Diversity assessment of small bitter gourd (*Momordica charantia* L. var *muricata*) genotypes based on phytochemical and quality traits using multivariate statistics

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Abstract

Momordica charantia var. *muricata,* being a small-fruited variant of the commercial large-fruited bitter gourd, has been exploited less with respect to its biochemical and antioxidant properties. However, given its immense potential as a crop rich in nutrients, a study was carried out at Central University of Tamil Nadu, Thiruvarur, to evaluate the variability and diversity of 10 genotypes of small bitter gourd based on phytochemical and nutrient quality. The genotypes were found to have significant (P≤0.05) variation for all the traits assessed in the study. Among the ten genotypes MCM-4 was found superior which recorded highest values for most of the traits viz. total phenol (79.57 mg GAE/g), total flavonoid (67.55 mg QE/g), total terpenoid content (2.49 mg/g), FRAP (44.618 mg/mL), DPPH activity (0.94 %), crude fat (2.43 %), ash content (9.23 %), crude protein (29.62 %), calcium (60.14 ppm), manganese (0.3 ppm) and zinc content (0.3 ppm). Antioxidant activity (both FRAP and DDPH) in the fruit showed a significantly strong positive relationship with the total terpenoid content, vitamin C, ash content, crude protein, calcium and manganese. The principal components found four independent components accounting for 88.93 % of the total variance among 10 genotypes. Among the first four principal components (PCs), PC1 had the highest variability (49.11 %), followed by PC2 (19.03 %), PC3 (11.86 %) and PC4 (8.92 %). Hierarchical cluster analysis grouped ten genotypes into four clusters. The genotype in cluster II recorded the highest cluster means for most traits under study except for the moisture content, magnesium, iron and copper. These genotypes from different clusters can further be selected as parents in the hybridisation program to get superior hybrids.

Keywords: biochemical; diversity; multivariate; muricata; PCA; small bitter gourd

Introduction

Bitter melon (*Momordica charantia* L.) is a sun-loving vegetable crop belonging to the Cucurbitaceae family, which possesses horticultural, medicinal and commercial importance. Its chromosome number is 2n = 2x = 22. The Latin word *momordica* means "to bite," alluding to the leaf margins' sharp, toothy appearance. India has been identified as the primary centre of diversity for this crop, with China being the secondary centre (1). Because of its enormous economic significance, bitter gourd is regarded as one of the major vegetable crops in the world (2). In India, bitter gourd is commercially consumed as a fresh vegetable and is grown in an area of 99,000 ha with an annual production of 1198000 MT (3).

Bitter gourd is edible from green fruits, having potential anti-oxidative effects due to the presence of higher phenolic content and a huge variety of phenolic derivatives (4). Consistently, various studies demonstrated that various phenolic compounds possessed activity of free radical scavenging and health-improving effects by reducing the ROS produced by oxidative stress and inhibition of macromolecular oxidation (5, 6, 7). The high anti-oxidative capacity

is responsible for the medicinal qualities of bitter gourd, which can be attributed to the presence of phenolics, flavonoids, isoflavones, terpenoids, anthraquinones and glucosinolates (8). Bitter melon is also abundant in vitamins (A and C) and minerals such as phosphorus, magnesium, iron, zinc and manganese, along with various therapeutic properties (9). The parts of bitter gourd are a source of over 60 phytochemicals that exhibit anti-tumour and anti-diabetic effects, making them useful in addressing numerous health conditions (10). As a result, it is often referred to as "vegetable insulin" and a "treasure trove of health" (11).

Considering the nutritional and therapeutic properties of bitter gourd, assessment of the genetic diversity of germplasm for nutritional qualities is of prime importance. A vast variety of bitter gourd genetic resources with different traits are widely available in India (12, 13). Earlier work was carried out on fifty genotypes of bitter gourd collected from different sources were studied for nutritional variation and genetic diversity with the objective to determine the superior genotypes for vitamin and mineral contents. These accessions can serve as beneficial genetic wealth to improve the nutritional qualities in bitter gourd (14). The crop improvement of

bitter gourd in India has predominantly evolved around the selection of individual plants from a broad range of germplasm resources, as it exhibits minimal inbreeding depression (15). The bitter gourd breeding is focused mainly on yield and its associated traits. Whereas, in recent times, many attempts were made in the crop improvement of bitter gourd, focusing more on the phytochemicals and nutrient content.

Momordica charantia comes in two varieties: the small-fruited wild form, known as *Momordica charantia* L. var. *muricata*. (Wild) and the large-fruited form, known botanically as *Momordica charantia* var. *charantia*. The small-fruited Bitter Gourd (SBG) is grown and consumed in certain pockets of Tamil Nadu and Kerala, West Bengal and the Andaman Islands (16, 17). Consumers love them for their flavour, taste and purported therapeutic qualities. Growers receive premium prices that are three to four times higher than those of large fruited cultivars. SBG, being the progenitor of commercially grown large-fruited bitter gourd reported to be rich in phytoconstituents and nutrients compared to commercial types. Despite several reports supporting muricata being the nutritious form of bitter gourd, no attempts to date were made to explore the variation and diversity of biochemical, proximate and mineral contents available exclusively among the small bitter gourd genotypes (18).

