RESEARCH ARTICLE

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Genetic Diversity Analysis by D^2 Clustering for Mineral Nutrient Composition of Watermelon Genotypes (*Citrullus* sp.)

Koushik Saha#, Jayanta Jamatia#, Harshawardhan Choudhary, Vinod Kumar Sharma

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ABSTRACT

Background: Watermelon is an important cucurbit with rich genetic diversity. A great proportion of this diversity remains largely unexploited in terms of mineral nutrient composition.

Methods: The contents of eight different mineral nutrients were determined in eighty diverse genotypes. D^2 statistics and principal component analysis were used to cluster genotypes and understand the underlying variations in mineral nutrient compositions of watermelon genotypes.

Result: Large variations in the content of many mineral contents such as Na, K and Zn were recorded. D^2 analysis placed the eighty watermelon genotypes into 8 distinct clusters. Cluster I comprised of maximum number of genotypes (68) followed by Cluster II (6). All other 6 clusters consisted of 1 genotype each. Among 8 clusters, cluster II showed highest intra cluster distance (342.14) followed by cluster I (279.07), whereas minimum intra cluster distance recorded in cluster III, cluster IV, cluster V, cluster VI, cluster VII and cluster VIII (0.00). Based on inter-cluster distance the maximum diversity was observed between clusters VIII and VI (3234.85). D^2 statistics showed that potassium content contributed highest (25%) to divergence followed by magnesium content (22.7%) and manganese content (11%). Principal component analysis revealed that the first two principal components (PCs) together controlled 52.92% of total variability. Based on higher genetic distance among clusters and higher mean value of genotypes for nutritional traits, DWM 164, DWM 129, DWM 165, DWM 115, DWM 142, DWM 117, DWM 45, DWM 196 and DWM 12 could be exploited in breeding programme as potential donors for developing nutrient- rich watermelon varieties/hybrids.

Key words: Clusters, D^2 statistics, Genetic diversity, PCA, Watermelon.

INTRODUCTION

Watermelon [*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. and Nakai] is a major cucurbitaceous vegetable rich in many health benefitting compounds including citrulline, lycopene, arginine and glutathione (Ren *et al*., 2012). Globally it is being grown over in about 100 countries which accounts for 7 per cent of the total area under vegetable crops. Watermelon is also excellent source of beta-carotene and vitamin C, while the seeds are high in vitamin E and antioxidants, minerals like zinc and selenium. Its fruits are more diverse in size, shape, rind thickness, rind colour, rind pattern, flesh colour, sugar content, carotenoid, flavonoid, mineral and nutrient composition. The genus *Citrullus* is comprised of four species (*C. lanatus*, *C. ecirrhosus*, *C. colocynthis* and *C. rehmii*) (Levi *et al*., 2001). *Citrullus colocynthis* (L.) a wild perennial species growing extensively in Northern Africa and South Western Asia. *Citrullus colocynthis* is considered as the putative ancestral or progenitor species of watermelon, which is commonly grown in north western parts of India (Jarret *et al*.,1997).

Presently, there is a worldwide interest in improving the nutrition content of fruits and vegetables. Although watermelon fruits have been improved for yield, sugar content, disease and pest resistance, efforts to improve the mineral nutrition content is limited, which requires identification of elite parents from diverse groups. Multivariate analysis techniques such as D^2 statistics and principal component analysis allow selection of elite genotypes from a group of diverse germplasm (Nalla *et al.*, 2014). The present study, genetic diversity of different watermelon genotypes were studied in terms of some mineral nutrient contents. Elite genotypes identified here may be used for improving the nutrition content of watermelon varieties.

MATERIALS AND METHODS

Experimental details

The study was conducted at the Research Farm of the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, in the 2017 *spring summer* and *kharif* seasons using eighty watermelon genotypes. The

Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, Delhi, India.

#These author contributed equally to this work.

Corresponding Author: Harshawardhan Choudhary, Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, Delhi, India. Email: harshahit2001@yahoo.co.in

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genotypes are procured from the United States Department of Agriculture and maintained in the division of vegetable science. The list of genotypes used in this study is mentioned in Table 1. The experimental site is located at an elevation of 228.61 metres above mean sea level (28°08'N, 77°12'E). The nursery was grown in polythene bags in poly house and 30 days old seedlings were transplanted. The plants were transplanted on raised beds of 2.5 m apart with 0.75 m spacing between the plants. The crop was grown following recommended agronomic practices along with adopting necessary plant protections measures.

