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GENETIC DIVERSITY IN SNAKE GOURD (*Trichosanthes cucumerina var. anguina* L.)

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Abstract

Multivariate analysis of fifty five genotypes of snake gourd was performed at the experimental field of Horticulture Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh from March to June, 2017 to estimate genetic diversity and choosing the potential parents for a successful hybridization program. The genotypes are grouped into six clusters according to an analysis of Principal Component Analysis (PCA) and Mahalanobis D^2. The highest inter-cluster distance was noted between clusters I and VI (205.66) and the lowest between clusters II and V (38.87). The maximum intra-cluster distance was in cluster V (31.23) while the minimum was in cluster VI (15.00). Among the six clusters the IV was the most important cluster, which contains most of the desirable parent selection characters. Taking into account the magnitude of genetic divergence, cluster distance and agronomic performance, 7 parents could be selected from the clusters for the future hybridization program. Crossing among the genotypes of the selected clusters would produce high heterotic progeny.

Keywords: Snake gourd, genetic diversity, principal component analysis, cluster analysis, principal coordinate analysis, canonical variate analysis.

Introduction

As a monoecious vegetable snake gourd (*Trichosanthes cucumerina var. anguina* L.) has a strong function of cross-pollination. Variability in this crop is always generated due to its outcrossing characteristics. Genetic diversity is an essential element of any crop improvement programme. This offers breeder the opportunity to combine desired genes into novel genotypes to improve the yield and stability of economically important crop plants. Therefore, under the existing environment, evaluation of germplasm is essential for selecting donors for features to be improved in the breeding program. The key constraints for achieving higher yields are inherently low yield potential of varieties, lacking genetic diversity, inefficient plant type and low yield potential, lack of suitable ideotypes for different cropping systems, poor harvest index, low crop management level, increased weed competition and susceptibility to biotic and abiotic stress (Srinives, 2006). The main problem in our existing varieties are low

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yield, lacking genetic diversity and the national average was 5.02 MT /ha (BBS, 2020). The figure shows that our cultivars have poor yielding capacity compare to other snake gourd growing countries (Ara *et al.*, 2013). Selection of high yielding parents with diversity for hybridization program may solve the existing problem. Yield is a dynamic, polygene-controlled trait that is highly sensitive to environmental factors and is therefore, not an efficient selection character. The effectiveness of selection depends on the existence and magnitude of the genetic variability in the breeding material for yield and yield contributing traits. Genetic diversity estimation is considered an important factor, which is also an essential prerequisite for the hybridization program in order to develop a high yielding variety. However, reliable knowledge on the degree of genetic divergence and character variability used for population differentiation is essential in any crop enhancement system (Ananda and Rawat, 1984; De *et al*., 1988). With the assistance of advanced biometric techniques, such as multivariate analysis (Rao, 1952) based on Mahalanobis' (1936) D^2 statistics, it has become possible to measure the magnitude of genetic variation between germplasm. Mahalanobis' D^2 statistics are an important method in quantifying the degree of genotype heterogeneity. The utility of multivariate analysis was strongly emphasized by Murty and Arunachalam (1966). Researches on genetic diversity, clustering patterns and the relative contribution of different characters to divergence and selection effectiveness were done by several researchers (Venkateswarlu, 2001; Manivannan, 2002; Bisht *et al*., 2005). Considering the above, genetic divergence information in these genotypes would help selecting potential parental materials which could be used developing a strong breeding program. The research was conducted to determine the degree of genetic variation among genotypes available and to choose different parents to conduct future genetic study.

