UDC 575. 630
https://doi.org/10.2298/GENSR2202829V
Original scientific article

ASSESSMENT OF PEPPER GENETIC RESOURCES FOR *VERTICILLIUM* WILT RESISTANCE

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Vasileva K. and V. Todorova (2022). *Assessment of pepper genetic resources for verticillium wilt resistance*. - Genetika, Vol 54, No.2, 829-840.

Study was carried out for determining the reaction of 83 peppers varieties, breeding lines, and local forms /landraces/ to fungal pathogen *Verticillium dahliae* Kleb. as they are the part of the Balkan *Capsicum* core collection which was deeply phenotyped in the recent years. The development of the disease was reported twice and high degree of resistance was reported for 41 accessions. The highest percentage of highly resistant group of genotypes was 72%, followed by resistant group with 23%. The infestation in the groups of medium sensitive, sensitive and highly sensitive was respectively 1%, 3%, and 1%. The highly sensitive (0 - 19%), sensitive (20 - 39%) and medium sensitive (40 - 59%) are consisted by the *var. blocky*, *var. ratundum* and *var. kapia* accessions. The group of resistant (60 - 79%) was dominated by *var. ratundum* and *var. blocky*. Materials from *var. kapia* prevail of highly resistant (80 - 100%). The group of pungent peppers accessions was more resistant to *Verticillium* infestation. On the base of current and previous results may concluded that four accessions were identified as resistant to TMV and non-infested by *Verticillium* while two ones possessed low infestation by green aphids, trips and cotton bollworm and non-attacked by *Verticillium*. Other seven genotypes combined lack of infestation by *Verticillium* wilt with high levels of three and more agronomic and biochemical traits.

Key words: Capsicum, disease, index of attack, soil-borne, *V. dahliae* Kleb, varietal type

INTRODUCTION

Verticillium wilt caused by *Verticillium dahliae* Kleb is an important soilborne disease of pepper (*Capsicum* species) worldwide (GURUNG *et al*., 2015). The disease has been reported in pepper in Europe, the Mediterranean, Canada and the United States (GOLDBERG, 2003).

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Demographic trends and changes to climate require more efficient use of plant genetic resources in breeding programs. Increases in pepper consumption have been observed in the past 20 years, and for maintaining this trend, the development of new resistant and high yielding varieties is demanded. The range of pathogens acting peppers is very broad and includes fungi, viruses, bacteria, and insects. In this context, the large number of accessions of domesticated and wild species stored in the world seed banks represents a valuable resource for breeding in order to transfer traits related to resistance mechanisms to various biotic stresses (PARISI *et al*., 2020). In the last decades, most of the pepper breeding programs have been addressed to the development of cultivars or hybrids against a wide range of pathogens and pests. Despite the reports made, the exploitation of *Capsicum* germplasm (pre-breeding materials, landraces, wild relatives and closed related species) and its use in breeding programs for biotic stress resistance still represent challenging tasks (SARATH *et al*., 2011). Unfortunately, although there are some tolerant pepper cultivars (i.e., Padrón and Yolo Wonder), resistance genes have not been identified for the *C. annuum*-*V. dahliae* interaction (POMAR *et al*., 2004).

Biofungicides based on plant oils and plant grow promoting rhizobacteria (PGPR) agents have some advantages compared to chemical fungicides, especially considering their harmful effect on the environment (GUENOUN *et al*., 2018; LUCOVICL *et al*., 2018). Application of activated yeasts for controlling *Verticillium* wilt under greenhouse conditions affected significantly the disease severity (EL-FAWAY and AHMED, 2015). Rhizobacterial strains as important biological control and PGPR agents capable of reducing or eliminating symptoms of *Verticillium* wilt in pepper plants (GUENOUN *et al*., 2018).

