DETECTION OF THE VIRUSES ON SELECTED LINES OF DWARF DRY BEAN (Phaseolus vulgaris var. nanus Martens) USING ELISA-TEST

Edyta HALUPECKI¹, Bogdan CVJETKOVIĆ², Dean BAN³, Josip BOROŠIĆ⁴

^{1,2}Department of Plant Pathology, Faculty of Agriculture, Zagreb, Croatia ³Institute of Agriculture and Tourism, Poreč, Croatia ⁴Vegetable Crops Department, Faculty of Agriculture, Zagreb, Croatia

ABSTRACT

Species from the genus *Phaseolus*, including *Phaseolus vulgaris*, can be naturally infected by viruses that can cause significant economic damage through the reduction of yield and quality. Some of the most important viruses of beans are Bean common mosaic (BCMV), Bean common mosaic necrosis (BCMNV) and Bean yellow mosaic virus (BYMV). In breeding it is very important to determine presence of viruses in newly bred lines, especially viruses that are transmitted through bean seed. Material resistant or tolerant to viral infection is favored in breeding programs. Plants were visually examined for five years. The symptoms on newly bred lines of the dwarf dry bean in the field were monitored. The frequency of viral infections was determined using ELISA-test. Results of these visual examinations and analysis enabled us to form a collection of newly bred lines of the dwarf dry bean, which are less susceptible to viral infections. Determined health conditions of these lines of dwarf dry bean would be, in presence of the satisfactory production characteristics, decisive for further breeding in order to produce new cultivars.

Key words: BCMV, BCNMV, bean, BYMV, ELISA

IZVLEČEK

DETEKCIJA VIRUSOV V SELEKCIONIRANIH LINIJAH NIZKEGA FIŽOLA (*Phaseolus vulgaris* var. *nanus* Martens) Z ELISA TESTOM

Vrste iz roda *Phaseolus*, vključno z navadnim fižolom (*Phaseolus vulgaris*), okužujejo virusi, ki lahko povzročijo znatno gospodarsko škodo (zmanjšanje pridelka in kakovosti). Nekateri najpomembnejši virusi fižola so Bean Common Mosaic (BCMV), Bean Common Mosaic Necrosis (BCMNV) in Bean Yellow Mosaic Virus (BYMV). Pri žlahtnjenju fižola je zelo pomembno, da ugotovimo viruse pri novih linijah, zlasti viruse, ki se prenašajo s semenom. V programih žlahtnjenja je uporaben zlasti rastlinski material, ki je toleranten na virusne okužbe. V 4-letnih raziskavah smo z vizualnim pregledovanjem znamenj okuženosti novo vzgojenih linij *P. vulgaris* L. var. *nanus* Martens na polju, v biotičnih testih in z ELISA testom, spremljali pogostnost virusnih okužb naštetih virusov. Rezultati teh analiz omogočajo, da ustvarimo fond novih linij nizkega fižola, ki so manj dovzetne za virusne okužbe. Zdravstveno stanje teh linij nizkega fižola bo, poleg ugodnih agronomskih lastnosti, odločilno pri nadaljnjem žlahtnjenju za pridobivanje novih kultivarjev.

Ključne besede: BCMV, BCNMV, fižol, BYMV, ELISA

1 INTRODUCTION

Viruses are one of the major problem in bean production since *Phaseolus vulgaris* can be naturally infected by viruses that can cause significant economic damage through the reduction of yield and quality (Hall, 1994). Some of the most important viruses of beans are Bean common mosaic virus (BCMV), Bean common mosaic necrosis virus (BCMNV)

³ Dr. sc., C. Huguesa 8, 52440 Poreč, Croatia

¹ M. Sc., Svetošimunska 25, 10000 Zagreb, Croatia

² Prof. dr. sc., ibid.

⁴ Prof. dr. sc., Svetošimunska 25, 10000 Zagreb, Croatia

and Bean yellow mosaic virus (BYMV). All of these three viruses belong to genus Potyvirus. In breeding it is very important to determine presence of viruses in newly bred lines, especially viruses that are transmitted through bean seed. The material resistant or tolerant to viral infection is favored in breeding programs.

BCMV is probably distributed worldwide wherever *Phaseolus* beans are grown. It is transmitted in non-persistent manner by an insect vectors (e. g. *Acyrthosiphon pisum*, *Aphis craccivora*, *A. fabae* and *Myzus persicae*) (Bos, 1971). It can be transmitted by mechanical inoculation and by seed (in *Phaseolus vulgaris* up to 83%) (Brunt *et al.*, 1996). Virions of the BCMV are filamentous, not developed of about 860 x 13 nm. Genome consists of single-stranded linear RNA. BCMV on bean cause systemic mosaic, malformation of leaves and pods and rugosity of lower leaves, sometimes even necrosis.

BCMNV (syn. Bean common mosaic virus - serotype A) is variety of Bean common mosaic virus but it cause more severe symptoms including severe mosaics that can evolve to vein banding or necrosis of whole leaves. All other characteristics of the virions and transmission are almost the same like in BCMV.

BYMV is present worldwide. It is transmitted in a non-persistent manner by an insect vectors (more than 20 spp. of Aphididae) (Bos, 1970). It can be transmitted by mechanical inoculation and by seed (according some authors; only up to 3%) (Brunt *et al.*, 1996). Viral particles of BYMV are filamentous, usually flexuous. Their dimensions are about 750 x 13 nm. Genome consists of linear single-stranded RNA. BYMV on beans cause severe yellow systemic mosaics, malformations and sometimes even tip necrosis.

