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Assessment of genetic variation at soybean mosaic virus resistance loci in Indian Soybean (*Glycine max* L. Merill) genotypes using SSR markers

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Abstract
Soybean mosaic virus (SMV) disease is one of the major diseases of soybean globally that hampers soybean productivity. In India, SMV disease is gaining importance and there is a need to breed the soybean cultivars resistant to SMV. In India, soybean cultivars resistant to SMV have been identified but source of resistance has not been characterized. Therefore, the current study was carried out to study the genetic variation at three SMV resistance loci in a set of SMV resistant and susceptible Indian soybean genotypes using the mapped SSR markers. A total of 13 SSR markers reported to be linked to three SMV resistance loci were screened on 23 soybean genotypes showing differential response to SMV disease. A total of 54 alleles were generated at 12 polymorphic SSR loci with an average of 4.5 alleles/locus. The number of alleles ranged from 2 to 7 and gene diversity for the polymorphic SSR markers ranged from 0.38 to 0.77 with an average of 0.60, indicating the presence of high genetic variation in the Indian soybean genotypes at SMV resistance loci. Cluster analysis grouped the 23 soybean genotypes into three major clusters and results of the Principal Coordinate Analysis also congruent well with the cluster analysis. In the clustering, most of the genotypes were grouped as per their pedigree suggesting that they may be carrying the same SMV resistance gene. This study showed that Indian soybean genotypes have sufficient genetic variability at the three SMV resistance loci and it would be helpful in the selection of suitable parents in breeding for SMV resistance.

Key words: Soybean, genetic variability, soybean mosaic virus, SSR

Introduction

Soybean (*Glycine max* L. Merill) is an important oilseed crop grown in India and is cultivated over an estimated area of 10.5 million hectares with a production of 14.7 million tonnes (Prajapat *et al.*, 2015). However, the average yield is very low as compared to other soybean producing countries of the world (Billore & Shrivastava, 2014). Diseases and pests are major constraints in realizing high yield potential in soybean. Soybean mosaic disease caused by soybean mosaic virus (SMV) is one of the major viral diseases prevalent in India and causes significant yield loss and also affects seed quality. Around the world, different SMV isolates have been classified into different strain groups based on their reaction to a set of soybean differentials. In USA, 7 SMV strain groups (G1-G7) have been reported (Cho & Goodman, 1979; 1982) and in China 21 strain groups (SC1-SC21) have been reported (Li *et al.*, 2010). However, in India little attention has been paid to the soybean mosaic disease and no such classification of isolate has been carried out despite the occurrence of SMV disease since 1960 in different parts of the country (Nariani & Pingaley, 1960; Singh *et al.*, 1976; Naik & Murthy 1997; Banerjee *et al.*, 2014).

Development of soybean cultivars resistant to SMV is the most economical, effective and environment friendly method of controlling the disease. However, breeding for resistance to SMV by conventional methods is a time consuming process because of the requirement to inoculate plants and screen disease phenotype. Molecular marker-assisted selection has proven to be a highly efficient strategy for selecting resistant lines (Mudge *et al.*, 1997; Prabhu *et al.*, 2009). Three independent single dominant SMV resistance loci, *Rsv1*, *Rsv3* and *Rsv4*, have been reported and mapped to soybean chromosomes 13 (F), 14 (B2) and 2 (D1b), respectively (Yu *et al.*, 1994; Hayes *et al.*, 2000; Gore *et al.*, 2002; Jeong *et al.*, 2002; Cregan, 2003; Hwang *et al.*, 2006; Shi *et al.*, 2008; Saghai Maroof *et al.*, 2010). The *Rsv1* locus confers resistance to strain G1, *Rsv3* locus confers resistance to strain G5-G7 and *Rsv4* locus

confers resistance to all 7 SMV strains. In India, different soybean genotypes showing resistance to SMV have been reported. However, no differential soybean lines have been identified to distinguish different SMV resistance genes.

The assessment of genetic variation is important to the plant breeders for the identification of diverse source of disease resistance and the deployment of these for crop improvement. The aim of the current study was to study the genetic variability at the three SMV resistance loci in Indian soybean genotypes differing in their reaction to SMV so that they can be used in soybean breeding programs for development of SMV resistant cultivars.

Materials and Methods

Plant material and DNA extraction

A total of 23 soybean released varieties used for studying the genetic diversity along with their pedigree and reaction to SMV are listed in Table 1. The seeds of these varieties were obtained from Regional Research Centre, Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Amravati and National Research Centre for Soybean, Indore, India. Total genomic DNA was extracted from two week old seedling using the DNeasy® Plant Mini Kit (Qiagen, Germany). The quality of DNA was checked on 0.8% agarose gel and the quantity was determined using NanoDrop ™ 1000 spectrophotometer.

