

Genetic Diversity Studies in Soybean (*Glycine max* (L.) Merrill) Germplasm Lines Based on Mahalanobis D² Distance

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ABSTRACT: Genetic divergence analysis is very helpful to compute the nature and extent of genetic diversity in a group of germplasm. The analysis helps in the identification of genetically diverse genotypes for hybridization programs to obtain desirable recombinants or transgressive segregants. Hence the present investigation was carried out to assess the genetic diversity present among 55 soybean germplasm lines along with 5 checks for 16 different quantitative traits. All the genotypes were grouped into 14 clusters by performing Tocher's clustering method using Mahalanobis D² distance. Cluster I was the largest, comprising of 32 genotypes, followed by Cluster II with 16 genotypes. The maximum genetic distance (D²) was observed between cluster VII and XIII (858.60), followed by clusters IX and XIV (807.51), clusters VII and X (794.75), clusters VII and IX (755.77) and clusters IV and X (697.77). Cluster XIV had the highest means for branches per plant (7.80) and clusters per plant (29.00). The results revealed that the highest contribution of seed yield per plant (31.26%) towards total diversity, followed by harvest index (21.18%), plant height (12.76%) and seed germination rate (10.00%). The soybean genotypes belonging to the clusters VII and XIII were found to be the most divergent, hence can be utilised in the recombination breeding programmes to exploit maximum heterosis.

Keywords: Soybean; Genetic diversity; Mahalanobis D² statistics, cluster.

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is a high-nutritional oilseed crop grown in India and around the world. It is a member of the Papilionaceae family and has the chromosome number 2n=40. It is known as the "miracle bean" or "golden bean" because of its properties such as protein content (40-42%) and 20% edible oil, in addition to minerals and vitamins. Soybean protein contains all of the essential amino acids, along with cardio-friendly oil that meets 30% of the world's vegetable oil requirements (Khan *et al.*, 2022). Therefore, soybean could be regarded as an ideal food crop for the people of poor and developing countries as it contains high quality protein and reasonable quantity of oil as a source of energy (Kumar *et al.*, 2018).

The first and most important step in any crop improvement programme is genetic diversity analysis. There are several important applications for genetic diversity among genotypes in crop improvement. Estimates of genetic divergence provide the extent of diversity existed within the available germplasm and moreover, evaluation of genetic diversity is important to know the sources of genes for a particular trait (Meena

et al., 2017). This diversity analysis information can be used to classify germplasm for cultivar identification, assist in parent selection for hybridization, and reduce the number of genotypes required to sample a wide range of genetic variability. A genetically diverse parent is required to increase the likelihood of selecting better segregants for various characters (Adsul and Monpara 2014). Multivariate analysis, such as Mahalanobis D² statistics, is extensively used in genetic divergence research findings to group genotypes so that more diverse genotypes are grouped into the most distant clusters. It is also useful in determining the relative contribution of each trait to total divergence (Swar *et al.*, 2021).

MATERIAL AND METHODS

The present investigation was carried out at the research farm, Agricultural Research Station (Adilabad) and PJTS Agricultural University, Hyderabad during 2021. The experimental material comprised of 55 soybean germplasm lines with five checks *viz.*, JS 93-05, JS 335, KDS-753, AISb-50 and Basara. Basara is the local check of Telangana. All the entries were evaluated in

Randomised Complete Block Design (RCBD) in three replications by keeping inter and intra-row spacing of 45 and 10 cm respectively.

The observations were recorded on whole plot basis for days to 1st flowering, days to 50 % flowering, days to 1st pod initiation, days to maturity, biomass and germination % whereas, data were recorded for plant height (cm), number of nodes on main stem of plant, number of branches plant⁻¹, number of clusters plant⁻¹, number of pods plant⁻¹, 100 seed weight (g), seed yield plant⁻¹ (g) and harvest index (%) traits based on five randomly selected plants of each entry per replication and protein content (%) and oil content (%) were estimated as per protocols of AOAC (1990). The recorded data was subjected to analysis of variance and Mahalanobis D² statistics were used for genetic divergence analysis. Analysis of variance for Randomised Complete Block Design (RCBD) was performed as per the method suggested by Panse and sukhatme (1978). The genotypes were clustered by using Tocher's method. The intra- and inter-cluster distances were calculated and were used to describe the genotype relationship with the help of the formula proposed by Singh and Chaudhary (1977). The dissimilarity coefficient between genotypes was organised into a reasonable hierarchical system and estimated using the D value proposed by Sneathe and Sokal (1973). All the above mentioned analyses were performed using WINDOWSTAT software.