Materials and Methods

A research work was conducted at the experimental field of Horticulture Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh from March to June, 2017 to study the genetic diversity. Fifty-five genotypes of snake gourd were collected from the Plant Genetic Resources Centre, BARI, Genetics and Plant Breeding Department of BSMRAU, different seed companies and various parts of Bangladesh. Seventeen days old seedlings were transplanted following a Randomized Complete Block Design (RCBD) with three replications in experimental plot on 10 April, 2017. Fertilizers were applied @ 5000-100.0-24.0-84.0-15.0-1.0-0.80 kg /ha of cowdung-N-P-K-S-Zn-B according to Fertilizer Recommendation Guide (FRG, 2012). The sources of N, P, K, S, Zn and B were Urea, Triple Super Phosphate (TSP), Muriate of Potash (MoP), Gypsum, Zinc Sulphate, Boric Acid (laboratory

grade). The entire amount of cowdung, P, S, Zn, B and one-third of K were applied during final land preparation as well as N and the rest of K were applied into four equal installments at 7, 21, 35, 49 days after transplanting. Necessary intercultural operations were done during the crop period for proper growth and development. The observations were recorded from all the plants in each plot for 13 characters. Mean data were subjected to multivariate methods of analysis for each character viz. Principal Component Analysis (PCA), Principal Coordinate Analysis (PCA), Canonical Variate Analysis (CVA) and Cluster Analysis (CLSA) using GENSTST 5 software. Principal components were derived from the matrix of correlation and the genotypical scores obtained for the first successor variable with latent roots greater than unity (Jager *et al.* 1983). Though using all dimensions of P, using the similarity matrix, it gives the minimum distance between each pair of N points (Digby *et al.* 1989). According to Singh and Chaudhury (1985), inter-cluster distance was determined using the values of inter-genotype distance from the PCO distance matrix. The complementary CVA to D^2 statistics is a kind of multivariate analysis in which canonical vectors and roots describing various axes of differentiation and the amount of variance accounted for by the axes are obtained, respectively. Cluster analysis was carried out using D^2 analysis (originally outlined by Mahalanobis in 1936 and expanded by Rao in 1952), which divides the genotypes into more or less homogeneous groups based on the data set. D^2 is the sum of squares of differences for each of the uncorrelated variables between any two populations (achieved through the transformation of associated variables by key condensation process). Clustering was done using non-hierarchical ranking.

Results and Discussion

Principal Component Analysis (PCA)

In this experiment, thirteen characters were taken into account for the study of genetic diversity. Table 1 presents eigen values of the thirteen principal axes and the percentage of total variance accounting for them derived from the study of the main variable. The findings showed that the first axis largely accounted for the genotypical variability (31.48%) followed by the second axis (15.53%). The first four axes (69.50%) accounted for the overall difference between the thirteen characters representing fifty five snake gourd genotypes, while the first three accounted for 60.10%. Ahmed *et al*. (2016) reported the first two components representing 52.57% of the total variation among the thirteen characters describing nineteen inbred lines of the pumpkin. Rasul and Okubo (2002) stated that 63.75% of the first two components and 73.38% of the overall variance was experienced in that of the three components.

Table 1. Eigen values and percentage of variation for corresponding 13 characters in 55 snake gourd genotypes

Principal component axis	Eigen values	% variation	Cumulative % variation
Days to male flower open	4.093	31.48	31.48
Days to female flower open	2.018	15.53	47.01
Node order of first male flower open	1.699	13.09	60.10
Node order of first female flower open	1.222	9.40	69.50
Main vine length (cm)	0.889	6.83	76.33
Number of nodes on main vine	0.779	5.99	82.32
Number of primary branches	0.658	5.06	87.38
Number of fruits / plant	0.539	4.14	91.52
Marketable yield / plant (kg)	0.463	3.56	95.08
Individual fruit weight (g)	0.272	2.08	97.16
Fruit length (cm)	0.237	1.82	98.98
Fruit diameter (cm)	0.119	0.92	99.90
100-seed weight (g)	0.012	0.10	100.00

Table 2. Factor loadings for corresponding 13 characters in 55 snake gourd genotypes

