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GENETIC DIVERGENCE IN YARDLONG BEAN (*VIGNA UNGUICULATA* (L.) WALP. SSP. *SESQUIPEDALIS* VERDC.)

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Abstract

Genetic divergence among 13 commercial yardlong bean (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* Verdc.) genotypes was investigated to select the parents for hybridization using Mahalanobis D^2 statistics. Cluster analysis was used for grouping 13 yardlong bean genotypes. The genotypes fall into four clusters. Cluster III had the maximum (5) and cluster I had the minimum (1) number of genotypes. Cluster III (D^2 = 1.439) had highest intra-cluster distance and the lowest in cluster I (D^2 = 0.000). The inter-cluster divergence ranged from 4.160 to 15.515 between clusters II and III and clusters I and II, respectively. The characteristics i.e. number of pods per plant, number of pods per cluster, days to first flowering, and vegetable pod yield per plant contributed maximum towards divergence among yardlong bean genotypes.

Introduction

Yardlong bean (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* Verdc.) is one of the most important leguminous vegetables grown widely in summer season in Bangladesh. It is well-known as Barboti in Bangladesh. At present yardlong bean is widely grown throughout Asia specially in the South Asian and South East Asian countries. But the production of this vegetable in Bangladesh is much lower than any other Asian countries with a national average of 3.64 MT/ha. (Anon. 2009, Anon. 1993)

It is a highly nutritive vegetable containing high percentage of digestible protein (23.52 - 26.27%) (Ano and Ubochi 2008) along with vitamin A, thiamin, riboflavin, calcium, phosphorus, sodium, potassium, magnesium and a very good source of vitamin C (Anon. 2006). It is also a good source of micronutrients containing 102.69 - 120.02 mg/kg of iron, 32.58 - 36.66 mg/kg of zinc, 2.92 - 3.34 mg/kg of manganese and 0.33 - 0.57 mg/kg of cobalt (Ano and Ubochi 2008). Although it is a highly nutritive summer vegetable, until now no commercial variety of yardlong bean with high yield and better pod quality has been released in Bangladesh and no proper research thrust has been given for the improvement of this vegetable. Better knowledge on genetic diversity or genetic similarity could help to get long term selection gain in plants (Chowdhury *et al.* 2002). Evaluation of genetic diversity is important to know the source of genes for a particular character within the available germplasms (Tomooka 1991).

The use of multivariate analysis for determining the degree of divergence and for assessing the relative contribution of different characters to the total divergence in self-pollinated crops has been established by several workers (Narayanankutty 2003, Kohli and Agarwal 2002, Bansal *et al.* 1999, Golakia and Makne 1992, Natarajan *et al.* 1988). This experiment was carried out to study genetic diversity and selection of suitable genotypes for future hybridization programme.

Materials and Methods

Thirteen commercial genotypes of yardlong bean (*V. uniguiculata* ssp. *sesquipedalis*) collected from different seed companies, Research Institutes and local seed markets were studied

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to measure the diversity among the genotypes. These are the best suited varieties released commercially through selection breeding technique and thus they could be good parents for hybridization programme. The source of genotypes is shown in Table 1. The experiment was laid out in a randomized complete block design (RCBD) with three replications during March, 2011 - June, 2011. The experimental field was divided into 39 plots where genotypes were randomly assigned. The unit plot was 2 m × 2 m in size having 0.5 m wide drains between two adjacent beds and 1 m wide drain surrounding the entire experimental field for proper drainage. Manures and fertilizers were applied as per recommended dose. Recommended agronomic practices were followed to raise a good crop. Weeding was done as and when necessary to keep the crops free from weeds. Flood irrigation was given to the plants when necessary.

Table 1. List of yardlong bean genotypes with their source.

Sl. No.	Genotypes	Place/source of collection
1.	Green Long	Masud Seed Co. (Dhaka)
2.	White Bean	Ripa Seed Co. (Dhaka)
3.	Kegarnatki	Bangladesh Agricultural Development Corporation (Dhaka)
4.	Toki	Lal Teer Seed Industry Ltd. (Dhaka)
5.	Lal Beni	Lal Teer Seed Industry Ltd. (Dhaka)
6.	Saba	Lal Teer Seed Industry Ltd. (Dhaka)
7.	Banalata	Lal Teer Seed Industry Ltd. (Dhaka)
8.	YB-465	Thailand
9.	Shada Sundari	Namdhari Seed Co. Ltd. (Dhaka)
10.	BARI Barboti-1	Bangladesh Agricultural Research Institute (Gazipur)
11.	Kashem King	Kashem Seed Co. (Dhaka)
12.	Sobujsati	Local Seed Market of Chittagong
13.	Lal Barboti	Banashree Agro Seed Co. (Dhaka)

