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Evaluation of Diversity among Soybean Genotypes via Yield Attributing Traits and SSR Molecular Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NM and MKT conceptualized the experiment. Author NM performed the experiment and data collection. Authors ST, NT and NG analyzed the data. Authors NT and MKT drafted the manuscript. Authors MKT, NT and RSS edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: As an important source of nutrients to humans and animals, soybean is considered to be a major crop.
Objective: The present study has been executed to identify diverse soybean genotypes on account of different morpho-physiological and microsatellite molecular markers.
Study Design: Data for Morpho-physiological traits were recorded from experiment conducted under field conditions in RBD design whereas molecular work was conducted in Laboratory.
Place and Duration of the Study: The present study was conducted at College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India during Kharif 2018-19.
Methodology: The study was conducted to document different morphological and physiological traits related to the yield and its attributing traits in soybean. Total 32 microsatellite markers were also used in laboratory to analyze the variability among soybean genotypes.

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Results: Morpho-physiological analysis among 53 genotypes revealed the presence of considerable level of variability. Phylogenetic tree based on morpho-physiological traits grouped the genotypes into major and minor cluster. Major cluster had fifty genotypes while minor cluster had only three genotypes. Among polymorphic 32 microsatellite markers, the highest genetic diversity (0.66) was recorded in Satt520 whilst lowest (0.037) was in Satt557 with an average of 0.35. The highest PIC value was 0.59 prearranged by Satt520 and lowest 0.036 by Satt557. An average major allele frequency was 0.69 while, an average PIC value was 0.32. Microsatellite markers-based data also grouped the genotypes into one major and one minor cluster.
Conclusion: Molecular analysis based on microsatellite markers confirms the presence of genetic variability among genotypes under the investigation. Data obtained in the present investigation may contribute towards improvement of soybean genotypes to develop high yielding varieties by considering diverse genotypes with good agronomical traits in hybridization programme.

Keywords: Breeding; microsatellites; sustainable agriculture; soybean; variability.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is among one of the major crops, consumed as human foods as well as animal feed [1-6]. It is also a rich source of essential amino acids in addition to oil. The role of soybean and its components as therapeutic agents, antioxidants, isoflavones *etc*. has been documented. Versatile nature of soybean makes it valuable in the field of industrial formulations, agriculture sector and pharmaceuticals [7-10]. Because of its high protein content, soybean flour is frequently used to supplement the nutritional value of cereal flours missing critical amino acids, such as sorghum and maize [8-10]. This indicates significant increase in demands of soybean in the future because of rapid growth in the population of India as well as the world. This demand can be fulfilled with high yielding varieties and this can be done through hybridization of genetically diverse parental genotypes.

A population base with wide genetic diversity is needed to develop new varieties. Soybean has narrow genetic base due to its self-pollinating nature [11]. Plant breeders have found difficulty in creation of genetic variation through hybridization process in soybean due to small size and fragile nature of flowers. This makes the emasculation process of flower very difficult [12]. The level of genetic variability present between and among developed elite materials and commercialized soybean varieties has not been done accurately. As a result, determining genetic diversity among released varieties and elite genotypes is critical for ensuring proficient selection and proposal of diverse lines as parents for expansion, commercialization, and future breeding efforts to get better soybean yield, quality, and pest and disease resistance [13-15].

Knowing the genetic diversity among and between available soybean genotypes can aid breeders to understand the structure of germplasm and prediction of best parental combinations [16]. This prediction will be suitable to achieve the goal of high yield as well as increment of genetic variability of breeding material for the selection in future. The developed or released variety should also be genetically different from the existing varieties of the same crop. Assessment of genetic variability also helps the breeders in crop variety protection [17].

The utilization of morphological and agronomic parameters, isozymes, pedigree information, and DNA markers have all been done to assess genetic diversity among different soybean accessions [1,3,16]. Moreover, the application of pedigree information is constrained by unclear or missing data, along with possibility of data entry errors. Because the use of isozymes limits the amount of data available, DNA markers can be used in genetic diversity investigations for more precise information [18].

Morpho-physiological characteristics have been used to analyze variability and relatedness between and among crop varieties and genetic resources. However, molecular markers have been reported as authentic tools in different crop species including soybean [19-24]. Several molecular markers have been used to characterize soybean genotype(s) as they are generally free from environmental situations and offer a new dimension, accurateness and excellence in divulgence of germplasm line(s). An array of molecular markers *viz.*, Random

Amplified Polymorphic DNA, Inter Simple Sequence Repeats, Amplified Fragment Length Polymorphism and Simple Sequence Repeat have been employed to study genetic diversity in soybean [25,14-16]. Among all the mentioned markers, SSRs have been extensively used in plants [16,26-28] because of their higher level of polymorphisms, higher polymorphic information content (PIC), co-dominant inheritance and dispersal in the whole genome [29,30,1]. Use of morphological characteristics and molecular markers in the detection of inherited variability is necessary for the management of crop genetic resources [22,30]. The present study was accomplished to screen soybean genotypes based on yield attributing morpho-physiological traits and SSR molecular markers.

2. MATERIALS AND METHODS

The present investigation was consisted of 53 *Glycine max* (L.) Merrill genotypes (Table 1). The seeds were acquired from College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur and Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India. The field trial was experimented at the experimental field, Department of Plant Molecular Biology & Biotechnology and the laboratory work at Molecular Biology Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, India during Kharif 2018-19. Crop was sown on 27th July 2018. The experiment was designated in Randomized Block Design in three rows and two replications and row to row distance was kept 30 cm. Fertilizer was applied in the ratio of 20 N: 60 P_2O_5: 20 K_2O:20S kgha^{-1}.

2.1 Morpho-Physiological Parameters to Screen Soybean Genotypes

Leaf area of three leaves of each observational plant was measured by Automatic Leaf Area Meter in cm^2 at a 30 days interval from 30 DAS to 60 DAS. The mean numbers of five arbitrarily chosen plants were documented for numbers of primary branches per plant. Number(s) of days required for beginning of flowering was considered from the date of sowing for days to flowering. Numbers of days required for 50 per cent of the plants to flower for 50 % flowering observation. Total number of seeds of 5 uprooted plants from each plot was recorded and then the average number of seeds per plant was computed for numbers of seeds per plant. Total weight of plants with pods of 5 uprooted plants from each plot was recorded and then the average weight per plot was computed for weight of plant with pod. The pods harvested from five observations threshed separately and grain yield was recorded and the average was worked out. Hundred-seed weight (SW) recorded on the basis of arbitrarily selected five plants of each plot by counting weight and averaging of 100 seeds. The weight of all harvested plant parts of 5 observational plants was recorded before threshing including the dry weight of leaves, stems and pods. Then the average biomass per plant was calculated. The data were analysed according to method proposed by Snedecor and Cochran [31].

