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VARIATION OF FRUIT MORPHOLOGY AND GENETIC DIVERGENCE OF INDIGENOUS POINTED GOURD

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Abstract

Thirty one indigenous pointed gourd genotypes were studied in *Terai* region of West Bengal, India to observe variation of fruit morphology and genetic divergence using Mahalanobis D^2 statistics and principal component analysis. The field experiment was designed in RBD with three replications at Uttar Banga Krishi Viswavidyalaya, West Bengal, India during the year 2018-19 and 2019-20. All the genotypes were arranged into eight divergent clusters in which cluster I included maximum number followed by cluster II. The highest contribution towards genetic diversity was shown for node at the first female flower (37.85%) followed by chlorophyll a content of leaf (16.77%) and seeds per fruit (12.04%). Principal component-1 had maximum variability (43.83%) with positive loading for the characters like node at the first female flower (0.298), appearance of first female flower (0.265), days to fruit maturity (0.194) and internodal length (0.137). Based on the inter and intra cluster distance cross combinations in between lines PCP-17 and PCP-19, PCP-17 and PCP-9, PCP-17 and PCP-28, PCP-19 and PCP-11, PCP-14 and PCP-9 and PCP-23 and PCP-10 may be utilized in future improvement programme of pointed gourd.

Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) is an important perennial, remunerative, dioecious nutritive cucurbitaceous vegetable commercially cultivated in different region of India and Indian subcontinent area (Bhardwaj 2011).This vegetable can help with a number of diet-related conditions, including alcoholism, dropsy, fever, skin, body, and liver problems. It also has positive effects on the heart and intestines (Khatua *et al.* 2016). There is great variation in the morho-physiological as well as qualitative traits (fruit form, color, tenderness and stripe) among the Indian genotypes of pointed gourds (Dora *et al.* 2001). For diversity study both principal component analysis and cluster analysis, which identify phenotypic similarities between genotypes of pointed gourd and categorize individuals with comparable types into the same group using a multivariate method (Kovacic 1994 and Hair *et al.* 1995) could be effective for evaluation. Few studies have been conducted on the genotypes of pointed gourds, however a variety of varieties are cultivated in farmer's fields in the *Terai* region of West Bengal in favorable climate circumstances and for commercial purposes with a high benefit-to-cost ratio. In light of the aforementioned considerations, the present study was initiated to evaluate the genetic diversity of pointed gourd genotypes in order to identify the most genetically divers genotypes for breeding purposes.

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Materials and Methods

A total thirty one pointed gourd genotypes was used for field experiment at the Instructional Field, Department of Vegetable and Spice Crops, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India (26 º 40′ N and 89 º 38′ E, and 43 meters above the mean sea level). The soil of the experimental plots was coarse, sandy loam, medium in water holding capacity with low pH and good organic matter content. One year old mature vines were collected as planting material from different corners of West Bengal and Bihar. Thirty genotypes of pointed gourds were collected from various locations in West Bengal, whereas the one genotype that was used as a check (Rajendra Parwal-1) was obtained from Bihar Agriculture University, Sabour, Bihar.

All the pointed gourd genotypes were evaluated during two seasons 2018-2019 and 2019-2020 designed in RBD with three replications. In November and December of first years, well-rooted stem cuttings were planted in the field with a 5.0 m x 2.0 m spacing. Recommended doses of organic manure as FYM @ 25 t/ha and fertilizers as N, P_2O_5 and K_2O @ 90:60:60 kg/ha were applied in the field. A complete dose of phosphorus and one-third of both nitrogen and potassium fertilizers were applied as basal, and the remaining were applied in two split doses at 45 and 90 days after planting.

The different observations, such as leaf blade length (cm), leaf blade width (cm), petiole length (cm), average internodal length (cm), days to first female flower appearance, node of the first female flower, fruit length (cm), fruit diameter (cm), number of fruits per plant, seeds per fruit, fruit weight (g), days taken for fruit set to harvest maturity, yield per plant (kg), yield per hectare (tonnes), ascorbic acid content of fruit (mg/100 g), beta carotene (IU/100 g), and the amount of chlorophyll (a ,b and total) in leaf tissue (mg/100 g tissue) were measured.

