



RESEARCH ARTICLE

# Magnitude of genetic variability and diversity analysis in bottle gourd accessions using agro-morphological descriptors

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## Abstract

Bottle gourd, a climbing vine crop exhibits potential genetic variability, offering opportunities for breeding new cultivars thorough characterization and evaluation of accessions. This study aimed to assess the extent of variability, genetic diversity and the relationships among various agro-morphological traits across 17 bottle gourd accessions. The present experiment was conducted at the Vegetable Research Farm, Department of Horticulture, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, India during 2020-2021 and 2021-2022. The accessions were organized using a randomized complete block design with three replications. The substantial mean square values observed across all studied traits confirm the presence of adequate genetic variability among the evaluated accessions. The bottle gourd yield per hectare exhibited a highly significant and positive correlation with branch count/vine (0.724), vine length (0.660), fruit diameter (0.608), fruit length (0.525), fruit weight (0.780), fruit/vine (0.917), ascorbic acid (0.890), soluble solids (0.858) and yield/plant (1.000). The highest positive direct effect on fruit yield per hectare was exerted by yield/plant (0.9722) and fruit/vine (0.0221). Principal component analysis demonstrated that 85.90 % of the total variance was contributed by the first three components, in which genotypes were characterized. Through multivariate analysis, the study effectively examined genetic divergence and classified the 17 accessions into five distinct clusters. The highest inter-cluster distance was observed between Cluster II and Cluster IV, highlighting considerable diversity among the accessions within these groups and holding potential for broad enhancements in subsequent crop breeding programs.

**Keywords:** character association; *Lagenaria siceraria* Molina L.; Mahalanobis D<sup>2</sup>; path coefficient; yield

## Introduction

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.), a member of the Cucurbitaceae family, is a widely cultivated vegetable crop known by various vernacular names such as Calabash, Doodhi and Lauki across different regions of India. Its nomenclature and usage reflect its deep cultural and culinary integration into Indian society. Based on the presence of diverse wild relatives and genetic evidence, the species is believed to have originated in Africa, although it has since spread globally and adapted to a wide range of agro-climatic conditions (1).

Botanically, bottle gourd is characterized by its monoecious reproductive system, wherein both male and female flowers are borne on the same plant. The species exhibits a predominantly cross-pollinated nature, facilitated by insect activity, which contributes to its genetic variability. Typically, male flowers are produced in greater numbers than female flowers, a trait that influences pollination dynamics and fruit set. The crop is primarily cultivated during the summer and Kharif seasons, thriving in warm temperatures and well-drained soils.

The fruit of bottle gourd is classified as a fleshy berry, varying significantly in size, shape and flavour profile (2). Its taste ranges from

mildly sweet to distinctly bitter, a variation attributed to the presence of cucurbitacin compounds-biologically active substances known for their bitterness and potential pharmacological properties. This diversity in fruit characteristics not only affects consumer preference but also plays a role in breeding strategies aimed at improving yield and quality.

In Indian cuisine, bottle gourd fruits are consumed in a variety of culinary preparations, including pickles, raita and sweet dishes, reflecting their versatility and cultural significance. Beyond their gastronomic appeal, bottle gourds are highly valued for their therapeutic properties. Traditionally recognized for their cooling effects, they are also attributed with a wide range of medicinal benefits, including anti-cancer, diuretic, cardioprotective, aphrodisiac and purgative actions (3). Additionally, they have been used as antidotes for certain poisons and scorpion stings, underscoring their role in indigenous healthcare practices.

Nutritionally, bottle gourd fruits are an excellent source of hydration and essential nutrients. Composed of approximately 93.5 % moisture, they offer a low-calorie profile (63 kcal per 100 g) while providing 3.69 % carbohydrates, 0.6 % protein and 1.2 % dietary fibre. Their mineral content includes 24 mg of calcium and 170 mg of

potassium, contributing to bone health and electrolyte balance. Furthermore, bottle gourds are a modest yet meaningful source of vitamins, including 8.5 mg of vitamin C, 0.029 mg of thiamine (B1), 0.022 mg of riboflavin (B2), 0.038 mg of vitamin B6, 0.39 mg of niacin (B3) and 0.144 mg of pantothenic acid (B5) per 100 g of raw fruit (4). These nutritional attributes make bottle gourd a valuable component of a balanced diet and a promising candidate for functional food development.

The development of high-performing bottle gourd hybrids necessitates the strategic identification of parental genotypes that exhibit desirable horticultural and agronomic traits (5). Key attributes such as vine length, the number of primary and lateral branches, fruit yield, earliness, fruit size and shape, as well as resistance to both abiotic and biotic stresses, are central to crop improvement efforts (6). These traits not only influence the overall productivity and adaptability of the crop but also determine its commercial viability and resilience under diverse environmental conditions.

Correlation analysis provides insights into the degree and direction of association between traits, helping breeders identify traits that are positively linked with yield. However, correlation alone does not reveal the causal relationships among traits. To overcome this limitation a path coefficient analysis quantifies the direct and indirect contributions of individual traits to yield, offering a more nuanced understanding of trait interdependencies.

To facilitate effective hybridization and selection, a comprehensive assessment of genetic diversity among available genotypes is essential. Such evaluations enable breeders to pinpoint genetically distinct and superior lines that can serve as potential parents in breeding programs. Understanding the extent of variation in yield-contributing traits and their interrelationships with morphological characteristics provides critical insights into the genetic architecture of the crop.

## Materials and Methods

### Place of study and planting material

The field experiment was performed at the Experimental Farm of the Department of Vegetable Science, Sam Higginbottom University of Agriculture Technology and Sciences (SHUATS), Prayagraj, Uttar Pradesh, India. The experiment was conducted in the months of February-May during the years 2020-2021 and 2021-2022 including seventeen accessions were collected from Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India and three accessions from local collections from SHUATS, Prayagraj, Uttar Pradesh, India (Supplementary Table 1).

### Data collection and field evaluation

The crop was grown in sandy loam soil, pre-ploughed and fertilized with urea and manure in a tropical climate during two growing seasons. The successful crop cultivation was followed by a package of practices suggested by the university (SHUATS, Prayagraj). The field trial was conducted in a randomised complete block design with three replications. The seeds were treated with 2 g/kg of Thiram to prevent fungal diseases. The seeds were sown at a spacing of  $3 \times 0.75$  m in a plot size of  $22.5\text{ m}^2$ .

During plant growth, observations were recorded from 10 selected plants from each genotype viz. days to 1<sup>st</sup> male bloom, days to 1<sup>st</sup> female bloom, number of nodes up to first male flower, node count to 1<sup>st</sup> female flower, branch count/vine, fruit length (cm), vine length (m), fruit weight (g), days to 1<sup>st</sup> harvest, fruit diameter (cm), fruit/vine, ascorbic acid (mg/100 g), total soluble solids (<sup>0</sup>Brix), yield/plant (g) and yield/ha (q).

