Comparative analysis of mineral nutrients composition of round gourd (*Praecitrullus fistulosus*) germplasm

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Abstract- *Praecitrullus fistulosus* is commonly known as a round gourd, a squash-type vegetable from the Cucurbitaceae family. 25 round gourd accessions were studied to estimate the variation in eight mineral nutrients. Accession 20248 possessed maximum nutrients with highest values for moisture contents, protein, nitrogen, and phosphorus at 95.67%, 23.75%, 3.80%, and 0.08 mg/g respectively. Potassium gave the maximum value of standard deviation (13.62) followed by protein contents (4.28) concluding that these two variables had maximum observed variability. PCA results of first three components for fruit mineral contents accounted maximum variation as PC1 34.766%, PC2 28.223%, and PC3 12.527% demonstrating a total variation of 75.516%. Cluster analysis classified the total variation as 18.58% variability within class and 81.42% variability between classes. All round gourd accessions exhibited significant amount of variation in the fruit mineral composition which could be utilized in breeding programs for developing the mineral-rich and high-yielding round gourd varieties.

Index Terms- round gourd, vegetable, mineral, nutrient composition, ascorbic acid, germplasm

I. Introduction

Praecitrullus fistulosus is herbaceous, annual with pointed, long, creeping, hairy, soft, and five angled stem with coiled long tendrils. Marr et al. (2007) reported cultivation of round and wax gourd from early ages in Asia but it is rarely cultivated in other countries of the world. Asian population consumes cooked round gourd fruits and it is sometimes candied and pickled as well (Sujatha and Seshadri, 1989; Grubben and Denton, 2004). The fruit is a berry, green colored, shape is globate, approximately the size of an apple, and weighs 50-60 grams (Tindall, 1983; Kirtikar and Basu, 1998; Levi et al., 2005; Gautam et al., 2011). The seeds of *Praecitrullus fistulosus* are roasted and eaten as snacks (Chaudhary, 1977; Hopkins and Thompson, 2002), used as fodder, for curing diabetes and many other diseases (Sujatha and Seshadri, 1989; Chadha and Tarsem, 1993). People in some parts of the world consume cooked leaves to control blood pressure (Sultana, 2006).

For identification, classification, and development of remarkable round gourd genotypes, it is mandatory to analyze germplasm for exploration of diversity in nutritional composition and biochemical properties and their transferability to advanced generations because this crop is a typical cultivated cucurbitaceous vegetable in Asia. For the advanced declaration of appropriate breeding strategy, comprehensive knowledge regarding the nature and degree of genetic variation existing in a particular population is extremely important. Numerous vegetable species are collected and grown from the wild to meet the nutritional requirements of the local community as these species have prime value as food and medicine in the lives of the local community. Cucurbits are a well-recognized source of mineral nutrients and secondary metabolites and are an important group of vegetable crops comprising both wild and cultivated species (Whitaker and Davis, 1962). Various plant parts including fruits of Cucurbitaceae have been established for their nutritive and pharmacological potential. In the series of medicinal plants, round gourd is one of the excellent plants, gifted by nature having composition of all the essential constituents that are required for normal and good human health.

Increasing the mineral contents in vegetables through breeding is considered a suitable strategy to combat mineral deficiencies in human populations. Developing countries have been fighting against malnutrition for the past recent years. Various evidences have proposed that nutraceuticals and nutrition-based research have centralized primarily on plant-based products mainly fruits, leaves, seeds, and roots, etc. (Yoshime et al., 2016). Lack of knowledge about proper nutrition, intake of macro and micronutrients, lack of maternal education, poor sanitary situation, unchecked infectious diseases, vaccination, and food insecurity are some of the factors contributing to childhood malnutrition. Among different plant-based food sources in Pakistan, vegetables play a critical role in meeting the nutritional necessities of the local population. People greatly rely on fruits and vegetables as a source of nutrition without knowing their mineral nutrient composition and quantity of nutrients which is leading to malnutrition in Pakistan. Most of the traditional food crops are grown and consumed without considering their nutrient constituents. So far little is known about the mineral nutrients of the commercially grown round gourd and its preserved germplasm in Pakistan.

For the quantification of plant therapeutic agents, information on the chemical constituents of different plant parts is mandatory for discovering potential remedies. For studying the pharmacological potential of phytochemical constituents, screening methods have been employed traditionally. For ages, plants have been playing a critical role in maintaining human health being a source of raw materials in formulating different medicines. In classical therapeutics, 80 % of the global population relies on different plant extracts as a source of folk medicine according to the World Health Organization (WHO) (Singh et al., 2012). Round gourd fruit extract contains diterpenes, phytosterols, flavonoids, alkaloids, resins, saponins, terpenoids, cardiac glycosides, and tannins (Ankita et al., 2012; Karandikar et al., 2014). Shah et al. (2011) reported the anti-inflammatory action of cardiac glycosides, the hemolytic role of saponins was reported by Rao and Sung (1995), and the anti-tumorous action of round gourd fruits was first reported by Nafiu et al. (2011) where saponins help reducing the risk of onset of cancer in humans by terminating the growth of cancer cells and Njoku and Obi (2009) reported the wound healing capability of tannins. The round gourd is traditionally used for curing heart diseases, strokes, controlling blood pressure, cancer, etc. (Archana et al., 2016). The anthelmintic and antioxidant property of round gourd fruit was reported by Gautam and Shivhare (2011) and Dixit and Kar (2010), respectively.

During numerous growth and developmental stages, minerals support different biological processes due to being a necessary portion of plant and human nutrition. To fulfill the dietary needs of humans, a suitable quantity of nutrition can be obtained from plant-based food. Previous studies on the nutrition composition of round gourd presents a detailed analysis report on mineral contents but there is not any information available on comparative mineral nutrient composition analysis of complete round gourd germplasm of Pakistan. This research gap is compelling the local population to nutritionally rely on the available non-approved cultivar of round gourd commercially cultivated. Presented research was conducted to evaluate the round gourd germplasm to assess the diversity in mineral nutrient composition and utilize the accession with the highest nutrient contents in the advanced breeding program to develop a new high-yielding variety with the high mineral nutrients.

