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Molecular Diversity Analysis and Cultivar Identification using Simple Sequence Repeat (SSR) Markers in Soybean [*Glycine max (L.) Merrill*]

Shipra Deo[1], Anto James[1], Sanjeev Kumar Singh[1], C. B. Singh[2] and Mukesh Kumar Rana[1]*

[1]ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012, India
[2]Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur, Bihar 812 007, India

**Corresponding author*

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A B S T R A C T

Soybean [*Glycine max (L.) Merrill*] is ranked number one among major oil crops and is the second most important oil seed crop of India. There has been significant growth in both area under cultivation and production, however, it still has lower productivity. In the present study, use of SSR markers has been made to study genetic diversity in the Indian soybean cultivars and for their identification. Ninety-six soybean cultivars were used and genetic diversity was assessed using 49 microsatellite markers distributed across the soybean genome. A total of 230 bands were amplified of which 229 bands were found to be polymorphic. The number of amplified bands ranged from 2 to 7 with an average value of 4.73. The PIC values of the primers ranged from 0.13 to 0.77 with an average of 0.61. The heterozygosity index values varied between 0.14 to 0.80 with an average of 0.66. The genetic similarity values in pair-wise comparison of cultivars ranged from 0.59 to 0.94 with an average similarity index of 0.71. Based on cluster analysis, soybean cultivars included in the study were grouped into two major clusters. However, all cultivars could be grouped into eight clusters. Among these eight clusters, cluster V was found to be the largest with 33 cultivars followed by cluster IV (30 cultivars), cluster I (16 cultivars), cluster III (4 cultivars), cluster VIII (03 cultivars) and cluster II (2 cultivars). Unique bands, present in one particular cultivar only and absent in all other cultivars, were observed for seven cultivars namely, Lee, MACS-58 KHsb-2 JS-97-52 PK-471, Bragg and Co-3. Six primers produced these seven unique bands with primer Gly SATT-586 producing two unique bands of sizes 170bp and 230bp in cultivars Lee and MACS-58, respectively. All the cultivars could be unequivocally discriminated from one another using SSR marker set included in the study.

Introduction

Soybean [*Glycine max (L.) Merrill*] is an important leguminous crop having 40-44% protein and 20% oil (Singh *et al*., 1999). Due to its versatile nutritional qualities and multiple uses, soybean is also known as the golden bean or miracle bean or wonder crop (Gopinath *et al*., 2015). In the international world trade, soybean is ranked number one among major oil crops such as rapeseed, groundnut, cotton seed, sunflower, linseed, sesame and safflower (Chung and Singh, 2008). Soybean is the second most important

oil seed crop of India, next to mustard. Although soybean has made significant strides for both area under cultivation and total production, it still suffers as far as productivity is concerned, particularly pertaining to climate, edaphic, production, and technology aspects that hinder its higher productivity. One way to enhance productivity is to enhance gains through genetic improvement, whereby to ascertain the existing levels of variability and quantifying genetic diversity is essential.

Genetic diversity in soybean has been assessed using various types of marker systems *viz.*, morphological, biochemical, cytological and molecular including DNA markers. Morphological and biochemical markers are limited in number, stage-specific and are highly influenced by the environmental conditions. Different types of DNA markers like RFLP (Botstein *et al*., 1980), RAPD (Williams *et al*., 1990), ISSR (Zietkiewicz *et al*., 1994), AFLP (Vos *et al.*, 1995) and microsatellite or SSR (Tautz and Renz, 1984) have been utilized for genetic diversity analysis at molecular (DNA) level in crop plants. DNA markers are more informative, stable and reliable, and hence are a powerful tool for diversity analysis and cultivar identification. Among different DNA marker types, Simple Sequence Repeat (SSR) or commonly called microsatellites are an excellent system. These SSR's can be mono-, di-, tri-, tetra- or penta-nucleotides with different lengths of repeats motifs and are abundant throughout the eukaryotic genome (Powell *et al.*, 1996). Over other techniques, SSRs have many advantages such as rapid and reliable detection, abundance in the genome, co-dominant inheritance, reveal high heterozygosity and high levels of polymorphism with high reproducibility (Akkaya *et al*., 1995, Lacape *et al.*, 2007). The hypervariable number of repeat units makes microsatellite markers an excellent tool for genotype differentiation and in evaluation of genetic diversity. High level of polymorphism at the SSR loci has been reported in soybean by various workers (Bisen *et al*., 2015; Rani *et al.*, 2016; Nawaz *et al*., 2017) and these microsatellite markers have been extensively used in genetic diversity analysis and in DNA fingerprinting of soybean genotypes as well (Ghosh *et al*., 2014; Chauhan *et al*., 2015; Pagar *et al*., 2017; Gupta and Manjaya, 2017; Zhao *et al*., 2018, Koutu *et al.*, 2019). The use of SSR markers has been made in the present study with the aim to prove the efficiency of microsatellite markers in evaluating genetic diversity of Indian soybean cultivars and for their molecular identification.

Materials and Methods

Plant material and DNA extraction

Ninety-six different cultivars of soybean released for different agro- climatic regions of the country over different years were selected for the present study. The details of the cultivars including pedigree, cultivation zone, year of release and breeding centre of the varietal material used in the study are provided in Table 1. Eighty-two SSR primer pairs selected from the 20 linkage groups of soybean from the previous study (Song *et al*., 2004) were initially screened in this study to find the ones giving sharp, scorable and polymorphic amplification products. The primer sequences with their linkage group location are available at https://www. soybean.org /d/page/# soybean data base.

The seeds of soybean cultivars used in the study were sown in plastic pots in the green house at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Five gram of leaf tissue from fifteen-days old seedlings was harvested in bulk from each cultivar for genomic DNA extraction.

Genomic DNA was extracted following CTAB (Cetyl-trimethyl-ammonium bromide) method as described by Saghai-Maroof *et al.*, (1984) with minor modifications. The concentration and quality of the isolated genomic DNA was estimated using the Nano-drop spectrophotometer (ND-1000, USA) and a final working concentration of 10ng/µL was made and stored at -4^{o}C for further analysis.

PCR amplification of SSR markers

Five different cultivars of soybean namely, Palam Soya, Harit Soya, Phule Kalyani, Improved Pelican and Alankar were used to screen the SSR primers. After screening of all the primers on this panel of five cultivars, 49 primers were finally selected for further study on the basis of clear polymorphic bands and good reproducibility (Table 2).

The reaction mixture (25 µL) consisted of 3.5ng genomic DNA, 2.5 µL of 10X PCR Buffer (10Mm Tris-HCL, pH-8.3 and 50mM KCL). 0.2mM dNTPs mix, 0.8 µM SSR primers (forward and reverse), 3mM $MgCl_2$ and 0.5 µL *Taq* DNA polymerase.

PCR amplification cycle was optimized at different temperatures and observed for sharp bands. PCR amplification consisted of initial denaturation at 95^{o}C for 5 min, followed by 40 cycles of 1 min at 94^{o}C,1 min at 55^{o}C,1 min at 72^{o}C and final extension at 72^{o}C for 8 min. All PCR reactions were carried out in thermal cycler from M/S MJ Research (Model PTC 200). At the time of electrophoresis PCR products were mixed with 1µl of gel loading dye (6x buffer: bromophenol blue, 0.25; xylene cyanol FF, 0.25; and glycerol in water 30%) and spun briefly in a microfuge before loading on to the gel.

The amplification products were electrophoresed and visualized on a 3% metaphor-agarose gel stained with ethidium bromide. The size of the amplified SSR fragments was estimated by running 100bp DNA ladder (M/S BR Biochem Life Sciences) in the gel as a standard size marker. After electrophoresis the gel was photographed under a UV light in a gel documentation system.