Total soluble solids were determined by using a HI96801 Digital Refractometer, which measures refractive index and displays results in <sup>0</sup>Brix which was calibrated at 20 °C temperature.

Ascorbic acid was calculated according to the 2,6-dichlorophenol-indophenol visual titration method (7). Use 3 % metaphosphoric acid, L-ascorbic acid standard solution and 2,6-dichlorophenolindophenol dye solution. Prepare metaphosphoric acid solution, standard ascorbic acid solution and dye

**Table 1.** Variability parameters for different traits of bottle gourd (pooled analysis)

TRAITS	Mean sum of squares			Mean	Range		Coefficient of Variance		h <sup>2</sup> (bs)	GA	GAM
	Replication (d.f. = 2)	Treatment (d.f. = 16)	Error (d.f. = 32)		Min.	Max.	PCV (%)	GVC (%)			
Days to 1 <sup>st</sup> male bloom	99.62	2.80 <sup>*</sup>	0.31	49.57	47.33	51.44	2.15	1.84	73.10	1.61	3.24
Days to 1 <sup>st</sup> female bloom	19.36	1.61 <sup>*</sup>	0.68	58.07	56.67	59.11	1.71	0.96	31.50	0.65	1.11
Number of nodes up to first male flower	0.59	5.43 <sup>*</sup>	0.30	11.50	9.22	13.33	12.32	11.38	85.20	2.49	21.64
Node count to 1 <sup>st</sup> female flower	0.34	3.17 <sup>*</sup>	0.69	13.35	11.33	14.78	9.22	6.81	54.60	1.38	10.36
Branch count/vine	0.78	2.47 <sup>*</sup>	1.19	15.73	14.56	17.89	8.08	4.16	26.60	0.70	4.42
Vine length (m)	0.36	1.44 <sup>*</sup>	0.27	4.11	3.15	5.64	19.72	15.21	59.50	0.99	24.17
Days to 1 <sup>st</sup> harvest	3.55	8.40 <sup>*</sup>	1.05	69.25	67.11	72.66	2.70	2.26	70.10	2.70	3.90
Fruit Weight (g)	3755.83	21342.25 <sup>*</sup>	1227.33	683.50	561.33	829.44	13.03	11.98	84.50	5.08	22.69
Fruit Diameter (cm)	1.01	4.37 <sup>*</sup>	1.14	15.20	13.11	17.44	9.79	6.82	48.50	1.49	9.79
Fruit Length (cm)	0.95	28.94 <sup>*</sup>	1.44	31.09	25.45	36.89	10.47	9.74	86.50	5.80	18.65
Fruit/vine	1.66	5.95 <sup>*</sup>	0.64	7.09	5.11	9.78	21.90	18.79	73.60	2.35	33.21
Ascorbic Acid (mg/100g)	0.03	9.39 <sup>*</sup>	0.30	30.89	28.22	34.11	5.91	5.63	90.90	3.42	11.06
Soluble solids ( <sup>0</sup> Brix)	0.01	0.43 <sup>*</sup>	0.04	3.22	2.68	4.18	12.75	11.31	78.70	0.67	20.66
Yield/plant (kg)	1.28	6.21 <sup>*</sup>	0.41	4.90	3.18	8.10	31.23	28.38	82.60	2.60	53.13
Yield/hectare (q)	2537.77	12271.92 <sup>*</sup>	807.39	217.85	141.51	360.14	31.23	28.38	82.60	5.71	53.11

\*Significant at  $P < 5\%$  level of significance

df: Degree of freedom, PCV: Phenotypic Coefficient of Variation, GCV: Genotypic Coefficient of Variation, h<sup>2</sup>: heritability, GA: Genetic Advance, GAM: Genetic Advance as percent of Mean

solution. Standardize the dye with a titration process. Crush 10 g of tender bottle gourd, dilute the juice with metaphosphoric acid and filter it. Titrate the filtered extract with dye to a pink endpoint. Repeat thrice and calculate the average dye volume used. Calculate the ascorbic acid content using the dye factor.

Ascorbic acid (mg/100 mL/g) =

$$\frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract} \times \text{Weight of sample}} \times 100$$

### Analysis of data

This investigation applied pooled ANOVA to discern significant differences among genotypes for selected traits (8), enabling a comprehensive partitioning of variance components. Traits exhibiting heritability estimates above 80 % were considered highly inheritable, indicating strong genetic control and promising prospects for selection.

To assess variability, phenotypic and genotypic coefficients of variation were estimated using Burton's method, offering insights into the relative influence of genetic versus environmental factors (9). Heritability (broad sense) and genetic advance as percent of mean were calculated following the formulas in previous studies to evaluate the efficiency of selection and expected genetic gain (10,11).

Further, genotypic correlations were computed to explore trait interrelationships, while path coefficient analysis was employed to quantify direct and indirect effects of independent variables on yield per hectare (q/ha), which served as the dependent variable. These analyses were conducted using OPSTAT software (12).

To visualize genotype performance and trait associations, a biplot was generated using SAS 9.3 (13) and a dendrogram was constructed via PAST version 4.15 (14) to classify genotypes based on hierarchical clustering.

For diversity analysis, Mahalanobis D<sup>2</sup> statistics were applied using TNAUSTAT within the DosBox interface (15), based on the classical method introduced by Mahalanobis (16). This multivariate approach facilitated the grouping of genotypes into distinct clusters, aiding in the identification of genetically diverse parental lines for breeding programs.

## Results and Discussion

### ANOVA and variability

The ANOVA, mean, range and estimates of various genetic parameters of 15 different traits of the 17 accessions of bottle gourd are presented in Table 1. ANOVA revealed significant differences among the accessions of bottle gourd for all 15 traits studied. Wide range of variation was observed for characters viz. fruit weight (561.33–829.44 g), fruit length (25.45–36.89 cm), ascorbic acid (28.22–34.11 mg/100 g), soluble solids (2.68–4.18 °Brix), yield/plant (3.18–8.10 kg) and yield/hectare (141.51–360.14 q). The presence of such high variability for these parameters will form the basis for effective selection of superior lines in bottle gourd. Similarly, high levels of variability for yield per plant, fruit weight, number of fruits per plant and days to first male flowering in this crop has been reported in a previous study (17). Higher variability for fresh pod yield per plant, seed yield per

plant, number of pods per plant, pod length, pod weight, number of seeds per pod, days to 50 % flowering and plant height were observed (18) and significant greater variability for traits, days to first male flowering, days to first female flowering, days to first harvest, fruit diameter, total yield and number of fruits per plant were well documented in a previous study (19).

