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Diseases of pepper in Serbia and results of breeding for resistance

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ABSTRACT

Pepper is one of the major vegetable crops grown in Serbia. According to the total area planted with pepper for fresh market, Serbia is among leading countries in Europe. However, pepper diseases, causing significant yield losses, are one of the limiting factors of successful production. A pepper breeding program in Serbia resulted in several breeding lines tolerant to *Verticillium albo-atrum*, where the most promising is line L-25. By breeding pepper for resistance to *Tobacco mosaic virus* TMV, strain P_0, genotypes carrying genes of resistance L^1L^1, registered as commercial cultivars were selected. Pepper genotypes carrying L^2L^2 genes for resistance to TMV isolates were selected as well. By crossing breeding lines

selected for tolerance to *Cucumber mosaic virus* CMV and lines with incorporated genetic sterility (gene *ms3*) hybrids of pepper tolerant to this virus were created. Breeding pepper for resistance to complex infections (CMV, TMV, *V. albo-atrum*) as well as breeding for resistance to *Xanthomonas axonopodis* pv. *vesicatoria*, races P7 and P8 is under way.

INTRODUCTION

Pepper is one of major vegetable crops grown in Serbia. Large area and intensive production, both in the open field and in greenhouses, make this country one of the leading pepper growing countries in Europe. However, very often the occurrence of pepper diseases, causing significant losses, is a limiting factor for successful production. The most important fungal diseases are verticilium wilt, caused by *Verticillium albo-atrum* (Aleksic et al. 1966, Marinkovic 1985) and pepper wilt caused by *Fusarium oxysporum* (Martinovic 1953, Acimovic 1960). Other fungal diseases, such as damping off the seedling, caused by soil-borne fungi *Rhizoctonia* spp., *Phytophthora* spp., *Fusarium* spp. and *Pythium* spp. also occur frequently (Acimovic 1960, Aleksic et al. 1990). In favourable conditions, *Phytophthora capsici* causes significant losses in the production (Aleksic et al. 1974). *Leveilula taurica* and *Botrytis cinerea* occur sporadically as pathogens of greenhouse grown pepper (Aleksic et al. 1990). The most important bacterial diseases of pepper in Serbia are leaf spot of pepper caused by *Xanthomonas axonopodis* pv. *vesicatoria* (sin. *Xanthomonas campestris* pv. *vesicatoria*) (Panic 1972, Arsenijevic 1997, Obradovic et al. 1997, 1999, 2000) and *Pseudomonas syringae* pv. *syringae* (Arsenijevic 1997, Obradovic et al. 1999, 2000). *Pectobacterium carotovorum* spp. *carotovorum* (sin. *Erwinia carotovora* subsp. *carotovora*) was described as a causal agent of pepper fruit soft rot (Arsenijevic 1972). Among the viruses infecting pepper in Serbia, the most severe losses are caused by *Cucumber mosaic virus* CMV (Delevic 1963, Miladinovic et al. 1985), *Tobacco mosaic virus* TMV (Sutic et al. 1978, Tosic et al. 1979, Mijatovic 1986, 1995), *Tomato mosaic virus* ToMV (Jasnic 1978), and recently by *Potato virus Y* PVY (Mijatovic et al. 1999). *Tomato spotted wilt virus* TSWV, *Alfalfa mosaic virus* AMV, *Potato virus X* PVX are also isolated from pepper in Serbia (Mijatovic et al. 1999). The diseases caused by *Pepper mild mottle virus* PMMV (Krstic et al. 1996), *Pepper mottle virus* PMV, *Broad bean wilt virus* BBWV (Mijatovic et al. 2000) appear sporadically. The yellow wilt of pepper caused by phythoplasma *stolbur* occurs periodically and causes significant losses in pepper production in Serbia. Since growing resistant cultivars is the most efficient control strategy, an intensive pepper breeding program for resistance to some of the most important pathogens was initiated during the last decade.