The control of these fungus is difficult because it can survive both in open field and under controlled conditions for several years (GOICOECHEA, 2006). Most commercial pepper cultivars lack resistance to *Verticillium* wilt (GURUNG *et al*., 2015). In addition, all cultivated species of *Capsicum* have 2n = 24 chromosomes and cross ability among the species is limited and breeders have only been able to make little differences in disease resistance. However, the studies of JUNG *et al*. (2003, 2005) constitute an important advance in the knowledge on genes involved in the response of pepper plants when subjected to pathogens, abiotic and biotic stress factors. JIANG (2015) reported that the inheritance of resistance to *V. dahliae* Kleb. is not well defined due to the limitation of resistant accessions and used molecular markers which would provide promising tools to investigate *Verticillium* wilt resistance, that would improve the efficacy of the breeding program in the chili pepper.

In recent years, one of the focuses of scientific research has been on a more in-depth and comprehensive study of the local pepper resources from Balkan region - Albania, Bulgaria, Greece, Macedonia, Serbia and Romania (NANKAR *et al*., 2020a). The whole or part of the collection was evaluated for phenotypic, agronomic, morphological (TODOROVA *et al*., 2019; NANKAR *et al*., 2020 a, b) and biochemical traits (DENEV *et al*., 2019), mineral composition (TRINGOVSKA *et al*., 2019), as well as for response to viruses (NANKAR *et al*., 2020 b), fungal diseases (VASILEVA *et al*., 2019) and for infestation by some important pest (YANKOVA *et al*., 2020, 2021).

The aim of the study was to establish the response of the next other non-tested pepper resources to an attack by *V. dahliae* Kleb and to summarize the results for the accessions from the completely Balkan *Capsicum* core collection.

MATERIAL AND METHODS

The Maritsa Vegetable Crops Research Institute (MVCRI), Plovdiv maintains a *Capsicum* core collection consisting of 180 accessions which originate from six Balkan countries – Albania, Bulgaria, Greece, North Macedonia, Serbia and Romania and was deeply investigated in various direction. In a previous study (VASILEVA *et al*., 2019), 97 accessions were investigated for resistance to *Verticillium* wilt. The current research continues the study on the rest of the 83 pepper resources and summarizes the results for the complete *Capsicum* core collection.

The same isolates and diagnostic scale were used to test the rest of the plant accessions in the collection, with an index of attack (%) and resistance (%), all described in detail in the first part of the study (VASILEVA *et al*., 2019). Isolates, which were conducted studies, are part of phytopathologycal collection of MVCRI. The inoculums were prepared from a one-month pure culture of the six isolates grown on a standard nutrient medium of Chapek and potato dextrose agar (PDA). The infection was carried out by root-dip technique, prior to planting. The roots of the plants were suspended in spores and mycelium suspension and then planted in infected soil for each of the 83 tested genotypes. Twenty plants from each accession were inoculated and grown in the field in a randomized block diagram. Periodical observations were carried out for the manifestation of disease symptoms (20.08.2018 и 14.09. 2018).

Using Multiple Range Test (DUNCAN, 1955) the results were statistically processed. Software products used for the investigation are "MS Excel Analysis Tool Pak Add-Ins" (https://support.office.com) and "R-3.1.3" in combination with "RStudio-1.1.447" and installed package "agricolae 1.2-2" (MENDIBURU, 2015).

RESULTS AND DISCUSSION

Significant differences between the tested materials in the attack index were established in the two observations. First rated of the disease was observed a lower attack index. Among the evaluated accessions, 41 did not show any symptoms, 45 were medium infected, 7 had an increase in the rate of infection and 3 had a strong symptoms manifestation (Table 1).

Table 1. Response of pepper accessions to attack by Verticillium wilt (Verticillium dahliae Kleb).