For 15 characters ANOVA showed significant differences among bottle gourd germplasm. The analysis revealed highly significant and positive variation obtained for fruit weight (21342.25) and the lowest was observed in soluble solids (0.43) (Supplementary Table 2). The similar variability in bottle gourd for total yield and number of fruits per plant were well-documented (19), fruit weight and vine length were observed in a previous study and fruit weight and fruit thickness were observed (20,21).

Yield for different genotypes showed variation that ranged from 141.51–360.14 q/ha with a mean of 217.85 q/ha. The production of 250–360 q/ha was achieved by VRBG-1, VRBG-2, LC-1 and LC-2 (Supplementary Table 3). The extent of variability can be judged by the magnitude of the Phenotypic Coefficient of Variance (PCV) and the Genotypic Coefficient of Variance (GCV) which is represented by different characters. GCV gives the maximum extent of genetic variability which is present in population ranging from 0.96 % in days to first female flower bloom to 28.38 % in yield/plant. Extensive variability was similarly documented for fruit length, fruit weight and fruit yield (10–25 %) (22). Whereas the range of GCV was also observed for fruit diameter, fruit weight and sex ratio (10.12–22.45 %) (23,24). However, number of nodes up to first male flower, branch count/vine, vine length (cm), yield/plant (kg) and yield/ha (q) showed wider variation between GCV and PCV. The PCV was higher than the GCV suggesting a slight impact from environmental factors. The minor differences between phenotypic and genotypic variation imply that these traits are stable despite environmental fluctuations (25).

The GCV alone is not sufficient to determine the extent of heritable variation. Therefore, heritability estimates are important as they indicate how effectively selection can be utilized to utilize the existing genetic variability. Heritability effectively indicates how traits are passed down from parents to their offspring (26). Out of 15 characters, days to 1<sup>st</sup> male bloom, number of nodes up to first male flower, days to first harvest, fruit weight, fruit length, fruit/vine, ascorbic acid, total soluble solids, yield/plant and yield/hectare exhibited high heritability whereas days to 1<sup>st</sup> female bloom, node count to 1<sup>st</sup> female flower, vine length and fruit diameter exhibit moderate levels of heritability and branch count/vine shows low levels of heritability among genotypes [ $<30$  % (low), 31–60 % (moderate) and  $>60$  % (high)] (27) represented in Table 1. The comparable results of high heritability of vine length, fruit length, fruit width and primary branches per vine were recorded (21) and also observed high heritability for vine length, number of primary branches, fruit length, fruit diameter and average fruit weight (20). Similar results for fruit length, fruit diameter, number of fruits per plant and fruit weight were also observed (24).

The high Genetic Advance over Mean percent (GAM) was exhibited by number of nodes up to first male flower, vine length, fruit weight, fruit/vine, total soluble solids, yield/plant and yield/hectare whereas moderate level of GAM was reported by node

count to 1<sup>st</sup> female flower, fruit length and ascorbic acid (Table 1). The lowest level of GAM was observed in days to 1<sup>st</sup> male and female bloom, branch count/vine, days to 1<sup>st</sup> harvest and fruit diameter. The recommended range of GAM was [ $<10\%$  (low), 10-20 % (medium) and  $>20\%$  (high)] (27). This implies that the presence of additive genetic factors is predominant in these characters. The branch count/vine shows a moderate increase in genetic advance relative to average, coupled with low heritability signifies the role of non-additive genetic factors. Comparable observations i.e., fruit length, fruit diameter, number of fruits per plant and fruit weight were reported (24), similar findings for fruit length, fruit weight and vine length were observed in previous studies (2,20).

### Correlation analysis

Correlation indicates the total influence of gene segregation; certain genes increase both characters, causing a positive correlation, whereas a negative correlation indicates that some increase one trait but reduce another (26). All genotypic correlation coefficients between fruit yield/hectare and its associated characters are presented in Table 2. Days to 1<sup>st</sup> female bloom demonstrated a highly significant positive correlation along with days to 1<sup>st</sup> male bloom (0.731<sup>\*</sup>). Number of nodes up to first male flower demonstrated a highly significant positive correlation along with days to 1<sup>st</sup> male bloom (0.524<sup>\*</sup>) and days to 1<sup>st</sup> female bloom (0.726<sup>\*</sup>). Node count to 1<sup>st</sup> female flower demonstrated a highly significant positive correlation along with days to 1<sup>st</sup> male bloom (0.551<sup>\*</sup>), days to 1<sup>st</sup> female bloom (0.696<sup>\*</sup>) and number of nodes up to first male flower (0.840<sup>\*</sup>).

Branch count/vine and vine length showed a highly significant negative correlation with days to 1<sup>st</sup> male bloom (-0.817<sup>\*</sup>, -0.770<sup>\*</sup>) and days to 1<sup>st</sup> female bloom (-0.610<sup>\*</sup>, -0.670<sup>\*</sup>) respectively. Vine length demonstrated a highly significant positive correlation along with branch count/vine (0.844<sup>\*</sup>). Days to 1<sup>st</sup> harvest demonstrated a highly significant positive correlation along with number of nodes up to first male flower (0.633<sup>\*</sup>) and node count to 1<sup>st</sup> female flower (0.511<sup>\*</sup>), days to 1<sup>st</sup> male bloom (0.567<sup>\*</sup>) but negatively significant correlated with branch count/vine (-0.484<sup>\*</sup>).

Fruit weight demonstrated highly significant positive correlation along with branch count/vine (0.690<sup>\*</sup>) and vine length

(0.529<sup>\*</sup>). Fruit diameter demonstrated highly significant positive correlation along with vine length (0.584<sup>\*</sup>) and fruit weight (0.702<sup>\*</sup>), branch count/vine (0.624<sup>\*</sup>). Fruit length demonstrated highly significant positive correlation along with vine length (0.511<sup>\*</sup>), fruit weight (0.658<sup>\*</sup>) branch count/vine (0.582<sup>\*</sup>) and fruit diameter (0.569<sup>\*</sup>) but negatively significant correlated with days to 1<sup>st</sup> male bloom (-0.500<sup>\*</sup>). Fruit/vine demonstrated highly significant positive correlation along with vine length (0.564<sup>\*</sup>) and branch count/vine (0.549<sup>\*</sup>) but negatively significant correlated with days to 1<sup>st</sup> male bloom (-0.688<sup>\*</sup>), days to 1<sup>st</sup> female bloom (-0.533<sup>\*</sup>), number of nodes up to first male flower (-0.761<sup>\*</sup>), node count to 1<sup>st</sup> female flower (-0.771<sup>\*</sup>) and days to 1<sup>st</sup> harvest (-0.801<sup>\*</sup>). Ascorbic acid demonstrated highly significant positive correlation along with fruit diameter (0.578<sup>\*</sup>), vine length (0.542<sup>\*</sup>), fruit weight (0.714<sup>\*</sup>), branch count/vine (0.617<sup>\*</sup>), fruit length (0.597<sup>\*</sup>) and fruit/vine (0.802<sup>\*</sup>) but negatively significant correlated with days to 1<sup>st</sup> male bloom (-0.532<sup>\*</sup>), number of nodes up to first male flower (-0.544<sup>\*</sup>), node count to 1<sup>st</sup> female flower (-0.595<sup>\*</sup>) and days to 1<sup>st</sup> harvest (-0.695<sup>\*</sup>). Total soluble solids demonstrated highly significant positive correlation along with fruit weight (0.886<sup>\*</sup>), vine length (0.643<sup>\*</sup>), branch count/vine (0.809<sup>\*</sup>), fruit diameter (0.705<sup>\*</sup>), fruit length (0.685<sup>\*</sup>), fruit/vine (0.627<sup>\*</sup>) and ascorbic acid (0.821<sup>\*</sup>) but negatively significant correlated with days to 1<sup>st</sup> male bloom (-0.648<sup>\*</sup>) and days to 1<sup>st</sup> harvest (-0.497<sup>\*</sup>).