The leaf colour along with six parameters related to fruit character (fruit shape, fruit colour, fruit stripes, fruit curvature, hardness and flesh colour) were recorded during fruit maturity period of pointed gourd. The volumetric approach, as described by Sadasivam and Balasubramanian (1987) was used to determine the ascorbic acid content.Beta-carotene content of fruit and leaf chlorophyll content were estimated as per guidelines of Ranganna (2000) and protocol suggested by Arnon (1949), respectively. Mahanalobis D^2 statistics and principal component analysis (PCA) method were adoptedto find out degrees of genetic divergence among the 31 pointed gourdgenotypes.Genetic diversity was studied by Mahalanobis (1936) employed generalized distance (D2) analysis, commonly known as nonhierarchical Euclidean cluster analysis (Beale (1969). A multivariate methodology known as principal component analysis (PCA) was also used for divergence analysis (Pearson 1904, Hotelling1933).All the analysis was done in R studio 4.2.2.

Results and Discussion

Among the pointed gourd genotypes, there was a wide range of variation in terms of several fruit and leaf characteristics (Table 1 and Fig.1). Based on the color of their leaves, all genotypes were divided into three categories. Among the genotypes the majority generated green to dark green leaves (21 numbers), followed by light green (6) and dark green (4).In this regard, variation of leaf colour dominated to dark green leaf in pointed gourd also found by Ara *et al.* (2012). Seven types of fruit shapes (club shaped,spindle tapering, spindle, oval, club shaped, spheriod and cylindrical) were recorded for all genotypes. Deviations in fruit shape for different genotypes mainly governed by genetic consequences (Sharma 2015). Based on the fruit colour, the dark green colour of primary skin producing genotypes were PCP-2, PCP-4, PCP-10, PCP-15, PCP-25 and PCP-30, while light green was produced by PCP-3, PCP-11, PCP-14, PCP-20, PCP-21, PCP-22, PCP-23 and PCP-26. Three types of striping pattern on fruit surface among the genotypes were recorded namely, uniform, mottled and striped**.** Maximum number of genotypes showed straight type of fruits except five (5) genotypes namely PCP-2, PCP-11, PCP-14, PCP-27 and PCP-30. It was also noted that mainly long fruited genotypes exhibited curvature type of fruits. Different fruit curvatures (curved, slightly curved and straight) were also characterized by Ara *et al*. (2012). Mainly two groups such as hard and soft fruited genotypes were found based on pericarp hardness of fruits.

Table 1. Fruit and leaf characteristics of 31 genotypes of pointed gourds.

Genotype	Leaf colour	Fruit shape	Fruit colour	Fruit stripes	Fruit curvature	Hardness	Flesh colour
PCP-1	Dark green	Club shaped	Green	Striped	Straight	Soft	White
PCP-2	Green to dark green	Spindle tappering	Dark Green	Striped	Curved	Hard	Creamy
PCP-3	Green to dark green	Spindle	Light Green	Striped	Straight	Soft	White
PCP-4	Green to dark green	Spindle tappering	Dark Green	Mottled	Straight	Soft	White
PCP-5	Green to dark green	Spindle tappering	Green	Striped	Straight	Soft	White
PCP-6	Light green	Spindle	Green	Striped	Straight	Soft	Creamy
PCP-7	Light green	Club shaped	Green	Striped	Straight	Soft	White
PCP-8	Light green	Club shaped	Green	Mottled	Straight	Hard	White
PCP-9	Green to dark green	Spheriod	Green	Striped	Straight	Soft	Creamy
PCP-10	Light green	Spindle	Dark Green	Striped	Straight	Hard	Creamy
PCP-11	Green to dark green	Cylindrical	Light Green	Striped	Curved	Hard	White
PCP-12	Light green	Oval	Green	Striped	Straight	Soft	White
PCP-13	Light green	Oval	Green	Mottled	Straight	Soft	White
PCP-14	Green to dark green	Cylindrical	Light Green	Striped	Curved	Soft	Creamy
PCP-15	Green to dark green	Club shaped	Dark Green	Mottled	Straight	Soft	White
PCP-16	Green to dark green	Spindle	Green	Striped	Straight	Soft	White
PCP-17	Dark green	Spindle	Green	Striped	Straight	Soft	White
PCP-18	Green to dark green	Spindle	Green	Striped	Straight	Soft	White
PCP-19	Green to dark green	Spheriod	Green	Striped	Straight	Soft	White
PCP-20	Green to dark green	Club shaped	Light Green	Uniform	Straight	Soft	White
PCP-21	Green to dark green	Club shaped	Light Green	Uniform	Straight	Soft	White
PCP-22	Green to dark green	Club shaped	Light Green	Mottled	Straight	Soft	White
PCP-23	Green to dark green	Spindle tappering	Light Green	Striped	Straight	Soft	White
PCP-24	Green to dark green	Spindle	Green	Striped	Straight	Soft	White
PCP-25	Green to dark green	Spindle tappering	Dark Green	Striped	Straight	Hard	Creamy
PCP-26	Dark green	Club shaped	Light Green	Mottled	Straight	Soft	White
PCP-27	Green to dark green	Cylindrical	Green	Striped	Curved	Hard	White
PCP-28	Green to dark green	Club shaped	Green	Striped	Straight	Soft	White
PCP-29	Green to dark green	Elongated spindle	Green	Striped	Straight	Soft	Creamy
PCP-30	Dark green	Cylindrical	Dark Green	Striped	Curved	Hard	Creamy
Rajendra Parwal-1	Green to dark green	Spindle	Green	Striped	Straight	Soft	White

