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in memoriam
Dr. Alain Palloix

PROCEEDINGS

Editors:
Katalin Ertsey-Peregi
Zsuzsanna Füstös
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Proceedings

of

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Sweet pepper breeding against bacterial spot (*Xanthomonas euvesicatoria*) in Serbia

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Abstract

Bacterial spot of pepper (BS) caused by *Xanthomonas euvesicatoria* is one of the most important pepper diseases in Serbia. Growing of resistant pepper genotypes is the most efficient way of BS control. The study was started in 2013, when eight pepper varieties from the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS), Cal wonder and isogenic line from Early Cal wonder (ECW-20R) were inoculated with *X. euvesicatoria* strain P8 in the field conditions. Intensity of infection was evaluated according to the Horsfall-Barratt (HB) scale. Nonparametric Kruskal-Wallis test was used for statistical analysis. All tested varieties were susceptible, except ECW-20R. In the same year, several IFVCNS pepper varieties were crossed with ECW-20R and also with line 30. After crossing 3 hybrids were sown in a greenhouse in October 2013 to obtain F2 generation. Those F2 transplants with resistant parents and Cal Wonder were planted 2014 in open field and inoculated. In the other field, IFVCNS varieties (Amfora, Una, Matica), line 30, ECW-20R and their 6 hybrids were evaluated under natural infection. Hybrids showed a significant resistance in relation to susceptible parents, except combination Matica x ECW-20R. Seeds of selected plants (F2 and F1 generation) from those two fields were sown for transplants producing in 2015 in order to obtain F3 and F2 generation. Transplants were inoculated in a greenhouse. Healthy plants were transplanted in the open field and after three months evaluated again. Progenies from ECW-20R were more resistant than progenies from line 30.

1. Introduction

Bacterial spot (BS), caused by *Xanthomonas euvesicatoria* (Xe-Group A) is a widespread and it is considered as one of the most common pepper diseases in Serbia (Ignjatov et al. 2010). Pathogen is seed-borne and during past few years, disease appeared regularly caused great damage and complete death of the plants (Ignjatov et al. 2010). Pepper ranked as the second highest vegetable crop in Serbia (grown on about 20.000 ha of open field and plastic covered greenhouses) and BS is considered to be an economically very important disease. Presence of diseased plants in certified pepper production fields affects the certification eligibility of the crop, as defined by certification rules and regulations. When weather conditions are favorable for disease development, pepper producers do not have adequate resources to control this pathogen. Copper based bactericides registered in our country are not effective enough (Šević et al. 2015). Breeding programmes for BS-resistance are considered as one of the most effective strategic measures for controlling the disease. However, the development of resistance has been limited by the high degree of genetic and phenotypic diversity within the *Xanthomonas* species complex. Resistance to BS of pepper is conferred by single dominant genes *Bs1*, *Bs2*, *Bs3* and *Bs4* (APS-ISF, 2010). So far, 11 physiological races of *X. euvesicatoria* were described around the world. Four of them (P1, P3, P7, P8) are recorded in Serbia, with P8 predominating (Obradović et al. 2004; Ignjatov et al. 2015). In Serbian growing conditions the most cultivated pepper genotypes showed various degree of susceptibility to *X. euvesicatoria* (pepper race 8) (Ignjatov et al. 2012). Considering that the most of the studied genotypes were sensitive to *X. euvesicatoria* (P8), with exception of the isogenic line ECW-20 carrier of *Bs2* resistance gene,

transfer of this gene into commercial varieties of pepper would be significant contribution to control of this economically important disease. At the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS) eleven pepper varieties with different fruit types were developed (Gvozdenović et al. 2002). So far IFVCNS pepper varieties were not resistant to bacterial spot. Since 2013 we have started breeding program to develop new varieties resistant to this disease because of the market demands.

2. Materials and Methods

The study was started in 2013, when eight pepper varieties (Amfora, Anita, Atina, Matica, Novosađanka, Plamena, Una and Vranjska) from the Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS), Cal Wonder and isogenic line from Early Cal Wonder (ECW-20R) were sown on 3 April 2013 in plastic tunnel. Young plants were transplanted two months later to the open field. Spacing between plants was 70 x 25 cm in all trials. Copper products or other bactericides were not applied in the trials. The inoculation was done a month and a half after transplanting of the plants. Plants were inoculated with *X. euvesicatoria* strain P8 with concentration 10^6 CFU/ml and covered with foil overnight. (Figure 1).

