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ASSESSMENT OF G2P-SOL PEPPER CORE COLLECTION TO *VERTICILLIUM DAHLIAE* KLEB. INFESTATION

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Abstract

Pepper (*Capsicum* spp.) is one of the major vegetable crops grown worldwide largely appreciated for its economic importance and nutritional value. The aim of study is to be assessing the G2P-Sol pepper core collection including 414 accessions from four cultivated *Capsicum* species to *Verticillium dahliae* Kleb. infestation. The use isolates VdCa13, VdCa15, VdCa17, VdCa18, VdCa19 and VdCa21, originally isolated from *Capsicum annuum* in the Plovdiv region, were used for inoculum. The strongest manifestation of the disease is found in accession GPC 118230 (88.54%). Among the evaluated tested accessions, 85 do not show any symptom of the disease. The average degree of attack by *V. dahliae* Kleb. is the highest in *C. frutescens* - 25.70%, and the lowest in *C. chinense* - 13.62%. The maximum attack index is reported in *C. annuum* - 89.58%, while *C. annuum*, *C. baccatum* and *C. chinense* all have a lack of infection. The standard deviation varied from 8.54 to 17.60. The coefficient of variation is the highest in *C. annuum* (106.25%), and the weakest in *C. frutescens* (39.08%).

The percentage distribution of the tested accessions according the attack index of all examined materials is as follows: *C. annuum* represented 94.43%, *C. baccatum* - 1.77%, *C. chinense* - 2.78% and *C. frutescens* - 1.01%.

Keywords: *Capsicum* sp., attack index, Verticillium wilt

INTRODUCTION

Pepper (*Capsicum* spp.) is one of the major vegetable crops grown worldwide largely appreciated for its economic importance and nutritional value. This crop belongs to the large *Solanaceae* family, which, among more than 90 genus and 2500 species of flowering plants, includes commercially important vegetables such as potato, tomato and eggplant. The genus *Capsicum* includes over 30 species, five of which (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*) are domesticate and mainly grown for consumption as food and for non-food purposes (Parisi et al., 2020).

Considerable diversity exists within cultivated and wild *Capsicum*, not only for agronomic characters such as fruit color, shape, and size, but for pest and disease resistance (Pickersgill, 1997; Walsh & Hoot, 2001). Five cultivate species are known. *Capsicum annuum* L., *C. frutescens* L., and *C. chinense* Jacq., are widely grown throughout the world, while production of *C. baccatum* L., *C. baccatum* var. *pendulum* Willd., and *C. pubescens* Ruiz and Pav. occurs mostly within South America. More than 20 wild *Capsicum* species are described, including *C. annuum* var. *glabriusculum* (Dun.) Heiser & Pickersgill and *C. baccatum* var. *baccatum* Eshbaugh, which are closely related to cultivated peppers (Nicola¨ı et al., 2013). Natural interspecific hybridization can occur between species and it is hypothesized to have contributed to the diversity observed in the present-day peppers (Perry et al., 2007).

The range of pathogens attacking the pepper plants is very broad and includes fungi,

viruses, bacteria. In this context, the large number of domesticated and wild species accessions 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). The main mechanism of *Verticillium* pathogenesis is the xylem vessel blockage and the toxin production. When the fungus invades the plant body, the mycelium blocks the xylem vessel, affecting the transport of water and nutrients in the plant (Klosterman et al., 2009; Shaban et al., 2018).

Resistance to Verticillium wilt can be controlled by several genes with minor effects or polygenes (Palloix et al., 1990). However, the plant immune receptor *Ve* was first discovered in the *Solanaceous* relative tomato (Diwan et al., 1999; Kawchuk et al., 2001) and may also exist in peppers, though this currently remains to be elucidated. The *Ve* and its homologs in other plant taxa have played critical roles in developing disease resistance to *V. dahliae* race 1 (Hayes et al., 2007). Nevertheless, *Ve* and its homologs offer inadequate protection against race 2 isolates because they lack the corresponding avirulence gene, *Ave1* (de Jonge et al., 2012).

