GENETIC DIVERGENCE AMONG INBRED LINES OF PUMPKIN
(*Cucurbita moschata* Duch Ex Poir)

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ABSTRACT

Genetic divergence among nineteen inbred lines of pumpkin (*Cucurbita moschata* Duch Ex Poir) was estimated through principal component analysis and Mohalanobis's D^2 analysis. Inbred lines were grouped into five clusters. Cluster I, II, III, IV and V were composed of five, three, four, six and one inbred lines respectively. Maximum inter cluster distance was observed between the cluster III and V (72.69) followed by the cluster I and V (63.33). Minimum inter cluster distance was observed between cluster II and IV (10.64). The mean value of cluster III indicated the importance of days to first male and female flower open, node order to 1st female flower open and TSS. Cluster IV showed the importance of days to first female flower open, fruit diameter, cavity diameter, flesh thickness and average fruit weight. Similarly, cluster V indicated the importance of yield per plant, fruits per plant and fruit length. Days to first male and female flower open, fruit length and cavity length had the highest contribution towards divergence among the inbred lines. Considering the magnitude of cluster distance, cluster means for different characters and contribution of characters towards divergence, the inbred lines from clusters I, III and IV could be selected as parent for hybridization program.

Keywords: Pumpkin, PCA, D^2 – statistics, genetic, diversity, yield

INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch. Ex Poir.) is one of the major cucurbitaceous vegetables grown all over Bangladesh. The crop is variously known as 'Misti kumra' or 'Misti lau' or 'Misti kadu' in different parts of Bangladesh. It is consumed by most of the people of the country. It is popular among the people owing to its good taste, high nutritive value, good storability and long time of availability and better transport potentialities (Hazra *et al.*, 2007; Rashid, 1999). About 14% (8% in *rabi* and 6% in *kharif* season) of the total vegetable production comes from pumpkin (BBS, 2013). It ranks 3rd in respect of both area and production next to brinjal and radish. It occupies an area of 27,500 ha with an annual production of 2,18,000 tons accounting to an average yield of 7.93 t/ha (BBS, 2013).

Pumpkin is a monoecious crop and has high cross pollination mechanism. Due to its out crossing characteristics, diverse type of genotypes are always generating in this

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crop. The desirable variations particularly for fruit types and quality have been preserved by the growers. Along with fruit morphotypes, variations for other plant characters are also available. Multivariate analysis is a useful tool in quantifying the degree of genotypic divergence in a biological population like an inbred population of pumpkin. It helps to assess the relative contribution of different components to the total divergence both at inter and intra-cluster levels (Das and Gupta, 1984). Efficient use of such genetically diverse parents has a bright scope for the development of high yielding open pollinated and hybrid pumpkin variety. From the plant breeding point of view the degree or extent of genetic diversity between two parents is an index of determining the hybridity over parents or nature of segregates in the follow-up generations. Keeping the importance of the above facts in mind, the present study was undertaken to analyze the genetic divergence of nineteen pumpkin inbred lines.

MATERIALS AND METHODS
Nineteen pumpkin inbred lines (PK01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19) were used in this study. These inbred lines were received from the breeding program of Olericulture Division, Horticulture Research Centre (HRC), BARI. The experiment was setup at the Experimental field of Olericulture Division, HRC, Gazipur during October, 2011 to May, 2012. The experiment was conducted in RCBD with three replications. Twenty five days old seedlings were transplanted in well prepared experimental plot. Fertilizers were applied @ 5000-35-75-18-4.3-2 kg/ha of cowdung-N-P-K-S-Zn-B. The sources of N, P, K, S, Zn and B were urea, TSP, MoP, gypsum, zinc sulphate and boric acid (laboratory grade). The entire amount of cowdung, P, S, Zn, B and $1/3^{rd}$ of K were applied during pit preparation as basal. The rest of K was applied in two equal installments at 20 and 35 days after transplanting (DAT). N was applied in four equal installments at 7, 20, 35 and 50 DAT (Anon., 2012).

