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Diversity in phytochemical composition of bitter gourd (*Momordica charantia* L.) genotypes based on principal component analysis

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Abstract
Bitter gourd is highly nutritious with high medicinal value vegetable crop due to their high contents of phytochemicals, especially-Anti oxidant, Vitamin C, Iron and phosphorus. The objective of this study was to evaluate the genetic diversity of bitter gourd based on phytochemical contents and Antioxidant activities in different *in vitro* assay. A total of 16 traits were studied for sixteen diverse bitter gourd genotype were collected from different part of India and grown in a randomized block design (RBD) with three replications, each replication consist of sixteen plants per treatment during *Kharif* -2015 at vegetable farm of Department of Horticulture (Vegetable and Floriculture), Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur (Bihar). The antioxidant activity was investigated by the water extract of fruit fractions by several *in vitro* systems of assay, namely DPPH radical-scavenging activity, ferric reducing/antioxidant power (FRAP) assay and total antioxidant capacity. Total phenolic content was measured by Folin–Ciocalteu reagent and TSS was measured by refractometer. Chlorophyll content was measured by spectrophotometer at different wave length. High variations were observed in these accessions for all phyto-chemical contents. Many of these traits are of significant economic importance and could be used as breeding targets to increase fruit yield and fruit quality. Principal component analysis of sixteen bitter gourd genotypes based on phyto-chemical traits was performed by using statistical software SPSS 15.0. Cluster analysis showed that the 16 genotypes of bitter gourd could be grouped into 5 clusters. The highest intra cluster distance was recorded for cluster II followed by cluster I. Genotype from this cluster could be utilized as parental line for hybrid breeding programme owing to their higher mean performance within group. Genotype from distance clusters could be used in crop improvement programme to produce populations with wide variability with transgressive segregants possessing nutrient rich fruits. Total antioxidant capacity (µmol TE/g) CUPRAC contribute maximum towards genetic diversity for phytochemical traits. The results are useful for germplasm conservation, utilization and management for breeding of bitter gourd in the future for quality improvement.

Keywords: bitter gourd, genetic divergence, phytochemicals, cluster, d^2 statistics

1. Introduction
Bitter gourd (*Momordica chrantia* L.) is known by different name such as Balsam pear or Bitter cucumber in English, *Karela* in Hindi, Gujarathi and Punjabi, Karla in Marathi, Beet Karela in Assames, *Kakara kaya* in Telugu, *Pavakai* in Tamil, *Hagala Kayi* in Kannada and *Pavakka* in Malayalam. It is a fast growing warm seasonal climbing annual, native to South Asia. It is widely cultivated in India, China, Malaysia, Africa, and South America (Raj *et al*., 1993) [11]. Although the general chemical composition of *M. charantia* immature fruit is similar to other cucurbits, bitter gourd possesses comparatively high concentrations of ascorbic acid and iron (Behera *et al*., 2004) [4]. Bitter gourd is grown for its bitter tender fruits. Fruits are covered with blunt tubercles. It bears simple; alternate leaves 4-12 cm across, with 3-7 deeply seprated lobes. Each plant bears separate yellow male and female flower. Compare to other cucurbits, bitter gourd has relatively high nutritional value, in respects of iron and ascorbic acid contents. The fruit are rich in iron, Vitamin A, B, C and are an inexpensive source of protein and minerals.