RESULTS AND DISCUSSION

A. Grouping of Accessions into Various Clusters

The Tocher's method was used to group 60 soybean germplasm lines based on D² values. A total of 14 clusters were formed, within which two clusters

contained multiple genotypes and the remaining 12 clusters contained only one genotype, indicating that genotypes are highly diverse in nature. The lines viz., KDS 1175, Z-5, PS 1682, Z-P1, Z-3, DLSb 3, KDS 1169, JS 20-03, PS 1675, Asb-62, KDS 1187, Z-16 belongs to clusters III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII and cluster XIV respectively which resembles that their distinct genotypic composition from the remaining genotypes. Cluster I was found to be the largest comprising of 32 genotypes followed by cluster II with 16 genotypes (Table 1, Fig. 1). Similar results are observed in findings of Pawar *et al.* (2015), Naik *et al.* (2016).

B. Average Inter and Intra-cluster Distances

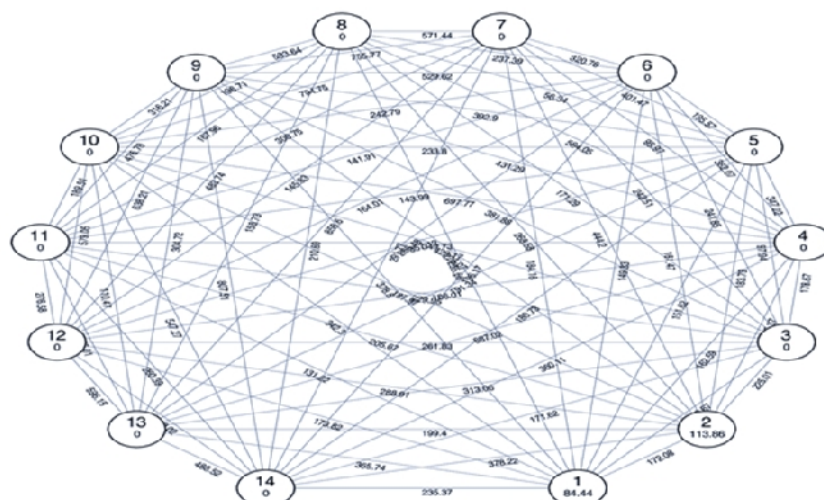
The intra-cluster D² values ranged between zero and 113.86. Cluster II (113.86) had the greatest intra-cluster distance among the 14 clusters, followed by Cluster I (84.44), indicating the presence of some amount of genetic divergence in the genotypes belonging to these clusters. The genotypes carefully selected from these clusters may be used in future recombination breeding programmes. The remaining ten clusters had zero intra-cluster distance as they each had a single genotype (Table 2).

The inter-cluster distance ranged from 56.34 to 858.60. Clusters VII and XIII had the longest and best inter-cluster distance (858.60), followed by clusters IX and XIV (807.51), clusters VII and X (794.75), clusters VII and IX (755.77), and clusters IV and X (697.77). Similarly, the relatively short inter-cluster distance was observed between clusters V and VIII (56.34), followed by clusters IV and VII (95.97), clusters III and V (97.94), clusters X and XIII (110.47) and clusters I and III (120.67) (Table 2).

Table 1: Grouping of all the soybean accessions into different clusters based on D² values.