CAPS-	Variety/Accession	Varietal type	Index of attack (%)				Resistance (%)	
			20-08-18		14-09-18			
96	Splendid	*ratundum*	0	c	0	c	100	a
151 A	94601049	*ratundum*	0	c	0	c	100	a
154	Ajvarka_B1E0318	*conicum*	0	c	0	c	100	a
165	Babura_B1E0413	*ratundum*	0	c	0	c	100	a
160	Baburi_B1E0372	*blocky*	0	c	0	c	100	a
151B	94601049	*kapia*	0	c	0	c	100	a
149	Krusnica	*corniforme*	0	c	0	c	100	a
79A	Silvia A	*conicum*	0	c	0	c	100	a
97A	Zelen Ratund A	*ratundum*	0	c	0	c	100	a
156	White_B1E0321	*conicum*	0	c	0	c	100	a
119	Vezen_B1E0242	*conicum*	0	c	0	c	100	a
145	Gorogled	*kapia*	0	c	0	c	100	a

CAPS-	Variety/Accession	Varietal type	Index of attack (%)				Resistance (%)	
			20-08-18		14-09-18			
143	Kalinkov_B1E0532	*blocky*	0	c	0	c	100	a
134	Kalinkov _B2E0048	*blocky*	0	c	0	c	100	a
135	Kalinkov _B2E0049	*blocky*	0	c	0	c	100	a
135A	Kalinkov _B2E0049 A	*ratundum*	0	c	0	c	100	a
163	Kamba_B1E0405	*ratundum*	0	c	0	c	100	a
169	Kambi_B0E0334	*ratundum*	0	c	0	c	100	a
152	Kambi _B1E0301	*shipka*	0	c	0	c	100	a
102	Kapia_B1E0041	*kapia*	0	c	0	c	100	a
122	Kapia _B1E0250	*kapia*	0	c	0	c	100	a
157	Kapia _B1E0327	*kapia*	0	c	0	c	100	a
140	Kapia _B1E0501	*kapia*	0	c	0	c	100	a
129	Kapia _B2E0034	*kapia*	0	c	0	c	100	a
133A	Konusovidni A	*kapia*	0	c	0	c	100	a
130	Ribki_B2E0035	*like fish*	0	c	0	c	100	a
113	Rogovidni_B1E0042	*shipka*	0	c	0	c	100	a
144	Rogovidni _B1E0533	*corniforme*	0	c	0	c	100	a
168	Rabesta kapia	*kapia*	0	c	0	c	100	a
173	Selska kapia_B4E0023	*kapia*	0	c	0	c	100	a
118	Sivria_B1E0241	*corniforme*	0	c	0	c	100	a
110	Topcheta_B0E0324	*cerasiforme*	0	c	0	c	100	a
126	Topcheta_B1E0271	*cerasiforme*	0	c	0	c	100	a
115	Chereshki_B2E0069	*cerasiforme*	0	c	0	c	100	a
174	Cherna shipka	*shipka*	0	c	0	c	100	a
109	Chorbadzhiyski _B0E0321	*shipka*	0	c	0	c	100	a
117A	Chorbadzhiyski _B1E0240 A	*conicum*	0	c	0	c	100	a
132	Chorbadzhiyski _B2E0041	*corniforme*	0	c	0	c	100	a
124	Shipka_B1E0257	*shipka*	0	c	0	c	100	a
137	Shipka_B1E0491	*shipka*	0	c	0	c	100	a
139	Shipka_B1E0494	*shipka*	0	c	0	c	100	a
159	Piza	*kapia*	0	c	3.65	bc	95	a
71A	Dzinka A	*corniforme*	2.3	bc	5.5	bc	90	a
150	Rotund zuti	*kapia*	7.8	bc	11.01	bc	90	a
127	Kambi_B1E0272	*ratundum*	0	c	3.25	bc	90	a
141	Kapia_B1E0504	*kapia*	0	c	5.3	bc	90	a
158	Kozi rog_B1E0330	*conicum*	4.36	bc	8.56	bc	90	a
121	Rogovidni_B1E0248	*corniforme*	0	c	8.15	bc	90	a
123	Sivria_B1E0257	*conicum*	0	c	5.14	bc	90	a
110A	Topcheta_B0E0324 A	*shipka*	11	bc	5.85	bc	90	a
166	Chorbadzhiyski _B1E0301	*corniforme*	4.51	bc	8.51	bc	88	a
149A	Krusnica A	*corniforme*	8.41	bc	14.2	bc	85	a
128	Kambi_B1E0280	*cerasiforme*	5.21	bc	11.25	bc	85	a