It contents considerable amount of water (83-92%), carbohydrates (4.0-10.5%), protein (1.5-2%), fat (0.2-1%), minerals (0.5-1%) and fiber (0.8-1.7%).The nutritive values of bitter gourd fruits vary with the varieties. The small-fruited (*Momordica charantia* var. muricata) types are found to be more nutritious than the large-fruited (*Momordica charantia* var. charantia) types (Tendulkar 1997) [16]. Ripe fruits are rich in vitamin A. White-fruited Indian varieties are relatively high in polypeptide-p, phenolics, polyphenolic compounds, and natural oxidants and antioxidants. The immature fruits contain high amounts of vitamin C, vitamin A, vitamin E, vitamins B 1,B2 and B3, as well as vitamin B9 (folate) (Bakare *et al*., 2010) [3]. Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flvonoids, isoflavones, terpens, anthroquinones, and glucosinolates, all of which confer a bitter taste (Snee *et al*., 2011) [15]. Bitter gourd (*Momordica charantia* L.) has long been regarded as a food and medicinal plant. Bitter melon contains a number of natural plant compounds, or phytochemicals, that have possible health benefits. Bitter melon contains biologically active chemicals such as essential oil, flavonoids, phenolic acids, glycosides, triterpenes, and alkaloids (Choi *et al*., 2012) [5].They include beta-carotene, lycopene and zeaxanthin, three carotenoids with antioxidant properties, as well as other active compounds called momordin, vicine and charatin, and several other natural constituents. The fruits, vines, leaves, and roots of *M. charantia* have been used to treat toothache, diarrhea, furuncle, and diabetes. Bitter gourd has been used as a traditional medicine for diabetes (Yeh *et al*., 2003) [17] and other health-related ailments (e.g., health promoting substances such as charantin (Yeh *et al*., 2003) [17] and vicine (Dutta *et al*., 1981) [7]. Bitter melon has been used for the treatment of diabetes mellitus due to their effective constituents such as charantin and peptides which are similar to insulin and several alkaloids. Bitter melon has some interesting biological and pharmacological activities, e.g. anti-cancer, anti-viral, anti-bacterial, analgesic, antiinflammatory, hypotensive, anti-fertility, and anti-oxidant (Mahmood *et al*., 2012) [9] and Sin *et al*., 2012) [14].

Genetic diversity is an important factor and also a pre-request in any hybridization programme. Inclusion of diverse parents in hybridization programme serves the purpose of producing desirable recombinants. Therefore, an attempt has been made in the present investigation to estimate genetic divergence among a set 12 genotypes including for 16 biochemical traits and antioxidant capacity in different *in vitro assay*. The biochemical characterization of bitter gourd fruit is important in providing information useful in defining its use. Genetic diversity and discrimination among individual accessions or groups of individuals or populations can be analyzed by a specific method or combination of method (*Coser et al.*, 2012) [6]. Information on heterosis and genetic divergence analysis is inadequate in bitter gourd. The information about the nature and magnitude of genetic divergence is essential for selection of diverse parent which upon hybridization can result in productive hybrids. In bitter gourd genetic diversity in term of phytochemical composition has not been fully explored yet. The objective of the study was to evaluate the diversity in phytochemical composition and antioxidant potential of 12 bitter gourd genotypes. The information generated will be helpful in the selection of potential bitter gourd genotype for future propagation and crop improvement based on their phytochemical composition and antioxidant potential to harvest the maximum health benefits.

2. Material and Methods

2.1. Plant Materials and Site of Study

In the present study sixteen genotypes of bitter gourd were collected from different parts of the country were grown in a Randomized Block design with three replications, each replication consist of twelve plants per treatment during *Kharif* 2015 at vegetable farm of department of Horticulture (Vegetable and Floriculture), Bihar Agricultural College, Bihar agricultural University, Sabour, Bhagalpur (Bihar).The seeds were sown after soaking in water for 24 hours at a spacing of 2m x 50 cm. The crop was managed as per recommended package of practice. Sixteen important biochemical and antioxidant activities traits viz, ascorbic acid content, acidity, total carotenoid, total phenol, chlorophyll a, chlorophyll b, total chlorophyll, total sugar, reducing sugar, non reducing sugar, TSS, flavonoid, radical scavenging activity, metal chelating activity, Total antioxidant by CUPRAC assay and Total antioxidant by FRAP assay, was recorded with the help of five randomly selected plant from each replications.

2.2 Biochemical and antioxidant analysis

Ascorbic acid

Ascorbic acid content of the juice was determined by titrating freshly extracted juice against 2, 6-dichlorophenol indophenols dye (A. O. A. C., 1975). For its determination 2 g pulp was crushed with 3.00 per cent Meta phosphoric acid (MPA) solution and volume made up to 100 ml with 3 % MPA in a volumetric flask. After 10 min. aliquot of filtrate was titrated against standard 0.025 per cent 2, 6-dichlorophenol indophenols dye solution. The end point was marked by the appearance of pink colour which persisted for 15 second. The content of ascorbic acid was expressed as mg/100 g of pulp.

