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DNA fingerprinting of soybean (*Glycine max* l.) Genotypes by using simple sequence repeats (SSR) markers

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Abstract
Thirteen soybean [*Glycine max* (L.) Merr.] genotypes were screened using 52 SSR markers. Out of that, 31 markers were recognized to be polymorphic across the thirteen soybean genotypes under study. A total of 103 polymorphic alleles with an average of 7.92 alleles per locus were detected with maximum eleven bands (primer Sat_406) and minimum one polymorphic allele (Satt126). The pair wise coefficient of genetic similarity between all soybean genotypes ranged from 0.792 to 0.929. Out of 31 polymorphic SSR primers, fifteen SSR primers (i.e. Satt184, Satt329, Satt335, BE806308, Satt077, Satt557, Satt194, Sat_409, Satt328, Satt406, Satt717, Satt329, Satt120, AW620 and Sct_189) successfully distinguished and discriminated soybean genotypes by generating unique allele for respective genotype. These unique bands can be used for the identification of specific soybean genotype.

Keywords: DNA Fingerprinting, SSR, genotype, allele, etc.

Introduction
Soybean, one of the legume species widely developed in China. In India, commercial cultivation of soybean started only few decades ago with unprecedented growth in the cultivated area and total production (Tiwari *et al*., 1999; Agarwal *et al*., 2013) [1, 2] and becomes the leading oilseed crop which is grown over 10.84 million hectare with total production and productivity of 14.66 million tons and 1353kg/ha during the year 2013-14 (Yashpal *et al*., 2015) [3]. Soybean has long been recognized as a high quality food because of high level of protein viz. defensin, glycinins, conglycinins and lunasins, known to provide health benefits (Anderson *et al*., 2011) [4].
Early studies have shown utilization of molecular markers for identification of genetically diverse genotypes to use in crosses in breeding program (Maughan *et al*., 1996; Thompson and Nelson 1998) [6, 7]. DNA based molecular markers meet the criteria of ideal marker and hence highly preferred in all species (Paterson 1996) [9]. National Active Germplasm Site (NAGS), holds 4248 soybean germplasm comprises of indigenous collections, land races, wild and exotic species from USA, Taiwan, Philippines, China, Brazil, Argentina and Thailand (Prabhakar and Bhatnagar 1995; Agarwal *et al*., 2013) [10, 2]. SSR markers have been widely used in the genetic diversity studies because of high levels of polymorphism reported for both number of alleles per locus and the gene diversity (Tantasawat *et al*., 2011) [11].

Material & Methods
Seeds of thirteen genetically pure soybean genotypes (Table No. 1) were collected from Regional Research Centre, Amravati, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.
DNA was extracted from young leaves by modified protocol given by Amani *et al*., (2011) [5] and the concentrations of aliquots were 50ng/µl. For screening of soybean genotypes, 52 simple sequence repeats (SSR) primers (Table No. 2) were chosen used by Mulato B. *et al*., (2010) [12] and Kumawat G. *et al*., (2015) [13] based on their ability to reveal high levels of polymorphism. The PCR reactions were performed for 20 µl volume containing 10x *Taq* buffer, 25mM $MgCl_2$, 10mM of each dNTP's, 0.5 µl of each primer, 50µg of DNA template and 1 unit of *Taq* DNA polymerase.
The DNA was denatured at 94 ºC for 2 min. followed by 35 cycles each consisting of denaturation at 94 ºC for 1 min., annealing for 2 min., elongation at 72 ºC for 1min.; and final

extension of 72 ºC for 7 minutes. Amplified products were analyzed on 8% PAGE with 100bp& 1kb ladder as molecular size standards. Results were analyzed on gel doc (Syngene Biorad) and data were recorded as presence (1) or absence (0) for each amplified band to construct the band matrix. A dendrogram for genetic similarity was constructed by Jaccard's similarity index following the UPGMA.

