

**GENETIC DIVERGENCE ANALYSIS IN INDIGENOUSLY DEVELOPED
INDIAN SOYBEAN (*Glycine max* L. Merrill) GERMPLASM**

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Knowledge of the naturally occurring diversity helps to identify diverse groups of soybean genotypes that can be useful for the breeding program. Therefore, this study aims to identify traits that influence the soybean genotypes in cluster formation using D² analysis. Hundred genotypes of soybean were studied for 15 characters in randomized block design with three replications and grouped into fifteen clusters. The cluster I was the largest with 55 genotypes followed by cluster III containing 17 genotypes and cluster IV containing 16 genotypes. The remaining clusters were solitary with single genotype each. The inter-cluster distance was the highest between clusters XIII and XIV (D=38.28) followed by clusters X and XII (D=33.64), XIII and XIV (D=32.71), III and XIV (D=32.06) and XII and XIV (D=31.65). Genotypes falling in these clusters may serve as potential parents for a hybridization programme. Pods per plant contributed the highest in manifestation of total genetic diversity. The presence of clear phenotypic and genotypic differences in the characters under consideration between or among clusters gives us an opportunity to bring about improvement through hybridization of genotypes between these clusters and subsequent selection in the segregating generations. The genotype JS (SH) 131 of cluster XIV, J 606 of cluster X, JS 46-75 of cluster V and Himso 1548 of cluster XV were identified as genetically diverse parents, which can be utilized for future crop improvement programme.

Key words: D² statistics, genetic diversity, soybean germplasm

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INTRODUCTION

Soybean (*Glycine max* L. Merrill) is one of the world's leading sources of vegetable oil and plant protein, both of which are very well adapted to the nourishment of human beings. It contains about 37-42% good quality protein and about 17-24% oil (ZAFAR *et al.*, 2010). Soybean tops in the world production of both oil seeds and edible oil. It is cultivated at broadly diverse geographical locations and under many different growing conditions. In India, it is grown in an area of 10.8 million hectares and accounting production of 11.5 million tones with productivity of 1065 kg/ha (FAO, 2012). Its capacity for protein and oil production makes it a significant contributor to human nutrition. Its characteristic symbiosis with root bacteroids makes it a very important crop in research. The observations of last 25 years with respect to production trend revealed that the soybean yield per hectare has made satisfactory increase in the United States, but not so happened in India. This is ascribed to narrow genetic base of soybean cultivars resulting in susceptibility to biotic (diseases and insects) and abiotic (unfavorable soils and erratic climatic conditions) stresses and finally in yield stagnation. This narrow genetic base with more than 50% of popular Indian soybean varieties may be the concern of trace back to only few ancestral lines. Several breeding devices including molecular marker have been suggested for broadening genetic base of soybean cultivar (LAL and RANA, 2000).

Soybean is well suited to semi-arid regions of the country. Indeed, it is one of the few crops that can produce sustainable yield in relatively harsh environments. In the crop like soybean, assessment of genetic diversity based on phenotypic traits is enhanced with the use of DNA markers in the last decade. Several workers studied genetic divergence among soybean genotypes for agronomic traits (SIHAG *et al.*, 2004; SHARMA, *et al.*, 2005; KAYANDE and PATIL, 2009; PAWAR *et al.*, 2013) and for molecular markers (BONATO *et al.*, 2006; FU *et al.*, 2007; WANG *et al.*, 2008; KURODA *et al.*, 2009). Due to the rapid developments in the field of molecular genetics, a variety of different techniques have emerged to analyze genetic variation, which provides many advantages that make it especially attractive in studies of diversity and relationships, such as independence from environmental and pleiotropic effects, a potentially unlimited number of available markers and selectively neutral nature of many molecular markers (SPOONER *et al.*, 2005). These advantages do not imply that other more traditional data used to characterize biodiversity are not valuable. On the contrary, morphological, ecological and other traditional data will continue to provide practical and often critical information needed to characterize genetic resources. In belief of SHRIVASTAVA *et al.* (2011), there is a possibility of increasing soybean yield potential upto 27% through developing four seeded plant ideotype.