The biochemical content of SBG fruits differs depending on the cultivar, production area and maturation stage (19). As a result, evaluating the genetic diversity of SBG with respect to phytochemical composition would help to find and choose cultivars with desired features to include in crop improvement programs. Hence, the present investigation was conducted to explore the variability and analyse the diversity in ten SBG genotypes for various biochemical and nutritional quality traits. The findings of the present study could provide input for identifying possible parents for further bitter gourd breeding initiatives.

Materials and Methods

Experimental site

Ten small bitter gourd (SBG) genotypes (Table 1), grown in the experimental plot following RBD design with three replications at the Department of Horticulture, Central University of Tamil Nadu (CUTN), Thiruvarur, Tamil Nadu, India (10°48'24.7" N 79°36'49.0" E), which falls under the Cauvery Delta agroclimatic zone of Tamil Nadu. The experimental site is characterized by fertile alluvial soils and a tropical climate. Throughout the growing season, the average temperature varied from 28 °C to 35 °C, with an annual rainfall of approximately 1,200 mm, predominantly during the monsoon months. Crop was raised under optimal production practices recommended by TNAU.

Table 1. Genotypes list and place of collection of SBG used in the study

IC number/Genotype code	Place of Collection
IC639012	Rankuva, Navsari, Gujarat
IC636486	Vellanikkara, Thrissur
IC639010	Rankuva, Navsari, Gujarat
IC638996	Vellanikkara, Thrissur
IC636495	Chalakudy, Thrissur
MCM-1	Lalgudi, Tiruchirapalli, TN
MCM-2	Attur, Salem, TN
MCM-3	Chengalpattu, TN
MCM-4	Ariyalur TN
MCM-5	Karunagaranalur, Koradacherry, Thiruvarur, TN

Among the ten genotypes, 5 genotypes (IC series) were obtained from NBPGR, Regional Station, Thrissur, Kerala and others (MCM series) are local collections. The selected genotypes under investigation represented a diverse range of wild, semi-domesticated and landrace forms. The genotypes were classified based on their fruit size, morphology and local cultivation practices.

Sample collection and preparation

Immature fruits at edible maturity were harvested 15 days after pollination from ten genotypes of var. *muricata*. The fruits of different genotypes were packed in zip-lock covers separately with labels and immediately brought to the laboratory for further processing. De-ionised water was used to thoroughly wash the samples and dried under the fan to remove the excess moisture. The fruits were divided into two groups; one group was stored in a freezer at -20 °C for biochemical analysis, whereas the other group of fruits were dried for 72 hr at 60 °C in a hot air oven to maintain a consistent dry weight. A mill grinder was subsequently utilised to grind the dried samples into a fine powder (UDY Corporation, Model: 3010-014) subsequently stored in airtight plastic containers for later analysis. The powder samples were used in proximate and mineral estimations.

Biochemical traits

Total phenol content

The total phenols in SBG were estimated using the Folin-Ciocalteu assay (20). 1 g of a fresh fruit sample was ground with 10 mL of 80 % methanol using a pestle and mortar. Collect the mixture and incubate it for an hour with frequent vortexing. After that, the mixture was centrifuged for 15 min at 10000 rpm while being kept at ambient temperature (27 °C). For the assay, the supernatant was collected. 0.5 mL of the methanolic extract, 0.2 mL of 1N Folin-Ciocalteu reagent, 3.25 mL of distilled water and 1 mL of 20 % sodium carbonate were poured into a test tube. The sample was incubated at room temperature for 30 minutes and the resulting bluish tint was measured using a UV spectrophotometer at 717 nm (Thermo-scientific, Model: Genesys 180 UV-Visible Spectrophotometer). Gallic acid equivalents (GAE) in milligrams per gram of fresh weight were computed to express the total phenol content.

Total flavonoid content

The total flavonoid content of the fruit was determined using the methodology with slight modification. 0.1 mL of aluminium chloride, 2.8 mL of distilled water, 0.1 mL of HCl and 0.1 mL of sodium acetate (1 N) were combined with one mL of fruit methanolic extract (21). After that, the samples were incubated for 30 min at room temperature. A spectrophotometer was used to test the solutions' absorbance at 425 nm (Thermo-scientific, Model: Genesys 180 UV-Visible Spectrophotometer). Total flavonoid content of the extracts was expressed as mg of Quercetin equivalents (QE) per g of fresh weight.

Total terpenoid Content

Total terpenoid content was determined following the protocol with a few modifications (22). The fresh, small bitter gourd fruit pulp (1 g) was extracted with 10 mL of ethyl acetate. The mixture was then sonicated for 30 min and centrifuged at 10,000 rpm at room temperature for 15 min. After removing the supernatant, 6 mL of perchloric acid and 1 mL of a 5 % vanillin-glacial acetic acid solution were added. The mixture was kept in a water bath at 70 °C for 15 min, then the mixture was cooled on ice. After adding 1.6 mL of acetic acid to the resultant mixture, it was placed at room temperature for half an hour. At 555 nm, the absorbance was determined.