Table 1: List of soybean genotypes used in study

Sr. No.	Soybean genotypes	Centre of development
1	MAUS-71	VNMKV, Parbhani, M. S
2	JS-93-05	JNKVV, Jabalpur, M. P
3	JS-95-60	JNKVV, Jabalpur, M. P
4	JS-97-52	JNKVV, Jabalpur, M. P
5	JS-335	JNKVV, Jabalpur, M. P
6	MAUS-162	VNMKV, Parbhani, M. S
7	MAUS-158	VNMKV, Parbhani, M. S
8	NRC-37	ICAR-DSR, Indore, M. P
9	AMS-1001	RRC, Dr. PDKV, Amravati and BARC, Mumbai, M. S
10	AMS-1002	RRC, Dr. PDKV, Amravati and BARC, Mumbai, M. S
11	AMS-1003	RRC, Dr. PDKV, Amravati and BARC, Mumbai, M. S
12	AMS-MB-5-19	RRC, Dr. PDKV, Amravati and BARC, Mumbai, M. S
13	AMS-MB-5-18	RRC, Dr. PDKV, Amravati and BARC, Mumbai, M. S

Table 2: Sequence of SSR primers used in study

Sr. No.	Primer name	Primer Sequence (Forward primer)	Primer Sequence (Reverse primer)
1.	Satt005	TATCCTAGAGAAGAACTAAAAAA	GTCGATTAGGCTTGAAATA
2.	Satt009	CCAACTTGAAATTACTAGAGAAA	CTTACTAGCGTATTAACCCTT
3.	Satt045	TGGTTTCTACTTTCTATAATTATTT	ATGCCTCTCCCTCCT
4.	Satt102	CACCTTGCTTCAAAATTC	AATAAGTGAGAGCATAGAAAATAC
5.	Satt126	GCTTGGTAGCTGTAGGAA	ATAAAACAAATTCGCTGATAT
6.	Satt165	CACGAATAACTTGACACATT	TAAAAACAAAGCAAACATAAA
7.	Satt173	TGCGCCATTTATTCTTCA	AAGCGAAATCACCTCCTCT
8.	Satt182	GGTCCACATGAAATGAAGGT	TCTCAGCCTGCAAAGAAAA
9.	Satt184	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT
10.	Satt191	CGCGATCATGTCTCTG	GGGAGTTGGTGTTTTCTTGTG
11.	Satt192	CACCGCTGATTAAGATTTTT	CGCTGAGTTGTTTTCATC
12.	Satt194	GGGCCCAACTGATATTTAATTGTAA	GCGCTTTGTGTTCCGATTTTGAT
13.	Satt307	GCGCTGGCCTTTAGAAC	GCGTTGTAGGAAATTTGAGTAGTAAG
14.	Satt308	GCGTTAAGGTTGGCAGGGTGGAAGTG	GCGCAGCTTTATACAAAAATCAACAA
15.	Satt329	GCGGGACGCAAAATTGGATTTAGT	GCGCCGAATAAAACGTGAGAACTG
16.	Satt335	CAAGCTCAAGCCTCACACAT	TGACCAGAGTCCAAAGTTCATC
17.	Satt509	GCGCTACCGTGTGGTGGTGTGCTACCT	GCGCAAGTGGCCAGCTCATCTATT
18.	Sat_001	GCGGATACGACCAAAAATTGTT	GCGAACTGCGAAGATACTACCC
19.	Sct_189	CTTTTCCTGGCAATGAT	AAAATCGCAAAACCTTAGT
20.	BE806	GCGACCCCTTTTGTCTTCTT	GCGGAGGCCAGAGATGAA
21.	AW310	GCGCAACTTTTTAGTAAATATTGCATAA	GCGCATACATCTTTTGGGATTTCT
22.	AW620774	GCGATTTCCCCTCTTACTC	GCGAAAAACCAAGTTC
23.	AW508	GCGCCCAATCCCAATCTCAC	GCGAAGCCAATAAATGATAAAAATC
24.	BF00890	GCGTCTGGCTCTTCA	GCGAGCAGTGATGTTGTT
25.	Satt406	GCGTGAGCATTTTTGTTT	TGACGGGTTTAATAGCAT
26.	Satt155	AGATCCAACACCTGGCCTAAT	GCTGCACAATTCATTCCATTT
27.	Satt200	GCGATAAATGGTTAATGTAGATAA	GCGAAAGGACAGATAGAAAGAGA
28.	Satt717	GCGTTTTGTGATTTGTTTTCCTCATTTACT	GCGGCTATCAAACATTTTTACATGATGGTTA
29.	Sat_406	GCGCGTGTGGTGGTTACATTA	GCGTTTGCAGCCATTTCCATTTAC
30.	Sat_409	GCGGAGGTTTGTGCATTTCTAGGTCTTC	GCGACGCGTATGTACATAAAATATGCTGTT
31.	BE806308	GCGATTTGACCCCGTTCATACAT	GCGGCAGAAATCCGCTCTCTTTA
32.	Satt484	GCGTTTAATAAAACTAATTTAATTGTACT	GCGTTCCCTTTCTCTCCTTTCTTTCTT
33.	Satt687	ACCGCAACTCACTCACCTT	GCGCCCAATTAACAGAAAC
34.	Satt164	CACCAATGGCTAAAGGTACATAT	AGGAGAAGAAAAAATCACATAAAATATC
35.	Satt396	GCGAAAAGGGATAAGTTTAAAAAT	GCGGGCCTGTAAAGGGATTCC
36.	Sat_252	GCGTTTTTCTGTCATGTCTTTGAATTTT	GCGGCAGGTCTCATACAAGTCATCATCT
37.	Satt557	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA
38.	Satt129	TTCAGTACAAGTCGGGTGAATAATAATA	TCACATGTTCGGGACTTAAGGTAT
39.	Satt077	GATCTAAAGTCTGATATTTTTAACTA	AAAAGGAGAAGGAATGC
40.	Sat_227	GCGCAAAATGATTTGGGAAAATAACTTAAA	GCGTTATATACTTTTGGCGAGTTATCC