SSR data scoring and analysis

Amplified fragments of different sizes were considered as different alleles. Only distinct and reproducible bands were scored as present (1) or absent (0) for each SSR primer pair. This scored data was recorded in an MS excel spread sheet and the resultant data matrix was subjected to further analysis.

Various band statistics were calculated which consisted of total number of bands, total number of polymorphic bands, percent polymorphism, number of bands per marker, allelic frequency, unique alleles, gene diversity, heterozygosity, polymorphic information content (PIC) [Botstein *et al.*, (1980)], Effective multiplex ratio (EMR), Resolving Power (RP), and Marker Index (MI). Marker Index (MI), the measure of marker utility was obtained as a product of PIC and Effective Multiplex Ratio and was calculated in accordance with Powell *et al.*, (1996).

Resolving Power (RP) of each primer pair was calculated as per Prevost and Wilkinson (1999). Resolving Power of a marker gives us an indication of the discriminatory potential of the marker. The data analysis was carried out using computer software NTSYS-pc version 2.2 (Rohlf, 2009) for calculating Simple Matching coefficient values and to generate a dendrogram to decipher genetic relationship among the genotypes. Principal co-ordinate analysis (PCoA) was also computed using the same NTSYS-pc software.

Results and Discussion

Genetic diversity assessment and cultivar identification is important for crop improvement management and protection of genetic variability (Chandra *et al.,* 2013). DNA markers nowadays are routinely used in such studies to provide authenticity to morphological markers and to arrive at accurate estimates of genetic relationships among genotypes. Moreover, DNA markers, particularly SSR markers, have been the preferred choice in breeding programs as these are more authentic and less influenced by environmental fluctuations (Vinu *et al*., 2013). Several previous investigators recommended the use of microsatellite markers as a rapid and powerful tool for genetic diversity assessment in soybean and different plant species.

SSR polymorphism survey

In the present investigation 82 SSR primers were initially chosen based on published research work (Song *et al*., 2004). Out of these 82 primers, 49 SSR primers were able to amplify the genomic DNA of soybean and were informative in discriminating the 96 soybean cultivars. The representative amplification profile of 96 soybean cultivars using SSR primer SATT-268 is shown in Figure 1.

A total of 230 bands were amplified by the 49 SSR primers, out of which 229 bands were found to be polymorphic and only one band was monomorphic which was by using the primer SATT-346 (Table 3). Forty-eight primers produced 100% polymorphic bands, while one primer SATT-346 produced 80% polymorphic bands. The percentage of polymorphism produced by SSR primers was, hence, found to be 99.59%. Further, it was observed that there was no correlation between per cent polymorphism and PIC values as SSR primer Sat-366 showed minimum PIC values but was 100 per cent polymorphic. The average percentage of polymorphism produced by the 49 SSR primer in our study was found to quite high compared to previous studies (Kumar *et al.,* 2009; Singh *et al.,* 2010; Bisen *et al.,* 2015) which might be due to inclusion of genetically more diverse material in our study.

The number of alleles amplified by each primer pair ranged from 2 to 7 with an average value of 4.73 (Table 3). The SSR primers Gly Satt-586 and Satt-038 each, generated the maximum number of seven alleles while the primer Sat-366 produced the minimum number of alleles which was two. The product size of the bands ranged from 80bp (Satt-038) to 480bp (Gly Satt-577).

The allele frequency obtained in earlier studies was quite similar to what we observed in our study (2 to 7 alleles) per locus. For example, Yun-Lai *et al.,* (2009) detected 2 to7 alleles per locus in 83 soybean cultivars with 43 SSR primers. A similar result was reported by Chauhan *et al.,* (2015) using 21 SSR markers in 48 Indian Soybean cultivars. The average value of alleles per locus (4.73) detected in our study was found to be similar with that observed by Tantasawat *et al.,* (2011) (4.8 allele/locus).

The PIC values of the primers ranged from 0.13 to 0.77 with an average of 0.61 (Table 3). The maximum PIC value was recorded for the primer Gly SATT-449, whereas the minimum value was recorded for primer Sat-366. Tantasawat *et al.,* (2011) found the polymorphic information content (PIC) among 25 soybean genotypes to be ranging from 0.13 to 0.88 with an average of 0.60. The results of this study were comparable to what we had observed in our study, with regards to polymorphic information content (PIC) and average number of alleles per

locus. Ghosh *et al.,* (2014) observed PIC among 32 soybean cultivars varies from 0.21 (S26) to 0.83 (S27) with an average of 0.51, which was quite low compared to what we have observed in our study. The heterozygosity index values ranged from 0.14 (Sat-366) to 0.80 (Gly SATT-449) with an average of 0.66 (Table 3).

The genetic similarity values (Simple Matching) ranged from 0.59 to 0.94 with an average similarity index of 0.71 (similarity matrix table not shown). The lowest average similarity coefficient value (0.59) was shown by the cultivar Hardee with Pant soybean and JS-71-05. The highest similarity coefficient value (0.94) was shown by MAUS-81 and Paratap Soya. The Marker Index (MI) values ranged from 1.348 to 5.082 with an average value of 3.30. Resolving Power values ranged from 2 to 2.23 with an average of 2.0 for the polymorphic markers.

The discriminatory power of SSR markers for varietal differentiation has been observed in earlier studies also. The gene diversity in our study ranged from 0.44 (Satt-243) to 0.80 (Gly SATT-449) with an average of 0.69. Diwan and Cregan (1997) reported a relatively large mean gene diversity of 0.80, which was much greater than that observed in our study.

Similarly, Cho *et al.,* (2006) reported the genetic diversity to range from 0.41 to 0.93 with an average of 0.69, which was quite similar to our findings. Similarly, Chotiyarnwong *et al*., (2007) reports the mean gene diversity of the soybean cultivars to be 0.831, which is quite high compared to our study. Yoon *et al*., (2009) reports similar levels of genetic diversity that range from 0.35 to 0.94 with an average value of 0.70. Nawaz *et al*., (2017) reports a relatively low average genetic diversity of 0.4 in 100 Korean wild soybean accessions, using 42 SSR markers, this was found to be far lesser than what was observed in our study.

The results of the present investigation are in disagreement with others with respect to SSR polymorphism surveys. For example, the study of Velusamy *et al.,* (2013) reported the number of alleles to range from 6 to 11 alleles, with a mean value of 9.11 per locus and the total number of alleles to be 82 in 178 wild soybean accessions using nine SSR markers. The discrepancy in the observations might be due to very less number of primers used in their study.

Kumawat *et al.,* (2014) assessed the genetic diversity in 82 soybean accessions using 40 SSR markers and observed, unlike in our study, the number of alleles for SSR loci to range from 2 to 4 with an average of 2.97 alleles per marker. PIC values in their study ranged from 0.10 to 0.74 with an average of 0.48. Rani *et al.,* (2016) characterized 102 Indian soybean cultivars using 10 SSR primers only and observed PIC values to range from 0.47 to 0.81 and with a total 50 alleles.

Genetic diversity and relationships among cultivars

A cluster analysis of all the cultivars included in the study was carried out based on the similarity indices which were derived from 49 SSR primers. Based on cluster analysis, soybean cultivars were grouped into two major clusters i.e. A and B (Figure 2).

