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RESPONSE OF PEPPER VARIETIES, F_1 HYBRIDS AND BREEDING LINES TO *VERTICILLIUM DAHLIAE* KLEB. IN TWO METHODS OF INFESTATION

S. MASHEVA and V. TODOROVA
Maritsa Vegetable Crops Research Institute, BG - 4003 Plovdiv, Bulgaria

Abstract

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During the period 2006-2007 in the Maritsa Vegetable Crops Research Institute, Plovdiv have been tested twenty breeding lines, varieties and F_1 hybrids from different variety types – *var. Conicum, var. Kapia* and *ser. var. Grossum*. Plants from each genotype were tested by two methods – direct planting in infected soil and infestation in transplanting by *root-dip technique* and planting in the same soil. The level of resistance to *Verticillium dahliae* Kleb in the studied materials is higher in planting in infected soil without additional infestation. Firmly low index of infestation (below 5 %) is found in line 820/03 from kapia type and in line 398/03 from *ser. var. Grossum*. Variety Yasen F_1 is more slightly susceptible to the pathogen in planting in infected soil without additional infestation. Lower index of infestation was determined in candidate-variety Milkana F_1 in infestation by *root-dip technique* and planting in infected soil. The breeding line 398/03 is with high level of resistance in the two methods of infestation and could be used as a source of resistance in the future breeding programs.

Key words: Capsicum annuum L., *Verticillium* wilt, index of infestation

Introduction

The pepper is one of the main vegetable crops in Bulgaria. Being one of the intensive crops, it is attacked from different diseases, which could cause significant losses depending on the degree of infestation. The *Verticillium* wilt, caused by the pathogen fungus *Verticillium dahlae* Kleb. is with the greatest economic importance among the whole complex of disease in pepper. The control of this fungus is difficult because it is able to survive both in open field and under controlled conditions for several years (Goicoechea, 2006). According to Goldberg (2003) at present there is no effective enough method in the world for control of this pathogen after the disease has been appeared in the sowing. The retrieval of alternative methods for control is extremely necessary for the agriculture in future (Goicoechea, 2006). Some of these possibilities are development and growing of resistant varieties, application of adequate strategies for control, development and use of the biological method for control. It is considered that the resistance is the most reasonable safety strategy for control to *Verticillium* wilt. Although there are several tolerant varieties, genes for resistance are not established in *Capsicum annuum* (Pomar et al., 2004). A commercial variety resistant to this pathogen is not still developed in the world (Goldberg, 2003).

In screening of 125 accessions, partial resistance to *Verticilium* wilt is established only in three accessions (Gonzalez-Salan and Bosland, 1991). The resistance to *V. dahliae* is polygenic determined. It depends also on the conditions of infestation: lines chosen in conditions of natural infestation (Fiume et al., 1982) have been susceptible to artificial inoculation. Varieties with high level of resistance reported by Pesti (1985) are susceptible in test with inoculum with higher concentration.

E-mail: smasheva@abv.bg

The purpose of the study was to determine the response of varieties, F_1 hybrids and breeding lines in pepper to *Verticillium dahliae* Kleb. using two method of infestation as well as the effect of the method of infestation on the index of infestation from *Verticillium* wilt.

Materials and Methods

During the period 2006-2007 were tested 20 breeding lines, varieties and F_1 hybrids from different type, according to fruit shape - *var. Conicum* (№ 828/03, variety Yasen F_1 and candidate-variety Milkana F_1), *var. Kapia* (№360/03, 747/03, 820/03, 568/03, 834/03, 869/03, 1103/03, 587/02, 1547/03 and Serbian variety Palanachko chudo) and from *ser. var. Grossum* (№ 334/03, 337/03, 350/03, 380/03, 388/03, 398/03 and 400/03). The plants from each genotype were infested by two methods: I – directly planted in infested soil in the *Verticilium* field; II – infestation in transplanting by *root-dip technique* (Goldberg, 2003) and then planted in the same soil. The *Verticillium* field has been established in the "Maritsa" Vegetable Crops Research Institute before more than 20 years. Pepper plants infested in transplanting by ***root-dip technique*** are planted there every year. Mixed infection containing chosen six aggressive isolates of *Verticillium dahliae* Kleb., from the collection of the institute has been used for the inoculating. Inoculum was prepared from one-month-old cultures of the isolates grown on standard nutritional Chapek medium. The plant roots were soaked in mixture from pathogen and soil for 30 minutes.