41.	Satt328	TGACCACCATGAGTTCATT	GGGGGTGGCTTTTAGATTC
42.	Satt310	GCGAGTTTTTATCTCATGACTTTT	GCGGGGGTATGGGACCTAAAGAAAC
43.	Satt411	TGGCCATGTCAAACCATAACAACA	GCGTTGAAGCCGCCTACAAATATAAT
44.	Satt230	CCGTCACCGTTAATAAAATAGCAT	CTCCCCCAAATTTAACCTTAAAGA
45.	Sat_390	GCGTAGATGCTTATAATCGACCCTAACAATT	GCGCGAGGATCCCATAAAAAAAGTAAAATAG
46.	Satt362	GCGTTGTTGTTTCAAATGTATTTTAGTT	GCGGACGGATCATCAAACCAATCAAGAC
47.	Satt163	AATAGCACGAGAAAAGGAGAGA	GTGTATGTGAAGGGGAAAAACTA
48.	Satt517	CTTGTTGCCTTTAACACACTTCAT	TCAACTGAAAAAGGAAACTAGAATAATG
49.	Satt666	TGGCTTGTCATCTCTACTTTTATTAG	TCATGCATCTAATTTGTTTTATCTATCA
50.	Sat_218	GCGCACGTTAAATGAACTGGTATGATA	GCGGGCCAAAGAGGAAGATTGTAAT
51.	Satt127	CGCTTGTGAACCCTGCTAAA	CCATCCTCTGAAACCGTTATCT
52.	Satt270	TGTGATGCCCCTTTTCT	GCGCAGTGCATGGTTTTCTCA

Results and Discussions

Out of 52 SSR primers, 31 primers resulted in scorable bands with clear amplified pattern [Fig. No. 1(a) and 1(b)]. The detailed information of polymorphic primers and unique band for particular genotypes is given in table no.3. From the data analyzed for 31 polymorphic primers, the variation between the amplification patterns across the genotypes ranged from 100bp – 1111bp with an average of 36% percent polymorphism across the thirteen soybean genotypes.

Four primers namely, Satt184, Satt329, Satt335 and BE806308 produced unique bands for genotype MAUS-71 of size 627bp, 105bp, 614bp and 650bp. Satt102 and Satt328 produced unique bands of size 237bp and 176bp respectively for genotype JS-93-05. For JS-97-52, Satt077 and Satt557 has produced unique amplicon of size 283bp and 180bp respectively. Satt194 produced two discriminating bands of size 133bp and 176bp and AW-620774 produced three unique bands of size 204bp, 301bp and 780bpfor genotype JS-335.

MAUS-158 has 47 alleles by all amplified primers but primer Sat_409 discriminated with a unique band of 590bp. Primer Satt328 and Satt406 produced amplicon of size 750bp and 228bp respectively whereas Satt717 generate three unique polymorphic bands of size 204bp, 240bp and 298bp for discrimination of NRC-37. For genotype AMS-1002 primer Satt329 and Sct_189 each produced unique band of size 118bp and 340bp respectively. Soybean genotypes AMS-MB-5-18, discriminated by primer Satt077 by unique alleles of size 272bp and 376bp.The SSR primers chosen in study failed to produce unique alleles for JS-95-60, MAUS-162, AMS-1001, AMS-1003 and AMS-MB-5-19.

