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Genetic diversity of country bean (*Lablab purpureus*) genotypes collected from the coastal regions of Bangladesh

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ABSTRACT

Purpose: Sixty one country bean genotypes collected from the coastal region of Bangladesh and were evaluated for the genetic divergence of yield and yield traits. **Research method:** Divergence analysis was performed by Mahalanobis D^2 statistics using Genstat 4.2 program. **Findings:** The results of the univariate analysis revealed that country bean genotypes differed significantly for 12 yield and related characters. Multivariate analysis grouped 61 genotypes into eight clusters. Cluster II and IV comprised the minimum number (2) of genotypes followed by V (4). Cluster VII contained the maximum number (16) of genotypes and sub-divided into five subgroups. The highest inter-genotypic distance was observed between the genotype CB-32, and CB-109 (2.238), and the lowest inter-genotypic distance between the genotypes CB-016 and CB-017 (0.252). The inter-cluster distance (D^2) was maximum between cluster I and V (22.15) followed by the distance between cluster IV and V (19.21). The highest intra-cluster distance (1.17) was observed in cluster V revealed the maximum heterogeneity, while the least variation (0.60) was noticed in cluster VI signifying the closeness of the genotypes. Data suggested that the pod length, pod width, and individual pod weight were major characters that contribute mostly towards genetic divergence. Both PC1-PC2 and PC1-PC3 biplot graphs clearly indicated that pod yield per plant had strong positive correlation with raceme per plant, pods per raceme, pods per plant, pod length and hundred seed weight. **Limitations:** There was no significant limitation to the report. **Originality/Value:** The genotypes from cluster I and V could be selected for future hybridization program.

INTRODUCTION

Country bean (*Lablab purpureus*) is a significant winter vegetable in Bangladesh known as "seem'' (Rashid, 1999). It belongs to the family Fabaceae with chromosome number 2n = 20, 22, 24 (Philip, 1982). The wild forms of country bean or lablab are believed to have originated in India (Deka & Sarkar, 1990) and were introduced into Africa from Southeast Asia during the eighteenth century (Kay, 1979). There have been frequent changes in the botanical name of this crop, which are Hyacinth bean, Dolichos bean, Indian bean, lablab bean, sem bean, labia bean (Sudan), and Egyptian Kidney bean (Pureseglove, 1968). Green country bean pod is an excellent source of different essential elements, minerals and vitamins. Young pods contain 83% water, 4.50g protein, 10.00g carbohydrate, 1.00 g fat, 2.00 g fiber, 0.05 mg thiamine, 0.01mg riboflavin per 100g, and little amount of vitamin C (Rubatzky & Yamaguchi, 1997). It has some excellent features which increase its value of cultivation. Drought tolerance, and its frequent growth in dry land area with limited rainfall are essential (Cameron, 1988). However, it has some drawbacks such as low salinity tolerance with symptoms being chlorotic leaves, reduced growth and plant death. But coastal area covers about 20% of the country, and over 30% of the net cultivable area where country bean seems to be grown vastly. Tidal floodplains occur in Satkhira, Khulna, Bagerhat, Pirojpur, Jhalokathi, Barisal, Patuakhali, Chittagong, and Cox's Bazar district (SRDI, 2001; Karim et al., 1990; Haque, 2004). It is also intolerant of moderate to heavy shading (Islam, 2008). Unlike great yielding varieties, the landraces maintained by the farmers are endowed with tremendous genetic variability, as they are not subjected to fine selection over a long period (Biswas et al., 2010). Indo-China region being a center of diversity, is endowed with significant variability in terms of morphological characters, especially growth habit, maturity including shape, size, the color of fruit and seed (Rai et al., 2006; Saravanan et al., 2013; Asaduzzaman et al., 2015; Kamotho et al., 2016; Hadavani Janaki et al., 2018; Dholakia et al., 2019). The present study was undertaken to find out the genetic diversity of collected *Lablab purpureus* germplasm to create ample scope for its genetic improvement and selection of diverse genotypes to facilitate the breeder to develop lablab bean varieties.