Amount of ascorbic acid mg/100 g sample

$$= \frac{0.5\text{ mg}}{\text{V1 ml}} \times \frac{\text{V2}}{5\text{ ml}} \times \frac{100\text{ ml}}{\text{Weight of the Sample}} \times 100$$

Titratable acidity (%)

Determined by using titration method.2 g of fruit sample was weighed and crushed. The homogenate was diluted up to 100 ml with distilled, then filtered and two drops of 1% phenolphthalein solution (indicator) was added to 10 ml of filtrate solution. Finally, it was titrated against 0.1 N NaOH solutions till pink colour appeared. The titre value was used for calculating acidity from the following formula and the results were expressed as percentage of citric acid.

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times \text{normality of alkali} \times \text{Vol. made up} \times 64 \times 100}{\text{Volume of sample taken} \times \text{Wt. of sample taken} \times 1000}$$

Total chlorophyll content

1 g of peel was taken and well mixed with 10ml of 80% acetone. It was kept under the cold condition for 2 days in dark. The sample was strained with fine muslin cloth. The mixture was centrifuged (5000 rpm for 5 min) and the supernatant was collected in volumetric flask. The supernatant (1ml) was taken in a test tube. The volume was made up to 10 times by adding 9ml of 80% acetone. From this 1ml was taken and diluted with 80% acetone to make volume up to 5ml.The optical density of the sample was recorded with the help of spectrophotometer with wave length of 663nm and 645nm.

Formula for estimation of total chlorophyll
Chl a (mg /100g) = [{(12.7 x A_{663})-(2.63XA_{645})} x D]/100
Chl b (mg /100g) = [{(22.9 x A_{645})-(4.68 x A_{663})} x D]/100
Total chlorophyll (mg /100g) = [{(20.2 x A_{645}) + (8.02 x A_{663})} x D]/100
Where, A_{663}= optical density at 663nm
A_{645}= optical density at 645nm
D= dilution.

Total sugar content of the pulp (Fehling's method)
5g crushed sample or 5ml of juice taken in a conical flask and add 3-4 drops of HCl. Heated till hydrolysis occurs, colour changes a bit and a burnt sugary smell is perceived, cooled 1(N) NaOH solution added to make the sample alkaline, tested by litmus paper. Volume made up to 50 ml by adding distilled water. Filled in burette and kept for heat titration. Meanwhile, 2ml Fehling solution A and 2ml Fehling's solution B taken in a conical flask and 16ml distilled water added. This conical flask is heated. When boiling starts methylene blue indicator 2-3 drops added. Heat titration with the sample in burette is done till end point. End point brick red colour precipitate. Titre value noted

$$\text{Calculation: Total sugar (\%) (G of glucose/100 g of fresh fruit)} = \frac{20}{x}$$

Where, x= burette reading/titre value

Reducing sugar
The reducing sugar was estimated by using copper titration methods as suggested by AOAC (1975) [1]. In this method Fehling's solution of A and B (5 ml each) were taken in a conical flask and 10 ml distilled water was added to it. The juice was then titrated against boiling solution of Fehling's. Methylene blue was used as an indicator. The appearance of brick red colour determined the end point. The reducing sugar was calculated in percentage in the juice considering 10 ml of Fehling's solution A and B equal to 0.05 g of glucose.

Non reducing sugar
Non-reducing sugar content was calculated by subtracting the value of reducing sugar from the total sugar.

TSS (%)
Bitter gourd juice was collected from green fruits. A drop of juice was placed over the prism of digital refractometer and value was noted in per cent.

Total carotenoid
Determined by Roy's (1973) method. 5 g of bitter gourd pulp was crushed in acetone till it became colourless, petroleum ether and small amount of sodium sulphate and shaken rigorously for mixing well. Then the separating funnel was kept undisturbed to separate the carotenoids from acetone to petroleum ether layer. After that, coloured solution was separated in a 50 ml volumetric flask and the value will be adjusted with petroleum ether. Finally, the sample absorbance was measured at 452 nm in a spectrophotometer, using petroleum ether as blank. The result was expressed as mg 100 g^{-1} FW (fresh weight) basis.

Prepration of Sample
1 gram composite sample from randomly selected three Bitter gourd fruits from each replication at edible maturity was crushed with 10 ml of 70% methanol in mortar pestle. Prepared sample was centrifuged at 10000 rpm at 4ºC. Supernatant was taken for further estimation.