S. No.	Cluster	Accessions	Total
1	Cluster I	Z-1, Basara, KSS 204, Z-19, Asb-80, JS 335, KDS 1149, Aisb 50, KDS 753, AMS 19-01, NRC 189, PS 1605, Asb-93, Asb-77, Asb-85, DSb 34, NRC 195, Asb-69, BAUS-116, Z-18, NRC 201, HIMSO-1694, Z-9, MAUS 791, Asb-82, Z-15, NRC 186, RVS 13-15, DSb 23, LOKSOY -1, SL 1282, AS 24	32
2	Cluster II	DS 1312, Z-11, DS 3124, PS 1689, Asb 15, Z-7, JS 23-09, KDS 1201, JS 93-05, SL 1230, AMS 115, DSb 39, NRC 196, NRC 203, Asb-84, KBS 21-1	16
3	Cluster III	KDS 1187	1
4	Cluster IV	Z-5	1
5	Cluster V	PS 1682	1
6	Cluster VI	Z-P1	1
7.	Cluster VII	Z-3	1
8	Cluster VIII	DLSb 3	1
9	Cluster IX	KDS 1169	1
10	Cluster X	JS 23-03	1
11	Cluster XI	PS 1675	1
12	Cluster XII	Asb 62	1
13	Cluster XIII	KDS 1187	1
14	Cluster XIV	Z-16	1

Tocher Method



Mahalanobis Euclidean Distance (Not to the Scale)

Fig. 1. Cluster diagram of soybean lines based on D^2 values by Tocher method.

Table 2: Average inter and intra-cluster distances (D^2) values of 60 soybean accessions.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII	Cluster XIII	Cluster XIV
Cluster I	84.44	172.08	120.67	162.59	151.62	149.33	184.16	255.66	378.80	342.30	131.22	173.82	365.74	235.37
Cluster II		113.86	225.01	315.37	183.79	161.47	444.20	268.58	226.07	187.97	205.67	288.91	199.40	378.22
Cluster III			0.00	178.67	97.94	241.85	242.51	171.29	381.88	429.17	229.95	261.83	313.06	171.62
Cluster IV				0.00	347.22	362.07	95.97	564.05	431.29	697.77	349.73	166.01	687.02	360.11
Cluster V					0.00	195.57	401.47	56.34	392.90	233.80	143.99	357.12	191.34	180.73
Cluster VI						0.00	320.76	237.39	529.62	242.79	141.91	164.01	286.48	196.17
Cluster VII							0.00	571.44	755.77	794.75	300.75	145.83	858.60	291.77
Cluster VIII								0.00	533.64	196.71	167.96	483.74	158.73	210.69
Cluster IX									0.00	316.21	476.78	638.21	304.72	807.51
Cluster X										0.00	189.51	579.05	110.47	547.27
Cluster XI											0.00	276.58	336.01	260.39
Cluster XII												0.00	596.15	220.02
Cluster XIII													0.00	488.52
Cluster XIV														0.00

* Diagonal bolded values indicate intra-cluster distances; *Above diagonal values are inter-cluster distances

The greater the distance (D^2) between clusters, the greater will be the genetic divergence between accessions belonging to that cluster (Ramyashree *et al.*, 2016). Crossing between genotypes from different clusters increases the variability of the gene pool. Soybean germplasm lines from clusters VII (Z-3) and XIII (KDS 1187) were found to be the most divergent, and thus may be used in recombination breeding programmes to maximise heterosis.

C. Cluster Means of the Characters

A considerable difference was noticed among the cluster means for all the characters. The genotypes in cluster IX had minimum mean performance for days to 1st flowering (23.00), days to 50% flowering (29.00), days to 1st pod initiation (36.00), days to maturity (86.00), plant height (40.00 cm), and germination percentage (71.67%). The genotypes in cluster VI had a maximum mean for days to 1st flowering (44.00), days to 50% flowering (47.00), days to 1st pod initiation

(66.00) and days to maturity (115.00). Cluster XIV had the highest means for branches per plant (7.80), clusters per plant (29.00) and nodes per plant (30.47), but it had the lowest mean for oil content (15.63). Cluster X had the lowest mean performance for branches per plant (3.20), clusters per plant (9.20), pods per plant (23.00), single plant yield (11.20) and biomass (388.45) and it had the highest value for 100 seed weight (15.33). For nodes per plant cluster, VIII recorded a minimum value of 10.93 and for 100 seed weight, XII (11.23) had a minimum value. Cluster VII had the highest mean for both seed germination percentage (87.00) and pods per plant (88.87). Cluster III had the lowest value for protein content (37.87). The traits like yield (25.40) and biomass (8054.37) showed maximum mean in cluster IV. For the harvest index, clusters XI (44.50) and XIII (35.07) recorded maximum and minimum mean values. Cluster XI had the highest mean for protein content (43.93), whereas cluster XIII had the highest mean for oil content (22.10) (Table 3).