Fig. 1. Variation in fruit and leaf appearance for 31 genotypes of pointed gourd

The D^2value was estimated for thirty one(31) pointed gourd genotypes and total eight numbers of clusters were formed (Table 2). Cluster I had the highest number of genotypes (13), followed by cluster II (6 genotypes), cluster III (5 genotypes) and cluster IV (3 genotypes). The remaining four (4) genotypes were equally distributed among the remaining four clusters.Verma *et al.* (2017) in an experiment on pointed gourd categorized into seven clusters as per the D^2value of thirty nine genotypes of pointed gourd. The present study indicated that the locational distribution of different genotypes were not related to genetic variation as the genotypes collected from same location were grouped into different distinct cluster.

Table 2. Clustering of 31 pointed gourd genotypes (Pooled basis).

Cluster No.	Genotype numbers in cluster	Genotypes
I	13	PCP-21, PCP-22 , PCP-18, PCP-24, PCP-3, PCP-1, PCP-20, PCP-6, PCP-16, PCP-29, PCP-26, PCP-4, PCP-23
II	6	PCP-2, PCP-14, PCP-30, PCP-27, Rajendra Parwal-1, PCP-11
III	5	PCP-10, PCP-25, PCP-8, PCP-9, PCP-28
IV	3	PCP-12, PCP-15, PCP-13
V	1	PCP-19
VI	1	PCP-17
VII	1	PCP-5
VIII	1	PCP-7

(PCP stands for Pointed gourd Collection Pundibari)

The largest inter-cluster distance was found between clusters V and VI (7001.52) followed by cluster III and VI (5259.40) according to the cluster distance analysis (Table 3). Clusters V and VI had the greatest inter-cluster distance, indicating a significant genetic variation between them. There appears to be more genetic variation among the genotypes between clusters as the average inter-cluster distances were greater than the average intra-cluster distances. The presence of genetic variation among the genotypes and their independent identity was inferred by larger inter-cluster distances than intra-cluster distances (Debata *et al*. 2017).

Table 3. Inter and Intra cluster (Diagonal) average distance in pointed gourd.

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**688.19**	1476.70	2495.60	1596.66	3694.54	1059.83	1101.63	2246.87
II		**708.28**	3752.75	2961.36	4448.79	1713.20	2308.75	2286.35
III			**460.33**	1079.03	809.35	5259.40	1122.67	1331.75
IV				**468.52**	1351.21	3577.85	1495.76	1816.61
V					**0.00**	7001.52	2650.86	1569.74
VI						**0.00**	2472.89	4180.11
VII							**0.00**	1628.90
VIII								**0.00**

