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RESEARCH ARTICLE

Assessment of genetic divergence in bottle gourd (*Lagenaria siceraria*) germplasm using Mahalanobis's D2 statistic for parental line selection

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Abstract

An investigation was conducted to assess the genetic divergence among 24 genotypes of bottle gourd (*Lagenaria siceraria*) to identify suitable parental lines for future breeding programs. The experiment was laid out in a randomized block design with three replications during the spring season of 2024. Quantitative data for 17 traits were analysed using Mahalanobis's D^2 statistic. The genotypes were grouped into 7 distinct clusters, with cluster 1 being the largest with ten genotypes, followed by cluster 2 with seven genotypes, followed by cluster 3 with three. The remaining four clusters were small, each containing one genotype. Analysis of cluster means revealed significant diversity across the genotypes. Cluster 4 exhibited the most desirable traits, showing the longest fruit length (42.567 cm) and the highest average fruit weight (1213.333 g). In contrast, cluster 6 had the highest number of fruits per plant (11.557) and the shortest harvest period (49.777 days). The average intra-cluster distance was lowest for the single-genotype clusters (0.000) and highest for cluster 2 (260.826). The inter-cluster distance, which indicates the level of diversity between groups, was found to be highest between cluster 3 and cluster 6 (2361.074), suggesting these groups are the most genetically distant. Conversely, the smallest inter-cluster distance was observed between cluster 5 and 6 (400.438). The character contributing most to the overall divergence was average fruit weight (34.84%), followed by days to first female flower anthesis (20.91%) and days to first fruit harvest (13.96%). These findings suggest that genotypes from the most divergent clusters, such as those from cluster 3 and 6, should be selected for hybridization programs to create superior recombinants with a broad genetic base.

Keywords: Bottle gourd, genetic divergence, D^2 statistics, cluster distance, parental selection.

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Introduction

Bottle gourd [*Lagenaria siceraria* (Mol.) Stand.] belonging to the family Cucurbitaceae is a warm season crop cultivated for its fruits. The cultivated species is commonly known as bottle gourd, lauki, calabash or white flowered gourd. It is a cross-pollinated crop with large amount of variation for many economically important traits. India is the secondary center of origin of bottle gourd endowed with a variety of diverse germplasm (Sultana et al., 2018). The average day temperature of 20 to 27°C with lower night temperature of 18 to 23°C is optimal for growth and fruiting.

Genetic diversity is necessary to select suitable parental lines from available indigenous germplasms, to carry out any successful breeding program. The study of genetic divergence is a popular method in parent selection for researchers involved in breeding programs of several crops, leading to a reduction in the number of crosses (Guerra et al., 1999). The progenies derived from diverse parents are expected to show a broad spectrum of genetic variability and provide better scope to isolate a superior recombinant. Therefore, genetically diverse genotypes should be used

in a hybridization program to get a superior recombinant. The multivariate analysis is the most widely used statistical tools to quantify the genetic divergence. Among the various methods developed to study the genetic divergence in the genotypes/accessions, the Mahalanobis D^2 (Mahalanobis, 1936) is the most reliable and widely used statistical tools to quantify the degree of genetic divergence by assessing the relative contribution of different characters to the total divergence. It is a very useful technique and has been used by several workers in case of cross-pollinated crops (Bashar et al., 2016; Hasan et al., 2015).

Material and Methods

The experimental material for the present investigation, which included 24 genotypes including check, was sown in a randomized block design with three replications during the spring season of 2024. About 15 advance lines from GBPUAT, Pantnagar were taken under the study including the check variety, *i.e.,* Pant Lauki - 3. One local cultivar from Uttarakhand, five local cultivars from Uttar Pradesh and three released varieties from IIVR, Varanasi (Kashi Ganga, Kashi Kirti and Kashi Kiran) were also taken under study. A spacing of 3 × 1 m was maintained, recommended and uniform agronomical practices were adopted to raise the crop. Observations were recorded on five randomly selected and tagged plants from each genotype for all the characters. The mean values measured from these five plants were used for further statistical analysis. Plot means for 17 quantitative characters were used for the statistical analysis. Genetic diversity was studied following Mahalanobis's (1936) generalized distance (D^2) extended by Rao (1952). Based on the D^2 values, the germplasm was grouped into clusters following the method suggested by Tocher (Rao, 1952). Genetic diversity was studied following Mahalanobis's (1936) generalized distance (D^2) extended by Rao (1952). Statistical analyses were carried out using R Studio software.