Relatively few secreted effector proteins are found to play a role in virulence. One of the effectors secrets by *V. dahliae*, Vd424Y, can trigger strong cell death in *Nicotiana benthamiana*, pepper, and *Arabidopsis.* After assessing the yeast signal trap assay system, the fusion of the signal peptide of Vd424Y into the invertase gene resulted in the secretion of invertase in yeast. This indicates that Vd424Y was most probably secreted into the extracellular space during an infection of the host plant (Liu et al., 2021). A VdSCP7-induced immune response was dependent on its nuclear localization. Similar research found that *Vd424Y*-induce immunity depends on its nuclear localization, while defense signaling is probably initiated in the host nucleus (Zhang et al., 2017).

Fungi can promote or inhibit plant growth, which is important; however, the most important relationship between plants and fungi is the host–pathogen relationship. Plants can become resistant to *V. dahliae* through diverse mechanisms such as cell wall modifications, extracellular enzymes, pattern recognition receptors, transcription factors, and salicylic acid (SA)/ jasmonic acid (JA)/ethylene (ET)-related signal transduction pathways (Song et al., 2020).

In the last decades, most of the pepper breeding programs are addressed to the development of cultivars or hybrids against a wide range of pathogens and pests. Despite the made reports, the exploitation of *Capsicum* germplasm (pre-breeding materials, landraces, wild relatives and closely related species) and its use in breeding programs for biotic stress resistance still represent challenging tasks (Sarath et al., 2011).

In recent years, one of the focuses of some scientific research programs has been on a more in-depth and comprehensive study of various peppers resources from all over the world.

The aim of study is to be assessing the G2P-Sol pepper core collection including four cultivated *Capsicum* species to *Verticillium dahliae* Kleb. infestation.

MATERIALS AND METHODS

The experiment for assessment of pepper genetic resources from G2P-SOL (global research alliance for *Solanaceae* crops) core collection to *Verticillium* wilt infestation caused by the soil borne pathogen *Verticillium dahliae* Kleb. was done at the Maritsa Vegetable Crops Research Institute (MVCRI), Plovdiv, Bulgaria from March to September 2021.

Pepper germplasm, sowing and seedling stage: The G2P-SOL pepper core collection was compiled and made available as part of G2P-SOL project: "Linking genetic resources, genomes and phenotypes of

Solanaceous crops" (ID 677379). It was originally composed of 414 accessions, comprising four species: *Capsicum annuum, C. chinense, C. frutescens and C. baccatum*. Fourteen seeds per accession were prepared and disinfected with a solution of 10% of trisodium phosphate.

The seeds were sown in polypropylene trays containing peat and perlite (1:1) and all next agrotechnical practices were conducted in the greenhouse during the seedling period. 35 accessions are not germinated and/or not survived before transplanting on the field. The seedlings were watered.

Inoculation procedure: Six isolates of *Verticillium dahliae* (Kleb.) - VdCa13, VdCa15, VdCa17, VdCa18, VdCa19 and VdCa21, originally isolated from *Capsicum annuum* in the Plovdiv region, were used for inoculum. The *V. dahliae* Kleb isolates were previously assessed for virulence (Vasileva et al., 2019). The isolates were grown on potato dextrose agar (PDA) and incubated at 27ºC for 3 weeks. The final aqueous inoculum was prepared by macerating the contents of the test tube with 150 millilitres of tap water in a Waring blender for 1 minute (Figure 1).

Fig. 1. Planting and growing of the inoculated pepper accessions in the *Verticillium* field.

The used method for inoculation was root-dip technique: before planting, the roots of the plants stayed in a suspension of spores (10^6 spores in 10ml) and mycelium of pure cultures of 6 isolates of the pathogen, after that they were planted in the *Verticillium* field (Figure 1).

Transplanting and growth conditions: The experimental plot is used for testing of different pepper genotypes to *Verticillium dahliae* Kleb more than 30 years as each year a new infection was inputted (Masheva & Todorova, 2013). G2P-SOL core collection

consisted of 414 accessions was transplanted in May in this open field area by randomized block design in two blocks of 5 plants per accession. The plants were grown from May to September 2021. The agrotechnical practices including irrigation, fertilization etc. were conducted. The pepper plants were observed at the fruit maturity stage (Figure 1).