Necessary intercultural operations were done during the crop period for proper growth and development. Observations were recorded from all the plants in each plot for 13 characters. Data were recorded for days to 1st male and female flower open, node order of 1st male female flower open, fruit length, fruit diameter, cavity length, cavity diameter, flesh thickness, average fruit weight, fruits per plant, yield per plant and TSS (%). Genetic diversity was studied following Mohalanobis's (1936) generalized distance (D^2) extended by Rao (1952). Clustering of genotypes was done according to Tocher's method (Rao, 1952) and Principal Component Analysis (PCA) for graphical representation of the genotypes. All the statistical analysis was carried out using GENSTAT 5 software.]

RESULTS AND DISCUSSION
Analysis of variance showed significant differences among the inbred lines for all the characters under study indicating the presence of notable genetic variability in these lines. The D^2 value varied widely and principal component scores also revealed a good degree of genetic diversity among the lines.

Principal component analysis (PCA)
Eigen values of thirteen principal axis and percentage of total variation accounting for them obtained from the principal component analysis are presented in Table 1. The results revealed that the first axis largely accounted for variation among the inbred lines (28.01%) followed by second axis (24.56%). The first six axis accounted for 92.45% of the total variation among the thirteen characters describing in nineteen pumpkin inbred lines while the first two accounted for 52.57%.

Table 1. Eigen values and percent of variation for corresponding 13 characters in 19 pumpkin inbred lines

Principal component axis	Eigen values	%	
		Total variation accounted for	Cumulative
Days to 1st male flower open	8.96	28.01	28.01
Days to 1st female flower open	1.78	24.56	52.57
Node order of 1st male flower open	0.99	15.01	67.58
Node order 1st female flower open	0.45	11.86	79.44
Fruit length (cm)	0.36	7.09	86.53
Fruit diameter (cm)	0.28	5.92	92.45
Cavity length (cm)	0.06	3.09	95.54
Cavity diameter (cm)	0.04	2.34	97.88
Flesh thickness (cm)	0.02	1.49	99.37
Average fruit weight (kg)	0.01	0.38	99.75
Fruits per plant	0.00	0.14	99.89
Yield per plant (kg)	0.00	0.08	99.97
TSS (%)	0.00	0.03	100

Cluster Analysis

Nineteen inbred lines were grouped into five different clusters (I - V) on the basis of D^2 analysis (Table 2). Cluster IV (6) had maximum inbred lines closely followed by cluster I (5) and cluster III (4). The highest intra-cluster distance (0.45) was recorded in cluster III containing four inbred lines. The second highest intra-cluster distance (0.29) was recorded cluster II and composed three inbred lines. The cluster IV contains six inbred lines and the cluster I contains five inbred lines. The cluster V contains only one inbred. Rasheed (2000) reported 7 clusters among 47 and Zahan (2009) reported 6 clusters among 49 pumpkin genotypes while Masud *et al*. (2003) reported 9 clusters among 46 pumpkin genotypes.

Table 2. Distribution of 19 pumpkin inbred lines in five clusters

Cluster	No. of inbred lines	Inbred lines
I	5	PK01,PK04, PK05, PK11,Pk19
II	3	PK14, PK16, PK18
III	4	PK08, PK09, PK15, PK17
IV	6	PK03, PK06, PK07, PK10, PK12, PK13
V	1	PK02

Principal coordinate analysis (PCO)

Ten of each lowers and higher inter-genotypic distance between pair of genotypes are presented in Table 3. The highest inter-genotypic distance was 2.63 which was observed between the inbred lines PK17 vs PK02. The lowest inter-genotypic distance 0.31 was observed between the indreds PK12 vs PK11. The difference between the highest and the lowest inter genotypic distance indicated the existence of variability among the nineteen pumpkin inbred lines.