D. The Contribution of each Traits towards Total Divergence

Each trait's contribution to total diversity is calculated based on the number of times the character appears in

the first rank. The seed yield per plant appeared 560 times in the first rank, indicating the greatest contribution to total diversity. Biomass, on the other hand, appeared once in the first rank, indicating a lower contribution to total diversity. The relative contribution of each traits towards total divergence is mentioned in Table 4 and Fig. 2.

The results revealed the highest contribution of seed yield per plant (31.26%) towards total diversity, followed by harvest index (21.18%), plant height (12.76%), seed germination rate (10.00%), while other traits like oil content (0.16%) were noticed as the lowest contribution towards diversity, followed by pods per plant (0.33%), biomass (0.5%) and days to 1st pod initiation (1.46%).

The result suggests that the soybean accession selected for the present study are mostly divergent for seed yield per plant, harvest index, plant height and seed germination rate as these four characters contributed 75.2 % to the total diversity. The present findings are in agreement with the results obtained by Chandel *et al.* (2013) for harvest index and pods per plant, Manav and Arora (2018) for seed yield per plant and Kachadia *et al.* (2014).

Table 3: Cluster means for sixteen characters.

	DDF	DDF2	DFPI	DM	PH (cm)	BR/PL	CL/PL	ND/PL	SG (%)	PD/PL	TW (g)	SY/PL (g)	HI (%)	PC (%)	OC (%)	BM (kg/ha)
Cluster I	38.82	41.86	53.32	107.24	54.90	4.18	20.72	22.01	80.07	58.67	13.40	20.64	41.55	42.15	18.43	6613.57
Cluster II	36.42	39.96	49.77	103.50	46.76	4.00	15.90	17.23	76.81	43.25	13.07	15.32	37.43	40.54	18.76	5460.30
Cluster III	36.33	39.67	54.00	102.33	90.67	5.20	21.20	22.63	74.67	71.07	13.53	21.03	41.03	37.87	21.90	6878.46
Cluster IV	35.67	38.67	50.00	102.67	40.17	6.40	28.27	29.30	78.67	81.87	12.40	25.40	41.67	38.40	19.70	8054.37
Cluster V	39.00	41.33	50.67	102.33	77.30	4.33	27.33	28.23	72.33	36.87	13.90	12.93	41.30	39.80	19.27	4151.06
Cluster VI	44.67	47.33	66.00	115.00	51.23	4.00	15.40	17.47	81.33	41.53	12.67	14.90	37.27	42.10	16.63	5280.35
Cluster VII	43.00	45.33	61.67	111.67	46.00	4.93	28.40	29.53	87.00	88.87	12.40	24.47	44.33	39.40	17.47	7325.54
Cluster VIII	39.00	41.67	50.33	108.00	99.93	4.33	19.73	20.47	77.67	37.67	15.10	13.5	40.80	38.63	21.80	4450.51
Cluster IX	23.00	29.00	36.00	86.67	40.03	4.00	11.07	13.40	71.67	47.80	12.80	15.83	39.50	37.90	20.70	5322.95
Cluster X	34.33	38.33	46.00	105.33	50.13	3.20	9.27	10.93	81.33	23.07	15.33	11.20	38.97	40.40	20.03	3858.45
Cluster XI	39.00	44.00	51.67	109.67	47.40	4.80	17.93	19.33	85.67	48.67	15.23	17.87	44.50	43.93	19.50	5366.27
Cluster XII	40.33	45.33	59.67	113.33	49.47	5.53	26.33	27.73	86.33	73.47	11.23	18.87	35.23	43.63	18.57	7077.27
Cluster XIII	35.67	39.00	49.00	100.00	84.50	3.40	10.40	11.23	72.00	36.40	15.20	12.77	35.07	42.30	22.10	4816.38
Cluster XIV	42.33	44.67	62.33	110.00	100.7	7.80	29.00	30.47	82.00	45.00	11.87	14.83	39.17	39.80	15.63	5020.40

