

## GENETIC DIVERGENCE AMONG BOTTLE GOURD GENOTYPES

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### Abstract

A field experiment was conducted at the farm of ACI Plant Genetics Research and Development Center (APGRDC), Mawna, Gazipur, during 2020 - 2021 to assess genetic diversity among 30 bottle gourd [*Lagenaria siceraria* (Molina) Standl.] genotypes across eleven agronomic traits. Multivariate analyses, including PCA, PCoA, CVA, and cluster analysis, were performed. Based on PCA scores and Mahalanobis' D<sup>2</sup> statistics, six distinct clusters were identified. Cluster I contained the most significant number of genotypes (15), while Cluster IV had only one. The significant inter-genotypic distance was recorded between BG10 and BG16, while BG8 and BG21 showed the least divergence. Maximum inter-cluster distance occurred between Clusters V and III, suggesting wide genetic variability, whereas the lowest was between Clusters V and VI. Cluster VI exhibited the highest intra-cluster variability. Trait-wise, Cluster VI was superior in yield per plant, average fruit weight, fruit length, and vine length; Cluster II excelled in flowering- and harvest-related traits; Cluster I had the longest internode and delayed female flowering; and Cluster III had the highest fruit number per plant. Genotypes BG14, BG19, BG20, Hajari, BG22, BG23, and BG25 were identified as promising parents for hybridization to exploit heterosis for yield improvement.

### Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.], locally known as lau, is an important vegetable crop of the family cucurbitaceae, widely cultivated across tropical and subtropical regions, including Bangladesh. It is grown almost year-round and consumed mainly at the tender stage as a cooked vegetable. It is also used in making sweets and pickles (Thakur *et al.* 2013) and in folk medicine (Rahman 2025).

Globally, hybrid vegetable seed adoption has advanced significantly in countries like the USA (92.8%), Europe (89.9%), Vietnam (73.8%), and India (72%), while Bangladesh lags at around 40% (AVRDC 2021, Euroseeds 2022, BADC 2023, NASS 2023). This highlights the need for systematic breeding programs to improve productivity and enhance genetic resources. Bangladesh ranks as the third-largest vegetable producer globally, following China and India, with an annual production exceeding 24.2 million metric tons (DAE 2025).

Singh *et al.* (1996) observed significant genetic variability among landraces and breeding lines across different agro-ecological zones in Bangladesh. However, many promising genotypes remain underutilized due to insufficient evaluation of their agronomic performance and adaptability. Understanding genetic diversity is critical for crop improvement, as it enables the selection of genetically divergent parents, maximizing heterosis and facilitating the development of improved cultivars (Bhatt 1973). The success of hybridization and varietal development largely depends on the extent of genetic divergence among available genotypes (Murty and Anand 1966). Quantitative techniques such as Mahalanobis' D<sup>2</sup> statistics (Rao 1952) and multivariate analyses (Anderson 1957) are widely used to assess genetic divergence based on multiple morphological and agronomic traits. These methods have been proven effective in bottle gourd as a cross-pollinated crops for evaluating variability and identifying superior parental combinations (Griffing

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and Lindstrom 1954, Gaur *et al.* 1977). Cluster analysis is particularly useful for grouping genotypes and determining genetic distances, providing insights into potential combinations for hybrid development (Das and Gupta 1984, Golakia and Makne 1992).

The objective was to assess the genetic divergence among 30 bottle gourd genotypes grown under the central plain agro-ecological conditions of Bangladesh, providing essential information to support future breeding and hybrid development efforts.

### Materials and Methods

This study evaluated thirty genotypes of bottle gourd [*Lagenaria siceraria* (Molina) Standl.]. The materials included two open-pollinated varieties from the Bangladesh Agricultural Research Institute (BARI), twenty-three advanced lines from Advanced Chemical Industries PLC, one released open-pollinated variety from Lalteer seeds, one local variety from Cumilla, two open-pollinated varieties from India, and one line from Thailand. The experiment was conducted at the ACI Plant Genetics Research and Development Center in Niz-Mawna, Sreepur, Gazipur, during the winter growing season 2020-2021. The genotypes were cultivated in a 47m × 10m experimental plot by following Randomized Complete Block Design (RCBD) with 3 replications. Twelve days old seedlings were transplanted at a spacing of 1.5 meters between plants and 1.75 meters between rows and 1 meter between replication. Standard agronomic and plant protection practices were followed uniformly as per recommendations of BARI. Observations were recorded from ten randomly selected plants per genotype per replication for eleven quantitative traits: internode length (cm), node of first female flower (No.), node of first male flower (No.), vine length (cm), days to 50% female flower opening, days to first edible harvest, fruit length (cm), fruit diameter (cm), average fruit weight (kg), fruit per plant (No.), and yield per plant (kg). Mean values across the three replications were used for analysis. Genetic divergence among the genotypes was estimated using Mahalanobis' D<sup>2</sup> statistic (Mahalanobis 1936), as extended by Rao (1952). Clustering was performed using Tocher's method, while Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), and Canonical Variate Analysis (CVA) were used for the graphical representation of genetic relationships, following the multivariate techniques described by Rao (1964). All statistical analyses were performed using R software version 4.0.5 a widely used open-source programming environment developed by Ihaka and Gentleman (1996).