Characters	PC1	PC2	PC3	PC4
Days to male flower open	0.299	-0.267	0.256	-0.075
Days to female flower open	0.280	-0.318	0.254	0.1641
Node order of first male flower open	0.267	-0.320	-0.031	-0.193
Node order of first female flower open	0.231	-0.432	-0.200	0.165
Main vine length (cm)	-0.344	-0.141	0.272	0.323
Number of nodes on main vine	-0.126	-0.250	0.387	0.102
Number of primary branches	-0.278	-0.131	0.211	0.395
Number of fruits / plant	-0.391	0.146	-0.019	-0.098
Marketable yield / plant (kg)	-0.437	-0.131	-0.050	-0.190
Individual fruit weight (g)	-0.283	-0.477	-0.067	-0.276
Fruit length (cm)	-0.261	-0.394	-0.304	-0.004
Fruit diameter (cm)	-0.033	-0.023	0.364	-0.712
100-seed weight (g)	0.0142	-0.120	-0.571	-0.037
Standard deviation	2.023	1.420	1.303	1.105

The factor loadings of the characters from PCA retained four components identified as the major characters responsible for the maximum variability (Table 2). A five-factor loading was positively correlated with the respective characters and the rest eight were negatively correlated with the principal component I (PC1).

In particular, the principal component I (PCI) can be considered as the most significant for days to 1st male flower open (0.299) followed by days to 1st female flower open (0.280), node number to 1st male flower open (0.267) and node number to 1st female flower open (0.231). All factor loading was negatively correlated except fruits/plant (0.146) with the respective characters in principal component II (PC2). The characters associated with the principal component III (PC3) depicted in nodes on main vine (0.387), fruit diameter (0.365), main vine length (0.272), days to 1st male flower open (0.256) and days to 1st female flower open (0.255) as well as primary branches/plant (0.396) and main vine length (0.324) as an important parameter for variation in the principal component IV (PC4). Kundu *et al.* (2019) stated that the principal component I (PC1) could be regarded as a portion of plant height, leaf, indicated by high plant height loads at 60 days following planting (0.983), leaf length (0.133) and leaf width (0.099). Principal component II (PC2) indicated the importance of tuber yield and foliage coverage. Strong loadings for foliage cover (0.113), tuber weight per hill (0.015) and tuber yield (0.967) were observed. Principal component III (PC3) indicated the importance of the number of leaflets and tuber per hill. High loadings for plant vigor (0.113), terminal blade length (0.150), lateral blade length (0.141) and tuber number per hill (0.196) were observed. On the other hand, the principal component IV (PC4) suggested the value of midrib pairs of days to 80% emergence and secondary frequency leaflets.

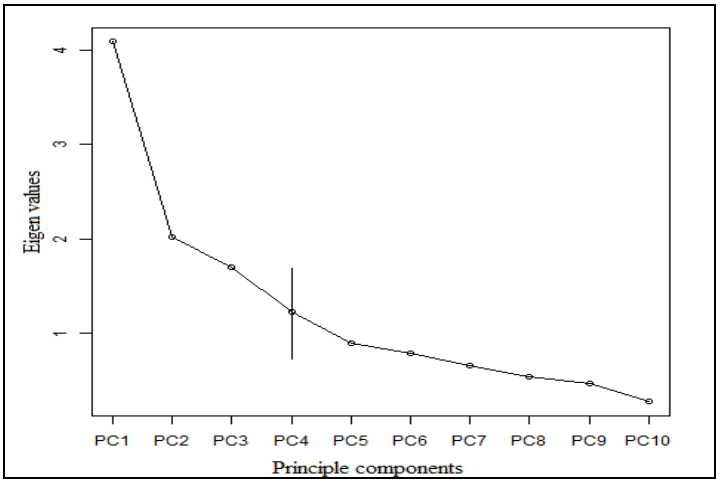

Fig. 1. Scree plot constructed from eigen values vs the number of principal components accounted for percent variation.

Scree plot is a valuable visual aid for the identification of a sufficient number of principal components. The magnitude of an eigen value versus its number with the eigen values ordered from the largest to the smallest. The researcher will look through an elbow (bend) in the scree plot to decide the number of components necessary. The number of components is assumed to be the point where the remaining eigen values are relatively small and all around the same size (Johnson

and Wichern, 2008). In this case, it could be observed from the scree plot that the first four principal components effectively summarized the total variance (Fig. 1). The first four principal axes described 69.50 percent of the overall variability among the characters representing 55 snake gourd genotypes.

