Received: 16 Sep 2021 | Revised: 29 Aug 2022 | Accepted: 01 Sep 2022

Electronic Journal of Plant Breeding

Research Article

Genetic diversity study in soybean [*Glycine max* L. Merrill] based on agro-morphological characters

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Abstract
Using Mahalanobis D^2statistics, the level of genetic diversity among soybean genotypes based on 12 agro-morphological traits was investigated in this study. A total of 100 soybean genotypes were grouped into six clusters. Cluster I had the maximum number of genotypes (53 genotypes), followed by cluster II (37 genotypes), cluster III (6 genotypes), clusters IV and V (1 genotype each), cluster VI (2 genotypes). Clusters I and VI had the greatest inter-cluster distance (117618.10). Intra-cluster distance ranged between 0.00 and 9830.83. The biggest contribution to total divergence came from character plot yield (25.0 %). Cluster VI had the highest cluster mean values for yield and its component traits. Based on inter-cluster distance, cluster mean values, and mean per se performance, it is recommended to utilize genotypes of cluster I and cluster VI, I and III, III and IV, II and VI, respectively, for future hybridization programs to develop high yielding genotypes.

Keywords: soybean, clusters, genetic diversity

INTRODUCTION

Soybean (*Glycine max* L. Merrill), also known as the "miracle crop," is a self-pollinating legume. Soybean is the world's most important seed legume, contributing around 25 % of worldwide edible oil and 65 % of global protein concentrate for livestock feeding, respectively. It is also a valuable commodity for food processors, pharmaceutical industry, and a variety of other industries. Soybean is the only source of veggie that contains a complete high-quality protein. Soy milk is less expensive than other milk sources and has a wide range of applications among the growing vegan community. Tofu is a good source of protein and goes well with paneer. Soybean also has many therapeutic usages like overcoming problems related to menopause due to the presence of estrogen like compounds and presence of flavones which protect from cancer. Soybean production was 128.97 lakh tonnes in the year 2020-21. The largest soybean producing states are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Gujarat, and Chhattisgarh (Anonymous, 2021). Soybean can be cultivated as a vegetable crop from April to July when green peas are not readily available (Anonymous, 2021). The versatile nature of this crop, its growing contribution to the industrial, agricultural and medicinal sectors, as well as the wide yield gap, necessitate concerted pre-breeding efforts to broaden the genetic base and the formulation of an effective breeding program to develop varieties that are suitable for specific agro-climatic conditions. The use of available genetic diversity is the most important factor in bringing genetic upgrading to a crop. A crop's

genetic diversity is significant because genetically divergent parents can yield substantial heterotic effects (Falconer, 1960; Arunachalam, 1981; Ghaderi *et al.,* 1984; Mian and Bahl, 1989). As a result, knowledge of genetic diversity among elite breeding materials has a substantial impact on crop plant improvement (Hallauer *et al.,* 1989). Mahalanobis D^2 statistics is a useful method for determining the degree of genotypic diversity (Michener and Sokal, 1957; Murty and Arunachalam, 1966 and Arunachalam, 1981). Therefore, the present study aimed to quantify the genetic diversity in elite germplasm obtained from IISR, Indore using D^2statistic.

MATERIALS AND METHODS

During *Kharif* 2020, the current investigation was conducted at Central Agricultural University Research Farm, Andro, Imphal East. Geographically, the farm is located in Manipur's Imphal East district at 24°45.89' north latitude and 94°03.46' east longitude, at an elevation of 806.86m above mean sea level and is part of the Eastern Himalayan Region (II) and the agro-climatic zone Sub-Tropical Zone (NEH-4) of Manipur. The experimental field's soil is clay in texture, with an acidic soil reaction that ranges from 5.5 to 5.6 pH. For the current study, 100 elite soybean genotypes were obtained from the Indian Institute of Soybean Research, Indore, India, (**Table 1**) and sown in an augmented design in five blocks with five checks (RKS-113, RSC 10-46, RVS2010-1, KDS-753, and MACS-1460). Each genotype was sown in a single row of 3 m length with a spacing of 45 cm x 10 cm by dibbling. All intercultural operations were carried out according to Mondal and Wahhab (2001). Data were recorded from ten randomly taken plants on plant height (cm), the number of nodes/plant, the number of pod clusters/plant, the number of pods/cluster, the number of pods/plant, the number of seeds/pod, seed yield/plant (g). Data on days to 50% flowering and days to maturity were recorded by visual observation on a whole plot basis. Data on oil content (%) was estimated by using the AOAC method (2000). Data on plot yield was also recorded on a net plot basis. The genetic divergence analysis was done as per Mahalanobis D^2 Statistic (1936) and genotypes were classified into distinct clusters using Tocher's method (Rao, 1952). Computation of D^2 statistic and cluster analysis was carried out with the help of a computer, as per Windostat version 9.3 from Indostat service, Hyderabad.