SSR marker analysis

A total of 13 soybean SSR markers reported to be linked to the three independent SMV resistance loci were used in this study. The details of the SSR markers along with the chromosomal location is given in Table 2. PCR reactions were performed in 25 µl volume containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 unit *Taq* DNA polymerase (Jonaki, Hyderabad, India), 50 ng template DNA, and 20 ng each of forward and reverse primers. PCR amplifications were performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the following thermal profile:1 cycle of 95^0C for 2 min, followed by 34 cycles of 94^0C for 30 sec, 58^0C for 30 sec, 72^0C for 30 sec and a final extension of 72^0C for 7 min. PCR products were resolved on 4% MetaPhore® agarose gel (Cambrex, USA) in 1X Tris-borate-EDTA buffer, stained with ethidium bromide and photographed on a gel documentation system (Syngene, UK).

Statistical analysis

In case of SSR markers, scoring of bands was done as presence (1) or absence (0) for each marker allele. Data were entered in a binary data matrix as discrete variables and pair-wise similarities were obtained using the Dice's similarity coefficients. Cluster analysis was performed using the software Darwin version 5.0 (Perrier *et al.*, 2003). The matrices of similarity coefficients were subjected to Neighbour Joining group method to estimate the genetic relatedness among the genotypes and generate the dendrogram. The support for grouping nodes and stability of the dendrogram was evaluated by bootstrap analysis with 2000 permutations. Principal coordinate analysis (PCO) was also carried out to highlight the resolving power of the ordination using software Darwin. The gene diversity and observed heterozygosity for the SSR markers was calculated by using PowerMarker v3.25 software (Liu & Muse, 2005).

Results and Discussion

The assessment of genetic diversity is a prerequisite and important step for the improvement of any crop plant (Barrett & Kidwell 1998; Thompson *et al.*, 1998). In this study, we used SSR markers identified as linked to the SMV resistance genes in previous studies (Table 2) to evaluate the genetic variation at the three SMV resistance loci (*Rsv1*, *Rsv3* and *Rsv4*) in a set of 23 diverse Indian soybean genotypes showing differential response to SMV disease. Five SSR markers (Sat_234, Sat_297, Sat_317, Sct_033, Satt334) identified as linked to SMV resistance locus *Rsv1*, four SSR markers (Satt063, Satt560, Satt726, Satt687) linked to SMV resistance locus *Rsv3* and four SSR markers (Satt266, Satt157, Satt542, Satt634) linked with SMV resistance locus *Rsv4* were used. All 13 SSR markers were successfully amplified and 11 SSR markers were found polymorphic. Two SSR markers, Sat_317 and Satt157 were found monomorphic. All SSR markers amplified a single locus except marker Satt687 which amplified two loci (Table 3). A total of 54 alleles were generated at twelve polymorphic SSR loci with an average of 4.5 alleles/locus, indicating high allelic diversity at three SMV resistance loci in Indian soybean genotypes (Table 3). For SMV resistance locus *Rsv1*, four polymorphic SSR loci yielded 25 alleles at an average of 6.2 alleles/locus. For locus *Rsv3*, five polymorphic SSR loci produced 18 alleles at an average of 3.6 alleles/locus. Similarly for locus *Rsv4*, 11 alleles were generated at three polymorphic SSR loci with an average of 3.7 alleles/locus. The lowest number of alleles observed were 2 for SSR markers Satt063 and Satt726, and highest number of alleles observed were 7 for SSR marker Sat_297. The amplification pattern of three SSR markers

(Sat_297, Satt063 and Satt266) is shown in Fig. 1. The polymorphism level of the markers used in this study was comparable to similar studies in other crops like chickpea (Hamwieh *et al.,* 2013) and rice (Das *et al.,* 2014).

