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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Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

https://doi.org/10.18047/poljo.29.1.3

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ISSN 1330-7142
UDK=635.657:632
https://doi.org/10.18047/poljo.28.1.3

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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Original scientific paper
Izvorni znanstveni članak

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

INTRODUCTION

Chickpeas, which are the edible legumes, are among the first plants to be cultivated. Chickpea (*Cicer arietinum* L.), whose wild relatives naturally grow in the Southeastern Anatolia Region (Özkılınç & Can, 2019), is a traditional plant species that is grown in almost every province in Turkey and provides significant economic income (Nalçacı et al., 2021). Recently, the nutritional value of chickpea in terms of nutrition and health benefits has been frequently emphasized by nutritionists in the health and food area in many countries around the world (Merga & Haji, 2019). Being one of the main food sources in human nutrition and used in rotation to enrich the soil structure (Can et al., 2016), chickpea, is a legume plant used in the roasted chickpea industry as well as having a high usage amount as dry legumes in Turkey (Çalışkan & Gemici, 2011).

The most important biotic stress factor affecting chickpea yield worldwide is blight disease caused by *Ascochyta rabiei* (Baite et al., 2020). The pathotypes of *Ascochyta rabiei* which infects all above-ground plant organs, including leaves, petioles, young branches and capsules, show a high pathogenic variation (Kabakçı

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& Özer, 2021; Salotti & Rossi, 2021). However, the disease can also appear at a very early crop growth stage under favorable environmental conditions. The initial symptoms appear as water-soaked lesions on the upper leaves. Later, these lesions become dark brown spots and spread rapidly on aerial parts of the plant—leaves, petioles, flowers, pods, branches, and stem. The spots on leaves and pods are circular, while on the stem and branches they are elongated. The apical twigs, branches, and stem often show girdling, and the plant parts above the girdled portion are killed or break off even before drying. On the seed coat, lesions formed often lead to the seed infection through the testa and infected cotyledons. Infected seed may be discolored and possess deep, round, or irregular cankers, sometimes bearing conidia visible with the naked eye. Infection during the pod formation stage results in the shriveled and infected seed (Pande et al., 2010). Overall, the data of several studies conducted in Turkey reveal the unstable nature of the aggressiveness of *A. rabiei* population in Turkey, which, in turn, explains a frequent resistance overcome in registered chickpea cultivars leading to epidemics (Nalçacı 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 (Nalçacı 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 (Aydın et al., 2016) and susceptible İzmir-92 (Mart et al., 2016) cultivars were used as control, total of 15 chickpea cultivars (Hisar, Işık-05, Yaşa-05, Çakır, Çağatay, Arda, Azkan, İzmir-92, Sarı-98, Akçin-91, Uzunlu-92, Gökçe, Aksu, İnci 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±1ºC 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 $2x10^5$ 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±1ºC, 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 (İlyas & 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±1ºC, 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 1-9 scale of Singh et al. (1989) ([**1**- No lesion: Immune ((1.0-1.2 Disease Severity Index (DSI))], [**2**- Small spots only on leaves and pods (5%): Highly resistant (1.3-1.9 DSI)], [**3**- There are lesions on leaves and stems (less than 10% of plants have lesions, lesions do not cover the stem): Resistant (2.0-3.4 DSI)], [**4**- Lesions are seen in plants up to 15%: Mid-resistant (3.5-4.8 DSI)], [**5**- Lesions occur in 25% of plants, lesions encircle the stem in less than 10% of plants, but little damages occurs: Tolerant (4.9-5.8 DSI)], [**6**- 50% of plants are infected, 25% have branch breakage: Mid-susceptible (5.9-6.9 DSI)], [**7**- 75% of plants are infected, 50% have branch breakage: Susceptible (7.0-7.9 DSI)], [**8**- 100% of plants are infected, 75% have branch breakage: Highly susceptible (8.0-8.9 DSI)], [9- Dead plant: Extremely susceptible (9.0 DSI)]).

Disease calculation

A Disease Severity Index (DSI) was calculated according to the following formula:

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

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.