MATERIALS AND METHODS

The field evaluation was conducted at the experimental farm of Genetics and Plant Breeding Department of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, Bangladesh. The soil type of the experimental field belongs to the shallow red-brown terrace type under the Salna series of Madhupur tract of Agro-Ecological Zone (AEZ) 28, which is characterized by silty clay with a pH value of 6.5 (Haider et al., 1991). Sixty-one (61) country bean genotypes were used as experimental materials collected from different coastal areas of Bangladesh. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. The unit plot size was 3.0 m x 2.0 m, accommodating two rows and four holes per bed. Plants were spaced at 1.5 m x 1.0 m spacing. Each hole received fertilizers in the following rates: 10.0 g Urea-, 30.0 g TSP (Triple Super Phosphate), 20.0 g MP (Muriate of Potash) (Anonymous, 2012). Three to four seeds were sown per hole. Seeds germinated in 5-6 days after sowing. Additional seedlings were thinned out, keeping one healthy plant in each hole when the seedlings are about a month old. The young growing plant was supported by a single bamboo stake in each pit. Weeding was done at 25 and 50 days after sowing. Irrigation was applied in the experiment field as and when necessary. The crop was protected from the attack of insect pests (mainly aphids and fruit borers) by regular spraying of Malathion and Ripcord at the rate of 1 ml.liter^{-1}. Fungicide

Bavistin and Noin at the rate of 2g.10 liter^{-1} were applied at an early stage to prevent the severe incidence of disease. Harvesting of green edible pods was started in the middle of February and continued up to March. The beginning and ending of pod harvest varied depending on the genotypes. Data were recorded from all experimental plants on leaf length, leaf width, leaflet length, pod length, pod width, particular pod weight, pods per raceme, racemes per plant, pods per plant, pod yield per plant, individual seed weight, and 100 seed weight. Divergence analysis was performed by estimating Mahalanobis D^2 statistic, and the genotypes were grouped into eight clusters using GenStat 4.2 computer program. Intra- and inter-cluster distances, cluster mean, and percent distribution of individual character towards divergence were also estimated (Mahalnobis, 1936).

RESULTS AND DISCUSSION

Descriptive statistics and correlation

Mean performance, maximum, minimum value, standard deviation and F-value of 12 agronomic characters are presented in Table 1. Analysis of variance showed a significant variation among the genotypes for 12 agronomic characters (Table 1). Standard deviation revealed maximum variation among the genotypes for individual pod weight, and minimum for particular seed weight. Pod yield per plant showed significant positive correlation with leaflet length, pod length, pod width, individual pod weight, pods per raceme, racemes per plant, and pods per plant. On the other hand, leaf length, leaf width, individual seed weight, and 100 seed weight had negative correlation with pod yield per plant (Table 2).

Table 1. Descriptive statistics for 12 agronomic characters of 61 country bean genotypes

Variables	Min.	Max	Mean	Std. Dev.	F-value
Leaf length (cm)	6.76	12.71	9.93	1.3	*
Leaf width	5.86	11.49	8.83	1.3	*
Leaflet length (cm)	7.37	19.67	12.97	3.16	*
Pod length (cm)	93	267	148.25	38.32	**
Pod width (cm)	5.15	13.85	8.03	1.97	*
Individual pod weight (g)	515	2172.1	1192.73	411.83	**
Pods per raceme	7.37	15.47	10.53	1.81	**
Racemes per plant	1.63	4.2	2.58	0.53	**
Pods per plant	5.38	19.2	9.84	2.86	**
Individual seed weight (g)	0.24	0.73	0.44	0.11	*
100 seed weight (g)	24	73	44.47	11.34	*
Pod yield per plant (kg)	5.19	28.11	11.28	4.17	**

Table 2. Correlation matrix 12 agronomic characters of 61 country bean

Variable	LW	LLL	RPP	PPR	PPP	PLT	PWD	IPW	ISW	HSW	PYP
LLT	0.889	0.678*	-0.282	-0.197	-0.346	0.210	0.194	0.335	0.095	0.095	-0.110
LWD		0.639*	-0.266	-0.247	-0.371	0.242	0.256*	0.351	0.094	0.094	-0.111
LLL			-0.220	0.013	-0.149	0.029	0.233	0.269	0.029	0.029	0.031
RPP				0.036	0.753*	-0.173	-0.170	-0.135	-0.157	-0.157	0.716*
PPR					0.675*	-0.506	-0.474	-0.501	0.245*	0.245*	0.198*
PPP						-0.429	-0.414	-0.397	0.040*	0.040*	0.678*
PLT							0.428	0.712*	-0.119	-0.119	0.095
PWD								0.680*	-0.090	-0.090	0.131*
IPW									-0.076	-0.076	0.353*
ISW										1.000	-0.063
HSW											-0.063