Plants were transplanted in alluvial meadow soil type by block method in three replications on furrow surface by 70/15 cm scheme on May 25 in 2006 and on May 18 in 2007. They were grown according to the accepted technology for mid-early field production. Control with plant protection products was performed on pepper insect only. The degree of infestation was read twofold during vegetation by five scales (0-4). The recording was done at the beginning and end of August ie 10 to 12 weeks after inoculation, as earlier reporting is not efficient enough for practical breeding. Index of infestation was calculated by Mc. Kinney.

Data were processed by three-way analysis of variance (Lidanski, 1988) and Duncan Multiple range test (1955). It was also determined the power of influence (η, %) of the variation factors (Plohinskii, 1970).

Results and Discussion

Based on the applied three-way analysis of variance was established that all systematic factors played significant effect on the variability of the infestation index (Table 1). Gordon et al. (2005) in test of strawberry varieties and lines established significant role of factors genotype, method of infestation and interaction year x method of infestation only.

The method of infestation is with the greatest power of influence (60.34%) in the present study followed by the interaction genotype x method of infestation (13.40%) and genotype (12.63%). The individual effect of the year is slight although it has been proven (3.15%). The role of the interaction genotype x method of infestation x year is similar (2.92%). Year as a factor depends mainly on the meteorological differences because the remaining conditions of the environment are identical – soil type, agricultural practices etc. It was established that the second experimental year was with higher average monthly temperature from both the first year and climatic standard for the period from planting of the crop until August (Figure 1). The rainfalls in Mai, June, August and October for the same year have been in greater amount. The abovementioned results in provocation of growth and multiplication of the patho-

Table 1
Three-way analysis of variance on the index of infestation (2006-2007)

Factors of variation	Degree of freedom *df*	MS	η %
Genotype (A)	19	638.85***	12.63
Method of treatment (B)	1	57998.71***	60.34
Year (C)	1	3024.81***	3.15
Interaction A x B	19	677.63 ***	13.40
Interaction A x C	19	263.65 ***	5.21
Interaction B x C	1	116.81**	0.12
Interaction A x B x C	19	147.79 ***	2.92
Residual	160	13.41	

* Significant at p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001

gen and therefore the average index of infestation during the second year was significantly higher in the two methods of infestation (Table 2).

Planting in infected soil free of additional infestation

The infestation index averagely for the studied breeding lines, varieties and F_1 hybrids of pepper is 10.53 % in this method for the whole period of investigation. In estimating of the Bulgarian pepper varieties in our previous study (Masheva and Todorova, 2012) was established that the average infestation index is 35.62%. The higher level of resistance of the studied materials is due to long breeding process in this direction (Masheva et al., 2001; Todorov and Todorova, 2002).

Infestation index 5.00 % averagely for the experimental year was read in four lines. Two of them belongs to *ser. var. Grossum* - 398/03 (i = 2.22%) and 350/03 (i = 4.38%). The remaining two lines are kapia type – 820/03 (i = 2.09%) and 360/03 (i = 5.00%). Among the early pepper conic genotypes the F_1 hybrid Yasen is described with the lowest index that has been proven - averagely 9.03% in this method of infestation. The lowest average index of infestation was recorded in *ser. var. Grossum* - 9.74% followed by *var. kapia* – 10.13% (Table 3). The most susceptible to the agent of *Verticillium* wilt are the genotypes belongs to *var. conicum*.

The lines with the lowest and the highest index of infestation among the studied materials are 820/03 (i = 2.09%) and 1547/03 (i = 21.05%), belonging to kapia type (Table 2). Index of infestation in 2006 is lower than the one read in 2007. A deviation of this tendency is observed in 869/03 and 1547/03 only which are from kapia type. Six lines in this year show plants free of symptoms of the diseases. Three of them belong to *ser. var. Grossum* and the remaining – from kapia type. In 2007 two lines being free of symptoms previous year demonstrate the highest of infestation – 568/03 (i = 27.68%) from kapia type and 400/03 (i = 25.00%) from *ser. var. Grossum*. These results confirm the fact that the meteorological differences in the years have a significant effect on the susceptibility of pepper plants to the agent of *Verticillium* wilt.

Fixedly low index of infestation in the two experimental was read in line 820/03 (i = 0.00 and 4.17%, respectively) from kapia type and 398/03 (i = 0.00 and 4.44%, respectively) from *ser. var. Grossum*. Breeding lines 350/03 and 334/03 from *ser. var. Grossum* and 747/03 и 834/03 from kapia type are with index of infestation lower than 10% in the two years.