RESULTS AND DISCUSSION

For all of the characters, the analysis of variance indicated extremely high significant differences across genotypes, showing the presence of genetic variability in the experimental material (**Table 2**). Following Tocher's method of clustering (Rao, 1952), the 100 soybean genotypes were grouped into six clusters based on the relative magnitude of D^2 values, with the criteria that intra-cluster average D^2 values be less than inter-cluster D^2 values. Cluster I had the maximum number of genotypes, (53 genotypes), followed by cluster II with 37 genotypes, cluster III with six genotypes, clusters IV and V with one genotype each and cluster VI with two genotypes (**Table 3**). Grouping genotypes from various regions in the same cluster revealed that genetic diversity is not always connected to geographic origin. Ganesamurthy and Sheshadri (2002), Iqbal *et al.* (2008), Patil *et al.* (2011), Shinde *et al.* (2013) and Jency and Kalaimagal (2015) all support this observation. According to Murty and Arunachalam (1966), the genetic diversity among the genotypes may have originated through genetic drift and selection, which cause higher diversity than geographical spread. However, the effect of geographical origin influenced clustering in several circumstances. As a result, genetic diversity was not solely determined by geographic distribution. This shows that for hybridization, it is not required to select parents from various geographical regions (Rashid, 2000). However, the effect of geographical origin influenced clustering in several circumstances (Baruah *et al.,* 2015). The distances between inter-clusters were greater than the distances between intra-cluster (**Table 4 & Fig.1**). Intra-cluster distances ranged from 0.00 (clusters IV and V) to 9830.83 (cluster VI). Cluster-VI (9830.83) had the greatest intra-cluster distance, followed by cluster III (5899.82), cluster II (5022.21) and cluster I (3572.42). The intra-cluster distances were 0.00 for clusters IV and V because they were having only a single genotype. Greater genotype heterogeneity was also suggested by higher intra-cluster distance (Shwe, 1972). Clusters I and VI (117618.10) were found to have the greatest inter-cluster distance, followed by clusters IV and VI (85,009.17), clusters I and III (59,071.16), clusters -III and IV (51,156.72), clusters -II and VI (50,409.88), clusters -I and V (49,477.39), clusters -V and VI (23,318.18). The maximum inter-cluster distance indicated that the grouping was diverse, whilst the shortest inter-cluster distance indicated a closeness (Singh and Chaudhury, 1985). Genotypes from different clusters separated by a large statistical distance could be employed in a hybridization programme to obtain a wide range of variance between segregations (De *et al.,* 1992). Furthermore, it is claimed that the degree of genetic variability in the paternal lines influenced the amount of heterosis (Roy and Panwar, 1993).

The data on cluster mean value, given in **Table 5**, demonstrated that different clusters differed significantly across all 12 characters examined. The characters with the greatest cluster mean values were plant height, the number of nodes/plant, the number of pod clusters/plant, the number of pods/plant, the number of seeds/pod, seed yield/plant, plot yield , 100 seed weight and oil content was found in cluster VI, indicating that the parental lines in this cluster have the genetic potential for yield maximisation. Cluster V was next for days to 50% flowering and the number of pods/cluster and cluster IV was next for days

