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Assessment of genetic diversity among the selected genotypes of soybean (*Glycine max* L. Merrill) by using SSR markers

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Abstract
An experiment was conducted to assess the genetic diversity in soybean using Simple Sequence Repeat (SSR) diversity of 24 elite soybean genotypes were selected for high yield potential. The four primers showed reliable polymorphism produced a total of 92 bands of which 68 (75%) were polymorphic. Genetic similarity estimates based on simple matching coefficients revealed more genetic diversity among all the 24 elite lines, ranging from 0.143 to 1 indicating the distinctness of these genotypes under study. The dendrogram constructed using the UPGMA method separated these genotypes in two main groups each having two sub groups. The study also demonstrates high reliability, ease of applicability and importance of SSR markers in evaluating genetic variation among the genotypes of soybean.

Keywords: Genetic diversity, soybean, SSR marker

Introduction
Soybean (*Glycine max* (L.) Merrill) is the world's largest oilseed crop and accounts for about 56% of edible oilseed production. Soybean is used both as a food and fodder crop because of its high protein content (40%) on a dry matter basis. Soybean is cultivated widely in the USA, Brazil, Argentina, China, and India (Gupta and Manjaiya, 2017) [5]. The global production of soybean continue to increase a record world production of soybean is 364.33 million tones from an area of 127.19 million hectares with the productivity of 2864 kg per ha. At present, India occupies 13.46 million ha of area with 10.96 million tons of production and 1228 kg per ha productivity (Anon., 2019) [1].

The soybean varieties in India have been developed from both introduced varieties and native local landraces, and about 108 improved varieties has been released for commercial cultivation. Soybean is a plant with narrow genetic base and for the detection of differences between varieties morphological markers may not be sufficient. In such cases, extra information about diversity of the existing germplasm is done through molecular markers (Kumawat *et al.* 2015) [7], since they are highly polymorphic and unaffected by environmental conditions. Different markers are used to carry out genetic diversity analysis to find out diverse parents required for initiating breeding programme to aid in the development of soybean genotypes. At the recent time, simple sequence repeats (SSRs) is used because of their co-dominance, polymorphic and reproducible properties and so have been employed to assess genetic diversity in different crops (Li *et al.*, 2010 and Tantasawat *et al.*, 2011) [8, 9].

Information of genetic diversity and relationships among the cultivars of a crop species will be an essential component in germplasm characterization and conservation along with that it acts as an indicator for soybean breeders to evolve varieties with diverse genetic background to achieve sustainability in soybean production.

Material and Methods
The experiment was carried out by randomized complete block design with three replications during *Kharif* 2018-19 at Research cum Instructional farm, IGKV, Raipur (C.G.) whereas laboratory experiment has been conducted in MAS Laboratory, RRL, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur (C.G.). The DNA was isolated from the 24 selected genotypes of soybean by rapid DNA extraction method following the protocol of Doyle and Doyle (1990) [4], with slight modifications of buffer composition and

concentration. The quality and quantity of DNA was ascertained through polyacrylamide gel electrophoresis. The master mix was prepared by using 40ng/µg DNA template, 10X PCR buffer, 10 mm dNTPs, 10 pMol SSR primers (forward and reverse) and 1 unit/µl Taq polymerase enzyme. A total of 4 SSR primers were used for genetic diversity analysis in selected genotypes of soybean (Table 1). PCR was performed using polyacrylamide gels which have better resolution for amplified products, so for better separation and visualization of PCR amplified microsatellite products, 5% polyacrylamide gels (vertical) were used. Gels were casted in CBS-Scientific electrophoresis unit. Gels were stained in ethidium bromide solution and visualized under UV light using the gel documentation system and saved in computer for further analysis. Markers were scored for the presence (1) or absence (0) of the corresponding band among the genotypes. A pairwise similarity index (SI) was calculated and the UPGMA based dendrogram (Fig. 1) of 24 genotypes was prepared.

Results and Discussion

Marker analysis helps to understand the genetic makeup of the accessions and also make it possible to analyze the genetic diversity within a species. The SSR patterns of genomic DNA of 24 genotypes were analyzed with respect to the fragments, in formativeness of the markers and polymorphism for the assessment of genetic diversity present among the genotypes. For the present study 4 SSR primers were used for molecular characterization and for assessment of genetic diversity. A total of 92 Scorable DNA fragments were produced and among them 68 DNA fragments were found to be polymorphic in the selected genotypes of soybean. The primers produced high degree of polymorphism with an average of 75 per cent. Average 23 bands per primer were amplified. Among the 4 primers Gm000272, Gm000195, Gm000539, Gm000625 revealed 100 per cent polymorphism with the maximum number of DNA fragments except one. Comparison of more primers generally provides additional confirmatory evidence for genetic variation (Chauhan *et al.*, 2015 and Kumar *et al.*, 2015.) [3, 6]. The Polymorphism Information Content (PIC) value calculated for the 4 SSR primers: In the present study the maximum PIC information produced by the primer Gm000625 (0.92) while, the minimum PIC value was given by the primer Gm000539 (0.70) whereas, the average PIC value obtained for each primer was 0.63 (Table 1). The overall range of the similarity among 24 selected genotypes of soybean was found to be very wide ranging from 0.143 to 1 which indicates there was high variability among the genotypes under study. The cluster analysis was carried out based on the SSR profile. The results based on the SSR profile broadly grouped the 24 genotypes into two main clusters (I and II). The first cluster further subdivided into two subclasses (I A and I B). In which, I A contain four genotypes and I B contain 11 genotypes. The second cluster further subdivided into two subclasses containing nine genotypes. It was observed that, the genotypes namely, PI204336, EC590225 and EC468597 occupied a unique position and were most diverse from rest of the 21 genotypes. The similar findings are also in agreement of with the results of Kumawat *et al.*, (2015) [7], and Bisen *et al.*, (2015) [2].