CAPS-	Variety/Accession	Varietal type	Index of attack (%)				Resistance (%)	
			20-08-18		14-09-18			
116	Kapia_B2E0070	*shipka*	9.36	bc	14.23	bc	85	a
151	94601049	*kapia*	11.8	bc	27.1	ab	80	a
120	Vezen_B1E0245	*corniforme*	9.15	bc	15.14	bc	80	a
143A	Kalinkov_B1E0532 A	*kapia*	5.64	bc	17.3	ab	80	a
101	Kambi_B1E0021+A4:A77	*ratundum*	10	bc	11.56	bc	80	a
138	Kapia_B1E0492	*kapia*	4.21	bc	11.4	bc	80	a
112	Ribki_B1E0013	*like fish*	0	c	7.51	bc	80	a
121A	Rogovidni_B1E0248 A	*blocky*	10.2	bc	15.63	ab	79	ab
142	Kalinkov_B1E0525	*kapia*	10.4	bc	22.6	ab	78	ab
153	Kapia_B1E0061	*corniforme*	6.52	bc	12.7	bc	78	ab
117	Chorbadzhiyski _B1E0240	*conicum*	17.56	ab	22.12	ab	74	ab
131	Kambi_B2E0040	*ratundum*	12.4	bc	18.4	ab	72	ab
161	Kapia_B1E0373	*blocky*	10.24	bc	22.6	ab	71	ab
146	Pt 443	*blocky*	14.4	bc	28.7	ab	70	ab
147	Pt 451	*blocky*	9.64	bc	18.4	ab	70	ab
125	Buketen	*shipka*	11.54	bc	28.9	ab	70	ab
107	Kambi_B0E0312	*ratundum*	10	bc	26.52	ab	70	ab
105	Kambi_B1E0062	*ratundum*	27.1	ab	28.56	ab	70	ab
171	Selska kapia_BE04019	*kapia*	6.7	bc	17.64	ab	70	ab
111	Shipka_B0E0326	*shipka*	8.62	bc	15.62	ab	70	ab
103	Kambi_B1E0059	*ratundum*	29.3	ab	30.64	ab	67	ab
108	Edri dalgi	*conicum*	28.56	ab	33.21	ab	65	ab
155	Kozi rog_B1E0320	*corniforme*	7.81	bc	18.5	ab	62	ab
136	Kambi_B2E0050	*blocky*	28.7	ab	36.2	ab	60	ab
133	Konusovidni	*blocky*	9.87	bc	31.2	ab	60	ab
114	Rozichki	*ratundum*	29.25	ab	33.2	ab	60	ab
164	Kamba_B1E0410	*ratundum*	16.2	ab	27.2	ab	55	bc
162	B1E0378	*kapia*	49.87	a	61.58	a	20	c
104	Kambi_B1E0061	*ratundum*	39.65	a	51.23	a	20	c
148	Pt 452	*blocky*	55.41	a	59.6	a	0	c

*Duncan test - different letters in the same column indicate significant difference

During the second observation, an increase in the degree of infection was recorded because the conditions during this period favor the development and accumulation of pathogen in the soil and the susceptible plants were increased. 41 of the studied genotypes were proven not infected during this period, and three had a strong manifestation of the disease. Of the tested pepper accessions with proven lack of disease were: CAPS-96, CAPS-151A, CAPS-154, CAPS-165, CAPS-160, CAPS-151B, CAPS-149, CAPS-79A, CAPS-97A, CAPS-156, CAPS-119, CAPS-145, CAPS-143, CAPS-134, CAPS-135, CAPS-135A, CAPS-163, CAPS-169, CAPS-152, CAPS-102, CAPS-122, CAPS-157, CAPS-140, CAPS-129, CAPS-133A, CAPS-130,

CAPS-113, CAPS-144, CAPS-168, CAPS-173, CAPS-118, CAPS-110, CAPS-126, CAPS-115, CAPS-174, CAPS-109, CAPS-117A, CAPS-132, CAPS-124, CAPS-137, CAPS-139.