Fruit yield per plant demonstrated highly significant positive correlation along with vine length (0.660<sup>\*</sup>), fruit weight (0.779<sup>\*</sup>), fruit diameter (0.608<sup>\*</sup>), fruit length (0.524<sup>\*</sup>), fruit/vine (0.918<sup>\*</sup>), branch count/vine (0.723<sup>\*</sup>), ascorbic acid (0.890<sup>\*</sup>) and soluble solids (0.857<sup>\*</sup>), but negatively significant correlated with number of nodes up to first male flower (-0.631<sup>\*</sup>), node count to 1<sup>st</sup> female flower (-0.628<sup>\*</sup>), days to 1<sup>st</sup> harvest (-0.741<sup>\*</sup>) and days to 1<sup>st</sup> male bloom (-0.721<sup>\*</sup>). Yield/ha demonstrated highly significant positive correlation along with vine length (0.660<sup>\*</sup>), fruit weight (0.780<sup>\*</sup>), fruit diameter (0.608<sup>\*</sup>), fruit length (0.525<sup>\*</sup>), fruit/vine (0.917<sup>\*</sup>), ascorbic acid (0.890<sup>\*</sup>), branch count/vine (0.724<sup>\*</sup>), soluble solids (0.858<sup>\*</sup>) yield/plant (1.000<sup>\*</sup>) but negatively significant correlated with node count to 1<sup>st</sup> female flower (-0.628<sup>\*</sup>), days to 1<sup>st</sup> male bloom (-0.721<sup>\*</sup>), days to first harvest (-0.740<sup>\*</sup>) and number of nodes up to first male flower (-0.631<sup>\*</sup>).

Days to first female bloom is positively correlated with

**Table 2.** Genotypic correlation coefficient of different traits in bottle gourd (pooled analysis)

TRAITS	DM	DF	NM	NF	BV	VL	DH	FW	FD	FL	FV	AA	SS	YP	YH
DM	1.000														
DF	0.731 <sup>*</sup>	1.000													
NM	0.524 <sup>*</sup>	0.726 <sup>*</sup>	1.000												
NF	0.551 <sup>*</sup>	0.696 <sup>*</sup>	0.840 <sup>*</sup>	1.000											
BV	-0.817 <sup>*</sup>	-0.610 <sup>*</sup>	-0.418	-0.429	1.000										
VL	-0.770 <sup>*</sup>	-0.670 <sup>*</sup>	-0.470	-0.471	0.844 <sup>*</sup>	1.000									
DH	0.567 <sup>*</sup>	0.320	0.633 <sup>*</sup>	0.511 <sup>*</sup>	-0.484 <sup>*</sup>	-0.462	1.000								
FW	-0.469	-0.093	-0.202	-0.180	0.690 <sup>*</sup>	0.529 <sup>*</sup>	-0.405	1.000							
FD	-0.454	-0.035	-0.080	-0.039	0.624 <sup>*</sup>	0.584 <sup>*</sup>	-0.374	0.702 <sup>*</sup>	1.000						
FL	-0.500 <sup>*</sup>	-0.219	-0.119	-0.173	0.582 <sup>*</sup>	0.511 <sup>*</sup>	-0.232	0.658 <sup>*</sup>	0.569 <sup>*</sup>	1.000					
FV	-0.688 <sup>*</sup>	-0.533 <sup>*</sup>	-0.761 <sup>*</sup>	-0.771 <sup>*</sup>	0.549 <sup>*</sup>	0.564 <sup>*</sup>	-0.801 <sup>*</sup>	0.473	0.397	0.288	1.000				
AA	-0.532 <sup>*</sup>	-0.287	-0.544 <sup>*</sup>	-0.595 <sup>*</sup>	0.617 <sup>*</sup>	0.542 <sup>*</sup>	-0.695 <sup>*</sup>	0.714 <sup>*</sup>	0.578 <sup>*</sup>	0.597 <sup>*</sup>	0.802 <sup>*</sup>	1.000			
SS	-0.648 <sup>*</sup>	-0.305	-0.340	-0.374	0.809 <sup>*</sup>	0.643 <sup>*</sup>	-0.497 <sup>*</sup>	0.886 <sup>*</sup>	0.705 <sup>*</sup>	0.685 <sup>*</sup>	0.627 <sup>*</sup>	0.821 <sup>*</sup>	1.000		
YP	-0.721 <sup>*</sup>	-0.447	-0.631 <sup>*</sup>	-0.628 <sup>*</sup>	0.723 <sup>*</sup>	0.660 <sup>*</sup>	-0.741 <sup>*</sup>	0.779 <sup>*</sup>	0.608 <sup>*</sup>	0.524 <sup>*</sup>	0.918 <sup>*</sup>	0.890 <sup>*</sup>	0.857 <sup>*</sup>	1.000	
YH	-0.721 <sup>*</sup>	-0.448	-0.631 <sup>*</sup>	-0.628 <sup>*</sup>	0.724 <sup>*</sup>	0.660 <sup>*</sup>	-0.740 <sup>*</sup>	0.780 <sup>*</sup>	0.608 <sup>*</sup>	0.525 <sup>*</sup>	0.917 <sup>*</sup>	0.890 <sup>*</sup>	0.858 <sup>*</sup>	1.000 <sup>*</sup>	1.000