Data on the 18 quantitative characters, namely plant height (cm), number of nodes per plant, number of primary branches per plant, terminal leaflet length (cm), terminal leaflet breadth (cm), days to first flowering, days to 50% flowering, days to 95% pod maturity, length of the harvesting time, length of cluster stalk, number of clusters per plant, number of pods per plant, pod length (cm), pod girth (mm), seed number per pod, 100 seed weight and pod yield per plant (g) were recorded from five randomly selected plants from each plot. Mean data for each character was subjected to multivariate Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO) and Cluster Analysis (CLSA) using GENSTAT 5.13 (Mahalanobis 1936 and Digby *et al.* 1989).

Results and Discussion

Analysis of variances for yield and yield contributing traits showed that the genotypes differed significantly for the traits (Table 2). This indicates that the materials were genotypically divergent. The D^2 value varied widely and principal component scores also revealed a good degree of genetic diversity among the genotypes.

The PCA yielded Eigen values of each principal component axes of ordination of genotypes with the first axes totally accounting for the variation among the genotypes (Table 3). The result revealed that the first axis largely accounted for the variation among the yardlong bean genotypes (29.00%) followed by second axis (27.00%). The first five axes accounted for 87.38% of the total variation among 18 characters describing 13 yardlong bean accessions, while the first two accounted 56.00%.

On the basis of principal scores I, II and III from the principal component analysis, a three dimensional scatter diagram using component score I as X-axis, component score 2 as Y-axis and component score 3 as Z-axis was constructed (Fig. 1). The distribution of genotypes in scattered diagram revealed that considerable diversity exists among the genotypes.

Table 2. Analysis of variance for various quantitative traits in yardlong bean.

Characters	Genotypes (df = 12)	CV%
Plant height (cm)	622.128 **	1.78
Number of nodes per plant	5.280 **	1.69
Number of primary branches per plant	1.885 **	2.08
Terminal leaflet length (cm)	0.891 **	2.07
Terminal leaflet breadth (cm)	0.445 **	1.67
Days to first flowering	25.108 **	0.96
Days to 50% flowering	25.605 **	1.10
Days to 95% pod maturity	23.370 **	1.47
Length of harvesting time (days)	22.790 **	4.67
Length of cluster stalk (cm)	37.972 **	8.30
Number of clusters per plant	4.202 **	6.71
Number of pods per cluster	0.129 **	5.98
Number of pods per plant	58.035 **	5.52
Pod length (cm)	57.490**	1.74
Pod girth (mm)	24.443 **	4.22
Seed number per pod	6.019 **	1.29
100 seed weight (g)	2.507 **	1.31
Pod yield per plant (g)	19660.403 **	5.21

** $p < 0.01$.

Inter-genotypic distance (D^2) were obtained from PCO for all possible combinations between pairs of genotypes. The highest inter-genotype distance was 2.4518 observed between White Bean and Sobujsati followed by the distance 1.8279 and 1.6524 observed between Sobujsati and Lal Barboti, and between White Bean and Kegarnatki, respectively (Table 4). The lowest distance was 0.3348 which was observed between Green Long and Banalata followed by the distance 0.3451 was observed between the Lal Beni and Saba. The difference between the highest and lowest inter-genotype distance indicated the presence of variability among the 13 genotypes of yardlong bean.

The intra-cluster distance was computed by using the values of inter-accession distance from distance matrix according to Singh and Chaudhary (1985). The magnitudes of the intra-cluster distances were not always proportional to the number of genotypes in the clusters, as cluster II, composed of three genotypes, showed higher intra-cluster (0.762) distance than the cluster IV (0.574) containing four genotypes (Table 5). The highest intra-cluster distance was computed for cluster III (1.439) composed of five genotypes followed by cluster II (0.762) and cluster IV (0.574). Cluster I showed zero intra-cluster distance due to containing only one genotype (Table 4).

Table 3. Latent root (Eigen value) and per cent of variation for corresponding quantitative traits in yardlong bean.