II. Materials and methods

This study was conducted at Vegetable Research Area, and Pomology Lab, Institute of Horticultural Sciences (IHS), University of Agriculture, Faisalabad (UAF), to determine biochemical variations in fruits of round gourd accessions. Accessions considered for this study were A20247, A20415, A20532, A20487, A30875, A31228, A20428, A20215, A20453, A20407, A32585, A31225, A19237, A20277, A20305, A20399, A19239, A20194, A20343, A20481, A20385, A20296, A20229, A20463 and A20441. Seeds of these accessions were obtained from the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Raised beds were prepared in the field-sized 10' x 5', where five seeds were sown per hole at a distance of 2' x 2' and were later thinned at 3-4 leaf stage to keep one healthy seedling per hole. Regular cultural practices viz irrigation, weeding, and fertilizer applications were performed as per recommendations of Baloch (1994); Bajwa and Rehman (1998). For the quantification of fruits mineral contents, fruits were harvested from the field in evening at crop marketable and consumable stage. Harvested fruits were immediately taken to the pomology lab for further analysis. The outcome of this study will help in breeding nutritionally better accessions for improving their yield potential and economic returns. Details of studied parameters related to fruit mineral analysis are given below.

Moisture contents (%)

This method relies on measuring the mass of water in a known mass of sample. The moisture contents are determined by measuring mass of food before and after water is removed by evaporation. For estimation of fruit moisture contents, fruits were harvested from field in evening to prevent abrupt moisture loss. After tagging, fruit's fresh weight was immediately taken in field using a digital weighing balance (Hytek SF-400 A). Then fruits were dried in microwave oven (Memmert 100-800) at 72ºC till their constant dry weight was achieved and again weighed for dry weight. Data was computed using the following equation to calculate moisture contents of fruit

$$\%\text{Moisture} = \frac{M_{\text{INITIAL}} - M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100$$

$M_{initial}$ = Mass of sample before drying
M_{dried} = Mass of sample after drying

Nitrogen contents (%)

For estimation of total nitrogen in fruit sample, protocol described by Chapman and Parker (1961) was followed. Digestion combination consisting of ferrous, potassium, and copper sulphates (10:01:0.5) and potent sulfuric acid was utilized for sample digestion. Digestion combination, sulfuric acid, and powdered fruit material (oven dried) @ of 5g, 30 ml, and 1g respectively were added in a Kjeldhal's digestion flask. Gradual heating of the mix was done initially, followed by vigorous heating continuously for three hours till a clear green fluid material was obtained. Methyl red, bromocresol green as indicators, and boric acid 4% and sodium hydroxide 40% were used in distillation of 10 ml aliquot in micro kjeldhal's apparatus. For restoration of original methyl red color, N/10 sulfuric acid was used for titration of distillate. Nitrogen in percentage was calculated and converted in µmol/g from amount of acid consumed in titration employing the below-mentioned formula

$$\text{N\%} = \frac{\text{A} - \text{B x C x 0.0014 x 100}}{\text{D}}$$

Whereas
A: Amount of N/10 H_2SO_4 consumed
B: Blank reading (N/10 H_2SO_4 consumed for blank reading)
C: Final volume after digestion (250 ml)
D: Digested sample volume used
0.0014 Factor (which is equal to g of N in 1ml of N/10 H_2SO_4)

A blank reading is obtained to quantify the exact nitrogen percentage in the sample. Blank samples were processed following the same digestion, distillation, and titration procedures.

Phosphorous contents (mg P/ g)

Protocol for measurement of phosphorus contents in fruit sample involved weighing of 1 gram dried powdered fruit material added in 20 ml of concentrated HNO_3, covered with watch glass, and placed on hot plate with low heat until solid material vanished. Transparent colorless mixture was obtained by cooling the mixture, added 10 ml per-chloric acid, and first gently heated and then vigorously. After reduction of volume to 3ml, the material was shifted in a 100 ml volumetric flask with the aid of filter paper after cooling the sample and making the final volume up to the mark. For quantification of other elements except nitrogen, this filtrate was preserved in plastic bottles. A spectrophotometer was utilized for the quantification of phosphorus (Chapman and Parker, 1961). A 100 ml volumetric flask was taken and below mentioned reagents were used for the development of sample color.

i. Digested fruit sample (5ml)
ii. H_2SO_4 (5ml)
iii. Distilled water (30ml)
iii. 5% ammonium molybdate (5ml)
iv. 0.25% ammonium vandate (5ml)
v. Final volume up to the mark with distilled water

Potassium dihydrogen phosphate was used with predetermined concentrations for creation of a standard curve. An ultraviolet spectrophotometer (Irmeco Germany Version 3.4 a Model: U2020) was used for quantification of the amount of phosphorus in samples at 470 nm wavelength. To determine phosphorus quantity in µmol/g, color intensity was recorded and correlated with the standard curve. The below-mentioned formula was then used to calculate the final phosphorus contents in percentage.

$$P\% = \frac{\text{ppm on graph x dilution}}{1000000} X100$$

Potassium contents (mg/ g)

A flame photometer (Sherwood Flame photometer 410) was employed for quantification of the amount of potassium in samples (Chapman and Parker, 1961). Potassium chloride with predetermined concentrations was used for the creation of a standard curve. The emission of flame photometer for solutions of standards and samples was compared with the standard curve to determine the element concentration in ppm (Hussain, 2014). Obtained values were then computed in percentages using the below-mentioned formula.

$$K\% = \frac{\text{ppm on graph x dilution}}{1000000} X100$$

Protein contents (%)

Protein from fruit samples was determined after the determination of nitrogen from the samples following the protocol described earlier and simply multiplying the nitrogen value with the factor 6.25.

Protein (g) = Nitrogen in food sample x factor 6.25

Ascorbic acid (Vit C) (mg/10 mL)

The estimation of vitamin C contents was performed as previously described by (Bashir et al., 2015), 10 ml of fruit juice from each variety was extracted using a National juicer/blender (Model: MJ-170N Japan). Juice and oxalic acid were added in a 100 ml volumetric flask in the amount of 10 and 90 ml respectively. To get a light pink color, a 5ml aliquot was titrated with 2-6 Dichlorophenol-indophenol (AOAC, 1995).

Calcium contents (mg/ g)

The protocol that was followed for the determination of calcium contents in fruits of 25 round gourd accessions with the aid of a flame photometer (Sherwood Flame photometer 410) was described by Haworth and Cleaver (1961). 1 gram of oven-dried powdered fruit sample was transferred to a 50 ml beaker having 20 ml of concentrated HNO_3, covered with watch glass, and placed on hot plate with low heat until solid material vanished. A transparent colorless mixture was obtained by cooling the mixture, adding 10 ml per-chloric acid, and first gently heated and then vigorously. After reduction of volume to 3ml, the material was shifted in a 100 ml volumetric flask with the aid of filter paper after cooling the sample and made the final volume up to the mark. From 1000 ppm concentration of calcium standard, standards of 100, 75, 50, and 25 ppm were prepared using deionized water as diluent. The blank solution was aspirated and set to zero. Then standards were aspirated into the flame photometer. The standard curve of calcium concentration against intensity was plotted. Then sample solutions were aspirated into flame and recorded the readings. From calibration graph, samples concentrations were determined from recorded reading.