For any crop improvement programme, analysis of genetic diversity is the first and foremost step. Information on genetic diversity among genotypes has several important applications for crop improvement. This information can be useful to classify germplasm for identification of cultivars, assist in selection of parents for hybridization and reduce number of genotypes needed to ensure sampling of a broad range of genetic variability. Genetically diversified parent is a pre-requisite to improve the chances of selecting better segregants for various characters. When such parents utilized in cross breeding programme, they are likely to produce high heterotic effect and wide spectrum of variability (BARH *et al.*, 2014). The challenge is to select which genotypes to be used in breeding programmes from available germplasm, those carrying favourable rare alleles absent in elite germplasm.

The information generated through divergence studies in past are vary due to influence of populations used and the environments in which they were evaluated. Inference made in these

studies would be invalid to other soybean lines. Therefore, further study is needed to know genetic variation within available gene pool through divergence study and to make strategies for incorporating useful diversity or to facilitate the introgression of genes of interest into commercial varieties. Thus, present study is especially important for semi-arid region of India, where moisture stress in rainy season is frequent during crop growth period. This study reports genetic divergence for agronomic traits in soybean genotypes collected from various research centres across the country along with some exotic accessions.

MATERIALS AND METHODS

The experimental material comprised of 100 genotypes of soybean. Genotypes used in the study were indigenous germplasm lines from different parts of India and some exotic cultures. The study was conducted on medium black soil during *khari*f 2011 in randomized block design with three replications at the Instructional Farm, Junagadh Agricultural University, Junagadh. Each entry was accommodated to single row plot of 4.0 m length with a spacing of 45 x 10 cm. Crop was fertilized with 30 kg N₂ and 30 kg P₂O₅ ha⁻¹. All other recommended cultural practices were followed for harvesting good crop. The data were recorded on five randomly selected plants from each plot for 15 characters, *viz.*, days to 50% flowering, days to maturity, plant height(cm), primary branches per plant, cluster per plant, pods per plant, pods per cluster, pod length(cm), seeds per pod, 100-seed weight(g), biological yield per plant(g), harvest index(%), protein content(%), oil content(%) and seed yield per plant(g). Protein content was determined according to Lowery method (SHAH *et al.*, 2010) and oil content by NMR technique. The mean values were subjected to statistical analysis using SAS/STAT software (SAS INSTITUTE, 2008). Multivariate analysis of variance and corresponding divergence analysis were applied to the data. Mahalanobis' generalized distance and Tocher's algorithm (BARBOSU *et al.*, 2005) was used in the analysis with the assumption that genotypes within the cluster have smaller D²-values among themselves than those from groups belonging to different clusters.

RESULTS AND DISCUSSION

Improvement of yield, oil and protein content in soybean is attributed to increased use of genetically diverse parents. However, in case of Indian soybean varieties, a narrow genetic base has been observed. This is probably due to continuous use of genetically less divergent few elite lines in crossing programme for evolving new varieties (LAL and RANA, 2000).

In present study, the 100 genotypes were grouped into 15 clusters based on D² values (Table 1). These genotypes of different parts of India along with some exotic lines representing diverse agro-climatic conditions were distributed at random among the clusters formed on the basis of their genetic distance. The genotypes of different origin clustered together, whereas, genotypes originating from same place were scattered widely in to separate groups. For instances, 'Bragg' is the introduction from America but fall in the cluster I with released varieties developed indigenously. On the contrary, JS 75-46-R, MACS 13 and JS 335 are the released varieties for same agro-climatic conditions, fall in three different clusters showing the existence of divergence among them. Grouping of exotic culture with indigenous material indicated the absence of relationship between genetic diversity and geographical origin. KAYANDE and PATIL (2009) and BARH *et al.* (2014) reported similar results in soybean. SHARMA *et al.* (2012) stated that genetic diversity may not necessarily be related to geographic diversity. This suggests that genetic constitution of genotypes plays major role for group constellation rather than their

geographical placement. Free exchange of breeding material from one place to another (SHARMA *et al.*, 2005) or due to the unidirectional selection practiced by the breeder of different location (LAL and RANA, 2000) may be the reason for that.