However, all cultivars could be grouped into total eight clusters. Among these eight clusters, cluster V was found to be the largest with 33 cultivars followed by cluster IV (30 cultivars), cluster I (16 cultivars), cluster III (4 cultivars), cluster VIII (03 cultivars) and cluster II (2 cultivars). Cultivars Pratap Soya and MAUS-81, although from different

sources and with different pedigrees, shared the maximum similarity based on the chosen set of SSR markers. However, cultivars JS-9305 and JS-9560 were also found to be genetically similar based on the clustering.

These two cultivars were from the same breeding center i.e. JNKVV Jabalpur and were found to share a common pedigree of secondary selection from PS-73-22. There was no discernible clear pattern in grouping of cultivars into clusters either year-wise or from different breeding centers or zones. Loose packaging of cultivars into different clusters was conspicuous with respect to cultivars from VPKAS, Almora in cluster I, MACS, Pune in cluster IV and from GBPUA & T, Pant Nagar in cluster V.

Cultivars sharing common pedigree also tended to remain together within the same cluster. For example, cultivars MACS-57, MACS-58, and MACS-124 sharing common pedigree JS2 X Improved Pelican were found to be in cluster IV. Cultivars Ahilya-1(NRC-2) and Ahilya-2 (NRC-12) grouped in cluster V have a common parentage. Similarly, four cultivars *viz.,* Hara soya, MACS-450, PS-1042 and Pusa 98-14 grouped in cluster V has a common parent 'Bragg', and similar finding have been observed by Bisen *et al.,* 2015. In clustering, the genotypes with similar pedigree were grouped together.

In earlier studies also, grouping of Indian soybean genotypes into two (Chotiyarnwong *et al*., 2007, Ghosh *et al*., 2014, Bisen *et al.,* 2015; Chauhan *et al.,* 2015, Wang *et al*., 2015, Koutu *et al.,* 2019) and three clusters (Kumawat *et al.,* 2014, Gupta and Manjaya, 2017) have been reported, indicating the close relatedness among cultivars. The cluster analysis shows that the cultivars which are quite distinct from the rest might be source of novel genes/ allels and can be utilized in soybean breeding programs. Principal Coordinates analysis (PCoA) results (not shown) corroborated the finding of cluster analysis strengthening the clustering of cultivars into different groups.

Soybean cultivar identification

Cluster analysis shows that all the cultivars could be discriminated from one another using the set of 230 markers from 49 primers (Figure 2). These findings are useful to establish the identity of cultivars in the court of law in case any dispute with respect to cultivar identification arises.

Presence of unique bands helped in the identification of specific genotypes and is useful for DNA profiling of cultivars. In the present study, we observed unique bands, present in one particular cultivar only and absent in all other cultivars, for seven cultivars namely, Lee, MACS-58 KHsb-2 JS-97-52 PK-471, Bragg and Co-3.

Six primers produced these seven unique bands with primer Gly SATT-586 producing two unique bands of sizes 170bp and 230bp in cultivars Lee and MACS-58, respectively (Table 3). Similar findings have been reported earlier by Tantasawat *et al.,* (2011) and Sahu *et al.,* (2012) who reported seven unique alleles in their study and Koutu *et al.,* (2019) obtained eight unique alleles in soybean genotypes using SSR primers.

Kumawat *et al.,* (2014) reported five unique alleles in a set of 82 soybean genotypes, whereas Rani *et al.,* (2016) reported four unique allele in a set of 102 Indian soybean cultivars in their study. These unique alleles can be utilized for the identification of soybean cultivars and for isolating distinguishing genes/alleles for further studies.

Table.1 List of Indian soybean cultivars [*Glycine max* (L.) Merrill] along with their pedigree, zone, year of release and releasing centre

S. No.	Cultivar	Pedigree	Zone	Year of release	Releasing Centre
1	JS 335	JS78-77 × JS71 -5	CZ	1994	RVSKVV, Sehore (MP)
2	Palam soya	JS72-451xPunjab-1	NHZ	2005	CSKHPKV, Palampur (HP)
3	JS 9560	Secondary selection from PS 73-22	CZ	2006	JNKVV, Jabalpur (MP)
4	JS-9305	Secondary selection from PS 73-22	CZ	2002	JNKVV, Jabalpur (MP)
5	Harit Soya	Himso 1520 × Bragg	NHZ	2001	CSKHPKV, Palampur- (HP)
6	VL Soya-2	Selection from VHC 856007	NHZ	1989	VPKAS, Almora (UP)
7	VL Soya-21	Selection from VHC 3055	NHZ	1996	VPKAS, Almora (UP)
8	VL Soya-59	(Pb1 × VLS2) × EC361336	NHZ	2008	VPKAS, Almora (UP)
9	VL Soya-63	VLS2× (Bragg × VHC3022)	NHZ	2008	VPKAS, Almora (UP)
10	VL Soya-47	Selection from KHSF- 3-1-1	NHZ	2000	VPKAS, Almora (UP)
11	VL Soya-1	Mutant of Bragg	NHZ	1985	VPKAS, Almora (UP)
12	VL Soya-65	Selection from local cultivar	NHZ	2010	VPKAS, Almora (UP)
13	RVS-2001-4	JS93-01 × EC390981	CZ	2014	RVSKVV, Gwalior
14	Phule Kalyani	JS335 × Ankur	SZ	2006	MPKV, Rahuri
15	Kalitur	Indigenous native cultivar	CZ	1969	JNKVV, Jabalpur (MP)
16	Lee	S-100 × CNS	NHZ	1975	VPKAS, Almora ((UP)
17	Hardee	D 49-772 × Improved Pelican	SZ	1976	GKVK, Bangalore
18	Improved Pelican	Tanloxi × PI60406	SZ	1969	VNMKV, Parbhani
19	Ankur	SPS from composite of 22 crosses	NPZ	1976	IARI, New Delhi
20	Type 49	Selection from indigenous material	CZ	1978	JNKVV, Jabalpur (MP)
21	Punjab 1	Selection from Nanking cultivar	NPZ	1978	PAU, Ludhiana
22	Bragg	Jackson × D 49-2491	CZ	1978	ICAR, Indore
23	Alankar	D 63-6094 × D61-4249	NPZ	1978	IARI, New Delhi
24	KHSb-2	Manloxi × EC 39821	SZ	1982	GKVK, Bangalore
25	Shilajeet	Selection from EC 9309	NHZ	1980	VPKAS, Almora (UP)
26	Pusa 40	8-3 × Lee	NPZ	1981	IARI, New Delhi
27	Js-2	Selection from Tehri Garhwal material.	CZ	1982	JNKVV, Jabalpur (MP)
28	Gaurav (JS 72-44)	D 60-9647 × EC 7034	CZ	1982	JNKVV, Jabalpur (MP)
29	Durga (JS 72-280)	EC14437 × Bragg	CZ	1982	JNKVV, Jabalpur (MP)
30	Co- 1	Selection from EC398321	SZ	1982	TNAU, Coimbatore
31	Pusa-22	Punjab 1 × Clark 63	NPZ	1983	IARI, New Delhi
32	Gujarat(J-202) soyabean -2	Selection from Geduld cultivar	SZ	1983	MAUS, Parbhani
33	Gujarat(J-231) Soyabean 1	Selection from Punjab-1	SZ	1983	MAUS, Parbhani
34	PK 327	UPSM 82 × Semmes	NPZ	1983	GBPUA & T, Pant Nagar
35	PK 262	UPSM97 × Hardee	NPZ	1983	GBPUA & T Pant Nagar
36	Birsa soybean -1	Spontanous mutant of ‘Sepaya Black’	NPZ	1983	Ranchi
37	JS-76-205	Kalitur × Bragg	CZ	1990	JNKVV, Jabalpur (MP)
38	Pusa-37	Bragg × Java 16	NPZ	1985	IARI, New Delhi
39	PK-308	T 31 × Hardee	NPZ	1985	GBPUA & T, Pant Nagar
40	Monetta	Exotic cultivar EC 2587	SZ	1985	MACS, Pune