Results and Discussion

Genetic divergence analysis (D^2 statistics)

The genetic divergence analysis for the yield, quality and yield contributing characters was done by Mahalanobis D^2 statistic.

Grouping of genotypes into different clusters

Seven major clusters were formed by clustering all the 24 genotypes such that genotypes within each cluster exhibited lower D^2 values relative to those in other clusters (Table 1). Cluster pattern reveals that cluster 1 consisting of ten genotypes (PBOG - 11, PBOG - 16, PBOG - 17, PBOG - 23, PBOG - 28, PBOG - 3, PBOG - 30, PBOG - 5, PBOG - 6, PBOG - 88) and cluster 2 consisting of seven genotypes (PBOG - 1, PBOG - 14, PBOG - 15, PBOG - 18, PBOG - 24, Kashi Ganga, Kashi Kirti) were the largest cluster, which was followed by cluster 3 consisting three genotypes (PBOG - 29, PBOG - 4, PBOG - 61), cluster 4 (PBOG - 7), cluster 5 (PBOG - 10), cluster 6 (Kashi Kiran), and cluster 7 (PBOG - 32) consisting one genotypes, respectively.

Table 1: Distribution of 24 genotypes of bottle gourd in different clusters

Cluster	*Number of genotypes*	*List of genotypes*
Cluster 1	10	PBOG - 11, PBOG - 16, PBOG - 17, PBOG - 23, PBOG - 28, PBOG - 3, PBOG - 30, PBOG - 5, PBOG - 6, PBOG - 88
Cluster 2	7	PBOG - 1, PBOG - 14, PBOG - 15, PBOG - 18, PBOG - 24, Kashi Ganga, Kashi Kirti
Cluster 3	3	PBOG - 29, PBOG - 4, PBOG - 61
Cluster 4	1	PBOG - 7
Cluster 5	1	PBOG - 10
Cluster 6	1	Kashi Kiran
Cluster 7	1	PBOG - 32

Cluster means of characters

It was observed that in days to first male flower anthesis, cluster 4 showed the earliest flowering at 45.333 days, followed closely by cluster 5 (43.714), cluster 2 (43.278), cluster 1 (43.224), cluster 6 (43.223), cluster 3 (42.337), and cluster 7 (42.333) (Table 2). The variation between clusters was relatively small, with all means falling within a 3-day range. The days to first female flower anthesis cluster 4 again had the highest value (47.223 days), with cluster 5 (46.715), cluster 2 (46.544), cluster 1 (46.073), cluster 3 and 7 (both 45.557), and the least was observed in cluster 6 (44.443). The spread was wider than for male flowering, spanning nearly 3 days. Node of first male flower appearance was observed that cluster 3 displayed the highest nodal position (11.330), followed by cluster 4 (11.223), cluster 6 (10.670), cluster 2 (10.467), cluster 5 (10.333), cluster 7 (10.330), and cluster 1 (9.816). This trait showed more variability between clusters than flowering time. Node of first female flower appearance was observed that cluster 7 had the highest nodal position (11.333), with cluster 3 (11.557), cluster 6 (11.220), cluster 2 (10.699), cluster 5 (10.968), cluster 1 (10.480), and the least was in cluster 4 (9.777). Interestingly, cluster 4 showed substantially lower values than other groups.

It was observed that days to first harvest, cluster 4 required the longest time (54.667 days), followed by cluster 5 (52.222), cluster 2 (51.867), cluster 1 (50.562), cluster 3 (50.447), cluster 6 (49.777), and cluster 7 (48.777). The range exceeded 5 days between extreme clusters. The diversity in the fruit length was observed that cluster 4 produced exceptionally long fruits (42.567 cm), followed by cluster 2 (29.538), cluster 6 (30.990), cluster 1 (21.744), cluster 3 (20.137), cluster 5 (11.009), and cluster 7 (10.287). The values showed extreme variation, spanning over 32 cm. In terms of fruit diameter, diversity ranged from the widest fruits in cluster