Cluster Analysis (CLA)

Based on Mahalanobis D^2 analysis fifty five genotypes were grouped into six different clusters (I-VI) in Table 3. Cluster III (19) had maximum genotypes followed by cluster II (12) and cluster IV (11). The largest number of genotypes included in the cluster III (TC 05, TC 07, TC 16, TC 19, TC 22, TC 23, TC 24, TC 27, TC 29, TC 30, TC 31, TC 32, TC 33, TC 37, TC 38, TC 39, TC 40, TC 45 and TC 47), while the second highest genotypes were in cluster II, which retained the genotypes (TC 03, TC 06, TC 14, TC 15, TC 26, TC 28, TC 34, TC 41, TC 43, TC 44, TC 50 and TC 52) and followed by the genotypes of cluster IV (TC 08, TC 09, TC 10, TC 12, TC 17, TC 20, TC 25, TC 36, TC 46, TC 48 and TC 51). Cluster I had eight genotypes (TC 01, TC 02, TC 04, TC 11, TC 13, TC 18, TC 42 and TC 49) and cluster V included two genotypes (TC 21 and TC 35) and cluster VI got three genotypes (TC 53, TC 54 and TC 55). Devi and Mariappan (2013) documented the use of the Mahalanobis D^2 statistics to investigate genetic diversity among 50 genotypes of snake gourds. Khatun *et al.* (2010) found that 38 genotypes of snake gourd were grouped into four separate clusters based on D^2 analysis, where cluster I had the highest number (21) of genotypes followed by cluster II (8), III (7) and IV (2).

Table 3. Distribution of 55 snake gourd genotypes into six clusters

Number of clusters	Number of genotypes	Genotypes
I	8	TC 01, TC 02, TC 04, TC 11, TC 13, TC 18, TC 42, TC 49
II	12	TC 03, TC 06, TC 14, TC 15, TC 26, TC 28, TC 34, TC 41, TC 43, TC 44, TC 50, TC 52
III	19	TC 05, TC 07, TC 16, TC 19, TC 22, TC 23, TC 24, TC 27, TC 29, TC 30, TC 31, TC 32, TC 33, TC 37, TC 38, TC 39, TC 40, TC 45, TC 47
IV	11	TC 08, TC 09, TC 10, TC 12, TC 17, TC 20, TC 25, TC 36, TC 46, TC 48, TC 51
V	2	TC 21, TC 35
VI	3	TC 53, TC 54, TC 55

Construction of scattered diagram

A two-dimensional scattered diagram (Z_1-Z_2) using component score 1 as the X-axis and component score 2 as the Y-axis was constructed and presented in Fig. 2 based on the values of the principal component scores 1 and 2 obtained from the principal component analysis. The genotyping position in the scattered diagram was apparently split into six groups.

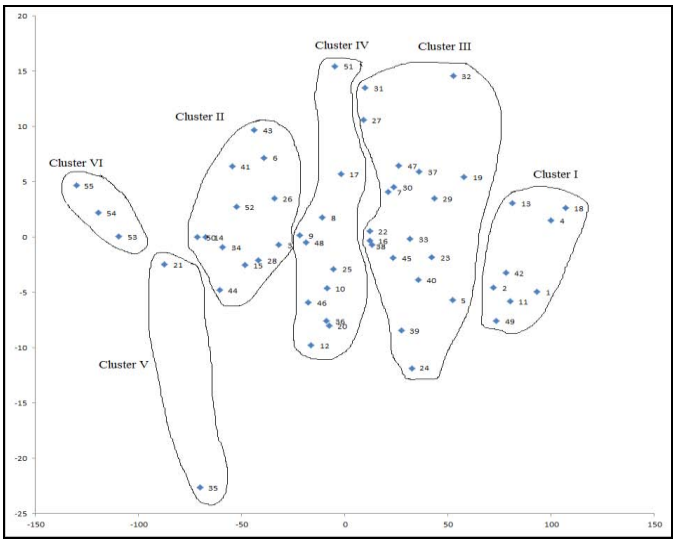

Fig. 2. Scattered distribution of 55 genotypes of snake gourd based on principal component scores superimposed with clustering.