\* Significant at  $P < 5\%$  level of significance. Means of three replications

DM = Days to 1<sup>st</sup> Male bloom, DF = Days to 1<sup>st</sup> Female flower, NM = Number of nodes to 1<sup>st</sup> Male flower, NF = Number of nodes to 1<sup>st</sup> Female flower, BV = Branch count/Vine, VL = Vine Length (m), DH = Days to 1<sup>st</sup> Harvest, FW = Fruit Weight (g), FD = Fruit Diameter (cm), FL = Fruit Length (cm), FV = Fruit/Vine, AA = Ascorbic acid (mg/100 g), SS = Soluble Solids (°Brix), YP = Yield/Plant (kg), YH = Genotypic correlation coefficient of yield/ha (q)

days to first male bloom. Node count to first flowers is strongly correlated with both male and female bloom timing and higher node counts are associated with delayed flowering. Similar findings suggest a positive correlation with days to 1<sup>st</sup> male bloom and higher node number (28). Vine length and branch count per vine are positively correlated with longer vines having more branches. Fruit traits like weight, diameter and length are positively correlated with vine length and branch count, indicating that larger fruits come from plants with longer vines and more branches. The results for positively correlated association with fruit length, fruit diameter with a greater number of branches is observed in a previous study (29). Ascorbic acid content and total soluble solids are positively correlated with larger fruit size, vine length and branch count. Fruit yield per plant and yield per hectare are strongly correlated with vine length, fruit size and the number of fruits per vine, highlighting these traits as key yield drivers. The comparable findings of positively correlated association with total soluble solids and yield per plant and related parameters (2). Branch count and vine length are negatively correlated with days to first male and female bloom, suggesting that plants with more branches and longer vines flower earlier (30). Fruit yield and quality traits like ascorbic acid and soluble solids are negatively correlated with later flowering and higher node counts, meaning earlier flowering and lower node counts contribute to better yield and quality. Early flowering, longer vines, more branches and lower node count to first bloom are associated with higher fruit yield, better fruit quality and higher nutritional content (ascorbic acid and soluble solids) in bottle gourd. These correlations can guide breeding efforts to improve overall productivity and fruit quality (24). Out of 15 characters the indirect selections for branch count/vine, vine length, fruit weight, fruit diameter, fruit length, fruit/vine, ascorbic acid, total soluble solids and yield per plant will improve fruit yield in bottle gourd. The similar results association with yield and related traits are observed in previous studies (31).

### Path analysis

Path analysis disaggregates correlation coefficients into indirect and direct effects. It is employed here to assess the impact of various traits on yield/ha (2). The examination of genotypic path coefficient and the accompanying data indicate that yield/plant (0.9722) has a direct and substantially positive effect towards

yield/ha and other traits viz. node count to 1<sup>st</sup> female flower (0.0011), fruit weight (0.0102), fruit/vine (0.0221), branch count/vine (0.0042), days to 1<sup>st</sup> harvest (0.0022), days to 1<sup>st</sup> male bloom (0.0014), soluble solids (0.0008) and fruit length (0.0027) (Table 3). Traits such as vine length (-0.0016), ascorbic acid (-0.0012), fruit diameter (-0.0009), days to 1<sup>st</sup> female bloom (-0.0006) and number of nodes up to first male flower (-0.0002) had a negative direct effect. The residual effect (0.0013) reflects the influence of an unmeasured trait on the yield per hectare. These results align with those of (2,32).

The path coefficient analysis reveals that yield per plant has the substantial positive direct effect on yield per hectare, making it a primary target for breeding programs. Traits like fruit weight, fruits per vine and branch count per vine also positively influenced yield. Previous studies similarly highlighted the positive direct effects of branch count per vine, fruit weight and fruits per vine on yield/ha (33,34). Conversely, traits like vine length, ascorbic acid and fruit diameter exhibit a minor negative impact, indicating they should be minimised to enhance yield. Another study also presents comparable results regarding the negative direct impact of fruit diameter on yield/ha (29). The residual effect suggests the possible influence of other unmeasured factors on yield.

### Principal Component Analysis (PCA)

PCA highlights the significance of the main element responsible for most of the variability along each axis of the associated character (35). The initial three principal components PC I, PC II and PC III, each with an eigenvalue >1, accounted for 85.90 % of the variation in total (Table 4). In PC I, the dominant factors contributing to 61.80 % of the total variation were days to 1<sup>st</sup> female bloom, node count to 1<sup>st</sup> female flower, number of nodes up to first male flower days to 1<sup>st</sup> male bloom and days to 1<sup>st</sup> harvest. These traits contribute the most to overall variation, indicating their significant role in the growth and development cycle of the bottle gourd. Similar findings were reported concerning flowering and harvest time (36).

In PCA II, number of nodes up to first male flower, node count to 1<sup>st</sup> female flower, fruit diameter, days to 1<sup>st</sup> female bloom, fruit length, soluble solids and fruit weight had the most influence, accounting for 15.50 % of the variation in total. These traits are mainly related to fruit size and quality, aligning with

**Table 3.** Genotypic path coefficient analysis of different traits in bottle gourd (pooled analysis)

Traits	DM	DF	NM	NF	BV	VL	DH	FW	FD	FL	FV	AA	SS	YP	YH
DM	0.0014	-0.0004	-0.0001	0.0006	-0.0034	0.0012	0.0012	-0.0048	0.0004	-0.0014	-0.0152	0.0006	-0.0005	-0.7005	-0.721*
DF	0.0010	-0.0006	-0.0002	0.0008	-0.0025	0.0011	0.0007	-0.0010	0.0000	-0.0006	-0.0118	0.0003	-0.0002	-0.4348	-0.448
NM	0.0007	-0.0004	-0.0002	0.0010	-0.0017	0.0007	0.0014	-0.0021	0.0001	-0.0003	-0.0168	0.0006	-0.0003	-0.6137	-0.631*
NF	0.0008	-0.0004	-0.0002	0.0011	-0.0018	0.0007	0.0011	-0.0018	0.0000	-0.0005	-0.0170	0.0007	-0.0003	-0.6105	-0.628*
BV	-0.0011	0.0004	0.0001	-0.0005	0.0042	-0.0013	-0.0010	0.0070	-0.0005	0.0016	0.0121	-0.0007	0.0006	0.7032	0.724*
VL	-0.0011	0.0004	0.0001	-0.0005	0.0035	-0.0016	-0.0010	0.0054	-0.0005	0.0014	0.0125	-0.0006	0.0005	0.6420	0.660*
DH	0.0008	-0.0002	-0.0001	0.0006	-0.0020	0.0007	0.0022	-0.0041	0.0003	-0.0006	-0.0177	0.0008	-0.0004	-0.7200	-0.740*
FW	-0.0007	0.0001	0.0000	-0.0002	0.0029	-0.0008	-0.0009	0.0102	-0.0006	0.0018	0.0105	-0.0009	0.0007	0.7578	0.780*
FD	-0.0006	0.0000	0.0000	0.0000	0.0026	-0.0009	-0.0008	0.0072	-0.0009	0.0015	0.0088	-0.0007	0.0006	0.5913	0.608*
FL	-0.0007	0.0001	0.0000	-0.0002	0.0024	-0.0008	-0.0005	0.0067	-0.0005	0.0027	0.0064	-0.0007	0.0005	0.5099	0.525*
FV	-0.0010	0.0003	0.0002	-0.0009	0.0023	-0.0009	-0.0017	0.0048	-0.0003	0.0008	0.0221	-0.0010	0.0005	0.8923	0.917*
AA	-0.0008	0.0002	0.0001	-0.0007	0.0026	-0.0009	-0.0015	0.0073	-0.0005	0.0016	0.0177	-0.0012	0.0006	0.8652	0.890*
SS	-0.0009	0.0002	0.0001	-0.0004	0.0034	-0.0010	-0.0011	0.0090	-0.0006	0.0019	0.0139	-0.0010	0.0008	0.8336	0.858*
YP	-0.0010	0.0003	0.0001	-0.0007	0.0030	-0.0010	-0.0016	0.0079	-0.0005	0.0014	0.0203	-0.0011	0.0007	0.9722	1.000*