Vitamin C

The 2, 6-dichlorophenol indophenol titration was used to measure the amount of vitamin C or ascorbic acid in edible fruits (23). After pipetting out 5 mL of the working standard solution, 10 mL of 4 % oxalic acid was added and the mixture was titrated against the dye (V_1 mL). After extracting 1 g of the fresh sample with 4 % oxalic acid and adjusting the volume to 100 mL, the sample was centrifuged. Titration was performed against the dye (V_2 mL) after 5 mL of this supernatant was removed and 10 mL of 4 % oxalic acid was added. The end state, which stretches for a few minutes, was confirmed with the appearance of pink colour. For all of the samples, the quantity of ascorbic acid found in the edible fruits was equal to the quantity of dye consumed and computed as given in Equation 1:

$$\text{Vitamin} - C \ (\text{mg } 100\text{g}^{-1}) = \frac{0.5}{V1 \ mL} \times \frac{V2 \ mL}{5 \ mL} \times \frac{100 \ mL}{Weight \ of \ the \ sample} \times 100$$

(Eqn. 1)

Total antioxidant activity

FRAP assay : The FRAP assay was used to measure reducing power capacity (24). 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid and 20 mM iron (III) chloride were blended in a 10:1:1 ratio to formulate the FRAP reagent. Ascorbic acid (1 mg/mL) was considered a positive control in varying concentrations. 1 mL of methanolic extract of the sample was mixed with 3.6 mL of FRAP reagent and 2.4 mL of distilled water. The solution was allowed to remain around room temperature for half an hour in the dark. The absorbance was measured at 593 nm using a UV spectrophotometer (Thermo-scientific, Model: Genesys 180 UV-Visible Spectrophotometer).

DPPH assay : Using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) test, the antioxidant activity was determined. 1 mL of 0.1 mM DPPH in methanol was added to each 200 µL aliquot of extracted content. For 30 to 40 min, the solution was incubated in total darkness. In a UV spectrophotometer, the absorbance of an immature fruit methanolic extract was measured at 517 nm. In this assay, vitamin C (10 mg/mL) acted as a positive control (25).

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

(Eqn. 2)

Determination of proximate composition

The proximate analyses (moisture, crude fibre, ash, crude fat and crude protein concentrations) of all the samples were determined. The standard techniques were used to determine the moisture and ash contents (23). The Soxhlet extraction method was used to ascertain the samples' fat content (23). A fibre analyser was used to determine the crude fibre content (26). The Kjeldahl technique was used to calculate the crude protein content (23). Total carbohydrates were estimated by the anthrone method (27).

Determination of mineral contents

Mineral contents were estimated using the standard procedure with minor modifications (14). Six mineral nutrients, viz. calcium, magnesium, iron, manganese, zinc and copper contents were estimated using an atomic absorption spectrophotometer (AAS, Shimadzu Model: AA-6880). A 0.5 g oven-dried sample was digested on a hot plate using 10 mL of a 9:4 di-acid mixture (70 % nitric acid and 70 % perchloric acid) until a clear solution formed. Whatman No. 42 filter paper was used to filter the digested samples after they were thoroughly diluted with distilled water. The samples were stored in bottles for further analysis. A combination of air (17 mL/min) and acetylene (2 mL/min) flame was used for the determination of mineral content. The hollow-cathode lamp of the respective mineral was used. Each metals' linear working range, calibration graph correlation coefficient and absorption wavelength were determined. A blank was also prepared by following the same procedure of digestion (diacid without sample) and the value in AAS was obtained in the same process as the sample for the respective minerals. Despite the device containing built-in algorithms to determine the mineral content, the results were distorted by the dilution factor. The concentration of the minerals was calculated by using the following formula.

Mineral concentration (ppm) = (Sample reading - blank reading) × Dilution factor   (Eqn. 3)

Statistical analysis

A one-way ANOVA was performed for statistical analysis and Duncans' multiple-range test was used to separate the mean values at a 5 % significance level ($p < 0.05$). Mean values and principal component analysis (PCA) for biochemical and other quality traits were carried out using the correlation matrix method in KAU - GRAPES 1.0.0, an online R-based tool (28). Cluster analysis employed the Ward method and squared Euclidean distance. It was carried out by IBM SPSS Statistics version 20.

Results and Discussion

Variations in traits among the genotypes

This study was conducted with an interest in exploring diversity in the biochemical contents, nutritional traits, mineral contents and antioxidant attributes of small bitter gourd. All the biochemical parameters, viz. total phenol content (TPC), total flavonoid content (TFC), total terpenoid content (TTC) and vitamin C content, studied across the genotypes showed considerable variation among the genotypes (Table 2). The genotype MCM-4 recorded a maximum total phenol (79.57 mg GAE/g), total flavonoid (67.55 mg QE/g) and total terpenoid content (2.49 mg/g). The minimum TPC and TFC were recorded in IC639010 (40.63 mg GAE/g and 30.91 mg QE/g, respectively) and the minimum TTC was reported in IC636495 (1.15 mg/g). The vitamin C content was highest in IC636486 (123.5 mg/100g), followed by MCM-4 (121.83 mg/100g). The least value of vitamin C was obtained in IC639012 (81.78 mg/100g). The FRAP and DPPH antioxidant activity was found to be maximum in MCM-4 (44.618 mg/ml and 0.94 %). The above results clearly indicate that the antioxidant activity increased with the increase in phytochemical contents. For the proximate and mineral contents, there was a significant difference among the genotypes; the genotype MCM-4 was found maximum for crude fat (2.43 %), ash content (9.23 %), crude protein (29.62 %), calcium (60.14 ppm), manganese (0.3 ppm) and zinc content (0.3 ppm) (Table 3-4). For crude fibre, the genotype MCM-1 (5.48 %) recorded the highest values, whereas for moisture content (90.75 %) and total carbohydrate (12.8 %), the fruits of IC639012 registered the highest values. The minerals like magnesium (15.96 ppm), iron (1.53 ppm) and copper (0.44 ppm) were found highest in IC636495, MCM-5 and MCM-3, respectively. The fruits of MCM-4 were found with maximum accumulation of most of the phytochemical and nutritional parameters. This might

