Genetic Diversity Studies in Ash Gourd [*Benincasa hispida* (Thunb.) Cogn.] from Northern India

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The genetic diversity, clustering pattern and ordination (principal components) analyses were undertaken in 26 ash gourd accessions. The accessions showed significant inter-population differences and wide variation for quantitative and qualitative morphological descriptors observed. Low level of difference between the magnitude of PCV and GCV indicated that the descriptors were least influenced by environment and are genetically controlled. High heritability coupled with high genetic advance was observed for descriptors such as primary branches, fruits/plant and fruit weight/plant. These accessions were grouped into four major clusters. The clusters were homogenous within themselves and heterogeneous among each other. Cluster IV was the most distinct with more primary branches, higher individual fruit weight, more fruits/plant and highest fruit weight/plant. The PCA in general confirmed the groupings obtained through cluster analysis. PCA revealed four informative components accounting for 67.71% variance. PC1 was related with petiole length, node number at which first female flower appears and yield contributing descriptors like primary branches, fruit length, fruit width and fruit weight.

Key Words: Ash gourd, *Benincasa hispida*, Germplasm evaluation, Cluster analysis, Principal component analysis, Diversity analysis

Introduction

Ashgourd [*Benincasa hispida* (Thunb. Cogn.)], also popularly known as wax gourd, white pumpkin or winter melon has been under cultivation in India since ancient times. Among the cucurbits, ash gourd/wax gourd is considered a prized vegetable because of its high nutritional value, long storage life and good transport qualities, besides its medicinal properties. The young leaves, flowers and both immature and mature fruits are consumed. The mature fleshy fruit is either eaten raw or cooked as vegetable marrow or 'candied' as sweetmeat popularly known as 'petha'. It is a good source of carbohydrate, vitamin A, vitamin C and minerals like iron and zinc (Randhawa *et al.*, 1983 and Sureja *et al.*, 2006). An enzyme extracted from ash gourd juice can be used in place of calf rennet for producing cheddar cheese (Gupta and Eskin, 1977). It is also used to treat a variety of ailments in ayurvedic and naturopathy systems of medicine (Ramesh *et al.*, 1989).

Ash gourd is a monotypic genus and no wild or related species are reported. De Candolle found it wild on the sea shore of Java and later it spread Northwards to Japan, Central America and West Indies. There are reports suggesting that cultivated forms might have originated in South East Asia. Indo-China region is the centre of diversity for ash gourd (Rubatzky and Yamaguchi, 1997). Indian cultivars were the earliest wax gourds introduced to Europe (Robinson and Decker-Walters, 1999).

In spite of its economic importance, no systematic efforts have so far been made to assess the genetic diversity of ash gourd germplasm. In India, the National Bureau of Plant Genetic Resources (NBPGR) has the responsibility of managing genetic resources of crop plants and their wild relatives. The present study was therefore undertaken to estimate the diversity present in the germplasm accessions with the objective of their potential use in crop improvement.

Materials and Methods

Twenty-six accessions of ash gourd germplasm representing diverse types from specific agro-ecological niches of ash gourd growing areas of Uttar Pradesh were used for the present study. These comprise 21 accessions from Eastern Uttar Pradesh, three from Orissa and one each from Jharkhand and Gujarat. Majority of these accessions were collected in the recent past under the National Agricultural Technology Project on Plant Biodiversity which was operational at NBPGR from 1999-2002. The germplasm accessions were grown in Complete Randomized Block Design (RBD) with two replications in three hills per accession and two plants per hill during 2002-03 at Vegetable Experimental Farm, Division of Vegetable Crops, IARI, New Delhi with a

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channel to channel spacing of 6 m and hill to hill spacing of 1m. Data were recorded on a set of distinct 20 qualitative and 12 quantitative morphological descriptors (Table 1) following the minimal descriptors (Srivastava *et al.*, 2001). Data for quantitative descriptors were recorded on four randomly selected competitive individuals per accession and subjected to Analysis of Variance (ANOVA).

The different variability parameters as well as the heritability (broad sense, H^2_{bs}) and genetic advance (GA) as per cent of mean were calculated using Statistical Package for Agricultural Research (SPAR) programme developed at the Indian Agricultural Statistics Research Institute (IASRI), Pusa Campus, New Delhi, India.

The frequency distribution for quantitative and qualitative descriptors was computed and the phenotypic frequencies were also analyzed by the Shannon-Weiner Diversity Index (H') in order to estimate the diversity of each descriptor (Weaver and Shannon, 1949).

$$H' = \sum_{i=1}^{n} p_i \log_e p_i$$

where, p_i is the proportion of the accessions in the ith class of *n*-class descriptors.