Table 1. Details of the genotypes used in the present study

S. No.	Name of the germplasm lines	Source	S. No.	Name of the germplasm lines	Source
1.	AGS 163 (B)	IISR, Indore, India	51.	UPSM 719	IISR, Indore, India
2.	EC 251506	IISR, Indore, India	52.	NRC 59	IISR, Indore, India
3.	EC 251531	IISR, Indore, India	53.	UPSL 742	IISR, Indore, India
4.	EC 251516	IISR, Indore, India	54.	EC 456549	IISR, Indore, India
5.	EC 457285	IISR, Indore, India	55.	PK 1038	IISR, Indore, India
6.	EC 251383	IISR, Indore, India	56.	PK 726	IISR, Indore, India
7.	AGS 32	IISR, Indore, India	57.	SL – 443	IISR, Indore, India
8.	AMS-MB 51-18	IISR, Indore, India	58.	NRC 42	IISR, Indore, India
9.	EC 309512	IISR, Indore, India	59.	EC 39177	IISR, Indore, India
10.	AGS 99	IISR, Indore, India	60.	EC 456527	IISR, Indore, India
11.	AGS 129	IISR, Indore, India	61.	UPSL 422	IISR, Indore, India
12.	EC 251682	IISR, Indore, India	62.	EC 389392	IISR, Indore, India
13.	EC 333872	IISR, Indore, India	63.	EC 24207	IISR, Indore, India
14.	EC 251470	IISR, Indore, India	64.	EC 313915	IISR, Indore, India
15.	NRC 43	IISR, Indore, India	65.	EC 391162	IISR, Indore, India
16.	PK 1220	IISR, Indore, India	66.	EC 309538	IISR, Indore, India
17.	EC 391012	IISR, Indore, India	67.	TNAU 20037	IISR, Indore, India
18.	SQL 12	IISR, Indore, India	68.	TUNIA	IISR, Indore, India
19.	NRC 79	IISR, Indore, India	69.	UPSM 57	IISR, Indore, India
20.	UPSL 470	IISR, Indore, India	70.	RKS 21	IISR, Indore, India
21.	TNAU 20049	IISR, Indore, India	71.	TNAU 20051	IISR, Indore, India
22.	NRC 41	IISR, Indore, India	72.	UPSM 783	IISR, Indore, India
23.	AGS 116	IISR, Indore, India	73.	EC 127503	IISR, Indore, India
24.	EC 393222	IISR, Indore, India	74.	GP 496	IISR, Indore, India
25.	EC 325103	IISR, Indore, India	75.	UPSL 326	IISR, Indore, India
26.	AGS 371	IISR, Indore, India	76.	EC 389170	IISR, Indore, India
27.	AMS 19 B	IISR, Indore, India	77.	RKS 30	IISR, Indore, India
28.	EC 457214	IISR, Indore, India	78.	UPSL 340	IISR, Indore, India
29.	EC 171194	IISR, Indore, India	79.	NRC 20063	IISR, Indore, India
30.	EC 37939	IISR, Indore, India	80.	NRC 37	IISR, Indore, India
31.	EC 172578	IISR, Indore, India	81.	JS 87 – 24	IISR, Indore, India
32.	UPSL 601	IISR, Indore, India	82.	EC 241780	IISR, Indore, India
33.	EC 389156	IISR, Indore, India	83.	UPSL 788	IISR, Indore, India
34.	NRC 57	IISR, Indore, India	84.	UPSL 162	IISR, Indore, India
35.	TNAU 200-23	IISR, Indore, India	85.	RKS 52	IISR, Indore, India
36.	EC 281462	IISR, Indore, India	86.	IMP – Z	IISR, Indore, India
37.	EC 280149	IISR, Indore, India	87.	JS 20 – 41	IISR, Indore, India
38.	PK 1220	IISR, Indore, India	88.	NRC 34	IISR, Indore, India
39.	EC 456599	IISR, Indore, India	89.	UPSL 72	IISR, Indore, India
40.	NRC 2320	IISR, Indore, India	90.	UPSL 479	IISR, Indore, India
41.	UPSM 780	IISR, Indore, India	91.	NRC 2007-1-3	IISR, Indore, India
42.	EC 393222	IISR, Indore, India	92.	Cat 2126(A)	IISR, Indore, India
43.	JS 75-30	IISR, Indore, India	93.	EC 48571	IISR, Indore, India
44.	Sehore 1	IISR, Indore, India.	94.	UPSL 786	IISR, Indore, India
45.	NRC 80-1	IISR, Indore, India	95.	RVS 200622	IISR, Indore, India
46.	RVS 2006-4	IISR, Indore, India	96.	GP 525	IISR, Indore, India
47.	JS 20 – 38	IISR, Indore, India	97.	UPSL 415	IISR, Indore, India
48.	EC 37183	IISR, Indore, India	98.	UPSM 695	IISR, Indore, India
49.	PS 1336	IISR, Indore, India	99.	UPSM 662	IISR, Indore, India
50.	TNAU 5-55	IISR, Indore, India	100.	JS 97 – 52	IISR, Indore, India