Table 2. Variations in biochemical and total antioxidant properties of SBG genotypes

Genotypes	Phenol	Flavonoid	TTC	VIT C	FRAP	DPPH
IC639012	47.944[de]	32.089[de]	1.4[def]	81.78[d]	30.11[cd]	0.54[def]
IC636486	47.732[de]	41.162[ce]	2.29[ab]	123.5[a]	43.628[ab]	0.89[ab]
IC639010	40.638[e]	30.912[e]	1.55[def]	106.6[abcd]	36.895[abc]	0.67[cde]
IC638996	54.962[cde]	44.444[bcd]	1.26[f]	98[abcd]	27.393[d]	0.48[f]
IC636495	68.486[abc]	47.537[bc]	1.15[f]	92.45[bcd]	29.613[cd]	0.51[ef]
MCM-1	78.947[a]	66.147[a]	1.84[bcd]	113.29[abc]	34.304[bcd]	0.62[def]
MCM-2	47.705[de]	31.634[e]	1.37[ef]	96.66[abcd]	30.46[cd]	0.58[def]
MCM-3	72.86[ab]	55.654[ab]	2.18[abc]	90.8[cd]	40.143[ab]	0.81[abc]
MCM-4	79.573[a]	67.552[a]	2.49[a]	121.83[a]	44.618[a]	0.94[a]
MCM-5	58.555[bcd]	40.921[cde]	1.73[cde]	118.6[ab]	37.433[abc]	0.72[bcd]
Mean	59.74	45.80	1.72	104.35	35.45	0.67
SE(m)	5.453	4.235	0.159	9.371	3.199	0.062
SE(d)	7.712	5.989	0.224	13.253	4.524	0.087
CV(%)	15.811	16.014	15.914	15.555	15.624	15.808

Treatments with same letters are not significantly different. TTC - Total terpenoid content, Vit C - Vitamin C, FRAP - Ferric Reducing Antioxidant Power, DPPH - 1,1-diphenyl-2-picrylhydrazyl,

Table 3. Variations in proximate composition of SBG genotypes

Genotypes	Fiber	Fat	Ash	Moisture	Protein	Carbohydrate
IC639012	4.826[ab]	2.18[ab]	5.74[de]	90.75[a]	26.14[ab]	12.8[a]
IC636486	4.701[ab]	2.11[ab]	8.62[a]	81.91[cde]	27.68[ab]	8.89[cd]
IC639010	3.823[bcd]	1.1[d]	7.53[abc]	84.7[bcd]	24.46[abc]	10.46[bc]
IC638996	3.467[cde]	0.92[de]	5.12[def]	80.7[de]	15.12[d]	6.14[ef]
IC636495	2.892[de]	0.88[de]	3.87[f]	86.74[abc]	19.23[cd]	5.61[f]
MCM-1	5.481[a]	2.21[ab]	8.12[ab]	87.64[ab]	25.89[ab]	8.32[cde]
MCM-2	2.467[e]	0.56[e]	6.01[cde]	80.58[de]	18.34[cd]	7.82[def]
MCM-3	4.482[abc]	1.81[bc]	4.56[ef]	79.15[de]	22.48[bc]	6.97[def]
MCM-4	5.361[a]	2.43[a]	9.23[a]	78.43[e]	29.62[a]	11.62[ab]
MCM-5	4.167[bc]	1.56[c]	6.36[bcd]	86.51[abc]	21.64[bc]	6.43[ef]
Mean	4.16	1.57	6.51	83.71	23.06	8.50
SE(m)	0.381	0.151	0.600	1.923	2.089	0.785
SE(d)	0.538	0.214	0.848	2.720	2.954	1.110
CV(%)	15.820	16.619	15.940	3.979	15.689	15.979

Treatments with same letters are not significantly different

Table 4. Variations in mineral nutrients of SBG genotypes

Genotypes	Calcium	Magnesium	Iron	Manganese	Zinc	Copper
IC639012	25.69[c]	11.48[bcde]	0.79[de]	0.13[e]	0.17[d]	0.24[c]
IC636486	43.15[b]	10.69[cdef]	0.84[de]	0.21[bc]	0.1[e]	0.19[cd]
IC639010	39.54[b]	9.95[def]	0.8[de]	0.21[bcd]	0.1[e]	0.14[de]
IC638996	23.01[cd]	12.98[abcd]	0.7[e]	0.19[bcd]	0.28[ab]	0.21[cd]
IC636495	15.42[d]	15.96[a]	1.04[cd]	0.16[cde]	0.26[ab]	0.34[b]
MCM-1	40.6[b]	8.65[ef]	0.84[de]	0.15[de]	0.19[cd]	0.23[c]
MCM-2	25.79[c]	13.65[abc]	1.11[bc]	0.22[b]	0.24[bc]	0.35[b]
MCM-3	22.18[cd]	13.12[abcd]	1.35[ab]	0.22[bc]	0.24[abc]	0.44[a]
MCM-4	60.14[a]	7.96[f]	0.73[e]	0.3[a]	0.3[a]	0.09[e]
MCM-5	25.93[c]	14.58[ab]	1.53[a]	0.25[b]	0.22[bcd]	0.22[c]
Mean	32.14	11.90	0.97	0.20	0.21	0.24
SE(m)	3.077	1.082	0.090	0.019	0.020	0.023
SE(d)	4.352	1.530	0.127	0.026	0.028	0.033
CV(%)	16.582	15.749	15.992	15.813	16.129	16.630

