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RESISTANCE OF PEPPER ACCESSIONS AND LINES TO ECONOMICALLY IMPORTANT DISEASES

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SUMMARY

Macedonian and Bulgarian pepper accessions and lines were tested for resistance to cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), paprika mild mottle virus (PMMoV) - (P1 pathotype), pepper mild mottle virus PMMoV - (P1.2 and P1.2.3 pathotypes) and *Xanthomonas vesicatoria* pepper-tomato pathotype (XvPT). The L57 was resistant to CMV and L15, L16 and L113 possessed complex resistance to CMV, TMV, ToMV and *P. capsici* and L64 were resistant to CMV, TMV, ToMV, PaMMV, PMMoV (pathotype P1.2) and *P. capsici*. The lines L16, L64 and L113 were additionally moderately resistant to XvPT. The Macedonian line MK6 showed strongly expressed high antocyanine having the lowest infection index and lacking defoliation.

Sources of resistance to XvPT (MK6) and sources of complex resistance to tobamoviruses, CMV, *P. capsici* and tolerant to XvPT have been established. The lines L16 and L64 possess complex resistance to CMV, tobamoviruses and *P.capsici*, tolerant to XvPT and with desired fruit form. These lines own valuable agronomic characteristics, e.g. potential yield, high vitamin C and dry matter content and may be used by the plant breeders in pepper improvement programs.

Key words: *cucumber mosaic virus, tobacco mosaic virus, paprika mild mottle virus, pepper mild mottle virus, Xanthomonas vesicatoria pepper-tomato pathotype, resistance, disease, pepper*

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ОТПОРНИ ЛИНИИ ПИПЕРКА НА ЕКОНОМСКИ ЗНАЧАЈНИ БОЛЕСТИ

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КРАТОК ИЗВАДОК

Македонски и бугарски линии пиперки се тестирани за да се утврдуви нивната отпорност на: вирусот на краставицата - (CMV), вирусот на мозаикот на тутунот (TMV), вирусот на мозаикот на доматот (ToMV), вирусите на пиперката: paprika mild mottle virus (PMMoV) - (P1 pathotype), pepper mild mottle virus PMMoV - (P1.2 and P1.2.3 pathotypes) и *Xanthomonas vesicatoria* pepper-tomato pathotype (XvPT). Линијата L57 е отпорна на CMV и L15, L16 и L113 и покажува комплексна отпорност на CMV, TMV, ToMV и *P. capsici* и L64 резистентана на CMV, TMV, ToMV, PaMMV, PMMoV (pathotype P1.2) и *P. capsici*. Линиите L16, L64 и L113 се дополнително умерено отпорни на XvPT. Македонската линија MK6 покажува силно присуство на антоцијан , понизок индекс на инфекција и отсуство на дефолијација.

Изворите на отпорност на XvPT (MK6) и изворите на комплексна отпорност на тобамовирусите, CMV, *P. capsici* и толерантност на XvPT се утврдени. Линиите L16 и L64 покажуваат комплексна отпорност на CMV, тобамовирусите и *P.capsici*, tolerantnost kon XvPT и со сакана форма на плодовите. Овие линии поседу-

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ваат вредни агрономски својства: висок принос, висока содржина на витамин С и суви материи и може да се користат за одгледување на пиперката во современите програми.

Клучни зборови: *вирус на мозаик на краставица, вирус на мозаик на тутун, вируси на пиперката, Xanthomonas vesicatoria патоген пипер-домат, отпорност, болест, пиперка*

INTRODUCTION

Plant diseases are main limiting factor in contemporary agriculture. The actual control of plant diseases - far from being effective - requires an extensive use of pesticides and herbicides in order to manage mainly the insect and herb populations that act as vector and natural reservoirs of plant pathogens. The application of chemical compounds has deleterious effect on human beings and also causes environmental contamination despite the fact that they become ineffective because resistance against them appears in weeds and insects. Therefore, and in order to minimize these environmental risks, it is necessary to breed disease-resistant horticultural crops. In addition, it is also needed to enhance economic competition of horticultural producers.

In Bulgaria and Macedonia sweet and hot Bulgarian pepper cultivars are prevalent due to their good savor. These cultivars are susceptible to cucumber mosaic virus (CMV), tobamoviruses, *Xanthomonas vesicatoria* (Xv) and *Phytophthora capsici* (*P. capsici*) which cause economically important diseases.

