



**Identification of *I*-gene for BCMV Resistance with Scar Marker SW13 in
Phaseolus vulgaris L. Generations, Derived from the Cross
(BAT 477 X Dobroudjanski Ran)**

^aVladimir KRASDEV, ^bGancho PASSEV, ^bDimitrina KOSTOVA-PROTOCHRISTOVA,
^aDiana SVETLEVA*

^aAgricultural University – Plovdiv, Bulgaria

^bMaritsa Vegetable Crops Research Institute – Plovdiv, Bulgaria

Corresponding author: svetleva@yahoo.com

Abstract

Resistance to BCMV (*Bean common mosaic virus*) was established in *Phaseolus vulgaris* variety BAT 477, following artificial inoculation with the virus. Crosses were performed between BAT 477 and BCMV susceptible parent Dobrudjanski ran, a widely spread Bulgarian variety, in order to transfer the resistance in the generations. By PCR, using SCAR marker SW 13, the presence of *I*-gene for BCMV resistance was proved in donor parent BAT 477, in all F₁ plants and in segregating F₂. The monogenic control of resistance was demonstrated by 690 bp fragment amplification in 75 plants and absent of fragment in the remaining 31 plants. The ratio of segregation was approximately 3:1, as evidenced by the criterion " χ^2 " at level of significance 0,50>P>0,20.

Key words: *Bean common mosaic virus* (BCMV), *I*-gene, *Phaseolus vulgaris* L.

Introduction

Bean common mosaic virus (BCMV) is widely spread pathogen on beans (*Phaseolus vulgaris* L.). The resistance towards BCMV is controlled by one dominant *I*-gene and five recessive genes: *bc-u*, *bc1*, *bc1²*, *bc2*, *bc2²*. Recessive genes are expressed and active only in the presence of strain-unspecific recessive gene *bc-u* or in the presence of *I*-gene (Drijfhout, 1978; Kelly et al., 1995). Bean varieties, possessing *I*-gene were resistant to mosaic strains and to temperature-dependent necrotic strains of BCMV (below 30°C). Latter necrotic strains appeared, causing hypersensitive systemic necrosis in varieties with *I*-gene, at all temperature. These so-called temperature-independent strains were separated as a new virus called *Bean common mosaic necrosis virus* (BCMNV) (Vetten et al. 1992; McKern et al., 1992; Khan et al., 1993). BCMNV caused severe necrosis and dead of the plants with *I*-gene.

Haley et al. (1994) identified and offered a marker for breeding purposes, which was linked with dominant *I*-gene. The Random Amplified Polymorphic DNA (RAPD) marker OW13 690 was at 1,3-5 cM in *cis*-position of *I*-gene. It amplified a

fragment of 690bp. But this marker appeared to be not enough reliable due to the low reproducibility of the results. It was converted in SCAR marker SW13, with longer primers and amplifying only one fragment with the same size (Melotto et al., 1996). SW13 was tested in collection of diverse bean genotypes, representing the two main centers of origin – Andean and Mesoamerican gene pools (Singh et al., 1991). In all cases presence or absent of SCAR marker SW13 in tested materials was connected with the presence or absent of *I*-gene which emphasized the importance of the marker in genetic investigations.

The aim of the current investigation was to identify *I*-gene in parents, F₁ and F₂ generations using SCAR marker SW13 in order to separate the resistant genotypes as starting materials in breeding programs.

Materials and methods

Plant material

Two bean varieties (BAT 477, as a female parent and Dobrudjanski ran, as a male parent) were used in the breeding program for resistance to

BCMNV. Parents were crossed to obtain F₁ and F₂ progenies.

For identification of dominant *I*-gene in parent varieties 10 days old bean plants of BAT 477 and Dobrudjanski ran were inoculated with NL3 strain of BCMNV. The inoculum was prepared from infected leaves of the sensitive variety Sutter pink with clear virus symptoms, ground in a buffer (1% K₂HPO₄ and 0,1% Na₂SO₃) in ratio 1:10 w/v with addition of carborundum 600 mesh. Experiment was performed in an insect proof growing chamber at 18-25°C and 14/10 h, photoperiod. Symptoms (primary and systemic) were assessed periodically up to 30-th day after inoculation.