Total phenolic content
Total phenolic content were estimated spectrophotometrically using Folin–Ciocalteau reagent (FCR).To the 100 µl of the sample extract (70% methanol), 2.9 ml of deionized water, 0.5 ml of Folin–Ciocalteu reagent and 2.0 ml of 20% Na_2CO_3 solution were added. The mixture was allowed to stand for 60 min and absorption was measured at 765 nm against a reagent blank in UV vis spectrophotometer (Varian Cary 50). Calibration curve was built with standard catechol and results were expressed as catechol equivalent (mg CE/100 g fw).

$$\text{Calculation: } \frac{Absorbance \times Volume\ made\ up \times dilution \times 100}{Aliquot\ taken \times sample\ weight \times 1000}$$

Flavonoid
Measurement of total flavonoid content in the investigated extracts was determined spectrophotometrically, using a method based on the formation of complex flavonoid aluminium with the absorbtivity maximum at 430 nm. The diluted sample solutions, in the amount of 1 mL, were separately mixed with 1 mL of 2 % AlCl3 × 6H2O. After incubation at room temperature for 15 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoid content was expressed as mg of quercetin equivalents (QE) per g of dried extract, by using a standard graph. All measurements were carried out in three replicates.

$$\text{Calculation: } \frac{Absorbance \times Volume\ made\ up \times dilution \times 100}{Aliquot\ taken \times sample\ weight \times 1000}$$

Radical scavenging activity
DPPH (2, 2-diphenylpicrylhydrazyl) assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH (Williams *et al.*, 1995). A 3.9 ml aliquot of a 0.039432 m mol/L of DPPH solution in methanol was added to 0.1 ml of each extract and shaken vigorously. Change in the absorbance of the sample extract was measured at 517 nm for 30 min. The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula:

$$\%\text{Inhibition} = \left(\frac{A0 - A}{A0}\right) \times 100$$

Where, A_0 was the beginning absorbance at 517 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 517 nm. Methanol absolute was used as a blank.

Metal chelating activity
The chelating effect was determined by adding 2 mL of various concentrations (50 to 250 µg/mL) of the extracts in methanol to a solution of 2 mM FeCl2 (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine-Fe2+ complex formation was calculated by using the formula given below:

$$\%\text{Inhibition} = \left(\frac{A0 - A}{A0}\right) \times 100$$

Total antioxidant CUPRAC

The cupric ion reducing antioxidant capacity of Bitter was determined according to the method of Apak *et al.* (2004) [2]. Briefly, according to the protocol 0.1 ml of sample extract was mixed with 1 ml each of $CuCl_2$ solution (1.0×10^{-2} mol/L), neocuproine alcoholic solution (7.5×10^{-3} mol/L), and NH_4Ac (1 mol/L, pH 7.0) buffer solution and 1 ml of water to make the final volume 4.1 ml. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. The results were expressed as µ mol TE/g, using molar absorptivity of Trolox as 1.67 × 104 L/mol/cm.

Calculation

$$\text{Total antioxidant activity (µ mol trolox equivalent per gram)} = \frac{\text{Absorbance} \times \text{Final volume made up} \times \text{Total volume of sample made with methanol} \times \text{Dilution}}{1.67 \times 10^4 \times \text{volume of sample taken for estimation} \times \text{Weight of sample}}$$

Total Antioxidant FRAP

The determination of antioxidant activity through FRAPreagent was prepared using *300* mM acetate buffer (pH 3.6); 10 mM TPTZ (2, 4, 6- tris (2-pyridyl)-s-triazine), in 40 mM HCl; and 20 mM FeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. About 0.5 mL FRAP reagent was added to 50 µL bitter gourd fruit extracts (50 mg/mL 50% ethanol) and the absorbances were taken at 595 nm wavelength with the spectrophotometer after 30 minutes. The calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dried sample (mg TE/100 g of FW).

2.3. Statistical Analysis

The experiment data for various characters, recorded in course of this investigation were subjected to statical analysis using suitable technique for different characters. The procedures for calculating the inter-cluster distance is first to measure the distance between various combinations of clusters and divide them by the product of the number of genotypes in the concerned cluster combinations. Each character is ranked on the basis of mean difference, i.e., d_i $d_i = Y_i^1 - Y_i^2$ value, rank 1 is given to the highest mean difference and rank p to the lowest mean difference where p is the total number of characters. Principal component analysis of sixteen bitter gourd genotypes based on phyto-chemical traits was performed by using statistical software SPSS 15.0.