The tobamoviruses prevail in the protected pepper crops and generate significant losses of yield (up to 60%). In the field the most widespread virus is CMV that infects up to 80-90% of the pepper plants (S t o i m e n o v a, 1995). Since 2000 to now Xv has widely spread in pepper in our countries and causes significant losses (Б о г а ц е в с к а, 2002; М и т р е в 2001).

A natural population of *Xanthomonas* causing bacterial spot on tomato and pepper is usually presented by various strains differing in their pathogenic reaction to the hosts. Thus three pathotypes can be distinguished: pepper-P (XvP), tomato-T (XvT) and pepper-tomato pathotype-PT (XvPT). All *Capsicum* tested genotypes are resistant to XvT strain, and conversely all *Lycopersicon* tested genotypes are resistant to XvP strains, some strains are virulent on plants of both genotypes - XvPT pathotype (M i n s a v a g e, *et al.*, 1990; J o n e s, *et al.*, 1998; O'G a r r o, 1998).

P. capsici is a major threat to pepper production and ranked as the second most devastating disease worldwide (B a r k s d a l e, *et al.*, 1984). In favourable conditions (high temperature, humidity and rainy weather) the pathogen may also attack the leaves and fruits thus destroying plants on a pepper plantation for a short period of time. The main symptoms of the disease are the root and stem rot followed by wilting and mortality of the plants. No cultural or chemical control and antagonistic micro-organisms for biological control have been found to be effective.

The aim of the present investigation was to select pepper accessions and lines for single and complex resistance to CMV, tobamoviruses, XvPT and *P. capsici* under artificial infection conditions.

MATERIAL AND METHODS

The following viruses and strains were used: tobacco mosaic virus (TMV) - U1; tomato mosaic virus (ToMV) - GM-0 ; paprika mild mottle virus (PMMoV) - P101 (P1 pathotype); pepper mild mottle virus PMMoV - GP_2 (P1.2 pathotype), GP_3 (P1.2.3 pathotype) and CMV – CMV-P. The purification of the tobamoviruses was performed according to the standard method and the plants were inoculated with 0.05 mg/ml virus. Inoculums of CMV-P were prepared just before inoculation by homogenizing CMV infected tobacco leaves in 0.01M phosphate buffer, pH 7 containing 0.1% Na_2S0_3 and 0.1% ascorbic acid, diluted 1:3 (w/v). The seeds of the test plants were sown in pots with sterile soil. The plants were inoculated with tobamoviruses in cotyledon-primary true leaf phase and with CMV in 3-4 leaves phase. The symptoms were recorded 7, 28 and 45 days after infection.

The pepper plants were inoculated in the phase 3-4 true leaves with suspension prepared from 36 h culture of XvPT (strain ?1/99) in a concentration of 10^8 cfu/ml by vacuum infiltration method (Б о г а ц е в с к а 2002). The spots on the foliage were recorded 5-6 days after inoculation. The average number of spots per leaf and grade of the disease (Infection and Defoliation index) were scored by the scale of P e t s i et al., (1990).

For inoculation an aggressive isolate of *P.capsici*, obtained from commercial fields of sweet pepper in the South-West of Bulgaria, was used. 30 plants from each line were inoculated by watering 100 ml of mycelium-sporangial suspension around the stem base of the plant 14 days after transplanting in soil. Inoculum was prepared by blending in a 7-day old petri-dish (9 cm) culture of isolate on oatmeal agar with 200 ml distilled water. The first recordings were done 20 days after the inoculation when

100% of the susceptible control plants showed symptoms of the disease. The following recordings were done 50 and 90 days after the inoculation when the plants were in technical and botanical maturity respectively.
The pepper cultivar Zlaten medal (susceptible control), the six Macedonian pepper accessions and the seven Bulgarian lines were tested.