### Results and Discussion

The genetic diversity of 30 bottle gourd genotypes was assessed through cluster analysis, which grouped into six different clusters. According to the Table 1, Cluster I, comprising of 15 genotypes, demonstrates significant phenotypic similarity among half of the accessions examined. This predominance indicates that these genotypes may have shared parental backgrounds, undergone similar environmental adaptations, or had convergent breeding histories. Conversely, Cluster IV comprised of BG16, a solitary genotype representing the greatest divergence from all other accessions. According to Mohammadi and Prasanna (2003), clustering analysis in crop germplasm allows for the identification of genetically distant individuals, which are optimal candidates for use in hybridization to harness heterosis. Clusters II, III, V, and VI, comprising 6, 2, 3, and 3 genotypes, respectively, exhibited moderate levels of genetic divergence. The variability among clusters signifies distinct genetic pools that can be utilized to improve the selection process in breeding operations. The presence of genotypes from various geographical regions across different clusters suggests that genetic diversity in bottle gourd is not strictly determined by geographical distribution, aligning with findings from Iqbal *et al.* (2014) in related cucurbit germplasm.

**Table 1. Distribution of 30 bottle gourd genotypes in six different clusters.**

Cluster No.	No. of genotypes	Types of genotypes	Genotypes designation
I	15	BG704, BG707, BG708, BG712, Khetlau (Lalteer), Hajari, BG721, BG722, BG723, BG725, Deballi (India), BG727, BHIMA (India), BARI Lau3, BARI Lau4	BG4, BG7, BG8, BG12, BG13, BG18, BG21, BG22, BG23, BG25, BG.26, BG27, BG28, BG29, BG30
II	6	BG701, BG702, BG703, BG709, BG711, BG724	BG1, BG2, BG3, BG9, BG11, BG24
III	2	Jhenai (Thailand), BG714	BG5, BG14
IV	1	BG716	BG16
V	3	BG710, BG715, BG717	BG10, BG15, BG17
VI	3	BG706, BG719, BG720	BG6, BG19, BG20

The eigenvalues and percentage of variation for the 11 components are shown in Table 2. Principal Component Analysis (PCA) was applied to reduce data dimensionality and identify traits that contribute most to genetic variability. The initial five components accounted for 82.16% of the overall variations, a substantial cumulative value indicating that most of the phenotypic diversity can be represented by a limited number of factors. The initial component alone accounted for 29.09% of the variation, primarily driven by internode length, underscoring its significance in differentiating genotypes. This is consistent with the findings of Hossain (2022), who identified internode length as a key characteristic affecting vine architecture and fruit load in bottle gourds. The second and third components, significantly shaped by the emergence of the initial female and male flowers, respectively are crucial flowering traits that influence earliness, a critical selection criterion in cucurbit breeding. As Kaiser's criterion suggests, retaining components with eigenvalues >1 (Kaiser 1960), the first five PCs are biologically meaningful and statistically robust. The significant contribution of flowering-related traits to the early principal components also aligns with the observations of Dhillon (2005), who emphasized flowering time as a key determinant of yield potential and adaptability in cucurbits.

**Table 2. Eigenvalues and percentage of variation for 11 traits in bottle gourd.**

Parameters	Eigenvalue	% of total variance covered	Cumulative percentage of variance
Internode length (cm)	3.20	29.09	29.09
Node of first female flower (No.)	1.85	16.84	45.93
Node of first male flower (No.)	1.62	14.69	60.62
Vine Length (meter)	1.27	11.53	72.14
Days to 50% female flower opening (days)	1.10	10.02	82.16
Days to first edible harvest (days)	0.71	6.49	88.65
Fruit length (cm)	0.42	3.78	92.43
Fruit diameter (cm)	0.36	3.31	95.74
Avg. fruit weight (kg)	0.29	2.65	98.39
Fruit per plant (No.)	0.17	1.57	99.96
Yield per plant (kg)	0.00	0.04	100.00

