Original Research Article

Genetic diversity of agro-morphogenic traits in soybean (Glycine max L. Merr).

Abstract

The genetic diversity of agro-morphogenic traits in 14 genotypes of soybean (Glycine max L. Merr.) were studied in an experiment using a Complete randomizes Design (CRD). Each trait's analysis of variance revealed a substantial difference between genotypes.Multivariate analysis based on thirteen characters of fourteen soybean genotypes was divided into four clusters. The maximum contribution of traits towards diversity was observed by days to 50% flowering, plant height, number of main branches per plant and leaf area index. As a result, these traits could be emphasized during selection of parents for hybridization. The highest inter cluster distance was observed between cluster II and IV and the maximum intra cluster distance was found in cluster III. Considering group distance and other agro-morphogenic performance, genotypes G1 (BADC SV1) from cluster II, G10 (Asset-95) and G7 (BS-29) from cluster VII, G4 (GMOT-43) and G12 (BADC SV2) from cluster IV found potential for future hybridization program.

Keywords: Soybean, Genetic diversity, Multivariate analysis, Cluster analysis

1. Introduction

Soybean (*Glycine max* L. Merr.) is a major grain and oil seed legume crop that has considerable potential in Bangladesh. *Glycine ussuriensis* is thought to be the progenitor of soybean (*Glycine*

max L. Merr.), which is said to have originated in China (Nagata, 1960; Vavilov, 1951). Regularly eating soy-based foods lowers cholesterol, calms hot flashes, prevents breast and prostate cancer, aids weight loss, and wards off osteoporosis. Soybean besides having high yielding potential (40-45 q/ha) also provides high quality protein (40-45%) and cholesterol free oil (18-20%) and provides around 60% of the world supply of vegetable protein and 30% of the oil (Fehr, 1989). The potential of soybean breeding is enormous, since, currently, a small fraction of the existing accessions in germplasm collections contribute to the genetic base of the present cultivars. The expansion of soybean genetic base may lead to the introduction of new favorable alleles to polygenic traits (Mulato*et al*., 2010; Guzman *et al*., 2007; Brown-Guedira*et al*., 2000).

Genetic diversity as a major factor for crop plant that determines yield security in future (Batugal, 1999). The importance of genetic diversity in the improvement of crop has been stressed on both self- and cross-pollinated crop (Gaur *et al.*, 1978; Murty and Anand, 1966; Griffing and Lindstrom, 1954). Knowledge of genetic diversity within a crop and correlation among the yield contributing characters is essential for the long-term success of a breeding program and exploration of germplasm resources. These indigenous types of soybeans contribute considerable degree of variability in respect to qualitative and quantitative characters. The quantification of genetic diversity through biometrical procedures (Anderson, 1957; Rao, 1952) has made it possible to choose genetically diverse parents for a successful hybridization program. A successful hybridization program for varietal improvement depends mainly on the selection of the parents having high genetic divergence (Upadhayay and Mehta, 2010). Moreover, evaluation of genetic diversity is important to know the source of genes for a particular trait within the available germplasm (Tomooka, 1991).

Total cropped area of soybean in Bangladesh is 5000 ha and the total production of the country stands at 4000 tons (Amin *et al.*, 2009). This amount of soybean is not enough as per demand of the increasing population of Bangladesh to meet the need of nutrition. Lack of adequate soybean production in Bangladesh is due to the lack of high yielding varieties. Enhancement of genetic diversity and enhancement of germplasm collection is crucial in future breeding program. Keeping this view in mind, for better genotype searching as well as find out a better parent for hybridization, a study was conducted to find out genetic diversity among genotypes of soybean

andcluster the genotypes according to their performance and to find out contribution of clusters to variability.

2. Materials and methods

2.1Lay out of the experiment

The experiment was laid out and evaluated in complete randomized design (CBD). The experiment was conducted in 3 replications and total 42 Pot sizewere used.Name and origin of used materials are provided in Table 1. Necessary watering and intercultural operations were provided as and when required.