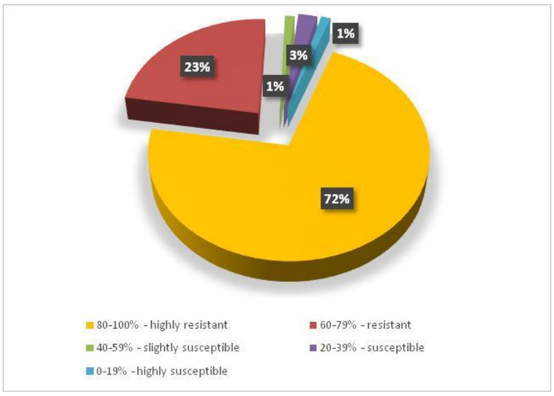

Figure 1. Grouping of the tested pepper materials by level of resistance (%).

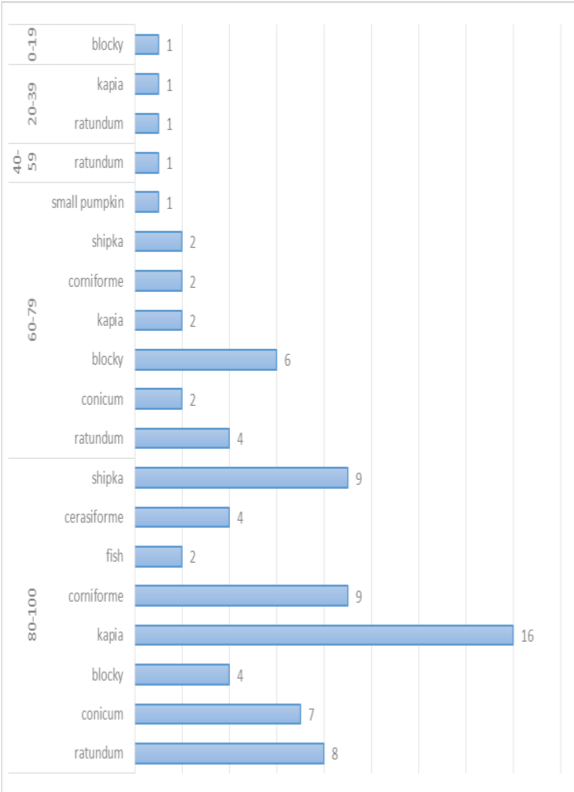

Figure 2. Resistance of the accessions to varietal type (%).

Based on the obtained results, were established that the group of highly resistant accessions was the biggest (72%), and followed by resistant 23%. The infestation in the groups' medium sensitive, sensitive and highly sensitive was respectively 1%, 3%, and 1%. (Figure 1). It was confirmed that in the groups of highly sensitive (0 - 19%), sensitive (20 - 39%) and slightly sensitive (40 - 59%) predominated resources from *var. blocky*, *var. ratundum* and *var. kapia*. The group of resistant (60 - 79%) was dominated by the *var. ratundum* and *var. blocky*. In the case of highly resistant (80 - 100%) *var. kapia* prevails (Figure 2).

