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## Original Research

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# Molecular signatures of elite brinjal varieties towards grouping and marker–trait association for shoot and fruit borer resistance

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## Abstract

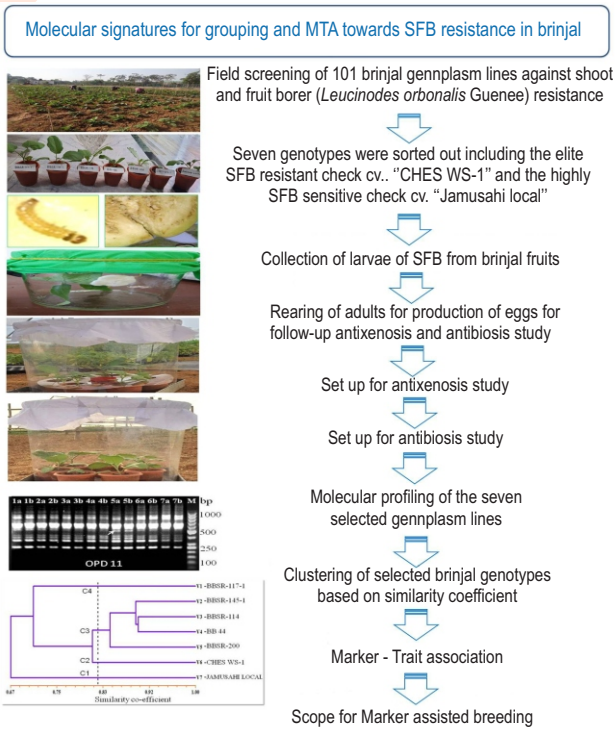
**Aim:** Molecular characterization of brinjal genotypes and exploring marker trait association with a number of antixenosis and antibiosis traits in relation to shoot and fruit borer (SFB) infestation.

**Methodology:** Seven elite brinjal genotypes including the most sensitive and highly resistant checks against SFB were sorted out from a set of 101 germplasm stocks. These were subjected to infestation with SFB in Mylar cages. Data were recorded on antixenosis and antibiosis traits related to SFB infestation. Molecular profiling was done using five RAPD and four SSR primers and the binary marker data (presence and absence of band) were analysed for clustering pattern of genotypes and marker-trait association if any for SFB infestation in brinjal.

**Results:** The RAPD primer OPN04 and two SSR primers smSSR03 and smSSR04 emerged as the highly informative, while OPD 16 (a RAPD primer) and smSSR01 and smSSR09 showed high Rp value. The clustering based on RAPD and SSR profiling was almost similar to the grouping based on antixenosis and antibiosis traits indicating good primer efficiency for screening against SFB infestation. A few RAPD markers revealed significant marker-trait association with a number of antixenosis and antibiosis traits, and more specifically with larval weight under SFB infestation. Besides, a 400bp molecular marker 'OPC05_8' had shown significant MTA with fruit infestation, trichome density and calyx length.

**Interpretation:** The above trait–specific molecular markers may be considered useful for screening of parental lines against SFB tolerance, and as such may be used for marker assisted selection in brinjal breeding programs.

**Key words:** Brinjal, Clustering pattern, Genotyping, Marker-trait association, Shoot and fruit borer infestation

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## Introduction

Brinjal or egg plant (*Solanum melongena* L., 2n=24) is an important solanaceous vegetable crop of tropics and subtropics. It is a versatile crop adapted to different agroclimatic regions and is being extensively grown in the warm areas of Far East (India, Bangladesh, Pakistan, China and Philippines). Brinjal is a rich source of minerals, vitamins, total water soluble sugars, free reducing sugars and amide proteins; but low in calories and fat. India ranks second next to China in area and production accounting 23.3% of the total global brinjal production (Manjunatha *et al.*, 2021). Brinjal is a source of cash income for resource-poor farmers and it assumes to be the lifeline of many Indian farm families due to ample demand by consumers. However, the crop is severely prone to lepidopteran insects, particularly the shoot and fruit borer (SFB) (*Leucinodes orbonalis* Guen.). The damage by this insect starts soon after transplanting seedlings till harvest of the fruits leading to loss up to 16% of shoots and 60% of fruit yield (Yadav and Kumayat, 2013). Asati *et al.* (2002) found that the borer infestation ranged from 20.11 to 51.96 per cent in different varieties. Arka Neelakanth (4.06%), CVK (4.66%), Shyamala (5.30%), PHB-909 (6.06%) and Arka Keshav (6.46%) are reported to have lower shoot damage by SFB (Umamahesh *et al.,* 2018).