Table 2: Cluster mean analysis of 24 genotypes of bottle gourd

	Cluster 1	*Cluster 2*	*Cluster 3*	*Cluster 4*	*Cluster 5*	*Cluster 6*	*Cluster 7*
DFMF	43.224	43.278	42.337	45.333	43.714	43.223	42.333
DFFF	46.073	46.544	45.557	47.223	46.715	44.443	45.557
NFMF	9.816	10.467	11.330	11.223	10.333	10.670	10.330
NFFF	10.480	10.699	11.557	9.777	10.968	11.220	11.333
DFH	50.562	51.867	50.447	54.667	52.222	49.777	48.777
F L	21.744	29.538	20.137	42.567	11.009	30.990	10.287
F D	6.024	6.261	9.970	6.160	10.650	5.993	10.830
AFW	848.519	728.750	820.833	1213.333	783.572	691.667	858.333
NFP	10.593	9.689	11.220	8.330	10.333	11.557	9.890
NPP	12.854	10.688	8.443	11.890	10.031	12.890	8.330
NNP	46.961	35.601	42.890	52.890	34.237	23.443	31.447
VL	4.931	3.091	3.797	5.020	3.604	1.920	1.893
IL	10.614	8.412	8.677	9.330	10.168	7.390	5.790
As A	4.707	6.907	8.893	6.803	6.429	4.703	8.373
An A	81.300	81.452	78.730	83.607	80.945	80.283	86.040
D.M	6.646	6.293	6.493	7.063	6.899	6.063	6.343
F Y	9.114	6.979	8.170	9.883	7.672	7.400	8.440

DFMF: days to first male flower anthesis, DFFF: days to first female flower anthesis, NFMF: node at which first male flower appears, NFFF: node at which first female flower appears, DFH: days to first fruit harvest, F L: fruit length (cm), F D: fruit diameter (cm), AFW: average fruit weight (g), NFP: number of fruits per plant NPP: number of primary branches per plant, NNP: number of nodes per plant, VL: vine length (m), IL: internodal length (cm), As A: ascorbic acid in fruit (mg/100 g), An A: antioxidant activity in fruit (%), D M: dry matter in fruit (%), and F Y: fruit yield per plant (kg).

7 (10.830 cm), followed by cluster 5 (10.650 cm), cluster 3 (9.970 cm), cluster 2 (6.261 cm), cluster 4 (6.160 cm), cluster 1 (6.024 cm), and cluster 6 (5.993 cm). Three clusters stood out with substantially larger diameters.

The average fruit weight was observed to be heaviest in the cluster 4 (1213.333 g), followed by cluster 7 (858.333), cluster 1 (848.519), cluster 3 (820.833), cluster 5 (783.572), cluster 2 (728.750), and cluster 6 (691.667). The range exceeded 500 g between the two extremes. The data represented that the number of fruits per plant was found to be with the highest count in the cluster 6 (11.557), followed by cluster 3 (11.220), cluster 1 (10.593), cluster 5 (10.333), cluster 7 (9.890), cluster 2 (9.689), and the least was observed to be in cluster 4 (8.330). Most clusters clustered between 9-11 fruits. Number of primary branches was observed, cluster 1 showed the most branches (12.854), followed by cluster 6 (12.890), cluster 4 (11.890), cluster 2 (10.688), cluster 5 (10.031), cluster 3 (8.443), and the least number of primary branches was observed in cluster 7 (8.330). The range was relatively large at over 4 branches. The number of nodes per plant cluster 4 had the highest node count (52.890), followed by cluster 1 (46.961), cluster 3 (42.890), cluster 2 (35.601), cluster 5 (34.237), cluster 7 (31.447), and cluster 6 (23.443). The variation was substantial, exceeding 30 nodes.

In vine length, cluster 4 showed the longest vines (5.020 m), followed by cluster 1 (4.931), cluster 3 (3.797), cluster 5 (3.604), cluster 2 (3.091), cluster 6 (1.920), and the smallest vine length was observed to be in cluster 7 (1.893). The range in the evaluated germplasm was dramatic, spanning over 3 m. It was recorded that the internode length was longest in cluster 1 (10.614 cm), followed by cluster 5 (10.168), cluster 4 (9.330), cluster 3 (8.677), cluster 2 (8.412), cluster 6 (7.390), and the shortest internodal length is observed in cluster 7 (5.790). The variation was nearly 5 cm between extremes.

