

E-ISSN: 2278-4136 P-ISSN: 2349-8234 JPP 2019; 8(4): 1324-1329 Received: 07-05-2019 Accepted: 09-06-2019

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Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



Analysis of genetic diversity of 120 genotypes of *Glycine max* (L.) merrill by using D² analysis

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Abstract

Soybean is an essential legume crop all over the world where it serves to meet the increasing demands for protein, edible oil and calories. In this study, we analysed genetic diversity of 120 soybean germplasm lines by using D² analysis. An experiment was conducted in randomized augmented block design at ICAR-Indian Agricultural Research Institute (IARI), during kharif-2016-17. The analysis of variance showed significant differences for seven morphological traits viz., days to 50% flowering, days to maturity, plant height (cm), primary branches per plant, pods/plant, 100- seed weight (g) and seed yield / row (g). Narrow differences between phenotypic and genotypic coefficients of variation (PCV and GCV) for all the characters coupled with high heritability were found for characters such as primary branches / plants, Pods/plant and seed yield /row revealed that additive gene action is predominant. The genetic advance in percentage of the mean was higher for all the characters under study except primary branches/plant and 100 seed weight (g). The genetic divergence assessed using D² statistics for characters enabled grouping of all the genotypes in eleven distinct clusters. Maximum (17) genotypes were grouped in cluster X and minimum (3) in clusters VI. The inter cluster distance in matrix ranged between 2.98 to 10.45.The maximum intra-cluster distance was recorded in cluster V, followed by cluster II while, minimum intra-cluster distance was recorded for cluster IX followed by cluster X. The maximum intercluster distance was found in the cluster VII (60.78) followed by the cluster IX (47.97), cluster VI (47.05), cluster VIII (43.98), cluster V (41.42 and 39.24) followed by the cluster X (39.70) respectively. The minimum inter - cluster distance was being observed between cluster IX (8.91) and cluster X (7.02) .The highest cluster mean for seed yield / row in (g) was recorded in case of cluster V followed by cluster XI whereas minimum cluster mean was recorded in cluster III.

Keywords: Soybean [Glycine *max*. L Merrill] genetic variability, genetic divergence, D² analysis, cluster mean analysis and Inter and intra-cluster matrix distance

1. Introduction

Soybean (Glycine max (L.) Merrill) is a member of Papilionaceae family having genus Glycine. The genus Glycine is divided into two sub-genera, Glycine max and G. soja. The cultivated soybean is progenitor of *Glycine soja* Sub. and Zucc (2n = 40). This crop is apply called as "Golden Bean" or "Miracle crop" of the 20th century. The soybean seed is composed of protein (40%), carbohydrates (20-30%), oil (20%), minerals -fibre (3-6%) and 5% ash (Liu et al. 1997; and Carrera et al. 2011)^[10, 3]. Soybean builds up the soil fertility by fixing large amount of atmospheric nitrogen through the root nodules, and also through leaf fall on the ground at maturity. It has a fairly wide range of adaptation involving a wide array of climatic, soil and growth conditions and known for its highly valued protein and oil owing to its use in food, feed and industrial applications (Kumar et al., 2015)^[9]. Soybean is known for 200 different uses including medicinal use. It contains oligo- saccharides, raffinose and stachyose which are significantly important for health by promoting growth of bifido-bacteria present in intestine. This bifido-bacterium play vital role in reduction of colon cancer (Birt et al., 2004) ^[1]. Currently world soybean production is 347.6 MT with an area coverage of 125.4 M ha. In India, it occupies 10.35 m ha with an average productivity of 905 Kg/h and total production of 8.35 MT (USDA: 2017/18). Madhya Pradesh, Rajasthan, Karnataka, Uttar Pradesh and Andhra Pradesh are important soybean growing states of India.

Collection of germplasm and assessment of genetic variability is a basic step in the entire crop improvement programme as this provides wider scope for selection. Knowledge of diversity patterns will allow breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion and to develop strategies to incorporate useful diversity in their breeding programs (Naik *et al.*, 2016)^[14]. Several methods including Morphological and agronomic traits have been used to investigate the genetic variation in soybean (Perry and McIntosh, 1991; Sneller *et al.*, 1997)^[16, 21]. For yield improvement, it is essential to have knowledge on the variability of different characters

namely days to 50% flowering, days to maturity, plant height (cm), number of branches/plant, number of pods/plant, number of seeds/plant, 100-seed weight (g), seed yield / row (g). Morphological traits can be used to assess phenotypic variation in growing environments and are also used as tools for the indirect analysis of genetic variability and diversity (Kaur *et al.*, 2016) ^[8]. The main objective of this study was to characterize soybean genotypes using morphological traits in order to evaluate the genetic variability and genetic diversity relationships among selected genotypes.