There was a remarkable deviation in the mean performance of eight clusters with respect to different attributes (Table 4). The maximum mean values for fruit weight (35.80 g), leaf length (9.88 cm), and fruit length (10.46 cm) were found in Cluster II. The parameters found maximum in cluster VI were the fruit yield (6.28 kg/plant), leaf width (8.40 cm), number of fruits per plant (219.75), ascorbic acid content (20.81 mg/100 g of fresh fruit), chlorophyll a (136.07 mg/100 g of tissue), chlorophyll b (41.72 mg/100 g of tissue), and total chlorophyll (174.66 mg/100 g of tissue). Besides, this cluster (VI) also exhibited minimum days taken for first female flower appearance (46.83 days), minimum number of seeds per fruit (11.42) and least duration of fruit set to harvest maturity (5.65 days). The biggest petiole length (4.85 cm), maximum days required to first flowering (63.33 days), second longest inter-nodal length (10.83 cm) and second highest chlorophyll content (Total) of leaf (159.45 mg/100 g of tissue) were found in cluster VII. Cluster V shown highest diameter of fruit (3.17 cm) and second highest numbers of node (15.43) required for first female flower appearance. Highest average inter-nodal length (13.15 cm), beta carotene content (154.36 μg/100g of fresh fruit) and second highest ascorbic acid content (19.33 mg/100 g of fresh fruit) were recorded in Cluster VIII. Similar trends were also explained by Debata *et al*. (2017) in pointed gourd. The contribution of each trait to total divergence is presented in Table 4. The highest contribution towards the divergence was recorded for node at the first female flower appearance (37.86%) followed by chlorophyll a content of leaf (16.77%).

The Principal Component-1 (PC 1) had 43.83% of total variability followed by PC2 (18.27%), PC 3 (12.47%) and PC 4 (7.06%) (Table 5). The Eigen vectors of the eight principal components have been scaled in such a way that the largest element in each of the vector is unity. As per the calculation of the Eigen root values, first four components had Eigen values more than 1represented 7.64 (PCA 1), 3.18 (PCA 2), 2.17 (PCA 3), and 1.23 (PCA 4), respectively.

Table 4. Cluster mean of individual character of pointed gourd genotype.

Characters	Clusters								Number of first rank	Contribution %
	I	II	III	IV	V	VI	VII	VIII		
LBL	8.65	9.88	7.77	8.72	8.31	9.17	8.28	9.21	0	0.00
LW	7.48	7.36	6.47	6.60	7.16	8.40	7.03	5.97	1	0.21
PL	3.40	4.11	2.71	2.46	2.41	4.16	4.85	3.21	0	0.00
AIL	8.59	10.13	11.23	8.28	11.13	7.68	10.83	13.15	49	10.54
DFF	52.87	53.81	60.83	54.39	61.00	46.83	63.33	57.17	1	0.22
NFF	11.74	7.95	16.08	14.93	15.43	9.73	14.80	11.37	176	37.86
FL	8.26	10.46	6.20	6.11	6.02	9.38	7.41	6.78	0	0.00
FD	2.73	2.33	2.40	1.88	3.17	3.09	2.41	2.37	0	0.00
NFP	160.72	154.74	74.23	96.99	71.20	219.75	132.58	121.65	2	0.43
NSF	14.86	25.74	20.17	14.00	25.90	11.42	17.38	15.05	56	12.04
AFW	28.78	35.80	24.13	22.02	21.06	29.62	27.86	21.70	1	0.21
DFHM	6.65	8.13	8.71	7.42	8.60	5.65	7.83	7.77	0	0.00
ACC	18.16	18.42	14.32	14.48	12.15	20.81	15.75	19.33	22	4.73
BC	139.56	141.29	133.41	143.34	136.38	150.46	137.47	154.36	0	0.00
CA	111.96	102.09	80.98	74.04	38.24	136.07	122.62	78.24	78	16.77
CB	34.15	32.63	26.12	23.78	15.77	41.72	38.87	27.22	0	0.00
TC	145.33	130.84	106.30	95.95	58.95	174.66	159.45	89.86	40	8.61
YLP	4.50	5.37	1.73	1.96	1.45	6.28	3.64	2.55	39	8.38