2 MATERIALS AND METHODS

The symptoms of virus infection on 30 newly bred lines of the dwarf dry bean *Phaseolus vulgaris* L. var. *nanus* Martens in the field were monitored. These lines of dwarf dry bean were bred at the Vegetable Crops Department of the Faculty of Agriculture in Zagreb. Plants were visually examined for five years in the field in Zagreb. In plants showing symptoms presence of the viruses was determined using DAS ELISA-test. Serums for detection of the Bean common mosaic virus (BCMV-serotype B), Bean common mosaic necrosis virus (BCMV-serotype A) and Bean yellow mosaic virus (Bean Virus 2) were obtained from Loewe Biochemica (Germany) and analysis were performed according to protocol of the manufacturer.

3 RESULTS AND DISCUSSION

In this study in newly bred lines of dwarf dry bean were found all of three above mentioned viruses (BCMV, BCMNV and BYMV) were found according symptoms in the field (Figs. 1 and 2) and using ELISA-test.

In five years of investigation (1998-2002) through visual examinations of the symptoms on bean plants in the field we have found that new lines of the dwarf dry beans, *Phaseolus vulgaris* L. var. *nanus* Martens, were more often infected with Bean common mosaic virus than with Bean yellow mosaic virus, followed by Bean common mosaic necrosis virus.

Results of these visual examinations of viral symptoms incidence, judged by the percentage of plants showing symptoms, and analysis enabled us to form a collection of newly bred lines of the dwarf dry bean, which are less susceptible to viral infections (Table 1).

Further studies are needed in order to describe the natural circle of surviving of the viruses in the field with emphasis on impact of virus transmission through the seeds and through the alternative host plant species and insect vectors in fields.



Fig. 1: Leaves of dwarf dry bean (Phaseolus vulgaris var. nanus Martens) infected with BCMV.



Fig. 2: Leaves of dwarf dry bean (left: healthy leaf, right: leaf showing symptoms of BYMV).

4 **CONCLUSIONS**

Newly bred lines of dwarf dry bean that were found less susceptible to viral infections in this study through five years were: 12-94-11, 12-94-3,12-94-3-2, 14-94-47, 15-94-X, 18-94-34, 18-95-22, 18-95-44 and 18-95-7 (ŠT).

Determined health conditions of these lines of dwarf dry bean would be, in presence of the satisfactory production characteristics, decisive for further breeding in order to produce new cultivars.

Table 1: Percentage of plants (mean values) from different lines of dwarf dry bean showing viral symptoms (overall results for symptoms caused by three viruses: Bean common mosaic, Bean common mosaic necrosis and Bean yellow mosaic virus).

Line/Year 10-94-22 10-94-45-1 11-94-14	1998. 2 2 1 1	1999. 3 3,33 3,33	2000.	2001. 0,33 0,33	2002. 1,75
10-94-45-1	2	3,33		· · · · · · · · · · · · · · · · · · ·	*
	1	*		0.33	0.67
11-94-14		3,33		0,55	0,67
	1	- ,		2	1
11-94-15		3		0,67	1,75
11-94-5	1,67	4,33		0,33	1
12-94-11*	0,33	2,33		0,67	2
12-94-13	2,67	3,67	3	1,33	2,33
12-94-3*	0,33	1,67	2	0	0,67
12-94-3-2*	0,33	3		1	0,67
12-94-3-3	1	4		0,33	2,33
12-94-6	1	4		0	2
13-94-18	0,5	2,33	2	0,67	1
14-94-18				0,33	1,82
14-94-47*	0	2,67		2	1
15-94-12	1	2,33	2	0,33	1,67
15-94-20	0,33	6		0,67	1
15-94-27	1,5	2,67		0	0
15-94-3	0,5	2,67		2,67	1,33
15-94-50	0,5	3		1,67	2
15-94-X*	0,33	3,67		1	1
16-94-19	0,5	8,33		6	1,67
18-94-14-2-Š	0,5	3		0,67	
18-94-34*	0	0,67		1	0,33
18-94-7-ŠT	0	1,5			0
18-95-22*	0	2,33		0,33	0,67
18-95-44*	0	2		0,33	1
18-95-45	0,6	1		0	0,75
18-95-45-1				2	1,67
18-95-7 (ŠT)*	0	6		1	1,5
9-94-29	2	2,67		1,33	1,67

^{* -} Newly bred lines of the dwarf dry bean that were less susceptible to viral infections in this study. (Note: empty cells stands for certain lines that were not analyzed in certain year.)

5 REFERENCES

Hall, R. 1994. Compendium of Bean Diseases, APS Press, St. Paul, Minnesota, USA.

Bos, L. 1970. Bean Yellow Mosaic Virus, C.M.I./A.A.B. Descriptions of Plant Viruses, Commonwealth Agricultural Bureaux and the Association of Applied Biologists, Wm. Culross and Son Ltd., Coupar Angus, UK.

Bos, L. 1971. Bean Common Mosaic Virus, C.M.I./A.A.B. Descriptions of Plant Viruses, No. 73, Commonwealth Agricultural Bureaux and the Association of Applied Biologists, Wm. Culross and Son Ltd., Coupar Angus, UK.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., Zurcher, E.J. 1996. Bean common mosaic *potyvirus*, Bean yellow mosaic *potyvirus* and Bean common mosaic necrosis *potyvirus*. In: `Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' (http://biology.anu.edu.au/Groups/MES/vide/).