Estimations of mineral nutrient contents

Fruit of each genotype in replicated trial were harvested at fresh marketable stage. For estimating mineral elements, 100 g of finely chopped fruit was oven dried at 60-65°C followed by grinding in a pestle-mortar and sieving using a 1 mm sieve. The dried sieved fruit powder was stored in an airtight aluminum container. Mineral nutrients were determined by digesting oven dried samples (1 g) overnight in a diacid solution of perchloric acid and nitric acid in a 1: 1 ratio and then heating to 200°C for 2-3 hours until the solution became colourless (Singh *et al*., 1999). The solution was filtered through Whatman No. 42 filter paper and the final volume was made up to 100 ml using double distilled water. On an Atomic Absorption Spectrophotometer (AAS-4141), the filtrate was used to determine the concentrations of Zn, Mn, Cu and Fe at wavelengths of 213.9 nm, 279.8 nm, 324.8 nm and 248.3 nm, respectively. The concentrations of K and Na were determined at 766.5 and 622 nm wavelengths respectively using a Flame photometer (ELICO CL-361). Ca and Mg contents were determined by EDTA titration method (Tandon, 1998).

Statistical analysis

The statistical analyses like ANOVA, principal component analysis (PCA) were carried out by using INDOSTAT software package Version 8.1, developed in the year 2010. Genetic diversity in the collection was assessed by determining Mahalanobis (1936) D^2 statistics. The sample similarities were calculated on the basis of pair-wise Euclidean distance (Rao, 1952).

RESULTS AND DISCUSSION

Analysis of variance indicated substantial amount of genetic diversity for the mineral nutrients (n=8) in the genetic materials used for this study. The range of variation was very high for many mineral contents such as Zn (0.03 to 18.11 mg/100 g), K (369.93 to 8326.2 mg/100 g) and Na (38.00 to 728.17 mg/100 g) reflecting high selection prospects for these traits to improve the performance through breeding programme (Data not presented). For an initial of successful breeding programme, it is desirable to select genetically divergent suitable parents based on information about the genetic variability and genetic diversity present in the available germplasm. D^2 statistics showed that potassium content contributed highest (25%) to divergence followed by magnesium content (22.7%) and manganese content (11%) (Table 2). Eighty watermelon genotypes were classified into 8 distinct clusters using D^2 statistics based on mineral nutrient contents. Among 8 clusters (Table 3 and Fig 1), cluster I comprised of maximum number of genotypes (68) and except few (13) all of them belong to *lanatus* group. Cluster II consist of 6 genotypes and except one (DWM 39) all of them belong to *lanatus* group. All other 6 clusters consisted of 1 genotype in each. Among 8 clusters (Table 4), cluster II showed highest intra cluster distance (342.14) followed by cluster I (279.07), whereas minimum intra cluster distance recorded in cluster III, cluster IV, cluster V, cluster VI, cluster VII and cluster VIII (0.00). Based on inter-cluster distance the maximum diversity was observed between clusters VI and VIII (3234.85), followed by clusters II and VIII (1347.37) and clusters III and VIII (983.62), suggesting wide divergence between these clusters. The genotypes of cluster III (Table 5) recorded maximum sodium (728.17 mg/100 g) and potassium (8326.20 mg/100 g), while cluster VII recorded minimum sodium (108.10 mg/100 g) and potassium (1159.80 mg/100 g). Cluster VI recorded maximum zinc (18.11 mg/100 g), while cluster IV recorded minimum zinc (0.04 mg/100 g); cluster VIII recorded maximum manganese (0.15 mg/100 g) and copper (0.07 mg/100 g), while cluster III recorded minimum manganese (0.01 mg/100 g) and copper (0.01 mg/100 g); the higher iron was found in cluster IV (2.34 mg/100 g), while it was lower in cluster I (0.88 mg/100g). Cluster VII recorded the maximum magnesium content (4.47 mg/100 g), while cluster III recorded minimum magnesium content (1.23 mg/100 g). The higher and lower calcium content were found in cluster V (19.92 mg/100 g) and cluster I (10.19 mg/100 g), respectively. Genotypes within the same clusters were more similar than those between clusters. Although grouping of watermelon genotypes on the basis of yield traits have been reported by Choudhary *et al*., 2012, Gbotto *et al*., 2016 and Singh *et al*., 2017, no studies considered grouping based on mineral nutrient contents. However, genetic diversity of muskmelon germplasm for nutrient composition has been reported by Bhimappa *et al*., 2018. In this study, we found that no clusters were superior in terms of all the traits, which indicates wide divergence of the traits among the studied genotype. Similar findings were reported by Bhimappa *et al*., 2018.