Principal Coordinate Analysis, utilizing Mahalanobis  $D^2$  statistics, was used to assess inter-genotypic distances and quantify pairwise genetic divergence. The most divergent pair was G10 and G16 ( $D^2 = 1.306$ ), followed closely by G3 and G16 ( $D^2 = 1.225$ ), and G5 and G16 (Table 3). This indicates the unique genetic identity of G16, which is further validated by its standalone

status in Cluster IV. This demonstrates that G16 may function as an effective donor parent in hybrid breeding, facilitating the introduction of novel alleles and enhancing heterotic potential. The variation in the highest intergenotypic distance indicates the presence of genetic variability among the 30 bottle gourd genotypes.

**Table 3.** Top ten inter-genotypic distance values among 30 bottle gourd genotypes.

Sl. No.	Genotype combination	10 higher $D^2$ values
01	G10 and G16	1.306
02	G3 and G16	1.225
03	G5 and G16	1.225
04	G5 and G19	1.138
05	G5 and G20	1.110
06	G10 and G24	1.109
07	G13 and G16	1.107
08	G5 and G25	1.106
09	G1 and G16	1.104
10	G10 and G23	1.103

Conversely, G8 and G21 showed the minimal genetic distance ( $D^2 = 0.202$ ), indicating possible redundancy or close genetic relationship, and may be considered for elimination in core collection development to avoid duplication. The effectiveness of  $D^2$  statistics in assessing divergence is well-documented in breeding literature, as evidenced by Singh and Chaudhary (1985), who highlighted the utility of inter-cluster distances in parental selection for optimal heterosis and transgressive segregation. The integration of PCoA with PCA and clustering offers a multidimensional understanding of the genetic structure of the germplasm, facilitating the identification of both genetically distinct and similar genotypes for strategic breeding initiatives. The genetic divergence among the 30 bottle gourd genotypes, assessed using Mahalanobis  $D^2$  statistics, demonstrated considerable intra- and inter-cluster variability, as outlined in Table 4. Intra-cluster distances, indicative of genetic variability within clusters, were lowest in Cluster III (2.500) and Cluster II (2.969), signifies more genetic similarity among genotypes within these groups. Conversely, Cluster VI (4.079) exhibited the highest intra-cluster distance, suggesting a relatively diverse set of genotypes. Inter-cluster distances were particularly high between Cluster V and Cluster IV ( $D^2 = 6.084$ ), Cluster VI and Cluster III ( $D^2 = 6.047$ ), Cluster VI and Cluster V ( $D^2 = 6.072$ ), signifying considerable genetic divergence among these clusters. The significant inter-cluster distances point to a broad genetic base and provide a rationale for selecting parental genotypes from maximally divergent clusters, as such crosses are more likely to yield superior segregants in subsequent generations (Singh and Chaudhary 1985). The low intra- and high inter-cluster distances highlight the efficacy of cluster analysis in identifying genetically diverse parents for heterosis breeding, consistent with the findings of Mohammadi and Prasanna (2003), who advocated Mahalanobis  $D^2$  analysis as a robust method for classifying genetically divergent germplasm.

Analysis of the Cluster mean for 11 morphological and yield-contributing traits revealed clear performance patterns among clusters (Table 5), facilitating targeted genotype selection. Cluster VI emerged as the most promising, exhibiting the highest yield per plant (16.91 kg), the most significant average fruit weight (1.93 kg), and the most extended fruit length (42.35 cm), indicating its potential for yield-focused breeding objectives. In contrast, Cluster III, despite having the lowest intra-cluster distance, showed the highest fruit per plant (11.80) and second

highest yield (16.43 kg), suggesting prolific fruiting and strong source-sink dynamics. Cluster II, although moderate in yield (11.58 kg), displayed early flowering characteristics, with the lowest node of first female flower (11.69) and male flower (7.98), making it a suitable source for earliness, a critical trait for escaping biotic and abiotic stresses (Dhillon *et al.* 2005). Clusters I and V showed moderate performance across most traits. At the same time, Cluster IV stood out for late maturity (66.65 days) but also had a high yield potential (14.79 kg), indicating value in combining earliness and yield through crossbreeding. The distinct trait profiles emphasize the importance of multi-trait selection and highlight the possibility of utilizing clusters like VI and II in hybridization programs to combine yield and early maturity. This approach is further supported by Sharma (2013), who advocated for such combinations in cucurbit improvement strategies.