2. Materials and Methods

2.1 Experimental site: The present study was conducted in the fields of ICAR-Indian Agricultural Research Institute

(IARI), during year *kharif*-2016-17. One hundred twenty germplasm lines of soybean were procured from Division of Genetics IARI, New Delhi. The details of the material used are given in Table 1. The experiment was conducted in augmented block design. The genotypes Pusa-9712, Bragg, PK-1347, SL-688 and PS-1092 were used as check .The average rainfall during the cropping season was 888.0 mm with average maximum day temperature was 35° C during *Kharif* season. All the germplasm lines were planted with a spacing of 45 cm row to row and 5 cm plant to plant distance. Fertilizers were applied (20 kg N/ha, 60 kg P₂O₅/ha 40 kg K₂O/ha and 5 kg S/ha) at the time of sowing. All the recommended agronomical practices and plant protection measures were adopted to raise a healthy crop.

Table 1: List of one hundred twenty genotypes used for morphological characterization.

SN	Genotypes	SN	Genotypes	SN	Genotypes	SN	Genotypes	
1	DS-76-1-35-4	31	EC-457398	61	DS-2614	91	PK-1240	
2	DS-76-1-2-1	32	EC-457274	62	DS-9812	92	PK-1060	
3	DS-9820	33	EC-458383	63	DS-9813	93	PK-1080	
4	EC-389179	34	EC-457415	64	DS-9816	94	PK-1135	
5	EC-472139	35	DS-9909	65	DS-9817	95	PK-1180	
6	EC-472141	36	MAUS-164	66	DS-9819	96	PK-1243	
7	EC-472145	37	VLS-74	67	DS-9820	97	PK-1169	
8	EC-472150	38	RKS-115	68	DS-9822	98	PK-1503	
9	EC-472154	39	RKS-9	69	DS-9814	99	AMS-56	
10	EC-472161	40	SL-710	70	DS-9720	100	SH-46	
11	EC-472173	41	UGM-20075	71	JS-20-5	101	SL-979	
12	EC-472184	42	NRC-53	72	DSB-19	102	EC-472184	
13	EC-472197	43	PK-1024	73	EC-4830578	103	VLS-61	
14	EC-472199	44	PUSA-16	74	EC-483559	104	LEE	
15	E-20	45	HIMSO-1574	75	HIMSO-1563	105	SL-958	
16	EC-472210	46	TS-148-22	76	HIMSO-1587	106	PS-1506	
17	EC-472221	47	MACS-231	77	HIMSO-1588	107	VLS-74	
18	EC-472214	48	MACS-869	78	HIS-1	108	AMS-353	
19	EC-472220	49	JS-20-19	79	JS-94-67	109	PS-1572	
20	EC-472222	50	TS-3	80	KB-222	110	SS-222	
21	MACS-1336	51	VLS-57	81	TGX1835-3E	111	DSB-9	
22	EC-472229	52	SL-688	82	MACS-450	112	DSB-10	
23	EC-472242	53	DS-2001	83	MAUS-666	113	UPSL-298	
24	EC-471720	54	DS-2003	84	SL-64-A	114	AMS-56	
25	EC-472173	55	DS-2004	85	MAUS-81	115	PHATERKHANI-2	
26	EC-456574	56	DS-2005	86	MACS-869	116	PK-1347	
27	EC-456597	57	DS-2006	87	NRC-53	117	DS-9712	
28	EC-456615	58	DS-2007	88	NRC-83	118	SL-688	
29	EC-45714	59	DS-12-13	89	NRC-89	119	PS-1092	
30	EC-457189	60	DS-2708	90	PS-1466	120	Bragg	

2.2 Observation recorded

At maturity ten competitive plants were randomly selected from each genotype to record observation, except for days to 50 per cent flowering and days to maturity. The recorded observations / data on different characters are as follow: days to 50% flowering, days to maturity, plant height (cm), primary branches per plant, Pods/plant, 100- seed weight (g) and seed yield / row (g). The recorded data were subjected to analysis of variance. Genetic divergence analysis was done by using Mahalanobis D^2 – statistics (Mahalanobis 1936) ^[12]. Clustering of genotypes was done according to Torcher's method (Rao, 1952) ^[17]. Intra and inter-cluster distances generated were used to describe the relationship among the genotypes, formula proposed by (Singh and Chaudhary, 1977) ^[19]. The dissimilarity coefficient between the genotypes was arranged into reasonable hierarchal system and estimated by using D^2 value as suggested by (Sneath and Sokal, 1973)^[20].