The study indicated that SSR markers are suitable for the assessment of genetic variability among different genotypes of soybean. The SSR analysis revealed substantial polymorphism in soybean. The results of the present study indicated the efficiency of SSR markers in investigating genetic variability at molecular level, which is important for detecting distinctness of genotypes and also for the identification of desirable genotypes and its utilization for further breeding programme.

Table 1: List of Polymorphic SSR Primers in Soybean.

S. No.	SSR Primers	Primer Sequences
1	Gm000272 F	TAATTGGTGGAAGCCAAAGG
	Gm000272 R	CCAGCATCAAAGTGGAGGAT
2	Gm000539 F	AACGAGAATCCCCCTCCTTA
	Gm000539 R	GTTCGTCGGTGGACATTTCT
3	Gm000625 F	TACTTTGCCCAATGATGCAC
	Gm000625 R	GCAGGGTCATCCAATCTAGC
4	Gm000195 F	TAAATCCGAAAACCTCGTCG
	Gm000195 R	CCGTTACCAACAAAGGCTGT

Table 2: Molecular information obtained by 4 SSR primers

S No.	Marker	Number of alleles	Total No. of bands	No. of polymorphic bands	% polymorphism	PIC
1	Gm000272	3	23	23	100	0.89
2	Gm000195	1	24	0	0	0
3	Gm000539	2	22	22	100	0.70
4	Gm000625	4	23	23	100	0.92
Total		10	92	68	-	-
Average		2.5	23	17	75	0.63

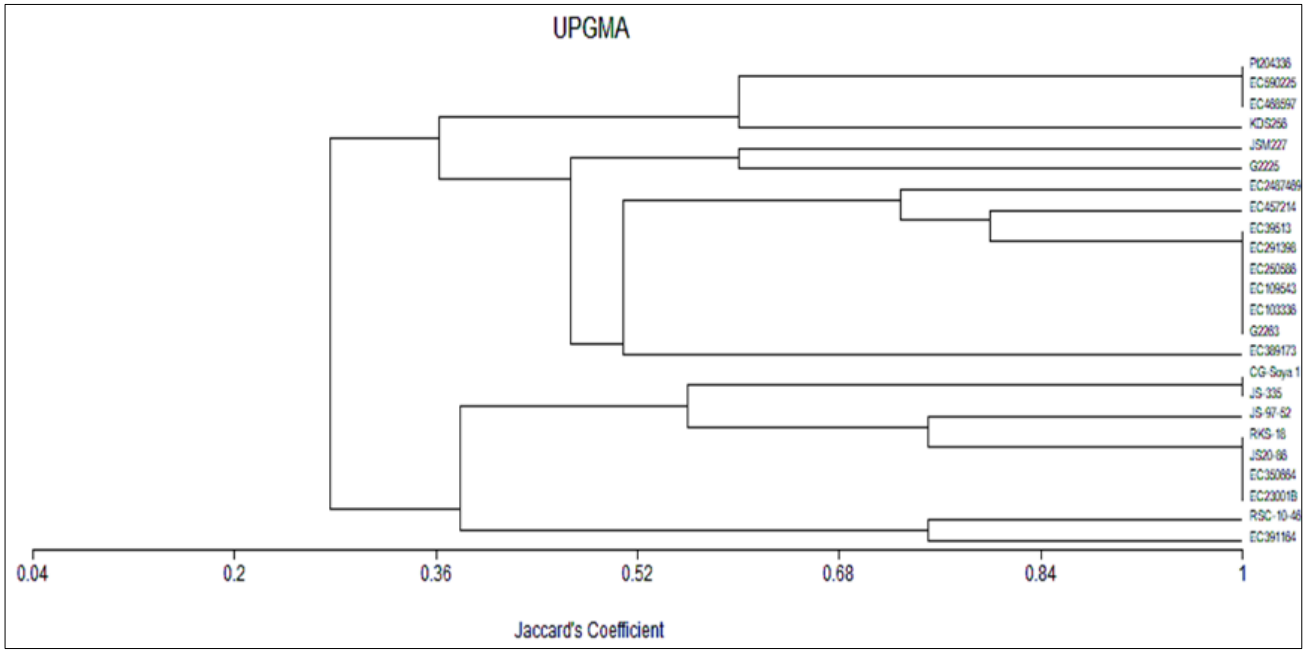

Fig 1: Dendrogram constructed using Jaccards Similarity Coefficient

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