DNA isolation

DNA was isolated from young leaves collected from parent genotypes BAT 477, Dobrudjanski ran, F₁ and 106 plants from F₂ progeny by standard CTAB procedure (Edwards et al., 1991). DNA pellet was resuspended in dH₂O. Concentration was determined on a UV-VIS spectrophotometer at 260 nm. All DNA samples were adjusted to 50 ng/μl.

Polymerase chain reaction (PCR)

PCR reactions were performed with SW13 SCAR marker primers (5'-CACAGCGACATTAATTTTCCTTTC-3' and 5'-CACAGCGACAGGAGGAGCTTATTA-3'). Reaction mixture contained 1x PCR buffer, 1,5 mM MgCl₂, 0,4 mM dNTPs, 0,3 μM primers and 0,2 U BioTaq™ Polymerase (Bioline reagents

Ltd., London, UK) and 1 μl genomic DNA (50 ng/μl). Amplification reaction was carried out in TGradient (Biometra GmbH, Germany) with PCR program consisted of initial denaturation step for 4

min at 95°C followed by 35 cycles for 30 sec at 94°C, annealing step for 40 sec at 60°C, elongation step for 2 min at 72°C; final extension for 5 min at 72°C.

Five μl of every PCR product were analyzed on 1% TAE agarose gel. Electrophoresis was performed for 45 min at 150 V. Fragments were stained with DNA StainG (Serva, Germany) and visualized under UV light.

Results and Discussion

Both parent genotypes expressed two different reactions after inoculation with NL3 strain of BCMNV. The female parent BAT 477 developed local necrotic lesions on inoculated leaves e.g. reacted as hypersensitive genotype, 3 to 4 days after inoculation. Top necrosis and dead of all plants was reported on the 6th – 7th day, similar to the control differential variety Widusa (carrier of a single dominant *I*-gene). Obviously such phenotype proved the presence of *I*-gene in female parent BAT 477. The male parent Dobrudjanski ran appeared to be virus susceptible by developing mosaic symptoms and leaves deformations.

Molecular analyses with SCAR marker SW13 identified presence of *I* gene in BAT 477. A fragment with expected size of 690 bp was amplified. Dominant gene was proved also in all tested F₁ progeny. Segregation of inheritance of SW13 marker linked to *I*-gene was observed in tested 106 F₂ progeny. Amplicon with expected size was scored in 75 plants, while in 31 plants PCR product was not observed (Fig. 1) (Melotto et al. 1996; Sharma et al. 2008).

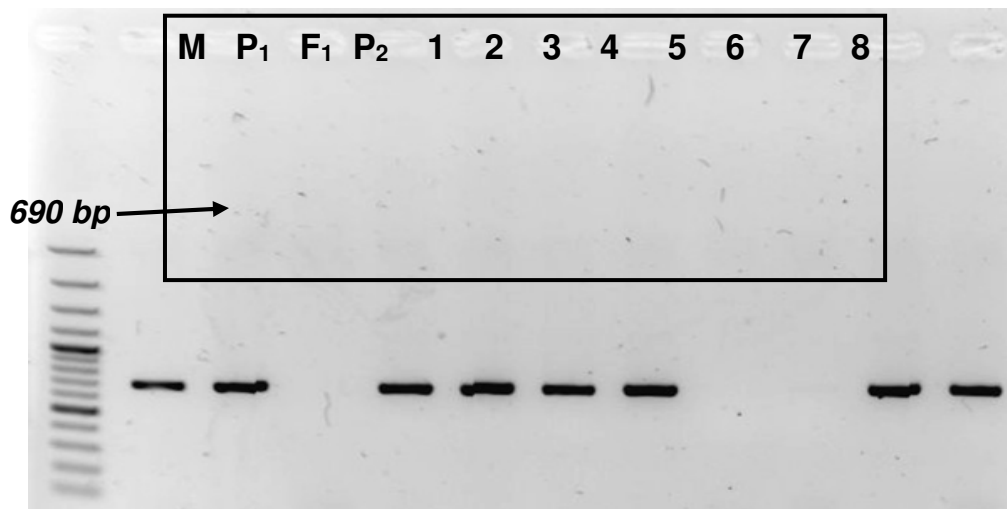


Fig. 1. PCR analysis with SCAR marker SW13 for identification of *I*-gene.

