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Genetic diversity analysis in soybean [*Glycine max* (L.) Merrill] using simple sequence repeat (SSR) markers

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SUMMARY

The genetic diversity studies using SSR markers revealed that among the primers used, Satt207 and Satt135 produced a maximum of four alleles. PIC was highest for the SSR primer Satt207 which indicated that the primer Satt 207 might be an effective and useful tool to determine the genetic differences among the soybean accessions and to study the phylogenetic relationship. The SSR marker profiles resulted in fifteen clusters at nearly 77 per cent similarity. Cluster V consisted of 15 accessions followed by VIII cluster with six genotypes. The formation of 15 clusters through SSR data revealed that the presence of genetic diversity at molecular level was high among the selected germplasm.

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Soybean [*Glycine max* (L.) Merrill] is a marvellous world's leading economic oilseed crop and ranks first among the oilseeds in the world. It is unique in being a legume-cum-oilseed crop. Soybean is the world's foremost provider of vegetable proteins (42%) and oil (20%), Hence it is called miracle golden bean of the 21st Century. A rapid advancement in DNA based marker techniques has proven to be powerful in genetic diversity estimation. These markers are highly heritable, available in high numbers and often exhibit enough polymorphism to discriminate even closely related genotypes. The DNA based markers have largely overcome the problems encountered with morphological and biochemical markers. Among the DNA markers, PCR based DNA marker like SSR share a number of general advantages over other markers. The major advantages are the speed with which results are generated, low amounts of genomic DNA required and the ability to share the information on primer sequences without the need to exchange DNA. Microsatellites or simple sequence repeats (SSRs) that consist of tandemly repeated core sequences which often vary in repeat number and are flanked by conserved DNA sequences. (Maughan *et al.,* 1995).Therefore, under present investigation, effort was made to understand molecular diversity present in a set of 50 elite soybean accessions.

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MATERIALS AND METHODS

The Experiment was conducted during *Rabi* 2009-10. Fifty accessions of soybean germplasm maintained in the Department of Pulses, Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University (TNAU), Coimbatore were utilized. The list of accessions taken for study is presented in Table 1.

SSR primers screening:

Eleven SSR primers from first base (Mumbai) were initially screened for their repeatable amplification with five accessions. Primers were selected for further analysis

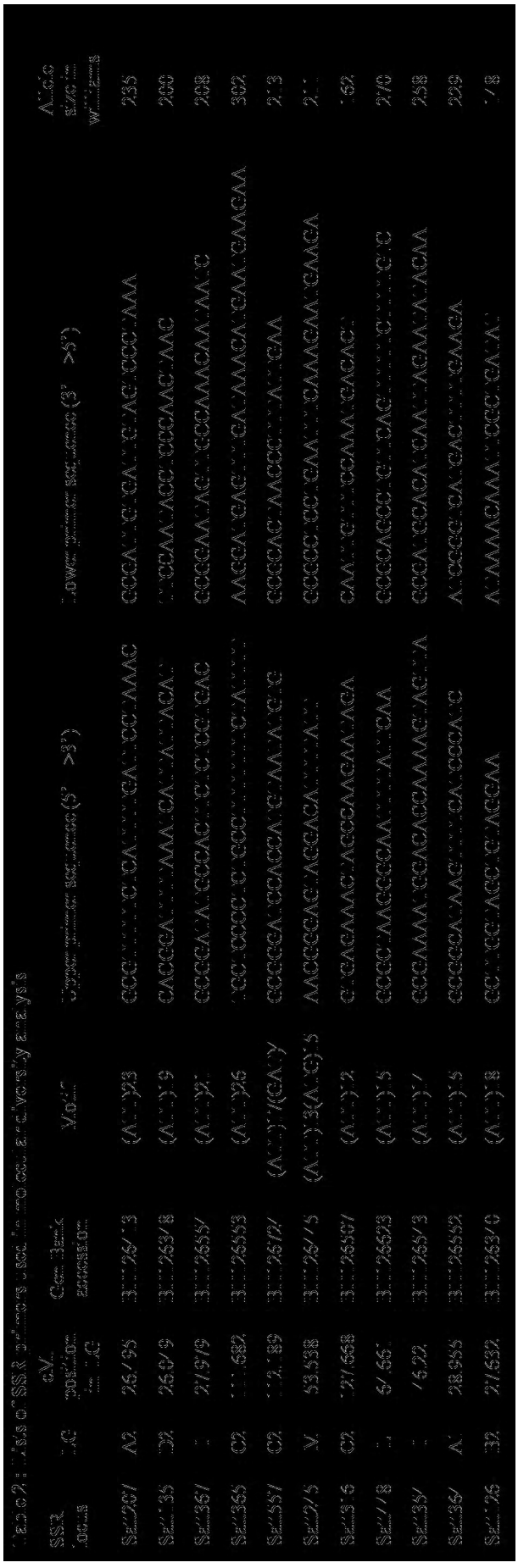
Table 1 : The list of soybean accessions used for molecular diversity study