41	MACS-13	Hampton × EC 7034	SZ	1985	MACS, Pune
42	PK-472	Hardee × Punjab-1	NPZ	1986	GBPUA & T, Pant Nagar
43	PK-416	UPSM 534 × S 38	NPZ	1986	GBPUA & T, Pant Nagar
44	Shivalik	Selection from segregating PK 73-55	NHZ	1990	CSKHPKV, Palampur- (HP)
45	Pusa-24	Shelby × Bragg	NPZ	1987	IARI, New Delhi
46	Pusa-16	CNS × Lee	NPZ	1987	IARI, New Delhi
47	JS-75- 46	Improved Palican x Semmes	CZ	1987	JKVV, Jabalpur (MP)
48	Pusa-20	Bragg × Lee	NPZ	1988	IARI, New Delhi
49	MACS-58	JS2 × Improved Pelican	SZ	1989	MACS, Pune
50	AD-1(UGM-33)	Selection from “Hill cultivar”	SZ	1990	TNAU, Coimbatore
51	Pant soybean 564	(UPSM 534 × Ankur) × Bragg	NPZ	1991	GBPUA & T, Pant Nagar
52	JS-80-21	JS75-1 × PK 73-94	CZ	1991	JANKVV, Jabalpur (MP)
53	JS-71-05	Selection from Lee type exotic material	CZ	1991	JANKVV, Jabalpur (MP)
54	MACS-124	JS 2 × Improve Pelican	SZ	1992	MACS, Pune
55	MACS-57	JS2 × Improved Pelican	SZ	1992	MACS, Pune
56	JS-79-81	Bragg × Harsoy-Deciduous	SZ	1994	IASRI
57	Aarti (MAUS 1)	Mutant from DS 87-14	SZ	1996	MAUS, Parbhani
58	SL-295	PK416 × PK564	NPZ	1997	PAU, Ludhiana
59	Sneh (KB 79)	Hardee × Monetta	SZ	1997	GKVK, Bangalore
60	Pooja (MAUS 2)	Selection from SH 84-14	SZ	2014	MAUS, Parbhani
61	Pant soybean 1042	Bragg × PK 416	NPZ	1997	GBPUA & T, Pant Nagar (UP)
62	Pant soybean 1029	PK 262 × PK317	NPZ	1997	GBPUA & T, Pant Nagar
63	Pant soybean 1024	PK 308 × PK317	NPZ	1997	GBPUA & T, Pant Nagar
64	Co- Soya -2	UGM21 × JS335	SZ	1995	TNAU,Coimbatore
65	Ahilya-1 (NCR-2)	Induced mutant of Bragg	CZ	1996	ICAR, Indore
66	Ahilya-2(NCR -12)	Induced mutant of Bragg	CZ	1996	ICAR, Indore
67	Ahilya-3(NCR -7)	Selection from S 69-96	CZ	1996	ICAR, Indore
68	MACS -450	Bragg × MACS 111	SZ	1999	MACS, Pune
69	JS-90-41	PS73-7 × Hark	CZ	1999	JNKVV, Jabalpur (MP)
70	Prasad (MAUS -32)	Selection from JS 80-21	SZ	2000	MAUS, Parbhani
71	Parbhani Sona (MAUS- 47)	PS73-7 × Hark	CZ	2000	ICAR, Indore
72	Pant soybean 1092	PK 327 × PK416	NPZ	1999	GBPUA & T, Pant Nagar
73	LSb 1	Selection from MACS 330	SZ	2001	ANGRAU Hyderabad
74	Indira soya -9	Secondary selection from JS 80-21	CZ	1999	IGKVV, Raipur
75	Hara soy (HIMSO - 1563)	(Ankur × Himso 330) × Bragg	NHZ	2001	CSKHPKV, Palampur- (HP.)
76	Ahilya-4 (NCR -37)	Gaurav × Punjab 1	CZ	2001	DSR Indore
77	Samrudhi (MAUS 71)	JS 71-5 × JS 87-38	CZ	2002	MAUS, Parbhani
78	Prtishta (MAUS-61-2)	JS80-21 X KB-60	CZ	2002	MAUS, Parbhani
79	Pratikar (MAUS-61)	JS71-1 × PK-73-94	CZ	2002	MAUS, Parbhani
80	Pratap soya (RAUS 5)	PUSA 16 x JS 335	CZ	2007	MPUA,Udaipur
81	MAUS 81(Shakti)	KB74 × JS335	SZ	2003	MAUS,Parbhani

82	Pant soybean 1241	PK 1039 × PK327	NPZ	2003	GBPUA&T, Pant Nagar
83	TAMS-38	Monetta × PK472	SZ	2004	PDKV, Amravati
84	SL 525	PK 416 × PK1023	NPZ	2007	PAU, Ludhiana
85	Co-3 (Co soya)	UGM 69 × JS335	SZ	2005	TNAU, Coimbatore
86	PRS -1	Selection from germplasm	NPZ	2009	GBPUA&T, Pant Nagar
87	Pusa 97-12 (DS-97-12)	Mutant of DS74	NPZ	2007	IARI, New Delhi
88	JS-95-60	Selection from PS 73-22	CZ	2007	JNKVV, Jabalpur
89	PS 1347	PS 1024 × PK472	NPZ	2006	GBPUA&T, Pant Nagar
90	Pusa-98-14	Bragg × DS 93-MM-39	NPZ	2006	IARI, New Delhi
91	RKS 18 (Pratap soya -2)	MACS 450 × Monetta	CZ	2007	MPUA, Udaipur
92	TAMS -98 -21	Mutant of JS80-21	SZ	2007	PDKV, Akola
93	PS -1225	PK515 × PK327	NPZ	2007	GBPUA&T, Pant Nagar
94	JS-97-52	PK 327 × L 129	CZ	2008	DSR, Indore and JNKVV, Jabalpur
95	SL 688	PK416 × SL317	NPZ	2008	PAU, Ludhiana
96	PK -471	Hardee × Punjab1	SZ	1988	VNMKV, Parbhani

NHZ: Northern Hill Zone, NPZ:Northern Plain Zone, NEZ: Northern Eastern Zone, CZ: Central Zone, SZ: Southern Zone. RVSVV: Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, CSKHPKV: Chaudhary Sarwan Kumar Himachal Pradesh University, JNKVV: Jawaharlal Nehru Krishi Vishva Vidhyalya, VPKAS: Vivekananda Parvatiya Krishi Anusandhan Sansthan, MPKV: Mahatma Phule Krishi Vidyapeeth, GKVK: Gandhi Krishi Vignana Kendra, VNMKV: Vasntrao Naik Marathwada Krishi Vidyapeeth, IARI: Indian Agricultural Research Institute, PAU: Punjab Agricultural University, ICAR: Indian Council of Agricultural Research, TNAU: Tamil Nadu Agricultural University, MAUS: Marathwada Agricultural University, GBPU&T: G.B Pant University of Agriculture and Technology, MACS: Maharashtra Academy for Cultivation of Sciences, IASRI: Indian Agricultural Statistics Research Institute, ANGRAU: Acharya N.G Ranga Agriculture University, IGKVV: Indian Gandhi Krishi Viswavidyalaya, MPUA: Maharana Pratap University of Agriculture and Technology, PDKV: Panjabrao Deshmukh Krishi Vidyapeeth, DSR: Directorate of Soybean Research