Sodium contents (mg/g)

Following protocol defined by Deal (1954), one-gram dried powder of fruit was covered with a watch glass and placed on hot plate with low heat until the solid material vanished. A transparent colorless mixture was obtained by cooling the mixture, added 10 ml per-chloric acid and first gently heated and then vigorously. After reduction of volume to 3 ml, the material was shifted in a 100 ml volumetric flask with the aid of filter paper after cooling the sample and made the final volume up to the mark. Standard solutions of sodium chloride were prepared by volumetric dilution of the stock solution. The following approximate concentrations were made: 5, 10, 25, 50, 75, and 100 mg/ml as Na. To set readout to zero on flame photometer (Sherwood Flame photometer 410), distilled water was used as a blank. Then emission intensity of each sodium standard solution and sodium unknown solution was measured, and a working curve was plotted from obtained data.

Statistical analysis

Plants were grown in field according to a Randomized Complete Block Design with four replications for each genotype selected. Multivariate analysis of variance (Principal component analysis and Cluster analysis) was performed for grouping round gourd accessions based on similarities in fruit mineral contents using XLSTAT (2018). One-way analysis of variance (ANOVA) was practiced for statistical analysis and significant variations between means were recorded by Duncan's multiple range (DMR) test at P<5% in the quantitative data of mineral parameters. Descriptive statistics (mean, standard deviation, and coefficient of variation), were generated using multivariate procedure. In this procedure, first a similarity matrix was calculated and was used to calculate eigen values and scores for the accessions. Selected 25 round gourd accessions were considered as treatment with four replications.

III. RESULTS

Eight above-mentioned fruit mineral attributes were considered to characterize round gourd germplasm and to assess the nutritional value and divergence of twenty-five accessions for fruit mineral contents. Descriptive statistics of fruit mineral analysis data including minimum, maximum, mean, standard deviation, and coefficient of variation values for observed attributes are presented in Table 1. Results explain high levels of divergence in mineral contents of round gourd germplasm. Among all said accessions, 20248 accession possessed maximum number of mineral nutrients with highest values for moisture contents, protein, nitrogen (N), and phosphorus (P) as 95.67%, 23.75%, 3.80%, and 0.08 mg/g respectively. Among all eight studied minerals, potassium gave maximum value of standard deviation which was 13.62 followed by protein contents with standard deviation value of 4.28 which clearly states that these two variables had maximum observed variability and there was huge difference observed in standard deviation values of these two variables. Contrary to this, lowest value of standard deviation 0.01 was found in mineral phosphorus indicating low levels of variation for this variable, followed by ascorbic acid with standard deviation value of 0.08. Similarly, maximum coefficient of variation value 185.62 was observed in mineral potassium, followed by protein contents with coefficient of variable value of 18.33 indicating greater variation discovered in these variables from all round gourd accessions, whereas calcium ranked lowest with minimum value of coefficient of variation (0.001). Recorded data of moisture contents demonstrated that maximum fruit moisture contents (95.67 %) were recorded from accessions 20428, 31225, 19239 and 20343 while minimum fruit moisture contents which were 92.33 %, recorded from accessions 20247, 32585, 20229, and 20463. Mean computed value for fruit moisture contents was 94.08 % and accessions which behaved moderately were 20415, 20532, 20487, 30875, 31228, 20407, 19237, 20399, 20481 and 20296 containing 94.67 % moisture contents in fruit. Maximum protein contents (23.75 %) were recorded from accession 20428, while minimum quantity of protein was 3.75 %, measured from accessions 20481, 20385 and 20463. Mean computed value for protein contents was 6.58 % and accessions that contained protein contents close to mean value were 31225 and 20194 having 6.88 % protein in their fruit. The maximum amount of nitrogen 3.80 % was recorded from accession 20428 while accessions 20481, 20385 and 20463 contained lowest amount of nitrogen 0.60 % in fruit. Accessions that contained nitrogen close to the average 1.05 % were 20247 and 20532 having nitrogen contents 1.00 %. Amount of phosphorus was maximum (0.08 mg/g) in accession 20428, while it was minimum (0.05 mg/g) in accessions 30875 and 20453. Mean value for phosphorus was 0.06 (mg/g) which was observed in accessions 20532, 20215, 19237, 19239, 20194, 20343 and 20385. Furthermore, maximum amount of potassium was 77.80 (mg/g), recorded from accession 20463, while minimum recorded amount of potassium was 16.30 (mg/g) in accession 20194. The average computed value for potassium was 32.88 (mg/g) and accessions containing moderate amount of potassium (33.1 mg/g) were 20532 and 20343. Maximum quantity of vitamin C (0.50 mg/ 10mL) was quantified from accessions 31228, 20453, 31225, 20481, 20463 and 20441, while minimum quantity (0.20 mg/ 10mL) was quantified from accession 20415. Average value for vitamin C contents was 0.39 (mg/ 10mL) and accessions containing moderate vitamin C were 20532, 20487, 30875, 20428, 20407, 20277, 20305, 20399, 20194, 20343, 20385 and 20229 containing 0.4 (mg/ 10mL) ascorbic acid. Maximum amount of sodium was 3.10 (mg/g), measured from accessions 20215 and 20385, while minimum amount of sodium 2.10 (mg/g) was measured from accessions 20415, 19237, and 20277. Accessions with moderate amounts of sodium were 20247, 20305, and 20229 containing 2.5 (mg/g) sodium which was similar to mean computed value. For calcium, maximum recorded amount was 0.20 (mg/g), and minimum amount recorded amount was 0.10. Accessions which maximum calcium contents were 20481, 20385, and 20463, while the rest of the accessions contained lowest amount of calcium.

Table 1. Descriptive statistics of eight fruit mineral contents of round gourd accessions.