3. Result and Discussion

Cluster analysis based on all the traits studied divided genotypes into 5 clusters for biochemical parameter with antioxidant activity. Composition of clusters based on Principal Component analysis analysis of 16 bitter gourd genotypes is represented in Table -1 and genetic similarity relationship of genotypes is represented in Fig-1. Among the different clusters, cluster II content maximum number of genotype (6 genotype) followed by Cluster I and Cluster III both have 4 genotypes followed by Cluster IV and Cluster V both having 1 genotypes based on biochemical and antioxidant capacity. The clustering pattern of the genotypes under this study revealed that the genotypes collected from the same district were grouped in to different clusters. Similar finding was given by Rasmi *et al*., (2012) [12] and Mausud *et al*., (1995) in Pumkin. It indicated that the geographic diversity is not always related to genetic diversity and, therefore, it is not adequate as an index of genetic diversity. Genetic drift and selection indifferent environment could cause greater diversity that geographic distance. The clustering pattern clearly reflects the presence of considerable extent of genetic diversity in the material under study.

Average of intra (diagonal) and inter cluster distance based on Principal component analysis is represented in Table - 2 and Fig-2. The highest intra cluster distance was recorded for cluster II (65950.340) followed by cluster I (62643.930). Genotype from this cluster could be utilized as parental line for hybrid breeding programmes owing to their higher mean performance within group. Inter cluster distance is more than intra cluster distance. Inter - cluster distance ranged from 205601.000 (between cluster IV and III) to 1329708.000 (between cluster V and II). The highest inter – cluster distance was observed between clusters V and cluster II (1329708.00). Scattered distributions of sixteen genotypes are shown in Fig-3. The crosses involving parents belonging to the maximum divergent clusters were expected to manifest maximum heterosis and also wide genetic variability. Genotypes from the distance clusters if involved in hybridization, may give a wide spectrum of segregating population, as genetic variation was very distinct among the groups. Selection of diverse parents from various clusters is considered to be an acceptable procedure in harnessing the heterosis. However, where the number of genotypes constituted by a cluster is more than one, an appropriate methodology need to be adopted to select a parents or parents from within a cluster (Kumari *et al*, 2017) [8]. Cluster mean for 16 biochemical character and antioxidant activity is presented in Table -3. Cluster I content maximum mean value for most of the biochemical trait and antioxidant activity like total phenolic content (345.77), acidity (100.38), flavonoid content (74.74), antioxidant by FRAP assay (65.77), radical scavenging activity (55.69), total antioxidant by CUPRAC assay (11.41), chl a (1.78) and chl b (0.72). To develop varieties with high amount of biochemical and antioxidant capacity genotypes of these groups can be used in hybridization program. Antioxidant by CUPRAC assay (55.00%) has maximum contribution towards diversity. It is interesting that greater divergence in the parent material due to these characters will offer a good scope for improvement of yield through rational selection of parents for producing heterotic bitter gourd genotypes.

Principal component analysis was used to identify the most significant variables in the data set (Table 5 and Table 6).The results indicated that vector 5 explain about 96.74 % of the total variability observed, whereby vector 4 accounted for 91.61%. Variables with higher scores on PC5 are related to Acidity % and flavonoid content. The highest contribution on PC4 corresponded to variables related to Ascorbic Acid. Data from Table 6 Indicating that PC2 contributing positively for genetic divergence from all genotypes to all traits studied.

Table 1: Composition of clusters based on Principal Component analysis analysis of 16 bitter gourd genotypes

Clusters	Members
Cluster I	Gangajali Small, Narendra Baramasi, L-514, L-814,
Cluster II	Improve Katahi, Jhalari, Meghdoot, L-214, Pusa Aushadhi, L-114
Cluster III	Preethi, Pusa do Mousami, Pusa vishesh, Swarna Yamini
Cluster IV	L-314
Cluster V	Green star bold

Table 2: Average of intra (diagonal) and inter cluster distance based on Principal component analysis

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	62643.930	225084.100	254549.800	336723.100	765932.300
Cluster II		65950.340	100941.100	345388.800	1329708.000
Cluster III			57826.860	205601.000	1035511.000
Cluster IV				0.000	550693.100
Cluster V					0.000