Table 1. Name and origin of 14 soybean genotypes used in the present study

Sl. No.	Genotypes No.	Name/Acc No. (BD)	Origin
1	G1	BADC-SV_1	BADC
2	G2	GP: Sj-1	PGRC, BARI
3	G3	BR-13	PGRC, BARI
4	G4	GMOT-43	PGRC, BARI
5	G5	BR-33	PGRC, BARI
6	G6	BR-29	PGRC, BARI
7	G7	BS-29	PGRC, BARI
8	G8	BR-14	PGRC, BARI
9	G9	GP-Djs-9207	PGRC, BARI
10	G10	Asset-95	PGRC, BARI
11	G11	BINA Soybean-1	BADC
12	G12	BADC-SV_2	BADC
13	G13	MTD-453	PGRC, BARI
14	G14	BS-13	PGRC, BARI

PGRC=Plant Genetic Resources Centre, BADC = Bangladesh Agricultural Development Corporation, BARI=Bangladesh Agricultural Research Institute

2.2 Nursery care and data collection

Weeding was performed in all pots as and when required to keep plants free from weeds. Diseases and pest are a limiting factor to soybean production. Experimental soybean plants were

treated with Bavistin DF and Cupravit 50WP to prevent unwanted diseases problem @1g/l and 2g/l respectively. Pests were controlled by Malathion 250 EC @ 0.5 ml/l. Those fungicide and pesticide were sprayed two times, first at vegetative growth stage and next to early flowering stage to manage pest and diseases. When plants were well established, stalking was done to each plant by bamboo stick between 25-30 DAT to keep the plants erect. Proper tagging and labeling were done for each plant.

2.3 Data collection and statistical analysis

Data were recorded from each pot based on different agro-morphogenic traits throughout the life cycle of the plant. Multivariate analysis was done by GENSTAT 5.13 and Microsoft Excel 2000 software through four techniques viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CA) and Canonical Vector Analysis (CVA).

2.4Multivariate analysis

Rao (1952) suggested that the quantification of genetic diversity through biometrical procedures had made it possible to choose genetically diverse parents for a hybridization program. Multivariate analysis viz. Principal Component analysis, Principal Coordinate analysis, Cluster analysis and Canonical Vector analysis (CVA), which quantify the differences among several quantitative traits, are efficient method of evaluating genetic diversity. These are as follows:

2.5 Estimation of genetic diversity

2.5.1 Principal Component Analysis (PCA)

Therefore, principal component was computed from the correlation matrix and genotype scores obtained from the first components (which has the property of accounting for maximum variance) and succeeding components with latent roots greater than the unity (Jager*et al.* 1983). Contribution of the different morphological characters towards divergence is discussed from the latent vectors of the first two principal components.

2.5.2 Principal Coordinate Analysis (PCO)

Principal coordinate analysis is equivalent to principal component analysis, but it is used to calculate inter-unit distances. Using all dimensions of P it gives the maximum distances between each pair of the n point using similarity matrix (Digby*et al.*, 1989).

2.5.3 Canonical Vector Analysis (CVA)

The canonical vector analysis computes a linear combination of original variabilities that maximize the ratio in between group to within group variation to be finding out and thereby giving functions of the original variabilities that can be used to discriminate between groups. Finally, a series of orthogonal transformations sequentially maximizing the ratio of the among groups to the within group variations.

2.5.4 Cluster diagram

Using the values of intra and inter-cluster distances ($D = \sqrt{D^2}$), a cluster diagram was drawn as suggested by Singh and Chaudhury (1985). It gives a brief idea of the pattern of diversity among the genotypes included in a cluster.

2.5.5 Clustering

To divide the genotypes of the study into some number of mutually exclusive groups clustering were done using non-hierarchical classification. Starting from some initial classification of the genotypes into required groups, the algorithm repeatedly transfers genotypes from one group to another so long as such transfers improve the criterion, the algorithm switches to a second stage which examine the effect of swapping two genotypes of different classes and so on.

2.12 Selection of varieties for future hybridization program

Divergence analysis is usually performed to identify the diverse genotypes for hybridization purposes. The genotypes grouped together are less divergent among themselves than those, which fall into different clusters. Clusters separated by largest statistical distance (D^2) express the maximum divergence among the genotypes included into these different clusters. Variety (s) or line(s) were selected for efficient hybridization program according to Singh and Chuadhury (1985). According to them the following points should be considered while selecting genotypes for hybridization program:

- Choice of cluster from which genotypes are selected for use as parent (s)
- Selection of genotype(s) from the selected cluster(s)
- Relative contribution of the characters to the total divergence
- Other important characters of the genotypes performance

3. Results and Discussion

The present study was carried out with a view to determine the genetic diversity among 14 genotypes of soybean. The data were recorded on different parameters such as plant height, days to 50% flowering, number of leaves per plant, leaf area index, number of pods per plant, days to maturity, number of primary branches per plant, number of pod per plant, number of seeds per pod, pod length, seed yield per plant and thousand seed weight. The data were statistically analyzed, and results obtained from statistical analysis are described below under the following sections.