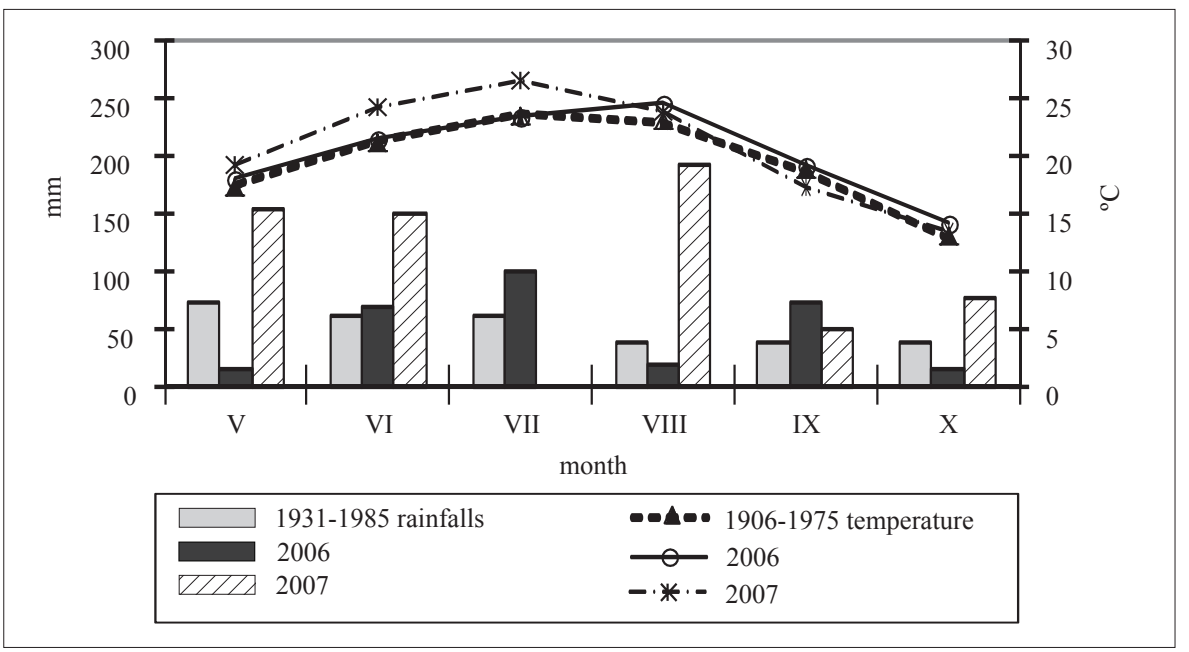

Fig. 1. Meteorological conditions during the experimental period

Infestation in transplanting by *root-dip technique* and planting in infected soil

Index of infestation in this method of test is considerably higher – averagely 41.62% than that recorded in non-infected materials planted in infected soil - 10.53% (Tables 2 and 3). Considerable susceptibility is demonstrated as a response of the additional infestation. These results corresponding total to the established by Gordon et al. (2005) in test on strawberry genotypes by *root-dip technique* and by inoculum input (microsclerotia) directly in the soil. It is considered that in the first method of infestation the plant roots are in direct contact with the inoculum and the systematic infestation was realized more rapidly. In the second method, the probability for contact between the plant roots and inoculum is smaller.

Genotypes from *ser. var. Grossum* are outlined with the lowest index of infestation (averagely 30.35%) during the completely experimental period also in this method of screening (Table 3). Kapia type materials – 50.26%, demonstrate the strongest susceptibility.

Table 2
Response of the estimated pepper genotypes to the *Verticilium dahliae* Kleb. in two methods of treatment

Breeding lines, variety and F_1 hybrids	I method			II method		
	2006	2007	Mean	2006	2007	Mean
828/03	13.89 j-n	18.75 k-o	16.32 j-m	19.44 i-k	51.04 d	35.24 ef
Yasen F_1	5.56 op	12.50 o-s	9.03 p-t	50.00 c	46.87 d-f	48.43 d
Milkana F_1	13.29 j-n	18.06 l-p	15.67 j-n	24.09 g-i	43.06 e-g	33.57 f
334/03	7.50 no	8.33 r-t	7.92 q-n	35.42 e	38.71 gh	37.07 ef
337/03	5.00 op	14.58 m-r	9.79 o-s	29.98 e-g	66.67 a-c	48.32 d
350/03	0.00 p	8.75 r-t	**4.38 uv**	23.76 g-i	47.65 d-f	35.71 ef
380/03	13.54 j-n	13.33 n-s	13.44 l-p	20.00 ij	36.28 h	**28.14 g**
388/03	15.00 j-m	20.83 j-m	17.92 i-l	22.92 hi	26.67 ij	**24.79 gh**
398/03	0.00 p	4.44 t	**2.22 v**	22.92 hi	16.67 m-q	**19.79 ij**
400/03	0.00 p	25.00 i-k	12.50 m-q	12.50 l-n	24.80 i-l	**18.65 i-k**
360/03	0.00 p	10.00 q-t	**5.00 t-v**	54.17 bc	46.67 d-f	50.42 cd
747/03	5.00 op	9.58 q-t	**7.29 r-u**	30.83 ef	36.11 h	33.47 f
820/03	0.00 p	4.17 t	**2.09 v**	41.75 d	61.54 c	51.65 cd
568/03	0.00 p	27.68 i	13.84 l-o	28.75 f-h	41.18 f-h	34.96 ef
834/03	5.00 op	8.12 r-t	6.56 s-v	56.35 ab	71.25 a	63.80 ab
869/03	16.11 j-l	6.66 st	11.39 n-r	57.08 ab	64.47 bc	60.78 b
1103/03	8.33 m-o	11.25 p-t	9.79 o-s	41.81 d	35.00 h	38.40 e
Palanachko chudo	8.75 m-o	20.00 j-n	14.38 k-o	62.28 a	68.56 ab	65.42 a
587/02	8.75 m-o	11.11 p-t	9.93 o-s	51.67 bc	49.07 de	50.37 cd
1547/03	27.72 f-h	14.38 m-r	21.05 hi	61.64 a	45.00 d-g	53.32 c
Average	7.67	13.38	10.53	37.37	45.86	41.62