Table 3: Mean values of five clusters for 16 morphological characters under study based on Principal Component analysis

Clusters					
Characters	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**
Ascorbic acid	100.380	74.816	61.149	44.055	46.903
Acidity	1.603	0.693	0.839	0.893	1.158
Total carotenoid	2.610	2.343	1.539	2.070	0.970
Total phenolic content	345.773	176.773	120.777	91.050	121.770
Chlorophyll a content	1.780	0.943	0.547	0.522	0.477
Chlorophyll b content	0.720	0.436	0.283	0.198	0.143
Reducing sugar	0.107	0.173	0.149	0.133	0.128
Non reducing sugar	0.080	0.101	0.121	0.120	0.122
TSS	4.450	6.100	5.220	4.625	5.900
Flavonoids	74.743	28.573	26.408	16.578	45.792
Radical scavenging activity	55.697	51.854	48.434	48.518	52.313
Metal chelating activity	73.910	65.555	40.987	41.643	58.118
Total antioxidant CUPRAC	11.413	9.044	7.185	5.167	5.808
Totalantioxidant FRAP	65.773	47.094	37.707	25.560	33.060

Table 4: Percentage contribution of each character towards total genetic divergence in 16 bitter gourd genotypes

Characters	Times Rank 1st	Contribution of characters (%)
Ascorbic acid (mg/100 g fw)	0.01	0.00 %
Acidity (%)	0.01	0.00 %
Total Carotenoids (mg/100g fw)	9	7.5 %
Total phenolic content (CE/100 g)	28	23.33 %
Chlorophyll- a (mg/100g fw)	0.01	0.00 %
Chlorophyll- b (mg/100g fw)	0.01	0.00 %
Total chlorophyll content (mg/100g fw)	0.01	0.00 %
Reducing sugar (%)	0.01	0.00 %
Non-reducing sugar (%)	0.01	0.00 %
TSS (%)	0.01	0.00 %
Flavonoids (mg/100g)	2	1.67 %
Radical Scavenging Activity (%)	13	11%
Metal chelating activity (%)	0.01	0.00 %
Total antioxidant capacity (µmol TE/g) CUPRAC	66	55.00 %
Total antioxidant capacity (mg TE/100g) FRAP	2	1.67 %

Table 5: PCA result based on phytochemical contents for bitter gourd genotypes

Canonical Roots Analysis (P.C.A)					
	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**
	Vector 1	**Vector 2**	**Vector 3**	**Vector 4**	**Vector 5**
Eigene Value (Root)	8.254	2.659	1.450	1.379	0.769
% Var. Exp.	55.030	17.725	9.665	9.194	5.126
Cum. Var. Exp.	55.030	72.755	82.420	91.614	96.740
Ascorbic Acid	-0.123	0.520	-0.246	0.208	0.022
Acidity %	-0.102	-0.241	-0.450	-0.114	0.745
Carotenoid	-0.113	0.290	0.257	-0.610	0.221
Total Phenol Content	-0.333	0.007	0.182	0.108	-0.090
Chlorophyll A	-0.319	0.008	0.035	-0.284	-0.160
Chlorophyll B	-0.325	0.003	0.060	-0.211	-0.196
Reducing Sugar	0.336	0.090	-0.078	-0.113	-0.076
Non Reducing Sugar	0.148	0.473	0.221	-0.279	0.235
TSS	0.304	0.022	-0.266	-0.254	-0.026
Flavonoid	-0.281	-0.032	0.184	0.186	0.375
RS	-0.342	-0.073	0.068	-0.046	-0.103
MC	0.330	0.063	-0.026	0.048	-0.100
Antioxident	-0.164	0.324	-0.536	-0.127	-0.231
FRAP	-0.290	0.146	-0.376	0.116	-0.061

Table 6: Principle component analysis loadings of phytochemical composition based on phenotypic distance of 16 bitter gourd obtain from different geographical regions