3.1 Genetic diversity

3.1.1 Principal component analysis (PCA)

Principal component analysis was carried out with fourteen genotypes of soybean which gives Eigen values of principal component axes of coordination of genotypes with the first axes totally accounted for the variation among the genotypes. First six Eigen values for six principal coordination axes of genotypes accounted for 98.45% variation showed in Table 2.Based on principal component scores I and II obtained from the principal component analysis (Appendix I), a two-dimensional scatter diagram (Z1-Z2) using component score I as X axis and component score II as Y axis was Constructed, which has been presented in Figure 1. The scatter diagram revealed that there were four apparent clusters. The genotypes were distantly located from each other, which indicated that considerable diversity existed among the genotypes.

3.1.2 Canonical variate analysis

Canonical Variate Analysis (CVA) was done to compute the inter-cluster distances. The intra and inter-cluster distance (D^2) values were shown in Table 3. In this experiment, the inter-cluster distances were higher than the intra-cluster distances thus indicating broader genetic diversity among the genotypes of different groups. The highest inter-cluster distance was observed between clusters II and IV (21.90), followed by between clusters I and II (16.41), III and IV (15.15). In contrast, the lowest inter-cluster distance was observed between cluster I and III (12.20).However, the maximum inter-cluster distance was observed between the clusters II and IV (21.90) indicating genotypes from these two clusters if involved in hybridization may produce a wide spectrum of segregating population.On the other hand, the maximum intra-cluster distance was found in cluster III (3.26), which contained of 2 genotypes, while the minimum distance was found in cluster I (0.79)that comprises 5 genotypes.

Table 2. Eigen values and yield percent contribution of 13 characters of 14 genotypes of soybean

Parameters	Eigen values	Percent variation	Cumulative % of Percent variation
I	8.74	61.91	61.91
II	1.89	13.36	75.27
III	1.74	12.29	87.56
IV	0.80	5.65	93.21
V	0.57	4.01	97.22
VI	0.17	1.23	98.45
VII	0.11	0.76	99.21
VIII	0.07	0.52	99.73
IX	0.02	0.16	99.89
X	0.01	0.06	99.95
XI	0.01	0.04	99.99
XII	0.00	0.01	100.00
XIII	0.00	0.00	100.00

Inter and intra cluster distances were showed in Table 3. Cluster I consist of nearest cluster with D^2 values cluster IV (8.53) and farthest cluster with D^2 values II (16.41) (Table 3). Cluster II consists of nearest cluster with D^2 values cluster III (7.90) and farthest cluster with D^2 values IV (21.90). Cluster III consists of nearest cluster with D^2 values cluster II (7.90) and farthest cluster with D^2 values IV (15.15). Cluster IV consists of nearest cluster with D^2 values cluster I (8.53) and farthest cluster with D^2 values II (21.90).A two-dimensional scatter diagram was constructed using component I as X-axis and component II as Y-axis, showing in the relative position.

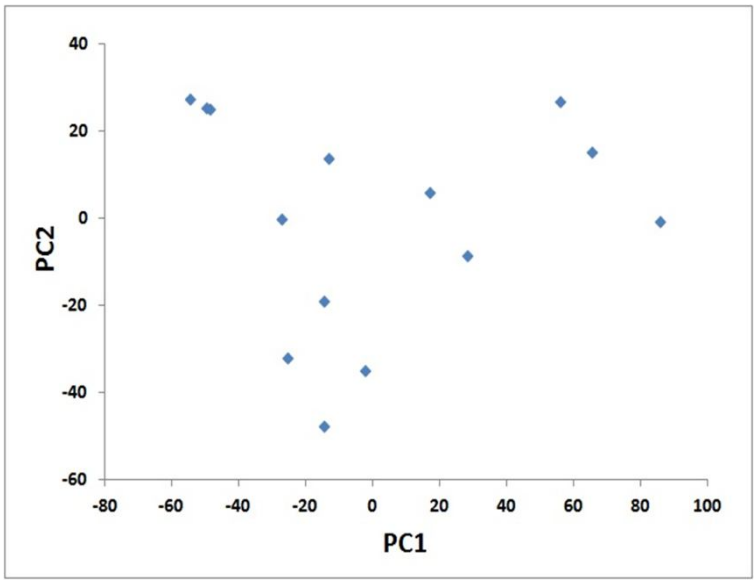

Figure 1. Scatter diagram of 14 genotypes of soybean based on their principal component scores.