Principal coordinate analysis (PCO)

Principal coordinate analysis (PCO) was done to get the inter-genotypic distance of the genotypes. Table 4 presents ten of each lower and higher inter-genotypical distance between pairs of genotypes. The highest inter-genotypical distance between the genotypes TC 55 and TC 18 was 237.28 and the lowest was 8.25 between genotypes TC 46 and TC 12. The difference between the highest and the lowest inter-genotypical distance indicates variability among the genotypes of fifty five snake gourds. The highest inter-genotypic distance obtained by Ahmed *et al.* (2016) was 2.63 between PK 17 and PK 02, whereas the lowest was 0.31 between PK 11 and PK 12.

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

Genotypic Combination	Ten maximum (D^2) values	Genotypic Combination	Ten minimum (D^2) values
TC 55 & TC 18	237.28	TC 46 & TC 12	8.25
TC 55 & TC 4	230.15	TC 31 & TC 27	8.41
TC 54 & TC 18	226.55	TC 39 & TC 24	8.42
TC 55 &TC 01	223.5	TC 49 & TC 42	10.28
TC 54 & TC 04	219.5	TC 37 & TC 33	10.58
TC 53 & TC18	216.84	TC 11 & TC 02	11.04
TC 54 & TC 01	212.7	TC 13 & TC 11	11.09
TC 55 & TC 13	211.54	TC 29 & TC 23	11.15
TC 55 & TC 11	210.55	TC 39 & TC 33	11.32
TC 53 & TC 04	209.39	TC 47 & TC 37	12.02

Canonical variate analysis (CVA)

The intra and inter-cluster values among the six clusters outlined in Table 5. Statistical distance represents the cluster indices of genetic diversity. The distance between the clusters was greater than the distance between the intra-clusters indicating broader genetic variation among the genotypes of different groups. In a multivariate analysis, Quamruzzaman *et al.* (2008) obtained greater inter-cluster distance than the intra-cluster distance in bottle gourd, bitter gourd and ridge gourd while Masud *et al.* (2003) reported similar results in pumpkin. The inter-cluster D^2 values ranged from 38.87 to 205.66 suggesting large genotypical diversity. The maximum distance between clusters I and VI (205.66) was noted, followed by clusters I and V (165.97), clusters III and VI (149.98), clusters I and II (136.98) and clusters IV and VI (109.56). The genotypes in these clusters were noted to have been widely divergent from one another. Lower inter-cluster distances were observed between cluster II and V (38.87), cluster II and IV (43.06), cluster III and IV (44.92) and cluster V and VI (46.47), suggesting a close relationship between these cluster pairs. The distance from the intra-cluster ranged from 15.00 to 31.23, the maximum being from cluster V (31.23) which consisted of two genotypes while the minimum distance was observed in cluster VI (15.00) which was composed of three genotypes.

Table 5. Average inter and intra (bold) cluster distance (D^2) for 55 snake gourd genotypes

Cluster	I	II	III	IV	V	VI
I	**22.02**					
II	136.98	**22.34**				
III	58.84	81.85	**23.59**			
IV	98.12	43.06	44.92	**20.37**		
V	165.97	38.87	111.10	71.67	**31.23**	
VI	205.66	70.64	149.98	109.56	46.47	**15.00**