Genotype	PC 1	PC 2	PC 3	PC 4	PC 5
	Vector 1	Vector 2	Vector 3	Vector 4	Vector 5
L-814	-395.942	232.323	-307.368	-80.455	-167.986
Gangajali Small	-471.042	293.276	-270.596	-162.821	-156.867
Green star bold	-855.961	244.535	-198.461	-95.766	-165.506
Improve Katahi	-345.589	224.775	-165.165	-95.094	-87.909
Jhalari	-316.421	227.758	-118.017	-84.159	-38.845
L-514	-443.961	261.352	-191.099	-146.618	-92.882
L-314	-533.225	177.191	-41.627	-142.842	-104.574
L-214	-309.375	219.666	-222.048	-50.730	-104.710
Meghdoot	-296.431	198.688	-170.375	-89.163	-83.798
Narendra Baramashi	-403.303	313.814	-258.225	-88.966	-110.531
Preethi	-356.582	186.296	-116.594	-27.619	-74.737
Pusa Aushadhi	-212.174	188.321	-121.450	-88.935	-45.828
Pusa do Mousami	-349.719	123.753	-104.393	-30.033	-68.112
Pusa vishesh	-301.103	154.973	-130.706	-49.234	-44.207
L-114	-240.925	131.684	-76.192	-93.309	-51.242
Swarna Yamini	-435.924	183.906	-85.478	-10.273	-74.699

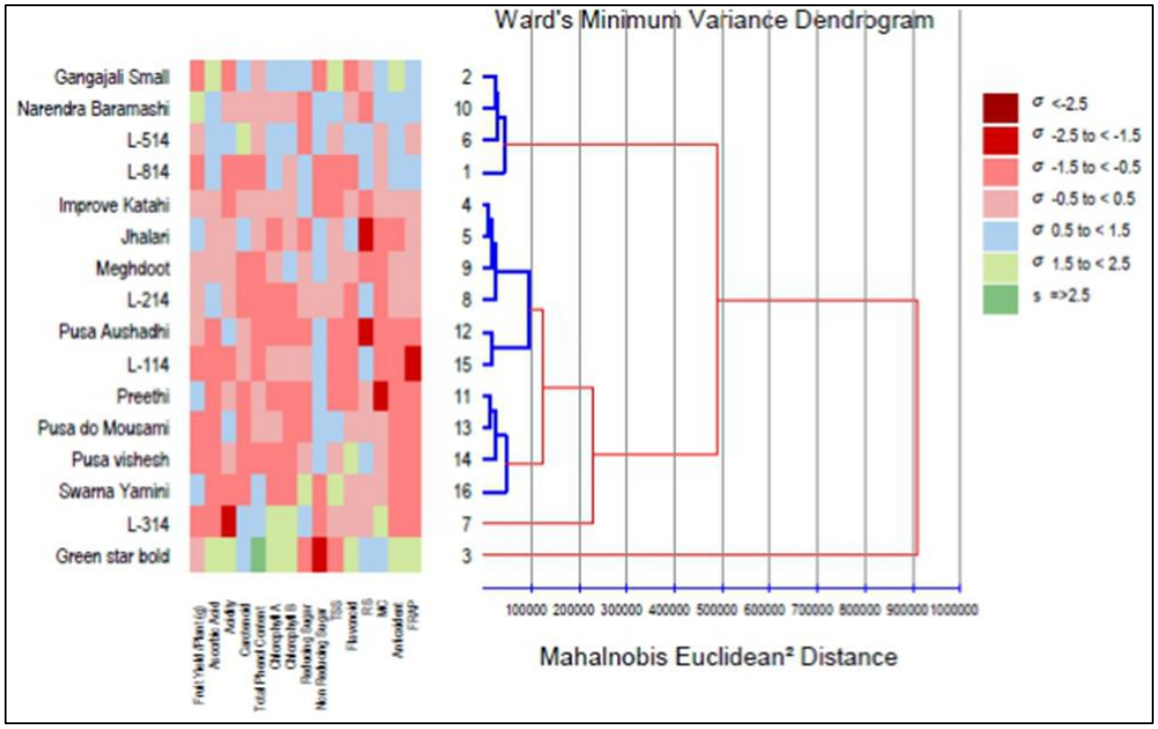

Fig 1: Genetic similarity relationship of sixteen genotypes after clustering by Principal component analysis