According to scatter diagram all the genotypes were apparently distributed into four clusters (Figure 2).It is assumed that maximum amount of heterosis will be manifested in cross combination involving the genotypes belonging to most divergent clusters.

Table 3. Intra (Bold) and inter cluster distances (D^2) for 14 genotypes of soybean

Cluster	I	II	III	IV
I	**0.79**			
II	16.41	**1.85**		
III	12.20	7.90	**3.26**	
IV	8.53	21.90	15.15	**1.23**

In the present study the maximum distance existence between cluster II and IV.So, the crosses between the genotypes belonging cluster II with cluster IV might produce high heterosis. Also, the crosses between genotypes from cluster II with IV might produce high level of segregating population. So, the genotypes belonging to cluster II and cluster IV might be selected for future hybridization program.

Table 4. The nearest and farthest clusters from each cluster between D^2 values in soybean

Sl. No.	Cluster	Nearest Cluster with D^2 values	Farthest Cluster with D^2 values
1	I	IV (8.53)	II (16.41)
2	II	III (7.90)	IV (21.90)
3	III	II (7.90)	IV (15.15)
4	IV	I (8.53)	II (21.90)

3.1.3 Principal coordinate analysis (PCO)

Inter genotypic distances as (D^2) as obtained by principal coordinate analysis (PCO) for all possible combinations between the pairs of genotypes. Inter genotypic distances, as obtained from principal coordinate analysis showed that the highest distance was observed between the G1 and G10 (Table 5). The lowest distance was observed between the G4 and G10. The difference between the highest and the lowest inter genotypic distance indicated the prevalence of variability among the 14 genotypes of soybean studied.

3.1.4 Nonhierarchical clustering

Fourteen *Glycine max* L. Merr. genotypes were grouped into four different clusters through non-hierarchical clustering (Table 6). These results confirmed the clustering pattern of the genotypes obtained through principal component analysis. Cluster I had highest number of five genotypes followed by cluster IV and cluster II constituted by four and three genotypes, respectively. On the other hand, cluster III constituted by two genotypes. Cluster I had maximum five genotypes namely G_3 (BR-13), G_4 (GMOT-43), G_7 (BS-29) G_8 (BR-14) and G_{10} (Asset-95). Cluster II represents 3 genotypes namely G_1 (BADC Sv_1), G_6 (BR-29) and G_9 (Gp-Djs-9207).Last of all cluster III had minimum 2 genotypes G_5 (BR-33) and G_{11} (BINA Soybean-1). The results confirmed the clustering pattern of the genotypes according to the principal component analysis.