2.2 SSR Molecular Marker Analysis

Forty-eight simple sequence repeats primer pairs, dispensed transversely the amalgamated linkage map of soybean [32] were procured from Imperial Life Sciences Pvt. Ltd, Gurgaon, Haryana, India. Genomic DNA was extracted from young leaves of 10 plants from each genotype perusing the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Saghai-Maroof et al. [33] with slight amendments as advocated by Tiwari et al. [3]. DNA was quantified by viewing the concentration of ethidium bromide-stained DNA bands on 0.8 % agarose gels. Concluding concentration was corrected to 50 ng µl^{-1} for later uses in PCR analysis. DNA was amplified by PCR in a total volume of 10 µl comprising 25 ng template DNA, 1×buffer (75 mM Tris.HCl (pH 9.0), 50 mM Kull, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 µM of each dNTP, 5 pmol each of forward and reverse SSR primers and 1-unit *Taq* polymerase (Fermentas). PCR reactions were performed in a Bio-Rad Thermocycler. Cycling parameters were initial denaturation step at 94°C for 5 min, tracked by 94°C, 30 s, 52–58°C, 30 s and 72°C, 30 s. This cycle was repeated 35 times, trailed by 5 min final extension at 72°C. The amplified artifacts were separated on 3.5% agarose gels and detected by ethidium bromide staining. Allele sizes were estimated in comparison with 100 bp DNA ladder (Fermentas).

2.3 Data Scoring

The PCR products generated by SSR were investigated by scoring qualitatively for presence or absence of bands. A genetic similarity between the genotypes was quantified by the similarity coefficient. In instance of SSRs,

Polymorphism Information Content (PIC) was computed perusing the equation: $PIC_j = 1 - \sum_{i=1}^{n} P_i^2$ Where, i = the i^{th} allele of the j^{th} marker, n = the number of alleles at the j^{th} marker and p = allele frequency.

3. RESULTS AND DISCUSSION

3.1 Morpho-Physiological Variability

The analysis of variance presented in Table 2 vibrantly designated existence of substantial magnitude of variations among 53 soybean genotypes for different yield attributing characters. Maximum leaf area in cm sq/plant was recorded for genotype: AMS-100-39 (75.18304cm sq/plant) closely followed by genotype MACSNRC-1575 (73.65035cm sq/plant) while the lowest area was covered by genotype PS1092 (11.02047 cm sq/plant) intimately tracked by a group of three statically *at par* genotypes, *namely*: NRC-132 (12.09476 cmsq/plant), SL-1123 (13.31485 cmsq/plant) and JS335 (14.65448 cm sq/plant). Number (s) of primary branches per plant varied in range of 2.3-6.1 with maximum numbers in genotype RSC-10-52 (6.1) intimately trailed by genotype SL-1068 (6.0), whilst the lowest numbers of primary branches (2.3) were recorded for the genotypes *viz*., RVS 2011-35, JS 93-05, JS 20-34 and RVS2007-6.

It is observed that the productivity of soybean is mostly reliant on flowering and maturity periods. Days to flowering play an imperative part in the productivity of crops and were found to be positively correlated with seed yield [34-37]. The longer days to 50% flowering trait was found to be associated with the higher economic productivity in several investigations. Kachare et al. [1] defined flowering behavior as the time and position of first flower and considered it to be an important trait of soybean cultivars. Days to initial flowering varied significantly in range of 33.5-45.5 days with maximum in genotype MACS-58 (45.5 days) and SKF-SPS-11(45.5 days) firmly tracked by genotypes VLS-94 (44.5 days) and MACS-15-20 (44.5 days), whereas the minimum days (33.5) to initiate flowering was taken by genotypes JS20-84, JS20-71, RVS 2001-4, NRC-130 and PS1613. Days to 50% flowering also varied significantly in range of 40.5-54.0 days highest 54 days taken by genotypes KDS980, MACS-58 and SKF-SPS-11, however the minimum days (40.5) engaged by genotype JS20-71 meticulously tracked by genotypes NRC-130 (41 days). It was also observed that around 70% of the genotypes exhibit flower initiation within 30-40 days and were categorized as early flowering genotypes.

Pod initiation is an important phenological stage sensitive to environmental conditions and determines the crop yield [38,39]. In the present study, range of days taken to pod formation varied between 36.5-55.1 days with highest by genotype KDS980 (55.1 days) trailed by VLS -94 (53.9 days), NRC SL-1 (52.5 days) and AMS 2014-1 (52.4 days), whereas the minimum days to pod formation was taken by genotype JS 93-05 (35 days) intimately tailed by genotype JS 95-60 (37 days). Earlier variations in numbers of days to pod initiation among genotypes were also reported by Jadhav [40], Kachare [8], Karam et al. [41], Meena [37] and Wei et al. [42].

Significant variations were observed for number of pods per plant and numbers of seeds per pod among the genotypes under taken for study. Numbers of seeds per pod varied significantly in array of 0.96-3.8. Maximum numbers of seeds per pod was formed by genotype JS 95-60 (3.8) persuaded by JS 93-05 (3.6). While, the minimum numbers of seeds per pod was formed by genotype PS1613 (0.96) closely tracked by genotypes EC457286 (1.2), AMS 2014-1(1.2), NRC-132 (1.3) and RVS 76 (1.4). In previous studies, difference in numbers of seeds per pod among different genotypes were also evidenced by Kachare [8], Meena [37], Feroud et al. [43], Jain et al. [44], Onemi [45], Oya et al. [46] and Salimi et al. [47].

Weight of plant with pod varied significantly in range of 12.20 g to 95.44 g. Maximum weight of plant with pod was documented for the genotype NRC-147 (95.44 g) tracked by NRC-125 (75.40 g) and KDS980 (70.90 g). However, the minimum weight of plant with pod was noted in genotype NRC-131 (12.24 g) trailed by two genotypes *namely*: G-29 (12.56 g) and RSC-10-71 (14.06 g).