**Table 4. Mean intra (bold) and inter-cluster ( $D^2$ ) distances among 30 bottle gourd genotypes evaluated across 11 characters.**

Cluster	CI	CII	CIII	CIV	CV	CVI
CI	<b>3.221</b>					
CII	3.924	<b>2.969</b>				
CIII	4.564	4.252	<b>2.500</b>			
CIV	5.234	4.666	5.682	<b>3.778</b>		
CV	4.739	4.435	5.370	6.084	<b>3.978</b>	
CVI	5.031	4.606	6.047	5.306	6.072	<b>4.079</b>

C indicates cluster.

**Table 5. Cluster mean for 11 characters of 30 bottle gourd genotype.**

Parameters	CI	CII	CIII	CIV	CV	CVI
Internode length (cm)	15.82	18.33	16.37	17.58	16.43	17.01
Node of first female flower (No.)	14.88	11.69	15.14	13.63	14.43	13.40
Node of first male flower (No.)	10.15	7.98	11.70	9.38	10.67	8.09
Vine Length (meter)	12.86	10.60	10.06	12.73	11.71	12.93
Days to 50% female flower opening (days)	52.55	61.04	54.82	55.88	59.40	60.24
Days to first edible harvest (days)	65.00	64.11	64.21	66.65	70.72	70.98
Fruit length (cm)	35.49	38.00	32.24	34.64	34.07	42.35
Fruit diameter (cm)	11.47	13.87	10.48	11.40	10.71	11.78
Avg. fruit weight (kg)	1.54	1.54	1.41	1.49	1.50	1.93
Fruit per plant (No.)	6.49	7.60	11.80	9.99	6.75	8.78
Yield per plant (kg)	9.93	11.58	16.43	14.79	10.02	16.91

The two-dimensional scatter plot of the PCA scores for 30 bottle gourd genotypes, with cluster assignments overlaid, illustrates the distinctiveness of the genes within the population and their arrangement. Fig. 1 shows a scatter plot of 30 different bottle gourd genotypes along the first two principal component (PC) axes. This plot was generated with canonical principal component analysis, which uses 11 quantitative variables. There are six cluster groups (I-VI) stacked on top of each other, representing the genotypes. This helps in observing both phenotypic divergence and genetic relatedness simultaneously. The axes, Vector 1 (PC1) and Vector 2 (PC2), are derived from Table 6, which presents the latent vectors that illustrate the extent to which each characteristic contributes to the primary component structure.

Vector 1 (PC1) explains the most variation and is primarily influenced by variables related to fruit size and biomass, including fruit length (0.4226), fruit diameter (0.4046), and average fruit

weight (0.4204). Days to 50% female flower opening (0.3265) and days to first edible harvest (0.2538) also have moderate positive loadings, which means that PC1 is a composite measure of fruit form and maturity. On the other hand, variables such as node of first male flower (-0.2943) and fruit per plant (-0.3309) have negative loadings, indicating that they are inversely related to size traits. This is a common phenomenon in cucurbit breeding, resulting from the trade-off between size and number when allocating resources (Liu *et al.* 2020, Zhao *et al.* 2022).

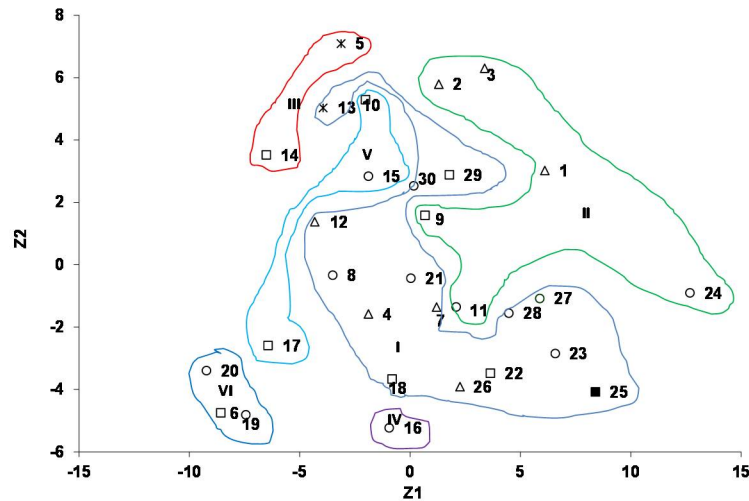


Fig. 1. Scatter distribution of 30 bottle gourd genotypes based on their principal component scores superimposed with clustering.