Note: DDF=Days to 1st flowering, DDF2 = Days to 50% flowering, DFPI=Days to 1st pod initiation, DM=Days to maturity, PH=Plant Height, BR/PL=Number of branches per plant, CL/PL= Number of clusters per plant, ND/PL= number of nodes per plant, SG (%) =Seed germination, PD/PL=Number of pods per plant, TW (g) = Test weight of seed, HI (%) =Harvest index, PC (%) =Protein content, OC (%) =Oil content, BM (kg/ha) =Biomass, SY/PL(g) = Seed yield per plant.

Table 4: Relative contribution of different characters towards total genetic diversity.

S. no.	Source	Times ranked 1 st	Contribution %
1	Days to 1 st flower	35	1.97
2	Days to 1 st pod initiation	26	1.46
3	Days to maturity	108	6.10
4	Plant height	226	12.76
5	Branches per plant	41	2.31
6	Clusters per plant	97	5.48
7	Seed Germination%	177	10.00
8	Pods/plant	6	0.33
9	100 seed weight	62	3.50
10	Seed plant yield	560	31.26
11	Harvest index	375	21.18
12	Protein content	53	2.99
13	Oil content	3	0.16
14	Biomass	1	0.5

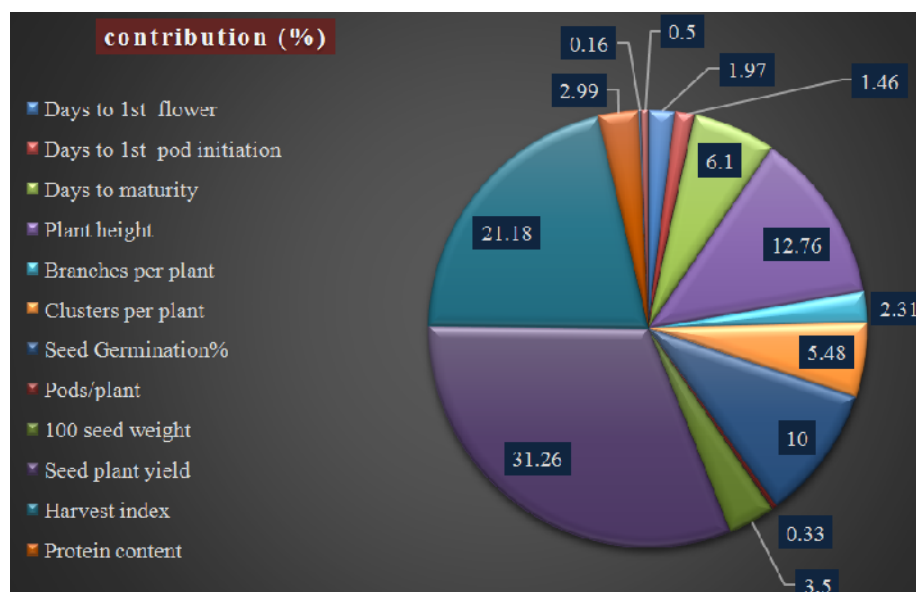


Fig. 2 Relative contribution of different characters towards total genetic divergence of soybean.

CONCLUSION

The current investigation's findings shows that the selected soybean lines are highly diverse and are formed into a total of 14 clusters. The accessions belong to the most distant clusters, namely (VII and XIII), (IX and XIV), (VII and X). These might be used directly or as parents in the soybean hybridization programme for yield improvement.

FUTURE SCOPE

Through the studies of genetic diversity, the genotypes that are desirable for hybridization programme and the traits that were desirable for yield improvement can be identified. Choosing of genotypes belonging to distant clusters was expected to execute maximum heterosis in crossing and to be used in hybridization program for improvement of quality traits.

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Conflict of Interest. None

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