Tablica 1. Analiza varijance za vrijednosti indeksa težine bolesti i srednja vrijednost zbroja kvadrata

Source / *Izvor*	DF	**Pathotype-I** / *Patotip I*		**Pathotype-II** / *Patotip II*		**Pathotype-III** / *Patotip III*		**Pathotype-IV** / *Patotip IV*	
		Sum of Squares	Prob> F	Sum of Squares	Prob> F	Sum of Squares	Prob> F	Sum of Squares	Prob> F
Replication / *Ponavljanje*	2	0,01944	0,9406	0,26944	0,3845	0,252778	0,3764	0,544444	0,1201
Cultivar / *Kultivar*	14	194,57778	<,0001	121,1611	<,0001	39,39444	<,0001	56,93611	<,0001
Error / *Greška*	28	4,43889		3,81389		3,497222		3,330556	
Total / *Ukupno*	44	199,03611		125,2444		43,14444		60,81111	

DF: Degree of Freedom, Pro: Probability, F: Frequency / *DF: Stupnjevi slobode, Pro: Vjerojatnost, F: Frekvencija*

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, Akçin-91, Gökçe and Sarı-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, Akçin-91, and Gökçe were found to have the T resistance levels, respectively. The DSI values of Uzunlu-99 (8.25), Yaşa-05 (7.67), and Hisar (7.58) cultivars were higher than the susceptible control Izmir-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

Tablica 2. Prosječne vrijednosti intenzitet bolesti kultivara slanutka

Cultivar / *Kultivar*	**Seed Type** / *Tip sjemena*	**Pathotype-I** / *Patotip I*		**Pathotype-II** / *Patotip II*		**Pathotype-III** / *Patotip III*		**Pathotype-IV** / *Patotip IV*	
		DSI	R. Level	DSI	R. Level	DSI	R. Level	DSI	R. Level
Uzunlu-99	Kabuli	8,92a[3]	HS	8,25a	HS	8,33a	HS	8,50a	HS
Hisar	Kabuli	8,42ab	HS	7,58b	S	7,33bc	S	7,17c-e	S
Yaşa-05	Kabuli	7,92b	S	7,67ab	S	7,25b-d	S	8,42a	HS
Işık-05	Kabuli	6,92c	MS	6,83c	MS	6,58ef	MS	7,83b	S
İzmir-92[1]	Kabuli	6,83c	MS	7,58b	S	7,50b	S	8,58a	HS
Azkan[2]	Kabuli	6,33cd	MS	6,00d	MS	6,67d-f	MS	7,00d-e	S
Hasanbey	Kabuli	6,00de	MS	6,25cd	MS	7,67b	S	6,33g	MS
Sarı-98	Kabuli	5,83de	T	5,92d	MS	7,25b-d	S	7,50b-d	S
Gökçe	Kabuli	5,58ef	T	5,75d	T	6,17fg	MS	6,83e-f	MS
Akçin-91	Kabuli	5,50ef	T	5,67d	T	6,75c-f	MS	7,50b-d	S
Arda	Kabuli	5,17f	T	4,67d	MR	6,42ef	MS	7,58bc	S
Çakır	Kabuli	3,33g	R	4,00f	MR	5,25h	T	7,50b-d	S
Çağatay	Kabuli	2,75gh	R	4,92d	T	5,75gh	T	6,58fg	MS
Aksu	Kabuli	2,42hı	R	2,25g	R	4,58ı	MR	5,75h	T
İnci	Kabuli	2,00ı	R	3,50f	MR	6,83c-f	MS	4,08ı	MR
Mean		5,59		5,79		6,69		7,14	
$F_{cultivar}$		**		**		**		**	
CV (%)		7,1		6,4		5,3		4,8	

[1] Susceptibility control, [2]Tolerant-control, [3]Numbers 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 / *[1]Kontrola osjetljivosti, [2]kontrola tolerancije, [3]brojevi s istim slovima su u istoj skupini, ** 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*