Treatments with same letters are not significantly different

be due to the smaller fruit size when compared to other genotypes. The wild bitter gourd and cultivated forms are cross-compatible and large forms are most preferred over the small fruits; hence, this may result in mixing of genes among the wild and cultivated forms, leading to semi-domesticated forms of an intermediate fruit size. Since the MCM-4 is smaller in size, it is closely related to or might be the wild forms without any genetic admixtures. Due to these reasons of being wild-natured and smaller fruit size, it has resulted in high-nutritional fruits when compared to the other genotypes under study. Research indicates that similar results were reported in bitter gourd guava (22, 29, 30).

Correlation between the biochemical and nutritional traits with antioxidant capacity

The correlation study (either positive or negative) between the traits is presented in Table 5. Antioxidant activity (both FRAP and DDPH

respectively) showed a significantly strong positive relationship with the total terpenoid content (r = 0.95 and 0.96), vitamin C (r = 0.70 and 0.67 respectively), ash content (r = 0.67 and 0.64), crude protein (r = 0.74 and 0.71), calcium (r = 0.72 and 0.71) and manganese (r = 0.65 and 0.70). The other traits like total phenol (r = 0.26 and 0.27), total flavonoid (r = 0.40 and 0.41), crude fiber (r = 0.60 and 0.57), crude fat (r = 0.63 and 0.62), total carbohydrate (r = 0.30 and 0.29) and iron content (r = 0.11 and 0.11) are positively correlated with the FRAP and DPPH respectively. Whereas, moisture content (r = -0.43 and -0.51), magnesium (r = -0.51 and -0.48), zinc (r = -0.21 and -0.12) and copper (r = -0.33 and -0.30) showed negative correlation with respect to FRAP and DPPH, respectively. It is clear from the present investigation that all the biochemical parameters have a positive correlation with the antioxidant activity, suggesting the high health benefits of the crop.

Table 5. Correlation analysis of Biochemical, proximate and mineral contents with antioxidant capacity

	TPC	TFC	TTC	Vit C	FRAP	DPPH	CF	CFat	AC	MC	CP	TC	Ca	Mg	Fe	Mn	Zn	Cu
TPC	1.00																	
TFC	0.95**	1.00																
TTC	0.42	0.58	1.00															
Vit C	0.18	0.36	0.64*	1.00														
FRAP	0.26	0.40	0.95**	0.70*	1.00													
DPPH	0.27	0.41	0.96**	0.67*	0.99**	1.00												
CF	0.45	0.59	0.72*	0.42	0.60	0.57	1.00											
CFat	0.43	0.55	0.75*	0.38	0.63	0.62	0.97**	1.00										
AC	0.06	0.29	0.66*	0.81**	0.67*	0.64*	0.63	0.59	1.00									
MC	-0.18	-0.28	-0.48	-0.26	-0.43	-0.51	0.12	0.11	-0.15	1.00								
CP	0.21	0.34	0.74*	0.47	0.74*	0.71*	0.82**	0.86**	0.76*	0.10	1.00							
TC	-0.19	-0.08	0.31	0.04	0.30	0.29	0.54	0.57	0.57	0.18	0.75**	1.00						
Ca	0.22	0.44	0.73*	0.74*	0.72*	0.71*	0.65*	0.62	0.95**	-0.30	0.78**	0.61	1.00					
Mg	-0.18	-0.42	-0.60	-0.48	-0.51	-0.48	-0.75*	-0.67*	-0.86**	0.11	-0.77**	-0.71*	-0.89**	1.00				
Fe	0.12	-0.08	0.03	-0.04	0.11	0.11	-0.23	-0.18	-0.41	0.00	-0.27	-0.53	-0.47	0.62	1.00			
Mn	0.21	0.27	0.60	0.60	0.65*	0.70*	0.08	0.09	0.42	-0.73*	0.19	0.03	0.53	-0.19	0.21	1.00		
Zn	0.60	0.47	-0.07	-0.18	-0.21	-0.12	-0.19	-0.17	-0.37	-0.40	-0.42	-0.37	-0.18	0.30	0.18	0.36	1.00	
Cu	0.11	-0.09	-0.28	-0.65*	-0.33	-0.30	-0.44	-0.37	-0.77**	-0.05	-0.50	-0.54	-0.76*	0.67*	0.59	-0.32	0.23	1.00

* and ** indicates that values are significant at 0.05 and 0.01 level of probability