Variables	Minimum	Maximum	Mean	SD	CV
Moisture contents (%)	92.33	95.67	94.08	1.11	1.24
Protein (%)	3.75	23.75	6.58	4.28	18.33
Nitrogen (%)	0.60	3.80	1.05	0.69	0.47
Phosphorous (mg/g)	0.05	0.08	0.06	0.01	6.11
Potassium (mg/g)	16.30	77.80	32.88	13.62	185.62
Ascorbic acid (mg/10 mL)	0.20	0.50	0.39	0.08	0.01
Sodium (mg/g)	2.10	3.10	2.51	0.29	0.08
Calcium (mg/g)	0.10	0.20	0.11	0.03	0.001

Abbreviation: SD = Standard deviation, CV = Coefficient of variation

To estimate correlation matrix among twenty-five round accessions, eight earlier mentioned fruit minerals were selected, and results of correlation analysis are presented in Table 2. Results demonstrated that moisture contents had negative correlation with potassium, ascorbic acid, sodium, and calcium with correlation coefficient values of -0.12, -0.01, -0.20 and -0.22, respectively, while it had a positive correlation with protein contents, nitrogen, and phosphorus with correlation coefficient values of 0.20, 0.20 and 0.004, respectively. Negative correlation of protein contents was observed with potassium, ascorbic acid, sodium, and calcium with correlation coefficient values of -0.04, -0.28, -0.07, and -0.25, respectively, whereas moisture contents, nitrogen, and phosphorus had a positive correlation with protein and correlation coefficient values were 0.20, 1.00 and 0.36, respectively. Negative correlation values of -0.04, -0.28, -0.07, and -0.25 were observed for minerals potassium, ascorbic acid, sodium, and calcium, respectively indicating negative correlation of nitrogen with these minerals while positive correlation values of 0.20, 1.00, and 0.36 for moisture contents, protein and phosphorus suggesting that these minerals are positively correlated with nitrogen contents. Potassium had a positive correlation with four minerals including phosphorus, ascorbic acid, sodium, and calcium with correlation coefficient values of 0.52, 0.26, 0.42, and 0.80, respectively. In contrast, moisture contents, protein, and nitrogen were negatively correlated to potassium with correlation coefficient values of -0.12, -0.04 and -0.04, respectively. Phosphorus had a positive correlation with all mineral contents including moisture contents, protein, nitrogen, potassium, ascorbic acid, sodium, and calcium with correlation coefficient values of 0.004, 0.36, 0.36, 0.52, 0.27, 0.18, and 0.36, respectively. Ascorbic acid was negatively correlated to moisture contents, protein, and nitrogen with correlation coefficient values of -0.01, -0.28, and -0.28, respectively. In contrast, a positive correlation was observed with phosphorus, potassium, sodium, and calcium with correlation coefficient values of 0.27, 0.26, 0.36, and 0.35, respectively. Four minerals were positively correlated to sodium including phosphorus, potassium, ascorbic acid, and calcium with correlation coefficient values of 0.18, 0.42, 0.36, and 0.42, respectively while moisture contents, protein, and nitrogen with correlation coefficient values of -0.20, -0.07 and -0.07, respectively were indicating a negative correlation with sodium. Calcium was negatively correlated to moisture contents, protein, and nitrogen with correlation coefficient values of -0.22, -0.25, and -0.25, while positively correlated to phosphorus, potassium, ascorbic acid, and sodium with correlation coefficient values of 0.35, 0.80, 0.35 and 0.42, respectively.

Table 2. Correlation coefficients among fruit mineral contents of round gourd accessions

Variables	Moisture contents (%)	Protein (%)	Nitrogen (%)	Phosphorou s (mg/g)	Potassium (mg/g)	Ascorbic acid (mg/10 mL)	Sodium (mg/g)	Calcium (mg/g)
Moisture contents (%)	-							
Protein (%)	0.2	-						
Nitrogen (%)	0.2	1	-					
Phosphorous (mg/g)	0.004	0.36	0.36	-				
Potassium (mg/g)	-0.12	-0	-0	0.52	-			
Ascorbic acid (mg/10 mL)	-0.01	-0.3	-0.3	0.27	0.26	-		
Sodium (mg/g)	-0.2	-0.1	-0.1	0.18	0.42	0.36	-	
Calcium (mg/g)	-0.22	-0.3	-0.3	0.35	0.8	0.35	0.42	-

Principle component analysis (PCA) was performed based on eight fruit mineral contents of twenty-five round gourd accessions. Eigen values for each component or factor is presented on a scree plot (Figure 1). Eigen values of first three factors were above 1 and eigen values of first two components were 2.78 and 2.26, respectively and were highest among all three factors depicting high levels of variation encountered by these two components therefore component 1 and 2 from total eight components were analyzed which explained 34.77 % and 28.22 % variability, respectively and cumulative 62.99 % variability. Additionally, principle component analysis explained complete variation in three components rather than eight encountering total 75.52 % variability among all round gourd accessions for all studied mineral contents. Results indicated that first two components explained more than half of total observed variations and were considered important components. In first principle component, which explained 34.77 % variability, moisture contents, protein and nitrogen contributed negatively towards variability with eigen vector values of -0.20, -0.30 and -0.30, respectively, while phosphorus, potassium, ascorbic acid, sodium and calcium contributed positively towards variability with eigen vector values of 0.20, 0.46, 0.37, 0.37 and 0.51, respectively. Second principle component explaining 28.22 % variability contained variable ascorbic acid with eigen vector value -0.001 which contributed negatively towards variability while all other variables contributed positively towards variability.