Grain yield per plant ranged from 7.52-21.67g. Maximum with genotype NRC-130 (21.67 g) perused by JS97-52 (17.57 g), whereas the minimum grain yield per plant was noted with genotype AMSMBC-18 (7.52 g) trailed by two genotypes *viz.,* VLS-94 (9.85 g) and EC457286 (9.65 g). Dissimilarity in grain yield also addressed by Kachare [8] and Meena [37] in previous studies.

Hundred-seed weight is the ultimately decider yield component associated with higher yield, thus, occupies key importance in the selection

criteria for improving yield potential of soybean. In the current study, 100- seed weight was ranged between 6.60-15.70 g. Maximum 100-seed weight was documented with genotype NRC-130 (15.70 g) tracked by genotypes MACS-1575 (13.65 g), SL-1123 (13.09 g), SL-1068 (11.91 g), G-29 (11.90 g), KDS 992 (11.47 g), JS 20-94 (11.23 g), NRC -125 (11.20 g) and PS 1092 (11.11 g), whilst the minimum was observed in genotype AMSMBC-18 (6.60 g) trailed by genotypes NRC-132 (6.85 g), MACS-1520 (8.42 g), NRC-127 (8.66 g), VLS -94 (8.68 g), EC457286 (8.80 g) and RSC-10-71 (8.88 g). Seed index is an important yield component directly correlated to seed yield in soybean. In past investigations, disparities in 100-seed weight among different soybean genotypes also documented by Kachare [8], Meena [37], Oya et al. [46], Manavalan et al. [48], Angra et al. [49], Demirtas et al. [50], Maleki et al. [51] and Khan et al. [52].

Biological yield is a prominent yield component which decides the fate of seed yield in soybean. Kunert et al. [53] and Ghanbari et al. [54] stated that biological yield is highly correlated with seed yield in soybean. Significant variations were also observed for biological yield among the genotypes undertaken in current experimentation. Biological yield varied between 14.30 and 69.61. Maximum with genotype KDS980 (69.61) closely tracked by genotypes SP37 (67.70), SL-1123 (63.63), NRC-132(62.80), NRC-125 (62.53) and SL-1068 (61.40), while the minimum biological yield was observed with genotype VLS -94 (14.30) tracked by genotypes NRC -131 (15.53), JS 95-60 (15.35), G-29 (16.60), RSC-10-71 (16.85) and JS 20-69(17.87).

3.2 Hierarchical Cluster Analysis

Rendering to the hierarchical cluster investigation and the traits values (Table 2), the dynamic countenance profile was depicted and is presented in Fig.1. Multivariate analysis constructed on diversity was accomplished *via* the UPGMA. A dendrogram of 53 genotypes of soybean acquired with simple flexible linkage was constructed commissioning different morpho-physiological traits of soybean genotypes. Agglomerative genotype hierarchical clustering assemblage the genotypes into two major cluster MC-1 and MC-2. Later MC-1 was subdivided into two sub clusters in that NRC-147 presented as a separate group (Fig.1). Subsequently, in MC-2 cluster, NRC-86, NRC-131 and AMS-100-39 genotypes are presented as an out group. This outline of clustering hence, show blended tendency of different morpho-physiological traits of genotypes.

3.3 Principal Component Analysis

Principal component analysis (PCA) was performed by taking morpho-physiological variables concurrently. The design of variations demonstrated by the PCA designated by correlation coefficients governed for pair-wise connotation of the traits. The PCA correlation illustrated that which variety possessed higher and lower content occupying unique position towards the graph (Fig. 2). Among ten variables, leaf area content has the highest variability (57.95 %).

3.4 SSR Markers-Based Screening

Diversity estimation of any plant species is a right initiator for enhancement of the crop since it offers base line data to correct selection of parental genotypes for a varietal improvement scheme [16,17,29]. Hybridization between genotypes belonging to the similar cluster will not prime to desired segregates as all the genotypes with genetic resemblance set into same cluster, while genotypes with genetic distance departed into clusters representing better diversity between the clusters [13,55]. Molecular markers-based profiling has been the ideal preference of hybridization as it proved its reliability and authenticity without influence of environmental conditions on it [1,3,13,16]. Molecular markers have also been utilized for finding of specific trait linked markers [1,3,12]. These studies are useful in the categorization of crop populations into miscellaneous assemblage which may be helpful in expansion of gene pool.

A total of forty-eight SSR markers were attempted to amplify 53 soybean genotypes selected under the current study in beginning experiment. Initially only twenty SSR markers amplified the template DNA across all 53 genotypes. However, remaining markers were tried two more times with different annealing temperatures and finally thirty-two SSR markers were successfully amplified across all soybean genotypes. These all thirty-two SSR markers (66.67%) were found to be polymorphic. However, in a previous study, Bisen et al. [16] reported less than 50% (23 out of 50 SSR markers) amplification and polymorphism efficiency of SSR markers in soybean. This may be due to trial of only one annealing temperature during PCR process.