Correlation between quantitative descriptors were computed for identification of descriptors of breeding importance.

Classification (cluster) and ordination (principal components) analyses were also performed. Skewed data on quantitative descriptors were transformed before multivariate analysis. Ward's minimum variance clustering method was used to classify accessions in discrete clusters (Sneath and Sokal, 1973). Principal components analysis was performed using quantitative descriptors. The statistical analysis was carried out using INDOSTAT statistical package developed at the INDOSTAT Services, Hyderabad, India.

Results and Discussion

The ANOVA (Table 2) showed significant differences as well as wide variation for all the quantitative descriptors studied. The variability parameters, heritability (H^2_{bs}) and genetic advance (GA) for 12 quantitative descriptors and frequency distribution for 20 qualitative descriptors are presented in Table 3. The difference between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were low for all the descriptors. This indicates that the descriptors were least influenced by environment and are controlled genetically. The phenotypic coefficient of variation for six descriptors namely, primary branches, petiole length, fruit weight, fruits/plant, seeds/plant and fruit yield/plant were in excess of 20% signifying skewed distribution, both positive and negative. Similar results were also observed in ash gourd by Singh *et al.* (2002). H' revealed presence of diversity (≥0.40) in respect of primary branches, petiole length, days to 50% flowering, fruit width, fruit weight, seeds/fruit, early plant vigour, plant growth habit, fruit skin colour and fruit ridge (rib) shape. The estimates of genotypic coefficient of variation (GCV) alone may not be adequate for selection. Hence, heritability estimates should also be considered (Burton, 1952). The descriptors *viz.* primary branches, node number at which first female

Table 1. List of descriptors studied

Quantitative descriptors		
1	PB	Primary branches
2	PL	Petiole length (cm)
3	NFFF	Node number at which first female flower appears
4	DFFF	Days to 50% flowering
5	PDL	Peduncle length (cm)
6	RF	Ridges/fruit
7	FL	Fruit length (cm)
8	FWD	Fruit width (cm)
9	FWT	Fruit weight (Kg)
10	FPP	Fruits/plant
11	SPF	Seeds/fruit
12	FWP	Fruit weight/plant (Kg)
Qualitative descriptors		
13	Early plant vigour: (3=Poor 5=Good 7=Very Good)	
14	Plant growth habit (3=Short vine 5=Medium vine 7= Long vine)	
15	Stem pubescence (1=Smooth 2=Pubescence)	
16	Stem shape (1=Rounded 2=Angular)	
17	Tendril (0=Absent 1=Present)	
18	Tendril type (1=Coiled 2=Straight)	
19	Tendril branching (1=Unbranched 2=Branched)	
20	Leaf margin (1=Entire 2=Serrate 3=Multifid)	
21	Leaf size (3=Small 5= Medium 7=Large)	
22	Leaf pubescence nature (3=Soft 5= Intermediate 7= Hard)	
23	Leaf pubescence density (0=No hairs 3=Sparse 5=Intermediate 7=Dense)	
24	Sex type(1=Monoecious 2=Gynoemonoecious 3=Andromonoecious 4=Hermaphrodite 5=Androecious 6=Dioecious)	
25	Peduncle shape (3=Round 5=Smoothly angled 7=Sharply angular)	
26	Fruit shape (1=Cylindrical 2=Spindle type 3=Round)	
27	Fruit skin colour (3=Light green 5=Intermediate 7=Dark green)	
28	Fruit skin lustre (3=Matty 5= Intermediate 7=Glossy)	
29	Fruit pubescence density (0=No hairs 3=Sparse 5= Intermediate 7= Dense)	
30	Fruit ridge (rib) Shape (1=Superficial 2=Rounded/Grooved 3= Intermediate 4= Deep grooved)	
31	Stem-end fruit shape (1=Depressed 3=Flattened 5=Rounded 7=Pointed)	
32	Blossom-end fruit shape (1=Depressed 3=Flattened 5=Rounded 7=Pointed)	