LLT= leaf length (cm), LWD= leaf width, LLL= leaflet length (cm), PLT= pod length (cm), PWD= pod width (cm), IPW= individual pod weight (g), PPR= pods per raceme, RPP= racemes per plant, PPP= pods per plant, ISW= individual seed weight (g), HSW= 100 seed weight (g) and PYP= pod yield per plant (kg).

Principal component and cluster analysis

Intergenotypic distances as obtained from the principal coordinate analysis showed in Table 3. The highest intergenotypic distances (2.238) were observed between CB-032 and CB-109, and the lowest length (0.252) was observed between CB-016 and CB-017. Differences between the highest and the lowest inter genotypic distances indicated the prevalence of genetic diversity among 61 country bean genotypes.

The intergenotypic distances were used in the computation of intra-cluster distances from the distance matrix of PCO, according to Singh and Choudhary (2001). The statistical distances represent the index of genetic diversity among the cluster. The highest intra-cluster distance (1.17) was observed in cluster V (Table 4), which composed of eight genotypes and it was favored to decide that the genotypes in cluster V are more heterogeneous. Whether cluster VI showed the lowest intra-cluster distance (0.60) having eight genotypes and the genotypes belongs to this group is comparatively homogeneous.

Inter-cluster distance, as obtained from the principal coordinate analysis, showed that the highest inter-cluster distance (22.12) was observed between cluster I and cluster V. The results indicated that the genotypes in these clusters have diverged than those of other clusters. The lowest inter-cluster distance (1.51) was observed between clusters VIII, and cluster VI revealed that an intimate relationship among the genotypes included within these clusters.

By the application of the covariance matrix for non-hierarchical clustering, the 61 genotypes were grouped into eight clusters. Different clusters with their corresponding genotypes are presented in Table 5. Cluster VII contained the highest (16) number of genotypes, followed by cluster 1 (11) and cluster VI (10). Cluster II and cluster IV contained a minimum number (2) of genotypes; and cluster III contained seven genotypes; cluster V contained four genotypes, and cluster VIII nine genotypes (Table 5).

Table 3. Five highest and five lowest inter genotypic distances among 61 genotypes of country bean

Sl. No.	Genotypic contribution	Distances
A. Five highest inter genotypic distances between		
01	CB-032 and CB-109	2.238
02	CB-060 and CB-109	2.237
03	CB-086 and CB-109	2.170
04	CB-086 and CB-104	2.148
05	CB-032 and CB-040	2.119
B. Five lowest inter genotypic distances between		
01	CB-016 and CB-017	0.252
02	CB-067 and CB-068	0.277
03	CB-031 and CB-037	0.291
04	CB-009 and CB-043	0.296
05	CB-068 and CB-085	0.316

Table 4. Intra-cluster and inter-cluster distances (D^2) for 61 country bean genotypes

	I	II	III	IV	V	VI	VII	VIII
I	0.78							
II	16.78	0.63						
III	6.35	10.97	0.66					
IV	4.03	13.49	2.55	0.76				
V	22.15	6.08	16.77	19.21	1.17			
VI	11.32	5.65	5.32	7.85	11.55	0.60		
VII	10.05	7.74	3.71	6.22	13.76	2.38	0.85	
VIII	12.79	4.15	6.82	9.36	10.11	1.51	3.67	0.86