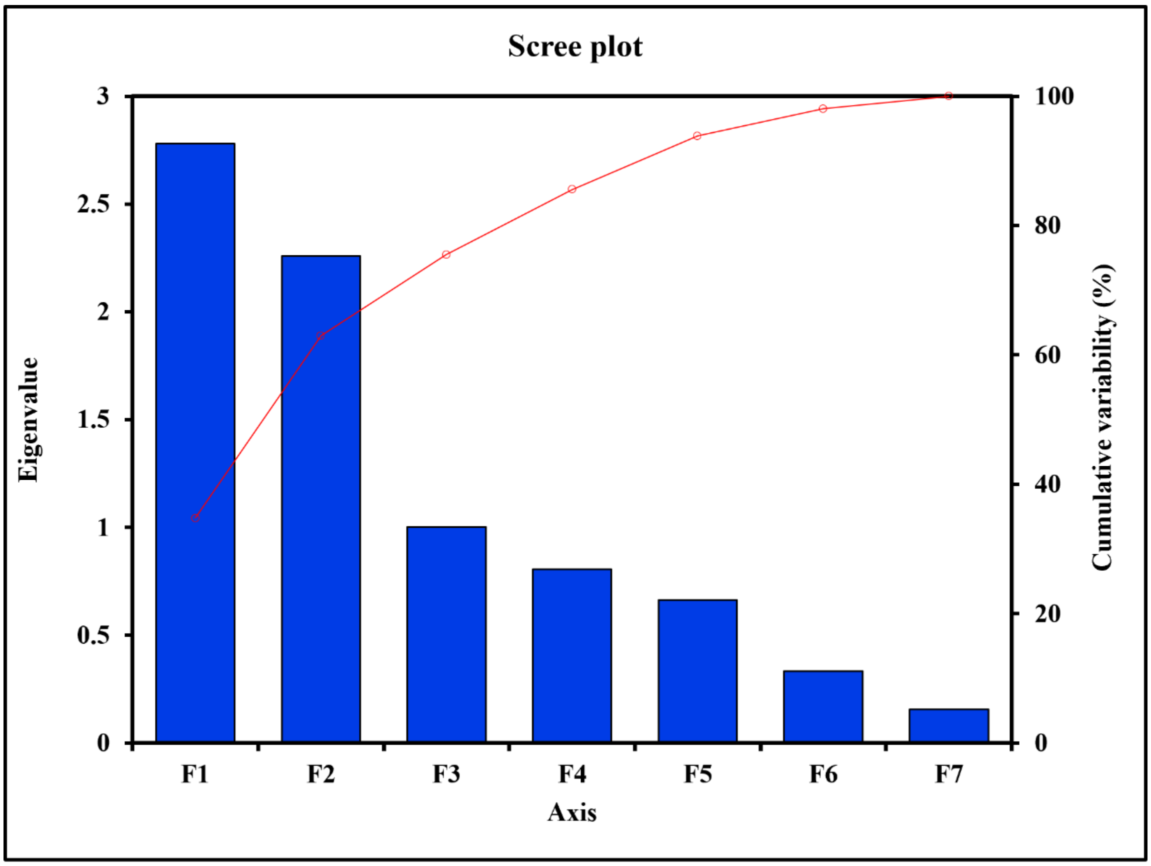

Figure 1: Scree plot showing eigen values of each component for fruit mineral contents of round gourd accessions.

From variables of first two principle components which explained cumulative variability of 62.99 %, a linkage map was constructed among eight mineral contents studied. Two-dimensional plot illustrates that variable like phosphorus, potassium, vitamin C, sodium and calcium had higher and positive correlation values of 0.34, 0.76, 0.61, 0.61 and 0.85, respectively than other variables studied (Figure 2). Results of principle component analysis are presented in a two-dimensional plot (Figure 3) based on first two principle components. All accessions are distributed on entire plot in all four planes. Accession 20428 was entirely different from all other accessions and was placed on extreme left top corner of PCA graph depicting high variation for all fruit mineral contents in this accession. Accessions 20463, 20453, 20215 and 20296 were entirely different and distinguished from other accessions and were placed in different planes of plot due to being distant in relatedness, whereas, accessions 20385, 20481, 20441 and 31225 also showed greater diversity but were placed close to one another probably due to close relatedness. Contrary to that, accessions 20305, 20277 and 30875 showed lower levels of diversity in fruit mineral contents and were placed closely in the same plane of 2D plot possibly due to being not diverse from one another. Similarly, accessions 20194, 19239, 20532 and 20343 were also placed close to one another in the same plane of 2D plot due to possessing lower levels of diversity in fruit mineral contents.

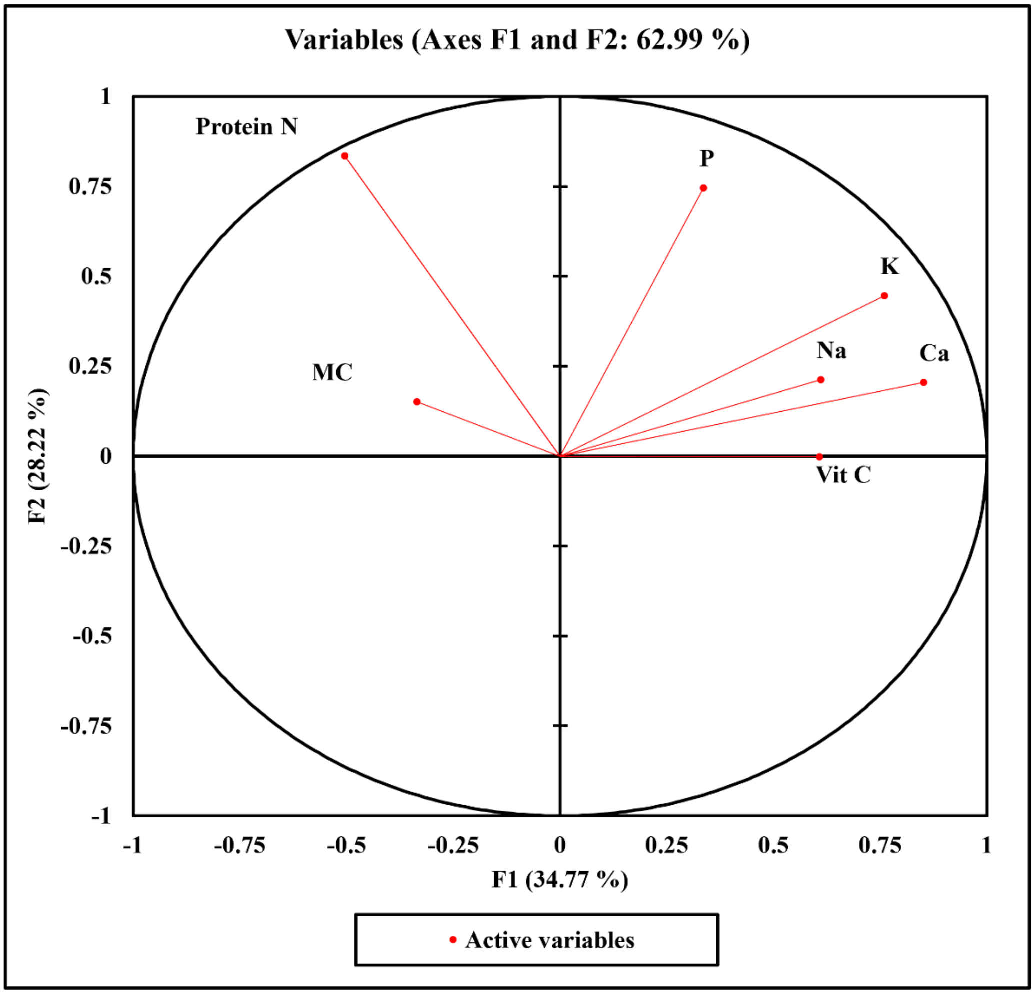

Figure 2. Projection of fruit mineral content variables based on component 1 and 2 factors showing the relationship among round gourd accessions.