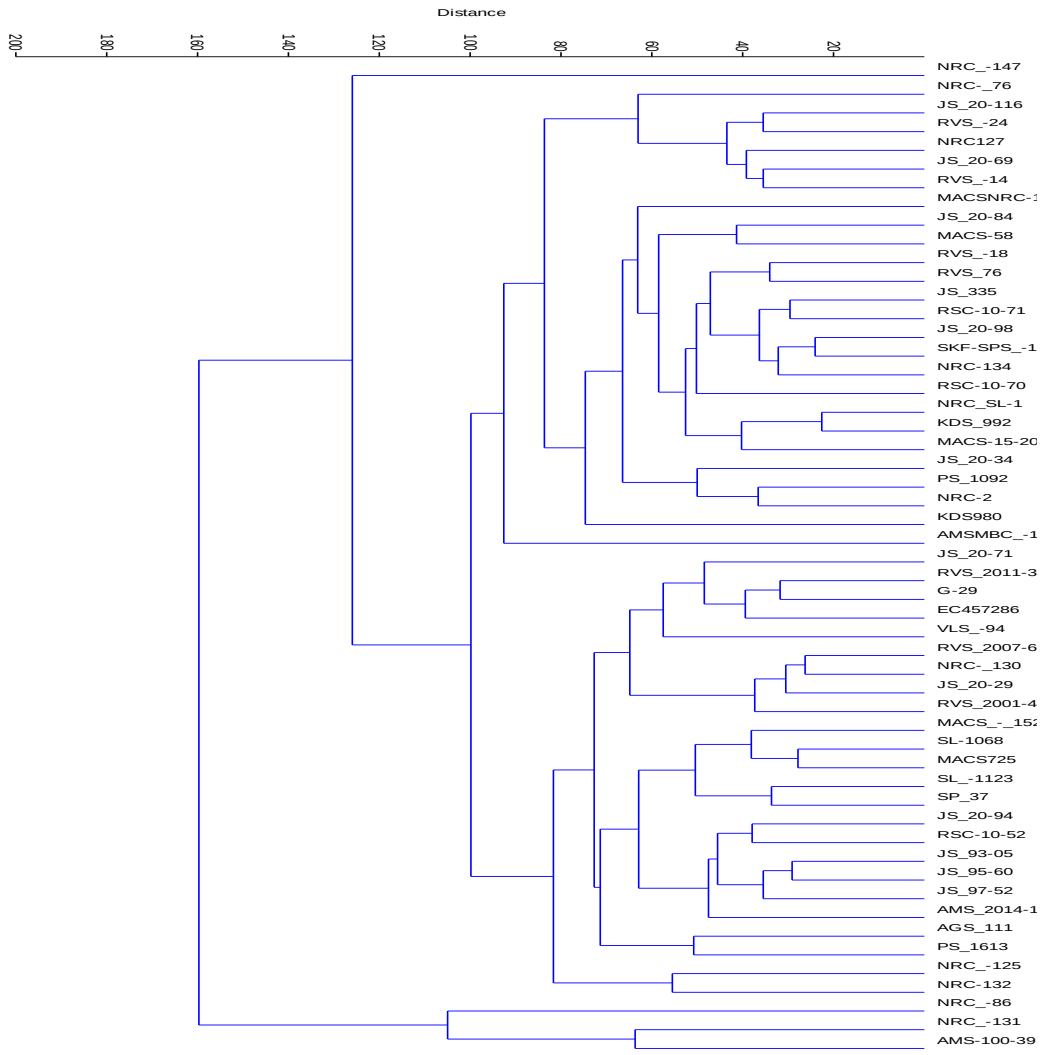

Fig. 1. Dendrogram based on different morpho-physiological traits showing relationship among 53 soybean genotypes

The total numbers of alleles amplified with all SSR markers were 76 and average polymorphic alleles were 2.38. Out of thirty-two microsatellites, two markers *viz*., Satt119 and Satt154 amplified maximum four alleles and ten markers were found to be able to amplify only three alleles each. Rests of the markers were found to be able to amplify only two alleles. The highest major allele frequency (0.98) was observed in Satt557 tracked by 0.95 in Satt518 while lowest (0.36) in Satt520. An average major allele frequency was 0.69. The highest genetic diversity (0.66) was demonstrated by Satt520 while lowest (0.04) was in Satt557. An average genetic diversity was 0.35. Among all thirty-two SSR molecular markers the highest PIC value was 0.67 demonstrated by Satt119 and lowest 0.04 by Satt557 with an average of 0.32 (Table 3). Similar to the present finding, the polymorphism of SSR loci perceived in this study match with the earlier data of Bisen et al. [16] and PIC values were in agreement with previous result of Sahu et al. [13]. Valliyodan et al. [56] also found an average PIC value of 0.36 with SSR markers in soybean. According to various other researchers, PIC values were ranged from 0.199 to 0.87 [13]. Higher value of PIC indicates the presence of various alleles in every locus, and is also important in the identification of molecular markers-based analysis of variability [12].

Owing to high level of reproducibility and co-dominant inheritance, SSR markers have been

practiced for distinguishing genotypes and investigating genetic relationships among 53 soybean genotypes. Microsatellites have been employed for genetic diversity analysis among soybean genotypes by various researcher groups [13,16,55]. The present study with 53 genotypes including a variety of imperative cultivars from India is the important study so far, to characterize the variation at molecular level. Thirty-two SSR markers employed in this study offered valuable evidence about genetic diversity present in soybean genotypes as they were linked with genotypes. For impressive genetic diversity analysis, allele frequency, genetic diversity and polymorphism information content for each SSR locus were computed. The PIC values were generally good for all the SSR loci tested with an average of 0.32. Two SSR loci revealed PIC values higher than 0.6 and, Satt154 and Satt119 were notable owing to their relatively higher polymorphism (four alleles). The average numbers of alleles per locus in our analysis was lesser than the past study conducted by Kaewwongwal et al. [57] where it was 9.05. However, Bisen et al. [13] detected an average of 1.97 alleles per locus across 38 Indian soybean genotypes. This higher rate of SSR polymorphism may be attributed to the selected set of SSR markers which were previously tested for polymorphism among a set of genotypes. Nevertheless, the lower allele number and PIC values designates low allelic diversity in present set of soybean accessions. The SSR allelic diversity distinguished among soybean genotypes in this experimentation was low comparison to previous experimentation [58].

Table 1. List of soybean genotypes with their parentage

S No	Genotypes	Source/Pedigree	S No	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	28	RSC-10-52	NRC37 x JS335
2.	JS 20-69	JS 97-52 x SL 710	29	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	30	SL-1068	SL755 x SL525
4.	JS 20-98	JS 97-52 x JS SL710	31	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	32	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	33	MACS725	JS93-05 x MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	34	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	35	NRC -125	EC54688 x ps1044
9.	JS 97-52	PK 327 x L 129	36	NRC-132	JS97-52 x PI086023
10.	JS 20-84	JS 98-63 x PK 768	37	NRC-134	NRC7 x AGS191
11.	JS 20-34	JS 98-63 x PK 768	38	NRC SL-1	JS335 x SL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	39	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	40	PS 1613	PS1225 x PS1042
14.	RVS 2011-35	JS 335 x PK 1042	41	AMS 2014-1	AMS99-33 x H6P5
15.	RVS 2001-4	JS 93-01 x EC 390981	42	KDS 992	JS93-05 x EC241780
16.	RVS -14	JS 93-05 x EC 390981	43	VLS -94	VL Soya59 x VS2005-1
17.	RVS -24	J P 120 x JS 335	44	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110 x JSM66	45	RVS 76	MAUS-162 x JSM-66
19.	NRC- 76	NRC-37 x L-27	46	NRC127	JS97-52 x PI542044
20.	NRC -86	RKS15 x EC481309	47	KDS980	JS93-05 x AMS1
21.	NRC- 130	EC390977 x EC538828	48	G-29	Germplasm
22.	NRC -131	EC390977 x EC538828	49	RSC-10-70	JS335 x Bragg
23.	NRC -147	Germplasm accessions C210	50	RSC-10-71	Bragg x JS335
24.	AMSMBC -18	Mutant of Bragg	51	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	52	MACS-15-20	NRC37 x Mohetta
26.	MACS – 1520	EC241780 x MACS330	53	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044 x JS9305			