Table 5. Distribution of 61 country bean genotypes in eight clusters

Cluster	Number of genotypes	Genotypes
I	11	CB-040 (Borguna), CB-100 (Cox's Bazar), CB-042 (Borguna), CB-047 (Borguna),CB-059 (Khulna), CB-064 (Khulna), CB-067 (Bagerhat), CB-068 (Bagerhat), CB-085 (Bagerhat), CB-091 (Patuakhali), CB-104 (Cox's Bazar),
II	2	CB-009 (Khulna), CB-034 (Patuakhali),
III	7	CB-003 (Khulna), CB-007 (Bagerhat), CB-043 (Borguna), CB-073 (Bagerhat), CB-095 (Cox's Bazar), CB-102 (Cox's Bazar), CB-105 (Cox's Bazar)
IV	2	CB-041 (Borguna), CB-109 (Cox's Bazar)
V	4	CB-001 (Khulna), CB-005 (Borguna), CB-012 (Khulna), CB-062 (Khulna)
VI	10	CB-011 (Khulna), CB-013 (Khulna), CB-029 (Patuakhali), CB-030 (Patuakhali), CB-032 (Patuakhali), CB-033 (Patuakhali), CB-035 (Patuakhali), CB-036 (Borguna), CB-086 (Patuakhali), CB-087 (Patuakhali)
VII	16	CB-010 (Khulna), CB-025 (Bagerhat), CB-026 (Patuakhali), CB-027 (Patuakhali), CB-031 (Patuakhali), CB-037 (Borguna), CB-045 (Patuakhali), CB-060 (Khulna), CB-061 (Khulna), CB-089 (Patuakhali), CB-090 (Patuakhali), CB-092 (Patuakhali), CB-093 (Cox's Bazar), CB-096 (Cox's Bazar), CB-097 (Cox's Bazar), CB-107 (Patuakhali)
VIII	9	CB-015 (Khulna), CB-016 (Khulna), CB-017 (Khulna), CB-018 (Khulna), CB-019 (Khulna), CB-020 (Khulna), CB-021 (Khulna), CB-022 (Khulna), CB-088 (Patuakhali)

Cluster analysis is one of the statistical techniques aimed at grouping objects in clusters so that the items in one cluster have high similarities to those in other clusters. The clusters represent uncorrelated groups which may be useful for future heterotic breeding as their traits, performance may be governed by different sets of alleles (Twumasi et al., 2018). Based on cluster analysis on 12 traits 61 country bean genotypes which were separated into eight groups (Table 5) further divided into subgroups (Fig. 1). Thus, Figure 1 illustrates the eight clusters formed by hierarchical clustering with subgroups, and Table 5 summarizes the number of genotypes in each cluster.

The first cluster was again divided into two subgroups; IA and IB (Fig. 1). Subgroup IA consisted of two genotypes; CP 091 and CP 100, while Subgroup IB comprised of nine genotypes; CP 040, CP 104, CP 042, CP 059, CP 064, CP 067, CP 047, CP 068 and CP 085. Cluster II consists of only two genotypes, CP 009 and CP 034, signifying its divergence from other than any member of the experimental population. Cluster III, IV, and V consist of seven, two and four genotypes, respectively. Cluster VII contains a maximum number of genotypes (16), and subdivided into five subgroups; VIIA comprises of two genotypes; VIIB consists of three genotypes; VIIC comprises of five genotypes; VIID comprises two genotypes and VIIE comprises of four genotypes. Cluster VII consists of nine genotypes.