2.3 Statistical Package

Recorded data for seven morphological traits were subjected to Analysis of variance by the software Windostat version 9.3 of Indostat services

3. Results and Discussion

3.1 Variability and genetic parameters: The Analysis of Variance showed that the existence of remarkable amount of genetic variability among the soybean germplasm line and advance breeding lines for yield and yield related parameters under study .The mean and range for all the seven morphological characters are presented in Table 2 and 3.

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Table 2: Estimation of Range, Mean, GCV, PCV, GA and h 2 for 7 morphological characters

Characters	GCV	PCV	h2 (broad sense)	GA
Days to Flowering	15.84	15.96	98%	20.75
Days to Maturity	7.81	8.0	94%	21.96
Plant Height cm	14.77	14.89	98%	28.17
Primary Branches/Pl	27.7	27.12	100%	3.8
Pods/ Plant	27.16	27.78	99%	27.27
100-seed -wt.(g)	11.86	12.01	97%	3.3
Seed Yield/Row(g)	59.23	59.33	99%	18.7

Table 3: Descriptive statistics for quantitative characters in soybean germplasm

Characters	Min	Max	Range	Mean	SE (d)	CV%
Days to 50% flowering	34.16	65.16	31.0	49.98	3.53	7.0
Days to maturity	93.7	125.3	31.6	109.5	11.0	10.0
Plant Height	50.13	101.53	51.4	72.80	11.7	16.0
Branches/plant	2.0	11.0	9.0	5.29	0.85	16.0
No. of pods per plant	14.41	95.99	81.6	38.52	1.41	3.66
100-seed weight	6.72	13.84	7.11	10.77	1.36	12.62
Seed yield/ row	14.96	514.98	501.95	117.17	43.2	26.86

3.2 Descriptive statistics for morphological traits

Days to 50% flowering ranged between 34.16 (SL-958) to 65.16 (EC-389179) days with a general mean value of 49.98 with coefficient of variance 7.0%. The estimates of PCV (15.96%) and GCV (15.84%) were moderate. The heritability (98%) was high with a genetic advance of 20.75. Days to maturity has been observed in the range of 93.7 (SS-222) to 125.3 days (DS-76-1-2-1) with a general mean value of 109.5 with a coefficient of variance 10%. The estimates of PCV (8%) and GCV (7.81%) were low. The heritability (94%) was high with a genetic advance of 21.96. Plant height (PH) ranged between 50.13 cm (EC-472173) to 101.53 cm (EC-456597) with an average mean of 72.80 cm with a phenotypic coefficient of variance (16%), GCV (14.77%) and PCV (14.89%) was moderate. The magnitude of heritability estimated was high (98.0%) with high genetic advance value of 28.17. Primary branches / plant ranged between 2 (PS-1503) to 11 (SL-688) with a mean value of 5.29 and coefficient of variance 16%. The magnitude of heritability was recorded high (100%) with high PCV (27.7%) and GCV (27.12%) along with low genetic advance 3.8.Pods/Plants ranged between 14.41 (EC-472222) to 95.99 (DS-2004) with a general mean value of 38.52 having the phenotypic coefficient of variance 27.78%. The estimates of higher PCV (27.78%), GCV (27.16%) was recorded along with high heritability (99%) was low with high genetic advance of 27.27.100-seed weight: ranged between 6.72 (DS-12-13) g to 13.84 (SL-46) with a general mean value of 10.77 having coefficient of variance 12.62%. The estimates of PCV

(12.01%) and GCV (11.86%) were moderate. The heritability (97%) was high with a low genetic advance of 3.3. Seed yield/ row: ranged between 14.96 g (EC-471720) to 514.98 g (PS-1092) with a general mean value of 117.17 having coefficient of variance 26.83%. The estimates of higher PCV (59.33%), GCV (59.23%) was recorded along with high heritability (99%) respectively. From the obtained results PCV was found higher than the GCV, showed narrow differences between PCV and GCV for all the traits indicating less influence of environmental factors on the expression of these traits and the chance of high selection gain. This indicates the characters control by additive gene action would respond very well to continuous selection. These results were in conformity with the reports of (Panse 1957; Bhairav *et al.* 2006 and Jagdish *et al.* 2000)^[2].