Table 1 Distribution of 100 Soybean Genotypes into Different Clusters

Cluster	No. of Genotypes	Name of the genotypes
I	55	JS 79-82, G SOY-2*, JS 79-41, SH 84-16, AGS 40, IC 47384, AGS 23, AGS 143, AGS 129, AGS 171, AKSS 66, DS 154, EC 95261, EC 93601, KB 85, VLS 20, JS 81-607, Himso 1549, SL 164, AGS 30, Himso 1525, PBN 106, AGS 167, JC 41686, MACS 58, DS 61, PBN 101, JC 498621, SL 165, G SOY-1, SL 236, SL 111, NRC 8, S 2, MACS 201, PBN 102, AMTS 1, JS 79 – 9, BR 6, EC 107009, SL 75, DS 112, AKSS 153, Bragg (R), EC 93605, Paredu 34, DS 84-6, PK 1015, MACS 57, KHSb 2, UGM 34, MACS 92, MACS 410, SH 8414, RPSP 728
II	1	PK 833
III	17	DS 393 – A, JS 87 – 07, MACS 10, JN 2750, AGS 84, BR 19, MACS 239, JS 82 -106, BK 10, AGS 110, J 218, DS 84 – 6, JS 319, MACS 329, EC 107606, Himso 494, Himso 5506
IV	16	JC 43336, MACS 125, DS 23, JS 81 -106, PK 1010, KB 68, MACS 349, KDS 5, J 751, AGS 53, J 563, JB 3-3, AMS 92-1, DS 41, DS 71-12-1, SL 88
V	1	JS 75-46-R
VI	1	JC 49863
VII	1	N 23 - A
VIII	1	VLS 25
IX	1	EC 149388
X	1	J 606
XI	1	MACS 13
XII	1	PK 854
XIII	1	JS 335
XIV	1	JS (SH) 131
XV	1	Himso 1548

*Light shaded are released varieties for different agro-climatic conditions in India

Cluster I was the biggest with 55 genotypes (Table 1), most of which are indigenous lines along with some released varieties in India and exotic cultures. The second largest was the cluster III belonging 17 genotypes comprising one exotic culture along with indigenous germplasm lines. The cluster IV having 16 genotypes all of them were indigenous germplasm lines. Remaining 12 clusters were solitary clusters. Among them, cluster V, XI and XIII each contained released variety and cluster IX possessed one exotic line. The formation of distinct solitary clusters may be due to the fact that geographic barriers preventing gene flow or intensive natural selection for diverse adoptive gene complexes must have been responsible for this type of genetic diversity.

Table 2. Average Inter and Intra-Cluster Distance ($D=\sqrt{D^2}$) Values of 100 Soybean Genotypes

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
I	12.81	15.10	16.07	16.38	15.53	15.46	16.06	18.05	16.35	19.55	19.09	18.59	23.26	23.97	20.27
II		0.00	13.84	19.89	14.67	20.39	21.01	26.23	20.94	20.58	21.91	17.83	24.79	29.93	23.03
III			14.06	21.47	19.48	18.05	20.93	24.50	17.66	24.64	21.17	18.42	19.61	32.06	24.89
IV				16.74	19.70	19.30	18.44	18.77	21.38	19.84	22.58	23.79	29.85	21.24	19.30
V					0.00	19.52	21.67	19.92	20.01	19.06	22.01	18.73	27.43	23.81	23.01
VI						0.00	19.19	18.42	11.88	21.46	25.52	23.64	21.70	28.91	23.55
VII							0.00	20.81	18.35	20.04	18.52	22.70	25.25	22.06	19.37
VIII								0.00	18.25	23.65	23.64	23.33	29.38	20.07	24.37
IX									0.00	25.64	26.04	24.90	20.67	29.68	25.53
X										0.00	23.76	22.96	33.64	23.72	21.87
XI											0.00	15.07	21.77	25.45	23.58
XII												0.00	22.59	31.65	28.67
XIII													0.00	38.28	32.71
XIV														0.00	22.56
XV															0.00