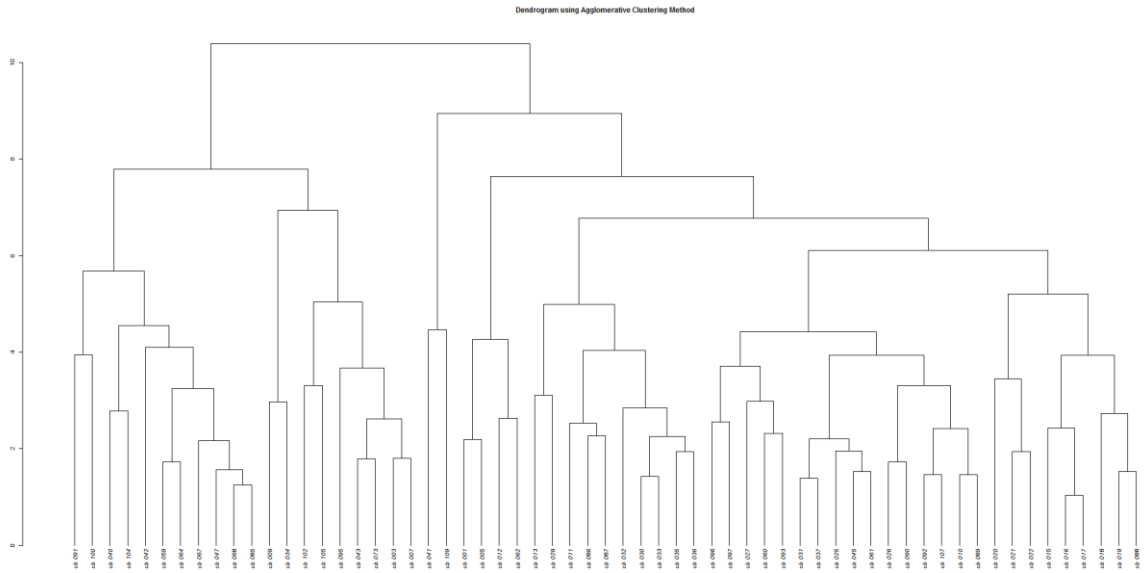

Fig. 1. Tree diagram of 61 genotypes of country bean based on 12 yield contributing traits.

Table 6. Cluster means for 12 characters of 61 country bean genotypes

Characters	I	II	III	IV	V	VI	VII	VIII
Leaf length	9.70	9.60	9.50	10.90 (H)	8.70 (L)	10.10	10.80	10.20
Leaf width	8.70	8.30	8.50	9.60 (H)	7.50 (L)	9.00	9.60	9.40
Leaf length (cm)	12.70	11.40 (L)	11.90	14.30 (H)	12.30	14.10	13.90	13.20
Raceme per plant	99.20 (L)	190.10	139.4	120.70	191.90 (H)	142.80	127.50	167.70
Pods per raceme	5.90 (L)	8.80	6.90	6.60	10.60 (H)	8.90	9.10	8.20
Pods per plant	589.60 (L)	1603.90	951.80	792.70	1941.50 (H)	1259.5	1128.00	1352.8
Pod length	12.00	9.40	10.80	12.10 H	10.00	9.50	9.10 (L)	10.00
Pod width	3.50 (H)	2.40	2.50	2.80	2.30 (L)	2.50	2.40	2.50
Individual pod weight	12.30	8.70	9.50	12.40 H	8.70	8.70	8.50 (L)	9.10
Pod yield per plant (kg)	7.20 (L)	14.00	9.00	9.80	16.90 (H)	11.00	9.60	12.30
Individual seed weight	0.40	0.40	0.40	0.40	0.40	0.50(H)	0.40(L)	0.50
100 seed weight	40.10	42.50	44.60	43.60	43.40	51.5(H)	39.20(L)	46.70(H)

Note: H= High, and L= Low.

The intra-cluster mean values for 12 characters along with the highest (H) and the lowest (L) markings for each cluster has been presented in Table 6. Cluster V produced the highest intra-cluster mean for raceme per plant (191.9) and the lowest intra-cluster mean (99.2) has been found in cluster I. Cluster V produced the highest intra-cluster mean for pod per raceme (10.6), and cluster I showed the lowest intra-cluster mean (5.9). The highest intra-cluster mean for pod per plant (1941.5) has been found in Cluster V, and the lowest (589.6) by cluster I. Cluster IV had the highest intra-cluster mean for pod length (12.1 cm). Cluster VII showed the lowest intra-cluster mean for pod length (9.1 cm). The highest intra-cluster mean

for pod width (3.5 cm) produced in Cluster I. Cluster V showed the lowest intra-cluster mean for pod width (2.3 cm). Cluster IV produced the highest intra-cluster mean for individual pod weight (12.4 g) and the lowest intra-cluster mean (8.5 g) by Cluster VII. The highest intra-cluster mean for Pod yield per plant (16.9 Kg) has been found in Cluster V. Cluster I showed the lowest intra-cluster mean for pod yield per plant (7.2 Kg).

Biplot analysis based on principal components

Biplot analysis is mostly used to determine the components which effect is more to create the genotypic variation. The highest values indicate the strongest influence of the trait on the total variation. It also provides a tool for visual comparison among genotypes based on multiple traits (Al-Naggar et al., 2020). Biplot in the principal component represents variables that are super imposed on a plot as vectors where relative length of vectors represents the relative proportion of variability in each variable defined on the biplot. If the angle between vectors of two traits is < 90°, both are positively correlated, whereas if the angle is > 90° there is a negative correlation and both vectors show no correlation if the angle is 90° (Sabaghnia et al., 2011). Genotype by Trait (GT) biplot is an effective tool for revealing the interrelationships among the maize traits. Also, it can be used in independent culling based on multiple traits and in comparing selection strategies (Alvi et al., 2003).