3.3 Cluster Analysis

Using all the seven morphological traits, accessions were grouped into 11 distinct clusters on the basis of nonhierarchical Euclidean cluster analysis. D^2 analysis was carried out using all the 7 characters and generalized distance (D^2) was calculated for each pair of genotypes among 60000 possible combinations. It was also found that, among the 11 clusters, cluster X was the largest and consisted of 17 genotypes followed by Cluster III (16) Cluster VII and Cluster VIII that consisted of 15 genotypes. The cluster II and IV (11genotypes), I (9), IX (8), V and XI (7) and VI possessed 3 genotypes respectively Table 4; fig 1.



Fig 1: Clustering of 120 genotypes of soybean (morphological)

Table 4: Clustering pattern of 120 genotypes on the basis of non-hierarchical Euclidean Cluster analysis for 7 morphological characters

Cluster Number	Number	Genotypes
Ι	9	EC-472150, PUSA-16, MACS-231, DS-2708, MAUS-666, SL-979, EC-472184, VLS- 61 and PUSA-9712.
II	11	EC-472145, EC-472220, EC-472222, EC-472173, DS-9812, DS-9813, DS-9814, MACS-450 & MAUS-81.
ш	16	DS-76-1-2-1, EC-472173, EC-472210, EC-472229, EC-457189, EC-457415, VLS-57, DS-12-13, AMS-56, PK-
111	10	1240, HIMSO-1588, HIS-1, HIMSO-1587, KB-222, TGX 1835-3E and DS-9822
IV	11	EC-457398, EC-458383, DS-9909, MAUS-164, RKS-9 VLS-74, SL-710, TS-3, PK-1060, PK-1503 and PS-1572
V	7	RKS-115, UGM-20075, NRC-53, MACS-869, PK-1080, PK-1243 & VLS-74
VI	3	HIMSO-1574, PK-1092 and BRAGG
VII	15	EC-472197, TS-148-22, DS-2003, DS-2004, DS-2005, DS-9816, NRC-53, PK-1466, PK-1169, LEE, SL-958,
V 11	15	DSB-9, UPSL-298, PK-1347 and SL-688.
VIII	15	EC-389179, EC-472141, EC-472199, E-20, EC-472221, EC-472214, MACS-1336, EC-472242, EC-456597, EC-
V 111	15	4566, JS-20-5, EC-4830578, EC-483559, HIMSO-1563 and JS-94-67.
IX	8	EC-389139, EC-472161, EC-457274, SL-688, SL-64-A, NRC-83, PK-1135, DSB-10
V	17	DS-76-1-35-4, DS-9820, EC-472184, EC-456574, EC-45714, DS-2001, DS-2006, DS-2007, DS-2614, DS-9817,
Λ	1/	DS-9819, DS-9802, DS-9720, DSB-19, NRC-89, AMS-56 and EC-472161
XI	7	EC-472154, PK-1023, PK-1180, PK-1506, AMS-353, SS-222, PATARKHANI-2

3.4 Cluster means

The genotypes were classified into homogenous groups by the non-hierarchical k-means technique of cluster analysis. The cluster means and general mean values for 7 characters are presented in Table 5. Cluster VI took maximum days to 50% flowering (59.66) followed by the clusters VIII (59.58), cluster 3^{rd} (55), I (54.0), while it was minimum in cluster X (37.4) with a general mean value of 49.92. The genotypes of cluster III showed highest mean for days to maturity (119.36)

followed by clusters VI (118.55) and cluster V (115) with general mean value of 109.51.The highest cluster means for Plant height was observed in cluster V (89.33) followed by cluster III (85.54) and cluster VI (82.22). While minimum cluster mean was found in cluster XI (53) with a general mean value of 72.86. Highest cluster mean for primary branches/plants was found in cluster V (9) followed by cluster IV (8.28) while lowest cluster mean was recorded into cluster VI (4.11) with a general mean value of 5.29.The highest

cluster mean for pods/plant was recorded in cluster V (70.17) followed by cluster II (63) with a genera mean value of 38.51, while lowest mean for pods/plant was recorded in cluster VII (27.7). Highest cluster mean for 100-seed wt. (g) was recorded in cluster V (12.75) followed by cluster X (12.41) and cluster XI (12.045) with a general mean value of 10.765, while lowest cluster mean was recorded in cluster VII

(6.595).The highest cluster mean for seed yield / row in (g) was recorded in case of cluster V (499.0g) followed by cluster XI (338.525g), with general mean value of 128.52, that indicating lines covering in these clusters showing very high seed yield/ row (g).The minimum cluster mean was recorded in cluster III (81.73**).