Table 2. Analysis of Variance for different descriptors studied

Source of Variation	df	PB	PL	NFFF	DFFF	PDL	RF	FL	FWD	FWT	FPP	SPF	FWP
Replication	1	1.86	1.24	6.11	64.69	0.55	1.61	3.5	5.92	0.14	1.44	29473.92	1.04
Treatment	25	21.70**	28.06**	101.53**	78.49**	6.66**	1.37**	45.50**	13.18**	2.11**	19.63**	139633.92**	267.10**
Error	25	1.38	3.87	3.73	3.77	0.85	0.39	1.53	2.20	0.26	0.32	30946.33	13.11
Total	51	11.35	15.68	51.72	41.59	3.69	0.89	23.13	7.65	1.16	9.81	84195.69	137.38

** Significant at $p = 0.01$

Table 3. Range of variation in quantitative descriptors and predominance of qualitative descriptors of the ash gourd collections

Descriptors	Range	Mean	*H2*	GCV (%)	PCV (%)	H^2_{bs}	GA as per cent of mean
Primary branches	6.0 - 18.0	11.5 ± 0.5	0.46	27.8	29.6	88.1	53.8
Petiole length (cm)	10.2 - 29.0	17.7 ± 0.6	0.44	19.6	22.3	75.7	35.2
Node number at which first female flower appears	33.0 - 66.3	45.6 ± 1.0	0.37	15.3	15.9	92.9	30.4
Days to 50% flowering	58.0 - 86.0	70.8 ± 0.9	0.42	8.6	9.1	90.8	16.9
Peduncle length (cm)	6.9 - 17.1	9.8 ± 0.3	0.38	17.4	19.8	77.5	31.6
Ridges/fruit	4.5 - 8.5	6.7 ± 0.1	0.33	10.4	13.9	56.0	16.0
Fruit length (cm)	28.8 - 51.7	36.1 ± 0.7	0.39	13.0	13.4	93.5	25.8
Fruit width (cm)	15.1 - 27.5	19.1 ± 0.4	0.40	12.3	14.5	71.4	21.4
Fruit weight (Kg)	2.0 - 6.3	3.6 ± 0.2	0.42	27.0	30.5	78.4	49.3
Fruits/plant	1.0 - 12.5	3.8 ± 0.4	0.36	81.2	82.5	96.8	164.5
Seeds/fruit	267.5 - 1538.0	836.8 ± 40.2	0.41	27.9	34.9	63.7	45.8
Fruit weight/plant	2.5 - 56.9	13.2 ± 11.7	0.30	85.6	89.9	90.6	167.8
Early plant vigour	Poor (3), Good (13), Very Good (10)		0.42				
Plant growth habit	Short vine (3), Medium vine (11), Long vine (12)		0.42				
Leaf size	Small (3), Medium (16), Large (7)		0.39				
Leaf pubescence nature	Soft (3), Intermediate (20), Hard (3)		0.30				
Leaf pubescence density	Sparse (4), Intermediate (18), Dense (4)		0.36				
Fruit shape	Cylindrical (21), Spindle type (2), Round (3)		0.27				
Fruit skin colour	Light Green (13), Intermediate (7), Dark green (6)		0.45				
Fruit skin lustre	Matty (14), Intermediate (12)0.30						
Fruit pubescence density	Sparse (19), Intermediate (4), Dense (3)		0.33				
Fruit ridge (rib) shape	Rounded/Grooved (11), Intermediate (3), Deep grooved (12)		0.42				
Stem-end fruit shape	Depressed (14), Rounded (12)0.30						
Blossom-end fruit shape	Depressed (6), Rounded (20)0.23						

flower appears, days to 50% flowering, fruit length, fruits/plant and fruit yield/plant showed high broad sense heritability (>80%) whereas petiole length, peduncle length, ridges/fruit, fruit width, fruit weight and seeds/fruit showed medium heritability (50-80%). Genetic advance over mean is the measure of genetic gain over mean. Higher genetic advance over mean was observed for descriptors namely, primary branches, fruit weight, fruits/plant, seeds/fruit and fruit yield/plant. High heritability along with high genetic advance (per cent of mean) is more helpful in establishing close relationship between genotypes and phenotypes (Johnson *et al*., 1955). High heritability coupled with high genetic advance was observed for primary branches, fruits/plant and fruit yield/plant indicating the influence of additive gene effects; hence, selection will be effective for genetic improvement. Similar results for primary branches and fruit yield/plant were also observed in bottle gourd (Singh and Kumar, 2002). High heritability and low genetic advance were observed for node number at which first female flower

