



Short communication

Identification of RAPD marker linked to Mungbean Yellow Mosaic Virus resistance in French bean (*Phaseolus vulgaris* L.)

K.V. Ravishankar¹, T.S.Aghora, N. Mohan, A.H. Naveen and M. Krishnareddy²

Division of Vegetable Crops
Indian Institute of Horticultural Research
Hessaraghatta Lake Post, Bangalore - 560 089, India
E-mail: aghor@ihr.ernet.in

ABSTRACT

Mungbean yellow mosaic virus (MYMV) causes yield loss up to 80 % and is becoming problematic in French bean growing areas. Molecular marker linked selection to MYMV resistance is helpful in rapid identification of genotypes carrying resistant genes. Hence, the present study was undertaken to identify the RAPD marker associated with MYMV resistance in French bean (*Phaseolus vulgaris* L.). Bulk segregant analysis (BSA) was used to identify RAPD marker linked to MYMV resistance. More than 140 random decamers were surveyed for identification of polymorphic markers between the DNA bulks of resistant and susceptible F₂ individuals and their contrasting parents. Ninety eight per cent of these primers amplified DNA in both parents and bulks. Twenty two primers produced specific bands for resistant parent which was absent in the susceptible parent. Out of 22 primers, four primers produced specific fragments viz., OPG 13₄₅₈, OPX 5₆₇₀, OPW 17₃₈₀ and OPP 07₇₃₀, respectively in resistant parent and bulk, which were absent in susceptible parent and bulk. Amplification of individual DNA samples of segregating F₂ resistant individuals using putative marker, OPP 07₇₃₀, a decamer revealed polymorphism in all four resistant and four susceptible F₂ segregants, indicating that the marker OPP 07₇₃₀ was associated with MYMV resistance in IC-525260, a resistant genotype.

Key words: Bulk segregant analysis, MYMV, *Phaseolus vulgaris* L., RAPD marker

French bean, *Phaseolus vulgaris* L. is a diploid (2n = 22), with relatively a small genome 633 Mbp (Arumuganatham and Earle, 1991). It is one of the most important legume vegetables, grown for its tender green pods either for fresh consumption or for processing as canned, frozen or freeze dried products. Though, French bean is a short duration crop, it is prone to several biotic and abiotic stresses. Diseases however, are the most important production constraints.

Among the diseases, mungbean yellow mosaic virus (MYMV) has become epidemic in bean growing areas, which is easily transmitted by whitefly, *Bemisia tabaci* (Nariani, 1960 and Thongmearkjom *et al*, 1981). Resistance breeding is the most effective strategy to overcome the yield loss due to this disease in beans (Beebe *et al*, 1995; Singh *et al*, 2000; Morales, 2001). It is preferred over other methods as it is least expensive and eco friendly. Identification of resistant lines through conventional methods under field screening is time consuming and it requires

evaluation of the resistant lines in hot spots. Moreover, the incidence of disease at the testing site may not be at the desired level. The disease incidence is seasonal and cannot be created as and when desired by artificial means. Therefore, identification of molecular marker linked to MYMV resistant genes helps in rapid identification of resistant genotypes, thus reducing the time required for the development of resistant varieties. Hence, the present study was carried out to identify Random Amplified Polymorphic DNA (RAPD) marker associated with MYMV resistant gene in french bean (*Phaseolus vulgaris* L.).

Population development: The present investigation was carried out at the Indian Institute of Horticultural Research, Bangalore. MYMV resistant parent IC-525260 (Aghora *et al*, 2008) was crossed with susceptible parent IC-525283 during *kharif* 2007. The F₁ seeds were collected and were advanced to F₂, which was screened for resistance during summer 2008. Disease scoring was done on 1-9 scale (Singh *et al*, 2004) at pod maturity stage and based on the score,

¹Division of Biotechnology

²Division of Plant Pathology

plants were grouped into resistant and susceptible segregants. Segregants with scores of less than 5 were grouped under resistant and 5.0 and above were grouped as susceptible. Leaves of these plants were used for DNA extraction. The genomic DNA of the parents and F₂ population was extracted by following modified CTAB (Cetyl trimethyl ammonium bromide) DNA extraction method and was quantified using UV-spectrophotometer at 260 nm absorbance (Ravishankar *et al*, 2000).

RAPD analysis and marker development: Genomic DNA was used as template for polymerase chain reaction (PCR). PCR amplification was carried out in 25 µl reaction volume, containing reaction buffer of pH 9.0, 15 mM MgCl₂, 2.5µl of 3µM primer (OPERON Primers), 2.5µl of 1mM dNTPs, 0.5 U of Taq DNA polymerase and 50 ng genomic DNA. Amplification was performed using thermocycler (Eppendorf- Master Cycler Gradient) with initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 38°C for 2 seconds and extension at 72°C for 90 seconds and final extension at 72°C for 8 minutes. PCR amplified products were separated on 1.5% agarose gel (1x TAE buffer). The amplified products were visualized under UV-transilluminator.