The gene diversity, often referred to as expected heterozygosity, for the polymorphic SSR markers ranged from 0.38 to 0.77 with an average of 0.60 (Table 3), indicating the presence of high genetic variation in the Indian soybean genotypes at SMV resistance loci. No correlation between the length of the SSR motif and number of alleles obtained was observed in this study. For example SSR marker Sat_317 [repeat motif $(AT)_{24}$] and Satt157 [repeat motif $(ATT)_{31}$] were monomorphic, whereas Satt334 [repeat motif $(ATT)_{16}$] and Satt560 [repeat motif $(ATT)_{12}$] were highly polymorphic. The absence of observed hetrozygosity (average 0.0) in the study is attributed to the self pollinating nature of soybean crop and resulting homozygosity at all the three SMV resistance loci. In this study, a total of 11 genotype specific alleles corresponding to seven soybean genotypes were also observed and thus would be a valuable tool for varietal identification and discrimination. The highest numbers of genotype specific alleles were observed with SSR marker Sat_234 (three genotype specific alleles). Among the genotypes, 'Hardee' had three genotype specific alleles, 'Bragg' and 'JS97-81' had two each, while 'KB-79', 'PK-564', 'Improved Pelican' and 'JS-335' each had one genotype specific allele. SSR markers have been shown to be useful in discrimination of genotypes in many other species including legumes and cereals (Eujayl *et al.,* 2001; Gupta *et al.,* 2009; 2010).

Based on cluster analysis, 23 soybean genotypes were grouped in 3 major clusters (Fig. 2). Cluster I consisted of 9 genotypes (JS-335, JS79-81, MACS-450, Lee, Kalitur, Type-49, SL-688, Pusa-37 and Hardee). Cluster II had 8 soybean genotypes (PK-1241, PS-1225, KB-79, PK-564, NRC-12, SL-295, PK-416 and SL-525) and cluster III consisted of 6 soybean genotypes (PK-472, MACS-124, Punjab-1, MACS-57, Improved Pelican and Bragg). Based on cluster analysis genotypes could not be clearly separated into SMV resistant and susceptible genotypes. However, there was some degree of separation based on SMV reaction and five of the seven SMV susceptible genotypes were grouped in cluster I (JS-335, JS79-01, Lee, Kalitur and Type-49) along with four SMV resistant genotypes. The remaining two susceptible genotypes (Bragg and Improved Pelican) present in Cluster III showed least similarity with the SMV resistant genotypes present in the cluster III and formed as a separate sub-cluster. Cluster II contained only SMV resistant genotypes. In clustering, most of the genotypes were showing grouping based on their pedigree suggesting that they are carrying the same SMV resistance gene. For example, genotypes SL-295, PK-416 and SL-525 which share the parentage were grouped together. The results of the PCO analysis congruent well with the cluster analysis and most of the genotypes were grouped in three clusters (Fig. 3). The first three Eigen vectors accounted for 53.2 % of the total molecular variation, with each component individually contributing 23.7%, 17.9% and 11.6%, respectively.

Most of the present day cultivars have been developed by repeated use of few cultivars/lines known to have wider adaptability or other agronomically important traits that may lead to low level of genetic diversity in the cultivated gene pool. Narrow genetic base increases vulnerability to pathogens/pest epidemic and reduces genetic gain from selection. However, result of this study showed that Indian soybean genotypes have good genetic variability at the three SMV resistance loci and would help the soybean breeders in the selection of suitable parents in breeding for SMV resistance. The SSR markers used in the present study were reported to be linked to three different SMV resistance loci located on three different linkage groups of soybean (Table 2). The advantage of these mapped markers is that genomic regions associated with SMV resistance were only analyzed and representation from other regions of genome was avoided, thus leading to more accurate estimates of genetic similarities between the individuals with reference to SMV resistance. Earlier, many researchers have emphasized that for genetic diversity studies markers should be chosen based on their map locations to ensure good genome coverage and reduce the marker sampling errors (Karp *et al.,* 1997; Singh *et al.,* 2004).

This study provides an insight on the genetic variability at the three SMV resistance loci in Indian soybean genotypes and would be helpful in the selection of suitable parents for developing SMV resistance soybean cultivars.

References

Banerjee, A., Chandra, S., Swer, E.K.P., Sharma, S.K. and Ngachan, S.V. 2014. First molecular evidence of Soybean mosaic virus (SMV) infection in soybean from India. *Australas Plant. Dis. Notes*, **9**: 150.

Barrett, B.A. and Kidwell, K,K. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop Sci*., **38**: 1261-1271.

Billore, S.D. and Srivastava, S.K. 2014. Soybean productivity and sustainability as affected by irrigation scheduling under different agro-ecological regions of India. *Soybean Res*., **12**: 75-82.

Cho, E.K. and Goodman, R.M. 1979. Strains of soybean mosaic virus: classification based on virulence in resistant soybean cultivars. *Phytopath*., **69**: 467-470.