Canonical variate analysis (CVA)

The intra and inter- cluster values among the five clusters are presented in Table 4. Statistical distance represents the index of genetic diversity among the clusters. The inter cluster distance were larger than the intra cluster distances suggesting wider genetic

diversity among the genotypes of different groups. Quamruzzaman *et al.* (2008) obtained larger inter cluster distance than the intra cluster distance in a multivariate analysis in bottle gourd, bitter gourd and ridge gourd while Masud *et al.* (2003) reported similar findings in pumpkin. The inter - cluster D^2 values varied from 10.64 to 72.69 indicating wide diversity among the inbred lines. The maximum inter-cluster distance was noted between cluster III and V (72.69) followed by cluster I and V (63.33). It is noted that the inbred lines of these clusters were highly divergent from each other. The intra cluster distance varied from 0.0 to 0.45, maximum being from cluster III (0.45) that comprised of four inbred lines while the minimum distance was observed in cluster V (0.0) that comprised of only one inbred lines.

Table 3. Ten of each lower and higher inter-genotypic distance between pair of inbred lines

10 lower D^2 values	Inbred lines combinations	10 higher D^2 values	Inbred lines combinations
0.31	PK12 vs PK11	2.63	PK17 vs PK02
0.35	PK19 vs PK04	2.08	PK17 vs PK07
0.35	PK05 vs PK 01	2.06	PK09 vs PK02
0.36	PK19 vs PK12	2.02	PK17 vs PK16
0.36	PK19 vs PK11	1.97	PK17 vs PK03
0.40	PK18 vs PK16	1.93	PK08 vs PK02
0.40	PK10 vs PK06	1.83	PK11 vs PK02
0.42	PK13 vs PK 12	1.80	PK17 vs PK06
0.45	PK18 vs PK 06	1.79	PK17 vs PK19
0.47	PK05 vs PK 04	1.71	PK18 vs PK17

Selection of parents from the highly divergent clusters is expected to manifest high heterosis in hybridization and to show wide variability in genetic architecture. Lower inter - cluster distances were observed between cluster II and IV (10.64), cluster I and III (10.81) and cluster I and II (17.92) suggesting that these cluster pairs had close relationship among themselves. The inter cluster distance were greater than intra cluster distance, revealing considerable amount of genetic diversity among the inbred lines studied (Table 4). Rasheed (2000) reported that the highest inter-cluster distance exists between cluster V and VI (11.30) and the lowest between the cluster III and VI (3.71). Masud *et al*. (2003) reported maximum inter - cluster distance (72.20) was observed between the genotypes of cluster II and VII and minimum inter cluster distance between clusters I and VI (12.73).

Table 4. Average inter and intra (bold) cluster distance (D^2) for 19 pumpkin inbred lines

Cluster	I	II	III	IV	V
I	**0.26**				
II	17.92	**0.30**			
III	10.81	26.51	**0.45**		
IV	22.54	10.64	27.49	**0.29**	
V	63.33	46.19	72.69	48.98	**0.00**

A comparison of five cluster means for thirteen characters is presented in Table 5. Differences in cluster means were noticed in number of characters. The maximum range of variability was recorded for fruit length (12.41 – 38.65 cm) among all the

characters in five clusters. Cluster II had the highest mean values for node order of 1st male flower open (7.46) and days to 1st male flower open (80.92). This cluster comprised late male flowering inbred lines. Cluster IV included inbred lines with the highest mean values for fruit diameter (22.89 cm), average fruit weight (4.39 kg), flesh thickness (4.14 cm) and cavity diameter (13.71 cm). Besides, this cluster took minimum days to 1st male flower open (76.49) and days to 1st female flower open (85.92) which indicated earliness. Thus, cluster IV possessed inbred lines with maximum desirable qualities for selection as parents. It was observed that the inbred lines under cluster III had the least mean values for yield per plant (9.34 kg), average fruit weight (2.08 kg), flesh thickness (2.96 cm), fruit length (12.41 cm) and node order of 1st male flower open (6.28). The inbred lines under this cluster were also more or less early type (76.75 days for 1st male flower open and 86.85 days for 1st female flower open). Inbred lines of cluster V had maximum mean values for fruit length (38.65 cm), fruits per plant (5.13), yield per plant (19.17 kg) and cavity length (30.33 cm). The maximum mean values for TSS (9.04 %) were noted in cluster II. Endang *et al*. (1971) stated that the clustering pattern could be utilized for choosing parents for cross combinations which likely to generate the highest possible variability for effective selection of various economic traits. Under such conditions, Choudhury *et al*. (1975) suggested that selection for one type from each cluster and testing them by a series of diallel analysis may prove to be highly fruitful.