Bioactive molecules like total phenols, lectins, a-amylase inhibitor, proteinase inhibitor, chitinase, peroxidise and polyphenol oxidase present in the plant are known to offer innate resistance to SFB (Singh *et al.,* 2000). High solasodine content (0.90-5.4%) and plant structure in wild species (*S. indicum, S. khasianum, S. viarum, S. Xanthocarpum* and *S. nigrum*) are the crucial factors in conferring nonpreference and antibiosis (Paturde *et al.,* 2002). Besides, plant structure, fruit skin thickness, fruit form and spines of leaves, branches, petioles, calyx of fruits act towards the pest nonpreference (Shaukat *et al.,* 2018). In fact, the young larvae of pest bore into tender shoots cause shoot tips to wilt and at flowering they bore into developing flower buds and fruits making them unsuitable for marketing. Continuous use of pesticides (several sprays) for controling of this insect has a negative impact on human health and environment besides pest resurgence and destruction of beneficial insects and microbiome (Nicolopoulou-Stamat *et al.,* 2016).

Cultivation of SFB resistant genotypes can address the problem. However, inspite of several attempts over last 40 years, no ruling non-transgenic varieties with resistance to SFB is currently available. The problem lies with the proper screening and selection of plants for SFB resistance. Transgenic brinjal with *Cry1Ac* though effectively control SFB (Shelton *et al.,* 2018) but not acceptable in the Indian context due to biosafety concern. However, the molecular marker-assisted selection can be a reliable option that involves selection of plants carrying genomic regions that are involved in the expression of trait. In this context, exploring marker –trait association for SFB in brinjal may incredibly help to achieve the target. Preliminary study revealed association of SSR marker(s) with SFB infestation in the crop. Besides, Ghante *et al.* (2013) detected genetic variation for SFB in brinjal using RAPD markers. The choice of germplasm is a key to the success of association study (Zhu *et al.,* 2008). Therefore, an experiment was set up with few elite brinjal germplasm stocks comprising resistant and most susceptible genotypes of brinjal for molecular characterization and exploring marker trait association with a number of antixenosis and antibiosis traits in relation to SFB infestation.

## Materials and Methods

**Plant material:** Out of 101 germplasm lines of brinjal tested for SBF resistance during Rabi, 2016-17 and 2017-18 at Horticultural Research Station, OUAT, Bhubaneswar; seven genotypes e.g., BBSR-117-1, BBSR-145-1, BBSR-114, BB-44, BBSR-200, CHES WS-1 (SFB resistant check) and Jamusahi local (SFB sensitive check) were sorted out. These test genotypes were laid out in CRD with three replications (Rabi, 2018-19) for antixenosis related morphological traits [fruit infestation (%), plant height(cm), fruit length(cm), fruit diameter(cm), fruit shape, fruit pericarp thickness(cm), pedicel length (cm), calyx length(cm), trichome density $cm^{-2}$ and number of seeds per 50g pulp] under SFB infestation. Besides, insect response to antixenosis related traits [adult orientation after 6 hrs, 12, 24 hrs; larval orientation after 6 hrs, 12, 24 hrs; oviposition] and antibiosis related traits [larval survival (%), larval duration (days) and larval weight] were assessed following infestation with SFB as per Heinrichs *et al.* (1985).

**Isolation of genomic DNA:** Genomic DNA was isolated from 2 g. tender young leaves of aseptically grown seedlings on the same day of collection using standard CTAB (Cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1990) with minor modification. The contaminating RNA was removed by treatment with 6µl RNAase (10mg/ml) for each ml of DNA extract following standard protocol and finally, the DNA pellets were washed twice with 70% ethanol and dried under vacuum. The dried DNA was dissolved in TE buffer (10mM Tris-HCl, pH 8.0 and 1mM EDTA) and stored in a deep freezer at -20°C for future use.

**Test for quality and quantity of purified DNA:** The genomic DNA was checked for purity using the ratio of absorbance at 260 and 280 nm wavelength and finally, quantified using UV-VIS Nano Drop (Thermo Fisher Scientific). After quantification, the DNA was diluted in TE buffer to a working concentration of 25ng $\mu l^{-1}$ for PCR analysis. DNA was also loaded in 0.8% agarose gel alongside diluted uncut lambda DNA as standard to recheck the quality and quantity of DNA.