Table 2. Analysis of variance of 12 different characters in soybean

Sources of variation	d. f.	Mean sums of squares											
		Days to 50% flowering	Days to maturity	Plant height	Nodes/ plant	Clusters/ Plant	Pods/ cluster	Pods/ plants	Seeds/ pods	Seed yield/ plant	Plot yield	100 seed weight	Oil content
Blocks	4	10.83	11.47	4.57	0.086	0.63	0.051	67.529	0.004	1.783	145.69	0.219	1.669
Entries	104	31.84**	17.36	311.86**	7.22**	197.99**	0.391**	1671.646**	0.084**	43.482**	7264.10**	7.071**	9.795**
Checks	4	4.24	4.65	109.06**	1.46	81.98**	0.599**	1665.459**	0.168**	96.479**	12151.4**	3.113**	18.473**
Varieties	99	27.78**	16.15	302.57**	7.33 **	204.01**	0.383**	1685.246**	0.080 **	40.294**	7099.53**	6.852**	9.504**
Checks vs. Varieties	1	544.63**	188.84**	2042.78**	18.69**	66.59 **	0.358**	349.983*	0.213**	147.131**	4007.90**	44.605**	3.851*
Error	16	5.386	14.56	18.03	1.22	1.61	0.033	48.669	0.019	1.22	143.03	0.546	0.763

** Significant at 1% level of probability, * significant at 5% level of probability

Table 3. Distribution of 100 soybean genotypes into different clusters

Cluster Group	Number of genotypes	Genotypes
I	53	EC 39177, TUNIA, TNAU 20037, NRC 2007-1-3, NRC 34, RKS 21, EC 457214, AMS 19 B, AGS 371, EC 313915, UPSL 479, EC 325103, EC 309538, EC 456527, UPSL 72, RVS 2006-22, UPSM57, Sehore-1, Cat 2126 (A), UPSL 786, NRC 59, UPSM 783, TNAU 20051, NRC 2320, EC 456599, EC 457285, GP 525, UPSL 422, UPSL 470, EC 172578, UPSM 780, EC 127503, EC 280149, EC 391162, JS 97 - 52, AMS-MB 51-18, NRC 43, EC 251531, UPSM 695, EC 389392, JS 20 - 38, EC 391316, SQL 12, EC 37183, EC 389170, EC 48571, EC 251470, UPSL 340, EC 456549, UPSL 742, PK 1038, EC 383165, and RKS 30
II	37	EC 333872, NRC 80-1, AGS 163 (B), EC 251516, EC 251383, TNAU 20049, NRC 41, JS 20 - 41, EC 391012, UPSM 662, NRC 57, PK 1220, UPSL 326, TNAU 200-23, RKS 52, IMP - Z, EC 457185, NRC 79, EC 37939, NRC 42, EC 251682, AGS 99, UPSL 415, RVS 2006-4, UPSL 601, PK 726, AGS 32, TNAU -5 - 55, EC 251506, GP 496, JS 87 - 24, UPSM 719, NRC 37, NRC 20063, UPSL162, JS 75-30, and MACS-303
III	6	EC 171194, EC 281462, EC 309512, AGS 116, EC 389156, and EC 241780
IV	1	UPSL 788
V	1	SL – 443
VI	2	EC 393222 and PS 1336

Table 4. Average intra and inter cluster distances

	Cluster distances					
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	3572.42	19722.19	59071.16	11646.80	49477.39	117618.10
Cluster II	19722.19	5022.21	18258.69	14693.58	15704.54	50409.88
Cluster III	59071.16	18258.69	5899.82	51156.72	21371.51	22933.82
Cluster IV	11646.80	14693.58	51156.72	0.00	22354.67	85009.17
Cluster V	49477.39	15704.54	21371.51	22354.67	0.00	23318.18
Cluster VI	117618.10	50409.88	22933.82	85009.17	23318.18	9830.83