The association between 12 characteristics among the 61 genotypes were visualized by the biplot analysis. Biplot analysis revealed the trait profiles of the genotypes, primarily, those genotypes positioned far away from the origin, and the results indicated a correlation between traits with genotypes. In PC1 and PC2, together differences among individual pod weight, 100-seed weight, raceme per plant, and pods per plant are well represented in the biplot, but leaflet length and pod yield per plant have minimum differences between PC1 and PC2 biplot (Fig. 2). These variations are sources of germplasm improvement in the respective quantitative trait, which contribute toward better agronomic performance and increase in yield potential.

In PC1 and PC2 biplot graph, it was indicated that pod yield per plant had a strong positive correlation with raceme per plant, pods per plant and pod length, whereas kernel width (mm) has a strong negative correlation with leaf length, leaf width, leaflet length, pod width, and 100-seed weight (Fig. 2). A principal component scatters plot of bean germplasm depicts the similarity among the performance of country bean genotypes in respect of 12 variables. In contrast, genotypes that are further apart from one another are different. Genotypes that are nearer to variable vectors are more significant for that trait and vice versa. Therefore, a scatter plot helped to select genotypes for the yield contributing trait or for the trait that helped in better agronomic performance. In PC1 and PC2 biplot it is indicated that genotypes CB 029, CB 040, CB 058 is suitable for raceme per plant; genotype CB 057 can be selected based on pods per raceme; CB 030, CB 040, CB 050, and CB 056 has higher number pods per plant; CB 02, CB 004, CB 005, CB 033, CB 052 and CB 053 can be selected for hundred seed weight (g) and CB 021, CB 041 and CB 042 can be chosen for pod yield per plant (Fig. 2). As PC1 and PC3 were imposed in the biplot, differences among leaf length (cm), leaf width, leaflet length (cm), individual pod weight (g), pods per plant, individual seed weight (g), and pod yield per plant (kg) are well represented, but pod length (cm), pod width (cm), pods per raceme, racemes per plant and 100 seed weight (g) have minimum differences between PC1 and PC3 biplot (Fig. 3).

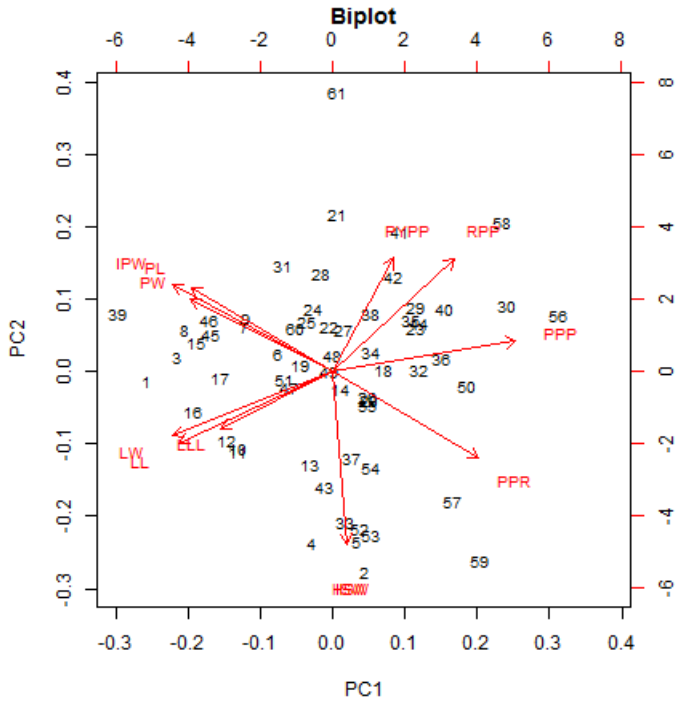

Fig. 2. Principal Components Analysis (PCA) ordination graph. Position of maize advance genotypes from the selected germplasm pools along first two axes obtained from PCA, where LL= leaf length (cm), LW= leaf width, LLL= leaflet length (cm), PL= pod length (cm), PW= pod width (cm), IPW= individual pod weight (g), PPR= pods per raceme, RPP= racemes per plant, PPP= pods per plant, ISW= individual seed weight (g), HSW= 100 seed weight (g) and PYPP= pod yield per plant (kg).