The genotypes at these clusters were therefore minimally divergent from one another. In hybridization, the selection of parents from highly divergent clusters is expected to manifest high heterosis and exhibit wide variation in genetic architecture. Wenxing *et al.* (1994) found that genetic variation was more effective if crossing among genotypes belonging to different groups with a greater genetic distance (D^2) was performed. Devi and Mariappan (2013) reported that in 50 genotypes of snake gourds the inter-cluster distance was greater than that of intra-cluster distance; there was an observation of the maximum distance between cluster III and cluster IV and the minimum distance between clusters I and cluster II. In the case of intra-cluster distance, the

maximum distance was observed in cluster III and that of minimum was observed in cluster IV. For pumpkin, Ahmed *et al.* (2016) recorded that the highest inter-cluster distance between clusters III and V was observed (72.69) followed by clusters I and V (63.33). The minimum inter-cluster distance was found between cluster II and cluster IV (10.64). Rashid (2000) also found that the highest inter-cluster distance between cluster V and cluster VI was observed in pumpkin (11.4) and the lowest between cluster III and cluster VI (3.7). The largest intra-cluster distance was found in Cluster VI (0.7) followed by Cluster VI (0.6). Ghosh *et al.* (2015) found that the bitter gourd genotypes showed a lot of substantial variabilities. The inter-cluster distance between II and III (12.97) was the largest and there was more or less moderate distance between other clusters as well as intra-cluster the distance was closely associated. The difference between clusters I and II (136.98), clusters I and V (165.97), clusters III and V (111.10), clusters III and VI (149.98), and clusters IV and VI (109.56) were found to be moderate. It is stated that there may also be significant and positive heterosis in the crosses involving parents who belong to the medium divergent (Mian and Bhal, 1989).

Cluster means for thirteen characters in snake gourd

A comparison of six cluster means for thirteen characters are presented in Table 6. Differences in the number of characters were found in cluster mean. Of all the characters in six clusters, the maximum variability range was recorded for individual fruit weight (108 to 303.25 g). Cluster IV had the lowest mean value for days to open the first male (45.12) and female (47.38) flower indicating earliness. This cluster also included the genotypes with the highest mean values for main vine length (5.26 m), number of primary branches (6.31), number of fruits per plant (14.79), marketable fruit yield per plant (4.51 kg), individual fruit weight (303.25 g) and fruit length (43.88 cm). Cluster IV, therefore, possessed the genotypes with the most desirable qualities for parent selection. Cluster III had the first male (11.75) and female (15.50) flower open with the lowest mean value for node order which also suggested earliness. The genotypes under cluster IV (14.79) produced the highest number of fruits per plant followed by cluster III (11.82). The heavy weighted fruits produced by the genotypes included in the cluster IV (303.25g) followed by the cluster II (266.00g) and cluster I (240.15g). The light weighted fruit was derived from the genotypes included in cluster III (108.00 g) but the number of fruits in the same cluster was higher. Maximum marketable yield of fruits per plant harvested from the cluster IV genotypes (4.51 kg) followed by cluster II (3.05 kg) and cluster I (2.58 kg). Cluster II (34.67) and cluster I (33.46) were found to have the highest number of nodes on the main vine.

Table 6. Cluster mean values for 13 characters of 55 snake gourd genotypes

Characters	Clusters					
	I	II	III	IV	V	VI
Days to first male flower open	47.38	45.83	47.50	45.12	46.73	47.00
Days to first female flower open	49.69	48.67	48.25	47.38	49.27	50.85
Node order of first male flower open	14.08	13.00	11.75	12.25	12.36	13.69
Node order of first female flower open	18.62	19.83	15.50	17.88	18.55	19.38
Main vine length (m)	4.49	4.83	3.82	5.26	4.47	4.78
Number of nodes on main vine	33.46	34.67	31.25	32.75	31.82	31.85
Number of primary branches	5.27	5.67	4.88	6.31	5.95	5.15
Number of fruits / plant	10.85	11.40	11.82	14.79	10.29	10.21
Marketable yield / plant (kg)	2.58	3.05	1.30	4.51	2.14	1.69
Individual fruit weight (g)	240.15	266.00	108.00	303.25	207.36	167.00
Fruit length (cm)	31.23	34.33	16.50	43.88	31.73	25.69
Fruit diameter (cm)	4.37	4.45	4.32	4.41	4.33	4.12
100-seed weight (g)	31.62	27.33	26.50	30.38	30.27	29.69