Cho, E.K. and Goodman, R.M. 1982. Evaluation of resistance in soybeans to soybean mosaic virus strains. *Crop Sci*., **22**: 1133-1136.

Das, B., Sengupta, S., Prasad, M., and Ghose, T.K. 2014. Genetic diversity of the conserved motifs of six bacterial leaf blight resistance genes in a set of rice landraces. *BMC Genetics*, **15**: 82.

Eujayl, I., Sorrells, M., Baum, M., Wolters, P. and Powell, W. 2001. Assessment of genotypic variation among cultivated durum wheat based on EST-SSRs and genomic SSRs. *Euphytica*, **119**: 39-43.

Gore, M.A., Hayes, A.J., Jeong, S.C., Yue, Y.G., Buss, G.R. and Saghai Maroof, M.A. 2002. Mapping tightly linked genes controlling potyvirus infection at the Rsv1 and Rpv1 region in soybean. *Genome*, **45**: 592-599.

Gupta, S.K. and Gopalakrishna, T. 2009. Genetic diversity analysis in blackgram [*Vigna mungo* (L.) Hepper] using AFLP and transferable microsatellite markers from azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. *Genome*, **52**: 120-128.

Gupta, S.K. and Gopalakrishna, T. 2010. Development of unigene-derived SSR markers in cowpea (*Vigna unguiculata*) and their transferability to other *Vigna* species. *Genome*, **53**: 508-523.

Hamwieh, A., Imtiaz, M., Hobson, K. and Kemal, S.A. 2013. Genetic diversity of microsatellite alleles located at quantitative resistance loci for Ascochyta blight resistance in a global collection of chickpea germplasm. *Phytopathol. Mediterr*., **52**: 183-191.

Hayes, A.J., Ma, G.R., Buss, G.R., Saghai Maroof, M.A. 2000. Molecular marker mapping of Rsv4, agene conferring resistance to all known strains of soybean mosaic virus. *Crop Sci*., **40**: 1434-1437.

Hwang, T.Y., Yu, S., Yang, K., Kim, H.M. and Jeong, S.C. 2006. Application of comparative genomics in developing molecular markers tightly linked to the virus resistance gene Rsv4 in soybean. *Genome*, **49**: 380-388.

Jeong, S.C., Kristipati, S., Hayes, A.J., Maughan, P.J., Noffsinger, S.L., Gunduz, I., Buss, G.R. and Saghai Maroof, M.A. 2002. Genetic and sequence analysis of markers tightly linked to the soybean mosaic virus resistance gene, Rsv3. *Crop Sci*., **42**: 265-270.

Karp, A., Edwards, K.J., Bruford, M., Funk, S. and Vosman, B. 1997. Molecular technologies for biodiversity evaluation: opportunities and challenges. *Nat. Biotech*., **15**: 625-628.

Li, K., Yang, Q.H., Zhi, H.J., Gai, J.Y. and Yu D.Y. 2010. Identification and distribution of soybean mosaic virus strains in southern China. *Plant Dis*., **94**: 351-357.

Liu, K. and Muse, S.V. 2005. PowerMarker:an integrated analysis environment for genetic marker analysis. *Bioinformatics*, **21**: 2128-2129.

Mudge, J., Cregan, P.B., Kenworthy, J.P., Kenworthy, W.J., Orf, J.H. and Young, N.D. 1997. Two micro satellite markers that flank the major soybean cyst nematode resistance locus. *Crop Sci*., **37**: 1611-1615.

Naik, R.G. and Murthy, K.K.V. 1997. Transmission of soybean mosaic virus through sap, seed and aphids. *Karnataka J. Agric. Sci*., **10**: 565-568.

Nariani, T.K. and Pingaley, K.V. 1960. A mosaic disease of soybean (Glycine max L.). Indian *Phytopath*., **13**: 130-136.

Perrier, X., Flori, A. and Bonnot, F. 2003. Data analysis methods.
In: Hamon, P., Seguin, M., Perrier, X., Glaszmann, J. C. Ed., Genetic diversity of cultivated tropical plants. Enfield, Science Publishers. Montpellier. PP 43-76.

Prabhu, K.V., Singh, A.K., Basavaraj, S.H., Cherukuri, D.P., Charpe, A., Krishnan, S.G., Gupta, S.K., Joseph, M., Koul, S., Mohapatra, T., Pallavi, J.K., Samsampour, D., Singh, A., Singh, V.K., Singh, A. and Singh, V.P. 2009. Marker assisted selection for biotic stress resistance in wheat and rice. *Indian J. Genet*., **69**: 305-314.