The D values obtained between the 100 genotypes showed a wide range from 0.00 to 38.28 (Table 2), suggesting wide diversity in the material studied. However, gradual increase in D values over the range without any sudden jumps in the values among the genotypes may be result of their evolution with the same ancestral parents or due to evolution under near identical ecological parameters or they might have been subjected to similar natural selection. Earlier studies also reported high degree of diversity among the soybean genotypes (PAWAR *et al.*, 2013). The inter-cluster distance was maximum between clusters XIII and XIV ($D=38.28$) followed by clusters X and XII ($D=33.64$), XIII and XIV ($D=32.71$), III and XIV ($D=32.06$) and XII and XIV ($D=31.65$). The minimum inter-cluster distance was observed between clusters VI and IX ($D=11.88$). The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregates. It is true that larger the divergence between genotypes, higher would be the heterosis when hybrid programme is planned to develop yield superior varieties (BEKELE *et al.*, 2012). In this context, genotypes from cluster XIII (JS 335), cluster XIV (JS (SH) 131), cluster X (J 606) and cluster XII (PK 854) should be selected as parents in hybridization programme. The intra-

cluster distance ranged from 0.00 to 16.74. This reveals that 16 genotypes in cluster IV, 17 genotypes in cluster III and 55 genotypes in cluster I to be the most heterogeneous.

Table 3. Cluster Means for 15 Characters of 100 Soybean Genotypes

Clusters	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches / plant	Clusters/ plant	Pods/ plant	Pods/ cluster	Pod length (cm)	Seeds/pod	100 seed weight (g)	Biological yield/ plant (g)	Harvest index (%)	Protein content (%)	Oil content (%)	Seed yield/ plant (g)
I	38.56	98.73	23.16	2.90	12.66	20.61	1.63	3.03	2.26	9.50	7.46	53.73	35.75	19.69	4.05
II	37.67	98.00	23.73	3.27	14.93	23.80	1.62	2.50	2.51	9.63	8.47	55.65	35.75	20.30	4.73
III	39.88	100.06	21.80	2.98	11.84	21.15	1.79	2.58	2.23	8.32	7.20	51.74	38.76	19.65	3.71
IV	37.08	95.77	20.72	2.55	10.63	17.41	1.59	3.29	2.20	10.67	6.66	54.02	37.05	20.10	3.69
V	35.67	100.33	31.20	4.40	18.27	35.80	1.97	3.11	2.67	8.90	12.80	56.73	32.25	19.93	7.27
VI	34.67	96.33	18.67	2.87	8.33	9.13	1.10	3.07	2.21	7.08	4.73	39.82	34.50	18.86	1.87
VII	41.33	98.33	17.17	1.07	7.07	14.40	2.04	3.08	2.21	12.07	4.40	51.45	31.25	18.89	2.27
VIII	39.33	97.33	29.40	2.67	18.80	26.87	1.44	3.80	2.07	8.36	8.60	49.52	36.00	19.68	4.27
IX	40.33	98.33	19.93	2.07	9.20	20.07	2.20	3.10	2.32	6.13	4.87	53.05	33.50	18.91	2.60
X	36.00	89.67	23.53	3.67	12.47	15.33	1.22	3.33	2.75	12.94	7.73	42.03	41.75	19.34	3.27
XI	47.67	106.67	25.93	4.07	13.93	22.87	1.64	3.00	2.12	12.30	10.67	51.99	41.50	19.43	5.60
XII	40.67	100.67	31.10	4.40	21.00	28.07	1.34	2.69	2.13	10.51	12.00	41.89	40.25	19.52	5.07
XIII	43.67	112.67	19.47	3.87	12.20	18.00	1.50	2.50	2.07	6.58	5.93	38.05	38.00	18.50	2.33
XIV	37.33	103.00	22.07	1.60	11.67	17.47	1.49	4.07	2.45	13.00	8.13	62.91	34.50	20.27	5.13
XV	37.67	92.67	23.00	3.20	17.27	42.47	2.45	3.24	2.20	11.70	12.47	82.70	38.25	19.93	10.33
Mean	38.64	98.58	22.61	2.88	12.33	20.46	1.66	3.00	2.26	9.54	7.40	53.25	36.56	19.72	4.00
SEm ±	0.63	0.63	1.32	0.23	1.25	2.16	0.09	0.24	0.03	0.21	0.45	2.81	0.71	0.07	0.41
C.V.%	2.84	1.12	10.11	13.75	17.53	18.26	9.57	13.46	2.32	3.80	10.50	9.14	3.38	0.63	17.70
Percentage contribution of characters towards total divergence															
Number of first rank	163	485	6	64	31	92	66	1524	426	618	319	56	448	641	11
Per cent of contribution	3.29	9.80	0.12	1.29	0.63	1.86	1.33	30.79	8.61	12.48	6.44	1.13	9.05	12.95	0.22