RESULTS AND DISCUSSION

The data for resistance to CMV, tobamoviruses and *P. capsici* of tested accessions and lines are presented in Table 1.
L113 (dark green blocky type fruits) was developed after 7 years of screening for resistance to CMV in a segregating Dutch material. The line L57 was selected from a local Bulgarian material as resistant to CMV and was consolidated after 6 years of testing (M i h a i l o v a et al., 2001). CMV resistance of L57 and L113 is expressed as limitation of the virus transport. On the rub-inoculated leaves of line L57 chlorotic spots developed and on L113 – necrotic lesions, followed by detachment of these leaves. Moreover, in the second line hypersensitive reaction appeared additionally. The data from the ELISA and Tissue Print of the symptomless zones of the rub-inoculated leaves and petiole reveal the presence of the virus. The plant stem and non-infected leaves do not contain the virus (S t o i m e n o v a et al., 2005).
The lines Alfi and Zalfi were resistant to *P. capsici* and anthocyanineless. These lines were developed by hybridization of the mutant variety Albena with the resistant line P51, one backcross with the line P51 followed by many years of screening for resistance and good agronomic characters. L114 possessed CMV resistance from L113, two backcrosses with the cultivar Zlaten medal – ms-8 followed by screening for resistance and good agronomic characteristics. Cv. Novares was a Hungarian cultivar with L3 gene-mediated resistance to tobamoviruses. Cv. Zlaten medal and all Macedonian accessions were susceptible to the viruses and *P. capsici*. L15 (F4[L114 x Alfi]), L16 (F4[L114 x Zalfi]) and L113 possessed complex resistance to CMV, TMV, ToMV and *P. capsici* and L64 (F5[F2(L114 x Alfi) x F2 (Novares x L57)]) was resistant to CMV, TMV, ToMV, PaMMV, PMMoV (pathotype P1.2) and *P. capsici*.
The results for resistance to XvPT of pepper accessions and lines are shown in Table 2. Summa of Di and Ii referred to the susceptibility of the pepper samples, while Di and Ii were forms of this susceptibility. No correlation between the defoliation index and the infection index was found. There was high percentage of defoliation in plants of low Ii (Alfi) as well

as in plants of high Ii (Zlaten medal). The high Ii percentage identified the plants as highly sensitive to XvPT, since defoliation resulted into strong yield reduction. The Macedonian lines MK1, MK2, MK3 and MK6, where ms was below 0.5, were tolerant to XvPT and without defoliation. Lines Alfi and Zalfi are closely related lines and differ by the fruit color. The first line had light green kapia fruits and the second – dark green kapia fruits. Di of Alfi was very high – 15.6% while the corresponding index of Zalfi was 0%. The most resistant Macedonian line MK6 was of strongly expressed antocyanine having the lowest Ii and lacking defoliation. These data suggest that high antocyanine availability increases pepper resistance to XvPT.

Climatic and meteorological peculiarities of every area determine the appearance and simultaneous distribution of a specific complex of viruses, bacteria and fungi, which attack the crops during different stages of vegetation. That is why the resistance against one disease is not a sufficient circumstance for high and stable yield because of the losses provoked by other pathogens. Therefore, the creation of cultivars with complex resistance to economically more important diseases for certain geographical region is the cheapest and efficient method to control them.

Sources of resistance to XvPT (MK6) and sources of complex resistance to tobamoviruses, CMV, *P. capsici* and tolerant to XvPT have been established. The lines L16, L64 and L113 with complex viruses and *P. capsici* resistance being tolerant to XvPT provide a particular interest.

CONCLUSIONS

The L57 was resistant to CMV and L15, L16 and L113 possessed complex resistance to CMV, TMV, ToMV and *P. capsici* and L64 was resistant to CMV, TMV, ToMV, PaMMV, PMMoV (pathotype P1.2) and *P. capsici.* The lines L16, L64 and L113 were additionally moderately resistant to XvPT. The Macedonian line MK6 was of strongly expressed antocyanine having the lowest Ii and lacking defoliation. The high antocyanine availability increases pepper resistance to XvPT. All disease resistant lines can be used in pepper breeding programs.

The lines L16 and L64 were developed with complex resistance to CMV, tobamoviruses and *P.capsici*, tolerant to XvPT and with desired fruit form. These lines own valuable agronomic characters, e.g. yield potential, high vitamin C and dry matter content and may be used by the plant breeders in pepper improvement programs.