Disease severity rating: The disease severity was recorded between August and September, when the symptoms of *Verticillium* were expressly manifested. An infestation index of McKiney (in %) was used and the rate of pathogen attack was reported on scale (0 to 5). The severity of *Verticillium* wilt was measured on a scale of 0 to 5, where 0 = no vascular discoloration observed, 1 = 1 to 25% of the vascular tissue discolored, 2 = 26 to 50% of the vascular tissue discolored, 3 = 51 to 75% of the vascular tissue discolored, 4 = 76 to 100% of the vascular tissue discolored, and 5 = 100% of the vascular tissue discolored, with foliar wilting also observed (Bhat et al., 2003; Vallad et al. 2006). Each plant of the field was inspected, isolations from wilted plants were carried out and pathogenicity tests were conducted for each isolate. In the laboratory, the symptomatic plants were cleaned using distilled water and 5% bleach, then dissected, and plated on PDA medium. After a 10-days incubation at ambient temperature, plates were examined under a microscope to detect the presence of conidiophores and microsclerotia, typical of *V. dahliae* Kleb (Inderbitzin et al., 2011).

Data analysis: MS Excel 2020 Descriptive Statistics analysis tool was used to generate reports of univariate statistics for the data, providing information about the central tendency and variability of our data. Standard deviations (SD) were calculated and the coefficients of variation (CV%) showed the extent of variability in relation to the mean of the population, expressed as a percentage.

The results for each individual type of pepper are summarized and processed. The table of all tested materials is hugely presented as a supplementary and, therefore, we present a summary of the results.

RESULTS AND DISCUSSION

Initially, the symptoms of the disease appeared on the lower leaves, which lost their turgor, slightly turned yellow, curled and dried out. Subsequently, the disease spread to the leaves of the upper floors. In the cross section of the root crown and the stem, browning of the conducting bundles of tubes was observed. When plants were highly infected at a young age, their growth was severely depressed, the internodes were shortened, and there were fewer leaves, which were also smaller (Figure 2).

The infected plants were examined by inspecting single plants throughout the field. Isolations from wilted plants were carried out (Figure 3).

The results in Table 1 are a representative sample of all test materials. Among the evaluated tested accessions, 85 do not show any symptoms of the disease.

The results in Table 2 include descriptive statistics of the index of attack for each of the four assessed *Capsicum* species. The average degree of attack by *V. dahliae* Kleb. is the highest in *C. frutescens* - 25.70%, and the lowest in *C. chinense* - 13.62%. The maximum attack index is reported in *C. annuum* - 89.58%, while *C. annuum*, *C. baccatum* and *C. chinense* all have had no infection. The standard deviation varied from 8.54 to 17.60. The coefficient of variation is the highest in *C. annuum* (106.25%), and the weakest in *C. frutescens* (39.08%).

The percentage distribution of the accessions according to the attack index is presented in Table 3. *C. annuum* represented 94.43% of all examined materials, *C. baccatum* - 1.77%, *C. chinense* - 2.78% and *C. frutescens* - 1.01%. The attack index of tested accessions ranged between 0% to 30 %. The disease is not registered in 21.01% of *C. annuum* accessions and in 0.25% in *C. baccatum* and *C. chinense*.

Fig. 2. Development and symptoms of the disease during the reporting period

Fig. 3. Isolations from wilted plants.

Table 1. Index of attack of some accessions to Verticillium wilt, %.

G2P-SOL code	Original code	Species	DS
GPC000140	PI 1238	*Capsicum annuum*	**0.00**
GPC000360	PI 807	*Capsicum annuum*	**0.00**
GPC000830	PI 2054-8	*Capsicum annuum*	**0.00**
GPC001050	PI 2054-43	*Capsicum annuum*	**0.00**
GPC001360	PI 2054-76	*Capsicum annuum*	**0.00**
GPC018610	CAP 831	*Capsicum baccatum*	**0.00**
GPC014350	CAP 383	*Capsicum baccatum*	**7.16**
GPC002870	PI 2055-242	*Capsicum baccatum*	**13.49**
GPC098270	VI028774	*Capsicum baccatum*	**15.17**
GPC000760	PI 1723-43	*Capsicum baccatum*	**18.25**
GPC058920	CGN22855	*Capsicum chinense*	**0.00**
GPC049520	14PT28	*Capsicum chinense*	**5.17**
GPC010350	CAP 1544	*Capsicum chinense*	**6.01**
GPC002650	PI 2055-180	*Capsicum chinense*	**9.71**
GPC020470	2016-CAP-AN-38	*Capsicum chinense*	**10.93**
GPC037620	BGV001829	*Capsicum frutescens*	**15.85**
GPC022660	PM0140	*Capsicum frutescens*	**20.48**
GPC057060	CGN22105	*Capsicum frutescens*	**25.38**
GPC006660	CAP 1198	*Capsicum frutescens*	**41.08**

Table 2. Disease severity by species – descriptive statistics.