Residual effect: 0.0013, (Bold diagonal values are direct effect)

DM = Days to 1<sup>st</sup> Male bloom, DF = Days to 1<sup>st</sup> Female flower, NM = Number of nodes to 1<sup>st</sup> Male flower, NF = Number of nodes to 1<sup>st</sup> Female flower, BV = Branch count/Vine, VL = Vine Length (m), DH = Days to 1<sup>st</sup> Harvest, FW = Fruit Weight (g), FD = Fruit Diameter (cm), FL = Fruit Length (cm), FV = Fruit/Vine, AA = Ascorbic acid (mg/100 g), SS = Soluble Solids (°Brix), YP = Yield/Plant (kg), YH = Genotypic correlation coefficient of yield/ha (q)

**Table 4.** Percent variation explained by the first 4 principal components of bottle gourd (pooled)

Traits	PC1	PC2	PC3	PC4
Days to 1 <sup>st</sup> male bloom	0.271	0.084	-0.325	0.197
Days to 1 <sup>st</sup> female bloom	0.195	0.375	-0.462	-0.089
Number of nodes up to first male flower	0.220	0.418	0.091	-0.107
Node count to 1 <sup>st</sup> female flower	0.220	0.404	0.061	-0.344
Branch count/vine	-0.278	0.101	0.348	-0.133
Vine length (m)	-0.262	0.008	0.399	-0.238
Days to 1 <sup>st</sup> harvest	0.240	0.135	0.334	0.457
Fruit Weight (g)	-0.244	0.358	-0.092	0.141
Fruit Diameter (cm)	-0.208	0.385	0.017	-0.371
Fruit Length (cm)	-0.200	0.316	0.193	0.545
Fruit/vine	-0.286	-0.202	-0.264	-0.102
Ascorbic Acid (mg/100g)	-0.287	0.074	-0.296	0.232
Soluble solids (°Brix)	-0.286	0.250	-0.015	0.147
Yield/plant (kg/ha)	-0.316	0.020	-0.192	0.000
Yield/hectare (q/ha)	-0.316	0.021	-0.191	0.001
Eigen values	9.269	2.331	1.291	0.597
Variation (%)	61.800	15.500	8.600	4.000
Cumulative Variation (%)	61.800	77.300	85.900	89.900

PC: Principal component

results (37), which also highlighted the importance of fruit length and weight.

In PC III, the branch count/vine, vine length and days to 1<sup>st</sup> harvest were the dominant factors, contributing to total variation of 15.99 %. These traits are more related to plant structure and growth, influencing the overall productivity of the plant. Similar findings were reported with branch count/vine, early harvesting and vine length (38,39). A previous study also demonstrated substantial genetic diversity using PCA analysis (40).

Biplot was created with the scores of Principal components I and II. This illustrates the diversity among genotypes by their distribution across different quadrants (Fig. 1). Traits with lower correlation are shown by vectors with larger angles. Genotypes are sorted into quadrants by their scores on principal components. However, they overlapped, showing similarities among the collection's genotypes. In the PCA biplot, quadrant I (+, +) features four genotypes with nine key traits i.e. fruit length, branch count/vine, soluble solids, fruit diameter, ascorbic acid, fruit weight, vine length, yield/plant and yield/ha, positioned centrally. These traits are important for both plant structure and fruit quality, making this quadrant significant for breeding high-yielding, quality fruit genotypes. The relationship between genotypes and fruit quality- and yield-related traits in bottle gourd was also demonstrated earlier (41). Quadrant II (-, +) contains eight genotypes linked to five traits viz. days to 1<sup>st</sup> female bloom, node count to 1<sup>st</sup> female flower, days to 1<sup>st</sup> harvest, number of nodes up to first male flower and days to 1<sup>st</sup> male bloom. These traits are essential for understanding the reproductive development and maturation of the plant. Quadrant III (-, -) contains three genotypes but lacks strong associations with specific traits. This suggests that these genotypes do not show significant divergence in key traits compared to others. Quadrant IV (+, -) includes two genotypes, primarily influenced by fruit per vine, a trait linked to overall productivity.

Traits with lower correlations are represented by vectors with larger angles between them in the biplot. This helps visualize

the relationships and diversity among genotypes based on their trait performance (42). The biplot analysis highlights diversity among bottle gourd genotypes, grouping them based on key traits across different quadrants. Genotypes in quadrant I are associated with high yield and quality traits, while quadrant II focuses on reproductive and harvest timing traits. Quadrant III genotypes lack strong trait associations and quadrant IV genotypes are influenced by fruit productivity. This visualization helps in identifying genotypes with desired traits for breeding purposes.