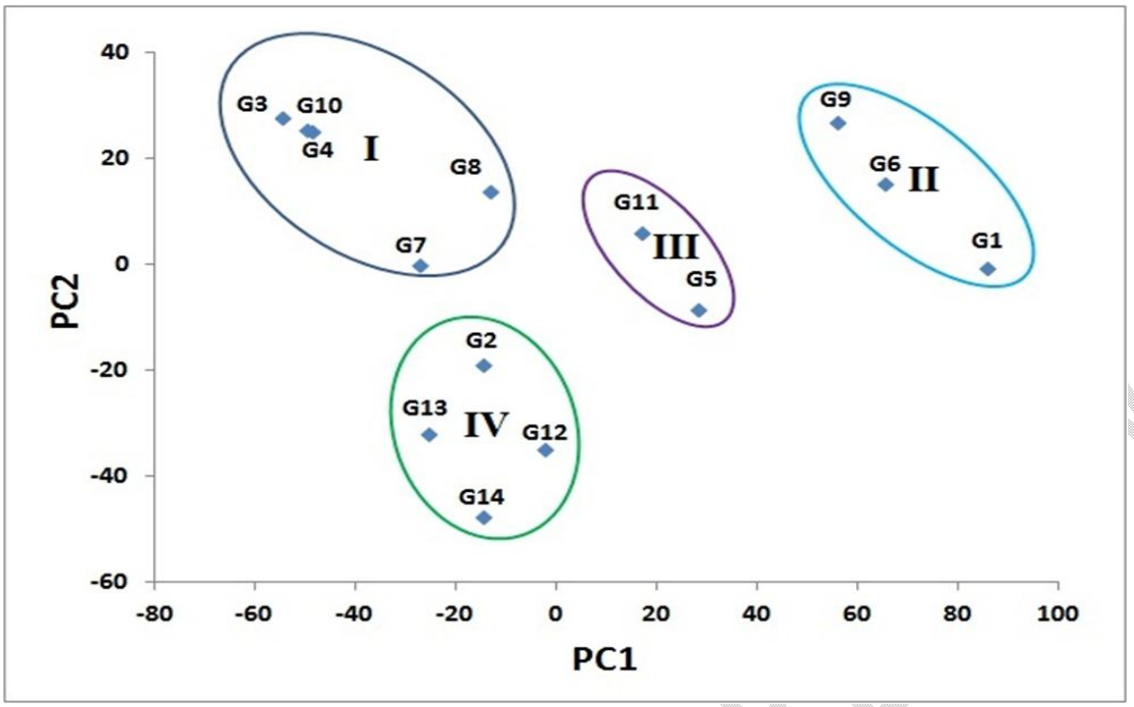

Figure 2. Scatter distribution of 14 genotypes of soybean based on their principal component scores super imposed with clustering.

The clustering pattern obtained coincided with the apparent grouping patterns performed by PCA. For that reason, it can be said that the results obtained through PCA were established by nonhierarchical clustering. Clustering pattern of 14 genotypes of soybean is presented in Figure 1 and 2.

3.1.5 Cluster mean analysis

The cluster means of 13 different characters (Table 7) were compared and indicated considerable differences between clusters for all the characters studied. Maximum days to 50% flowering were observed in cluster I (79), whereas minimum days to 50% flowering in cluster IV (58). Thenmaximum plant heights were observed in I (45.47) whereas minimum plant height was observed in cluster IV (34.34). Maximum number of main branches was observed in cluster I and minimum (5.11) in cluster II. Number of secondary branches per plant was observed maximum in cluster I (17.80) and minimum to cluster II (9.00).Cluster I showed highest number of leaf (56.53) and cluster II showed lowest (25.89). Maximum (0.10) and minimum (0.04) number leaf area index were observed in cluster I and II, respectively.Maximum number of flowers per plant was observed in cluster I (39.20), whereas minimum number of flowers per

plant was observed in cluster II (27.00).

Table 5. Ten highest and ten lowest inter genotypic distance among 14 genotypes of soybean

Sl.	Genotype combination	Distance
10 highest inter genotypic distances		
1	G10-G1	2.824
2	G3-G1	2.817
3	G4-G1	2.788
4	G6-G3	2.338
5	G10-G6	2.309
6	G7-G1	2.274
7	G6-G4	2.143
8	G8-G1	2.138
9	G12-G10	2.121
10	G12-G3	2.099
10 lowest inter genotypic distances		
1	G10-G4	0.456
2	G10-G3	0.613
3	G9-G6	0.641
4	G8-G2	0.65
5	G4-G3	0.671
6	G11-G2	0.726
7	G11-G5	0.739
8	G13-G2	0.747
9	G14-G5	0.756
10	G12-G2	0.776

Maximum (31.54) and minimum (19.33) number of pods per plant were observed in cluster II and II, respectively. The maximum pod length (4.23) was observed in the cluster IV, whereas minimum pod length (3.63) was observed in cluster III.Maximum (148.50) and minimum (54.33) days to 1st pod maturity were observed in cluster IV and II, respectively. Number of seeds per plant was maximum in cluster I (95.27) and minimum number in cluster II (38.67). Weight of 100 seed was highest in cluster IV with a mean value of (7.85) and it was least in genotypes belongs to the cluster I (5.56). The maximum yield was observed in cluster I (1.16), whereas minimum yield was observed in cluster III (0.70). Cluster IV mainly an early flowering genotype

whereas it produces the lowest mean values for 50% flowering and weight of 100 seed and pod length was maximum in genotypes belonging to this cluster.

Table 6. Distribution of genotypes in different clusters

Cluster	Number of genotypes	Genotypes
I	5	G3, G4, G7, G8 and G10
II	3	G1, G6 and G9
III	2	G5 and G11
IV	4	G2, G12, G13 and G14

Cluster I has late flowering, maximum plant height, maximum number of main branches, maximum number of leaf and leaf area index, maximum number of pod and highest yield among the genotypes studied. Again, cluster II was matured early, lowest leaf area index and minimum number of flowers per plant. The genotypes belonging to the cluster III were minimum of yield and pod length. To develop high yielding varieties these groups can be used in hybridization program.

