR, whereas Çakır and Çağatay manifested the T resistance level. Uzunlu-99 (8.33) and Hasanbey (7.67) had higher values than all the other cultivars. The DSI values caused by the Pathotype-IV in the cultivars used in the experiment were strikingly high. Considering all the results of the pathotypes, in Pathotype-I, Pathotype-II, and Pathotype-III, the highest DSI values were detected in Uzunlu-99 cultivar, and in the Pathotype-IV in the İzmir-92 cultivars. The lowest DSI values in pathotype-I and pathotype-IV were found in the İnci cultivar, while the Pathotype-II and the Pathotype-III were determined in Aksu (Figure 1). The Azkan, which was used as the tolerant control in the experiment, produced the MS values against other pathotypes, except for the Pathotype-IV, and the S values against the Pathotype-IV (Table 2). This result can be explained by a decrease in the tolerance level over time.
### Table 2. The average values of disease severity for chickpea cultivars

| Cultivar / | Seed Type / | Pathotype-I / | Pathotype-II / | Pathotype-III / | Pathotype-IV / |
| Kultivar | Tip sjemena | Patotip I | Patotip II | Patotip III | Patotip IV |
|          |             | DSI | R. Level | DSI | R. Level | DSI | R. Level | DSI | R. Level |
| Uzunlu-99 Kabuli | 8,92a² | HS | 8,25a | HS | 8,33a | HS | 8,50a | HS | 8,71c-e |
| Hisar Kabuli | 8,42ab | HS | 7,58b | S | 7,33bc | S | 7,17c-e | S | 8,42a |
| Yaga-05 Kabuli | 7,92b | S | 7,67ab | S | 7,25b-d | S | 7,17c-e | S | 8,42a |
| Işık-05 Kabuli | 6,92c | MS | 6,83c | MS | 6,58ef | MS | 7,83b | S | 8,58a |
| İzmir-92 kabuli | 6,83c | MS | 7,58b | S | 7,50b | S | 8,58a | HS | 8,58a |
| Azkan² Kabuli | 6,33cd | MS | 6,00d | MS | 6,67d-f | MS | 7,00d-e | S | 8,58a |
| Hasanbey Kabuli | 6,00de | MS | 6,25cd | MS | 7,67b | S | 7,33g | MS | 8,33g |
| Sarı-98 Kabuli | 5,83de | T | 5,92d | MS | 7,25b-d | S | 7,50b-d | S | 8,50b-d |
| Gökçe Kabuli | 5,58ef | T | 5,75d | T | 6,17f | MS | 6,83e-f | MS | 8,58e-f |
| Akça-91 Kabuli | 5,50ef | T | 5,67d | T | 6,75-c-f | MS | 7,50df | S | 8,50df |
| Arda Kabuli | 5,17f | T | 4,67d | MR | 6,42ef | MS | 7,58bc | S | 8,50bc |
| Çakır Kabuli | 3,33g | R | 4,00f | MR | 5,25h | T | 7,50b-d | S | 8,50b-d |
| Çağatay Kabuli | 2,75gh | R | 4,92d | T | 5,75gh | T | 6,58gh | MS | 8,58gh |
| Aksu Kabuli | 2,42hi | R | 2,25g | R | 4,58i | MR | 5,75h | T | 8,50h |
| İnci Kabuli | 2,00hi | R | 3,50f | MR | 6,83c-f | MS | 4,08i | MR | 8,08i |
| Mean | 5,59 | 5,79 | 6,69 | 7,14 | ** | ** | ** | ** | ** |
| F<sub>cultivar</sub> | 7,1 | 6,4 | 5,3 | 4,8 | ** | ** | ** | ** | ** |
| CV (%) | 7,1 | 6,4 | 5,3 | 4,8 | ** | ** | ** | ** | ** |

¹ Susceptibility control, ²Tolerant-control, ³Numbers with the same letters are in the same group, ** p < 0.01, DSI: Disease severity index, HS: Highly susceptible, S: Susceptible, MS: Mid-Susceptible, T: Tolerant, MR: Mid-Resistant, R: Resistant, R. Level: Resistance Level, CV: Coefficient of Variation / Kontrola osjetljivosti, ** p<0,01, DSI: indeks intenziteta bolesti, HS: visoko osjetljiv, S: osjetljiv, MS: srednje osjetljiv, T: tolerantno, MR: srednje otporno, R: otporno, R. Level: razina otpornosti, CV: koeficijent varijacije