Abbreviations: MC; Moisture contents, N; Nitrogen, P; Phosphorus, K; Potassium, Vit C; Vitamin C, Na; Sodium, Ca; Calcium

Table 3. The first two principle components (PC) of the 8 mineral nutrients

Variable	PC 1	PC 2
Moisture contents (%)	-0.201	0.101
Protein (%)	-0.303	0.555
Nitrogen (%)	-0.303	0.555
Phosphorous (mg/g)	0.202	0.496
Potassium (mg/g)	0.455	0.297
Ascorbic acid (mg/10 mL)	0.365	-0.001
Sodium (mg/g)	0.366	0.142
Calcium (mg/g)	0.511	0.136
Eigen value	2.781	2.258
Variability	34.77	28.22
Cumulative	34.77	62.99

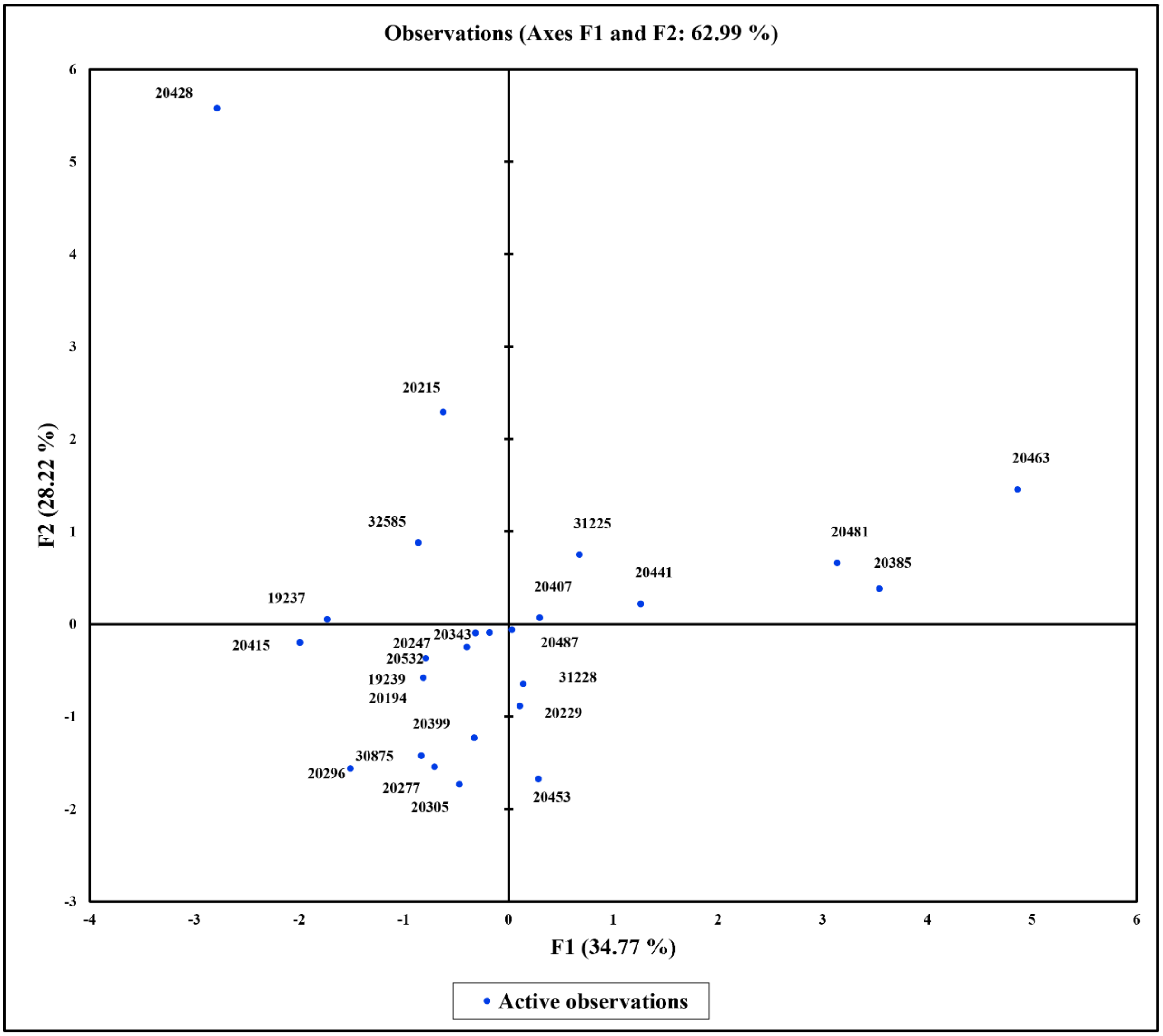

Figure 3. Two dimensional PCA plot based on first two component factors showing relationship among twenty-five round gourd accessions.

Cluster analysis for fruit mineral contents of round gourd accessions

Agglomerative hierarchical clustering (AHC) was done to assess variation in fruit mineral contents of round gourd germplasm considering twenty-five accessions. A dendrogram was constructed which is presented in Figure 4. Cluster analysis categorized variability as within class and between classes and explained 18.58 % variability in within class and 81.42 % variability between classes. All studied accessions were clustered in eight different classes. Class V, VI and VIII contained one accession each in the order 20428, 20215 and 20463, respectively. Class III ranked top with maximum number of accessions and highly genetically diverse class containing accessions 20532, 20487, 31228, 20407, 31225, 19239, 20343 and 20441 having within class variance value of 31.72. Class IV ranked second containing accessions 30875, 20453, 20277, 20305 and 20399 having within class variance value 14.66. Furthermore, class I contained accessions 20247, 32585, 20194 and 20229 having within class variance value of 54.10. Class II contained accessions 20415, 19237 and 20296 having highest within class variance value of 96.12 whereas class VII contained only two accessions 20481 and 20385 having within class variance value 14.55. Class III due to being largest class was further sub-divided into three sub-classes in which first sub-division contained accessions 19239, 20532, 20407 and 20343, while second sub-division contained accessions 20487 and 31228 and third sub-division was comprised of accessions 31225 and 20441. Accessions 19239 and 20441 from Class II were distant from one another due to being diverse in their mineral contents, whereas, accession 20532 was closest to accession 20407 in their fruit mineral contents and had lowest genetic distance. Similarly, in class I accession 32585 was at lower distance from accession 20194 depicting close relatedness with one another. Contrary to this, accession 20428 was at maximum distance from 20441 due to least relatedness depicting maximum diversity existing between these accessions.