Table.2 List of SSR primers used for genetic diversity estimation and cultivar identification in soybean [*Glycine max* (L.) Merrill]

S. No.	Marker name	Linkage group	Chromo some number	Repeat motif	Forward primer	Reverse primer
1	SAT 185	G	18	(AT)31	GCGGCTGGAGAAAACCTTTTATG	GCGAATAAAAACCGAGAATGATTT
2	SAT 366	J	16	(AT)8	GCGGCACAAGAACAGAGGAAACTATT	GCGGACATGGTACATCTATATTACGAGTATT
3	SATT002	D2	17	(ATT)25	TGTGGGTAAAATAGATAAAAAT	TCATTTTGAATCGTTGAA
4	SATT005	D1b	2	(ATT)19	TATCCTAGAGAAGAACTAAAAAA	GTCGATTAGGCTTGAAATA
5	SATT030	F	13	(ATT)21	AAAAAGTGAACCAAGCC	TCTTAAATCTTATGTTGATGC
6	SATT038	G	18	(ATT)17	GGGAATCTTTTTTTCTTTCTATTAAGTT	GGGCATTGAAATGGTTTTAGTCA
7	SATT082	D2	17	(ATT)13	AATTCATTTAGGGAGTTGAT	CTAGCCAATGTCATATGACT

8	SATT173	O	10	(ATT)18	TGCGCCATTTATTCTTCA	AAGCGAAATCACCTCCTCT
9	SATT177	A2	8	(ATT)16	CGTTTCATTCCCATGCCAATA	CCCGCATCTTTTTCAACCAC
10	GlySATT 180	C1	4	(ATT)16	TCGCGTTTGTCAGC	TTGATTGAAACCCAACTA
11	SATT181	H	12	(ATT)18	TGGCTAGCAGATTGACA	GGAGCATAGCTGTTAGGA
12	SATT183	J	16	(ATT)13	TAGGTCCCAGAATTTCATTG	CACCAACCAGCACAAAA
13	GlySATT 184	D1a	1	(ATT)13	GCGCTATGTAGATTATCCAAATT ACGC	GCCACTTACTGTTACTCAT
14	SATT197	B1	11	(ATT)20	CACTGCTTTTTCCCCTCTCT	AAGATACCCCCAACATTATTTG TAA
15	SATT243	O	10	(ATT)17	GCGCATTGCACATTAGGTTTTCT GTT	GCGGTAAGATCACGCCATTATT TAAGA
16	SATT244	J	16	(ATT)27	GCGCCCCATATGTTTAAATTATA TGGAG	GCGATGGGGATATTTTCTTTAT TATCAG
17	SATT245	M	7	(ATT)13(ATG)15	AACGGGAGTAGGACATTTTATT	GCGCCTCCTGAATTTCAAAGAA TGAAGA
18	SATT250	M	7	(ATT)16	CGCCAGCTAGCTAGTCTCAT	AATTTGCTCCAGTGTTTTAAGT TT
19	SATT264	K	9	(ATT)14	CCTTTTGACAATTATGGCATATA	GCATAGAAGGGCATCATTCAG AT
20	GlySATT 267	D1a	1	(ATT)16	CCGGTCTGACCTATTCTCAT	CACGGCGTATTTTTATTTTG
21	SATT268	E	15	(ATT)17	TCAGGGGTGGACCTATATAAAA TA	CAGTGGTGGCAGATGTAGAA
22	GlySATT 277	C2	6	(ATT)40	GGTGGTGGCGGGTTACTATTACT	CCACGCTTCAGTTGATTCTTAC A
23	GlySATT 279	H	12	(ATT)28	GCGCAAAAGGACGCCCACCAAT AG	GCGGTGATCGGATGTTATAGTT TCAG
24	SATT285	J	16	(ATT)19	GCGACATATTGCATTAAAAACAT ACTT	GCGGACTAATTCTATTTTACAC CAACAAC
25	SATT286	C2	6	(ATT)17	GCGGCGTTAATTTATGCCGGAAA	GCGTTTGGTCTAGAATAGTTCT CA
26	SATT288	G	18	(ATT)17	GCGGGGTGATTTAGTGTTTGACA CCT	GCGCTTATAATTAAGAGCAAA AGAAG
27	GlySATT 300	A1	5	(ATT)19	GCGCCCACACAACCTTTAATCTT	GCGGCGACTGTTAACGTGTC
28	SATT308	M	7	(ATT)21	GCGTTAAGGTTGGCAGGGTGGA AGTG	GCGCAGCTTTATACAAAAATC AACAA
29	SATT309	G	18	(ATT)13	GCGCCTTCAAATTGGCGTCTT	GCGCCTTAAATAAAACCCGAA ACT
30	SATT324	G	18	(ATT)19	GTTCCCAGGTCCCACCATCTATG	GCGTTTCTTTTATACCTTCAAG
31	SATT335	F	13	(ATT)12	CAAGCTCAAGCCTCACACAT	TGACCAGAGTCCAAAGTTCATC
32	SATT337	K	9	(ATT)19	GCGTAAATCTGATATATGTTACC ACTGA	GCGTAATACGCAAAACATAAT TAGCCTA
33	GlySATT 345	O	10	(ATT)27	CCCCTATTTCAAGAGAATAAGG AA	CCATGCTCTACATCTTCATCAT C

34	SATT346	F	13	(ATT)18	CGTCGCCATCACTATGAGAA	CCATCTTGAGCAGAGTTTGAAGTT
35	SATT373	L	19	(ATT)21	TCCGCGAGATAAATTCGTAAAAT	GGCCAGATACCCAAGTTGTACTTGT
36	SATT406	J	16	(ATT)31	GCGTGAGCATTTTTGTTT	TGACGGGTTTAATAGCAT
37	SATT431	J	16	(ATT)21	GCGTGGCACCCTTGATAAATAA	GCGCACGAAAGTTTTTCTGTAACA
38	SATT440	I	20	(ATT)14	TGAGAACGTTTGAAAAGAGAT	GAAGAGATTAAGCATAAAGAATACTT
39	GlySATT449	A1	5	(ATT)21	GCGTGCTTCTTATATTAGGTGTTAGT	GCGCATTGGAGTTTTTGCTTTT
40	GlySATT453	B1	11	(ATT)13	GCGGAAAAAAAACAATAAACAACA	TAGTGGGGAAGGGAAGTTACC
41	GlySATT530	N	3	(ATT)12	CATGCATATTGACTTCATTATT	CCAAGCGGGTGAAGAGGTTTTT
42	SATT534	B2	14	(ATT)25	CTCCTCCTGCGCAACAACAATA	GGGGGATCTAGGCCATGAC
43	SATT562	I	20	(ATT)18	GCGGATTGACTGAGATGTTTAT	GCGGCGGCAGGTTAAATGGATTGA
44	GlySATT577	B2	14	(ATT)12	CAAGCTTAAGTCTTGGTCTTCTCT	GGCCTGACCCAAAACTAAGGGAAGTG
45	GlySATT586	F	13	(ATT)19	GCGGCCTCCAAACTCCAAGTAT	GCGCCCAAATGATTAATCACTCA
46	GlySATT588	K	9	(ATT)18(AT)10(CT)14	GCTGCATATCCACTCTCATTGACT	GAGCCAAAACCAAAGTGAAGAAC
47	SATT590	M	7	(ATT)26	GCGCGCATTTTTTAAGTTAATGTTCT	GCGCGAGTTAGCGAATTATTTGTC
48	SOYPRP-1	-	-	(TAT)20	CGTGCCAAATTACATCA	TGATGGGAACAAGTACATAA
49	SOYSATT-005	D1b+ W	-	(ATT)19	TATATCCTAGAGAAGAACTAAAAAA	GTCGATTAGGCTTGAAATAATAC