Principal component axis	Eigen values	% of total variation	Cumulative per cent
Plant height (cm)	5.221	29.00	29.00
Number of nodes per plant	4.860	27.00	56.00
Number of primary branches per plant	2.307	12.82	68.82
Terminal leaflet length	1.797	9.98	78.80
Terminal leaflet breadth	1.545	8.58	87.38
Days to first flowering	0.964	5.35	92.73
Days to 50% flowering	0.640	3.56	96.29
Days to 95% pod maturity	0.279	1.55	97.84
Length of harvesting time (days)	0.179	0.99	98.83
Number of clusters per plant	0.118	0.65	99.48
Length of cluster stalk (cm)	0.068	0.38	99.86
Number of pods per cluster	0.024	0.13	99.99
Number of pods per plant	0	0	99.99
Pod length (cm)	0	0	99.99
Pod girth (mm)	0	0	99.99
Seed number per pod	0	0	99.99
100 seed weight (g)	0	0	99.99
Vegetable pod yield per plant (g)	0	0	99.99

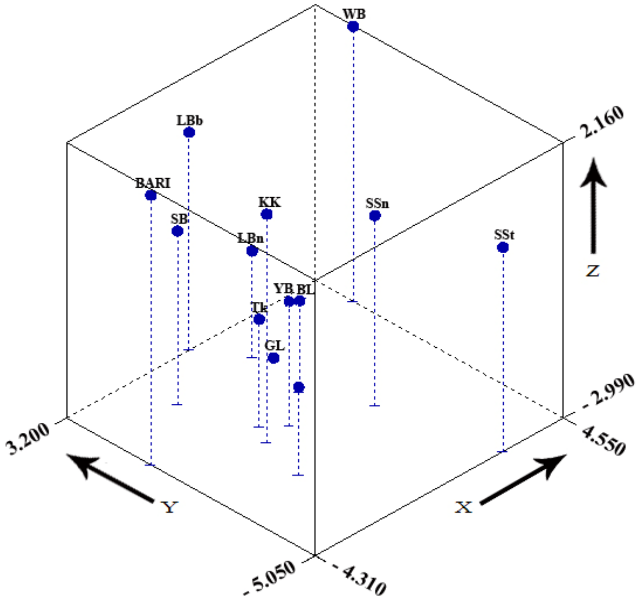

Fig. 1. Scattered diagram of 13 yardlong bean genotypes based on their principal component score. GL = Green Long, WB = White Bean, Kgn = Kegarnatki, Tk = Toki, LBn = Lal Beni, Sb = Saba, Bl = Banalata, YB = YB-465, SS = Shada Sundori, BARI = BARI Barboti-1, KK = Kashem King, S = Sobujsati, LBb = Lal Barboti.

The inter-cluster distances were calculated by averaging all possible D^2 values among all genotypes belonging to different clusters concerned, divider being the number of pairs involved. The values of inter-cluster distance (D^2) are presented in Table 4. Inter-cluster distances represent the index of genetic diversity among the clusters. The values of inter-cluster distances were larger than the intra-cluster distances suggesting wider genetic diversity among the genotypes of different groups (Tables 4, 5).

Table 4. Ten of each higher and lower inter-genotypes distances between pairs of genotypes.

Sl. No.	Genotypic combination	Distance (D^2) Value	Sl. No.	Genotypic combination	Distance (D^2) Value
Higher-inter genotypic distances			**Lower-inter genotypic distances**		
1.	White Bean and Sobujsati	2.4518	1.	Green Long and Banalata	0.3348
2.	Sobujsati and Lal Barboti	1.8279	2.	Lal Beni and Saba	0.3451
3.	White Bean and Kegarnatki	1.6524	3.	Banalata and YB-465	0.3674
4.	White Bean and Kashem King	1.2754	4.	Kegarnatki and YB-465	0.3727
5.	White Bean and YB-465	1.0959	5.	Saba and Banalata	0.4151
6.	White Bean and Banalata	1.0871	6.	Kegarnatki and Toki	0.4277
7.	Kegarnatki and Lal Barboti	1.0512	7.	Saba and BARI Barboti-1	0.4296
8,	Green Long and White Bean	1.0468	8,	Kegarnatki and Kashem King	0.4309
9.	Lal Beni and Sobujsati	0.9855	9.	Kegarnatki and Shada Sundori	0.4398
10.	White Bean and BARI Barboti-1	0.9830	10.	Toki and Shada Sundori	0.4416

Table 5. Average intra - (bold face) and inter-cluster distance (D^2) for 13 yardlong bean genotypes.