Prajapat, K., Vyas, A.K. and Dhar, S. 2015. Effect of cropping systems and nutrient management practices on growth, productivity, economics and nutrient uptake of soybean (Glycine max). *Indian J. Agric. Sci*., **85**: 1138-1143.

Saghai Maroof, M.A., Tucker, D.M., Skoneczka, J.A., Bowman, B.C., Tripathy, S. and Tolin S.A. 2010. Fine mapping and candidate gene discovery of the soybean mosaic virus resistance gene, Rsv4. *Plant genome*, **3**: 14-22.

Shi, A., Chen, P., Zheng, C., Hou, A. and Zhang, B. 2008. A PCR-based marker for the Rsv1 locus conferring resistance to soybean mosaic virus. *Crop Sci*., **48**: 262-268.

Singh, B.R., Singh, D.R. and Saksena, H.K., 1976. A mosaic disease of soybean at Kanpur, *India. Sci. Cult*., **42**: 53-54.

Singh, R.K., Sharma, R.K., Singh, A.K., Singh, V.P., Singh, N.K., Tiwari, S.P., Mohapatra, T. 2004. Suitability of mapped sequence tagged

microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. *Euphytica*, **135**: 135-143.

Song, Q. J., Marek, L.F., Shoemaker, R.C., Lark, K.G., Concibido, V.C., Delannay, X., et al., 2004. A new integrated genetic linkage map of the soybean, *Theor. Appl. Genet*., **109**: 122-128.

Thompson, J.A., Nelson, R.L. and Vodkin, L.O. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Sci*., **38**: 1348-1355.

Yu, Y.G., Saghai Maroof, M.A., Buss, G.R., Maughan, P.J. and Tolin, S.A. 1994. RFLP and micro satellite mapping of a gene for soybean mosaic virus resistance. *Phytopathol*., **84**: 60-64.

Table 1. Soybean genotypes used in the study with pedigree and response to soybean mosaic virus (SMV)

S. No.	Genotype	Pedigree	Reaction to SMV
1.	KB-79 (Sneh)	Hardee x Monetta	Resistant
2.	MACS-57	JS-2 x Improved Pelican	Resistant
3.	MACS-124	JS-2 x Improved Pelican	Resistant
4.	MACS-450	Bragg x MACS-111	Resistant
5.	NRC-12 (Ahilya-2)	Mutant of Bragg	Resistant
6.	PK-416	UPSM534 x S-38	Resistant
7.	PK-472	Hardee x Punjab-1	Resistant
8.	PK-564	(UPSM534 x Ankur) x Bragg	Resistant
9.	PS-1225	PK-515 x PK-327	Resistant
10.	PK-1241	PK-1039 x PK-327	Resistant
11.	Bragg	Jackson x D49-2491	Susceptible
12.	Hardee	D49-772 x Improved Pelican	Resistant
13.	Improved Pelican	Tanloxi x PI-60406	Susceptible
14.	JS79-81	Bragg x Harsoy	Susceptible
15.	JS-335	JS78-77 x JS71-5	Susceptible
16.	Kalitur	Indigenous native variety	Susceptible
17.	Lee	S-100 x CNS	Susceptible
18.	Punjab-1	Selection from Nanking variety	Resistant
19.	Pusa-37	Bragg x Java-16	Resistant
20.	SL-295	PK-416 x PS-564	Resistant
21.	SL-525	PK-416 x PK-1023	Resistant
22.	SL-688	PK-416 x SL-317	Resistant
23.	Type-49	Selection from indigenous material	Susceptible

Table 2. Details of SSR markers used in the study, indicating their chromosome location, repeat motif and the linked SMV resistance locus

