Vegetable Science (2019) 46 (1&2) : 50-55

Diversity analysis of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) germplasm by multivariate analysis

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Received: October 2019 / Accepted: December 2019

Abstract

Thirteen morphological traits of leaves, fruit and seed were employed to discriminate 127 bottle gourd germplasm, as well as to assess their morphological diversity. Results showed that bottle gourd accessions were highly diversified in characteristics of leaves, fruit and seed. Ten quantitative traits exhibited high degrees of variability with significant differences between accessions. Correlation analysis revealed that characters such as fruit length (0.888), fruit width (0.690), fruit weight (0.538), total number of fruits per plant (0.891) and vine length (0.329) had strong correlation with yield per plant at 5% level of significance. Principal component analysis (PCA) revealed that the first two components were responsible for 36.77% of the phenotypic variability. The first principal component (PC) had accounted for 24.11% of the total variability followed by second PC accounted for 12.64% of the total variability. The genotypes variability evaluated by hierarchical cluster analysis conducted on the quantitative traits, grouped the genotypes into three clusters and cluster-I comprise of medium vine length accessions with lower days to take 50% flowering, high fruit width, number of fruits per plant, 100 seed weight, vine length and yield per plant.

Keywords: Bottle gourd, diversity analysis, principal component analysis, correlation, Cluster analysis

Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is an important crop vegetable crop of India and grown in both rainy and summer seasons. It is also known as white-flowered gourd is a diploid (2n = 2x = 22) vine crop mainly grown for its fruit. The genus *Lagenaria* contains five wild species, namely: *L. abyssinica* (Hook f.) Jeffrey, *L. breviflora* (Benth.) Roberty, *L. ruffa* (Gilg.) Jeffrey, *L. sphaerica* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey. All the six species are found in Africa, which is believed to be the centre of genetic diversity for *L. Siceraria.* However largest variability among *Lagenaria* spp. is reported from India. Most fascinating variability is encountered in its fruit shape and size. Variability in fruit characteristics exhibited by bottle gourd has been described by Sirohi and Sivakami. It provides food, medicine, decoration, to make household utensils, fishing floats and musical instruments (Jeffrey 1976). Fresh bottle gourd fruit juice is used as medicine to cure various diseases including flatulence, constipation, diabetes mellitus, hypertension, liver diseases and as a diuretic (Ghule et al. 2007). The seeds of this crop are rich in essential amino acids and oil. Some bottle gourd types are exclusively grown for their seeds (Achigan-Dako et al. 2008). Further, bottle gourd serves as a rootstock in watermelon breeding to control soil-borne diseases and to manage low soil temperature stress (Lee 1994; Yetisir and Sari 2003). Recently, crosses were made between watermelon and bottle gourd as pollen (male) parent, which led to the production of seedless watermelon (Sugiyama et al. 2014). Bottle gourd exhibits significant genetic variation for fruit colours, shapes and sizes (Morimoto et al. 2005; Yetiºir et al. 2008; Xu et al. 2014), fruit shell thickness, fruit length and fruit width (Morimoto et al. 2005; Harika et al. 2012), seed colours and sizes (Morimoto et al. 2005; Yetiºir et al. 2008) and other agro-morphological characteristics (Morimoto et al. 2005; Sivaraj and Pandravada 2005; Morimoto et al. 2006; Achigan-Dako et al. 2008; Xu et al. 2014). Well-characterised germplasm is essential for bottle gourd improvement programs and its strategic conservation. Morphological traits have been used to characterise and evaluate bottle gourd accessions as morphological traits are quick, intuitive and simply distinguishable. The present study was initiated to assess the morphological diversity of different horticultural traits in 127 bottle gourd accessions, and then used the morphological traits to explore the phylogenetic relationships among the

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accessions. Thirteen quantitative traits of leaf and fruit were recorded and analyzed using multivariate applications, including the vine length and 100 seed weight. Multivariate analysis is conceptualized by tradition as the statistical study of experiments in which multiple measurements are made on each experimental unit and for which the relationship among multivariate measurements and their structure are important to the experiment's understanding.