A wide range of variation for several characters in single or multi-genotypic clusters was observed (Table 3). The characters contributing maximum to the divergence should be given greater emphasis to deciding the clusters for the purpose of further selection and choice of parents for hybridization. Contribution of each character towards genetic divergence was estimated based on number of times it appeared in the first rank. The results depicted that the

most important trait that contributed maximum to total genetic divergence were: pod length (30.79), oil content (12.95), 100-seed weight (12.48), days to maturity (9.80), protein content (9.05), seeds per pod (8.61) and biological yield per plant (6.44). They accounted for about 90% of total genetic divergence in the material. Looking to these results, pod length should be considered as an important trait when selecting varieties for high yield potential for the environments of this region where crop experiences frequent moisture stress during its growth period. Longer pod may have the ability to compensate for total seed mass by producing more number of seed in it under moisture stress conditions. Seeds per pod play a role in explaining greater seed number per unit area (DE BRUIN and PEDERSEN, 2009), while genetic yield compensation occurs between seeds per unit area and seed size (KAHLON *et al.*, 2011). Major contribution toward total genetic diversity by oil content (BEKELE *et al.*, 2012), 100-seed weight (PAWAR *et al.*, 2013), days to maturity (SHARMA *et al.*, 2012) and protein content (SHARMA *et al.*, 2005) have also been reported earlier in soybean.

On considering cluster means, the importance of cluster V for biological yield per plant, cluster X for days to maturity, seeds per pod and protein content and cluster XIV for pod length, 100-seed weight and oil content became obvious. The crosses involving parents belonging to most divergent clusters are expected to manifest maximum heterosis and also wide variability in genetic architecture. Thus, crosses among the genotype(s) of these clusters would exhibit high heterosis and is also likely to produce new recombinants with desired traits in soybean.

Practical implications

The clustering pattern could be utilized in selection of parents for crossing and deciding the best cross combinations which may generate the highest possible variability for various traits. The genotypes with high values of any cluster can be used either for direct adoption or for hybridization followed by selection. In the present study, pod length, an important contributing trait to genetic diversity, is larger in the genotype of cluster XIV (JS (SH) 131). This genotype was also high in 100-seed weight and oil content. The genotype in the cluster XV (Himso 1548) differed from other clusters in respect of higher seed yield per plant, pods per plant and harvest index. The genotype grouped in the cluster V (JS 46-75) was high in biological yield per plant with tall stature and profuse branches. The genotype of cluster X (J 606) was early maturing with high seed number per pod and high protein content. Therefore, genotypes of these clusters may be utilized in future breeding programme for creating wide spectrum of variability for different yield contributing characters. This will facilitate to isolate superior genotypes with higher seed yield.

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ANALIZA GENETIČKE DIVERGENTNOSTI GERMLAZME DOMAĆIH SORATA SOJE INDIJE (*Glycine max* L. Merrill)Hemant Rakhamaji ADSUL¹ i Bachubhai Arjanbhai MONPARA²¹ Odeljenje za genetika i oplemenjivanje biljaka, Junagadh, Poljoprivredni Univerzitet, Junagadh-362001, (Gujarat), India² Poljoprivredna istraživačka stanica, Junagadh Poljoprivredni fakultet, University, Keriya Road, Amreli-365601 (Gujarat), India**Izvod**

Vršena je identifikacija osobina koje utiču na grupisanje genotipova soje primenom. D² analize. Ispitivano je 15 osobina kod 100 genotipova u slučajnom blok sistemu u tri ponavljanja, grupisanih u 15 klastera. Prikazani su podaci za sve ispitivane klasterne. Genotipovi koji su se grupisali u klasterne mogu da budu korišćeni kao potencijalni roditeljski parovi u programu hibridizacije, posebno u segregirajućim generacijama. Genotipovi JS (SH) 131 klastera XIV, J 606 klastera X, JS 46-75 klastera V i Himso 1548 klastera XV su identifikovani kao genetički različiti roditelji koji mogu da se koriste u budućim programima oplemenjivanja.

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