Sr. No.	ACC. NO	Sr. No.	ACC. NO
1.	IC2574	26.	NRC 51
2.	PK 731	27.	IC25166
3.	NRC 21	28.	HIMS0 1565
4.	IC 9311	29.	JS 92-22-A
5.	IC 18596	30.	IC7361
6.	IC 110399	31.	PK 258
7.	TNAU 20049	32.	IC 2048
8.	SL 525	33.	JS(SH)93-37
9.	JS 92-22	34.	VLS 53
10.	JS 335	35.	JS (SH) 91-93
11.	RKS -18	36.	IC 216380
12.	AMSS 34-A	37.	JS 90-29
13.	IC14467-A	38.	MACS 715
14.	JS 148	39.	JS 95-98
15.	UGM-73	40.	IC18277
16.	PLSO 90	41.	IC 62376-A
17.	JS99-02	42.	JS (SH) 89-49
18.	PK257	43.	IC 39873
19.	IC2572-A	44.	IC16040
20.	IC25167	45.	IC34057
21.	IC100790	46.	IC15969
22.	TNAU 20037	47.	IC18736
23.	JS (SH) 99-02	48.	IC93751
24.	JS 89-24	49.	IC93752
25.	IC 1024	50.	IC93656

based on their ability to detect polymorphic amplified products across the accessions. To ensure reproducibility, the primers generating weak products were discarded. The primers used for genetic diversity analysis are presented in Table 2.

PCR amplification:

The DNA was extracted from the forty-five soybean accessions using CTAB method. The PCR amplification was performed in a total volume of 15.0 µl of 1x assay buffer, 1.20 µl of 2.5 mM dNTPs, 0.20 µl of 5 to 10 unit/ µl of Taq polymerase, 1.5 µl of 20 mM SSR primers (0.75 µl of FP and RF), 3.0 µl of 50.0 ng/µl DNA (50.0 ng/µl), after a denaturation step for 12 min at 94^{0}C, the amplification reaction were carried out for 42 cycles. Each cycles comprised of 12 min at 94^{0}C, 30 sec at 52^{0}C and 45sec at 68^{0}C, the final elongation step was extended to 5 min these amplification products were separated on 6.0 % polyacrylamide gel TBE buffer and stained with ethidium bromide and photographed under UV light.



Statistical analysis:

Clearly resolved, unambiguous polymorphic bands were scored visually for their presence or absence. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands in each species, respectively. The binary data scored were used to construct a Dendrogram. Polymorphism information content (PIC) or expected heterozygosity scores for each SSR marker was calculated based on the formula $Hj = 1-\Sigma pi^2$ where Pi is the allele frequency for the *i*-th allele (Nei, 1973). The binary data scored were used to construct a dendrogram. The genetic associations between accessions were evaluated by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Jaccard, 1908). Similarity matrix was generated using the SIMQUAL programme of NTSYS-pc software, version 2.02 (Rohlf, 1998). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted Pair-Group method (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Eleven SSR primer pairs amplified and produced thirty three alleles, out of which thirty one alleles were polymorphic and used to generate marker profiles. The number of alleles produced by different primers ranged from 2 to 4 with an average of 3 alleles per primer and level of polymorphism was found to be 96.96% (Table 3). Among the primers used, Satt207 and Satt135 produced a maximum of four alleles. Polymorphism information content (PIC) value of each SSR marker is a measure of marker diversity. PIC provides an estimate of discriminatory power of a locus by taking into account not only the number of alleles expressed, but also the relative frequency of those alleles. PIC was highest for the SSR primer Satt207 (0.843), and was lowest (0.280) for the primer Satt448. Hence, primer Satt207 is highly informative in the present study. This indicated that the primer Satt 207 might be an effective and useful tool to determine the genetic differences among the soybean accessions and to study the phylogenetic relationship. The SSR marker profiles of 50 accessions generated by the primers Satt367 and Satt135 are given in Fig. 1 and Fig. 2, respectively.

Table 3 : SSR marker profile across soybean accessions

Primer code	Number of alleles	Number of polymorphic alleles	Polymorphism (%)	PIC
Satt207	4	4	100	0.843
Satt135	4	4	100	0.656
Satt367	3	2	80.00	0.557
Satt365	3	3	100	0.532
Satt557	3	3	100	0.587
Satt245	3	3	100	0.646
Satt316	3	3	100	0.632
Satt448	2	2	100	0.280
Satt354	2	2	100	0.457
Satt364	3	3	100	0.438
Satt126	3	2	66.66	0.338

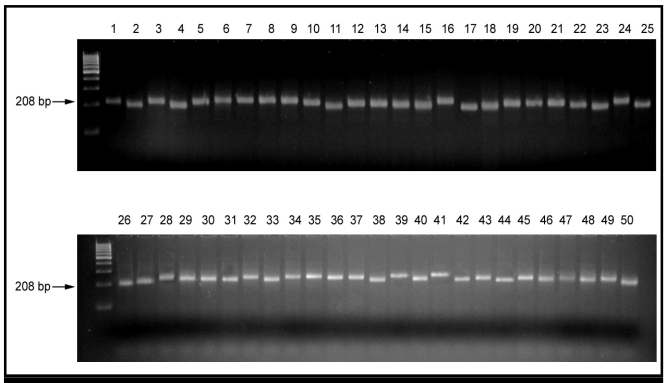


Fig. 1 : SSR marker profile of soybean genotypes generated by the primer satt 367

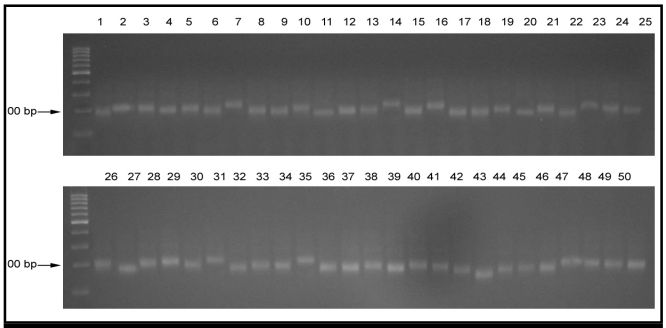


Fig. 2 : SSR marker profile of soybean genotypes generated by the primer satt 135

George *et al.*(2003) worked with 35 North American soybean ancestors (NASA) and used five AFLP primer-pairs which produced 90 polymorphic (27%) and 242 monomorphic AFLP fragments. In the present study, the polymorphism observed in SSR markers among the soybean accessions showed the effectiveness of this method in determining genetic variation. All the SSR markers used were found to be highly informative in revealing the genetic diversity among the genotypes studied suggesting their potentiality in future genetic diversity analysis.