In the present paper, the authors give the review of breeding work to often causal agents of pepper diseases, *V. albo-atrum*, *Cucumber mosaic virus*, *Tobacco mosaic virus* and *X. axonopodis* pv. *vesicatoria*.

MATERIAL AND METHODS

Choosing parents in breeding pepper for resistance to Verticillium albo-atrum

In breeding pepper for resistance to verticilium wilt, *Capsicum frutescens* L. (L-606) was used as a donor of resistance genes (Marinkovic et al. 1983). The interspecies hybridization between *C. frutescens* (L-606) and *C. annuum* L. was achieved by using *Capsicum* spp. (L-674), an unidentified species from Columbia, as mediator. By crossing the commercial cultivars a progeny tolerant to this pathogen was obtained. In further breeding process, after few back-crosses and three generations of self-pollination, pure lines tolerant to this pathogen were obtained. Line L-25 was used in further breeding process as a donor for resistance and different types of commercial pepper varieties as receptors (Miladinovic et al. 1989). The resistance level of the progeny (F_1, F_2, and F_3) was permanently tested by immersing the four-leaf plant roots into the pathogen (V-P isolate) spore suspension.

Choosing parents in breeding pepper for resistance to Cucumber mosaic virus and Tobacco mosaic virus

In breeding pepper for resistance to CMV, genotypes of *C. baccatum* var. *pendulum* (C-117, C-131), and *C. annuum* ('Punjab Lal', S 118-2, 'Laichi', and 'Perenial') were used as the sources of resistance (Mijatovic et al. 1997). The receptors were some commercial cultivars, as well as lines L-12/84 and L-14/84 partially tolerant to CMV (Miladinovic and Mijatovic 1986). Direct and reciprocal crossings as well as back-crossings with sensitive parents were performed. The resistance of the obtained progeny (phase 6 to 8 leaves) was tested by using the isolate CMV-6.

The TMV (isolate P-11) resistance donors were genotypes *C. annuum* 'Yolo Wonder' and 'Yolo Y' carrying L^1L^1 genes, and genotypes 8/1, 8/2, 8/3, 10/1, 10/6 carrying L^2L^2 genes of *C. chinense* species (Mijatovic 1995). Domestic pepper cultivars, sensitive to TMV, were used as gene receptors. Hybridization was direct and reciprocal. Reaction of the F_1, F_2, F_3 and the following plant generations was tested by mechanical inoculation of the plants with TMV isolate M-5 (P_0) and P-11 (P_1) (Rast 1979).

In order to obtain F_1 hybrid of pepper resistant to TMV and F_1 hybrids tolerant to CMV male sterile mother lines were used. Three breeding lines were selected

for hybridization (two sweet and one hot pepper) with introduced male sterility (gene *ms3*) as mother parent, and four TMV resistant and CMV tolerant father lines. The obtained hybrids were tested for resistance by mechanical inoculation with the TMV isolate M-5 and CMV isolate CMV-6.

C. annuum lines resistant to TMV, tolerant to CMV and line L-25 tolerant to *V. albo-atrum* were selected for breeding of pepper for resistance to complex infections (CMV, TMV, *V. albo-atrum*). Direct and reciprocal crossing as well as back-crossing we applied. The obtained progeny was tested for resistance to all three pathogens. The cotyledon leaves of tested plants were inoculated with TMV isolate M-5. After reaching four-leaf stage, TMV-resistant plants were inoculated with *V. albo-atrum* isolate V-P by dipping the roots into spore suspension 10^6 spores per ml. These plants were inoculated with CMV isolate CMV-6 at six to eight-leaf growth stage. Plant reaction to these pathogens was evaluated 45 days after last inoculation.