Table 4. Correlation matrix for quantitative descriptors

Descriptors	PB	PL	NFFF	DFFF	PDL	RF	FL	FWD	FWT	FPP	SPF	FWP
PB	**1.00**	0.15	0.13	0.13	0.06	-0.03	0.22	0.40**	0.22	-0.01	-0.07	0.03
PL		**1.00**	0.39**	-0.21	-0.20	0.17	0.41**	0.50**	0.30*	-0.21	0.31*	-0.10
NFFF			**1.00**	0.05	-0.01	0.01	0.01	0.24	0.37**	-0.05	0.29*	0.09
DFFF				**1.00**	-0.18	-0.14	-0.14	-0.18	0.03	-0.21	0.11	-0.10
PDL					**1.00**	0.01	-0.14	0.08	-0.16	0.05	-0.22	-0.07
RF						**1.00**	-0.09	0.24	-0.01	0.12	0.19	0.15
FL							**1.00**	0.57**	0.25	-0.15	-0.17	-0.10
FWD								**1.00**	0.46**	-0.14	0.12	-0.03
FWT									**1.00**	0.07	0.18	0.38**
FPP										**1.00**	0.29*	0.91**
SPF											**1.00**	0.36**
FWP												**1.00**

, * significant at $p = 0.05$ and 0.01 respectively.

appears, days to 50% flowering and fruit length indicating the influence of non-additive gene effects. These results are in conformity with the findings of the earlier workers in ash gourd (Singh *et al.*, 2002) and ridge gourd (Prasad and Singh, 1989).

Correlations between important descriptors are presented in Table 4. The fruit yield/plant was positively and significantly correlated with fruit weight, fruits/plant and seeds/fruit. Number of primary branches was positively and significantly correlated with fruit width where as petiole length was positively and significantly correlated with node number at which first female flower appears, fruit length, fruit width, fruit weight and seeds/fruit. Node at which first female flower appears was positively and significantly correlated with fruit weight and seeds/fruit. Fruit length and fruit width was positively and significantly correlated with fruit width and fruit weight respectively. Fruits/plant was positively and significantly correlated with seeds/fruit. Positive and significant correlation between fruit weight/plant and number of fruits/plant and in between fruit length and fruit width was also reported in ridge gourd (Prasad and Singh, 1989). Correlations between important descriptors help in selection of desirable types, if high correlations exist or are established by breeding techniques between complex descriptors like yield that has a low heritability with other simply inherited component descriptors.

Ward's clustering technique clearly defined and characterized the clusters based on morphological descriptors. The accessions were grouped into four clusters based on distance ranges for the tree shown in Figure 1. Clusters I, II, III and IV comprised 9, 8, 7 and 2 accessions, respectively. The maximum inter cluster distance (6.24) was observed in between cluster I and IV (Table 5). Cluster means of different quantitative descriptors and predominance of qualitative descriptor states for individual clusters are presented in Table 6. Critical examination of the clusters revealed that clusters were homogeneous within themselves and heterogeneous between each other based on the major descriptors. Cluster IV was most distinct from other three clusters as evident from inter cluster distances (Table 6). This cluster is characterized by accessions having more primary branches, shortest peduncle length, more ridges/ fruit, higher fruit weight, more fruits/plant, more seeds/fruit, highest fruit yield/plant, more early plant vigour, medium to long vine length, medium leaf size, soft to intermediate leaf pubescence nature, sparse to intermediate leaf pubescence density, cylindrical fruit shape, green to dark green fruit skin color, matty fruit skin lustre, dense fruit pubescence, rounded/grooved fruit ridge (rib) shape with rounded blossom end fruit shape. Cluster I was characterized by accessions having long petiole, higher node number at which first female flower appears, greater fruit length and width, good to very good early plant vigour, medium to long vine, medium to large leaf size, intermediate to hard leaf pubescence nature and matty to

Table 5. Inter and Intra cluster (underlined) distances

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**4.23**	5.04	4.99	6.24
Cluster II		**4.28**	4.55	5.68
Cluster III			**3.66**	6.03
Cluster IV				**5.31**