Table.3 SSR primers and their characteristics for diversity assessment and cultivar identification in soybean [*Glycine max* (L.) Merrill]

S. No.	Primer name	Size range (bp)	NSB	NPB	PPB	Gene diversity	RP	MI	Unique band size (Cultivar)	PIC	H_e
1	SATT-268	300- 360	4	4	100%	0.72	2	2.884	-	0.67	0.72
2	SATT-002	120-150	5	5	100%	0.70	2	3.488	-	0.65	0.70
3	SATT-038	170-480	7	7	100%	0.73	2	5.082	470bp (PK-471)	0.69	0.73
4	SATT-590	260-340	4	4	100%	0.65	2	2.612	-	0.61	0.67
5	SATT-082	100-130	4	4	100%	0.68	2	2.713	-	0.62	0.68
6	SATT-286	200-230	4	4	100%	0.66	2	2.629	-	0.55	0.59
7	SATT-197	140-180	5	5	100%	0.59	2	2.964	-	0.51	0.59
8	SATT-005	140-190	6	6	100%	0.71	2	4.306	140bp (JS-97-52)	0.67	0.72
9	SATT-335	140-250	4	4	100%	0.62	2	2.499	-	0.55	0.63
10	SATT-285	200-240	3	3	100%	0.46	2	1.392	-	0.33	0.39
11	SOY SATT-	150-190	5	5	100%	0.72	2	3.603	-	0.67	0.72

	005										
12	SOYPRP -1	120-150	4	4	100%	0.54	2	2.146	-	0.47	0.54
13	Gly SATT-267	230-250	3	3	100%	0.62	2	1.872	-	0.51	0.57
14	Gly SATT-279	180-200	3	3	100%	0.61	2	2.451	-	0.43	0.51
15	Gly SATT-577	80-130	5	5	100%	0.76	2	3.815	-	0.72	0.76
16	Gly SATT-586	170-230	7	7	100%	0.69	2	4.853	170bp (Lee), 230bp (MACS-58)	0.66	0.69
17	SOY SATT-183	200-270	6	6	100%	0.78	2	4.662	-	0.66	0.69
18	SATT-373	200-290	6	6	100%	0.79	2	4.728	-	0.76	0.79
19	SATT-177	100-200	6	6	100%	0.77	2	4.647	-	0.74	0.78
20	Gly SATT-453	240-280	4	4	100%	0.68	2	3.416	-	0.60	0.64
21	SATT-534	160-190	5	5	100%	0.73	2	3.657	175bp (Co-3)	0.68	0.73
22	SATT-244	120-180	6	6	100%	0.65	2	3.88	-	0.53	0.60
23	SATT-346	170-210	5	4	80%	0.65	2	2.58	-	0.59	0.64
24	SATT-309	120-150	4	4	100%	0.62	2	2.488	150bp (Bragg)	0.54	0.62
25	SATT-173	150-220	6	6	100%	0.75	2	4.519	-	0.72	0.75
26	Gly SATT-588	120-190	5	5	100%	0.73	2	3.668	-	0.69	0.73
27	SATT-243	270-300	3	3	100%	0.44	2	1.348	-	0.38	0.45
28	SAT-366	190-200	2	2	100%	0.60	2	1.804	-	0.13	0.14
29	SATT-245	190-210	3	3	100%	0.64	2	1.917	-	0.49	0.57
30	SATT-264	190-220	4	4	100%	0.66	2	2.621	-	0.59	0.66
31	SATT-288	190-220	4	4	100%	0.68	2	2.737	-	0.63	0.68
34	SATT-406	240-320	5	5	100%	0.72	2	3.607	-	0.68	0.72
35	SATT-440	170-220	6	6	100%	0.70	2.23	4.174	-	0.72	0.76
36	SATT-562	190-220	4	4	100%	0.70	2	2.814	-	0.40	0.42
37	SATT-030	130-170	5	5	100%	0.74	2	3.690	-	0.69	0.74
38	SATT-181	180-210	4	4	100%	0.73	2	2.94	-	0.68	0.74
39	SATT-324	220-240	3	3	100%	0.66	2	1.968	-	0.58	0.66
40	SATT-250	180-210	4	4	100%	0.59	2	1.761	-	0.57	0.62
41	SATT-431	180-230	5	5	100%	0.78	2	3.903	-	0.74	0.78
42	SAT-185	200-270	6	6	100%	0.77	2	4.624	-	0.74	0.77
43	GlySATT-277	170-250	6	6	100%	0.76	2	4.570	-	0.73	0.76
44	Gly SATT-184	140-180	4	4	100%	0.70	2	2.787	-	0.64	0.70
45	Gly SATT-449	230-280	6	6	100%	0.80	2	4.777	-	0.77	0.80
46	Gly SATT-300	250-270	3	3	100%	0.64	2	1.932	-	0.46	0.52
47	Gly SATT-530	190-250	6	6	100%	0.78	2	4.651	-	0.74	0.78
48	Gly SATT-345	210-260	6	6	100%	0.73	2	4.388	210bp (KHsb-2)	0.69	0.73
49	Gly SATT-180	230-290	6	6	100%	0.73	2	4.364	-	0.69	0.73

NSB: Number of scored band, NMB: Number of monomorphic bands, NPB: Number of polymorphic band, PPB: Percentage of polymorphic band, PIC: Polymorphism information content, RP: Resolving power, MI: Marker index, and H_e: heterozygosity

Figure.1 PCR amplification profile of 96 soybean [*Glycine max* (L.) Merrill] cultivars generated using SSR marker SATT 268. The numbers 1 through 96 on top of each lane corresponds to the cultivar name as indicated in Table 1. M is 100-base pair molecular weight standard