**DNA amplification and agarose gel electrophoresis:** Five decamer RAPD primers and four SSR primers were used for amplification using PCR (polymeric chain reaction) technique. Amplifications were performed in a reaction volume containing 10mM Tris-HCl,pH 9.0, 1.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 100µM each of dNTPs, 10ng of single random primer, 10ng of genomic DNA and 1 unit of Taq polymerase (Genei, Bangalore). Amplifications were performed in a GeneAmp PCR (Applied

Biosystems), programmed for 5 min at 940C, 40 cycles of 1min at 92°C, 1min at 35°C and 2 min at 72°C and final extension for 3 min at 72°C. The amplified products were loaded in 2% agarose gel (for RAPD) and 3% agarose gel (for SSR) containing 0.5mg $ml^{-1}$ of ethidium bromide and electrophoresed in a constant voltage at 55V. The amplifications were checked for their reproducibility.

**Visualization, scoring of bands and statistical analysis:** The gels were documented by Gel Doc 2000 (BioRad, USA) for scoring (1 and 0 for presence and absence) the bands. The size of the amplicons was determined by comparing with 50 bp DNA ladder (Thermo Fisher Scientific). Polymorphic information content (PIC) and resolving power of each primer was calculated as per formula PIC =Σ $(1 – Pi^2)/n$, where Pi is the frequency of the $i^{th}$ band amplified by the primer and n= total no. of bands produced by the primer. Resolving power was derived as Rp=Σ Ib, where Ib is band informativeness, Ib,=1–[2×(0.5–pi)] and pi is the proportion of accessions containing band i (Prevost and Wilkinson, 1999). Besides, estimation of similarity coefficients and construction of Dendrogram as per Jaccard (1908) was done using computer program for Numerical Taxonomy and Multivariate analysis system (NTSYS-PC), Version 2.02 (Rohlf, 2002). Similarity matrix was also used for principal coordinate analysis (PCA) comprising first three Eigen vectors (PCA 1, PCA 2 and PCA 3) to reveal spatial distribution of test genotypes in three dimensional spaces. Besides, the Marker-Trait Association (MTA) of different molecular markers (PCR bands or amplicons) with each biophysical and morphological trait was established using regression analysis (Elakhdar *et al.,* 2016) as per SPSS (Statistical Package for Social Sciences) Software, version 16. The Marker-Trait Association with probability value at least less than 0.05 is considered a statistically significant relationship between a marker with the trait concerned.

## Results and Discussion

Molecular characterization is immensely helpful in selective breeding although it is limited in eggplant compared to other crops of the same family (Jo *et al.,* 2010). The success depends upon generating wide range of polymorphic loci. SSR and RAPD are reported to be useful to reveal molecular diversity in *S. melongena* and its related species (Verma *et al.,* 2012, Parvin *et al.* 2020). In the present study, PCR amplification with five RAPD and four SSR primers revealed 48 and five amplicons respectively (Table 1). The number of bands per RAPD primer ranged from 7-12 with an average of 9.6 bands per primer as also reported by Verma *et al.* (2012), while SSR primers produced narrow range of 1-2 unique bands (180-320bp) with an average of 1.25 bands per primer in contrast to Sunseri *et al.* (2010) revealing as high as 7 alleles with an average of 4.5 per SSR locus). RAPD Primers e.g., OPD 11 and OPD 16 produced maximum 12 bands each, but the later showed maximum nine