S. No.	Marker	Linkage group	Position (cM)	Motif Repeat	Resistance locus	Reference
1.	Sat_297	LG-13 (F)	59.6	$(AT)_{23}$	*Rsv1*	Song et al. 2004
2.	Sat_234	LG-13 (F)	66.6	$(AT)_{22}$	*Rsv1*	Song et al. 2004
3.	Sat_317	LG-13 (F)	73.0	$(AT)_{24}$	*Rsv1*	Song et al. 2004
4.	Sct_033	LG-13 (F)	74.1	$(CT)_{12}(AT)_{9}$	*Rsv1*	Song et al. 2004
5.	Satt334	LG-13 (F)	78.1	$(ATT)_{16}$	*Rsv1*	Song et al. 2004
6.	Satt063	LG-14 (B2)	93.5	$(ATT)_{20}$	*Rsv3*	Jeong et al. 2002, Song et al. 2004
7.	Satt560	LG-14 (B2)	97.9	$(ATT)_{12}$	*Rsv3*	Song et al. 2004
8.	Satt726	LG-14 (B2)	100.6	$(ATT)_{20}$	*Rsv3*	Song et al. 2004
9.	Satt687	LG-14 (B2)	113.6	$(ATT)_{9}$	*Rsv3*	Song et al. 2004
10.	Satt157	LG-2 (D1b)	37.0	$(ATT)_{31}$	*Rsv4*	Song et al. 2004
11.	Satt634	LG-2 (D1b)	46.6	$(ATT)_{13}$	*Rsv4*	Hwang et al. 2006; Song et al. 2004
12.	Satt542	LG-2 (D1b)	53.0	$(ATT)_{19}$	*Rsv4*	Hayes et al. 2000; Song et al. 2004
13.	Satt266	LG-2 (D1b)	59.6	$(ATT)_{22}$	*Rsv4*	Hayes et al. 2000; Song et al. 2004

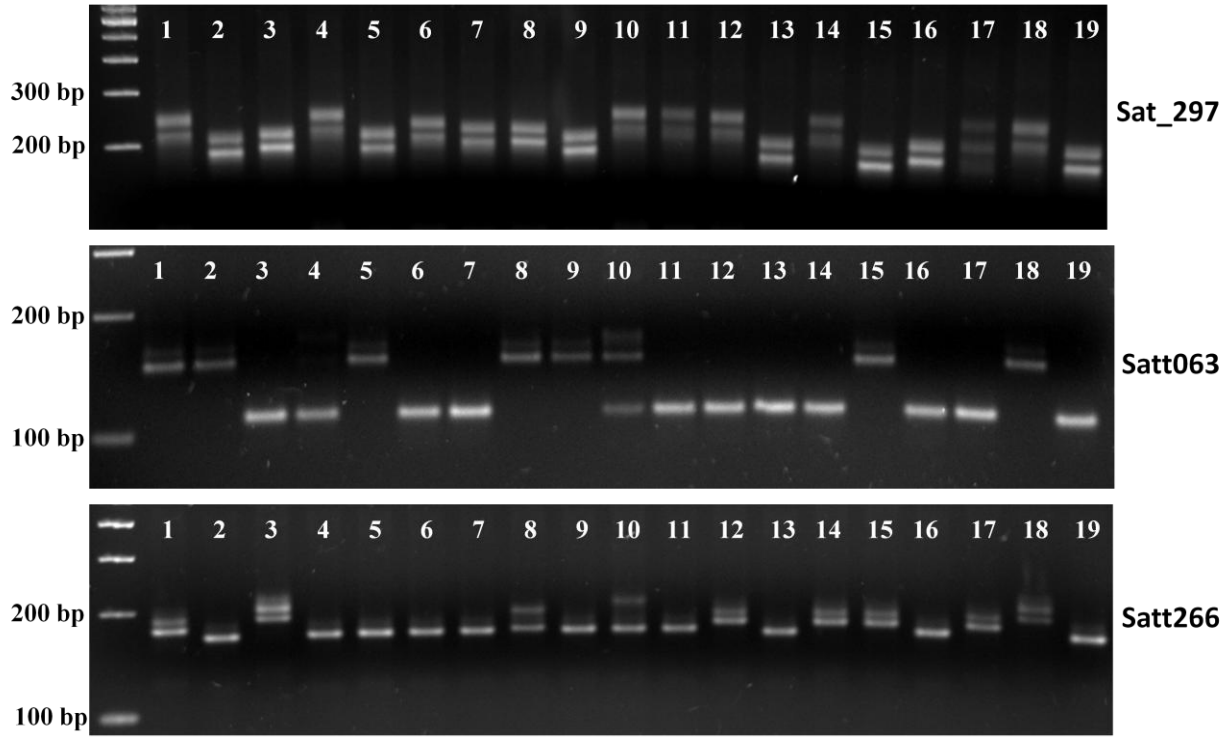
Table 3. Variation at polymorphic SSR marker loci used for studying the genetic diversity at SMV resistance loci in soybean genotypes

S.No.	Marker	Number of alleles observed	Gene Diversity	Observed hetrozygoisty
1.	Sat_234	6	0.59	0.0
2.	Sat_297	7	0.77	0.0
3.	Sct_033	6	0.77	0.0
4.	Satt334	6	0.67	0.0
5.	Satt063	2	0.49	0.0
6.	Satt560	6	0.76	0.0
7.	Satt726	2	0.38	0.0
8.	Satt687-1	4	0.58	0.0
9.	Satt687-2	4	0.48	0.0
10.	Satt266	5	0.62	0.0
11.	Satt542	3	0.55	0.0
12.	Satt634	3	0.51	0.0

Fig. 1

The amplification profile of SSR markers Sat_297, Satt063 and Satt266 linked to SMV resistance loci *Rsv1*, *Rsv3* and *Rsv4*, respectively on 19 Indian soybean genotypes.