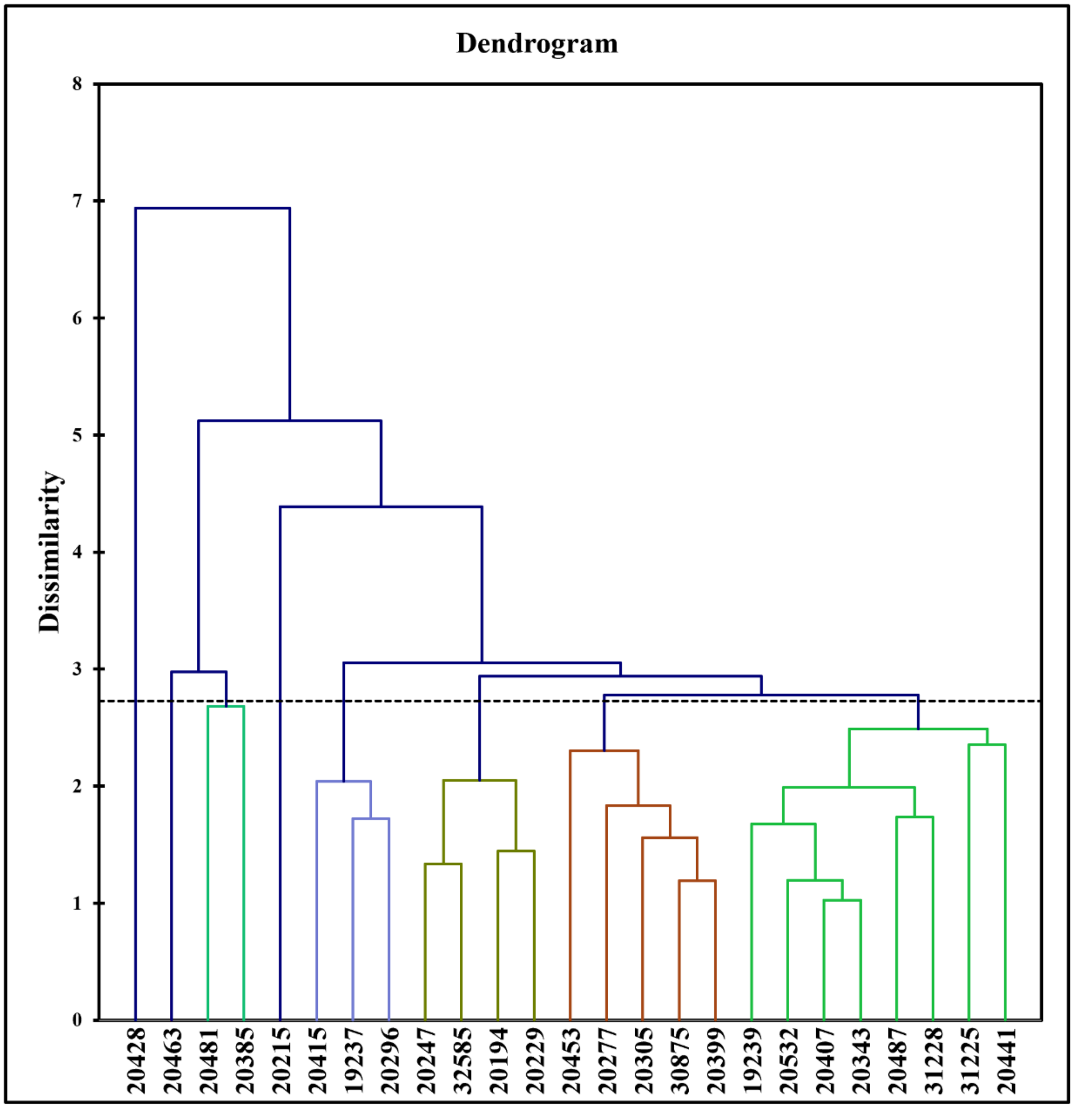

Figure 4. Dendrogram explaining fruit mineral contents diversity in twenty-five round gourd accessions.

IV. Discussion

Health and diseases are directly related to nutrition which is familiar as science of food, which guides us about the action and interaction of nutrients and additional substances (Clamp, 2007). Water, minerals, vitamins, proteins, lipids, and carbohydrates are the six major classifications of nutrients. Major operations of the above-mentioned nutrients are the governance and command of body processes, constructing body structural materials, and provision of energy (body fuel) (Kcal) (Akinwande, 1999). Living organisms have distinctive life cycle stages for which they require different micro and macronutrients including molybdenum, boron, cobalt, copper, manganese, zinc, iron, sodium, sulfur, magnesium, calcium, potassium, phosphorous and nitrogen to govern different biological processes. Hidden hunger is the term used for billions of people living in underdeveloped countries which are malnourished due to unavailability of micronutrients in their diet.

Round gourd fruit minerals contents per 100 g contains water, protein, fat, carbohydrate, fiber, calcium, iron, phosphorous, magnesium, carotene, thiamin, riboflavin, niacin, ascorbic acid, sodium, potassium, copper, vitamin A, chlorine, nicotinic acid and oxalic acid at the amount of 93.5 g, 1.4 g, 0.2 g, 3.6 g, 1.6 g, 21-25 mg, 0.9 mg, 24 mg, 14 mg, 13 μg, 0.04 mg, 0.08 mg, 0.3 mg, 18 mg, 35 mg, 24 mg, 0.12 mg, 23 I.U., 44 mg, 0.3 mg and 2 mg respectively (Chaudhary, 1977; Holland et al., 1991, Antonious and Kochhar, 2009; Hussain et al., 2010). Limited studies on horticultural traits inheritance in round gourds have been documented (Khan et al., 2001; Munawar et al., 2015) which is not sufficient information whereas available information regarding antioxidant activity and genetic variability in mineral composition of round gourds is limited (Hussain et al., 2010; Gautam and Shivhare, 2011; Tyagi et al., 2014; Mehreen, 2015).

The nutritional significance of fruits and vegetables is assessed by considering nutrients and proximate factors that play a prime role in germplasm evaluation (Pandey et al., 2006). The concentration of biochemical contents and/or proportion of other variants is obtained by proximate analysis. The potential of fruit species to encounter deficiencies of micronutrients like minerals and vitamins, food nutrition and security, and prime role in enhancing incomes of people has enhanced the wealth of agricultural biodiversity. Numerous clinical investigations and epidemiological examinations have demonstrated that possibility of onset of chronic pathological diseases like different cancer types, diabetes, and cardiovascular diseases reduces with intake of high dietary fruits and vegetables due to containing many bioactive phytochemicals and antioxidants like vitamin C (Liu, 2003; Arts and Hollman, 2005; Erdman et al., 2007; Henriquez et al., 2012). Prevention from such chronic diseases is only possible with the consumption of at least four hundred grams of fruits and vegetables daily eliminating tubers (cassava and potato) (WHO, 2003). Due to heavy prices of generally consumed food commodities and loss of access to many native fruits has forced developing populations to consume limited number of crops to fulfil their dietary needs which is considered as the reason behind decreased intake of vegetables and fruits. Many species among the gourds and other cucurbits are still unidentified, underutilized, and neglected and globally few species could get significantly commercialized (FAO, 1997).