**Table 1:** Amplified products and polymorhic information of a set of RAPD and SSR primers used in the study

| Primer | Source | Primer Sequence (3'-5') | GC Con-tent (%) | Tm (°C) | Tan (°C) | Mono-mor-phic alleles | Poly-mor-phic alleles | Total No. of alleles | % of poly-mor-phism | PIC | Rp | Range of ampli-con size (bp) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RAPD Primers** | | | | | | | | | | | | |
| OPM 03 | Europhin genomics | GGGGGATGAG | 70.0 | 34.5 | 31.0 | 3 | 4 | 7 | 57.14 | 0.285 | 11.14 | 600-1050 |
| OPN 04 | -do- | GACCGACCCA | 70.0 | 38.6 | 35.0 | 3 | 5 | 8 | 62.50 | 0.597 | 8.00 | 530-1400 |
| OPC 05 | -do- | GATGACCGCC | 70.0 | 37.6 | 34.0 | 6 | 3 | 9 | 33.33 | 0.254 | 14.57 | 320-1320 |
| OPD 11 | -do- | AGCGCCATTG | 60.0 | 37.1 | 33.0 | 6 | 6 | 12 | 50.00 | 0.320 | 18.28 | 250-1610 |
| OPD 16 | Jakatimath *et al.,* 2017 | AGGGCGTAAG | 60.0 | 33.9 | 31.0 | 9 | 3 | 12 | 25.00 | 0.217 | 20.00 | 280-1750 |
| | **Total** | - | | | | **27** | **21** | **48** | **43.75** | **Av. 0.33** | **Av. 14.4** | **250-1750** |
| **SSR Primers** | | | | | | | | | | | | |
| smSSR 01 | Tumbilen *et al.,* 2011 | GTGACTACGGTTTCACTGGT<br>GATGACGACGACGATAATAG | 50.0<br>42.9 | 54.5<br>51.4 | 55.0 | 0 | 1 | 1 | 100 | 0.670 | 1.14 | 310 |
| smSSR 03 | -do- | ATTGAAAGTTGCTCTGCTTC<br>GATCGAACCCACATCATC | 40.0<br>50.0 | 51.2<br>50.2 | 55.0 | 0 | 1 | 1 | 100 | 0.920 | 0.57 | 180 |
| smSSR 04 | -do- | CTCTGCTTCACCTCTGTGTT<br>CCATGAAAGAGAAGATCGAG | 50.0<br>45.0 | 54.6<br>50.2 | 55.0 | 0 | 2 | 2 | 100 | 0.950 | 0.85 | 265-320 |
| smSSR 09 | -do- | CACATGGGAACCTACTTACC<br>GACGACCATCAAACAAGAAT | 50.0<br>40.0 | 52.2<br>50.6 | 55.0 | 0 | 1 | 1 | 100 | 0.490 | 1.43 | 310 |
| | **Total** | | | | | **0** | **5** | **5** | **100** | **Av. 0.76** | **Av. 0.99** | **180-320** |

Tm: Primer melting temperature, Tan: Primer annealing temperature, PIC: Polymorphic information content and Rp: Resolving power

**Table 2:** Marker –trait association (MTA) for antixenosis and antibiosis traits using RAPD and SSR markers in relation to fruit and shoot borer infestation in brinjal

| Phenotypic traits | Primer | Molecular marker | Marker length (bp) | Beta-value | P-value | $R^2$ |
|---|---|---|---|---|---|---|
| Larval survival | OPM03 | OPM03_1 | 1050 | -0.955 | 0.001 | 0.913 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.955 | 0.001 | 0.913 |
| Larval weight | OPM03 | OPM03_1 | 1050 | -0.967 | 0.0001 | 0.934 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.967 | 0.0001 | 0.934 |
| Fruit Infestation % | OPM03 | OPM03_1 | 1050 | -0.891 | 0.007 | 0.794 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.891 | 0.007 | 0.794 |
| | OPC05 | OPC05_8 | 400 | 0.806 | 0.029 | 0.649 |
| Trichome density | OPC05 | OPC05_8 | 400 | -0.787 | 0.036 | 0.620 |
| Oviposition | OPM03 | OPM03_1 | 1050 | -0.864 | 0.012 | 0.747 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.864 | 0.012 | 0.747 |
| Adult orientation 6h | OPM03 | OPM03_1 | 1050 | -0.842 | 0.017 | 0.709 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.842 | 0.017 | 0.709 |
| Adult orientation 12h | OPM03 | OPM03_1 | 1050 | -0.752 | 0.051 | 0.566 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.752 | 0.051 | 0.566 |
| Larval orientation 6h | OPM03 | OPM03_1 | 1050 | -0.906 | 0.005 | 0.821 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.906 | 0.005 | 0.821 |
| Larval orientation 12h | OPM03 | OPM03_1 | 1050 | -0.835 | 0.019 | 0.698 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.835 | 0.019 | 0.698 |
| Larval orientation 24h | OPM03 | OPM03_1 | 1050 | -0.815 | 0.026 | 0.664 |
| | OPM03 | OPM03_6 | 650 | | | |
| | OPD11 | OPD11_6 | 890 | | | |
| | OPD11 | OPD11_1 | 1610 | 0.815 | 0.026 | 0.664 |
| Calyx length | OPM03 | OPM03_2 | 1000 | -0.782 | 0.038 | 0.611 |
| | OPN04 | OPN04_4 | 1010 | 0.782 | 0.038 | 0.611 |
| | smSSR03 | smSSR03_1 | 180 | | | |
| No. of seeds $50g^{-1}$ pulp | OPC05 | OPC05_8 | 400 | -0.797 | 0.032 | 0.635 |