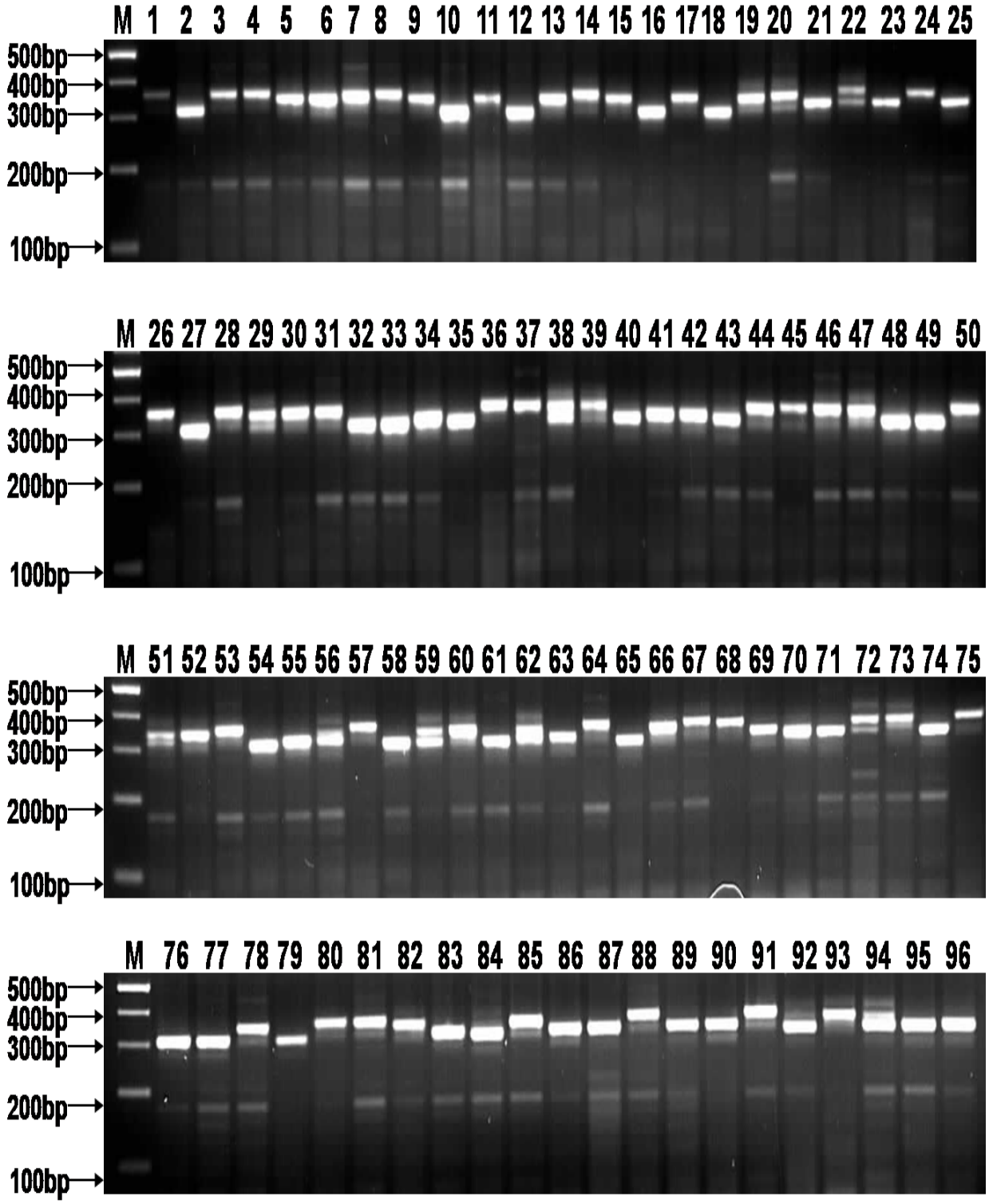
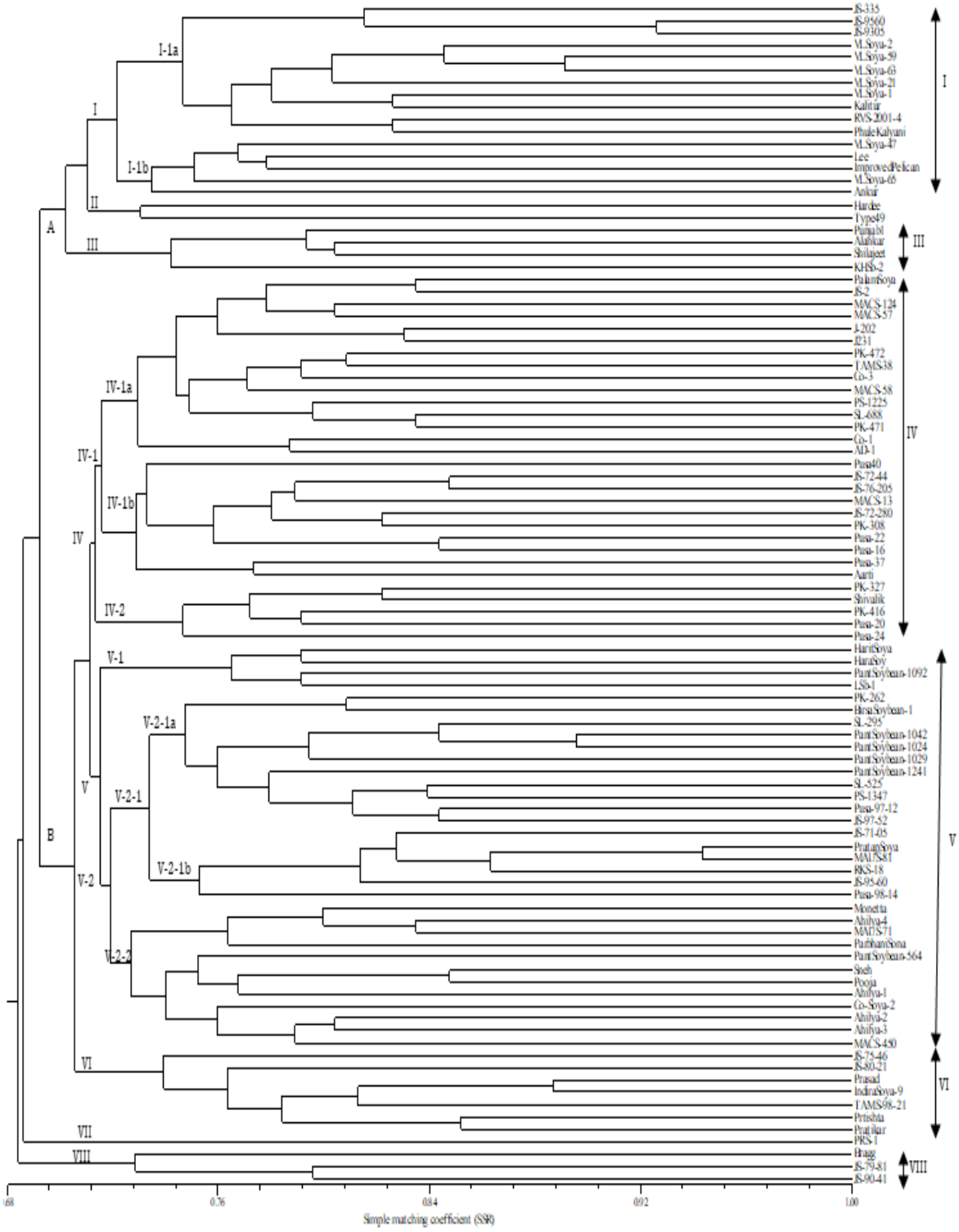


Figure.2 Dendrogram depicting genetic relationships (Simple Matching coefficient) among 96 soybean [Glycine max (L.) Merrill] cultivars generated using 230 markers from 49 SSR primer-pairs



The present study revealed the genetic diversity of 96 Indian soybean cultivars using SSR markers. The data revealed that 29% genetic dissimilarity existed in the material. Cluster analysis showed the genetic relationships among the cultivars and diverse cultivars as parents from different clusters can be selected. The results obtained in our study could be of practical application in breeding of soybean to produce better cultivars with high yielding characters. SSR markers were quite efficient in discriminating the cultivars from one another and the presence of unique bands highlighted the importance of their utility in IPR protection.

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References

Akkaya, M. S., Shoemaker, R. C., Specht, J. E., Bhagwat, A. A., and Cregan, P. B. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Science 35: 1439-1445.

Bisen, A., Khare, D., Nair, P., and Tripathi, N. 2015. SSR analysis of 38 genotypes of soybean (*Glycine Max* (L.) Merr.) genetic diversity in India. Physiology and Molecular Biology of Plants. 21: 109-115.

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 32: 314–331.

Chandra, V., Pant, U., Bhajan, R. and Singh, A.K. 2013. Studies on genetic diversity among *Alternaria* blight tolerant Indian mustard genotypes using SSR markers. The Bioscan. 8:1431–1435

Chauhan, D. K., Bhat, J. A., Thakur, A. K., Kumari, S., Hussain, Z., and Satyawathi, C. T. 2015. Molecular characterization and genetic diversity assessment in soybean [*Glycine max* (L.) Merr.] cultivars using SSR markers. Indian Journal of Biotechnology. 14: 504-510.