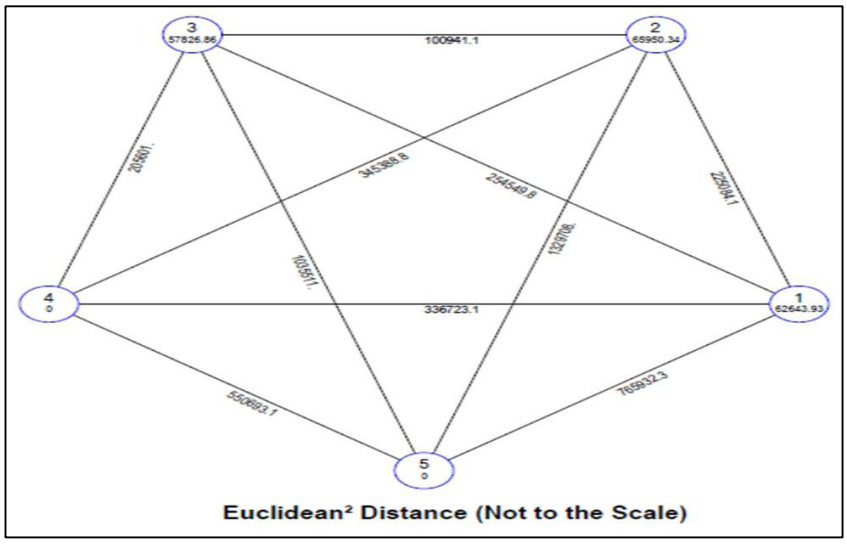

Fig 2: Diagram showing intra and inter-cluster distance sixteen genotypes of bitter gourd based on PCA analysis

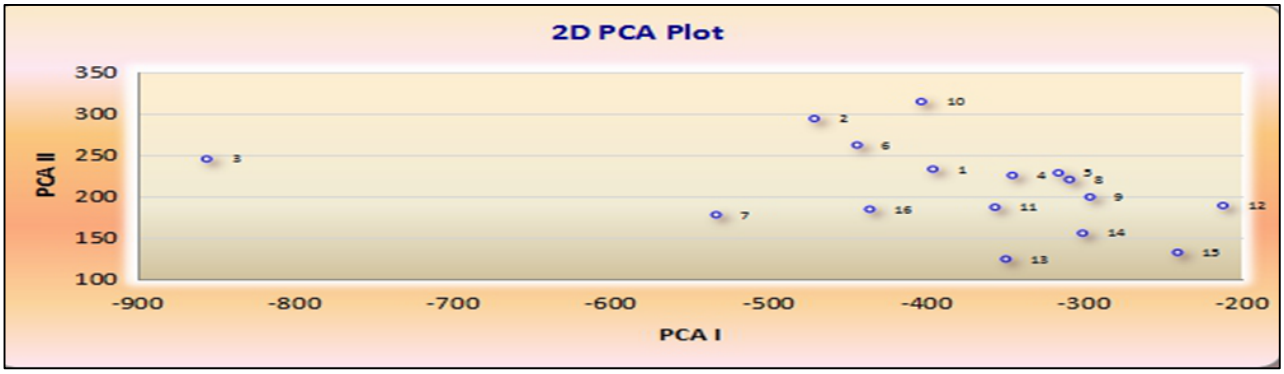

Fig 3: Scattered distribution of sixteen genotypes of bitter gourd based on their principal component scores superimposed with clusters

4. Conclusion

PCA analysis should be accounted as the powerful methods in managing multiple traits for plant breeding, especially in landrace populations. They could define many traits into few factors, and then cluster plant accessions into groups, that is convenient for plant breeders to consider the information and use in breeding programs. The phytochemical composition of bitter gourd genotypes exhibited genetic diversity in phytochemical composition in flesh of bitter gourd and this variation may be due to genotype, level of phytochemical present in genotypes, agro-climatic condition and other agricultural practices. In the present study inter-cluster distances were found higher than intra-cluster distances for principal component analysis revealing, a considerable amount of genetic diversity among the genotypes studied. Selection of genotypes from distant clusters may be used in crop improvement programmes to produce populations with wide variability with transgressive segregates possessing high yielding for future breeding and crop improvement program. The genetic diversity in the phytochemical composition in the flesh of bitter gourd showed the presence of a wide range of phytochemicals presence in the genotypes. It is suggested that the selection of potential bitter gourd genotypes for future breeding program should therefore be based on their phytochemical composition and antioxidant potential properties to harvest the maximum health benefits.

5. References

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