The estimates of genetic similarity for all marker systems used in screening based on Jaccard's coefficient ranged from 0.792 to 0.929. Genotype AMS-1001 and AMS-1002 showed closest relationship. Two main clusters are formed. The first cluster includes two sub-clusters having seven genotypes namely JS-335, JS-97-52, JS-93-05, JS-95-60, MAUS-71, MAUS-158 and MAUS-162 whereas the second cluster of six genotypes i.e. AMS-MB-5-18, AMS-MS-5-19, AMS-1002, AMS-1001, AMS-1003 and NRC-37 include two sub-clusters (Fig.2).

Conclusion

A total of thirteen soybean genotypes were screened by 52 SSR primers of which 31 primers were found polymorphic. The data obtained from the primary screening was analyzed for unique allele produced by polymorphic primers across the soybean genotypes. It is found that, very few primers produced unique allele discriminating the genotype from the others (Table No. 3). Such as MAUS-71 have four unique bands of size 627bp, 614bp, 650bp and 105bp by primer Satt184, Satt335, BE806308 and Satt329 respectively. Satt329 also produce a unique allele of 118bp for genotype AMS-1002. A band of 180bp amplified by primer Satt557 becomes a unique band for genotype JS-97-52. Satt077 produced a band of size 283bp for JS-97-52 genotype and two bands of size 272bp and 376bp for AMS-MB-5-18. Two primers namely Satt194 and AW620 were highly polymorphic for genotype JS-335 producing two and three bands of size 133bp, 176bp, 204bp, 301bp and 780bp respectively. MAUS-158 showed an amplicon of size 590bp by primer Sat_409 whereas Satt328produced a band of 750bp. Satt717 produced bands of 204bp, 240bp and 298bp for genotype NRC-37.

Table 3: Statistics of soybean genotypes and unique bands

Genotypes	Amplified primers (Out of 52 SSR used)	Poly-morphic bands	Percent poly-morphism	Name of primers associated with genotypes with their unique allele size (bp)
MAUS-71	22	49	42.3%	Satt184 (627), Satt329 (105), Satt335 (614) & BE806308 (650)
JS-93-05	19	43	36.5%	Satt102(237) & Satt328 (176)
JS-97-52	22	53	42.3%	Satt077 and Satt557(283 and 180)
JS-95-60	19	38	36.5%	No unique band
JS-335	22	58	42.3%	Satt194(176and133)AW-620774(780, 301 and 204)
MAUS-162	20	58	38.4%	No unique band
MAUS-158	22	47	42.3%	Sat_409 (590)
NRC-37	27	58	51.9%	Satt328(750), Satt406(228) Satt717(298, 240 and204)
AMS-1001	22	32	42.3%	No unique band
AMS-1002	25	51	48%	Satt329(118) Sct_189(340)
AMS-MB-5-18	23	45	44.2%	Satt077 (376and 272)
AMS-1003	16	50	30.7%	No unique band
AMS-MB-5-19	19	34	36.5%	No unique band

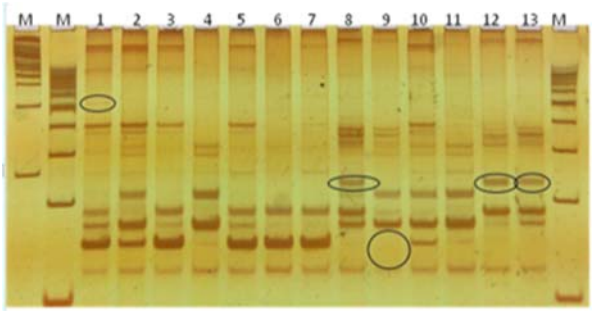


Fig 1(a): Amplification by primer Satt184

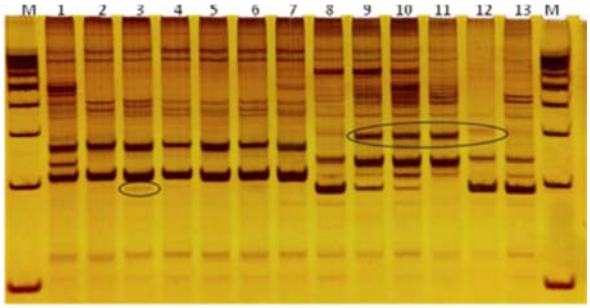


Fig 1(b): Amplification by primer Satt191

Where:			
1.	MAUS-71	2.	JS-93-05
3.	JS-97-52	4.	JS-95-60
5.	JS-335	6.	MAUS-162
7.	MAUS-158	8.	NRC-37
9.	AMS-1001	10.	AMS-1002
11.	AMS-1003	12.	AMS-MB-5-19
13.	AMS-MB-5-18	M	Ladder