Figure 1:
Inoculation with X. euvesicatoria in field conditions

Strain was isolated from pepper (variety Amfora) in 2011 locality Rimski Šančevi (near Novi Sad). Prior to the inoculation plants were irrigated by sprinklers to obtain optimal conditions for infection. The leaf spot evaluation was done three times. Data from third evaluation in the beginning of October were shown. In the same year, several IFVCNS pepper varieties were crossed with ECW-20R and line 30. After crossing 3 hybrid combination (Anita x ECW-20R, Amfora x ECW-20R and Matica x ECW-20R) were sown in the end of October 2013 in a greenhouse to obtain seed for F2 generation during winter period. Those F2 seed with ECW-20R resistant parents and Cal Wonder were sown in the beginning of April 2014 in plastic tunnel. Plants were transplanted in the beginning of June in open field and inoculated 40 days

later according to the same procedure. The leaf spot evaluation was carried out three times and final evaluation which was done in the end of September was shown (Figure 2, 3 and 4).

Figure 2:
Inoculated plant of Cal Wonder variety in 2014

Figure 3:
Inoculated plant of ECW-20R in 2014

Amfora, Una, Matica, line 30, ECW-20R and their 6 hybrids were sown in plastic tunnel together with plants for artificial inoculation. These plants were transplanted in the end of May in the other field. The intensity of bacterial leaf spot on these plants was evaluated under natural infection. The final evaluation in the end of September 2014 was shown.

Seeds of selected plants (F2 and F1 generation) from those two fields were sown on 6 April 2015 for producing transplants in order to obtain F3 and F2 generation. Transplants were inoculated in a greenhouse 40 days later. Evaluation of plants was done 18 days after inoculation. Plants without bacterial leaf spot were transplanted in the beginning of June to the open field and after three months evaluated again.

Figure 4:
Pepper line 30 after inoculation in 2014

Intensity of infection was evaluated according to the Horsfall-Barratt (HB) scale. Nonparametric Kruskal-Wallis test was used for statistical analysis because values from HB scale were not normally distributed. Data were analyzed using STATISTICA for Windows version 12.

3. Results and Discussion

According to results in 2013 all tested varieties were moderately susceptible (medians 5-6), while ECW-20R was resistant (Figure 5). Number of evaluated plants was recorded on the upper part of each graph as a second number in the bracket.

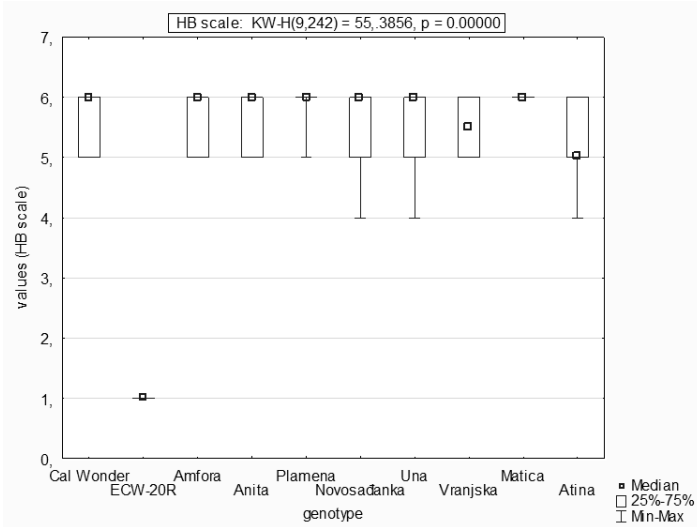

Figure 5:
HB values of pepper genotypes 84 days post inoculation (DPI) with X. euvesicatoria in 2013

Since a large amount of precipitation in 2014 we did not performed artificial infection in a selection plot for F1 progenies, because there were favorable conditions for the development of BS. Hybrids developed by crossing susceptible parents with resistant parents (ECW-20R and line 30) were more resistant comparing to susceptible parents in the condition of natural infection except combination Matica x ECW-20R (Figure 6). Researched the heritability to BS, Riva et al. (2004) reported that this parameter for reaction to diseases were somewhat variable as depend on the population and the environment studied. Our research confirmed this claim because values in HB scale for Matica x ECW-20R F1 varied from 1-4.

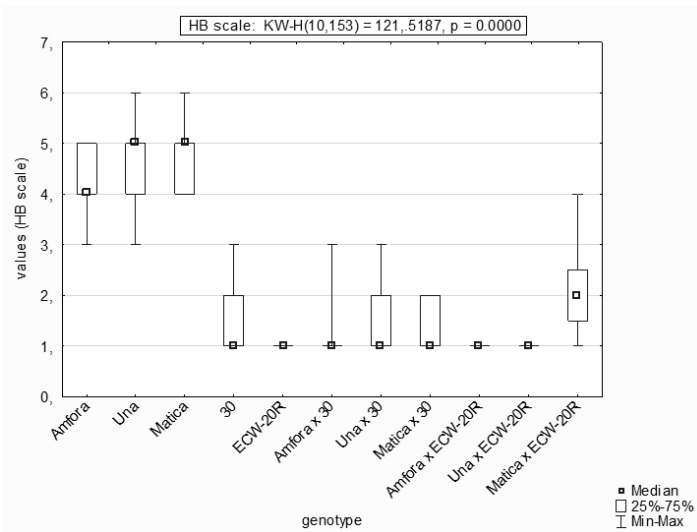

Figure 6:
HB values of pepper genotypes in natural infection with X. euvesicatoria in 2014