Choosing parents in breeding pepper for resistance to Xanthomonas axonopodis pv. *vesicatoria*

Breeding pepper for resistance to *X. axonopodis* pv. *vesicatoria* started in 2003. Pepper 'Early Calwonder' (ECW) and its isogenic lines ECW-10R, ECW-20R, ECW-30R, carrying genes of resistance *Bs-1, Bs-2,* and *Bs-3* respectively, and six commercial domestic pepper varieties sensitive to the pathogen, were selected for breeding program. In order to check the reaction, the F_1 generation plants were inoculated by spraying with bacterial suspension 10^6cfu per ml using pressure 0.1 bar cm^{-2}.

RESULTS AND DISCUSSION

Pepper resistance to Verticillium albo-atrum

Marinkovic et al. (1983) started crossing of C. annuum (L-724) and C. frutescens (L-606) with Capsicum spp. (L-674) as a mediator. After series of back-crossings and three generations of self-pollination, three lines highly tolerant to verticilium wilt were obtained. By crossing C. annuum 'Morava' with C. frutescens line L-606 and Capsicum spp. line L-674, Marinkovic et al. (1992) selected line L-25 with high tolerance to V. albo-atrum and satisfying agro-botanical characteristics. This line was used as a source of resistance in further breeding process. By crossing L-25 and some domestic commercial cultivars ('Palanacka Babura', 'Zeleni Rotund') new breeding lines with high tolerance to this pathogen and good agro-botanical traits were obtained (Marinkovic et al. 1994).

Pepper resistance to Cucumber mosaic virus and Tobacco mosaic virus

As a result of breeding pepper for resistance to CMV using (*C. annuum* × *C. chinense*) × *C. pendulum* progeny, line L-12 tolerant to the virus was obtained. L-12 was further used as a parent component in creating commercial cultivars 'Palancanka' and 'Severija' (Zecevic et al. 1997). By crossing *C. annuum* 'Perenial', as a donor of resistance, with lines tolerant to this virus (L-12 and L-14) and some commercial cultivars (Mijatovic et al. 1997), in two back-crossings and three generations of self-pollination, four lines with high tolerance to CMV were selected. In order to improve resistance and marketability two back-crossings were performed and plants were grown in few generations in self-pollination. Resulting lines were highly resistant and possessed good production traits (Mijatovic et al. 2001). Besides the mechanical inoculation with CMV-6 isolate, selected lines were tested for resistance in aphid-transmitted infection in open field conditions, and showed high resistance to this virus. Out of all, the lines L-11 C_2, L-4 C_2, L-6 C_2 and L-25 C_2 had the best agro-botanical characteristics such as fruit weight up to 125 g, fruit length 15.5 cm and pericarp thickness 0.5 cm.

Since 1986 (Miladinovic and Mijatovic 1986), many lines of different types of pepper (bell pepper, elongated pepper, pepperoni and pepper with conic fruits) resistant to *Tobacco mosaic virus* (P_0) were obtained. Some of these lines are registered commercial cultivars, such as 'Danica' and 'Virgina'. These lines possess good agro-botanical traits, such as yield, shape, size and colour of fruits and thickness of pericarp. Most lines resistant to this virus are in the final phase of the breeding process. Breeding pepper for resistance to P-11 strain of TMV is also in the final phase and supposed to result in commercial varieties of hot pepper. Hybridization between three lines with introduced genetic male sterility (gene *ms3*) and five lines resistant to TMV resulted in 15 hybrids F_1. All F_1 hybrids were resistant to TMV and four of them (2HMT, 7HMT, 9HMT, 10HMT) were selected according to their commercial characteristics (earliness, yield, shape, size and colour of fruits). By crossing these lines with four lines tolerant to CMV new 8 F_1 hybrids were obtained, and three of them (3HMC, 10 HMC, 26 HMC) were considered suitable for future breeding.

After applying two back-crossings and growing three generations in self-pollination, 4 lines L-700, L-12VO, L-18VO, and L-22VO in F_7 generation were resistant to *Tobacco mosaic virus*, and tolerant to CMV and *V. albo-atrum* L-70.