Lane M: 100 bp Ladder, 1: KB-79; 2: MACS-57; 3: MACS-124; 4: MACS-450; 5: NRC-12; 6: PK-416; 7: PK-472; 8: PK-564; 9: PS-1225; 10: PK-1241; 11: Bragg; 12: Hardee; 13: Improved Pelican; 14: JS79-81; 15: JS-335; 16: Kalitur; 17: Lee; 18: Punjab-1; 19: Pusa-37



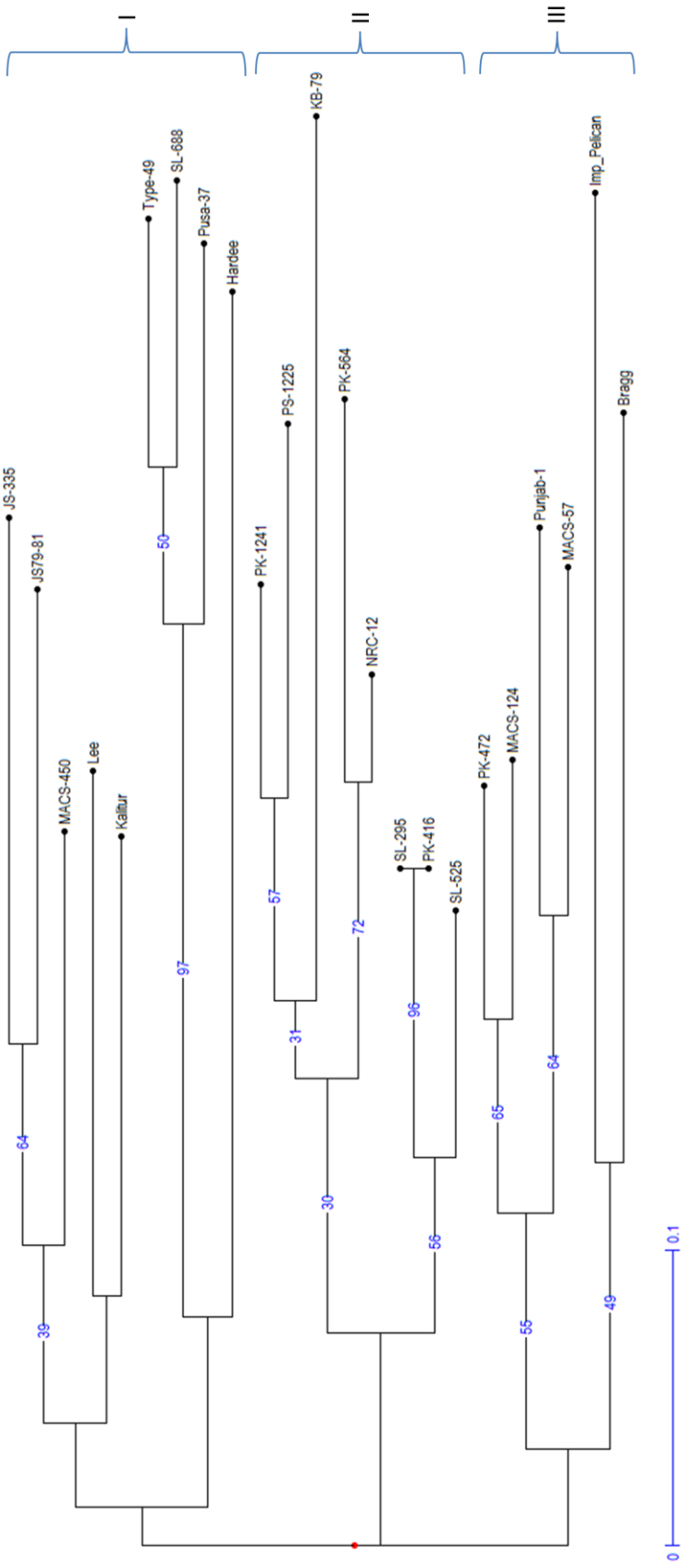

I
II
III
KB-79
Type-49
SL-688
Pusa-37
Hardee
Imp_Pelican
PS-1225
PK-564
Bragg
JS-335
JS79-81
PK-1241
Punjab-1
MACS-57
NRC-12
MACS-450
Lee
Kalbur
PK-472
MACS-124
SL-295
PK-416
SL-525
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Fig. 2