Material and Methods

The germplasm used in this experiment consisted of 127 accessions collected from different parts of India as largest variability in its fruit shape and size among *Lagenaria* spp. is reported from India. All germplasm used in this study were conserved in National Gene Bank at National Bureau of Plant Genetic Resources, New Delhi. The present investigation was carried out at Research Farm of the National Bureau of Plant Genetic Resources, in Augmented Block Design with four checks (Pusa Samridhi, Pusa Santushti, Pusa Naveen and Pusa Sandesh) for phenotypic evaluation during kharif season in the year 2016. The experimental site was situated at 28°35'N latitude and 77°12' E longitudes with an altitude of 228.6 m above mean sea level. The soil is sandy loam in texture. The crop raised in well-prepared hills with a spacing of 60 cm between hills and 3m between channels. The recommended dose of fertilizer and agronomic practices were followed to raise a successful crop. There were five hills per replication out of which three plants were selected for taking observation on 13 traits, viz., leaf length (cm), leaf width cm), petiole length (cm), days to 50% flowering, peduncle length (cm), internodal length (cm), fruit length (cm), fruit width (cm), fruit weight (kg), 100 seed weight (g), no. of fruit per plant, vine length (m) and yield per plant (kg/plant).

Most statistical analyses were performed using SPSS 13.0 statistical software. The percent of each qualitative characteristic in bottle gourd germplasm was calculated. Coefficients of variance (CV%) were used as indicators of variability in the studied accessions. Correlation coefficient for quantitative traits was calculated using method of Pearson correlation coefficient. Relationships among morphological characters in bottle gourd accessions determined by principal component analysis (PCA). Cluster analysis performed based on Euclidean distance using Wards method by cluster function in Excel stat software. Scatter plot was prepared according to the PC1 and PC2 using Excel stat software.

Results and Discussions

The analysis of variance showed that all quantitative parameters were significantly differed between accessions ($P < 0.01$; Table 1). Mean, standard deviation (SD), range and coefficient of variation (CV) values for studied variables in studied bottle gourd germplasm presented in Table 1. Among thirteen quantitative variables, coefficients of variation for leaf width, petiole length, peduncle length, internodal length, fruit length, fruit width, fruit weight, 100 seed weight, no. of fruit per plant and yield per plant were more than 20%. The greatest variance obtained in number of fruits per plant and the CV value was 66.74%. While CV values of leaf length and days to 50% flowering were less than 20%. The minimum coefficient of variation recorded in days to 50% flowering and was 16.71%. These characteristics showed significant morphological diversity in bottle gourd accessions. The days to 50% flowering ranged from 57 to 91 days. Among fruit characters, *viz*, fruit length (10.5-39.7 cm), fruit width (4.4-15.5 cm), fruit weight (0.3-1.4 kg), 100 seed weight (5.3-30.7 g) and number of fruits per plant (1-16), wide range of variations observed. Similarly, yield per plant varied from 0.23 to 19.12 kg.

Correlation coefficient analysis (Table 2) revealed that characters such as fruit length (r=0.888), fruit width (r=0.690), fruit weight (0.538), total number of fruit

Table 1. Estimates of variance for the traits studied in bottle gourd genotypes.

S. No.	Variables	Mean	SD	Variance	SE Mean	CV	Minimum	Maximum
1	Leaf length (cm)	15.89	2.5	6.1	0.21	15.54	11.5	21.9
2	Leaf width (cm)	20.17	4.4	19.26	0.38	21.75	11.8	51.3
3	Petiole length (cm)	14.02	3.8	14.73	0.33	27.37	6.7	23.3
4	Days to 50% Flowering	74.32	7.1	50.6	0.62	9.57	57	91
5	Peduncle length (cm)	9.39	2.5	6.31	0.21	26.75	4.4	16.6
6	Internodal length (cm)	11.89	2.8	7.5	0.24	23.15	5.6	19
7	Fruit length (cm)	24.39	6.1	36.82	0.53	24.87	10.5	39.7
8	Fruit width (cm)	7.13	2.2	4.77	0.19	30.63	4.4	15.5
9	Fruit weight (kg)	0.62	0.3	0.07	0.02	44.33	0.3	1.4
10	100 Seed weight (g)	15.53	4.7	22.31	0.41	30.7	5.3	30.7
11	No. of fruit per plant	4.48	3	8.9	0.26	66.74	1	16
12	Vine length (m)	6.36	1	1.03	0.09	16.23	3.1	9.9
13	Yield per plant (Kg/plant)	2.6	2.6	6.78	0.22	38.6	0.23	19.12

Table 2. Correlation coefficients amongst thirteen morphological variables under study