Pepper resistance to Xanthomonas axonopodis pv. *vesicatoria*

Hybridization between lines ECW-20R and 5 commercial pepper cultivars resulted in 10 lines of F_1 generation. Reaction of 40 plants from each line was tested by artificial inoculation at four-leaf stage. The lines with high resistance to this bacterium, i.e. to P-7 and P-8 races of the pathogen, were selected for further breeding.

CONCLUSION

Breeding for resistance to the most important pepper pathogens resulted in commercial varieties of pepper resistant to *Tobacco mosaic virus* and lines tolerant to *Cucumber mosaic virus* and *V. albo-atrum*. Pepper hybrids F_1 resistant to *Tobacco mosaic virus* and hybrids F_1 tolerant to CMV were also obtained. Lines, possessing resistance to complex infections (TMV, CMV, *V. albo-atrum*), were selected as well. At the moment, the research is focused on breeding pepper for resistance to *X. axonopodis* pv. *vesicatoria*.

REFERENCES

ACIMOVIC M., 1960. Neki paraziti povrtarskih kultura u Vojvodini. Zastita bilja 62: 83-89.

ALEKSIC Z., SUTIC D., ALEKSIC D., 1966. Neki rezultati proucavanja uvenuca paprike u Srbiji. Savremena poljoprivreda 10: 877-890.

ALEKSIC Z., ALEKSIC D., SUTIC D., 1974. Neki rezultati proucavanja *Phytophthora capsici* (Leonian) parazita paprike. Zastita bilja 128-129: 229-240.

ALEKSIC Z., SUTIC D., ALEKSIC D., 1990. Bolesti povrca i njihovo suzbijanje. Nolit, Beograd.

ARSENIJEVIC M., 1972. Prilog proucavanja patogenosti nekih izolata bakterije zbirne vrste *Erwinia carotovora* (Jones), Holland. Zastita bilja 119-120: 183-192.

ARSENIJEVIC M., 1997. Bakterioze biljaka. III izmenjeno i dopunjeno izdanje, Sprint, Novi Sad.

DELEVIC B., 1963. Viroze paprike u SR Srbiji. Zastita bilja 74: 347-437.

JASNIC S., 1978. Proucavanje epidimiologije virusa mosaika paradajza na paprici. Doktorska disertacija, Poljoprivredni fakultet, Novi Sad.

KRSTIC B., KRNJAJA V., MIJATOVIC M., TOSIC M., 1996. Virus blagog sarenila paprike prisutan u Srbiji. Zbornik kratkih sadrzaja. Ist Balkanski simpozijum "Povrce i krompir", Beograd: 164.

MARINKOVIC N., MILADINOVIC Z., ALEKSIC Z., 1983. Development of pepper lines resistant to *Verticillium spp.* Proc. of 5th Eucarpia Meeting on Genetics and Breeding on Capsicum and Eggplant, Plovdiv, Bulgaria: 175-178.

MARINKOVIC N., 1985. Otpornost paprike *Capsicum* spp. prema *Verticilluim albo-atrum* Reinke et Berth. Doktorska disertacija, Poljoprivredni fakultet, Beograd.

MARINKOVIC N., ALEKSIC Z., OBRADOVIC A., MIJATOVIC M., 1992. New pepper line resistant to Verticillium Wilt. Proceedings of VIIIth Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant, Roma, Italy: 195-201.

MARINKOVIC N., MIJATOVIC M., OBRADOVIC A., 1994. Znacaj i problemi selekcije povrca na otpornost prema bolestima. Radovi VI simpozijuma sa medjunarodnim ucescem "Povrce i krompir" , Savremena poljoprivreda, vanredni broj: 67-72.

MARTINOVIC M., 1953. Bolesti povrtarskih biljaka. Zadruzna knjiga, Beograd.

MIJATOVIC M., 1986. Reakcija nekih sorata paprike prema virusu mozaika duvana. Magistarska teza, Poljoprivredni fakultet, Beograd.