Species	Disease severity				
	Average	**Max**	**Min**	**SD**	**CV%**
Capsicum annuum	16.57	89.58	0.00	17.60	106.25
Capsicum baccatum	14.42	28.74	0.00	8.54	59.25
Capsicum chinense	13.62	38.57	0.00	9.69	71.15
Capsicum frutescens	25.70	41.25	14.25	10.04	39.08

*Max – maximum value; Min – minimum value; SD – Standard Deviation; CV –coefficient of variation (%).

Table 3. Distribution of the accessions from four species according to the attack index, (%).

Infestation index	***Capsicum annuum***	***Capsicum baccatum***	***Capsicum chinense***	***Capsicum frutescens***	**Total percentage**
0.00	21.01 %	0.25 %	0.25 %	0.00 %	21.52 %
0.01-10.00	21.52 %	0.25 %	0.76 %	0.00 %	22.53 %
10.01-20.00	24.56 %	1.01 %	1.27 %	0.25 %	27.09 %
20.01-30.00	10.89 %	0.25 %	0.25 %	0.51 %	11.90 %
30.01-40.00	5.57 %	0.00 %	0.25 %	0.00 %	5.82 %
40.01-50.00	4.81 %	0.00 %	0.00 %	0.25 %	5.06 %
50.01-60.00	1.52 %	0.00 %	0.00 %	0.00 %	1.52 %
60.01-70.00	2.78 %	0.00 %	0.00 %	0.00 %	2.78 %
70.01-80.00	1.52 %	0.00 %	0.00 %	0.00 %	1.52 %
80.01-90.00	0.25 %	0.00 %	0.00 %	0.00 %	0.25 %
90.01-100.00	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
Grand Total	**94.43 %**	**1.77 %**	**2.78 %**	**1.01 %**	**100.00 %**

In the range from 0.01 to 10% attack rate, 21.52% were *C. annuum*, 0.25% - *C. baccatum* and 0.76% - *C. chinense*. In up to 20%, the disease manifestation is the strongest in *C. annuum* - 24.56% followed by *C. baccatum*, *C. chinense* and *C. frutescens*, 1.01%, 1.27% and 0.25%, respectively. In the range of up to 30% a smaller number of infected accessions was reported - *C. annuum* - 10.89%, *C. baccatum*, *C. chinense* - 0.25% and *C. frutescens* - 0.51%. In up to 40%, the disease symptoms manifested for *C. annuum* - 5.57% and *C. chinense* - 0.25%. An attack index of up to 50% is reported in 4.81% of *C. annuum* and 0.25% for *C. frutescens*. In the attack ranging from 50% to 90%, *C. annuum* materials predominated.

The conducted research to determine the reaction of pepper accessions to *Verticillium dahliae* Kleb established that the degree of infection is mostly pronounced in the phase of mass fruiting, as conditions during this period favoured the development of the pathogen. There is a large variability in the reaction of the core collection to *Verticillium dahliae*.

The most radical method of control is creating of resistant varieties. This method is considered to be the most reliable for disease prevention and control because of its effectiveness, ease of use and lack of possible adverse effects on the environment (Bosland, 2003). In MVCRI-Plovdiv, a purposeful pepper breeding work with continuous screening of the available gene bank is being carried out to search for sources of resistance to this important pathogen and genotypes have been identified and successfully used in the selection process (Masheva et al., 2001) for the development of new varieties with a high level of *Verticillium dahliae* resistance (Masheva & Todorova,

2013). Selection lines and some varieties are the result of hybridization between Bulgarian or foreign resources, relatively resistant to *V. dahliae* Kleb. As a result of purposeful breeding work at MVCRI the following original varieties Hebar, Kapya 1300, Kapya UV (Vertus), Maritsa, Stryama and Buketen 50 (Todorov and Todorova, 2002) and Kaloyan (Todorova, 2019) have been bred and all of them possess field resistance to Verticillium wilt.

In deep complex evaluation of Balkan pepper resources some accessions show good resistance to testing pathogen which is some combined with another important 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*, a 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*, a 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 (Vasileva & Todorova, 2022).

