

Journal of Biological Control, 38(3): 363-366, 2024, DOI: 10.18311/jbc/2024/36313

Research Note

Validation of molecular markers linked to bruchid resistance in green gram (*Vigna radiata*)

M. SURYA, P. JAYAMANI* and S. MANJU DEVI

Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India

**Corresponding author E-mail:* jayamani1108@gmail.com

ABSTRACT: The pulse beetle, *Callosobruchus* spp., poses a threat to legumes by consuming the protein content of the grain, resulting in potential losses in storage ranging from 12 to 30%. Molecular marker technology helps to mitigate the breeding constraint in the development of bruchid resistance in green gram breeding programmes. In the present study, validation of locus-specific STSbr1 and STSbr2 markers linked with bruchid resistance was done using fifteen genotypes *viz*., VBN (Gg) 2 (susceptible) and *Vigna radiata* var. *sublobata*/2 (resistant), F_1 hybrid, two susceptible and ten resistant RILs derived from the above parents. The marker STS br1 behaved as a dominant marker and produced an approximate allele size of 225 bp in the resistant parent, F_1 hybrid and resistant RILs and absent in susceptible parent and susceptible RILs, which showed cent per cent co-segregation with bruchid resistance locus. Hence, it is concluded that STS br1 is linked with bruchid-resistant genes. The marker STS br2 behaved as a co-dominant marker and produced an approximate allele size of 470 bp in all the 15 genotypes evaluated (monomorphic) and did not differentiate the resistant and susceptible genotypes. The marker STS br1 could be used in marker-assisted selection to develop bruchid-resistant varieties and to screen the germplasm to identify bruchid-resistant donors in green gram.

KEYWORDS: Bruchid resistance, green gram, STS br1, STS br2

(Article chronicle: Received: 20-01-2024; Revised: 29-06-2024; Accepted: 02-07-2024)

Green gram (*Vigna radiata* (L). Wilczek) holds significant importance as a pulse crop in India, playing a crucial role in the cereal-based diet. Its widespread cultivation, facilitated by its short duration, contributes to enhancing soil fertility through the fixation of atmospheric nitrogen in various regions. The area under green gram cultivation in India is 5.5 million hectares with the production and productivity of 3.17 million tonnes and 570 kg/ha in 2022 (https://iipr.icar.gov.in), respectively. One of the major constraints in the storage of green gram is susceptibility to bruchid beetles (*Callosobruchus* sp.). A total of 20 species of bruchids belonging to six different genera infest different pulse crops (Southgate, 1979). Among them, cowpea weevil (*Callosobruchus maculatus*) and adzuki bean weevil (*Callosobruchus chinensis*) are the most destructive storage pests. It is commonly regarded as the field carryover pest where it's initial minor infestation starts in the field and is followed by a major secondary infestation during the storage period (Somta *et al*., 2007). Bruchids have the potential to cause complete loss of the seed within a period of three to four months (Bato & Sanchez, 1972) in storage. Bruchid infection leads to loss of mass, and limited germination of seeds renders them unsafe for human consumption due to an increase in trypsin inhibitor by 25 % (Modgil & Mehta, 1997) and for agricultural as well as commercial usage (Rees, 2004; Oke & Akintunde, 2013; Duan *et al*., 2014). Management through conventional approaches *viz.,* physical, chemical and biological methods check infestation to some extent. Chemical methods of control are found to be effective but are not advocated due to issues concerning food safety and environmental hazards. To overcome these problems, breeding resistant cultivars paves an economical and effective solution. Inducing host plant resistance through antibiosis and anti-xenosis is the only choice to arrest the growth and development of bruchids (Edwards & Singh, 2006). For this purpose, it is important to find sources of resistance among cultivars and different wild species available in the germplasm. A successful breeding programme will depend on a suitable source of resistant genes. The development of bruchid-resistant cultivars involving the selection process and bruchid evaluation is time-consuming and more expensive. Hence, molecular marker technology, which provides an indirect means of selection, has significantly accelerated breeding programmes for the enhancement of

numerous traits, including disease and pest resistance in diverse crops. Using molecular markers linked to resistant genes will be helpful, in the rapid identification of genotypes carrying these genes without subjecting them to screening. The wild progenitor of green gram, *Vigna radiata* var. *sublobata,* possesses genes resistant to bruchids (Fujii *et al.,* 1989). The genetic localization of the resistance gene and its linked molecular markers have been identified by several authors using the wild progenitor species. Thus, this research work has progressed to elucidate the validation of molecular markers linked to bruchid-resistant genes in green gram.