**Figure 1. Most resistant and most susceptible cultivars for pathotypes in the pot experiment**

*Slika 1. Najotporniji i kölvatur najosjetljiviji patotipovi pokusa u posudama*
The differences in the AB (Ascochyta blight) severity’s variation were greater among the kabuli cultivars than among the desi cultivars. A positive correlation in the AB severity on leaves, stems, and pods was observed, suggesting a lack of organ-specific reaction (Chandirasekaran et al., 2009). The results of these studies are in agreement with those of Benzohra et al. (2018), who reported that the Pathotype-I is the least virulent, Pathotype-II is moderately virulent, the Pathotype-III is more virulent, and the Pathotype-IV is highly virulent. The cultivars used in the experiment were bred and registered by different organizations for different geographical regions. However, the resistance levels of the cultivars used in the regions against the pathotypes differed. The results in the present study showed that the cultivars which were used in the experiment and registered in the early period and the cultivars which were registered in the recent period gave different responses to the pathotypes. Although Akçin-91, registered in 1991, and Aksu, registered in 2009, had the T and R resistance levels in the Pathotype-I and Pathotype-II, respectively, Azkan, which was registered in 2009, and Hisar, which was registered in 2008, manifested an almost S-typed against all pathotypes. The results presented in this study are inconsistent with Chongo & Gossen (2001), who reported that the resistance decreases with the plant age in partially resistant chickpea cultivars, and this resistance alone cannot provide an adequate disease control. According to the results of our study, the aggression status of the pathotypes changes, and the new pathotypes are more aggressive and are consistent with Vail & Banniza (2008), who reported that their results confirm that disease severity represents a continuum indicative of the quantitative inheritance of aggressiveness in the pathogen. The quantitative chickpea resistance to a necrotrophic fungal pathogen, Ascochyta rabiei, is conferred in a pathotype-dependent manner (Cho et al., 2004). Although the cultivars manifested an MR resistance against the Pathotype-III and Pathotype-IV, they were similar to the results of Kabağı & Öz (2021), who reported that none of the cultivars were resistant to the Pathotype-III. Our results are not similar to those of Bişer et al. (2017a, b) and Mart et al. (2017), who found the Azkan, Çağatay, and Hasanbey cultivars to be resistant in their studies. However, our findings partially agreed with the results reported that the Akçin-91 cultivar manifested the T value, Gökeç cultivar manifested the R value (Düzdemir et al., 2007), and the Arda cultivar manifested the MR value (Aydin et al., 2016), while the Gökeç and Arda cultivar manifested the R/T value (Bişer et al. 2017b). The reaction degrees of the chickpea cultivars registered in Turkey regarding the chickpea blight are quite different. As can be seen from the results, the variation between the resistance levels of cultivars against the A. rabiei pathotypes is quite wide. As a result of the absence of a gene that would provide resistance to all physiological races in plants and the interactions between the cultivars exhibiting resistance and the fungus, there is a decrease in the resistance levels of the plants over time (Aydin et al., 2016).

CONCLUSION

The resistance status of the cultivars against the Pathotype-IV was not sufficient in general; however, the resistance levels of the cultivars against the Pathotype-I and the Pathotype-II were high. The cultivars lose their resistance levels over time and become more sensitive to the emerging new pathotypes. These results can be explained by the fact that the new pathotypes (Pathotype-III and Pathotype-IV) are more virulent than other pathotypes. Although it is inevitable that a decrease in resistance will occur over the years, it is possible to say that the genetic basis of the genetic cultivar has an effect on the amount and duration of this decrease. For this reason, continuous testing of promising chickpea genotypes against current pathotypes of A. rabiei is of great importance in chickpea breeding studies.

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PROMJENE OSJETLJIVOSTI STARIH I NOVOREGISTRIRANIH KULTIVARA SLANUTKA (Cicer arietinum L.) NA SNIJET PROUZROČENU PATOTIPOVIMA Ascochyta rabiei (Pass.) Labr.

SAŽETAK


Ključne riječi: Ascochyta rabiei, patotip, intenzitet zaraze, razina otpornosti, slanutak

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