M - Gene Ruler™ 100 bp Plus DNA ladder; P₁ – BAT 477; F₁; P₂ – Dobrudjanski ran; 1 to 8 - F₂ plants.

Table. 1 Segregation of SCAR marker SW 13 in 106 F₂-plants

PCR products	Observed	Expected	χ^2	P
presence	75	3	0,25	0,50>P>0,20
absence	31	1	0,76	
Total:	106	4	1,01	

Segregation ratio was approximately 3:1, proved at probability level 0,50>P>0,20 by criterion " χ^2 ". This segregation confirmed the monogenic control of BCMV resistance in tested materials (Table. 1).

All 75 F₂ plants with *I*-gene phenotypically resembled BAT 477.

The application of molecular markers in bean breeding for virus resistance is of great importance. SCAR marker SW 13 can be used successfully for *I*-gene identification in initial materials as well as in progenies (Melotto et al., 1996; Sharma et al., 2008). Our results are in conformity with those of other authors as Drijfhout, 1978 and Kelly et al., 1995.

Conclusion

SCAR marker SW 13 successfully identified the presence of *I*-gene for BCMV resistance in donor parent BAT 477, in all F₁ plants and in segregating F₂.

The existence of monogenic control of BCMV resistance in tested materials was demonstrated by amplification of 690 bp fragment in 75 plants and absent of such in the remaining 31 plants (ratio of segregation approximately 3:1), supported by the criterion " χ^2 " at level of significance 0,50>P>0,20.

References

Drifhout, E., 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. *Agric Res Rep* 872: 98 pp.

Edwards, K., Johnstone, C., and Thompson, C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* 19(6):1349.

Haley, S.D., Afanador, L., and Kelly, J.D. 1994. Identification and application of a random amplified polymorphic DNA marker for the *I* gene (potyvirus resistance) in common bean. *Phytopathology*, 84: 157-1 60.

Kelly, J.D., Afanador, L., and Haley, S.D. 1995. Pyramiding genes for resistance to bean

common mosaic virus. *Euphytica*, 82: 207-2 12.

Khan, J. A., Lohuis, D., Goldbach, R., Dijkstra, J., 1993. Sequence data to settle the taxonomic position of bean common mosaic virus and blackeye cowpea mosaic virus isolates. *Journal of General Virology* 74:2243–2249.

Mc Kern, N.M., Mink G.I., Barnett O.W., Mishra A., Whittaker L.A., Silgernagel M.J., Ward C. W., Shukla D. D., 1992. Isolates of Bean Common Mosaic Virus Comprising two distinct Potyviruses. *Phytopathology* 82:923–929.

Melotto, M., Afanador, L., Kelly, J. D., 1996. Development of a SCAR marker linked to the *I* gene in common bean. *Genome* 39(6):1216–1219.

Sharma, P. N., Pathania, A., Kapil, R., Sharma, P., Sharma, O. P., Patial, M., & Kapoor, V. (2008). Resistance to bean common mosaic potyvirus strains and its inheritance in some Indian land races of common bean. *Euphytica*, 164(1), 173–180.

Singh. S.P., Gepts, P., and Debouck, D.J. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Econ. Bot.* 45: 379-396.

Vetten, H. J., Lesemann, D. E., Maiss, E., 1992. Serotype A and B strains of bean common mosaic virus are two distinct potyviruses. *Archives of virology Supplementum*. 5:415–431.