Table 2. Evaluation of all 180 pepper resources from Balkan collection to Verticillium infestation according to varietal type and pungency in the fruits

Varietal Type	Count of Fruit taste	Average of Resistance %
blocky	17	47.65
pungency	1	100.00
sweet	16	44.38
cerasiforme	6	89.17
pungency	6	89.17
conicum	25	40.80
pungency	6	92.33
sweet	19	24.53
conicum for powder	1	100.00
sweet	1	100.00
cordatum	2	15.00
sweet	2	15.00
corniforme	18	74.00
pungency	15	74.27
sweet	3	72.67
for powder	3	96.67
sweet	3	96.67
kapia	53	43.93
pungency	1	80.00
sweet	52	43.23
ratundum	24	53.27
pungency	4	70.00
sweet	21	50.40
ratundum-cerasiforme	1	80.00
pungency	1	80.00
shipka	21	61.19
pungency	17	75.59
sweet	4	0.00
x fasciculatum D conicum for powder	1	100.00
sweet	1	100.00
x fasciculatum M conicum for powder	1	100.00
sweet	1	100.00
like fish	6	61.67
pungency	4	60.00
sweet	2	65.00
Total	180	53.99
pungency		46.00
sweet		54.00

*The evaluation of 97 accessions was reported by Vasileva et al., 2019.

Data in Table 2 showed the average resistance for each individual varietal type and summarizing the results for the whole collection for all genotypes (180). For greater precision, the varietal types were divided into two groups - sweet (54%) and pungent (46%), which gave additional information of the impact of the combination cultivar-pungency to resistant. The varietal types, where pungent pepper materials predominated, were more resistant to *Verticillium* infestation. The only exception was in *var. like fish* where sweet accessions were more resistant to disease.

Similarly, GONZÁLEZ-SALÁN and BOSLAND (1992) tested 125 *Capsicum* accessions and did not found out accessions with desirable levels of resistance to *Verticillium* wilt although three accessions showed partial resistance.

One of the main tasks of the pepper breeding work at the Maritsa VCRI, Plovdiv is permanent screening of available germplasm to looking for the sources of resistance to this fungal pathogen as some resistant genotypes are identified and are included in breeding program (MASHEVA *et al*., 2001; VASILEVA *et al*., 2019). The development of resistant lines and cultivars is the most successful and harmless way to deal with important pathogens and pests. The cultivars Hebar, Kapia 1300, Kapia UV (Vertus), Maritsa, Stryama, Buketen 50, Milkana F1, Yasen F1, Kaloyan and Ivaylovska kapia are developed as a result of a long breeding work as they possess high level of resistance to *Verticillium dahliae* Kleb (TODOROV and TODOROVA, 2002; MASHEVA and TODOROVA, 2013, TODOROVA and PEVICHAROVA, 2018; TODOROVA, 2019).

During the last years, the focuses of scientific research have been on complexes and comprehensive study of the local pepper resources from Balkan Peninsula. The evaluation of the pepper collection towards the response to *Tobacco Mosaic Virus* (TMV) and *Pepper Mild Mottle Mosaic Virus* (PMMoV) showed that 24 and 1 accession were resistant, respectively while no *Tomato spotted wilt virus* (TSWV) resistance was found (NANKAR *et al*., 2020b). The screening for the answer of studied resources to the attack of some important pest in natural background showed that a relatively weak infestation by the three pests - green peach aphid, trips and cotton bollworm was observed in 8 accessions (YANKOVA *et al*., 2020; YANKOVA *et al*., 2021). In study carried out with the first part of *Capsicum* collection for determining the response of the 97 accessions to the pathogen *Verticillium dahliae* Kleb it was established absence of the pathogen in five of them (VASILEVA *et al*., 2019). On the base of all results, it may be summarized that the accessions CAPS-122, CAPS-126, CAPS-135, CAPS-135A are with complex resistance to *Verticilium* wilt and TMV while others - CAPS-67 and CAPS-174 possess low infestation by green peach aphids, trips and cotton bollworm and non-attacked by *Verticillium dahliae* Kleb. These six accessions could be used as donors for resistance to these biotic factors.