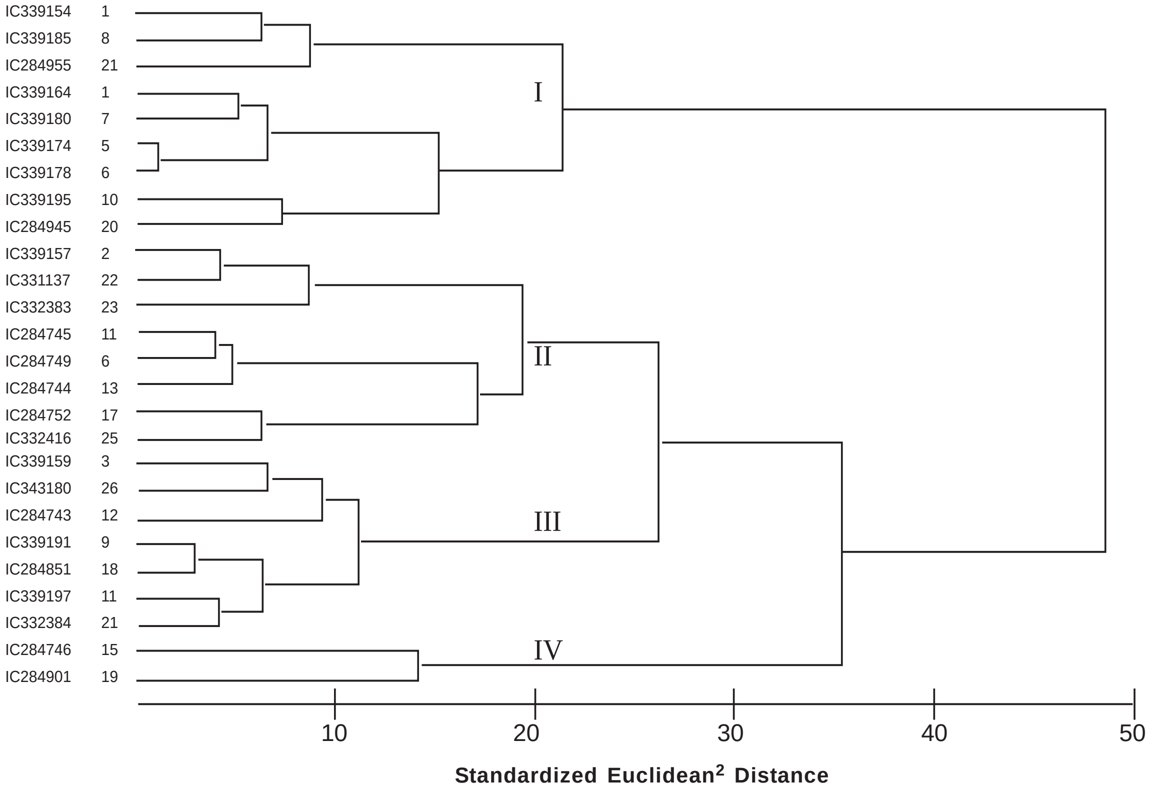

Fig. 1: Ward's Minimum Variance Dendogram

Table 6. Means of quantitative descriptors and predominance of qualitative descriptors' states in different clusters

Descriptors	Cluster I	Cluster II	Cluster III	Cluster IV
Primary branches	12.64	12.42	8.43	12.92
Petiole length (cm)	21.62	15.73	15.40	16.10
Node number at which first female flower appears	48.97	45.10	40.93	48.67
Days to 50% flowering	69.22	68.19	73.93	77.00
Peduncle length (cm)	9.26	10.96	9.74	7.84
Ridges/fruit	7.08	6.27	6.57	7.58
Fruit length (cm)	38.55	35.65	35.12	30.53
Fruit width (cm)	21.43	18.41	17.34	17.14
Fruit weight (Kg)	3.71	3.59	3.08	4.53
Fruits/plant	2.16	6.16	1.90	8.75
Seeds/fruit	936.90	762.79	664.58	1285.47
Fruit yield/plant (kg)	7.99	19.18	5.82	38.10
Early plant vigour	Good to very good	Poor to very good	Poor to very good	Good to very good
Plant growth habit	Medium to long vine	Short to long vine	Short to long vine	Medium to long vine
Leaf size	Medium to large	Small to medium	Medium to large	Medium
Leaf pubescence nature	Soft to hard	Soft to intermediate	Soft to hard	Soft to Intermediate
Leaf pubescence density	Sparse to Dense	Sparse to Dense	Sparse to Dense	Sparse to Intermediate
Fruit shape	Cylindrical to round	Cylindrical to round	Cylindrical and round	Cylindrical
Fruit skin colour	Light to dark green	Light to dark green	Light to dark green	Green to dark green
Fruit skin luster	Matty to intermediate	Matty to intermediate	Matty to intermediate	Matty
Fruit pubescence density	Sparse to Dense	Sparse to intermediate	Sparse	Dense
Fruit ridge (rib)	Roundeed/grooved to deep	Rounded/grooved to deep grooved	Rounded/grooved and grooved	Rounded/grooved deep grooved
Blossom-end fruit shape	Depressed and rounded	Depressed and rounded	Depressed and rounded	Rounded