Greater resistance to Verticillium wilt in cultivars of bell pepper relative to cultivars of chili pepper (Bhat & Subbarao, 2003, lower inoculums levels in fields cropped to jalapeno and bell pepper than in fields with other types of pepper, and a positive correlation between inoculum density and incidence of Verticillium wilt may account for relatively low incidences of Verticillium wilt in bell pepper crops.

Current management strategies to reduce the impact of Verticillium wilt in commercial pepper production include soil fumigation and planting resistant cultivars. None of the accessions were significantly better or worse than partially resistant PI 215699 but 26 PIs were significantly better than cv. California Wonder against isolate VdCa59. In the experiments with VdCf45, 14 PIs were significantly worse than the partial resistant PI 215699 and 28 PIs were more resistant compared with cv. California Wonder (Gurung et al., 2015).

Resistance to isolate VdCa59 was found in *C. annuum*, *C. baccatum*, and *C. frutescens* from Mexico, Costa Rica, and Peru. Similarly, resistance against VdCf45 was found in *C. annuum*, *C. annuum* var. *glabriusculum*, *C. baccatum*, and *C. frutescens* from Mexico and Costa Rica. More importantly, eight *Capsicum* accessions (Grif 9073, PI 281396, PI 281397, PI 438666, PI 439292, PI 439297, PI 555616, and PI 594125) were resistant to both isolates and re-isolation of the *V. dahliae* from these resistant pepper plants was unsuccessful. *Capsicum annuum* accession PI 555616 was also previously reported to have lower disease severity scores (Gonz´alez-Sal´an & Bosland, 1992). However, some of the pepper lines (PI 555614, PI 260550) reported to have lower disease severity were susceptible to both isolates (Gurung et al., 2015).

Despite efforts made in the past decade to evaluate and characterize resistance to V. dahliae, the available pool of Verticillium wilt resistant chili pepper appears inadequate, justifying the evaluation of additional sources of resistance to *V. dahliae* for use in *Capsicum* breeding programs. Previously, *C. annuum* accessions PI 555614, PI 555616, and *C. baccatum* var. *microcarpum*. *C. annuum* accession PI 215669 were characterized as resistant to *V. dahliae* in the United States (Gonz´alez-Sal´an & Bosland, 1992), while Woolliams et al. (1962) failed to identify any *V. dahliae*-resistant *Capsicum* accessions tested in Canada.

As the interactions between *V. dahliae*

isolates and pepper cultivars is evaluated, the highest disease severity is recorded in Sera Demre-8 x Kahramanmaraş interaction with 53%, and the lowest disease severity (no disease) is recorded in Karpuz-2 x Doru-16 interaction. When the leaf symptoms are evaluated, varieties resistant to wilt disease were Ergenekon F_1 and Bafra F_1, while Sera Demre-8 was determined as a susceptible variety. According to the browning symptoms of the stem cut, while the resistant variety was Ergenekon F_1, Sena was determined as the sensitive variety (Coşkun et al., 2021).

CONCLUSION

The average degree of attack by *V. dahliae* Kleb. was the highest in *C. frutescens* - 25.70%, and the lowest in *C. chinense* - 13.62%. The maximum attack index was reported in *C. annuum* - 89.58%, while a lack of attack in *C. annuum*, *C. baccatum* and *C. chinense*.

There is a large variability in the reaction of assessed accessions from the G2P-Sol pepper core collection to *Verticillium dahliae* Kleb.

Among the tested accessions, 85 did not show any symptom of Verticillium wilt and could be useful in breeding programs as sources of resistance to this fungal pathogen.

The percentage distribution of the accessions according to the attack index on *C. annuum* is 94.43% of all examined materials, *C. baccatum* - 1.77%, *C. chinense* - 2.78% and *C. frutescens* - 1.01%. The attack index of tested accessions ranged between 0% to 30 %. The disease is not registered in 21.01% of *C. annuum* accessions and in 0.25% in *C. baccatum* and *C. chinense*.

The conducted research to determine the reaction of pepper accessions to *Verticillium dahliae* Kleb established that the infection degree is mostly pronounced in the phase of mass fruiting, as conditions during this period favoured the development of the pathogen. There is a large variability in the reaction of assessed accessions from the core collection to *Verticillium dahliae* and the revealed resistant genotypes could be useful in breeding programs as sources of resistance to this fungal pathogen.

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