Variables	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Days to 50% Flowering	Peduncle length (cm)	Internodal length (cm)	Fruit length (cm)	Fruit width (cm)	Fruit weight (kg)	100 Seed weight (g)	No. of fruit per plant	Vine length (m)	Yield per plant (Kg/ plant)
Leaf length (cm)	1	0.494	0.452	-0.104	0.096	0.361	0.037	-0.035	0.093	0.056	0.287	0.311	0.291
Leaf width (cm)		1	0.311	-0.168	0.081	0.282	-0.050	0.006	0.146	0.250	0.181	0.188	0.210
Petiole length (cm)			1	-0.045	0.035	0.248	0.119	-0.129	0.107	0.185	0.180	0.170	0.245
Days to 50% Flowering				1	0.033	-0.318	0.074	-0.157	-0.113	-0.094	-0.204	-0.268	-0.198
Peduncle length (cm)					1	-0.116	-0.075	-0.004	-0.068	-0.068	0.018	0.010	0.013
Internodal length (cm)						1	-0.068	0.033	0.102	0.099	0.188	0.848	0.146
Fruit length (cm)							1	-0.416	0.150	0.107	0.008	-0.172	0.888
Fruit width (cm)								1	0.104	0.083	0.080	0.184	0.690
Fruit weight (kg)									1	0.094	0.064	0.004	0.538
100 Seed weight (g)										1	0.096	0.080	0.160
No. of fruit per plant											1	0.443	0.891
Vine length (m)												1	0.329

Values in bold are different from 0 with a significance level alpha=0.05

per plant (0.891) and vine length (0.329) had strong correlation with yield per plant at 5% level of significance, suggesting that fruit yield could be improved by making selection on the basis of the afore said characters, which are considered as major yield attributes in bottle gourd as reported by Raja *et al.* (2006) and Sunil *et al*. (2014). While negative correlation was observed for days to 50% flowering with yield per plant. Leaf length had significant correlation with leaf width (r=0.494), petiole length (r=0.452), internodal length (r=0.361) and vine length (r=0.311). Vine length had strong positive correlation with internodal length (r=0.848) and number of fruit per plant (r=0443). Days to 50% flowering had significant negative correlation with peduncle length (r=-0.78), total number of fruits per plant (r=-0.44) and seed oil content (-0.47). Fruit length recorded significant negative correlation with fruit width (r=-0.68). Also, fruit weight was found to be negatively correlated with the total number of fruits per plant (r=-0.79). Significant positive correlation was observed for total number of fruits and number of marketable fruits per plant (r=0.69). The trait 100-seed weight was significantly correlated with fruit yield per plant (r=0.65). Pradeep and Syamal (2010) reported similar findings in their character association studied in bottle gourd. 100 seed weight had significant positive correlation(p>0.05) with leaf width peduncle length suggesting that greater leaf width and peduncle length more will be the translocation of photosynthetic assimilates produced in the peduncle which are directed for seed development.

Principal component analysis (PCA) is a variable reduction procedure to develop a smaller number of artificial variables (called principal components) that will account for most of the variation in the observed variables. For the current study, PCA performed for bottle gourd germplasm to observe any possible cluster within analysed samples. A new set of 13 orthogonal variables (PCs) was generated by PCA. The first six principal components of data accounted for 65.07 % of the total variance among accessions with Eigen values >1 (Table 3). Rest of the PCs showed progressively smaller eigen values and counted less towards total variability (Fig. 1). The first principal component had the highest eigen value of 3.13 and accounted for 24.11%

Table 3. Eigen values, accumulated variance and correlations between original variables and the first six PCs representing variability of bottle gourd accessions

Variable	PC1	PC 2	PC 3	PC 4	PC 5
Leaf length (cm)	0.372	-0.189	-0.328	0.115	0.029
Leaf width (cm)	0.323	-0.146	-0.329	-0.110	0.317
Petiole length (cm)	0.291	-0.345	-0.226	0.014	0.061
Days to 50% Flowering	-0.239	-0.267	0.010	0.303	0.190
Penducle length (cm)	0.009	0.004	-0.189	0.566	0.484
Internodal length (cm)	0.298	0.031	-0.275	-0.348	-0.283
fruit length (cm)	-0.006	-0.569	0.296	-0.063	-0.167
Fruit width (cm)	0.085	0.577	0.036	-0.149	0.340
Fruit weight (kg)	0.214	-0.064	0.474	-0.264	0.390
100 Seed weight (g)	0.167	-0.126	0.023	-0.369	0.319
No of fruit per plant	0.393	0.095	0.268	0.368	-0.252
Vine length (m)	0.330	0.257	-0.101	0.194	-0.285
Yield per plant (Kg/plant)	0.426	0.011	0.474	0.192	0.049
Eigen value	3.13	1.65	1.42	1.2	1.05
Accumulated variance	24.08	36.77	47.74	56.97	65.07