### Cluster analysis

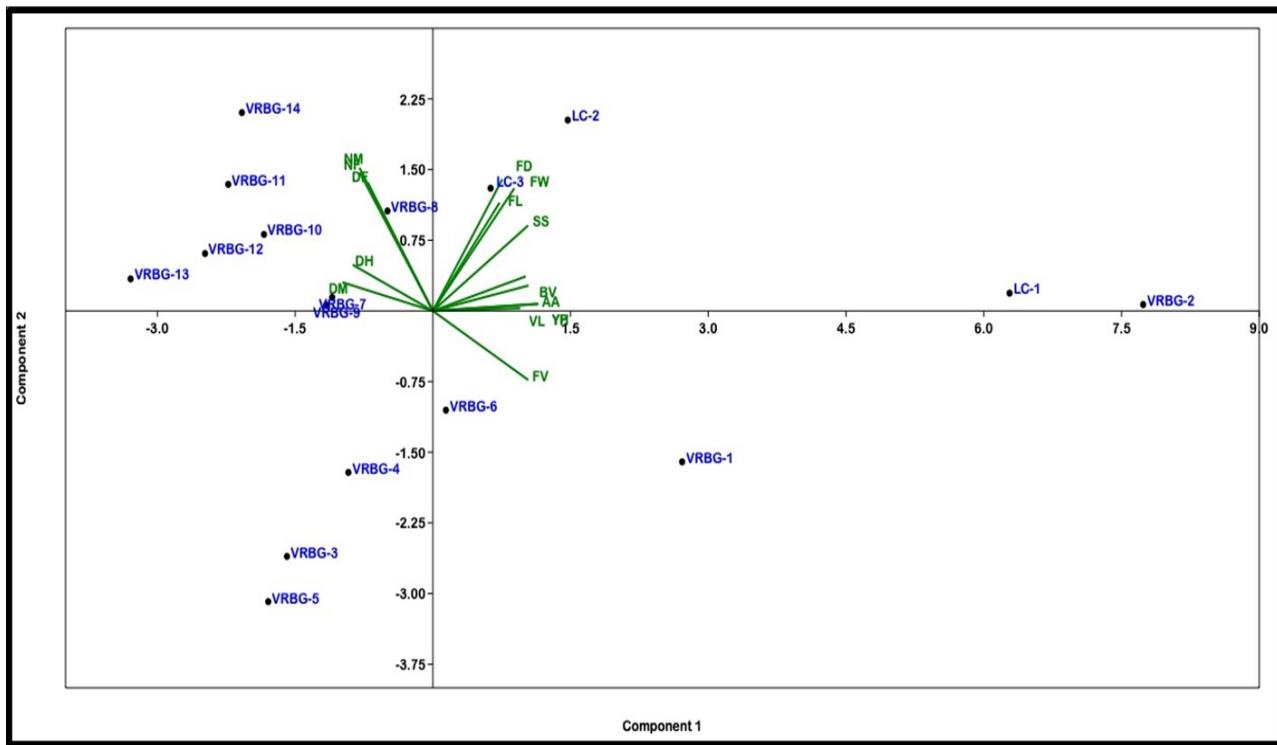
In the breeding programmes, the quantification of genetic diversity is very important because it gives the genetic structure of a population (18). The 17 accessions were grouped into five clusters based on genetic similarities. Accessions within the same cluster had smaller  $D^2$  values, indicating greater genetic homogeneity, while accessions in different clusters exhibited more genetic variation. Cluster I was the largest, containing 7 accessions (41 %) which exhibited high homogeneity or the least genetic variation, followed by clusters IV, II, III and V consisting of 5, 2, 2 and 1 accessions, respectively (Table 5). It was evident from the distinct cluster patterns that there was no parallelism between the patterns and the geographical diversity (43). In line with this, another study clustered 21 bottle gourd genotypes into six distinct clusters using Mahalanobis distance (44).

### Intra and inter-cluster analysis

The intra-cluster distance among the five clusters represented that cluster I had the highest intra-cluster distance (8.35), indicating significant genetic divergence within the accessions which is influenced by both natural and artificial selection forces (Table 6). This suggests that, although clustered together, the accessions in cluster I show notable genetic variation (43). The clusters IV and III also had relatively high intra-cluster distances, suggesting moderate diversity among their accessions. Despite the variation within these clusters, the overall intra-cluster distances suggest limited diversity within the clusters. The

**Table 5.** Clustering pattern of 17 genotypes of bottle gourd based on genetic variability

Clusters	Number of genotypes in cluster	Genotypes
I	7	VRBG-1, VRBG-4, VRBG-6, VRBG-7, VRBG-8, VRBG-9, LC-3
II	2	VRBG-2, LC-1
III	2	VRBG-3, VRBG-5
IV	5	VRBG-10, VRBG-11, VRBG-12, VRBG-13, VRBG-14
V	1	LC-2



**Fig. 1.** Biplot analysis for different traits responsible for variability in bottle gourd genotypes.

highest inter-cluster distance was observed between cluster II and cluster IV, indicating substantial genetic diversity between the accessions in these clusters. This makes them ideal candidates for breeding programs, as crossing accessions from these clusters could produce progenies with a wide range of desirable traits (45). The lowest inter-cluster distance was noted between cluster I and cluster V, implying that the accessions in these clusters are genetically more similar to each other, offering less potential for generating diverse offspring.

The highest genetic divergence within accessions was found in cluster I, while the most significant diversity between clusters was seen between cluster II and cluster IV, highlighting their potential as superior parental lines for breeding. Conversely, the low inter-cluster distance between cluster I and cluster V indicates genetic similarity. This analysis can help

breeders select the best parental combinations for enhancing genetic variability in bottle gourd breeding programs.

#### Cluster mean analysis

The mean values of all traits for each cluster are prepared in Table 7. Cluster V is characterized by later blooming of both male and female flowers compared to the other clusters. Cluster IV exhibits the highest node number for the appearance of the first male and female flowers and the maximum days to the first harvest. For crop improvement in bottle gourd, vine length and the number of branches is vital factors determining the yield potential of accessions on which the selection could be made (44).

Cluster II, which includes the highest-yielding accessions, is characterized by early blooming of both female and male flowers, maximum vine length, number of branches, fruit weight, fruit

**Table 6.** Average inter and intra cluster distance ( $D^2$ )

Clusters	I	II	III	IV	V
I	8.35				
II	17.60	4.27			
III	10.97	22.34	5.11		
IV	11.33	22.73	12.54	7.52	
V	9.71	16.44	16.55	11.94	0

**Table 7.** Cluster means for different characters among 17 genotypes of bottle gourd

Characters	Clusters				
	I	II	III	IV	V
Days to 1 <sup>st</sup> male bloom	49.76	47.50	49.72	49.96	50.11
Days to 1 <sup>st</sup> female bloom	58.22	56.83	57.66	58.33	59.00
Number of nodes up to first male flower	11.33	9.27	10.39	12.86	12.55
Node count to 1 <sup>st</sup> female flower	13.25	11.77	12.33	14.42	13.89
Branch count/vine	15.49	17.72	15.05	15.57	15.55
Vine length (m)	3.98	5.46	3.90	3.85	3.98
Days to 1 <sup>st</sup> harvest	68.57	67.16	69.66	71.11	68.11
Fruit Weight (g)	670.39	824.88	567.38	671.04	786.89
Fruit Diameter (cm)	15.04	17.27	13.50	15.06	16.22
Fruit Length (cm)	31.03	36.33	26.83	30.57	32.11
Fruit/vine	7.35	9.50	7.22	5.44	8.33
Ascorbic Acid (mg/100g)	31.49	33.94	29.28	29.15	32.44
Soluble solids (°Brix)	3.13	4.01	2.78	3.10	3.64
Yield/plant (kg)	4.94	7.83	4.10	3.64	6.56
Yield/hectare (q)	219.93	348.38	182.35	162.19	291.51

diameter, fruit length, fruits per vine, ascorbic acid content, total soluble solids, yield per plant and yield per hectare. Given its yield attributes, this cluster can be utilized to develop bottle gourd accessions with high yield, substantial fruit weight, extended vine length and early blooming traits.