MIJATOVIC M., 1995. Proucavanje otpornosti paprike prema virusu mozaika duvana. Doktorska disertacija, Poljoprivredni fakultet, Beograd.

MIJATOVIC M., OBRADOVIC A., STEVANOVIC D., MILADINOVIC Z., ZECEVIC B., 1997. Recent research results of pepper breeding for resistance to cucumbre mosaic virus. Proc. 10th Congres of the Mediterranean Phytophatological Union, Monpellier, France: 593-596.

MIJATOVIC M., OBRADOVIC A., IVANOVIC M., STEVANOVIC D., 1999. Rasprostranjenost i intezitet nekih virusa parazita paprike u Srbiji. Zasita bilja 228: 151-159.

MIJATOVIC M., IVANOVIC M., OBRADOVIC A., ZECEVIC B., 2000. Potato virus Y on pepper in Serbia. Book of Abstracts from 2nd Balkan Symposium on Vegetables and Potatoes, Thessaloniki, Greece: 152.

MIJATOVIC M., ZECEVIC B., STEVANOVIC D., MILADINOVIC Z., 2001. Virus diseases of pepper in Yugoslavia and breeding for resistance. Proceedings of the 11th Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant. Antalya, Turkey: 308-313.

MILADINOVIC Z., MIJATOVIC M., ALEKSIC Z., 1985. Reakcija nekih sublinija međuvrsnih hibrida paprike prema virusu mozaika krastavca. Zastita bilja 171: 33-38.

MILADINOVIC Z., MIJATOVIC M., 1986: Rezultati selekcije paprike na otpornost prema virusu mozaika duvana. Zastita bilja 178: 335-341.

MILADINOVIC Z., STEVANOVIC D., MIJATOVIC M., 1989. Breeding of pepper for resistance to tobacco mosaic virus, cucumber mosaic virus and verticillium wilt. Proc. of 7th Eucarpia Meeting on Genetics and Breeding on *Capsicum* and Eggplant. Kragujevac, Yugoslavia: 217-221.

OBRADOVIC A., ARSENIJEVIC M., MIJATOVIC M., 1997. Bakterioze paprike. Zbornik rezimea III jugoslovensko savetovanje o zastiti bilja. Zlatibor: 33-34.

OBRADOVIC A., ARSENIJEVIC M., MIJATOVIC M., 1999. Odredjivanje rasa bakterija *Xanthomonas campestris* pv. *vesicatoria* patogena paprike u Jugoslaviji. IV jugoslovensko savetovanje o zastiti bilja. Zlatibor: 86.

OBRADOVIC A., MAVRIDIS A., RUDOLPH K., ARSENIJEVIC M., MIJATOVIC M., 2000. Bacterial diseases of papper in Yugoslavia. Book of abstracts Xth International Conference on Plant Pathogenic Bacteria. Charolottetown, Canada: 50.

PANIC M., 1972. Vaznije bakterioze gajenih biljaka. Skripta Poljoprivrednog fakulteta Beograd.

RAST A. T. B., 1979. Pepper strains of TMV in the Netherlands. Med. Fac. Landb. Wet. Ryksinev Gent. 44/2: 617-622.

TOSIC M., IVANOVIC M., MITROVIC G., KRSMANOVIC Z., KOJIC Z., 1979. Prilog poznavanju viroza paprike u nasoj zemlji. Zastita bilja 150: 335-343.

SUTIC D., TOSIC M., PESIC Z., 1978. Virus mozaika duvana prouzrokovac nekroze paprike. Zastita bilja 146: 309-315.

ZECEVIC B., STEVANOVIC D., MIJATOVIC M., 1997. Koriscenje genetickih resursa roda *Capsicum* u oplemenjivanju paprike (*Capsicum annuum* L). Savremena poljoprivreda 3-4: 66-72.

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