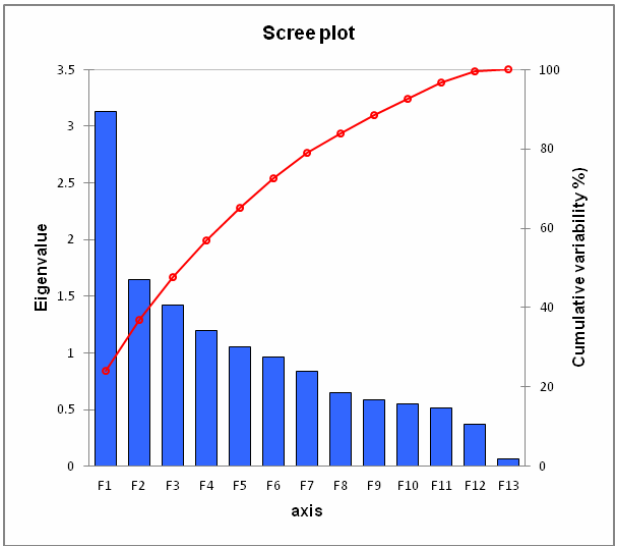

Fig. 1. Scree plot depicting Eigen values and cumulative of principle

of the total variability which was contributed by leaf length, leaf width, petiole length, days to 50% flowering, peduncle length, internodal length and number of fruit per plant, vine length and yield per plant. The second PC had the eigen value of 1.64 and accounted for 12.64% and featured fruit length and fruit width. A loading plot of 13 morphological parameters was also presented in fig 2.

The germplasm variability evaluated by hierarchical cluster analysis (dendrogram) conducted on the quantitative traits, grouped the genotypes into three clusters (Fig 3). Ward's Cluster method used in this study and grouped I, II and III having 41, 49 and 41 accessions, respectively (Table 5). The cluster I comprise of medium vine length accessions with lower days to take 50% flowering, high fruit width, numbers of fruit per plant, 100 seed weight, vine length and yield per plant (Table 4). The variety in cluster II is essentially low in fruit length, fruit weight, number of

Table 4. Clusters means for 13 quantitative traits of bottle gourd accessions

Variable/ Character	I	II	III
Leaf length (cm)	16.799	15.031	16.005
Leaf width (cm)	22.559	19.011	19.196
Petiole length (cm)	15.713	11.746	15.054
Days to 50% Flowering	67.537	74.265	81.195
Penducle length (cm)	9.196	9.729	9.151
Internodal length (cm)	13.629	10.986	11.217
fruit length (cm)	24.579	20.762	28.530
Fruit width (cm)	7.530	7.457	6.312
Fruit weight (kg)	0.662	0.574	0.641
100 Seed weight (g)	18.267	13.279	15.485
No. of fruit per plant	6.000	3.429	4.220
Vine length (m)	7.027	6.039	6.085
Yield per plant (kg/plant)	3.811	1.714	2.580