The maximum diameter of the fruit was obtained in the cluster II genotypes (4.45 cm), followed by cluster IV (4.41 cm). For cluster I, the maximum weight of 100-seeds was observed (31.62 g) followed by cluster IV (30.38 g). Cluster I was observed to contain the genotypes the second highest days needed for the opening of the male and female flower, maximum node order of the opening of the first male flower, the second highest number of nodes on the main vine, moderately marketable fruit yield per plant and individual fruit weight and a maximum of 100-seed weight. Cluster II had the highest node order of the opening of the first female flower, the maximum number of nodes on the main vine, moderate individual fruit weight, and the number of marketable fruits per plant and maximum fruit diameter. Cluster V genotypes produced second highest number of primary branches as well as cluster VI genotypes responsible for the maximum days required for opening the first female flower, second highest node order for opening the first female flower leading to late type, and the lowest number of fruits per plant and fruit diameter. Endang *et al.* (1971) reported that the pattern of clustering could be used to select parents for cross combinations which are likely to produce the highest possible variability for the effective selection of different economic traits. Under such a scenario, Choudhury *et al.* (1975) suggested that selecting from each cluster for one form and evaluating them through a series of diallel analyzes could prove highly beneficial.

Contribution of characters towards divergence

The contribution of characters towards divergence was obtained from canonical variate analysis (CVA) which is outlined in Table 7. The values for vector 1 and vector 2 revealed that both the vectors had positive values for the number of fruits per plant and 100-seed weight. Results suggested that for 55 snake gourd genotypes, these two characters had the highest contribution towards divergence among the thirteen characters. In vector 1, the other significant characters responsible for genetic divergence in the main axis of differentiation were the number of days needed for opening the first male and female flower, node order of first male and female flower open, and marketable fruit yield per plant, while in vector 2 (second axis of differentiation) individual fruit weight and fruit length contributed positively to divergence. Negative values for both vectors were for the length of the main vine, the number of nodes on the main vine, the number of primary branches and the diameter of the fruit showed the lowest contribution to the overall divergence. Ahmed *et al.* (2016) reported that there was a considerable contribution to divergence in the number of fruits per plants and fruit yield per plant. Similar results for pumpkin are also disclosed by Masud *et al.* (1995).

Table 7. The relative contribution of the thirteen characters to the total divergence in snake gourd

Characters	Vector 1	Vector 2
Days to first male flower open	0.2990	-0.2671
Days to first female flower open	0.2805	-0.3181
Node order of first male flower open	0.2672	-0.3203
Node order of first female flower open	0.2311	-0.4328
Main vine length (m)	-0.3442	-0.1417
Number of nodes on main vine	-0.1267	-0.2509
Number of primary branches	-0.2789	-0.1311
Number of fruits / plant	0.3913	0.1464
Marketable yield / plant (kg)	0.4377	-0.1308
Individual fruit weight (g)	-0.2832	0.4774
Fruit length (cm)	-0.2619	0.3947
Fruit diameter (cm)	-0.0332	-0.0236
100-seed weight (g)	0.0143	0.1205

Conclusion

The distance between clusters I and VI, clusters I and V, clusters III and VI, clusters I and II, and clusters IV and VI were greater among the inter-clusters.

The crossing between genotypes belonging to different groups with genetic distance (D^2) had a greater beneficial effect. Thus, genetically distant parents can generate higher heterosis in crosses. Taking into account the magnitude of the cluster distance, the contribution towards divergence, and the magnitude of the cluster means for different characters and considering yield and certain qualitative traits (size, shape, color, taste), as well as agronomic performance, the genotypes TC 01 and TC 02 from cluster I, TC 05, TC 24 and TC 33 from cluster III, TC 46 from cluster IV and TC 53 from cluster VI could be selected for the future hybridization program.

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