The experimental material comprised of two parents, VBN (Gg) 2 (P1Susceptible) and *Vignaradiata* var. *sublobata*/2 (P2Resistant), F_1, 10 resistant (< 10 % damage) and two susceptible (100 % damage) RILs (Table 1). The experiment was conducted at the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The resistant and susceptible parents and RILs were identified in the previous study by no choice method of screening using *Callasobruchus chinensis* (Data not shown). The young leaves of 10-day-old seedlings collected from the above materials were subjected to genomic DNA extraction by the modified CTAB (mini-prep) method (Doyle & Doyle, 1987). The DNA was quantified by using a Nanodrop. One µl of genomic DNA was used for quantification and the absorbance was measured at 260 nm. The quality of DNA was checked by using 0.8 % agarose gel electrophoresis using 1X TBE buffer and Ethidium bromide (5 µl /100 ml of Agarose). The two STS markers, STS br1 (F: CAGAAAACAAATCACAAGGC; R: GTAAGCATTGAAAAAGGGTG) and STS br2 (F: CCACCCTATTCAATGCTTAC; R: ACACTTCAATGGCGGACG) (Miyagi *et al.,* 2004) were employed for validation of markers linked to bruchid resistance gene. The PCR reaction mixture consisted of 10 µl (2.0 µl of 50 ng genomic DNA, 2.0 µl of 5 µM forward and reverse primers, 3 µl of SMART prime master mix and 3.0 µl of sterile water) was amplified in Master cycler gradient PCR (Eppendorf- Nexus). The amplified PCR products were analyzed through gel electrophoresis in a 3% agarose gel with 1X TBE, running at 100 volts for 3 hr in a gel electrophoresis unit. The resulting gels, stained with Ethidium bromide, were documented using a BIO-RAD Gel documentation unit (Gel DocTM XR+, BIO-RAD, USA).

The breeding for bruchid resistance in green gram is one of the major thrusts in the green gram breeding programme. A total of 15 green gram genotypes (P1, P2, F_1 and 10 resistant and two susceptible RILs derived from the cross VBN (Gg) 2 (Susceptible) and *V. radiata* var. *sublobata*/2 (Resistant) were subjected to validation for bruchid resistance gene using

Table 1. List of green gram genotypes used for validation of STS markers linked with bruchid resistance in green gram

S. No.	Genotype	Reaction to bruchid	STS br1	STS br2
1	VBN (Gg) 2	Susceptible	-	+
2	*Vigna radiata* var *sublobata*/2	Resistant	+	+
3	F_1 hybrid derived from VBN (Gg) 2 X *Vigna radiata* var. *sublobata/2*	Resistant	+	+
4	RIL 1	Resistant	+	+
5	RIL 2	Resistant	+	+
6	RIL 3	Resistant	+	+
7	RIL 4	Susceptible	-	+
8	RIL 5	Resistant	+	+
9	RIL 6	Resistant	+	+
10	RIL 7	Resistant	+	+
11	RIL 8	Resistant	+	+
12	RIL 9	Resistant	+	+
13	RIL 10	Susceptible	-	+
14	RIL 11	Resistant	+	+
15	RIL 12	Resistant	+	+

Note :
RIL- Recombinant inbred lines derived from the cross VBN (Gg) 2 X *Vigna radiata* var *sublobata*/2
+ □ Presence of allele
- □ Absence of allele

two STS markers which were tightly linked with bruchid resistance in green gram. STS markers, originating from the BAC library of green gram, exhibited co-segregation with bruchid resistance observed in the Australian collection of *V. radiata var. sublobata*, ACC41 (Miyagi *et al*., 2004).