Clusters	I	II	III	IV
I	**0.000**			
II	15.515	**0.574**		
III	12.335	4.160	**1.439**	
IV	9.245	7.765	4.234	**0.762**

The clusters I and II were more diverse as indicated by maximum inter-cluster distances between them (15.515, Table 5) followed by the distance between clusters I and III (12.335), I and IV (9.245), and between II and IV (7.765). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster I were far away from those of cluster II. Similarly, the higher inter-cluster values between clusters I and III, I and IV and II and IV indicated that the genotypes belonging to each pair of these clusters were far diverse. The minimum inter-cluster distance was observed between clusters II and III (4.160) indicating that the genotypes of these clusters were genetically closed. Higher inter- and intra-cluster distances indicate higher genetic variability among genotypes between and within clusters, respectively. The minimum inter- and intra-cluster distance indicates closeness among the genotypes of two clusters and within the clusters also. These relationships were also reflected in the scattered diagram (Fig. 1).

By applying non-hierarchical clustering using covariance matrix, 13 genotypes of yardlong bean were grouped into 4 different clusters (Table 6). Cluster III comprised of maximum number of genotypes (5) followed by cluster IV (4) and cluster II (3). While cluster I comprised of only one genotype. These results were confirmatory with the clustering pattern of the genotypes obtained through principal component analysis. The clustering pattern of the genotypes under this study revealed that the genotypes collected from two different countries were grouped into same clusters and the genotypes collected from same district were grouped into different clusters. As for example, YB-465 collected from Thailand and Green Long, Banalata, Saba and BARI Barboti-1 collected from Bangladesh were grouped into same cluster (cluster III). Whereas genotypes collected from Gazipur district (BARI Barboti-1 and Toki) were grouped into 2 clusters (cluster III and cluster IV) in spite of being collected from the same district. Similar opinions were given by Renganayaki and Sreerengasamy (1991) and Kohli and Agarwal (2002) in cowpea, Masud *et al.* (1995) in pumpkin and Singh and Singh (1979) in okra. It indicates that the clustering pattern did not follow their geoclimatic zonal distribution and taxonomic labels, suggesting that factors other than regional boundaries and taxonomic characters are also responsible for divergence. Murthy and Anand (1966) stated that genetic drift and selection in different environment could cause greater diversity than geographic distance.

Table 6. Distribution of 13 yardlong bean genotypes in four different clusters.

Cluster	No. of genotypes	Genotypes with original place of collection
I	1	Sobujsati (Chittagong)
II	3	White bean (Dhaka), Lal Beni (Dhaka) and Lal Barboti (Dhaka)
III	5	Green Long (Dhaka), Saba (Dhaka), Banalata (Dhaka), YB-465 (Thailand) and Bari Barboti - 1 (Gazipur).
IV	4	Kegarnatki (Dhaka), Toki (Gazipur), Shada Sundori (Dhaka) and Kashem King (Dhaka).

The genotypes from cluster I earned the highest cluster mean value for length of harvesting time (33.40), number of clusters per plant (55.09), number of pods per cluster (6.33), pod girth (27.2), seed number per pod (21.4), 100 seed weight (13.12) and vegetable pod yield per plant (508.51), but minimum cluster mean for days to first flowering (42.33), days to 50% flowering (45.13) and days to 95% pod maturity (50.33) (Table 7). On the other hand, cluster II integrated three genotypes produced highest mean for plant height (cm) (320.48) and terminal leaflet length (10.95) but lowest mean for length of harvesting time (30.47). Cluster III had the highest mean value for days to first flowering (48.57), days to 50% flowering (51.08) and days to 95% pod maturity (56.01). While cluster IV produced maximum mean for length of cluster stalk (25.17) and number of pods per plant (35.24).

The first position in respect to number of cluster per plant, number of pods per cluster, number of pods per plant, pod length (cm), pod girth (mm) and vegetable pod yield per plant (g) was established in the cluster I. These characters should be taken under consideration in breeding programme as suggested by Jana *et al.* (1983), Chattopadhyay *et al.* (1997), Resmi (1998), Vardhan and Savithramma (1998) and Vidya *et al.* (2002). The second position was occupied by cluster IV in respect to these characters. Again from the cluster mean value, cluster I was recorded as early flowering and early maturing with prolonged harvesting time and higher yield but cluster IV was recorded as moderately late flowering and late maturing with remarkable yield. So, to develop high yielding varieties, genotypes of these two groups can be used further in hybridization programme.