Table 5: Cluster means for different chara	cters of soybean
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Cluster Means										
S. No.	Days to Flowering	Days to Maturity	Plant Height	Primary Branches/ Plant	Pods/ Plant	100-seed weight (g)	Seed Yield/ Row(g)			
1 Cluster	54.059	114.412	60.176	5.000	40.588	11.033	114.641			
2 Cluster	48.667	111.333	78.778	4.778	63.000	10.217	149.689			
3 Cluster	55.000	119.368*	85.211	5.053	36.526	11.405	81.737**			
4 Cluster	46.214	111.571	74.643	8.286	35.714	11.551	107.786			
5 Cluster	51.000	115.500	89.335*	9.000*	70.170*	12.750*	499.500*			
6 Cluster	59.667*	118.556	82.222	4.111**	32.667	8.451	159.444			
7 Cluster	39.333	108.333	69.333	4.667	27.667**	6.959**	125.267			
8 Cluster	59.583	101.250	75.333	6.083	33.500	9.562	117.500			
9 Cluster	44.083	100.458**	64.208	4.167	35.667	10.903	99.875			
10 Cluster	37.714**	100.714	79.571	4.286	32.286	12.416	156.429			
11 Cluster	39.000	102.500	53.000**	5.750	42.500	12.045	338.525			
Mean	49.992	109.508	72.806	5.292	38.519	10.765	128.533			
TMSS	563.885	668.000	1112.420	21.745	915.029	16.336	54181.27			
EMSS	32.754	39.633	56.647	1.260	87.905	0.718	4279.88			
F Ratio	17.216	16.855	19.638	17.257	10.409	22.741	12.660			

3.5 Inter and intra-cluster matrix distance

The intra- and inter-cluster-matrix distances values between 11 clusters are presented in Table 6; figure 2. The maximum intra-cluster distance was recorded in cluster V (10.451) followed by cluster II (9.41), cluster V (8.458), cluster XI (7.91), cluster VI (7.49) and cluster VIII (7.09). Genotypes belonging to theses clusters are more divergent and hybridization between divergent clusters is likely to produce wide variability with desirable segregants (Maloo and Bhattacharjee, 1999)^[13]. Minimum intra-cluster distance was recorded for cluster IX (4.22) followed by cluster X (2.98). The maximum inter-cluster distance was found in the cluster VII (60.78) followed by the cluster IX (47.97), cluster VI

(47.058), cluster VIII (43.98), cluster V (41.42) and cluster X (39.708) respectively. The genotypes grouped in these clusters indicated them to be of diverse nature.

The least inter - cluster distance was observed between cluster IX (8.91) and cluster X (7.029) which indicating that the genotypes possess in these clusters were genetically very close to each other and less divergent . Similar results were also reported in soybean and other crops by Cui *et al.* 2001^[4]; Yu *et al.* 2005^[23]; and Latif *et al.* 2011^[11]. In 1994 Jay Lal also grouped forty genotypes of soybean into nine clusters on the basis of chlorophyll components and seven clusters with respect to yield attributes.



Fig 2: Matrix of distance between inter and intra –cluster distance ~ 1328 ~

Table 6: Matrix of distances between the intra and inter cluster distance for 11 clusters.

Clusters	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster	7 Cluster	8 Cluster	9 Cluster	10 Cluster	11 Cluster
Ι	6.296	13.687	10.09	13.122	41.416	14.408	18.304	11.843	8.910	14.065	17.383
II		9.416	13.642	18.249	31.73	17.262	21.532	16.592	14.614	16.696	22.618
III			4.724	12.188	39.241	11.316	21.247	12.223	13.134	12.831	27.104
IV				8.458	35.017	21.467	22.351	14.263	13.998	13.667	20.534
V					10.451	47.058	60.775	43.985	47.971	39.708	30.063
VI						7.485	14.717	12.269	17.698	22.074	32.138
VII							6.834	15.852	13.669	19.899	27.350
VIII								7.096	11.421	16.118	24.498
IX									4.221	7.029	14.737
Х										2.977	14.521
XI											7.918

*Bold figures represent intra-cluster distance

4. Conclusion

From this study it is evident that the wide range of variability for different traits coupled with high heritability and high genetic advance for important yield traits was detected hence selection will be effective for these traits in the population derived from cross between contrasting parents selected from the germplasm lines studied.

5. Acknowledgment

The financial support obtained from the Post Graduate School, Indian Agricultural Research Institute (IARI) to carry out the research work is fully acknowledged.

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