Table 2. Mean performance of different morpho-physiological traits of soybean genotypes

S No	Parameter Genotypes	Leaf area (cm sq/plant)	Numbers of primary branches per plant	Days to initial flowering	Days to 50% flowering	Days to pod formation	Numbers of seeds per pod	Weight of plant with pod (g)	Grain yield per plant (g)	100-seed weight (g)	Biological Yield
1.	JS 20-29	15.16	3.3	38.5	48.5	49.2	2.5	25.05	16.07	10.30	24.70
2.	JS 20-69	33.58	3.9	35.5	43.0	49.0	2.8	14.06	15.16	9.19	17.87
3.	JS 335	14.65	4.7	38.5	48.0	45.4	1.8	28.20	14.54	10.10	30.64
4.	JS 20-98	31.89	5.7	35.0	43.5	47.1	2.7	41.80	12.34	9.79	32.21
5.	JS 20-94	37.04	37	36.5	47.0	50.6	2.5	26.04	16.17	11.23	30.82
6.	JS 93-05	39.14	2.7	36.0	46.0	36.5	3.6	24.42	16.54	10.88	27.43
7.	JS 20-116	32.91	4.3	34.5	44.5	50.4	2.8	25.56	11.14	9.77	26.50
8.	JS 95-60	30.91	3.3	35.0	44.0	37.4	3.8	12.60	10.57	10.07	15.35
9.	JS 97-52	22.03	5.2	35.5	44.5	50.5	2.7	19.52	17.57	10.10	21.57
10.	JS 20-84	22.86	2.5	33.5	44.5	42.5	2.5	18.12	10.24	9.48	18.70
11.	JS 20-34	19.70	2.7	34.5	44.5	41.1	2.7	21.34	10.35	9.58	21.29
12.	JS 20-71	20.38	4.7	33.5	40.5	44.6	2.5	18.06	10.80	9.47	18.62
13.	RVS 2007-6	20.18	2.7	35.5	46.5	47.6	2.4	33.60	11.74	9.32	31.32
14.	RVS 2011-35	33.41	2.3	34.5	42.0	45.7	2.6	31.30	10.29	9.80	27.75
15.	RVS 2001-4	33.22	5.5	33.5	44.5	45.1	2.3	14.30	14.54	10.10	16.72
16.	RVS -14	54.71	3.7	34.5	44.0	44.7	2.3	13.96	12.59	9.12	18.00
17.	RVS -24	39.76	3.3	35.0	44.5	48.2	2.7	17.84	10.95	9.36	19.55
18.	RVS -18	44.36	2.8	34.5	44.5	47.1	2.7	54.12	13.13	9.12	22.96
19.	NRC- 76	48.06	3.9	36.5	47.0	49.9	2.6	22.00	13.03	9.05	21.61
20.	NRC -86	52.13	5.2	34.5	45.5	49.1	2.8	43.84	12.38	9.38	23.32
21.	NRC- 130	29.04	3.3	33.5	41.0	41.9	2.4	26.40	21.67	15.70	27.90
22.	NRC -131	76.25	2 8	34.5	47.0	45.8	2.2	12.24	11.23	9.85	15.33
23.	NRC -147	43.91	4.9	35.5	43.5	48.3	2.1	95.44	14.93	10.37	28.57
24.	AMSMBC -18	50.54	4.2	34.5	41.5	50.4	2.0	17.80	7.52	6.60	19.70
25.	AMS-100-39	75.18	4.5	35.5	43.5	50.5	2.2	19.00	11.57	9.18	37.10
26.	MACS – 1520	62.38	5.1	38.5	49.0	49.7	2.6	53.60	10.61	8.42	49.00
27.	MACSNRC-1575	73.65	3.4	36.0	46.0	43.6	2.5	35.00	14.74	13.65	35.30