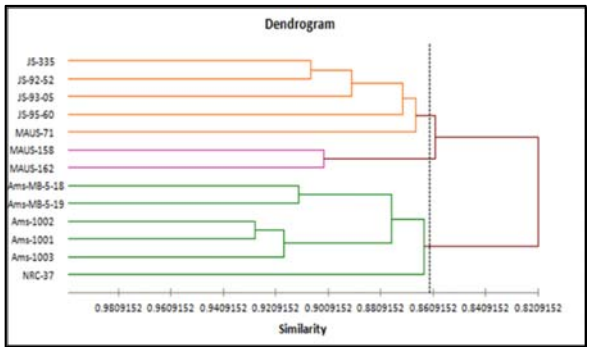
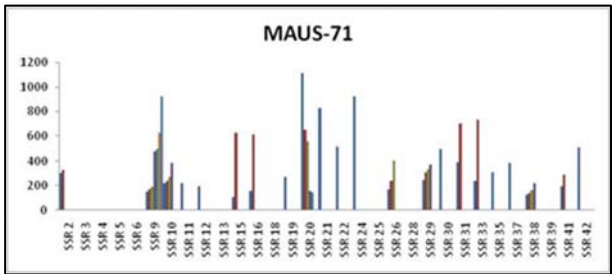
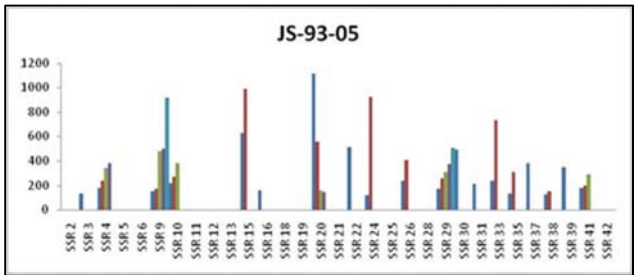
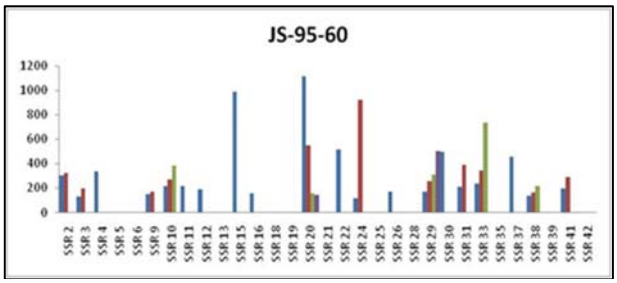
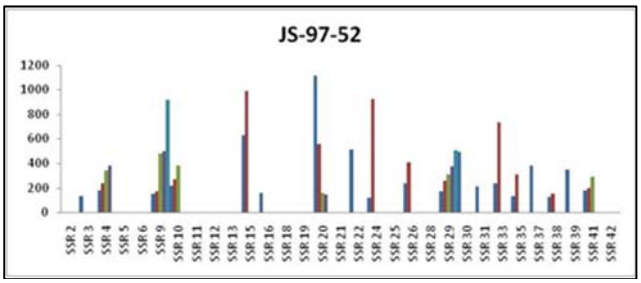


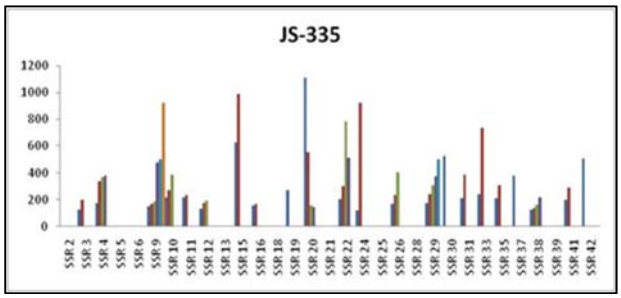
Fig 2: Dendrogram of 31 polymorphic SSR primers