Agroclimatic circumstances, variety and soil physical and chemical characteristics existing in a specified place collectively determine the deposition of nutrients in fruits. Bitter gourd germplasm was analyzed for fruit mineral content diversity and found significant differences in mineral quantities across the entire collection. Maximum folic acid, ascorbic acid, moisture and protein with observed values 0.10 µg/ml, 98.2 mg, 90.40 % and 2.06 g, respectively was observed from cultivar light green big. Light green small cultivar contained maximum amount of fiber content and carbohydrate as 1.21 g and 8.22 g, respectively, whereas, light green big contained maximum amount of sodium, phosphorus, and calcium as 20.12 mg/100 g, 79.64 mg and 25.44 mg/100 g respectively. Nei paval cultivar possessed maximum quantity of iron and potassium as 2.14 mg and 174.46 mg, respectively (Krishnendu and Nandini, 2016). Various cucurbits were analyzed for differences in their fruit mineral contents conducting a proximate analysis. Results demonstrated that maximum crude protein ranging between 1.87-7.88 g/100g and calcium 99.4 mg/kg was quantified from ash gourd, whereas cucumber contained maximum quantity of carbohydrates and sodium as 76.12 g/100g and 1046 mg/kg, respectively. Oriental gourd possessed maximum amount of manganese and iron as 1.7 mg/kg and 6.5 mg/kg, respectively. Cucumber and ash gourd contained variable quantity of ascorbic acid ranging between 21.82 – 60 mg/100 g, whereas ash gourd possessed the minimum value of energy as 977.69 kJ/100 g (Bello et al., 2014). Ridge gourd fruits were analyzed for variation in phytochemical and antioxidants contents and observed variability in studied contents. Values on fresh weight basis for moisture content, vitamin C, flavonoid, phenolics, soluble sugar, protein, carotenoids, and chlorophyll contents were variable as 90.47-92.78 %, 8.64-14.13 mg/100 g, 0.77-1.59 mg/g, 0.416-0.742 mg/100 g, 1.21-1.58 %, 0.175-0.253 %, 14.5-36.1 mg/100 g and 1.59-1.85 mg/g, respectively (Kandoliya et al., 2016). Various gourds were analyzed for fruit moisture contents among which *C. maxima* and *C. moschata* contained maximum moisture in the fruits (Fedha et al., 2010). In previously discussed results, almost all studies presented high water contents in fruits of gourds typically at the maturity stage of fruit (Umoh, 1998). It is a well understood phenomena that more the moisture in fruits, lesser will be the yield of dry matter as water contents of fruits have linear relationship with dry matter contents. Due to bearing small calorific value and raised moisture contents in brinjal, bottle, sponge and ridge gourds, these vegetables are considered as diet food vegetables.

Almost all metabolic activities are driven by the moisture available from food which is a crucial factor in retaining water level in body (Kandoliya et al., 2016). Human disorders and diseases are protected or treated with the intake of fiber food which is declared as bioactive composites and nutraceuticals having practical effects as in promotion of physiological processes in human body (Wildman, 2001). Prevention from colorectal cancer and constipation like disorders, improvement in antioxidants based defensive system, raised minerals absorption, decreased synthesis and absorption of cholesterol, enhanced bowel motions and fermentation in digestive path are all driven with the intake of fibrous food (Anderson et al., 2009). To increase the nutritional value and dietary fiber contents of food commodities, pulps of fruits can be added as ingredients of foods into various generally utilized food commodities such as breads. Diets consumed for weight reduction usually contains fruits because of containing reduced fat contents. Many complications regarding health arises due to consumption of such diets which contains high fat contents. Therefore, people looking for low fat diet must consume gourd fruits as bottle gourds contain higher amount of protein (0.2 %) when compared with baobab fruit pulp (Osman, 2004). All living organisms require protein as fundamental dietary component for their survival and the elementary function of proteins is to provide amino acids in an appropriate quantity according to body requirements (Pugalenthal et al., 2004). Body maintenance and repair (restoration of wear and teared tissues) and growth is controlled by the production of different hormones determined by protein contents in fruits as demonstrated in a study conducted on ridge gourd (Kandoliya et al., 2016). Accumulation of fluids in body, abnormal inflammation on the belly, edema, wastage of muscles and retardation of growth are some of the health-related problems arises due to the deficiency of protein in body (Perkins-veazie et al., 2005). Thirty-five grams of protein is required by an adult on daily basis weighing 70 kgs which can be obtained by consuming 19 g, 5 g, 16 g and 15 g of oriental gourd, ash gourd, marrow and cucumber, respectively to meet the least required amount of protein (Fagbemi and Oshodi, 1991). To avoid raising of blood pressure, diet with low sodium quantity must be consumed (Lichtenstein et al., 2006). Round gourd fruits contained satisfactory amount of calcium as well whose deficiency causes under-development of bones (Nelson et al., 2005; Helena, 2008). In a particular cell, transmission of nerve impulse and fluid balance is controlled by the concentration of potassium (Whitney and Rolfe, 2005). Scurvy can be avoided by the intake of vitamin C on daily basis in small quantities because it is extremely useful as an antioxidant. Vitamin C even in minute quantities protect DNA, RNA (nucleic acids), carbohydrates, lipids, and proteins from injuries caused by reactive oxygen species (ROS) and free radicals which are the results of plants vulnerability to pollutants and toxins and even during processes of normal metabolism (Caunii et al., 2010).

Concluding the presented study, the investigation of fruit mineral contents of round gourd indigenous germplasm revealed a significant variation among all studied accessions. 20248 accession contained the maximum number of mineral nutrients with the highest values for moisture contents, protein, nitrogen (N), and phosphorus (P) as 95.67%, 23.75%, 3.80%, and 0.08 mg/g respectively making it a valuable genetic resource to utilize in the breeding program to enhance its yield potential. Variations detected in fruit mineral contents across the studied germplasm give an idea to utilize accession(s) holding the maximum amount of nutrients or nutrients of interest to be advanced in the breeding programs for hybrid development. For efficient utilization of the round gourd indigenous germplasm, further investigations on phytochemical screening, antioxidant potential, and estimation of fruit's medicinal values are needed to make it a more valuable vegetable crop to be utilized as a healthy source of food for the local community. In conclusion, it is proposed that a huge asset of promising genetic material from diversified regions of Pakistan still seeks identification, evaluations, usage, and conservation to protect round gourd indigenous germplasm from the adverse effects of urbanization and/or domestication. The discoveries of this research should be beneficial for additional exploration and cultivation of this cucurbit crop.

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Declaration of interest statement

The authors report there are no competing interests to declare.

Data Availability

The data will be available on request.

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