S No	Parameter Genotypes	Leaf area (cm sq/plant)	Numbers of primary branches per plant	Days to initial flowering	Days to 50% flowering	Days to pod formation	Numbers of seeds per pod	Weight of plant with pod (g)	Grain yield per plant (g)	100-seed weight (g)	Biological Yield
28.	RSC-10-52	29.30	6.1	39.5	50.0	42.5	2.0	33.20	16.15	9.79	32.15
29.	SL -1123	13.31	5.1	39.0	46.5	50.6	2.8	64.60	14.25	13.09	63.63
30.	SL-1068	53.67	6.0	35.5	44.0	50.4	2.0	58.20	12.10	11.91	61.40
31.	AGS 111	25.17	3.7	39.0	48.0	50.0	2.1	26.36	10.95	10.43	32.11
32.	EC457286	26.85	5.5	39.5	48.5	49.6	1.2	43.20	9.65	8.80	44.38
33.	MACS725	42.05	3.8	35.0	42.5	51.7	1.6	41.80	11.37	10.83	46.60
34.	SP 37	39.57	5.1	37.5	47.0	49.8	1.7	61.20	11.60	10.00	67.70
35.	NRC -125	29.69	5.4	41.0	47.5	50.4	1.7	75.40	18.48	11.20	62.53
36.	NRC-132	12.09	5.3	44.0	50.0	51.0	1.3	63.20	10.48	6.85	62.80
37.	NRC-134	14.20	5.3	42.0	49.0	48.8	1.5	51.80	14.91	9.56	33.74
38.	NRC SL-1	27.48	3.9	38.5	47.0	52.5	1.5	49.96	12.61	10.25	39.82
39.	PS 1092	11.02	3.0	40.0	49.0	47.3	1.5	27.80	14.00	11.11	26.72
40.	PS 1613	28.32	3.9	33.5	42.5	49.1	0.96	30.60	12.20	10.70	32.01
41.	AMS 2014-1	62.22	5.6	43.0	46.0	52.4	1.2	30.20	13.25	10.06	29.20
42.	KDS 992	39.96	5.4	43.5	47.0	51.5	1.6	44.40	15.48	11.47	34.42
43.	VLS -94	24.59	4.5	44.5	53.0	53.9	1.7	12.20	9.85	8.68	14.30
44.	SKF-SPS -11	28.68	4.8	45.5	54.0	51.5	1.7	41.66	11.74	10.30	27.54
45.	RVS 76	28.13	4.6	43.5	53.5	51.1	1.4	47.16	14.89	10.13	34.90
46.	NRC127	38.30	4.8	43.0	49.5	50.1	1.5	30.48	10.91	8.66	27.79
47.	KDS980	45.01	5.0	43.5	54.0	55.1	1.5	70.90	13.32	9.25	69.61
48.	G-29	29.98	3.1	42.0	53.0	48.8	1.7	12.56	13.57	11.90	16.60
49.	RSC-10-70	33.71	5.8	44.0	52.5	44.9	2.5	25.05	11.70	10.15	22.38
50.	RSC-10-71	24.57	4.2	43.0	50.0	42.5	2.8	14.06	9.89	8.88	16.85
51.	NRC-2	22.94	4.4	43.0	51.5	44.3	2.5	28.20	10.33	9.31	34.36
52.	MACS-15-20	37.15	5.2	44.5	50.5	42.0	2.5	41.80	11.61	10.18	38.12
53.	MACS-58	22.52	4.6	45.5	54.0	44.2	1.7	26.04	12.23	9.71	35.67
Range		11.02-76.25	2.3-6.1	33.5-45.5	40.5-54.0	36.5-55.1	0.96-3.8	12.20-95.44	7.52-21.67	6.60-15.70	14.30-69.61
SE (m)		0.691	0.623	0.730	1.127	1.114	0.452	0.175	0.740	0.379	2.747
$CD_{0\ 05}$		1.964	1.769	2 074	3.201	3.164	1.284	0.498	2.052	1.078	7.801

Table 3. Details of SSR markers used for diversity analysis among soybean genotypes

Marker	Forward 5'-3'	Reverse 5'-3'	AN	MAF	GD	PIC
Sat_044	AAAAAATATTTATAGGTTACATGTG	TTACCACTAAGAATTAGGTCTAA	2	0.66	0.45	0.35
Sat_171	GCGCTCCTCTTTTTTTCACTTTC	GCGCGTGGGATTTTGGTATTTTT	2	0.57	0.49	0.37
Sat_205	GCGCCTTTTCGTCTGTTCTGTTC	GCGAGCTTTTAAAAATTTAGAAATCAAT	2	0.83	0.28	0.24
Sat_375	GCGTGTTAATGATTGCATAAGGTTCG	GCGTGTCAAAAGAAACTCAATAAAGAAAAAT	2	0.87	0.23	0.20
Satt174	TTTCATTTCTTTGCCTTCT	TTCGTAGTCCGTCTTTCAT	2	0.89	0.21	0.18
Satt226	GCGAAACAACTCACTTAAGCAATACAT	GCGTCCTCCTACCTTTCTTATC	3	0.58	0.51	0.39
Satt244	GCGCCCCATATGTTTAAATTATATGGAG	GCGATGGGGATATTTTCTTTATTATCAG	2	0.60	0.48	0.36
Satt500	GCGAACGACCATGATAATCACA	GCGCTCATTTGAAAGCATTGTTATA	3	0.75	0.38	0.32
Satt520	GCGGTGTGCAAGAGTGACA	GCGCATTTGGACTTTCTA	3	0.36	0.66	0.59
Satt540	CTGGCGAATCAAGCTTTGTAAC	CCGTGATTGCGAAGAGGATATT	2	0.68	0.44	0.34
Satt551	GAATATCACGCGAGAATTTTAC	TATATGCGAACCCTCTTACAAT	2	0.77	0.35	0.29
Satt557	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA	2	0.98	0.04	0.04
Satt119	TGTGCCAGTGTTGATAGTTA	CTGATCCCCAATAAATCTG	4	0.78	0.56	0.67
Satt245	AACGGGAGTAGGACATTTTATT	GCGCCTCCTGAATTTCAAAGAATGAAGA	2	0.58	0.33	0.43
Satt281	AAGCTCCACATGCAGTTCAAAAC	TGCATGGCACGAGAAAGAAGTA	3	0.83	0.54	0.29
Sat_076	GCGTAATTAACACCAATATATGACATG	GCGGGGTTAAAAATTCAAAATGT	3	0.67	0.45	0.44
Satt510	GCGAGTTTCGCCGTTACCACCTCAGCTT	CCCTCTTATTTCACCCTAAGACCTACAA	2	0.59	0.32	0.22
Satt114	GGGTTATCCTCCCCAATA	ATATGGGATGATAAGGTGAAA	3	0.69	0.29	0.32
Satt275	GCGGGATAATTGGTTTTACGAAAATGC	GCGCCTAATCACCTAAAAAAACGTTTA	2	0.85	0.21	0.23
Satt280	GCGGAATCTGCTTATTCATTGTGTG	GCGCCATGCTGTAACACGTCAAT	2	0.77	0.19	0.25
Satt292	CGGAATTAGAACTCCAGTAAAGA	GCGAGGCCAACATTGAAAAGT	2	0.93	0.33	0.19
Satt130	TAAACGAAATTTAGTTTTAAGACT	TGAATGGCTAAAAACGTGATT	3	0.53	0.43	0.37
Satt042	GACTTAATTGCTTGCTATGA	GTGGTGCACACTCACTT	2	0.68	0.33	0.30
Satt442	CCTGGACTTGTTTGCTCATCAA	GCGGTTCAAGGCTTCAAGTAGTCAC	2	0.49	0.19	0.28
Satt154	AGATACTAACAAGAGGCATAAAACT	AAAGAAACGGAACTAATACTACATT	4	0.88	0.58	0.66
Satt518	GCGCATATCAAATTGCATATAAAAATACG	GCGGGAATATAAAATAAAAATGCTCACTT	2	0.95	0.27	0.26
Satt418	GCGAAAGCACATATGGGTTTGAAT	GCGAGGGCATATATATGATGAGGTA	3	0.82	0.35	0.31