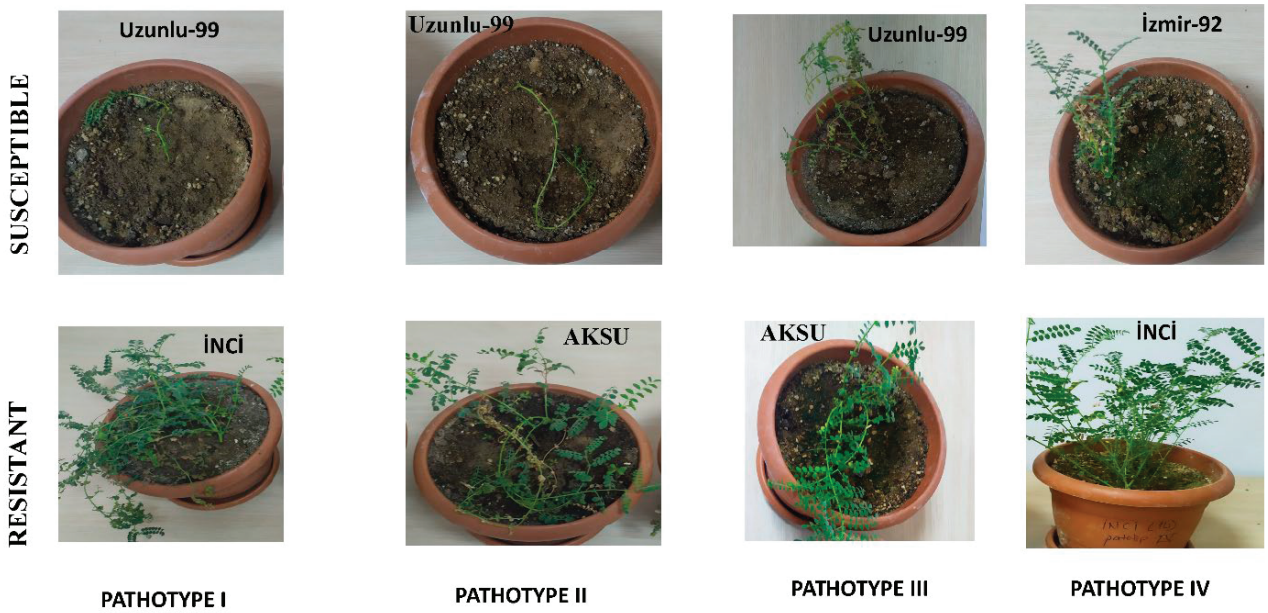

Figure 1. Most resistant and most susceptible cultivars for pathotypes in the pot experiment

Slika 1. Najotporniji i kultivari 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 study 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 Kabakçı & Özer (2021), who reported that none of the cultivars were resistant to the Pathotype-III. Our results are not similar to those of Biçer 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, Gökçe cultivar manifested the R value (Düzdemir et al., 2007), and the Arda cultivar manifested the MR value (Aydin et al. 2016), while the Gökçe and Arda cultivar manifested the R/T value (Biçer 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 (Aydın 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.

ACKNOWLEDGMENTS

This study was supported by the Scientific Research Coordination Unit of Pamukkale University under the project number 2020BSP014 (Determination of Reactions of Some Chickpea (*Cicer arietinum* L.) Cultivars against *Ascochyta rabiei* (Pass.) Labr. Causal Agent of Ascochyta Blight Disease. The author expresses his gratitude to Prof. Dr. Canan CAN (Faculty Member of Gaziantep University, Faculty of Arts and Sciences, Department of Biology) for kindly providing the fungal isolate and to the Research Institutes for kindly providing the chickpea cultivars. Also, the author expresses his gratitude to Prof. Dr. Oktay Erdoğan for his kind assistance in disease testing.

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

SAŽETAK

Snijet, koju uzrokuje Ascochyta rabiei (Pass.) Labrousse (teleomorf Didymella rabiei), je jedan od najvažnijih stresnih biotičkih čimbenika koji zahvaćaju proizvodnju slanutka širom svijeta. Uslijed visoke varijacije u intenzitetu bolesti i opadanja otpornosti kultivara, potrebna su kontinuirana istraživanja. Svrha je ovoga istraživanja bila odrediti i ocijeniti osjetiljvost kultivara slanutka uzgajanih u Turskoj na patotipove A. rabiei tijekom različitih godina. Tijekom 2021. proveden je pokus u posudama s trima ponavljanima u randomiziranom plot dizajnu u klimatskoj komori. U istraživanju je korišteno petnaest registriranih kultivara slanutka (uključujući jedan osjetljiv kultivar i kontrolni osjetljiv kultivar) te četiri patotipa A. rabiei. Iako je patotip IV utvrđen kao najagresivniji, slijedili su ga patotip III, patotip II te patotip I. Kultivar Azkan, uključen u pokus kao tolerantni kultivar (T), ocjenjen je kao srednje osjetljiv/osjetljiv (MS/S), što je moguće objasniti postupnim opadanjem otpornosti protekom vremena. Međutim, smatra se kako je glavni uzrok veće otpornosti Akçin-91, registriranoga 1991., te Gökçe, registriranoga 1997., genska osnova tih kultivara u odnosu na nedavno registrirane kultivare.

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

(Received on August 10, 2022; accepted on February 6, 2023 – *Primljeno 10. kolovoza 2022.; prihvaćeno 6. veljače 2023.)*