polymorphic amplicons with a wider range of 280-1750 bp (Fig 1a, 1b, Table 1). However, all SSR primers revealed 100% polymorphic alleles (bands) as compared to RAPD with a maximum 62.5% polymorphism shown by OPN 04 (Table 1) indicating higher genetic variation in the selected test genotypes. A molecular signature in each genotype invariably is expected to differ if the test genotypes are genetically different. A primer with polymorphic information content (PIC) more than 0.5 is considered to reveal high allelic diversity. All SSR primers (particularly smSSR 04) displayed superiority over RAPD (Ahmed *et al.,* 2019) to assess available genetic variation although RAPD primer OPN 04 also seems to be an ideal candidate (Table 1). In contrast, RAPD primers particularly OPD 16 showed invariably high resolving power than SSR primers to distinguish between genotypes. The presence or absence of specific band is the inherent status of each genotype.

The presence or absence of specific band is the inherent status of each genotype. The fruit and shoot borer sensitive variety 'Jamusahi local' revealed the amplicon OPD11_1

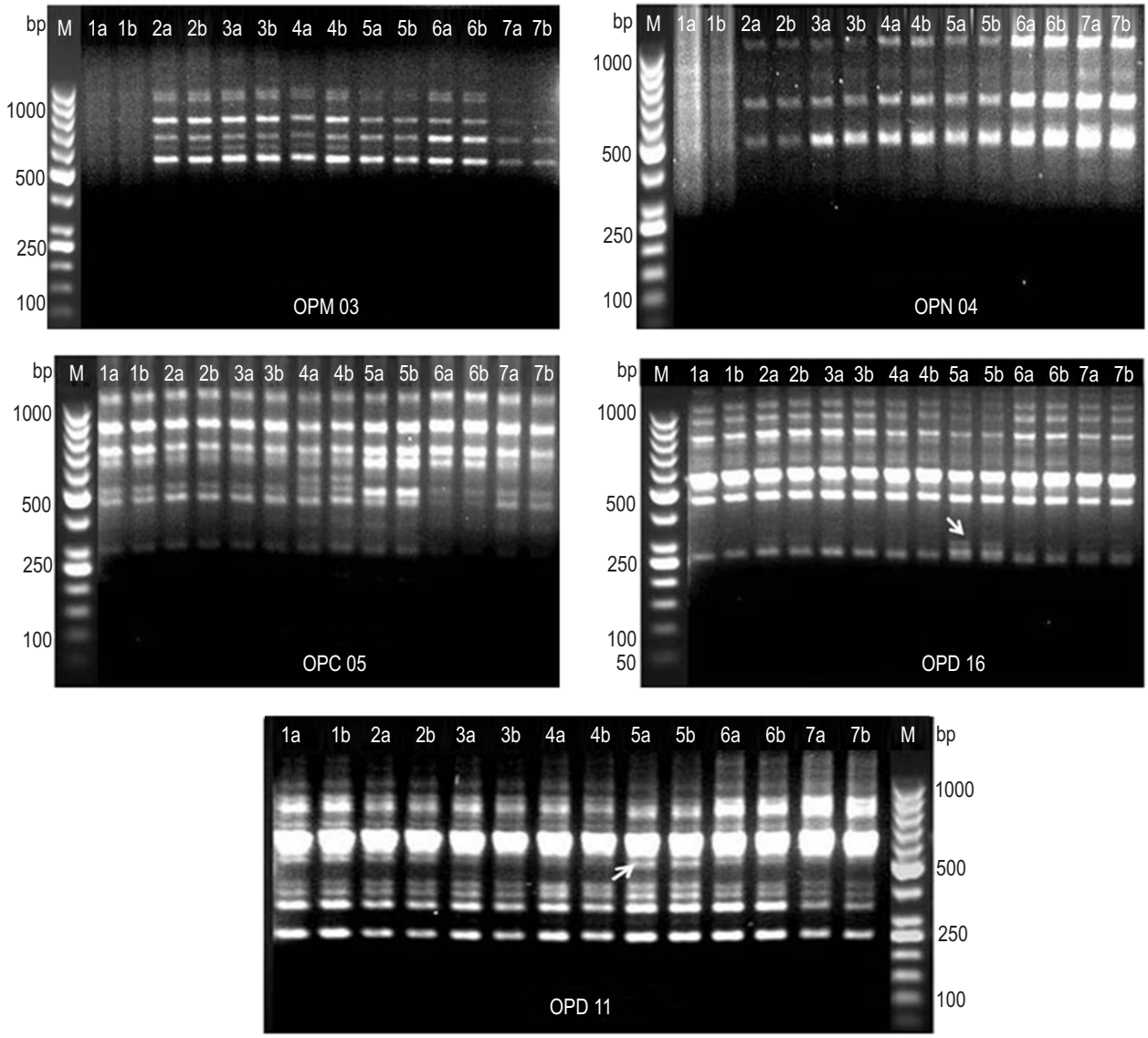


**Fig. 1a:** RAPD profiling of primer OPM 03, OPN 04, OPC 05, OPD 16 and OPD 11 in a set of seven selected brinjal varieties having differential response to fruit and shoot borer resistance. Lane 1-7: V1-BBSR-117-1 (1a & 1b), V2-BBSR-145-1 (2a & 2b), V3-BBSR-114 (3a & 3b), V4-BB-44 (4a & 4b), V5-BBSR-200 (5a & 5b), V-6CHES WS-1 (6a & 6b) and V7-Jamusahi local (7a & 7b) (each variety loaded in duplicates marked by a & b),. M: DNA 50 bp marker (Thermo Fisher).