Tab. 1 - Resistance to CMV, TMV, ToMV, PaMMV, PMMoV (P1.2 and P1.2.3 pathotype) and *P. capsici* in pepper accessions and lines

Таб. 1 - Отпорност на CMV, TMV, ToMV, PaMMV, PMMoV (P1.2 и P1.2.3 патотип) и *P. capsici* во линии од пиперка

samples/viruses/pathotypes примерок/вируси/патогени	CMV	TMV	ToMV	PaMMV	PMMoV		Ph
					P1.2	P1.2.3	
C.annuum Alfi (light green kapia)	S	S	S	S	S	S	R
C.annuum Zalfi (dark green kapia)	S	S	S	S	S	S	R
C.annuum L.57 (dark green kapia)	R	S	S	S	S	S	S
C.annuum L.113 (dark green blocky type)	R	R	R	S	S	S	R
L.15 [L.114 x Alfi] (light green kapia)	R	R	R	S	S	S	R
L.16 [L.114 x Zalfi] (dark green kapia)	R	R	R	S	S	S	R
L.64 [F2(L.114 x Alfi) x F2(Novares x L.57)]	R	R	R	R	R	S	R
C.annuum MK1	S	S	S	S	S	S	S
C.annuum MK2	S	S	S	S	S	S	S
C.annuum MK3	S	S	S	S	S	S	S
C.annuum MK4	S	S	S	S	S	S	S
C.annuum MK5	S	S	S	S	S	S	S
C.annuum var. *microcarpum* MK6	S	S	S	S	S	S	S
cv. Zlaten medal (dark green kapia), control	S	S	S	S	S	S	S

Ph – *Phytophthora capsici* (M i h a i l o v a et al., 2001and unpublished data) S – susceptible, R - resistant

Ph – *Phytophthora capsici* (M i h a i l o v a et al., 2001 и непечатени податоци) S – osetliva, R - отпорна

Tab. 2 - Reaction and grade of disease in pepper accessions and lines after vacuum infiltration with XvPT

Таб. 2 - Реакција и степен на заболување кај различни линии пиперка по вакуум инфилтрација со XvPT

samples/value примерок/ вредност	T	Number of plants with value Број на растенија со вредност						ms	Ii%	Di%	Ii+Di in %
		0	1	2	3	4	5				
C.annuum Alfi	77	52	22	3	0	0	0	0.36	7.2	15.6	22.8
C.annuum Zalfi	43	21	19	3	0	0	0	0.58	11.6	0	11.6
C.annuum L.57	57	21	21	10	5	0	0	0.98	19.6	14.0	33.6
C.annuum L.113	64	21	29	14	0	0	0	0.98	19.6	0	19.6
C.annuum L.15	85	52	29	4	0	0	0	0.41	8.2	10.6	18.8
C.annuum L.16	80	29	38	8	5	0	0	0.86	17.2	0	17.2
C.annuum L.64	39	10	25	4	0	0	0	0.85	17.0	0	17.0
C.annuum MK1	29	20	7	2	0	0	0	0.38	7.6	0	7.6
C.annuum MK2	30	21	9	0	0	0	0	0.30	6.0	0	6.0
C.annuum MK3	69	41	23	5	0	0	0	0.48	9.6	0	9.6
C.annuum MK4	47	21	17	7	2	0	0	0.79	15.8	17.5	33.3
C.annuum MK5	74	38	36	0	0	0	0	0.49	9.8	2.6	12.4
C.annuum MK6	85	71	14	0	0	0	0	0.16	3.2	0	3.2
cv. Zlaten medal	135	40	37	26	19	11	2	1.56	31.2	12	43.2

T - total number of tested leaves, ms – average degree of disease, Ii – infection index in %, Di – defoliation index in %. Infection scale value from 0 to 5 (Petsi et al. 1990)

T - вкупен број на тестирани листови, ms – просечен степен на болеста, Ii – индекс на инфекција во %, Di – индекс на дефолијација во %.Скала на вредност на инфекција од 0 до 5 (P e t s i et al. 1990).

Fig. 1 - TMV mosaic symptoms on pepper cv. Zlaten medal (susceptible control)

Сл. 1 - TMV симптоми на мозаик на пиперка Златен медал (осетлива контрола)

Fig. 2 - XvPT (left) symptoms on the leaves and defoliation on pepper L57 and *P. capsici* (right) wilt symptoms on pepper cv. Zlaten medal (susceptible control) and L64 resistant plant

Сл. 2 - XvPT (лево) симптоми на листови и дефолијација на пиперката од линија L57 и *P. capsici* (десно) симптоми на венење на пиперката кај сортата Златен медал (осетлива контрола) и L64 отпорно растение

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