Table 5. Cluster mean values for 13 characters of 19 pumpkin inbred lines

Characters	Clusters				
	I	II	III	IV	V
Days to 1st male flower open	80.74	80.92	76.75	76.49	78.25
Days to 1st female flower open	91.68	89.16	86.85	85.92	88.00
Node order of 1st male flower open	6.31	7.46	6.28	6.88	5.50
Node order of 1st female flower open	20.18	18.59	19.19	19.63	17.25
Fruit length (cm)	15.50	22.95	12.41	19.01	38.65
Fruit diameter (cm)	20.20	18.61	18.00	22.89	14.04
Cavity length (cm)	8.29	14.16	6.44	10.47	30.33
Cavity diameter (cm)	12.59	12.83	11.49	13.71	6.96
Flesh thickness (cm)	3.44	3.29	2.96	4.14	3.49
Average fruit weight (kg)	3.07	3.30	2.08	4.39	3.73
Fruits per plant	4.55	4.25	4.54	4.15	5.13
Yield per plant (kg)	13.77	14.13	9.34	17.98	19.17
TSS (%)	8.71	9.04	8.90	8.84	8.13

Contribution of characters towards divergence of the inbred lines

Contribution of characters towards divergence was obtained from canonical variate analysis (CVA) and is presented in Table 6. It is noted that the important characters responsible for genetic divergence in the major axis of differentiation were days to 1st male flower open (0.32), days to 1st female flower open (0.32), node order of 1st male flower open (0.28), node order of 1st female flower open (0.32), fruit length (0.26) and fruit diameter (0.25) in vector I. However, cavity length (0.07) also contributed moderately to divergence in vector I. Vector 2, the second axis of differentiating the characters, where fruit length (0.42), cavity length (0.63), average fruit weight (0.28) and yield per plant (0.56) played a significant role towards genetic divergence. It was observed that days to 1st male flower open, days to 1st female flower open, fruit length and cavity length in both the vectors exhibited important components of divergence. These results indicated that these four characters had maximum

contribution towards the divergence among 19 pumpkin inbred lines. Negative values in both the vectors indicated that these characters had the lowest contribution to the total divergence. Due importance should be put on these characters to increase productivity in pumpkin. Masud *et al*. (1995) reported that number of fruits per plant and yield per plant had considerable contributions to divergence.

Table 6. Latent vectors for 13 characters of 19 pumpkin inbred lines

Characters	Vector 1	Vector 2
Days to 1st male flower open	0.32	0.03
Days to 1st female flower open	0.32	0.02
Node order of 1st male flower open	0.28	-0.11
Node order of 1st female flower open	0.32	-0.02
Fruit length (cm)	0.26	0.42
Fruit diameter (cm)	0.25	-0.11
Cavity length (cm)	0.07	0.63
Cavity diameter (cm)	-0.32	-0.03
Flesh thickness (cm)	-0.32	0.01
Average fruit weight (kg)	-0.25	0.28
Fruits per plant	-0.32	0.03
Yield per plant (kg)	-0.06	0.56
TSS (%)	-0.32	-0.01

CONCLUSION

Considering the magnitude of cluster distance, cluster means for different characters, contribution of characters towards divergence, for quantitative and qualitative traits superior inbred lines like PK01 from cluster I, PK08 and PK09 from cluster III, and the PK06, PK07 and PK10 from cluster IV were selected for future hybridization program.

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