3.5.6 Cluster diagram

With the help of D^2 values within and between clusters, an arbitrary cluster diagram (Figure 3) was constructed, which showed the relationship between different genotypes. However, the diagram was not following exact scale. It was apparent from the Figure 3 that the genotypes included in the cluster IV was far diverse from the genotypes of the cluster II and where the genotypes belonging to II and III were the least diverse. Genotypes of cluster I-II and III-IV were moderately diverse from each other. The similar diverse genotypes were included between the cluster I-III and I-IV.

Table 7. Cluster mean values of 13 different characters of 14 genotypes of soybean

Parameters	I	II	III	IV
Days to 50% flowering	79	59	77	58
Plant height (cm)	45.47	35.22	39.84	34.34
Number of main branches/plants	8.07	5.11	7.17	6.67
Number of secondary branches/plants	17.80	9.00	14.00	11.75
Number of leaf/plants	56.53	25.89	44.84	34.58
L.A.I.	0.10	0.04	0.06	0.07
Number of flower/plants	39.20	27.00	30.50	28.42
Number of pod/plants	31.54	19.33	20.50	20.08
Pod length (cm)	4.20	4.22	3.63	4.23
Days to 1st pod maturity	135.60	54.33	101.00	148.50
Number of seed/plants	95.27	38.67	41.00	53.25
Weight of 100 seed (g)	5.56	7.43	6.70	7.85
Yield ton/ha	1.16	0.71	0.70	1.04

3.5.7 Contribution of characters towards divergence of the genotypes

Contribution of characters towards the divergence obtained from canonical variate analysis is presented in Table 8. In this method vectors were calculated to represent the varieties in the graphical form (Rao, 1952). This is helpful in cluster analysis as it facilitated the study of group constellation and serves as a pictorial representation of the configuration of various groups. The latent vectors (Z_1 and Z_2) obtained from principal component analysis (PCA). The important characters responsible for genetic divergence in the axis of differentiation in vector I (Z_1) were days to 50% flowering (0.001), number of secondary branches per plant (0.142), plant height (0.134), number of leaves per plant (0.144), leaf area index (33.087), number of flowers per plant (0.107) and yield (1.512).These characters were important because all these characters had positive signs in first axis. Plant height (0.054), number of main branches per plant (0.084),

number of leaves per plant (0.047), leaf area index (40.279), number of pods per plant (0.056), pod length (2.688), number of seeds per plant (0.103) and yield (1.77) had positive sign in vector II (Z2), second axis of differentiation.