In this plot, pod yield per plant showed a positive correlation with raceme per plant, pods per raceme, pods per plant, and hundred seed weight, whereas pod length and pod width (mm) showed negative correlative. PC1 and PC3 biplot illustrated that the CB 035 could be selected for raceme per plant, CB 059 for pods per raceme, CB 030 and CB 058, CB 001, CB 016, CB 017 for pod length, CB 012 for pod width CB 032 for pod yield per plant.

The genotypes from cluster 1 (CB-040: Borguna, CB-042: Borguna, CB-047: Borguna, CB-059: Khulna, CB-064: Khulna, CB-067: Bagerhat, CB-068: Bagerhat, CB-085: Bagerhat, CB-091: Patuakhali, CB-100: Cox's Bazar, CB-104: Cox's Bazar) and cluster V (CB-001: Khulna, CB-005: Borguna, CB-012: Khulna, CB-062: Khulna) showed maximum divergence as the cluster distance was found maximum for these clusters. Genetic diversity was also reported by Saravanan et al. (2013), Kamotho et al. (2016), Hadavani Janaki et al. (2018), Dholakia et al. (2019). On the other hand, pod length, pod width and individual pod weight showed the highest contribution towards the divergence among 61 country bean genotypes.

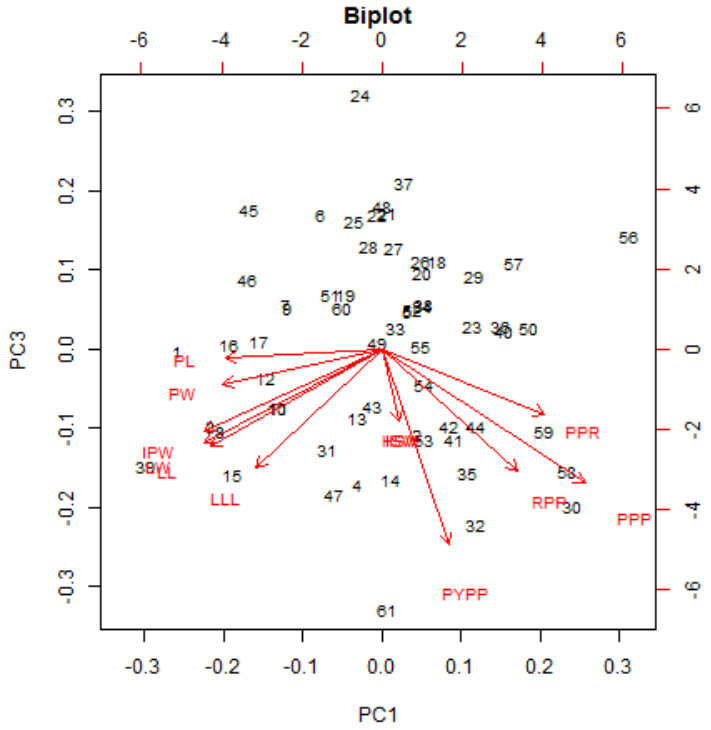

Fig. 3. Principal Components Analysis (PCA) ordination graph. Position of maize advance genotypes from the selected germplasm pools along first and third axes obtained from PCA where LL= leaf length, LW= leaf width, LLL= leaflet length, PL= pod length, PW= pod width, IPW= individual pod weight, PPR= pods per raceme, RPP= racemes per plant, PPP= pods per plant, ISW= individual seed weight (g), HSW= 100 seed weight (g) and PYPP= pod yield per plant (kg).

Conclusion

The study found that Lablab bean genotypes collected from the coastal regions of Bangladesh are distantly related. Therefore, selected genotypes of desirable agronomic traits from these germplasms could be used in hybridization program to produce genotypes with high heterosis. In the PC1 and PC2 biplot graph, it was indicated that pod yield per plant had a strong positive correlation with raceme per plant, pods per plant, and pod length. On the other hand, PC1 and PC3 biplot graph showed pod yield per plant had a positive correlation with raceme per plant, pods per raceme, pods per plant, and hundred seed weight. These results are expected to benefit Lablab bean breeding in Bangladesh as well as aid in the conservation of Lablab germplasm.

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