Cho, Y. H., M. S. Yoon, J. Lee, H. Y. Baek, C. Y. Kim, T. S. Kim, E. G. Cho, and H. B. Lee. 2006: Diversity and geographical relationships by SSR marker in subgenus Soja originated from Korea. Korean J. Crop Sci. 51: 239—247.

Chotiyarnwong, O., Chatwachirawong, P., Chanprame, S., and Srinives, P. 2007. Evaluation of genetic diversity in Thai indigenous and recommended soybean cultivars by SSR markers. Thai Journal of Agricultural Science. 40: 119-126.

Chung, G., and Singh, R. J. 2008. Broadening the genetic base of soybean: a multidisciplinary approach. Critical Reviews in Plant Sciences. 27: 295-341.

Diwan, N., and Cregan, P. B. 1997. Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theoretical and Applied Genetics. 95: 723-733.

Ghosh, J., Ghosh, P.D., and Choudhury, P. R. 2014. An assessment of genetic relatedness between soybean [*Glycine max* (L.) Merrill] cultivars using SSR markers. American Journal of Plant Sciences. 5: 3089.

Gopinath, P., and Pavadai, P. 2015. Morphology and yield parameters and biochemical analysis of soybean [*Glycine max* (L.) Merrill) using gamma rays, EMS and DES treatment. International Letters of Natural Sciences. 35: 50-58.

Gupta, S.K., and Manjaya J.G. 2017. Genetic diversity and population structure of Indian soybean [*Glycine Max* (L.) Merr.]

revealed by simple sequence repeat markers. J Crop Sci. Biotech. 20:221-231 DOI No. 10.1007/s12892-017-0023-0

Koutu, G.K., Shrivastava A., Singh Y., and Tiwari, S. 2019. Molecular characterization and genetic diversity assessment of soybean cultivars using SSR markers. *Int. J. Curr. Microbiol. App. Sci.*, *8*: 173-182 https://doi.org/10.20546/ijcmas. 2019.804.018

Kumar, J., Verma, V., Goyal, A., Shahi, A.K., Sparoo, R., Sangwan, R.S., and Qazi, G.N. 2009. Genetic diversity analysis in *Cymbopogon* species using DNA markers. Plant Omics J. 2:20–29.

Kumawat, G., Singh, G., Gireesh, C., Shivakumar, M., Arya, M., Agarwal, D. K., and Husain, S. M. 2015. Molecular characterization and genetic diversity analysis of soybean (*Glycine max* (L.) Merr.) germplasm accessions in India. Physiology and Molecular Biology of Plants. 21: 101-107.

Lacape, J. M., Dessauw, D., Rajab, M., Noyer, J. L., and Hau, B. 2007. Microsatellite diversity in tetraploid *Gossypium* germplasm: assembling a highly informative genotyping set of cotton SSRs. Molecular Breeding. 19: 45-58.

Nawaz, M.A., Yang, S.H., Rehman, H.M., Baloch, F.S., Lee, J.D., Park, J.H., and Chung, G. 2017. Genetic diversity and population structure of Korean wild soybean (*Glycine soja* Sieb. and Zucc.) inferred from microsatellite markers. Biochemical Systematics and Ecology 71: 87-96

Pagar, T. A., Akhare, A. A., Gahukar, S. J., Khwaja, M. S., and Gawande, A. M. 2017. DNA fingerprinting of soybean (*Glycine max* L.) genotypes by using simple sequence repeats (SSR) markers. International Journal of Chemical Studies. 5: 674-679.

Powell, W., Machray, G. C., and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. Trends in Plant Science. 1: 215-222.

Prevost, A., and Wilkinson, M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics. 98: 107-112.

Rani, A., Kumar, V., Shukla, S., Jha, P. and Rawal, R. 2016. DNA barcoding of Indian soybean cultivars as constructed through SSR markers. Seed Sci. & Technol. 44: 357-369. http://doi.org/10.15258/sst.2016.44.2.18

Rohlf F. James 2009. NTSYS-pc: Numerical taxonomy and multivariate analysis system version 2.2. Department of Ecology and Evolution State University of New York Stony Brook, NY 11794-5245 ISBN: 0-925031-31-3

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Science. 81: 8014-8018.

Sahu, P., Khare, D., Tripathi, N., Shrivastava, A.N., and Saini, N. 2012. Molecular screening for disease resistance in soybean. J. Food Leg. 25:200– 205.

Singh, R. J., and Hymowitz, T. 1999. Soybean genetic resources and crop improvement. Genome. 42: 605-616.

Singh, R.K., Bhatia, V.S., Bhat, K.V., Mohapatra, T., Singh, N.K., Bansal, K.C., Koundal, and K.R. 2010. SSR and AFLP based genetic diversity of soybean germplasm differing in photoperiod sensitivity. Genet and Mol Bio. 33: 319–324.

Song, Q.J., Marek, L.F., Shoemaker, R.C., Lark, K.G., Concibido, V.C., Delannay, X., Pecht, J.E.S., and Cregan, P.B. 2004. A new integrated genetic linkage map of the Soybean. Theor. Appl. Genet., 109: 122–128.

Tantasawat, P., Trongchuen, J., Prajongjai, T., Jenweerawat, S., and Chaowiset, W. 2011. SSR analysis of soybean (*Glycine max* (L.) Merr.) genetic relationships and cultivar Identification in Thailand.

Australian Journal of Crop Science. 5:283.

Tautz, D., and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research. 12: 4127-4138.

Velusamy, V., Toan, V. D., and Park, E. 2013. Evaluation of genetic diversity in wild soybean (*Glycine soja* Sieb. and Zucc.) accessions based on SSR markers. International Journal of Agronomy and Plant Production. 4: 1241-1246.

Vinu, V., Singh, N., Vasudev, S., Yadava, D.K., Kumar, S., Naresh, S., Bhat, S.R., and Prabhu, K.V. 2013. Assessment of genetic diversity in *Brassica juncea* (Brassicaceae) genotypes using phenotypic differences and SSR markers. Rev Biol Trop 61:1919–1934.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters ,A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research. 23: 4407– 4414.

Wang, Y.H., Zhang, X.J. and Fan, S.J. 2015. Genetic diversity of wild soybean populations in Dongying, China, by simple sequence repeat analysis. Genetics and Molecular Research 14: 11613-11623.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18: 6531-6535.

Yoon, M. S., Lee, J., Kim, C. Y., Kang, J. H., Cho, E. G., and Baek, H. J. 2009. DNA profiling and genetic diversity of Korean soybean [*Glycine max* (L.) Merrill] landraces by SSR markers. Euphytica. 165: 69-77.

Yun-Lai, G.A.O., Rong-Sheng, Z.H.U., Chun-Yan, L.I.U., Wen-Fu, L.I., Jiang, H.W., Can-Dong, L.I., and Qing-Shan, C.H.E.N. 2009. Constructing molecular identity for soybean cultivars from Heilongjiang Province, China. Acta Agronomica Sinica. 35: 211-218.

Zhao, H., Wang, Y., Xing, Fu, L., X., Yuan, C., Qi, G., Guo, J., and Dong, Y. 2018. The genetic diversity and geographic differentiation of the wild soybean in Northeast China based on nuclear microsatellite variation. *Hindawi International Journal of Genomics*: 2018, Article ID 8561458, 9 pageshttps://doi.org/10.1155/2018/8561458.

Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 20: 176-183.

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