Where, TPC - Total phenol content, TFC - Total flavonoid content, TTC - Total terpenoid content, Vit C - Vitamin C, FRAP - Ferric Reducing Antioxidant Power, DPPH - 1,1-diphenyl-2-picrylhydrazyl, CF - Crude Fiber, CFat - Crude Fat, AC - Ash content, MC - Moisture content, CP - Crude Protein, TC - Total Carbohydrate, Ca - Calcium, Mg - Magnesium, Fe - Iron, Mn - Manganese, Zn - Zinc, Cu - Copper

Correlation analysis is a statistical method that measures the degree of association between different traits in the studied datasets, giving a quantitative overview of the strength and direction of relationships. Positive associations were found among biochemical and quality parameters, including total phenol, total flavonoid, vitamin C, total terpenoid, ash content, crude protein and calcium and manganese. The correlation is a reliable indicator of the level of association between biochemical traits and antioxidant properties, which can provide additional criteria for selection in a breeding program. Nine quantitative traits of high economic value were correlated and the relative relationship of different phenotypic traits was established, indicating that while breeding for one trait can cause correlated changes in the others. This is consistent with the findings in large bitter gourd, bottle gourd and guava (22, 30, 31).

Principal component analysis

Multivariate analysis-based approaches enable the identification of genetic diversity and the clustering of genotypes. Out of the following approaches, PCA is a numerical method for classifying several subjects into significant uncorrelated variables. The multivariate numerical approach of the considered genotypes for phytochemical contents, antioxidant properties, proximate contents and mineral parameters assessed in the present study illustrated that significant variation between the selected genotypes existed. Biplot possessed principal components of the variances occupied by each accession and overall component response of the accessions on the characters (Fig. 1 and Table 6). The scree plot is used to determine the number of factors to retain in an exploratory factor analysis (FA) or principal components to keep in a principal component analysis (PCA) (Fig. 2). The first four principal components showed an eigenvalue greater than one, accounting for 88.93 % of the total variance among 10 genotypes. Among the 4 principal components (PCs), PC1 recorded the maximum variability (49.11 %), subsequently PC2 (19.03 %). The first principal component, PC1, was exhibiting 13 quality attributing traits including total phenol (0.33), total flavonoid (0.53), total terpenoid (0.88), vitamin c (0.73), FRAP activity (0.85), DPPH (0.83), crude fiber (0.82), crude fat (0.80), ash content (0.89), crude protein (0.88), total carbohydrate (0.57), calcium (0.94) and manganese (0.50). This indicates that most traits under study, i.e. biochemical, antioxidant and proximate traits, contributed to variability in nutrition. Hence, advancing a given quality parameter will lead the way for the
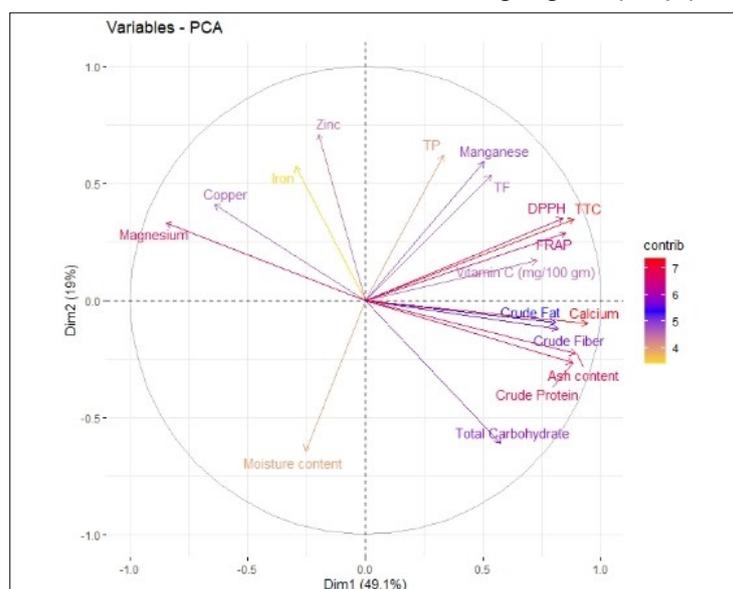

Fig. 1. PC1 and PC2 Biplot for 10 Small bitter gourd accessions.

Table 6. Principal component axis of ten small bitter gourd genotypes

Observations	PC1	PC2	PC3	PC4
Total phenol content	0.3311	0.6195	0.6615	0.1637
Total flavonoid content	0.5334	0.5338	0.57	0.2514
Total terpenoid content	0.886	0.3455	-0.021	-0.2376
Vitamin C	0.7307	0.1694	-0.3527	0.0486
FRAP	0.8513	0.287	-0.2223	-0.3385
DPPH	0.837	0.3503	-0.2366	-0.3024
Crude Fiber	0.8201	-0.1216	0.4719	-0.1578
Crude Fat	0.8077	-0.0952	0.4624	-0.2511
Ash content	0.8946	-0.2283	-0.221	0.1286
Moisture content	-0.2568	-0.6456	0.4353	-0.2863
Crude Protein	0.8807	-0.2685	0.1624	-0.2653
Total Carbohydrate	0.5745	-0.6112	0.054	0.0216
Calcium	0.9427	-0.1009	-0.1433	0.2504
Magnesium	-0.8493	0.3309	-0.1113	-0.2557
Iron	-0.2994	0.5725	-0.0837	-0.6526
Manganese	0.5028	0.5909	-0.52	0.15
Zinc	-0.2006	0.7091	0.2645	0.4931
Copper	-0.6422	0.408	0.2407	-0.4169
Eigen Values	2.973	1.851	1.461	1.267
Proportion of Variance (%)	49.11	19.03	11.86	8.92
Cumulative Proportion (%)	49.11	68.15	80.01	88.93