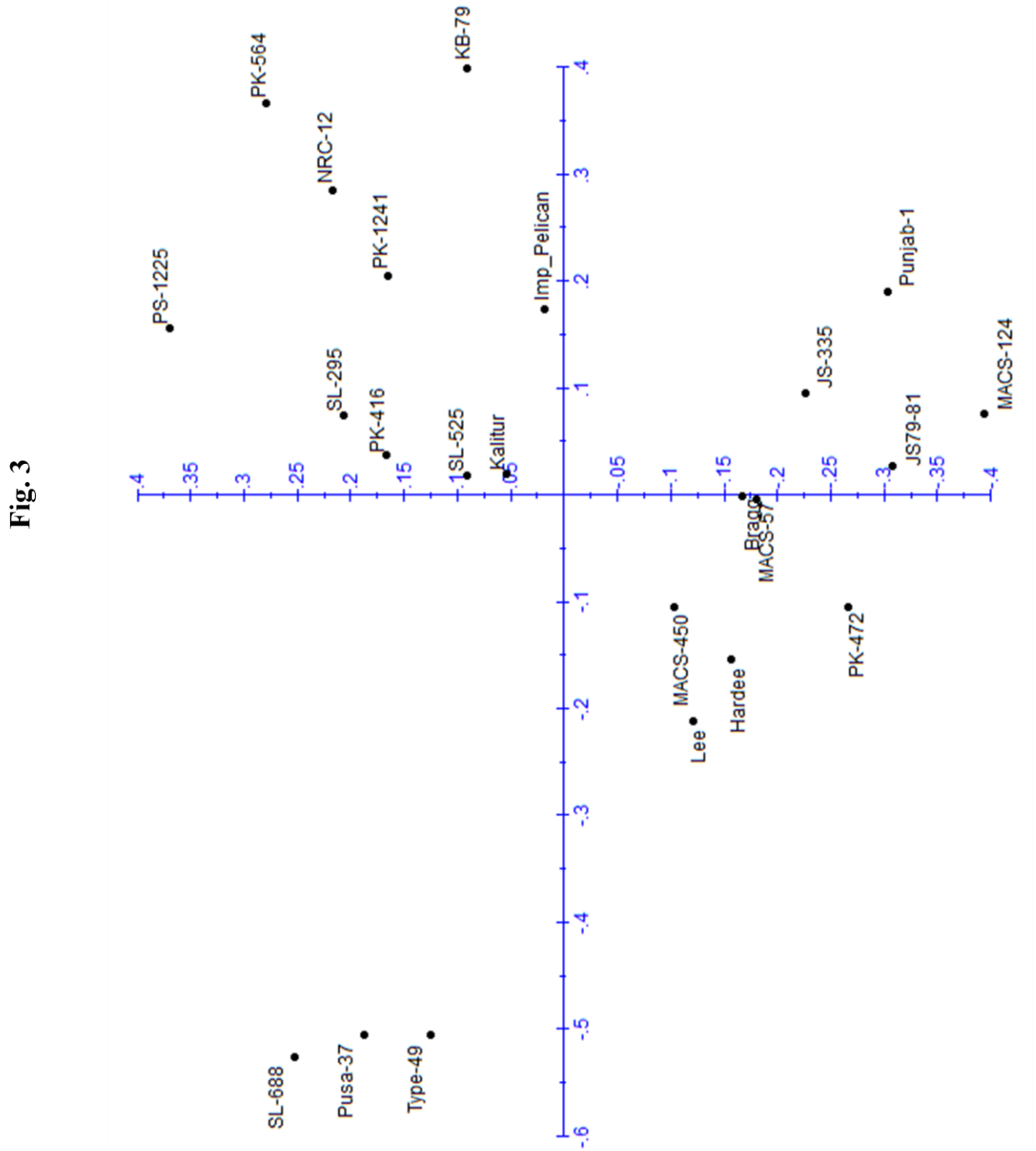

Fig. 3

Figure 3. Principal Coordinate analysis map showing relationship among 23 soybean genotypes based on SSR markers linked to three SMV resistance loci.