The molecular markers based on difference in DNA

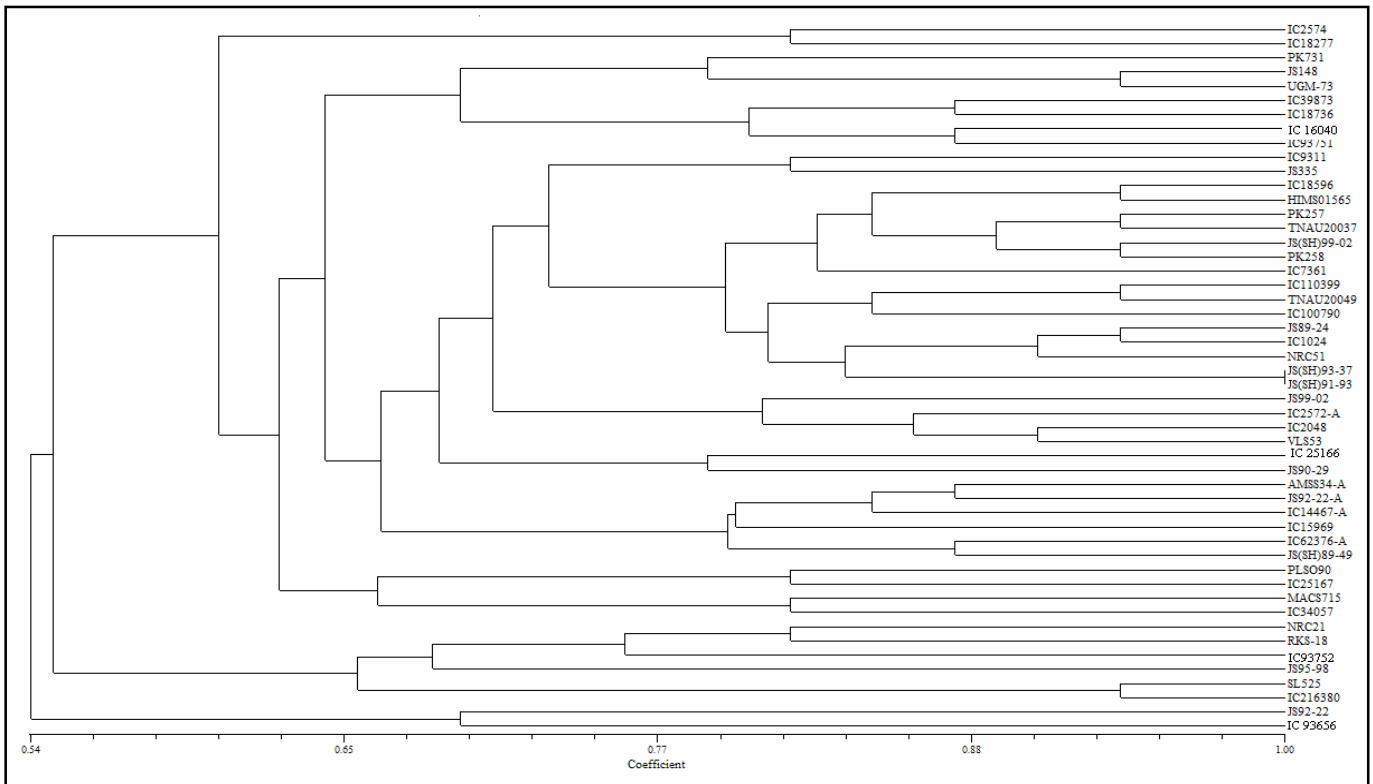


Fig. 3 : Dendrogram of soybean genotypes based on the SSR marker data

sequence between individuals generally detect more polymorphism than morphological and proteins based markers and constitute a new generation of genetic markers (Migouna *et al.,* 1998). In the present study, apart from using the morphological traits, an attempt has been made to use the SSR markers to determine the extent of diversity among accessions. SSR protocol generally involved three major steps *viz.*, isolation of genomic DNA, and amplification of the DNA by SSR primers and analysis of amplified products. The SSR marker profiles resulted in fifteen clusters at nearly 77 per cent similarity (Fig 3).