however, it was absent in all other genotypes. In contrast, the amplicon e.g., OPM03_1, OPM03_6 and OPD11_6 were specifically amplified in all $V_1$-$V_6$ test genotypes (moderately resistant and resistant genotypes), except cv. Jamusahi local ($V_7$) used as sensitive check (Fig 1a). In this context, the SSR band smSSR03_1 was specifically amplified in the SFB resistant varieties CHES WS-1 and BBSR-117-1 (Fig 1b). Hence, the above genotype specific allele(s) may be useful as potential molecular marker for screening SFB, varietal identification and elimination of duplicates within *Solanum melongena* (Verma *et al.,* 2012) and among *Solanum* species (Caguiat and Hautea, 2014). Genetic diversity and relatedness may be informative for varietal identification and genetic improvement of brinjal. Broadly, all the test genotypes were grouped into four distinct clusters (Fig. 2a) at about 81.4% similarity level using RAPD and SSR markers. Similarly, Boureima *et al.* (2018) revealed moderate genetic variability within the germplasm collection of African egg plant, which structured into three molecular groups based on EST-SSRs markers. In the present study, the fruit and shoot borer sensitive variety 'Jamusahi local' followed by BBSR 117-1 and CHES WS-1 forming monogenotypic clusters, were separated from the remaining test materials (varieties). In contrast, the

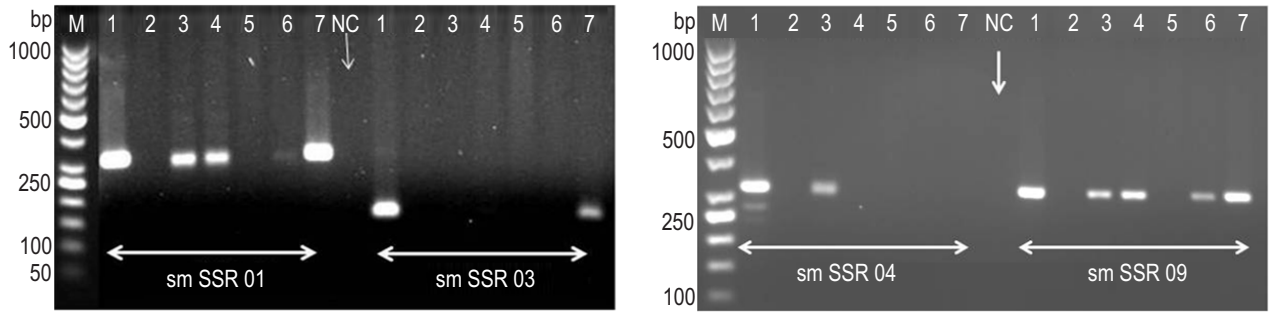


**Fig. 1b:** SSR profiling of primer smSSR 01 & smSSR 03 and smSSR 04 & smSSR 09 in a set of seven selected brinjal varieties having differential response to fruit & shoot borer resistance. Lane 1-7: V1-BBSR-117-1, V2-BBSR-145-1, V3-BBSR-114, V4-BB-44, V5-BBSR-200, V6-CHES WS-1 and V7-Jamusahi local,. M: DNA 50 bp marker (Thermo Fisher), NC- negative control (PCR mix without genomic DNA).