Bulk segregant analysis (BSA): BSA was employed to identify RAPD marker linked to MYMV resistance. DNA was extracted from each of 15 resistant and susceptible F₂ segregants, separately. The parents were screened along with resistant and susceptible bulks using 140 RAPD random primers (OPG 01-20, OPP 01-20, OPR 01-20, OPS 01-20, OPW 01-20, OPX 01-20, OPY 01-20).

In conventional breeding, selection of resistant genotype depends on artificial screening. This process is complex and time consuming. Whereas, molecular marker assisted selection helps in tagging the resistant gene. Once the gene of interest is tagged with molecular marker, selection for that gene can be made based on the marker rather than the phenotype. Moreover, selection can be performed at the seedling stage itself thus it helps in rapid screening (Tanklesy, 1983; Beckmann and Soller, 1986). An attempt was made to identify the RAPD marker associated with MYMV resistance in IC 525260 using bulk segregant analysis. For marker identification, screening of each segregant in mapping population with all the primers is time consuming. Hence, bulk segregant analysis was employed. The screening of contrasting bulks (resistant and susceptible) made from individuals of same phenotype of segregating population suggested that testing the entire population is

required only when polymorphism between the bulks is detected. This helps in considerable saving of time particularly when used with PCR based technique such as RAPD (Williams *et al*, 1990). Bulk segregant analysis was used to detect markers linked to many disease resistant genes including *Uromyces appendiculatus* resistance in common bean (Haley *et al*, 1993), leaf rust resistance in barley (Poulsen *et al*, 2000) and angular leaf spot in common bean (Nietsche *et al*, 2000).

In the present study, 140 RAPD primers were surveyed for identification of polymorphic markers between the DNA bulks of resistant and susceptible F₂ individuals and their parents. Ninety eight per cent of these produced DNA amplification in both parents and bulks. Twenty two primers produced specific bands for resistant parent which was absent in the susceptible parent. Out of 22 primers, four primers viz., OPG 13, OPX 5, OPW 17 and OPP 7 produced specific fragments viz., OPG 13₄₅₈, OPX 5₆₇₀, OPW 17₃₈₀ and OPP 7₇₃₀, respectively in resistant parent and resistant bulk, which were absent in susceptible parent and susceptible bulk. Amplification of individual DNA samples out of the bulk with putative marker, OPP 07₇₃₀ a decamer with sequence GTCCATGCCA revealed polymorphism in all four resistant and four susceptible F₂ segregants (Fig. 1). From the results, it was concluded that the RAPD marker OPP 07₇₃₀ was associated with resistance to MYMV in IC 525260. This marker can be effectively used for rapid screening of large scale population for resistance to MYMV in French bean. Similarly, RAPD markers linked to disease resistance in various crops were identified and reported earlier namely, BGMV resistance in french bean (Urrea and Mikals, 1996); MYMV resistance in mung bean (Selvi *et al*, 2006); powdery mildew resistance (pm1) in wheat (Hu *et al*, 1997); TMV resistance (Tm-2) in tomato (Ohmori *et al*, 1995) and tomato spotted wilt virus resistance (Sw-5) in tomato (Stevens *et al*, 1995).

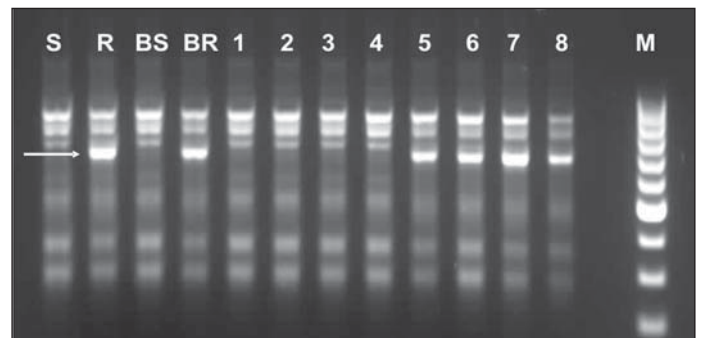


Fig 1. RAPD marker OPP 07₇₃₀ linked to MYMV resistance using bulk segregant analysis

Lane, S- susceptible parent; R - resistant parent; BS- susceptible bulk; BR- resistant bulk; 1, 2, 3, 4 - individual susceptible F₂ segregants; 5, 6, 7, 8 - individual resistant F₂ segregants and M - DNA marker 100 bp.

Arrow indicates the polymorphic band of size approximately 730 bp associated with MYMV resistance in *Phaseolus vulgaris*.

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(MS Received 15 June 2009, Revised 26 October 2009)