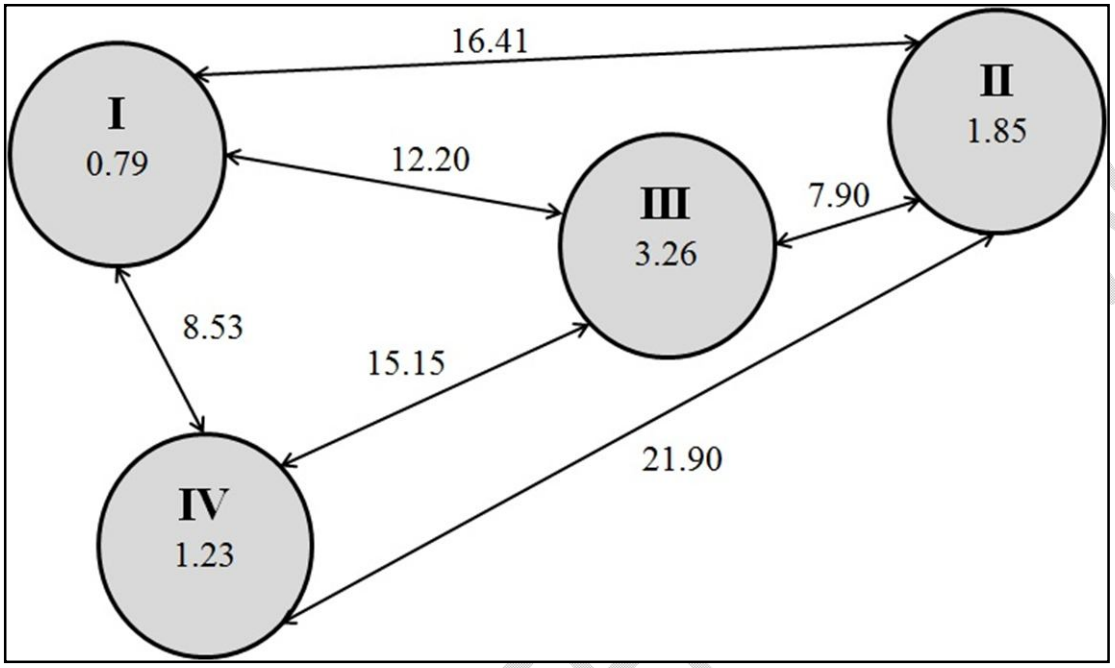

Figure 3. Intra and inter cluster distances (D^2) of 14 genotypes in soybean.

On the other hand, number of main branches per plant, number of pod per plant, pod length, days to 1st pod maturity, number of seeds per plant and weight of 100 seeds possessed the negative sign in the first axis of differentiation and days to 50% flowering, number of secondary branches perplant, number of flower per plant, days to 1st pod maturity and weight of 100 seed possessed negative signs in the second axis of differentiation that means these had minor role in the genetic divergence. Plant height, number of leaves per plant, leaf area index and yield had positive sign in both the axis, which indicated that they were the important component characters having higher contribution to genetic divergence among the genotypes studied.

3.5.8 Selection of genotypes as parent for hybridization program

Selection of genetically diverse parents is an urgent step for hybridization program. So, in the present study genotypes were to be selected based on of specific objectives. From the crosses between genetically distance parents a high heterosis could be produced. Considering the

magnitude of cluster mean and agronomic performance the genotype G_1 (BADC SV_1) for minimum days to 50% flowering from cluster II; for maximum plant height, pod length and yield G_7 (BS-29) from cluster I; G_{12} (BADC SV_2) for maximum weight of 100 seed from cluster IV, G_4 (GMOT-43) for maximum leaf area index from cluster I; G_{10} for maximum days to 50% flowering, maximum number of secondary branches and maximum weight of 100 seed from cluster I were found promising.

Table 8. Relative contributions of the ten characters of 14 genotypes of soybean to the total divergence

Parameters	Vector-1	Vector-2
Days to 50% flowering	0.001	-0.071
Plant height (cm)	0.134	0.054
Number of main branches/plants	-0.613	0.084
Number of secondary branches/plants	0.142	-0.047
Number of leaf/plants	0.144	0.047
L.A.I.	33.087	40.279
Number of flower/plants	0.107	-0.132
Number of pod/plants	-0.278	0.056
Pod length (cm)	-1.777	2.688
Days to 1st pod maturity	-0.239	-0.068
Number of seed/plants	-0.056	0.103
Weight of 100 seed (g)	-0.409	-0.255
Yield ton/ha	1.512	1.777

Therefore, considering group distance and other agronomic performance the inter-genotypic crosses between G_1 and G_{10}; G_1 and G_7; G_4 and G_{12} might be suggested for future hybridization program.

4. conclusion

From the findings of the present study, the following conclusions could be drawn:

- Wide range of genetic diversity existed among 14 soybean genotypes which were grouped into four clusters and most diverse genotypes were G_1 (BADC SV_1) and G_{10}

(Asset-95). That variability could be used for future breeding program of soybean in Bangladesh.

- The genotypes of clusters II were more diverse from the genotypes of cluster IV.
- Days to 50% flowering, plant height, number of main branches per plant, number of leaves per plant and leaf area index were found responsible for the maximum diversity. On the other hand, yield ton per hectare, weight of 100 seed, pod length, number of seed per plant and days to 1st pod maturity have the least responsibility of both the primary and secondary differentiation of genotypes.
- Further collection of soybean germplasms would be continued for getting more variability and desired traits in soybean.

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Appendix I. Z1-Z2 score of 14 genotypes of soybean

Genotype	PCA 1	PCA 2
G1	85.68	-0.45
G2	-14.80	-18.62
G3	-54.63	27.69
G4	-48.85	25.22
G5	28.02	-8.40
G6	65.29	15.51
G7	-27.33	0.20
G8	-13.53	14.01
G9	55.91	26.89
G10	-49.80	25.58
G11	16.81	6.19

G12	-2.41	-34.72
G13	-25.74	-31.68
G14	-14.62	-47.42