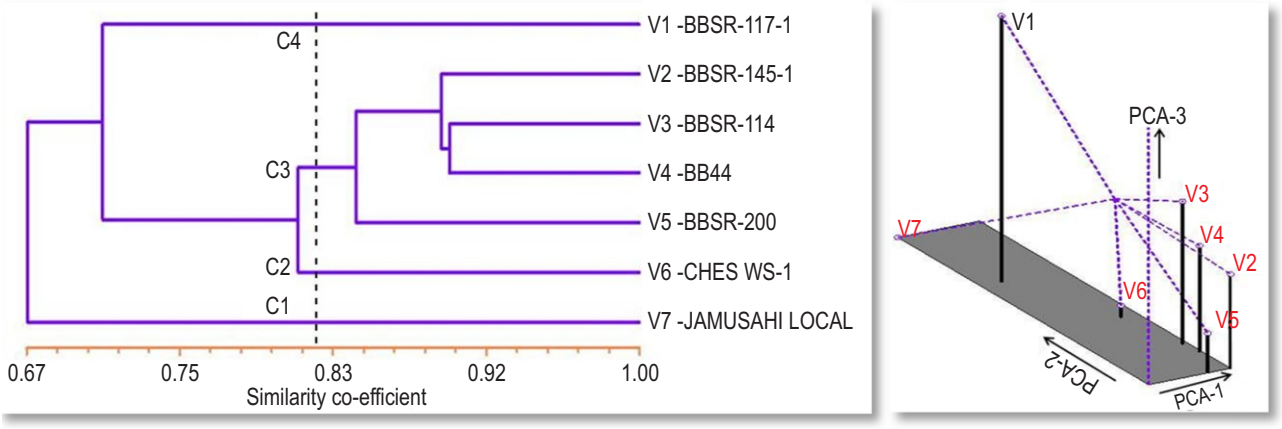


**Fig. 2a:** Dendrogram (A) and three dimensional scaling of PCA(B) showing genetic diversity in a set of 7 brijal test genotypes including susceptible check based on RAPD & SSR markers.

dendrogram revealed higher genomic homology between BBSR 114 ($V_3$) and BB 44 ($V_4$). Khorsheduzzaman *et al.* (2008) developed SSR profiles of five brinjal varieties. The genotype TURBO and BL009 were identified as diverse genotypes which showed a maximum of 17% dissimilarity from EG058, EG075 and ISD006. In the present study, the grouping of genotypes using three-dimensional scaling based on PCA values was found to be more or less consistent with that of UPGMA analysis (Fig. 2a).

The above divergent SFB sensitive (Jamusahi local) and resistant test genotypes (CHES WS-1 and BBSR 117-1) may serve as valuable materials for further genetic improvement in brinjal using recombination breeding. Further, it is worth to mention that three modes of groupings based on DNA profiles (Fig 2a), SFB antixenosis and antibiosis traits (Fig 2b) and all morphological attributes including SFB related traits (Fig 2c) reflected similar pattern of divergence with clear separation of sensitive genotype 'Jamusahi local'. This corroborates the findings of Ansari and Singh (2015). Further, the dendrogram based on antixenosis and antibiosis traits of SFB revealed almost similar clustering pattern to that of DNA based grouping indicating right choice of primers to separate the most sensitive genotype from resistant cultivars. This further gives a clue that there might be some relationship of DNA markers with the status of tolerance vs sensitivity to SFB infestation. The narrow genetic base makes the productivity of brinjal vulnerable to various biotic stresses including SFB infestation. Tolerance to SFB has its own genetic basis (Ahmad and Mustafa, 2018) with involvement of more than one genes/QTLs playing the inherent role for resistance mechanism. The long-awaited breeding goal can be accomplished by identifying tightly linked DNA markers for the trait of interest (Devsharma *et al.* 2020, Elakhdar *et al.,* 2016) even at early growth stage. Therefore, marker-trait association was explored if any for each of the amplicon with 19 antixenosis and antibiosis related traits using regression analysis. Until now, the available literature does not reveal any marker-trait association (MTA) for traits related to SFB infestation. The RAPD primers OPM03 and OPD11 amplified seven and twelve amplicons, respectively. Two polymorphic amplicons of primer OPM03 at 1050bp (OPM03_1) and 650bp (OPM03_6); and one amplicon of primer