FRAP - Ferric Reducing Antioxidant Power, DPPH - 1,1-diphenyl-2-picrylhydrazyl
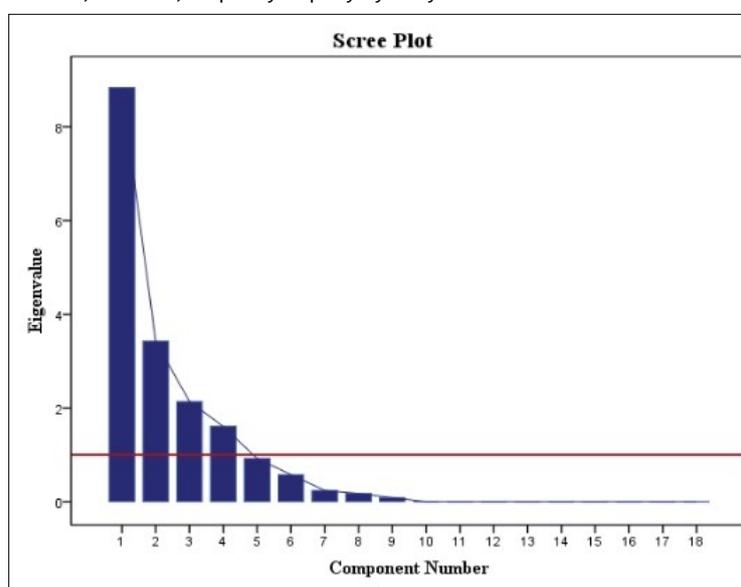
Fig. 2. Scree plot for ten small bitter gourd genotypes.

improvement of other quality attributing characteristics collected in the same PC. PC2 was found with positive contribution for all biochemical, antioxidant and mineral traits except calcium and proximate attributes, which showed negative contribution in PC2. This suggests a decrease in proximate attributes such as crude fibre, crude fat, crude protein, ash, moisture and carbohydrate might help in improving biochemical and mineral parameters. The PC3 contributed to 11.86 % of the overall variation and was primarily ascribed to total phenol, total flavonoid, crude fibre, crude fat, moisture, zinc and copper. PC4 resulted in 8.92 % of the total variance and was primarily associated with zinc, total flavonoid and total phenol. It is crucial to take into account how particular features (characters) tend to be connected in various principal components (PCs) when choosing traits for a breeding program. These characteristics frequently exhibit a propensity to stick together. Therefore, concentrating on these factors during the breeding process can result in quicker improvements in quality and nutritional traits. In simple terms, correlated characteristics can be enhanced in parallel to produce superior overall results in the desired traits. The first 4 PCs revealed maximum variation for the biochemical, antioxidant and proximate composition considered for the study and characteristics could be taken into consideration to improve

SBG. Variables were represented as vectors in the biplot and each vectors' length represented the level of variation in that parameter. The genotypes that were most diverse and least comparable to other types were those that were farthest distant from their place of origin. When assessing the cultivars, PC1 and PC2 showed that they were dispersed among the four quadrants, emphasising the highest level of genetic variation (Fig. 3). Research indicates a similar observation under the various crops (32-34). The traits of bitter gourd that demonstrated a positive association with PCs have a major role in variance contribution by the respective principal component (PC).