Initially, parents along with F_1 hybrids were analysed with both the STS markers. The marker STS br1 produced an allele size of approximately 225 bp in resistant parent and F_1 hybrids. The susceptible parent did not show any amplification. The marker STS br2 produced approximately 470 bp alleles in the parents and F_1 hybrids and was found to be monomorphic (Figure 1). For further confirmation, the marker STS br1 was analysed with parents VBN (Gg) 2 (Susceptible) and *Vigna radiata* var. *sublobata*/2 (Resistant), F_1 hybrid, 10 resistant RILs and two susceptible RILs. The marker STS br1 behaved as a dominant marker and produced an allele with an approximate size of 225 bp only in the resistant parent, F_1 and resistant RILs and absent in the susceptible parent and susceptible RILs (Table 1 and Figure 2). Similar results were also observed by Sarkar *et al*. (2011) for the marker STS br1 in the resistant parent, Sub2 in green gram. However, Miyagi *et al*. (2004) reported that STSbr1 behaved as a co-dominant marker, as it produced 390 bp in Berken (susceptible parent) and 370 bp in ACC41 (resistant parent), which is an Australian *V. radiata* var. *sublobata* accession.

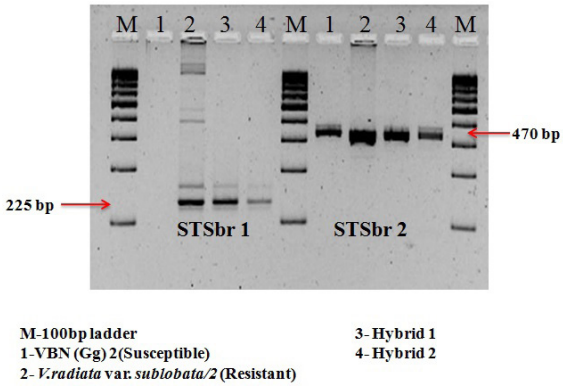


Figure 1. Validation of STS marker for bruchid resistance in green gram.

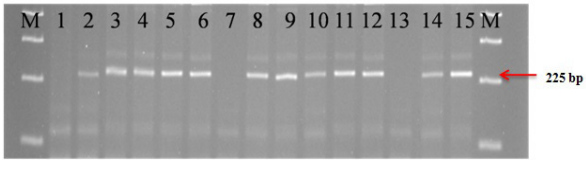


Figure 2. Validation of STS br1 marker for bruchid resistance. M-100 bp ladder. For lanes 1-15 refer to Table 1.

The marker STS br2 was also analysed with parents VBN (Gg) 2 and *Vigna radiata* var. *sublobata*/2, F_1 hybrid, 10 resistant RILs and two susceptible RILs. It produced an approximate allele size of 470 bp in all genotypes and was found to be monomorphic (Table 1 and Figure 3). The findings of Sarkar *et al*. (2011) for the marker STS br2 in green gram also observed a monomorphic banding pattern among the parents, B1 (susceptible) and Sub 2 (resistant) and also did not show any polymorphism among the green gram cultivars used in the study. However, STS br2 produced a 230 bp allele only in the resistant parent, ACC41 and behaved as a dominant marker in the earlier study by Miyagi *et al.* (2004) (Table 2). The results from the present study and that of Sarkar *et al.* (2011) are similar (STS br1 behaved as a dominant marker and STS br2 behaved as a co-dominant marker among the parents B1 (susceptible) and Sub 2 (resistant), whereas for Miyagi *et al.* (2004) the results were different (STS br1 behaved as co-dominant marker and STS br2 behaved as dominant marker between susceptible parent (Berken) and resistant parent (ACC 41). This might be due to the *sublobata* accession used in this study might be different from the Australian *sublobata* accession.

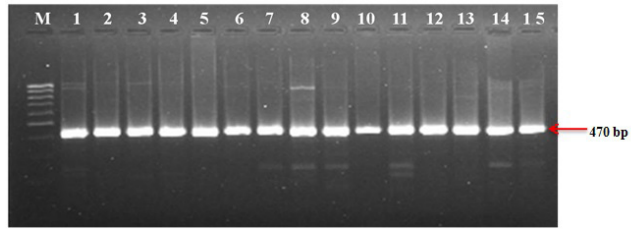


Figure 3. Validation of STS br2 marker for bruchid resistance. M-100 bp ladder. For lanes 1-15 refer to Table 1.