All ECW-20R plants were resistant (median 1) in the conditions of artificial inoculation in 2014, while in other genotypes there were plants with different level of resistance (Figure 7). Especially in F2 generation as it was expected the values ranged from 1-4 or 2-6. There was no significant difference between line 30 and ECW-20R, but line 30 was more variable.

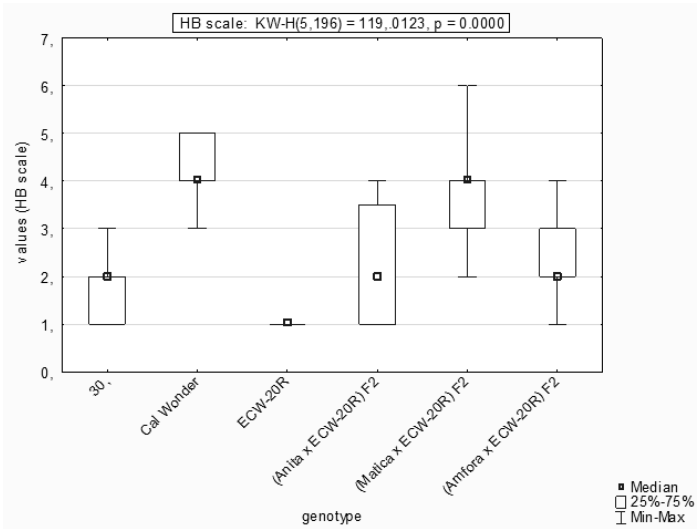

Figure 7:
HB values of pepper genotypes 73 DPI with X. euvesicatoria in 2014

Moderately susceptible were Cal Wonder and (Matica x ECW-20R) F2. Plants (Anita x ECW-20R) F2 were between those two groups. Since there were no plants with grade 1 in the progeny (Matica x ECW-20R) F2 this combination was discarded from further breeding.

ECW-20R was the most resistant genotype in 2015 trial (Figure 8). Plants from combination (Una x ECW-20R) F2, (Anita x ECW-20R) F3 and (Amfora x ECW-20R) F3 were not significantly different from ECW-20R. According to results from artificial infection progenies from the combination of susceptible parents and ECW-20R were more resistant (medians 1) than progenies from line 30 (medians 3 and 5). Line 30 was more susceptible (median 4) in 2015 than in 2014 (median 2). This could be explained that inoculation in 2015 was done when plants were in younger phase.

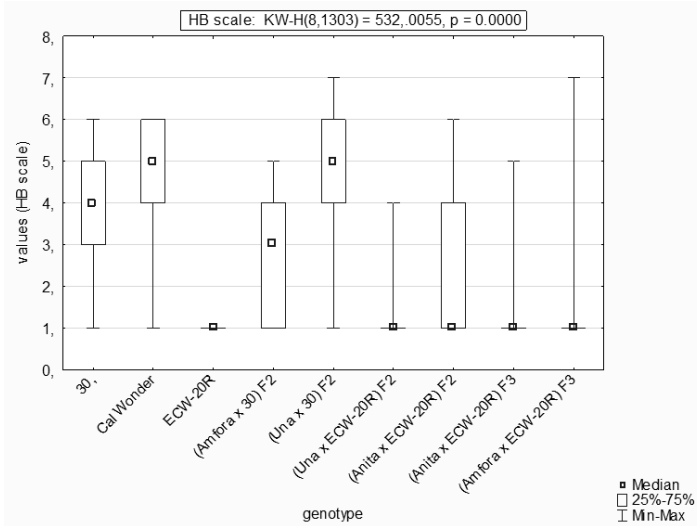

Figure 8:
HB values of pepper genotypes 18 DPI with X. euvesicatoria in 2015

Three months after inoculation median values were lower because of discarded susceptible plants (Figure 9). Also in repeated BS evaluation progenies from ECW-20R were more resistant (medians 1) than line 30 and progenies from line 30 (medians 2). At the moment ECW-20R (*Bs*$_2$ gene) is good donor for BS resistance in Serbia conditions. This gene *Bs*$_2$ is still functioning in Korea and may be used in breeding for resistance (Wai et al. 2015).