Marker	Forward 5'-3'	Reverse 5'-3'	AN	MAF	GD	PIC
Satt235	GCGGGCTTTGCCAAGA AGTTT	GCGGTGAGGCTGGCTAT AAG	3	0.65	0.31	0.29
Satt236	GCGTGCTTCAAACCAA CAAACAACTTA	GCGGTTTGCAGTACGTA CCTAAAATAGA	3	0.55	0.34	0.38
Satt184	GCGCTATGTAGATTATC CAAATTACGC	GCCACTTACTGTTACTCA T	2	0.64	0.44	0.29
Satt686	ACGGAAAATAAATGAAA CTAAGA	GCGCTATCAGATAGAGA AGCAGAAGAAT	2	0.67	0.32	0.31
Total			76	22.09	11.3	10.16
Average			2.38	0.69	0.35	0.32

AN-Allele numbers, MAF-Major allele frequency, GD-Genetic diversity, PIC-Polymorphism information content

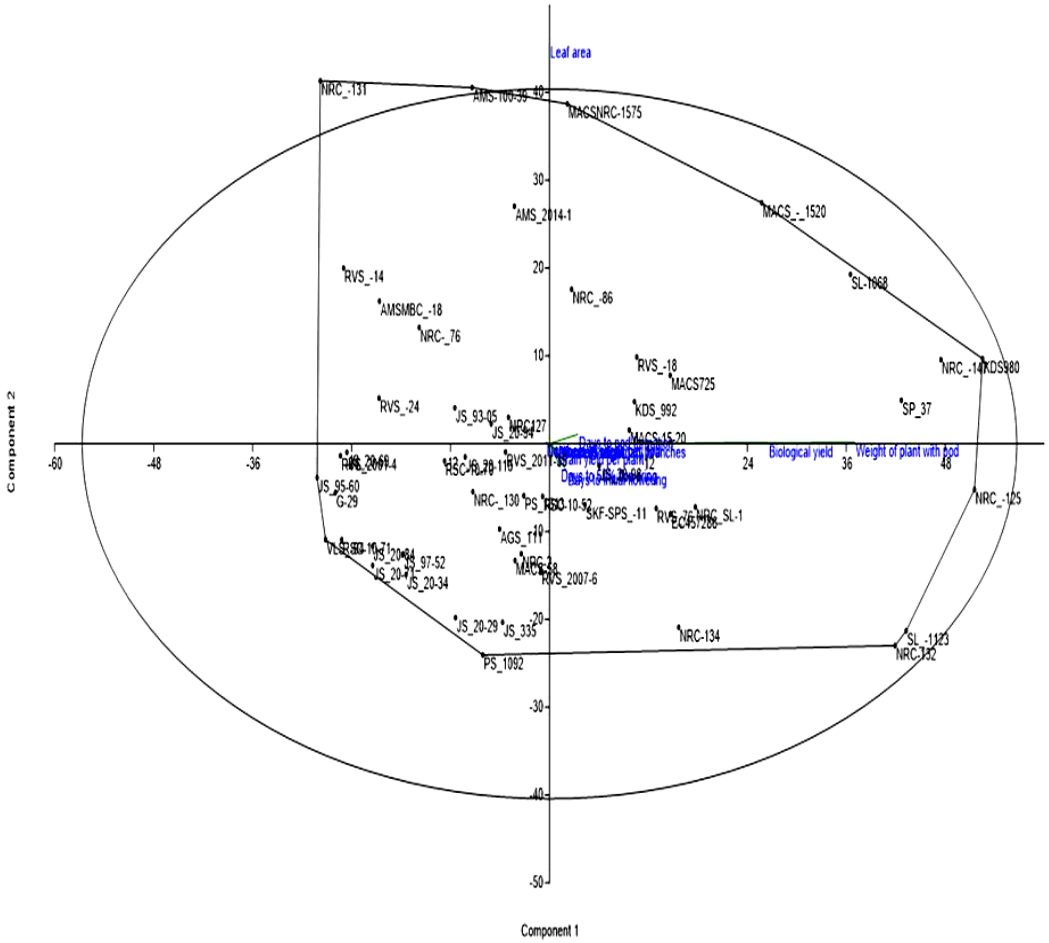

Fig. 2. Principal component analysis based on different morpho-physiological traits showing relationship among soybean genotypes

The UPGMA cluster analysis was accomplished employing SSR data. The clustering was done on the basis of genetic similarity between and among studied soybean genotypes. Initially 53 soybean cultivars were divided into two clusters one minor and one major (Fig. 3). Minor cluster contained six genotypes, *viz.,* PS-1092, RVS-14, AMS-100-39, SL-1068, NRC-125 and JS97-52. Among these six genotypes RVS-14 and AMS-100-39 have similar parental source. PS-1092 showed lower similarity with rest of the five genotypes and grouped distantly. This may be due to its geographical origin and parentage also. The major cluster contained 47 genotypes and further divided into two groups one minor and one major. Minor group had ten genotypes,