Table 7. Eigen values, cumulative variance of the first four principal components (PCs) and factor loading between PCs and descriptors studied

	PC1	PC2	PC3	PC4
Eigen value	2.87	2.39	1.52	1.34
Percent of variance	23.94	19.91	12.68	11.19
Cumulative variance	23.94	43.85	56.52	67.71
Descriptors	Factor loadings			
Primary branches	0.41	0.14	0.11	0.57
Petiole length (cm)	0.81	0.11	–0.06	–0.37
Node number at which first female flower appears	0.55	–0.15	–0.37	–0.02
Days to 50% flowering	–0.14	0.12	–0.72	0.36
Peduncle length (cm)	–0.21	0.04	0.53	0.07
Ridges/fruit	0.18	–0.31	0.22	–0.59
Fruit length (cm)	0.59	0.39	0.31	0.18
Fruit width (cm)	0.83	0.21	0.30	0.01
Fruit weight (Kg)	0.68	–0.23	–0.15	0.35
Fruits/plant	–0.08	–0.88	0.31	0.23
Seeds/fruit	0.34	–0.60	–0.44	–0.33
Fruit weight/plant (Kg)	0.11	–0.91	0.15	0.29

intermediate fruit skin lustre. Cluster II was characterized by accessions having less number of days to 50% flowering, small to medium leaf size, soft to intermediate leaf pubescence, matty to intermediate fruit skin lustre, sparse to intermediate fruit pubescence density. Cluster III was characterized by lowest node number at which first female flower appears, lowest seeds per fruit, sparse to intermediate leaf pubescence density, matty to intermediate fruit skin lustre, sparse fruit pubescence density, rounded/grooved and deep grooved fruit ridge (rib) shape. The minimum intra-cluster distance was recorded for cluster III (3.66) followed by cluster I (4.23) and cluster II (4.28).

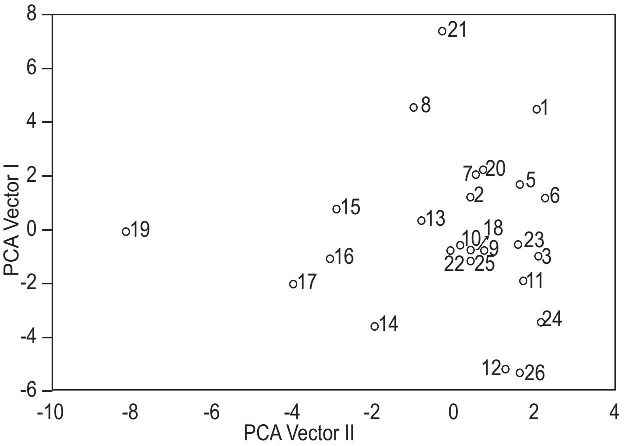

Fig. 2: 2D Principal Components Scatter Plot

Principal components analysis performed on quantitative descriptors revealed that the first four most informative components accounted for 67.71% variance (Table 7). It also presented the descriptors with greater weightings in each of the four principal component axes. Characteristics of each Principal Component were determined on the basis of estimated factor loadings. Descriptors with greater weightings were petiole length, node number at which first female flower appears, yield contributing descriptors like primary branches, fruit length, fruit width, and fruit weight in PC1; fruit length in PC2; peduncle length, ridges/fruit, fruit length, fruit width and fruits/plant in PC3; primary branches, days to 50% flowering, fruit weight and fruit yield/plant in PC4.

The scatter plot of PC scores of first two PC axes is presented in Figure 2. The Principal Components Analysis (PCA) in general confirmed the groupings obtained through cluster analysis. A few distinct accessions could be marked. In some cases the pairs of accessions originating from the same locations fall into tight groups within clusters whereas in other accessions from the same location, are far apart in ordination.

The multivariate analysis might be effective in indicating high yielding accessions in different clusters which could be usefully intercrossed. The PCA ordination revealed that while the scatters of points for the clusters have a central focus, there are significant outliers to some groups. This presumably comes from the third PC. The outliers and central clusters provide the opportunity to identify representatives from the central as well as outliers for use in breeding.

The present study helped in understanding the diversity in the accessions studied and indicates the need for evaluating large number of accesions so as to identify desired germplasm for crop improvement. Being a potential crop of the future, collection programmes from major ash gourd growing areas as well as non-traditional areas should be planned along with introduction from South East Asian and South Asian countries like China.

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