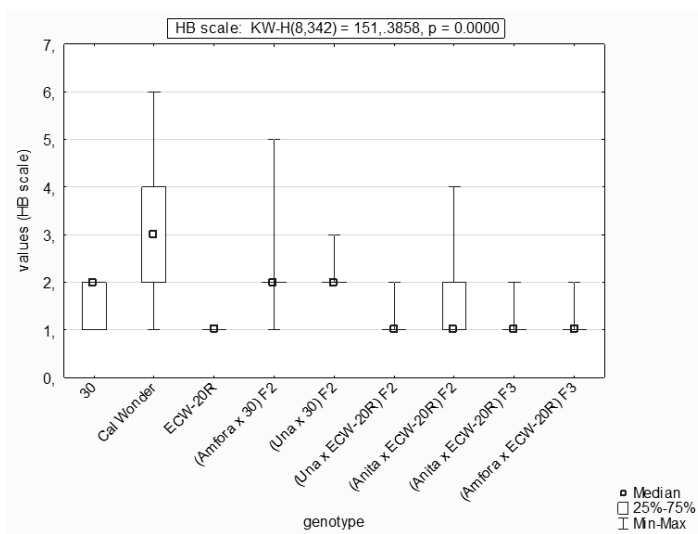

Figure 9:
HB values of selected pepper genotypes 90 DPI with X. euvesicatoria in 2015

4. Conclusion

In further selection process, plants will be also inoculated and measured for fruit traits and yield. Progenies from line 30 will not be used in breeding program because of lower resistance than progenies from ECW-20R. Backcross breeding with IFVCNS varieties will be applied if fruits traits from selected plants will not be commercially suitable. Also we plan to use marker assisted selection to speed up the breeding process.

References

APS-ISF (2010). Guideline for identification of pepper bacterial leaf spot races using differential hosts (Kurowski C., Conn K., Himmel P.)

Gvozdenović Đ., Bugarski D., Takač A., Červenski J. (2002). Amfora - nova sorta paprike. Zbornik radova Instituta za ratarstvo i povrtarstvo u Novom Sadu 36: 69-73.

Ignjatov M., Gašić K., Ivanović M., Šević M., Obradović A., Milošević M. (2010). Karakterizacija sojeva Xanthomonas euvesicatoria, patogena paprike u Srbiji. Pesticidi i fitomedicina, 25 (2): 139-149.

Ignjatov M., Šević M., Gašić K., Jovičić D., Nikolić Z., Milošević D. Obradović A. (2012). Proučavanje osetljivosti odabranih genotipova paprike prema prouzrokovaču bakteriozne pegavosti Rat/Pov 49 (2): 177-182.

Ignjatov M., Šević M., Gvozdanović-Varga J., Gašić K., Milošević M., Obradović A. (2015). Race differentiation within strains of Xanthomonas euvesicatoria causal agent of bacterial spot of pepper in Serbia. Proceedings of the 7th Congress on Plant Protection „Integrated Plant Protection – a Knowledge-Based Step towards Sustainable Agriculture, Forestry and Landscape Architecture" (November 24-28, Zlatibor, Serbia. Plant Protection Society of Serbia, IOBC-EPRS, IOBC-WPRS, Belgrade, 297 – 300.

Obradović A., Mavridis A., Rudolph K., Janse J. D., Arsenijević M., Jones J. B., Minsavage G. V., Wang J. F. (2004). Characterization and PCR-based typing of Xanthomonas campestris pv. vesicatoria from peppers and tomatoes in Serbia. European Journal of Plant Pathology, (110): 285–292.

Riva E. M., Rodrigues R., Pereira M. G., Sudré C. P., Karasawa M. (2004). Inheritance of bacterial spot disease in Capsicum annuum L. Crop Breeding and Applied Biotechnology 4: 490-494.

Šević M., Gašić K., Đorđević M., Ignjatov M., Mijatović M., Zečević B., Obradović A. (2015). Integration of biological and chemical methods in control of pepper bacterial spot. Proceedings of the 7th Congress on Plant Protection „Integrated Plant Protection – a Knowledge-Based Step towards Sustainable Agriculture, Forestry and Landscape Architecture" (November 24-28, Zlatibor, Serbia. Plant Protection Society of Serbia, IOBC-EPRS, IOBC-WPRS, Belgrade, 49 – 51.

Wai K. P., Siddique M. I., Mo H. S., Yoo H. J., Byeon S. E., Jegal Y., Mekuriaw A. A., Kim B. S. (2015). Pathotypes of Bacterial Spot Pathogen Infecting Capsicum Peppers in Korea. Plant Pathology Journal 31(4):428-432.