Cluster III has the lowest fruit length, fruit weight, fruit diameter and total soluble solids considering low performing accessions in this cluster.

Cluster IV, which comprises the lowest yielded accessions which has characteristics of later node counts to both male and female flowers, later days to harvesting, lowest fruit/vine and ascorbic acid content. Cluster V includes later blooming of male and female flowers and comprises highest vine length among all clusters (46).

#### Hierarchical cluster analysis

Cluster analysis evaluated the assessments (hierarchically) based on yield associated characteristics, categorizing them into two main clusters (Fig. 2). The formation of these clusters did not correlate with the genotypes' geographical origins, indicating that factors other than location influenced the grouping (47). Cluster I carry 3 genotypes (LC-1, LC-2 and VRBG-2) and cluster II comprised of 14 genotypes (VRBG-1, VRBG-3, VRBG-4, VRBG-5, VRBG-6, VRBG-7, VRBG-8, VRBG-9, VRBG-10, VRBG-11, VRBG-12, VRBG-13, VRBG-14 and LC-3).

Cluster analysis was used to evaluate the diversity and distinctiveness of genotypes, grouping them into one category based on distance (48). Genotypes spaced farther apart show more genetic diversity, beneficial for breeding better cultivars. Greater distance indicates broader genetic variation, while proximity suggests morphological similarity. The largest distance within a cluster was noted between LC-2 and LC-1 in the first sub-cluster and between VRBG-4 and VRBG-11 in the second sub-cluster. The greatest distance between inter-cluster was found

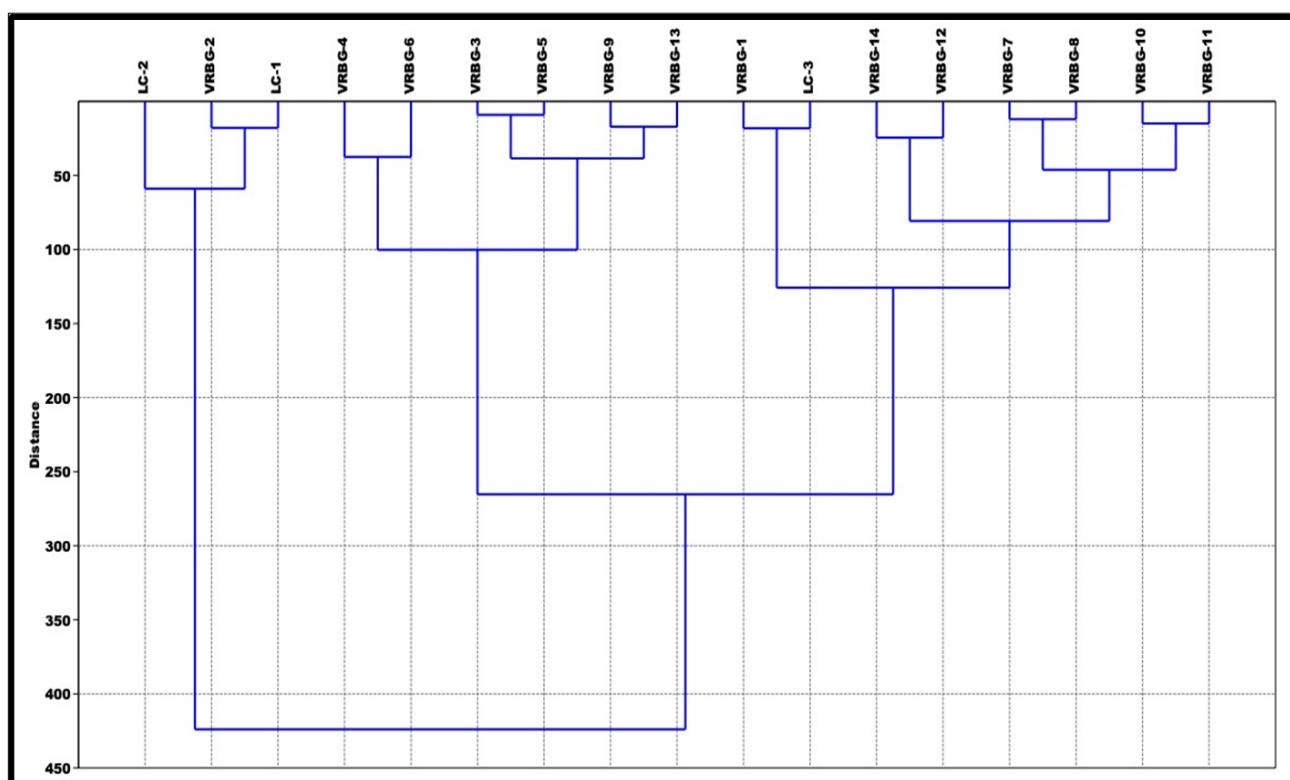
between LC-2 and VRBG-11. The comparable findings were also reported (36,41).

#### Conclusion

The study emphasizes the importance of fruit yield/plant, yield/hectare and fruit per vine as key traits with significant variability, with fruit/vine exhibiting a strong positive direct effect on yield/hectare, making them critical selection criteria for high-yielding bottle gourd accessions. Additionally, traits such as branch count/vine, days to first harvest, fruit weight, ascorbic acid content and soluble solids are essential for genetic enhancement in bottle gourd yield. Cluster analysis has proven to be an effective method for classifying accessions and facilitating their utilization in crop improvement programs through conventional breeding techniques. The highest inter-cluster distance was observed between cluster II and cluster IV, suggesting that accessions from these clusters possess high genetic divergence and should be considered potential parental lines for future breeding programs. Cluster II, comprising the highest-yielding accessions while developing superior-yielding cultivars. The analytical approaches adopted in this study offer a valuable framework for selecting genetically diverse parents and indigenous gene pools of bottle gourd, ultimately contributing to the advancement of breeding strategies aimed at enhancing productivity and genetic resilience in future cultivars.

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**Fig. 2.** Dendrogram of cluster analysis of genotypes.

## Authors' contributions

AS and VB conceptualized the research and curated the experimental data. BSD and AK performed the formal statistical analysis. VB secured the funding for the study. SD and SK developed the methodology. Project administration was carried out by VB and AS. Resources were provided by VB, AS and AK. The manuscript was written and reviewed by AS, SK, SD and BSD. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors have no conflict of interest to declare.

**Ethical issues:** None

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