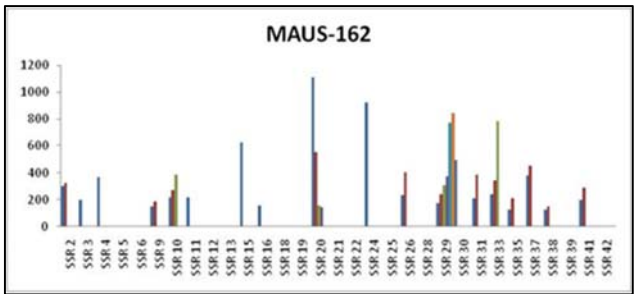


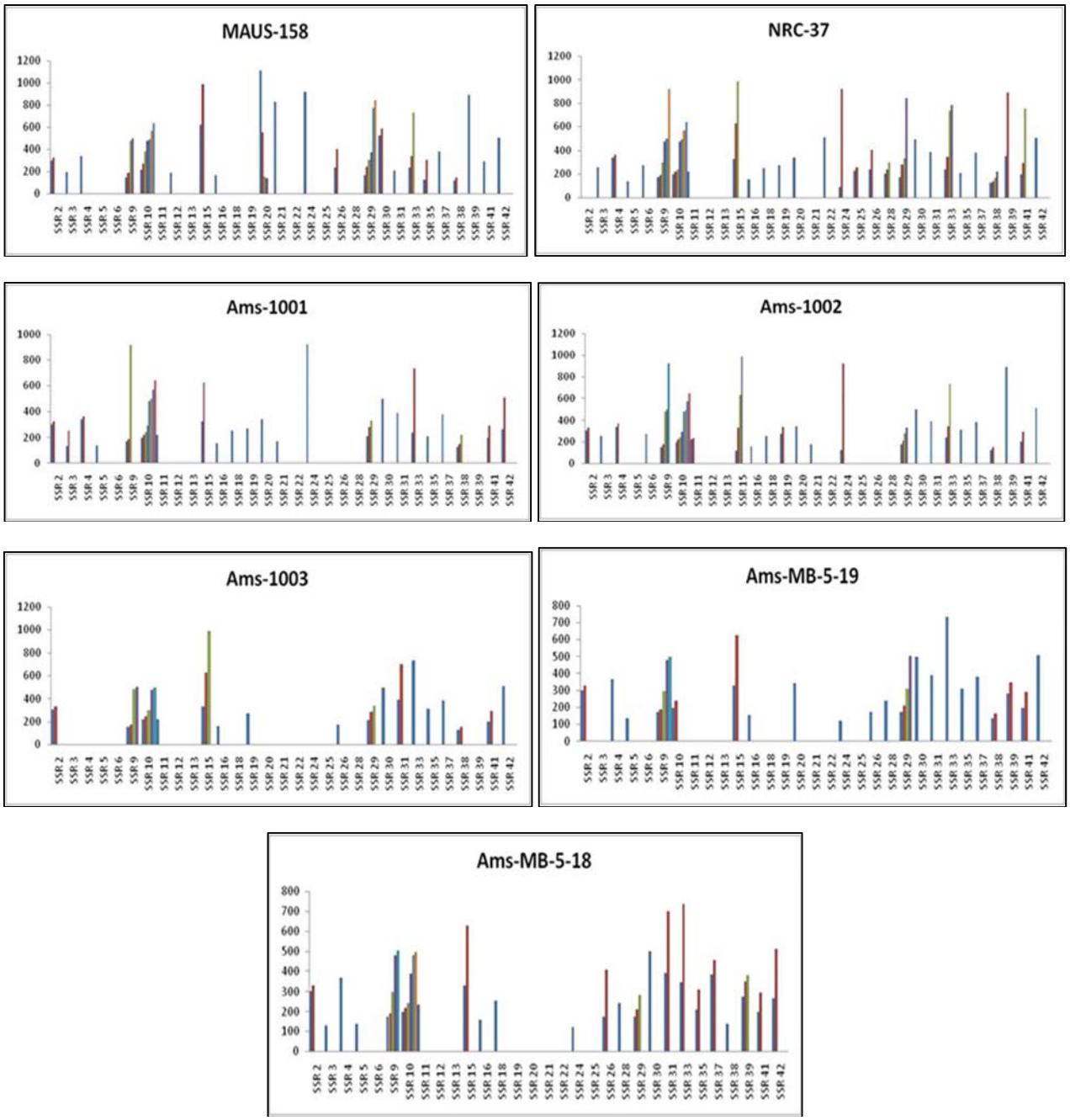












(**Fig:** Graphs of thirteen soybean genotypes under study with allelic bands of respective amplified primers).

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