Farmers are looking for cultivars resistant to diseases and pests, which are highly productive with very good market appearance of the fruit but consumers on the other hand, are mostly interested in the good appearance, quality and health effect of the pepper fruits. Based on the current and previous results about the characterisation of the studied accessions (VASILEVA *et al*., 2019; YANKOVA *et al*., 2020; NANKAR *et al*., 2020b; YANKOVA *et al*., 2021) could be generalized that some accessions worth 3 and more valuable traits:

CAPS-62: lack of infestation by *Verticillium*, high values for vitamin C, Total Phenols (TP) and Ferric Reducing Antioxidant Power (FRAP)

CAPS-67: lack of infestation by *Verticillium*, low attack by green peach aphid, trips and cotton bollworm and high values for TP;

CAPS-122: lack of infestation by *Verticillium*, resistant to TMV, high values for fruit width and fruit weight;

CAPS-135A: lack of infestation by *Verticillium*, high values for vitamin C, and FRAP and resistant to TMV;

CAPS-163: lack of infestation by *Verticillium*, high values for productivity, and fruit wall thickness;

CAPS-173: lack of infestation by *Verticillium*, high values for vitamin C, TP and FRAP;

CAPS-174: lack of infestation by *Verticillium*, low attack by green peach aphid, trips and cotton bollworm;

All of these seven accessions could be used as parental components in the future pepper breeding program.

CONCLUSION

From the tested 83 pepper accessions, representing to the second part of *Capsicum* collection 72% were high resistant, 23% - resistant, 1% - low susceptible 3% susceptible and 1% - high susceptible.

The results of all 180 pepper genetic resources tested to *Verticillium* wilt were summarized and discussed according to varietal type and pungency in the fruits.

It was established four accessions possessed complex resistance to TMV and *Verticilium dahliae* Kleb. and others 2 were with low attack by three important pest and non-infested by *Verticillium*.

Seven accessions were revealed which combined lack of infestation to *Verticillium* wilt with high levels of 3 and more agronomic and biochemical traits.

ACKNOWLEDGEMENT

This work was funded by the National Science Fund under the Bulgarian Ministry of Education and Science (grant No. DN06/4 16.12.2016).

The author Velichka Todorova acknowledges the financial support by Horizon 2020 PlantaSYST project under grant agreement No 739582.

Received, September 4th, 2021

Accepted March 28th, 2022

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PROCENA GENETIČKIH RESURSA PAPRIKE ZA OTPORNOST NA VENJENJE IZAZVANO VERTICILLIUM-OM

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Izvod

Urađena je studija za određivanje reakcije 83 sorte paprike, oplemenjivačkih linija i lokalnih populacija na gljivični patogen *Verticillium dahliae* Kleb. jer su deo balkanske Capsicum *core* kolekcije koja je poslednjih godina detaljno fenotipizirana. Razvoj bolesti je zabeležen dva puta, a visok stepen rezistencije zabeležen je za 41 uzorak. Najveći procenat visokorezistentne grupe genotipova bio je 72%, a sledi rezistentna grupa sa 23%. Infestacija u grupama srednje osetljivih, osetljivih i visoko osetljivih iznosila je 1%, 3% i 1%. Visoko osetljive (0 - 19%), osetljive (20 - 39%) i srednje osetljive (40 - 59%) čine *var. blocky*, *var. ratundum* i *var. kapia* uzorke. U grupi rezistentnih (60 - 79%) dominirala je *var. ratundum* i var. *blocky*. Kod materijala iz var. *kapia* preovlađuju visokootporni uzorci (80 - 100%). Grupa uzoraka ljute paprike bila je otpornija na infestaciju *Verticillium*. Na osnovu sadašnjih i prethodnih rezultata može se zaključiti da su četiri uzorka identifikovana kao otporna na TMV i nezaražene *Verticillium*-om, dok su dve posedovale nisku infestaciju zelenim lisnim vašima, tripsima i pamučnim sovicama i nisu napadnute *Verticillium*-om. Ostalih sedam genotipova je kombinovalo nedostatak infestacije verticilijumskim uvenućem sa visokim nivoima tri i više agronomskih i biohemijskih osobina.

Primljeno 4.IX.2021.

Odobreno 28. III. 2022.