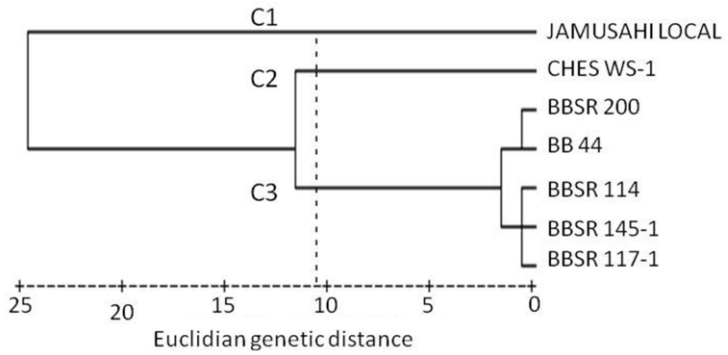


**Fig. 2b:** Dendrogram showing genetic diversity in a set of seven brinjal test genotypes including susceptible check based on antixenosis and antibiosis traits of fruit and shoot borer.

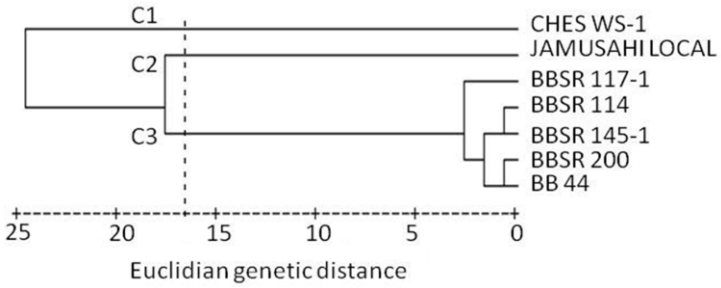


**Fig. 2c:** Dendrogram showing genetic diversity in a set of seven brinjal test genotypes including susceptible check based on total antixenosis and antibiosis traits of SFB and morphological traits of brinjal related to SFB infestation.

OPD11 at 890bp (OPD11_6) were found to have significant negative (absence of band specifically in sensitive genotype) marker–trait associatio with a number of antixenosis and antibiosis traits e.g., larval survival, larval weight, fruit infestation percent, oviposition, adult orientation (both 6 and 12 hr), larval orientation (6, 12 and 24 hrs) related to fruit and shoot borer infestation in brinjal (Table 2). In contrast, OPD11_1 revealed a strong significant positive (presence of band typically in the sensitive reference variety) MTA for all above traits related to SFB infestation.

Significant MTAs of so many traits with the same above four markers seems to be due to the fact that all such traits more or less directly reflect the extent of SFB infestation. However, among the above traits, MTA for larval weight had shown highest significant association (extremely low p-value =0.0001) with marker OPM03_1, OPM03_6, OPD11_6 and OPD11_1. Such MTA for larval weight also explained largest proportion of phenotypic variation as compared to other antixenosis and antibiosis traits. Hence, the presence or absence of above bands (amplicons) may be reliably considered for marker based screening for tolerance/resistance to SFB infestation in brinjal. Besides, a single 400 bp amplicon (OPC05_8) produced by primer OPC05 in the most sensitive cultivar 'Jamusahi local' as well as relatively less tolerant cv. BB44 and BBSR 200, had shown significant positive MTA with fruit infestation by SFB. But, the said marker (OPC05_8) established strong negative MTA with both trichome density and number of seeds per 50g pulp having negative impact on SFB infestation (Amin *et al.,* 2014). In fact, calyx which covers most part of the fruit at early stage of development has a role for SFB tolerance in brinjal (Hanur *et al.,* 2011). Significant positive MTA of calyx length was observed with a RAPD marker 'OPN04_4' as well as with a SSR marker 'smSSR03_1' which explained about 61.1% phenotypic variation. Thus, the molecular markers could be effectively used to gauge the extent of genetic variation, selection of most divergent genotypes and more particularly for selection of SFB resistant parental lines and their wise utilization in brinjal breeding programs by marker assisted selection.

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**Add-on Information**

**Authors' contribution: L. Dash:** Carried out the experiment and recorded data; **L.K. Rath:** Conceptualized and designed the

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