Hierarchical cluster analysis

The comprehensive clustering diagrams for SBG were constructed on the basis of all the quantitative traits (Fig. 4). Aiming to distinguish the genotypes based on their biochemical, antioxidant, proximate and mineral content traits, cluster analysis was performed using the ward method. And it helped to pinpoint the sources farthest out and closest for breeding the following round. Genotypes were grouped into four clusters using Squared Euclidean distances for the attributes measured, demonstrating that genotypes selected in this study had significant variability (Table 7). Cluster I had four genotypes (MCM-1, IC639012, IC639010, IC636486), followed by cluster II with one genotype (MCM-4), cluster III with two genotypes
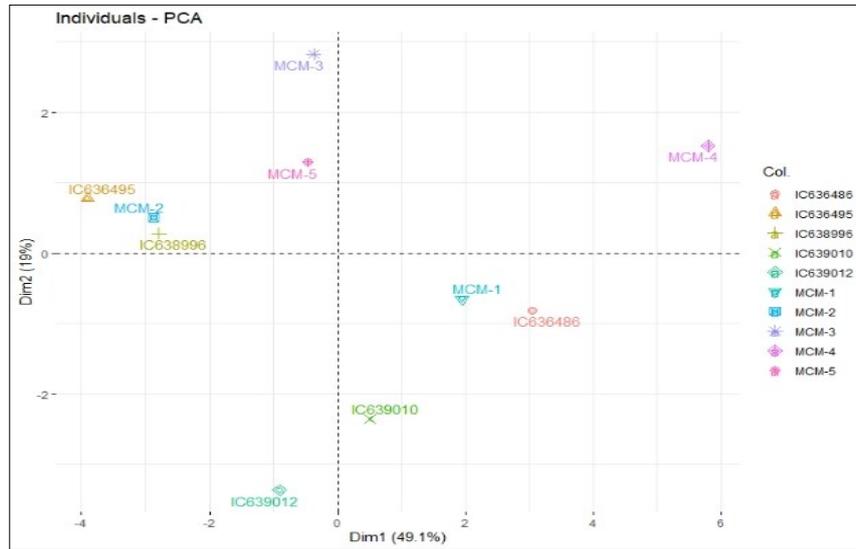
Fig. 3. Biplot distribution of ten small bitter gourd genotypes depending on principal component axes PC1 and PC2.
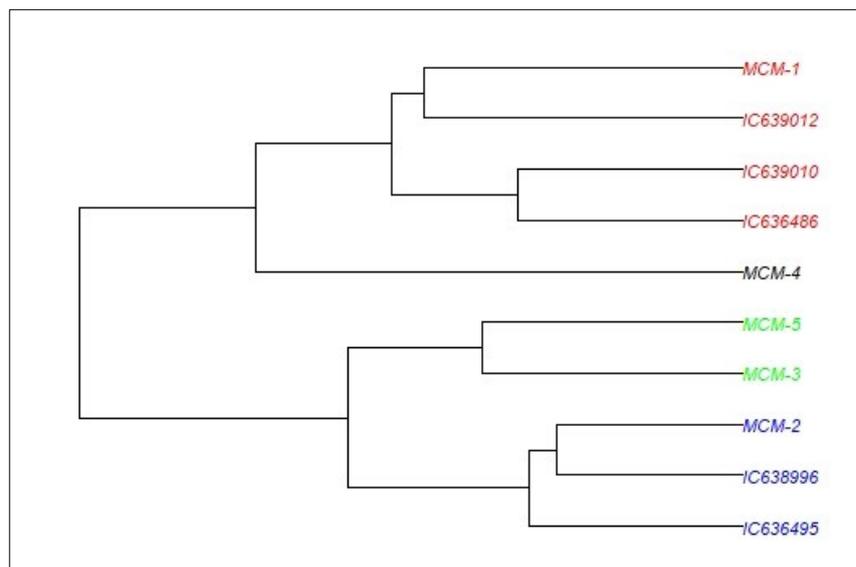

Fig. 4. Dendrogram showing cluster analysis of ten small biter gourd black pepper cultivars based on qualitative traits.

Table 7. Genotype distribution among different clusters

Cluster number	No of genotypes	Genotypes
I	4	MCM-1, IC639012, IC639010, IC636486
II	1	MCM-4
III	2	MCM-5, MCM-3
IV	3	MCM-2, IC638996, IC636495

(MCM-5, MCM-3) and cluster IV with three genotypes (MCM-2, IC638996, IC636495). The genotype in Cluster II recorded the highest cluster means for most traits under study except for the moisture content, magnesium, iron and copper (Table 8). The classification of thirty accessions of *Ceiba pentandra* into clusters was observed to match our results (32). A similar pattern of classification in bitter gourd was reported, where 70 genotypes are grouped into 7 clusters (35). Cluster analysis revealed that the selected populations were genetically diverse, though populations from the same geographical region clustered together. The cluster analysis offers an easily accessible avenue for improvement through hybridisation using different combinations of populations from the different clusters. The hybridisation for maximum heterosis in a short duration must be through high diversity, i.e. choosing maximum divergent parents.

Conclusion

A significant variation and diversity were observed among the genotypes for biochemical and quality traits. Genotype MCM-4 outperformed significantly for most of the traits. Genotypes such as IC636486, MCM-1 were found as better lines that can be included in bitter melon breeding programs concerning vitamin C and crude fibre content. The results of this study imply that various principal components could be used to group the significant characters responsible for variation in the bitter gourd genotypes. Clusters showed a lot of differences that were statistically significant. This demonstrated the potential for genotype improvement through hybridisation between two clusters. This increases the chances of obtaining desirable F_1 recombinants.

Table 8. Cluster means for different traits under study

FG Observations	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Total phenol content	53.82	79.57	65.71	57.05
Total flavonoid content	42.58	67.55	48.29	41.21
Total terpenoid content	1.77	2.49	1.96	1.26
Vitamin C	106.29	121.83	104.70	95.70
FRAP	36.23	44.62	38.79	29.16
DPPH	0.68	0.94	0.77	0.52
Crude Fiber	4.71	5.36	4.32	2.94
Crude Fat	1.90	2.43	1.69	0.79
Ash content	7.50	9.23	5.46	5.00
Moisture content	86.25	78.43	82.83	82.67
Crude Protein	26.04	29.62	22.06	17.56
Total Carbohydrate	10.12	11.62	6.70	6.52
Calcium	37.25	60.14	24.05	21.40
Magnesium	10.19	7.96	13.85	14.20
Iron	0.82	0.73	1.44	0.95
Manganese	0.17	0.30	0.23	0.19
Zinc	0.14	0.30	0.23	0.26
Copper	0.20	0.09	0.33	0.30

FRAP - Ferric Reducing Antioxidant Power, DPPH - 1,1-diphenyl-2-picrylhydrazyl

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Authors' contributions

AH performed the field and lab experiments and prepared the skeleton of the manuscript. KS provided their expertise in laboratory analysis. SS carried out data analysis. MK and RKA conceptualized and supervised the research. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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