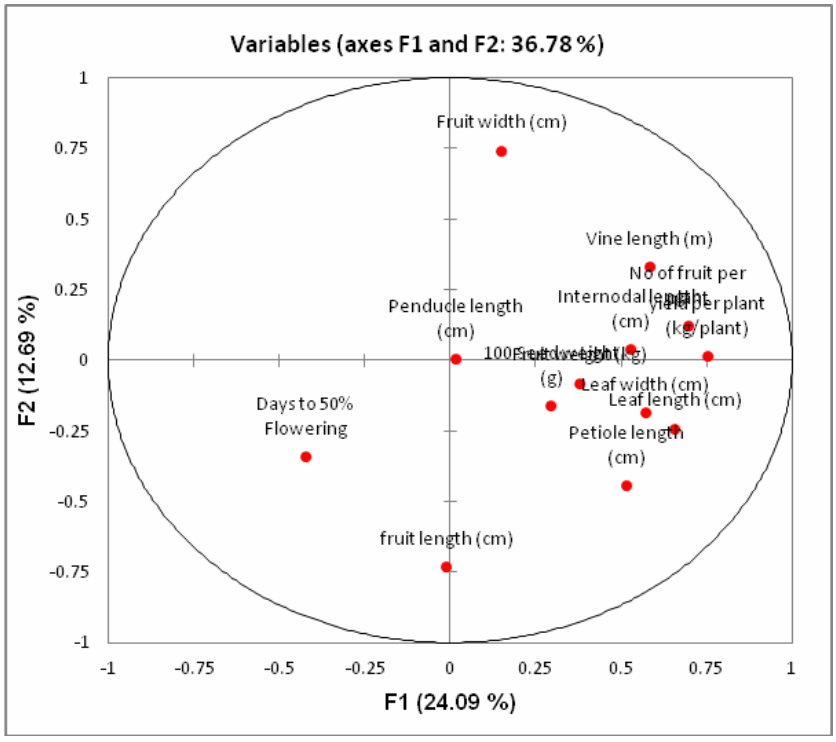

Fig. 2. Loading plot of 13 morphological parameter components.

Table 5. Accessions including in three different clusters

Cluster I	Cluster II	Cluster III
Pusa Samridhi, Pusa Santushti, Pusa Naveen, Pusa Sandesh, IC284826, IC204890, IC522210-A, IC276413, IC343153, IC336826, IC256043, IC294891-1, IC256051, IC385814, IC344759, IC522878, IC353408, IC318883-1, IC256043-1, IC276405, IC276552, IC321592, IC337078, IC052256, IC297512, IC262868, IC536894-1, IC316017-1, IC536594-A, IC539696, IC308864, IC342079, IC284895, IC318883, IC546165, IC336820, IC276353, IC276153, IC538163, IC522868, IC319840	IC546166, IC339209-A, IC424502-A, IC316018, IC536894, IC522210, IC522874, IC546167, IC539711, IC330999, IC306128-A, IC550725, IC346128, IC322278, IC567390, IC398533, IC421947 , IC306128-1, IC550741, IC331981, IC316017, IC546148 IC398543, IC339222 IC284952, SKY/AC-273, IC284830, IC418491, IC339199, IC310206, IC321559, IC541393-1, IC371675, IC424502 IC306422, IC284809, IC522858, IC321591, IC278793, IC371602, IC284933, IC522857, IC276328, IC284874, IC536518, IC397276	IC308564, IC307077, IC372079, IC047045, IC339206, IC331088, IC417704, IC308563, IC382240, IC417538, IC385816-1, IC331121, IC397253, IC546151, IC385816, IC522859 IC470445, IC284816, IC398534, IC306128, IC343153-A, IC592210-1, IC411915, IC331175 IC546143, IC371745 IC397291, IC321589, IC411811, IC538142, IC538196 , IC382188 IC522866, IC331025, IC308564-A, IC539701, IC383252, IC522210-2, IC330987 , IC020462, IC339199-A