Where, LBL=Leaf blade: Length (cm), LW=Leaf width (cm), PL=Petiole: length (cm), AIL=Average internodal length (cm), DFF=Days taken for appearance of first female flower, NFF= Node of the first female flower, FL=Fruit length (cm), FD=Fruit diameter (cm), NFP=No of fruits/plant, NSF=Number of seeds/fruit, AFW=Average fruit weight (g), DFHM=Days taken from fruit set to harvest maturity, ACC=Ascorbic acid content of fruit (mg/100 g), BC=Beta carotene (μg/100g), CA=A Chlorophyll content of leaf: mg/100 g tissue (A), CB=B. Chlorophyll b content of leaf: mg/100 g tissue (B), TC=Total Chlorophyll content of leaf: mg/100 g tissue, YLP=Yield/plant (kg),

In each component, these were regarded as the variables' respective weights. The contributions of the yield component traits for the principal component are presented in Table 5. The parameter produced high positive or negative loading values are contributed maximum variability in pointed gourd diversity study. Node at the first female flower (0.298), days taken for appearance of first female flower (0.265), days to fruit maturity (0.194) and internodal length (0.137) showed positive loadings in PC 1 and the remaining parameters showed negative loadings. In PC2, fruit diameter (0.250) number of fruits per plant (0.242), leaf width (0.149) and node at the first female flower (0.131) showed high positive loadings and the remaining factors showed negative loadings. The third principal component exhibited high positive values to leaf length (0.311), beta carotene content (0.258), number of seeds per fruit(0.176) and leaf width (0.155) in the major axis of differentiation. Whereas, PCA 4 showed high positive responses to diversity regarding fruit diameter (0.639), leaf width (0.338), appearance of first female flower (0.161) and fruit yield per plant (0.124). Eigen values are frequently used to decide how many variables to retain. Principal component analysis revealed the significance of the largest contributor to the overall variance at each axis of differentiation (Sharma 1998).

The percentage of variation was explained by a graph between the Eigen values and Principal Components using a scree plot (Fig. 2). The graph presents a clear picture of the highest variation that was noted in PC1, PC2, PC3 and PC4.The wide range of scores for the eighteen attributes in the Principal Component Analysis was demonstrated by the distributions of the scores. Debata (2016) observed that the first seven principal components (PC) out of eight PC provided 79.014% of the overall variation in the pointed gourd, and these eight components could express more than 83% of the divergence, which was consistent with the current findings. The distribution and kind of diversity for both variables and genotypes were described by the bi-plot diagram (Fig. 3) that shows the relationship between PCs 1 and 2. Nearly all of the genotypes and covariates displayed large degrees of variance, as the loading plot revealed. There was a lot of variation in the bi-plot diagram between the parameters and genotypes. The PC analysis of this experiment finally revealed the degree of trait variability among genotypes that might be exploited to improve pointed gourd.

Table 5. Eigenvalues, proportion of variability that contributed to the first eight principal components (PCs) of pointed gourd genotypes.

Eigenvectors	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Leaf length (cm)	-0.232	-0.206	**0.311**	-0.232	-0.249	-0.182	0.080	-0.083
Leaf width (cm)	-0.268	**0.149**	**0.155**	**0.338**	-0.151	-0.119	**0.233**	-0.133
Petiole: length (cm)	-0.281	-0.183	**0.034**	**0.029**	**0.153**	-0.196	**0.425**	**0.548**
Average internodal length (cm)	**0.137**	-0.363	**0.091**	**0.040**	**0.481**	-0.367	-0.245	**0.151**
Days taken for appearance of first female flower	**0.265**	-0.245	-0.177	**0.161**	**0.166**	-0.192	**0.147**	**0.141**
Node of the first female flower	**0.298**	0.**131**	-0.185	-0.073	0.008	-0.089	**0.330**	-0.065
Fruit length (cm)	-0.312	-0.229	**0.084**	**0.029**	-0.002	0.152	0.019	-0.077
Fruit diameter (cm)	-0.081	**0.250**	**0.126**	**0.639**	0.046	-0.428	-0.243	-0.058
No of fruits plant^{-1}	-0.166	**0.242**	**0.123**	-0.102	0.635	0.039	0.476	-0.366
Number of seeds fruit^{-1}	0.003	-0.476	**0.176**	**0.137**	-0.098	-0.105	0.024	-0.555
Average fruit weight (g)	-0.284	-0.275	**0.009**	**0.132**	-0.087	**0.265**	0.059	0.210
Days taken from fruit set to harvest maturity	0.194	-0.430	**0.041**	-0.025	0.077	**0.101**	0.068	-0.149
Ascorbic acid content of fruit (mg/100 g):	-0.277	0.033	-0.049	-0.107	**0.437**	**0.247**	-0.495	-0.071
Beta carotene (μg/100g)	-0.155	0.134	0.258	-0.552	-0.081	-0.509	-0.133	0.012
Chlorophyll content of leaf: mg/100 g tissue (A)	-0.228	-0.049	-0.468	-0.059	-0.021	-0.167	0.002	-0.221
Chlorophyll b content of leaf: mg/100 g tissue (B)	-0.219	-0.092	-0.447	-0.077	-0.022	-0.177	-0.085	0.062
Total Chlorophyll content of leaf: mg/100 g tissue	-0.226	-0.052	-0.490	-0.002	-0.068	-0.178	0.061	-0.199
Yield/plant (kg)	-0.342	0.005	0.074	**0.124**	0.044	0.159	-0.009	0.111
Eigenvalues	7.64	3.18	2.17	1.23	0.90	0.63	0.43	0.34
% of total variance	43.83	18.27	12.47	7.06	5.15	3.61	2.49	1.96
Cumulative variance %	43.83	62.11	74.57	81.64	86.79	90.40	92.90	94.85