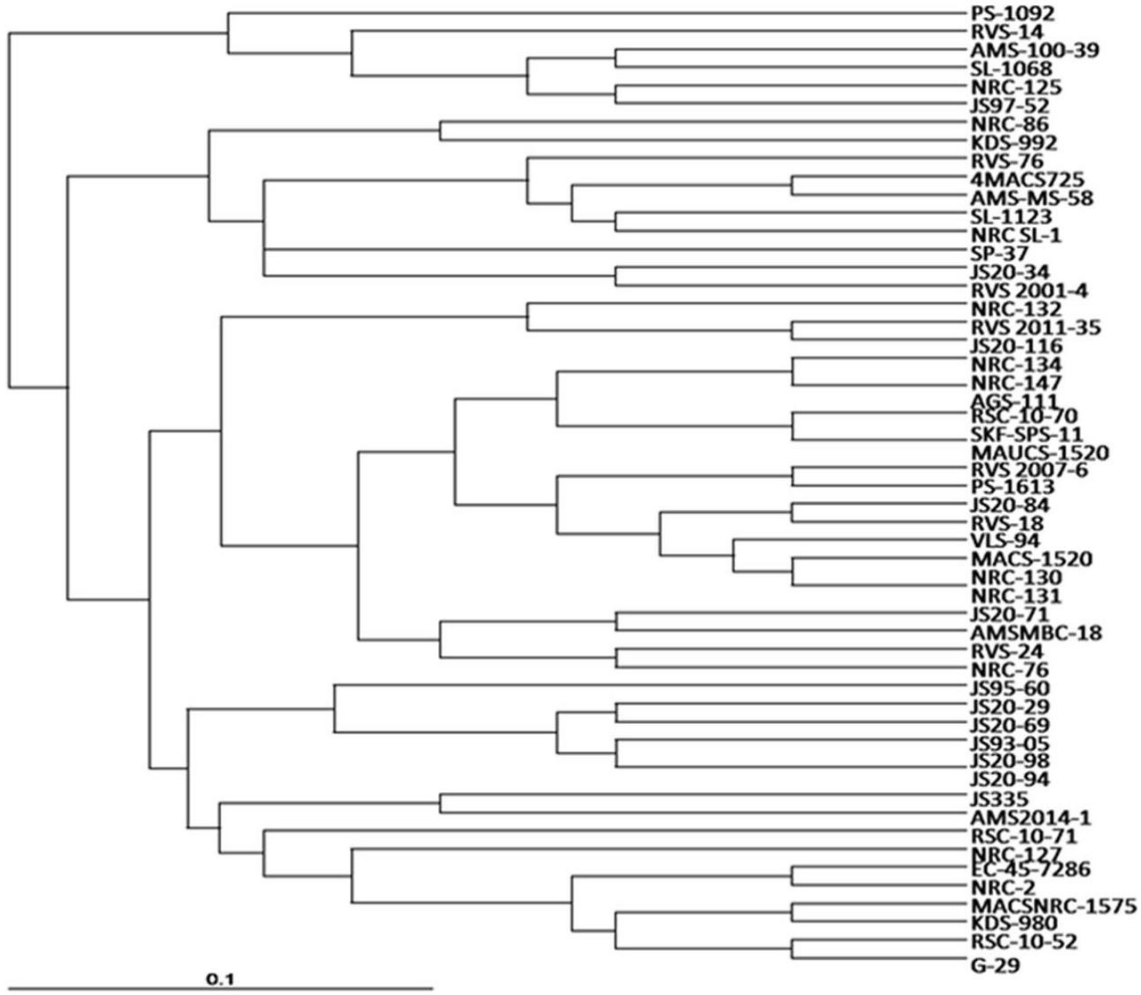

Fig. 3. Dendrogram representing SSR markers-based relationship among 53 genotypes of soybean

viz., NRC-86, KDS-992, PS-1613, MACS725, AMS-MS-58, SL-1123, NRC-SL-1, SP-37, JS20-34 and RVS2001-4. The major group contained 37 genotypes and it was later splinted into two sub groups. Major sub group contained 21 genotypes including NRC-132, RVS2011-35, JS20-116, NRC-134, NRC-147, AGS-111, RSC-10-70, SKF-SPS-11, MACS-1520, RVS2007-6, PS-1613, JS20-84, RVS-18, VLS-94, NRC130, NRC-131, JS20-71, AMSMBC-18, RVS-24 and NRC-76. Among these genotypes NRC130 and NRC131 showed higher similarity with each other and grouped closely. This may be due to the similarity in their parentage (EC390977 x EC538828). However minor group contained 16 genotypes. *namely*: JS95-60, JS20-29, JS20-69, JS93-05, JS20-98, JS20-94, JS335, AMS2014-1, RSC-10-71, NRC-127, EC-45-72-86, NRC-2, MACSNRC-1575, KDS980, RSC-10-52 and G-29. This clustering indicates the grouping of seven soybean varieties *i.e.,* JS95-60, JS20-29, JS20-69, JS93-05, JS20-98, JS20-94, JS335 developed in the same Centre. Among these seven varieties four (JS20-29, JS20-69, JS20-98 and JS20-94) share one of the common parents (JS97-52). Similar clustering was found in previous studies conducted on microsatellite-based diversity analysis among Indian soybean genotypes [1,3,13,16,59]. The clustering of bulky numeral of soybean germplasm lines in a single cluster indicates that soybean germplasm assemblage is having high genetic affiliation among genotypes.

During the present investigation the genotypes grouped in different clusters having large genetic distance can be used as parental candidates for cross breeding to produce progeny with added value or heterotic effect from each parent. In contrast, the genotypes grouped in the same cluster demonstrated their close genetic relation and should not be used as parents in cross-

breeding to prevent the occurrence of inbreeding depression. Inbreeding depression, the opposite of heterosis, is the decreased progeny vigour due to the increased homozygosity level as a result of crosses between two individuals with close genetic relationship [60]. Inbreeding depression in plants will cause the plants to be stressed, which is identified by the decrease of plant height, less vigour, sensitive to pest and disease attack, decrease of fruit number and increased fruit abortion, and the appearance of various unwanted characters due to the combination of recessive alleles [61]. Overall, the results of this study suggested the possibility to select parental lines for heterotic crosses in breeding programme using SSR molecular markers.

When we compared morpho-physiological and microsatellite markers-based clustering pattern of the soybean genotypes under the present study, no close association was found. The minor harmony between morpho-physiological and microsatellite dendrogram may be due to limited expression of morpho-physiological characters in the field conditions. Limited association between both of the data may also be due to substantial consequences of environment on these characters and use of less numbers of microsatellite markers. Markers are not enough to represent whole genome of soybean so, more numbers of markers with extensive coverage may be useful in clear understanding of association between morpho-physiological and molecular data.

4. CONCLUSION

Genetic diversity analysis based on yield attributing traits and SSR markers confirms the availability of variations among soybean genotypes under the study. Genetic diversity detected among soybean genotype collection in current examination dictates the necessity of expansion genetic diversity by familiarizing more exotic germplasm lines along with employment of wild relatives. The preferred method for breeding is genetic diversity analysis based on molecular markers, because of its authenticity and reliability. The sundry genotypes detected in present experimentation may assist as basis of new alleles in soybean breeding scheme in India.

DATA AVAILABILITY STATEMENT

The data presented in this study is available within this article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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