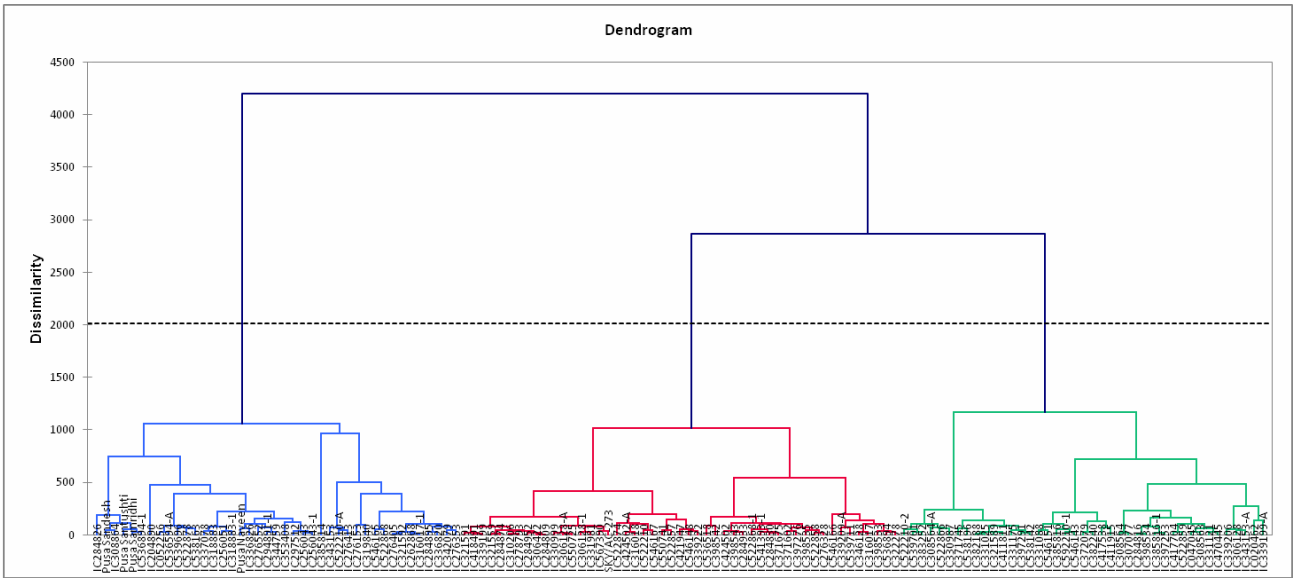

Fig 3. Dendrogram of grouping for 127 bottle gourd accessions and four checks based on 13 investigated morphological traits using Ward's method

Table 6. List of superior bottle gourd accessions

Traits	Superior Accessions
Days to 50% Flowering	IC284826, IC308864, IC536894, IC204890, IC336826
Fruit length	IC522866, IC339199, IC330987, IC538163, IC020462
Fruit width	IC522210, IC470445, IC522868, IC276413, IC339209
Fruit weight	IC020462, IC319840, IC397276, IC321589, IC339199
No. of fruit per plant	IC020462, IC047045, IC052256, IC204890, IC256043
Vine length	IC256043, IC276153, IC276552, IC047045, IC052256

fruit per plant and 100 seed weight. The cluster III took highest days to 50% flowering. It could be concluded that the genotypes in cluster I are promising in the production of high fruit width, number of fruit per plant, 100 seed weight, vine length and yield per plant. Therefore, accessions of cluster I could be recommended for selection and for further breeding program and in seed production programme.

Conclusion

In the present study, 127 bottle gourd accessions showed extensive genetic diversity based on thirteen morphological traits. Five accessions (IC522866, IC339199, IC330987, IC538163, IC020462) recorded high fruit length in marketable size. Highest number of fruits per plant found in accession IC020462, IC047045, IC052256, IC204890 and IC256043. Accessions number IC020462, IC319840, IC397276, IC321589 and IC339199 recorded high fruit weight. These cultivars are an important source of diversity, which could be used in future breeding programs.

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पत्तियों, फलों और बीजों के 13 रूपात्मक लक्षणों के आधार पर लौकी के 127 जननद्रव्यों का विविधता आंकलन किया गया। परिणाम से स्पष्ट हुआ कि लौकी के पत्तियों, फलों और बीजों की विशेषताओं में अत्यधिक विविधता विद्यमान है। जननद्रव्यों के बीच कुल दस मात्रात्मक लक्षणों में उच्च स्तर की सार्थक विविधता पाई गयी। सहसंबंध विश्लेषण से स्पष्ट हुआ कि फलों की लम्बाई (0.888), फलों की चौड़ाई (0.690), फल का वजन (0.538), प्रति पौध फलों की संख्या (0.891) और लता की लम्बाई (0.329) जैसे गुणों के प्रति उच्च सहसंबंध पाया गया। प्रमुख घटक विश्लेषण एवं 5 प्रतिशत सार्थकता के आधार पर बाह्यदृश्य प्रारूप परिवर्तनशीलता हेतु 36.77 प्रतिशत की सहभागिता है। प्रथम प्रमुख घटक का उच्चतम मूल्यांक 3.13 था और कुल परिवर्तनशीलता 24.11 प्रतिशत था एवं इसके बाद प्रमुख घटक मूल्यांक 1.64 के परिप्रेक्ष्य था और कुल परिवर्तनशीलता 12.64 प्रतिशत था। मात्रात्मक गुणों के लिए पदानुक्रमित क्लस्टर विश्लेषण द्वारा प्रभेदों का मूल्यांकन किया गया और परिणाम के आधार पर प्रभेदों को 3 समूहों और उप समूहों में वर्गीकृत किया गया जिसमें मध्यम लता की लम्बाई, कम दिनों के साथ 50 प्रतिशत पुष्पन, फल की अधिकतम चौड़ाई, प्रति पौध फलों की संख्या, 100 बीजों का भार, लता लम्बाई और प्रति पौध उपज समूह–1 में रखा गया।

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