The ascorbic acid content in the fruit of the evaluated genotypes was observed that in cluster 3, the highest content was recorded (8.893 mg/100 g), followed by cluster 7 (8.373), cluster 2 (6.907), cluster 4 (6.803), cluster 5 (6.429), cluster 1 (4.707), and cluster 6 (4.703). The range was substantial at over 4 mg/100 g of fresh fruit. The antioxidant activity in fruit was found to be in cluster 7 consisting of the highest percentage (86.040%), followed by cluster 4 (83.607), cluster 2 (81.452), cluster 1 (81.300), cluster 5 (80.945), cluster 6 (80.283), and the least in cluster 3 (78.730). The range was relatively narrow at under 8%. With the highest percentage of dry matter in cluster 4 (7.063%), followed by cluster 5 (6.899), cluster 1 (6.646), cluster 3 (6.493), cluster 7 (6.343), cluster 2 (6.293), and the least amount was observed in cluster 6 (6.063). The variation was minimal, under 1%.

The fruit yield per plant was observed that cluster 4 had the highest yield (9.883 kg/plant), followed by cluster 1 (9.114), cluster 7 (8.440), cluster 3 (8.170), cluster 5 (7.672), cluster 6 (7.400), and cluster 2 (6.979) was found to be with

Table 3: Analysis for intra and inter cluster distances for 24 genotypes

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	224.273	496.378	771.486	504.071	525.755	967.376	524.036
Cluster 2		260.826	765.211	776.438	615.139	756.103	1114.586
Cluster 3			242.708	542.215	1615.308	2361.074	657.929
Cluster 4				0.000	1542.524	1879.411	868.266
Cluster 5					0.000	400.438	1057.276
Cluster 6						0.000	2157.093
Cluster 7							0.000

the lowest fruit yield per plant. The range in the evaluated genotypes was observed to be nearly 3 kg between the two extremes. Several workers Masud et al. (2002), Morimoto et al. (2001) and Shubha et al. (2002) worked on divergence analysis in different accessions of bottle gourd and found similar results for cluster means of the characters.

Average intra and inter cluster distances

The distance between the two observations that belong to the different cluster is the intercluster distance (Table 3); on the other hand, the distance between the two objects belonging to the same cluster is the intracluster distance. In intracluster distance, the lowest value was observed in cluster 4, cluster 5, cluster 6, and cluster 7 (0.000), following cluster 1 (224.273), cluster 3 (242.708), and the highest value was observed in cluster 2 (260.826).The highest intercluster distance was found between cluster 3 and cluster 6 (2361.074), followed by cluster 6 and cluster 7 (2157.093). On the other hand, the smallest intercluster distance was observed between cluster 5 and cluster 6 (400.438), followed by cluster 1 and cluster 2 (496.378). Several workers Masud et al. (2002), Ram et al. (2001) and Rasheed et al. (2002) worked on divergence analysis in different accessions of bottle gourd and found similar results for average intra and inter cluster distances.

Contribution of characters towards divergence

The present study with 24 genotypes of bottle gourd was evaluated and concluded that the average fruit weight contributed maximum in the diversity (34.84%) followed by days to first female flower anthesis (20.91%), and days to first fruit harvest (13.96%) (Table 4). Moderate contribution was observed from days to first male flower anthesis (7.60%), number of nodes per plant (5.49%), number of primary branches per plant (3.79%), node number of first male flower appear (2.54%), antioxidant activity in fruit (2.52%), fruit yield per plant (2.37%), node number of first female flower appear (1.71%) internodal length (1.55%), and fruit length (1.53%). Low contribution was observed from fruit diameter (0.8%), ascorbic acid in fruit (0.12%), vine length (0.11), dry matter content (0.07%), and number of fruits per plant (0.06). Similar results were observed by Masud et al. (2002), Rathore et al. (2001) and Sunil et al. (2002) worked on divergence analysis in different accessions of bottle gourd for D^2 statistics.