a, b, c… - Duncan's multiple range test ($p<0.05$)

Table 3
Average index of infestation

Genotypes	I method			II method		
	2006	2007	Mean	2006	2007	Mean
var. Conicum	10.91	16.44	13.67	31.18	46.99	39.08
ser. var. Grossum	5.86	13.61	9.74	23.93	36.78	30.35
var. Kapia	7.97	12.30	10.13	48.63	51.89	50.26

Similar to the results that read in the first method of infestation the index in *root-dip* inoculation was lower in 2006 compared to that in 2007, which gives a reason to make a relation between plant susceptibility and meteorological characteristics (Table 2). These results correspond to the relationships established by Moser and Sackston (1973) and Palloix et al. (1990). It is observed some deviations concerning the genotype. Breeding line 1547/03 is the only line that is described with higher index of infestation in 2006 in the two methods of infecting compared to the index in 2007, which is a deviation from the total tendency.

The strongest infestation in this method of test was observed in variety Palanachko chudo (i = 62.28% and 68.56%, respectively), line 834/03 (i = 56.35% and 71.25%, respectively) and 869/03 (i = 57.08% and 64.47%, respectively). Breeding line 834/03 among them is with index of infestation below 10.0% when the planting is in the conditions of natural infestation.

Candidate-variety Milkana F_1 from the conic genotypes demonstrate in this method of infestation more slight susceptibility (proven) to the pathogen towards variety Yasen F_1.

In this study, the two methods show significant and proven differences in susceptibility of the studied breeding lines, varieties and F_1 hybrids to the infestation by *V. dahliae*. The differences between the two methods are clearly expressed in the two years of investigation. The presence of proven interaction between the factors genotype, treatment and year defines some differences in the ranking of the studied materials in the two methods and in different years of investigation.

The lowest index of infestation, average for the two years of the study, was recorded in four lines belongs to *ser. var. Grossum* among preliminary treated genotypes. In two of them 400/03 and 398/03 the index is below 20% - 18.65% and 19.79%, respectively. In the remaining two lines – 388/03 and 380/03 the index of infestation is 24.79% and 28.14%, respectively. Line 398/03 is with low index of infestation in the two methods of infestation 2.22% in the first and 19.79% in the second, respectively. It is described with high level of resistance and this line could be used as a source of resistance in the future breeding programmes.

Conclusions

All systematic factors have proven effect on the variability of the index of infestation by *Verticillium dahliae* Kleb. In the tested pepper varieties, F_1 hybrids and breeding lines. The greatest effect demonstrates the method of infestation (60.34%) followed by the interaction genotype x method of infestation (13.40%) and genotype (12.63%). The average level of resistance to *Verticillium dahliae* Kleb of the studied materials is significantly higher than the one of the Bulgarian pepper varieties when test was made by planting in infected soil. In the first method of infestation, lines 820/03 from kapia type and 398/03 from *ser. var. Grossum* are with firmly low index of infestation in the two year – below 5 % while the index of 350/03 and 334/03 from *ser. var. Grossum* and of kapia type lines 747/03 and 834/03 is lower than 10%. Variety Yasen F_1 is with proven lower susceptibility to the pathogen in planting of materials in infected soil without additional infestation while candidate-variety Milkana F_1 is with significantly lower index in infestation by *root-dip technique* and planting in infected soil. Breeding line 398/03 is with high level of resistance in the two methods of infestation and could be used as a source of resistance in breeding program.

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