The cluster I consisted of IC2574 and IC18277. The cluster II consisted of PK731, JS148 and UGM-73. The cluster III consisted of IC39873, IC18736, IC16040 and IC93751. The cluster IV consisted of accessions, IC93751 and JS835. The cluster V consisted of accessions, IC18596, HIMSO1565, PK257, TNAU20037, JS (SH) 99-02, PK258, IC7361, IC10399, TNAU20049, IC100790, JS89-24, IC1024, NRC-51, JS (SH) 93-37 and JS (SH) 91-93. The cluster VI consisted of accessions, JS99-02, IC2572-A, IC2048 and VLS53. The cluster VII consisted of accessions, IC25166 and JS90-29. The cluster VIII consisted of accessions, AMSS34-A, JS92-22-A, IC14464-A, IC15969, IC62376-A and JS (SH) 89-49. The cluster IX consisted of accessions, PLSO-90 and IC25167. The cluster X consisted of accessions, MACS-715 and IC34057. The cluster XI consisted of accessions, NRC-21, RKS-18 and IC93752. The cluster XII consisted of accession JS95-98. The cluster XIII consisted of accessions, SL525 and IC216380. The cluster XIV consisted of accession JS92-22. The cluster XV consisted of accession IC93656 (Table 4).

The Four accessions, (IC18596, IC7361, IC10399 and IC10070) of Directorate of Soybean Research, Indore, two accessions (TNAU20049 and TNAU20037) from Tamil Nadu Agricultural University (TNAU), Coimbatore and three accessions, (JS (SH) 93-37, JS (SH) 97-93 and JS89-24) released from Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, were grouped into a single cluster (Cluster V). These results indicated that there was a narrow genetic base of above soybean accessions, which were from different soybean breeding centers. The accessions, PK731 (Cluster II) and PK258 (Cluster V), were from Govind Ballah Pant University of Agriculture and Technology (GBPAU), Pantnagar showed wider genetic diversity. Similarly NRC 51 (Cluster V) and NRC 21 (Cluster XI) released from Directorate of Soybean Research, Indore, and JS95-98 (Cluster X11) and JS 148 (Cluster II) showed wider genetic diversity.

The SSR marker data were able to differentiate the following accessions into separate clusters IC2574

Table 4 : Cluster composition of soybean accessions for SSR markers

Cluster No.	Number of genotypes	List of genotypes
I	2	IC2574,IC18277
II	3	PK731,JS148, UGM-73
III	4	IC39873, IC18736, IC16040, IC93751
IV	2	IC93751, JS835
V	15	IC18596, HIMSO1565, PK257, TNAU20037, JS(SH)99-02, PK258, IC7361, IC10399, TNAU20049, IC100790, JS89-24, IC1024, NRC-51, JS(SH)93-37, JS(SH)91-93
VI	4	JS99-02, IC2572-A, IC2048, VLS53
VII	2	IC25166,JS90-29
VIII	6	AMSS34-A, JS92-22-A, IC14464-A, IC15969, IC62376-A, JS(SH)89-49
IX	2	PLSO-90, IC25167
X	2	MACS-715, IC34057
XI	3	NRC-21, RKS-18, IC93752
XII	1	JS95-98
XIII	2	SL525, IC216380
XIV	1	JS92-22
XV	1	IC93656

(Cluster I), JS835 (cluster IV), VLS 53 (Cluster VI) when compared to the morphological data. This elucidated the potentiality of SSR markers for the characterization of germplasm accessions. SSR marker data also revealed that the geographical diversity and genetic diversity are not related. Ghatge and Kadu (1993) grouped 48 soybean genotypes from different eco-geological regions of India into seven clusters and the clustering pattern revealed that genetic diversity was not associated with geographical diversity in soybean.

The formation of 15 clusters through SSR data revealed that the presence of genetic diversity at molecular level was high among the selected germplasm. But there was a large deviation between the clustering pattern obtained from morphological and molecular data. This showed that the variation observed at DNA level has not been expressed at phenotypic level.

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