The PCA revealed that in vector I (Z_1), the most important characters responsible for genetic divergence in the major axis of differentiation were number of nodes per plant (0.4798), number of primary branches per plant (0.3746), days to first flowering (0.1989), number of pods per plant (0.1112), number of pods per cluster (0.0778), plant height (0.0756), length of cluster stalk (0.0193) and vegetable pod yield per plant (0.0304) (Table 8). In vector II, which was second axis of differentiation, number of pods per cluster (0.6873), number of pods per plant (0.5917), 100 seed weight (0.1498), days to first flowering (0.1102), length of cluster stalk (0.0693), seed number per pod (0.0272), vegetable pod yield per plant (0.0239), plant height (0.0172), pod length

Table 7. Cluster mean values for various quantitative characters in yardlong bean.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV
Plant height (cm)	289.84	320.48	318.09	315.43
Number of nodes per plant	12.33	13.11	14.56	15.03
Number of primary branches per plant	6.47	6.31	7.07	7.12
Terminal leaflet length	10.44	10.95	10.58	10.68
Terminal leaflet breadth	3.61	4.38	4.39	4.31
Days to first flowering	42.33	45.87	47.95	48.25
Days to 50% flowering	45.13	48.40	51.08	50.03
Days to 95% pod maturity	50.33	53.24	56.01	54.82
Length of harvesting time (days)	33.40	30.47	33.27	33.18
Number of clusters per plant	55.09	44.69	44.55	49.87
Length of cluster stalk (cm)	22.98	22.52	22.02	25.16
Number of pods per cluster	6.33	2.98	4.44	4.75
Number of pods per plant	30.44	29.53	30.42	35.24
Pod length (cm)	3.13	2.75	3.02	3.13
Pod girth (mm)	27.20	15.11	22.31	21.06
Seed number per pod	21.40	18.08	17.74	19.51
100 seed weight (g)	13.12	12.10	12.01	12.46
Vegetable pod yield per plant (g)	508.51	230.94	321.53	388.74

Table 8. Latent vectors for various principal component characters of yardlong bean.

Characters	Vector I (Z_1)	Vector II (Z_2)
Plant height (cm)	0.0756	0.0172
Number of nodes per plant	0.4798	–0.8003
Number of primary branches per plant	0.3746	–0.6049
Terminal leaflet length	–1.2239	–0.1217
Terminal leaflet breadth	1.3638	–0.0592
Days to first flowering	0.1989	0.1102
Days to 50% flowering	–0.0755	0.0012
Days to 95% pod maturity	–0.2827	–0.3293
Length of harvesting time (days)	–0.1609	–0.2297
Number of clusters per plant	–0.0384	–0.0209
Length of cluster stalk (cm)	0.0193	0.0693
Number of pods per cluster	0.0778	0.6873
Number of pods per plant	0.1112	0.5917
Pod length (cm)	–3.97	0.0081
Pod girth (mm)	–0.0414	–0.031
Seed number per pod	–0.3362	0.0272
100 seed weight (g)	–0.4254	0.1498
Vegetable pod yield per plant (g)	0.0304	0.0239

(0.0081) and days to 50% flowering (0.0012). The role of number of pods per cluster, days to first flowering, vegetable pod yield per plant and number of pods per plant for both the vectors were high and positive across two axes indicating the important components of genetic divergence in these genotypes.

Considering the magnitude of genetic distance, contribution of characters towards the divergence and magnitude of cluster means for different characters, the following genotypes presented in the Table 9 were considered to perform better if used in hybridization programme. The genotypes Sobujsati of cluster I could be selected for earliness, number of pods per cluster and vegetable pod yield per plant. The genotypes Kegarnatki, Toki, Shada Sundori and Kashem King of the cluster IV could he selected for number of pods per plant.

Table 9. Finally selected genotypes for important traits.

Sl. No.	Selection traits	Genotypes	Cluster form	Mean value
1.	Days to first flowering (earliness)	Sobujsati	I	42.33
2.	Number of pods per cluster	Sobujsati	I	6.33
3.	Number of pods per plant	Keagarnatki, Toki, Shada Sundori and Kashem King	IV	35.24
4.	Vegetable pod yield per plant (g)	Sobujsati	I	508.51

Vegetable pod yield is the principal character in yardlong bean in terms of economic return. This is a complex quantitative character. So, the selection for desirable types should not only be based on yield, the other yield components should also be considered. Direct selection for yield is often misleading in yardlong bean because vegetable pod yield is polygenically controlled. Thus, knowledge about the degree of interrelationship that exists among different component characters and with vegetable pod yield is important for devising an efficient selection criterion for fruit yield and a basis for planning and efficient breeding programme future.

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