The results of the present study revealed that both markers got amplified and produced amplicons. Among the two markers screened, the marker STS br1 produced an approximate allele size of 225 bp, and behaved as dominant, either with the presence or absence of an allele. The RILs and parent VBN (Gg) 2 which are susceptible to bruchid infestation had a null allele or absent whereas, resistant parent *V. radiata* var. *sublobata*/2, F_1 hybrid and resistant RILs produced an allele with a size of 225 bp, which showed cent per cent co-segregation with resistance locus.

Thus, validation of STS markers revealed that the marker STS br1 could be further used in the screening of germplasm and segregating progenies to identify donors for bruchid resistance. This marker ought to be used in marker-assisted selection to develop bruchid-resistant varieties of green gram.

Table 2. Comparison of amplification pattern of STS markers used in this study with other studies

	Co-dominant/ Dominant	Polymorphic/ Monomorphic	Resistant	Susceptible
STSbr1				
Present study	Dominant	Polymorphic	225 bp	-
Sarker *et al.* (2011)	Dominant	Polymorphic	225 bp	-
Miyagi *et al.* (2004)	Co-dominant	Polymorphic	370 bp	390 bp
STSbr2				
Present study	Co-dominant	Monomorphic	470 bp	470 bp
Sarker *et al.* (2011)	Co-dominant	Monomorphic	-	-
Miyagi *et al.* (2004)	Dominant	Polymorphic	230 bp	-

Note: (-) null allele

REFERENCES

Bato, S. M., and Sanchez, F. F. 1972. The biology and chemical control of *Callosobruchus maculatus* (L.). *Phillip Ent*, **2**: 169-182.

Doyle, J. J., and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*, **19**(1): 11-15.

Duan, C. X., Zhu, Z. D., Ren, G. X., Wang, X. M., and Li, D. D. 2014. Resistance of faba bean and pea germplasm to *Callosobruchus chinensis* (Coleoptera: Bruchidae) and its relationship with quality components. *J Econ Entomol*, **107**(5): 1992-1999. https://doi.org/10.1603/EC14113 PMid:26309291

Edwards, O., and Singh, K. B. 2006. Resistance to insect pests: What do legumes have to offer? *Euphytica*, **147**(1-2): 273-285. https://doi.org/10.1007/s10681-006-3608-1

Fuji, K., Ishimoto, M., and Kitamura, K. 1989. Patterns of resistance to bean weevils (Bruchidae) in Vigna radiata-sublohata complex inform the breeding of new resistant varieties. *Appl Entomol Zool*, **24**: 126-132. https://doi.org/10.1303/aez.24.126

Miyagi, M., Humphry, M., Ma, Z. Y., Lambrides, C. J., Bateson, M., and Liu, C. J. 2004. Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). *Theor Appl Genet*, **110**, 151-156. https://doi.org/10.1007/s00122-004-1821-7 PMid:15490104

Modgil, R., and Mehta, U. 1997. Effect of *Callosobruchus chinensis* (Bruchid) infestation on antinutritional factors in stored legumes. *Plant Foods Hum Nutr*, **50**: 317-323. https://doi.org/10.1007/BF02436078 PMid:9477426

Oke, O. A., and Akintunde, E. M. 2013. Reduction of the nutritional values of cowpea infested with *Callosobruchus maculatus* (Coleoptera: bruchidae). *Int J Agric Sci*, **3**(1): 30-36.

Rees, D. P. 2004. Insects of stored products. CSIRO Publishing. https://doi.org/10.1071/9780643101128

Sarkar, S., Ghosh, S., Chatterjee, M., Das, P., Lahari, T., Maji, A., ... Bhattacharyya, S. 2011. Molecular markers linked with bruchid resistance in *Vigna radiata* var. Sublobata and their validation. *J Plant Biochem Biotech*, **20**: 155-160. https://doi.org/10.1007/s13562-011-0039-4

Somta, P., and Srinives, P. 2007. Genome research in mungbean [*Vigna radiata* (L.) Wilczek] and black gram [*V. mungo* (L.) Hepper]. *Sci Asia*, **33**(Suppl 1): 69-74.

Southgate, B. J. 1979. Biology of the Bruchidae. *Annu Rev Entomol*, **24**(1): 449-473. https://doi.org/10.1146/annurev.en.24.010179.002313