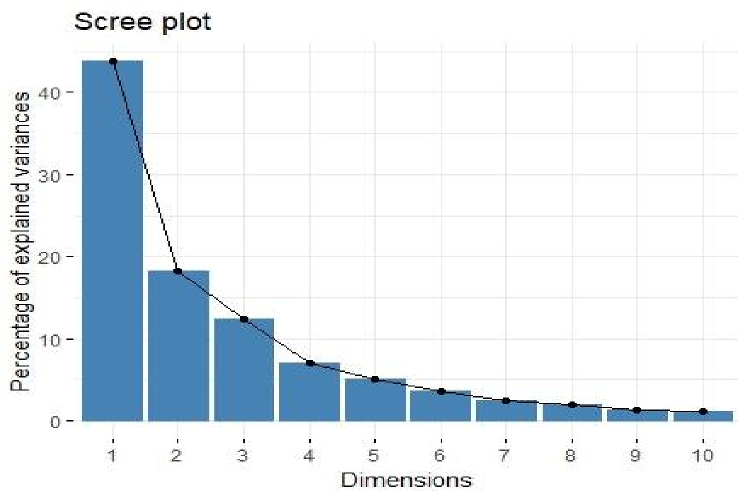

Fig. 2. Scree plot diagram constructed using principal components

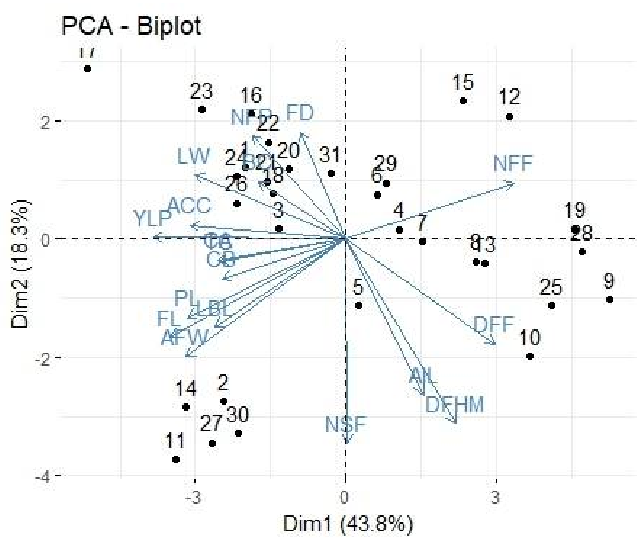

Fig. 3.Bi-Plot diagram of principal components

Based on genetic distance, contribution of traits towards divergenceand *Par se* performance of the cross combinations in between genotypes PCP-17 and PCP-19, PCP-17 and PCP-9, PCP-17 and PCP-28, PCP-19 and PCP-11,PCP-14 and PCP-9 andPCP-23 and PCP-10 may be utilized in future improvement programme. The PC analysis ultimately showed the amount of variability for the traits among the genotypes that could be used for the improvement of pointed gourd. The parents involved in the crosses belonging to the maximum divergent clusters were expected to manifest maximum heterosis and also wide variability.

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