**Table 6. Latent vectors for 30 bottle gourd genotypes across eleven characters.**

Parameters	Vector-1 ( $Z_1$ )	Vector-2 ( $Z_2$ )
Internode length (cm)	0.1888	0.2967
Node of first female flower (No.)	-0.2234	0.0993
Node of first male flower (No.)	-0.2943	0.11
Vine Length (meter)	0.1394	-0.244
Days to 50% female flower opening (days)	0.3265	0.091
Days to first edible harvest (days)	0.2538	0.1071
Fruit length (cm)	0.4226	0.2046
Fruit diameter (cm)	0.4046	0.1028
Avg. fruit weight (kg)	0.4204	0.1975
Fruit per plant (No.)	-0.3309	0.521
Yield per plant (kg)	-0.0941	0.6692

Vector 2 (PC2) is substantially influenced by yield per plant (0.6692) and fruit per plant (0.521), making it the axis of productivity and reproductive efficiency. Internode length (0.2967) also has a favorable effect, whereas vine length (-0.244) has an adverse impact (Table 6). This suggests that compact plant architecture may help with production. Genotypes in the top right corner of the plot do well on both vectors, making them the best ideotypes for high yield and good fruit quality. On the other hand, genotypes in the bottom left or lower right quadrants may exhibit

trade-offs, such as producing a large number of flowers but limited fruit production. This allows for the selection of specific traits.

The genetic diversity structure is strengthened by the clusters being spaced apart: Cluster IV (G16) is alone, which supports its status as a genetically unique line. Cluster III is tightly packed and located in the quadrant associated with abundant fruiting. In contrast, Cluster VI spans both axes, exhibiting genotypes with a good balance of high yield and fruit quality. This picture fits with recent plant breeding methods that recommend using multi-trait PCA to choose parents to take advantage of heterosis and transgressive segregation (Jolliffe and Cadima 2016, Gao 2023). Fig.1, based on the latent vectors from Table 6, provides a robust multivariate framework for analyzing the genetic and phenotypic structure of bottle gourd genotypes. It facilitates precise parental selection and ideotype design by correlating genotype placement with quantitative trait variance. This strategy is becoming more critical in data-integrated crop development pipelines (Mohammadi and Prasanna 2003, Yuan 2023).

Several strategic cross combinations are suggested to advance bottle gourd hybrid breeding. These are based on a thorough look at genetic divergence, principal component loadings, cluster mean traits, and latent vector patterns. One of the best combinations is between G16 (Cluster IV), which is the most genetically distinct genotype, and genotypes from Cluster VI, such as G6, G19, or G20, which have yielded better, larger, and heavier fruits. These crossings are meant to maximize heterosis by using both genetic divergence and phenotypic superiority. This could lead to hybrids with more variation and better performance. Additionally, it is suggested that crossings between G16 and Cluster III genotypes, such as G5 and G14, combine new genetic traits with the ability to produce a large amount of fruit, resulting in hybrids with consistently high yields and possibly new phenotypic manifestations. Crosses like G6 × G3 (VI × II) are recommended to enhance earliness while maintaining yield potential. The goal is to combine genes that cause plants to flower early with those that increase fruit production. This is especially important in areas with short-duration cropping systems or those that are under considerable stress. Similarly, pairing G19 (long, heavy fruits) with G14 (high fruit number) aims to boost both the number and biomass of fruits simultaneously, which aligns with modern breeding goals of integrating yield components. Additionally, crosses between early-blooming lines, such as G2 or G9, and stable-yielding lines, like G5 or G20, can produce hybrids that perform well in sequential cropping systems, striking a balance between earliness and production. Current breeding research (Zhao *et al.* 2022, Gao *et al.* 2023, Mohammadi and Prasanna 2003) supports the idea of using divergent × elite trait crosses to capitalize on both transgressive segregation and heterotic advantage. These carefully chosen hybridization paths work well together to create a robust framework for developing high-yielding, flexible, and market-ready bottle gourd cultivars.

The multivariate studies indicate that G16, G6, G19, G20, G5, G14, and G3 are the most suitable genotypes for a hybridization strategy targeting high yield, fruit quality, and genetic diversity. Crossing genetically distinct G16 with high-performing clusters (VI or III) offers maximum heterosis and a broader genetic base, while early genotypes like G3 add value to multi-parent programs by enhancing earliness. The plant's cross-pollinated nature and broad variability make it highly suitable for exploiting heterosis, enabling the development of hybrids that integrate yield, quality, and adaptability. Strategic use of both phenotypic performance and genetic divergence, ensuring that hybrid combinations possess both agronomic and genetic value.

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