Table 5. Cluster mean value of 12 different characters of soybean genotypes

	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of nodes/ plant	Number of clusters/ plant	Number of pods/ cluster	Number of pods/ plant	Number of seeds/ pod	Seed yield (g)/ plant	Plot yield (g)	100 seed weight (g)	Oil content (%)
Cluster I	47.76	107.31	51.46	11.78	14.76	2.87	46.08	1.75	7.38	79.23	8.88	13.30
Cluster II	46.05	107.51	62.58	12.37	29.99	3.12	92.19	2.03	14.65	193.15	10.32	13.97
Cluster III	48.67	106.50	63.20	12.17	27.50	3.12	89.07	2.28	18.94	308.49	12.71	12.43
Cluster IV	52.00	113.00	77.60	14.20	35.20	2.45	137.80	2.10	16.14	94.15	6.30	9.20
Cluster V	53.00	107.00	70.40	16.80	50.80	3.90	198.50	2.50	23.29	229.29	9.83	10.80
Cluster VI	52.50	109.50	77.90	18.00	55.60	3.55	201.30	2.80	32.19	372.84	14.12	18.70
Contribution of individual characters towards total genetic divergence												
Number of times appearing first in ranking	107	53	299	160	213	320	715	320	640	1333	640	533
Percentage contribution toward total divergence	2.0	1.0	5.6	3.0	4.0	6.0	13.4	6.0	12.0	25.0	12.0	10.0

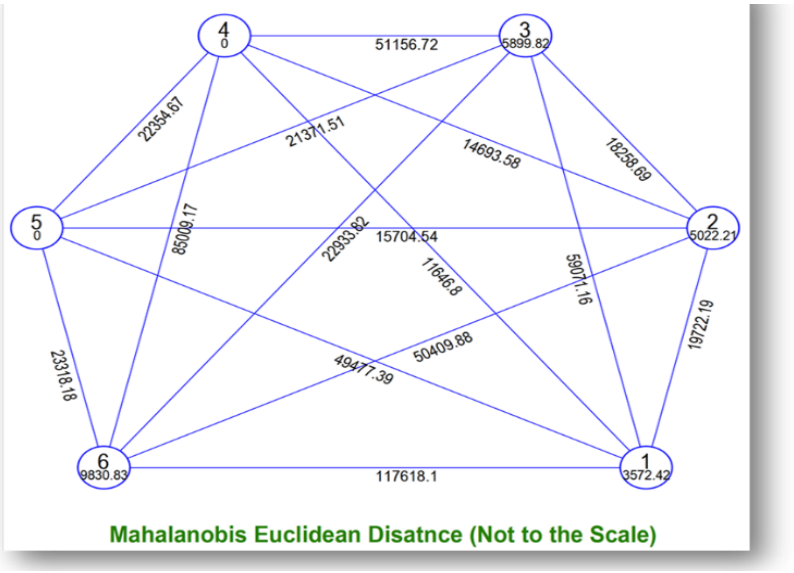

Fig. 1. Cluster diagram with inter and intra cluster distances

to maturity. Plot yield (25.0 %) contributed the maximum to total genotype divergence, followed by the number of pods/plant (13.4%), seed yield/plant and 100-seed weight (12.0 %) as shown in **Table 5**. The largest contribution of yield and pods/plant noticed in this investigation is consistent with the earlier findings of Shadakshari *et al.* (2011) and Promin *et al.* (2014). Days to maturity and days to 50% flowering were the least contributors for the divergence. On the contrary, Barh *et al.* (2014) and Kumar *et al.* (2018) reported that flowering and maturity traits are the essential factors for genetic divergence. It is possible that the differences in the reported results are attributable to the genotypic variations.

From the numerous genetic diversity studies conducted in soybean, it is understood that the cluster distance and characters that contributed the maximum to divergence should be given priority, while choosing parents for hybridization to achieve a high level of heterosis. Accordingly, the genotype, UPSM 719 was identified for plant height and clusters/plant and the genotype EC 393222 for days to 50% flowering, the number of pods per plant, the number of seeds per pod, seed yield per plant, oil content. The genotypes Cat 2126 (A), EC 251531, PS-1336, and EC 171194 were identified for for the number of nodes/plant, the number of pods/cluster, plot yield and 100 seed weight, respectively (**Table 6**).

Table 6. Promising genotypes for different characters

S. No.	Name of genotypes	Character(s) to be considered
1.	UPSM 719	Plant height, number of pod clusters/plant
2.	EC 393222	Days to 50% flowering, number of pods/plant, number of seeds/pod, seed yield/ plant, oil content.
3.	EC 37183, IMP-Z, EC 127503	Days to maturity.
4.	Cat 2126 (A)	Number of nodes/plant.
5.	EC 251531	Number of pods/cluster.
6.	PS-1336	Plot yield
7.	EC 171194	100 seed weight (g).

Based on inter-cluster distance, cluster mean values and mean per se performance, it is recommended that for future hybridization programs to develop high yielding soybean genotypes, the crossings should be attempted between genotypes of cluster I and clusters VI, I and III, III and IV, II and VI, respectively.

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