Table 4: Percentage contribution of various traits under study towards genetic divergence in bottle gourd

S. No.	*Source*	*Contribution (%)*
1	Days to first male flower anthesis	7.60
2	Days to first female flower anthesis	20.91
3	Node number of first male flower appear	2.54
4	Node number of first female flower appear	1.71
5	Days to first fruit harvest	13.96
6	Fruit length	1.53
7	Fruit diameter	0.80
8	Average fruit weight	34.84
9	Number of fruits per plant	0.06
10	Number of primary branches per plant	3.79
11	Number of nodes per plant	5.49
12	Vine length	0.11
13	Internodal length	1.55
14	Ascorbic acid in fruit	0.12
15	Antioxidant activity in fruit	2.52
16	Dry matter content	0.07
17	Fruit yield per plant	2.37
Total		100

Conclusion

Genetic divergence among bottle gourd genotypes was assessed using Mahalanobis' D^2 statistics based on 17 characters. The genotypes exhibited substantial genetic variability and were grouped into seven distinct clusters following Tocher's method. The maximum inter-cluster distance was observed between cluster III, comprising genotypes PBOG-29, PBOG-4 and PBOG-61, and cluster VI containing genotype PBOG-27, suggesting a high degree of genetic divergence. Therefore, crosses between genotypes belonging to these divergent clusters are expected to be

promising for exploiting heterosis and generating superior recombinants in bottle gourd improvement programmes.

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सारांश

आगामी प्रजनन कार्यक्रमों के लिए उपयुक्त पैतृक लाइनों की पहचान करने हेतु लौकी [*Lagenaria siceraria* (Mol.) Standl.] के 24 जीनोटाइप्स के बीच आनुवंशिक विविधता का आकलन किया गया। अध्ययन वर्ष 2024 की वसंत ऋतु के दौरान तीन प्रतिकृतियों वाले यादृच्छिक ब्लॉक डिजाइन (Randomized Block Design) में किया गया। सत्रह लक्षणों से संबंधित मात्रात्मक आंकड़ों का विश्लेषण महालनोबिस D^2 सांख्यिकी का उपयोग करके किया गया। अध्ययन में जीनोटाइप्स को सात अलग-अलग क्लस्टरों में विभाजित किया गया। क्लस्टर 1 सबसे बड़ा था, जिसमें 10 जीनोटाइप्स शामिल थे, इसके बाद क्लस्टर 2 में 7 तथा क्लस्टर 3 में 3 जीनोटाइप्स शामिल थे। शेष चार क्लस्टर छोटे थे, जिनमें प्रत्येक में एक जीनोटाइप शामिल था। क्लस्टर माध्यों के विश्लेषण से जीनोटाइप्स के बीच पर्याप्त विविधता का पता चला। क्लस्टर 4 में सबसे वांछनीय लक्षण पाए गए, जिसमें फलों की अधिकतम लंबाई (42.567 सेमी) तथा फलों का सर्वाधिक औसत वजन (1213.333 ग्राम) दर्ज किया गया। इसके विपरीत, क्लस्टर 6 में प्रति पौधा फलों की संख्या सर्वाधिक (11.557) तथा फसल कटाई की अवधि सबसे कम (49.777 दिन) पाई गई। औसत अंतः-क्लस्टर दूरी एकल-जीनोटाइप वाले क्लस्टरों में न्यूनतम (0.000) तथा क्लस्टर 2 में अधिकतम (260.826) थी। अंतर-क्लस्टर दूरी, जो क्लस्टरों के बीच आनुवंशिक विविधता की सीमा को दर्शाती है, क्लस्टर 3 और क्लस्टर 6 के बीच सर्वाधिक (2361.074) पाई गई, जिससे इन दोनों क्लस्टरों के जीनोटाइप्स के बीच अधिकतम आनुवंशिक विविधता का संकेत मिलता है। इसके विपरीत, क्लस्टर 5 और क्लस्टर 6 के बीच सबसे कम अंतर-क्लस्टर दूरी (400.438) दर्ज की गई। कुल विविधता में सर्वाधिक योगदान फलों के औसत वजन (34.84%) का रहा, इसके बाद प्रथम मादा फूल आने के दिनों (20.91%) तथा प्रथम फल तुड़ाई के दिनों (13.96%) का योगदान रहा। इन निष्कर्षों से संकेत मिलता है कि व्यापक आनुवंशिक आधार वाले श्रेष्ठ पुनर्योगजों (recombinants) के विकास के लिए अधिकतम आनुवंशिक रूप से भिन्न क्लस्टरों, विशेष रूप से क्लस्टर 3 और क्लस्टर 6 के जीनोटाइप्स को संकरण कार्यक्रमों में पैतृक लाइनों के रूप में उपयोग किया जा सकता है।