Principal component analysis revealed that the first two principal components (PCs) together controlled 52.92 % of total variability (Table 6 and Fig 2). PC1 and PC2 individually explained about 31.32% and 21.60% of the total variance, respectively. This result is similar to the findings of Bhimappa *et al.* (2018), in which first two PCs explained cumulative 48 percent of total variations for mineral nutrient contents in muskmelon. PC1 showed positive factor loading for manganese (0.55), copper (0.54), magnesium (0.36), iron

Table 1: List of watermelon genotypes studied.

Sl.no	Genotypes	Horticultural group	Sl.no	Genotypes	Horticultural group	Sl.no	Genotypes	Horticultural group
1	DWM 4	*Citrullus lanatus* var. *citroides*	28	DWM 56	*Citrullus lanatus* var. *lanatus*	55	DWM 122	*Citrullus lanatus* var. *lanatus*
2	DWM 7	*Citrullus lanatus* var. *lanatus*	29	DWM 59	*Citrullus lanatus* var. *lanatus*	56	DWM 129	*Citrullus lanatus* var. *lanatus*
3	DWM 8	*Citrullus lanatus* var. *lanatus*	30	DWM 61	*Citrullus lanatus* var. *lanatus*	57	DWM 134	*Citrullus lanatus* var. *lanatus*
4	DWM 9	*Citrullus lanatus* var. *lanatus*	31	DWM 62	*Citrullus lanatus* var. *lanatus*	58	DWM 136	*Citrullus lanatus* var. *lanatus*
5	DWM 10	*Citrullus lanatus* var. *lanatus*	32	DWM 63	*Citrullus lanatus* var. *lanatus*	59	DWM 142	*Citrullus lanatus* var. *lanatus*
6	DWM 12	*Citrullus lanatus* var. *lanatus*	33	DWM 64	*Citrullus lanatus* var. *lanatus*	60	DWM 143	*Citrullus lanatus* var. *lanatus*
7	DWM 13	*Citrullus lanatus* var. *lanatus*	34	DWM 65	*Citrullus lanatus* var. *lanatus*	61	DWM 152	*Citrullus lanatus* var. *lanatus*
8	DWM 15	*Citrullus lanatus* var. *lanatus*	35	DWM 66	*Citrullus lanatus* var. *lanatus*	62	DWM 162	*Citrullus lanatus* var. *lanatus*
9	DWM 16	*Citrullus lanatus* var. *lanatus*	36	DWM 67	*Citrullus lanatus* var. *lanatus*	63	DWM 164	*Citrullus lanatus* var. *lanatus*
10	DWM 25	*Citrullus lanatus* var. *lanatus*	37	DWM 68	*Citrullus lanatus* var. *lanatus*	64	DWM 165	*Citrullus lanatus* var. *lanatus*
11	DWM 26	*Citrullus lanatus* var. *citroides*	38	DWM 70	*Citrullus lanatus* var. *lanatus*	65	DWM 169	*Citrullus lanatus* var. *lanatus*
12	DWM 27	*Citrullus lanatus* var. *citroides*	39	DWM 76	*Citrullus lanatus* var. *lanatus*	66	DWM 171	*Citrullus lanatus* var. *lanatus*
13	DWM 28	*Citrullus lanatus* var. *citroides*	40	DWM 77	*Citrullus lanatus* var. *lanatus*	67	DWM 174	*Citrullus lanatus* var. *lanatus*
14	DWM 30	*Citrullus lanatus* var. *citroides*	41	DWM 90	*Citrullus lanatus* var. *lanatus*	68	DWM 176	*Citrullus lanatus* var. *lanatus*
15	DWM 32	*Citrullus lanatus* var. *citroides*	42	DWM 98	*Citrullus lanatus* var. *lanatus*	69	DWM 178	*Citrullus lanatus* var. *lanatus*
16	DWM 34	*Citrullus lanatus* var. *citroides*	43	DWM 99	*Citrullus lanatus* var. *lanatus*	70	DWM 184	*Citrullus lanatus* var. *lanatus*
17	DWM 35	*Citrullus lanatus* var. *citroides*	44	DWM 100	*Citrullus lanatus* var. *lanatus*	71	DWM 189	*Citrullus lanatus* var. *lanatus*
18	DWM 36	*Citrullus lanatus* var. *citroides*	45	DWM 102	*Citrullus lanatus* var. *lanatus*	72	DWM 195	*Citrullus lanatus* var. *lanatus*
19	DWM 39	*Citrullus lanatus* var. *citroides*	46	DWM 108	*Citrullus lanatus* var. *lanatus*	73	DWM 196	*Citrullus lanatus* var. *lanatus*
20	DWM 40	*Citrullus lanatus* var. *citroides*	47	DWM 109	*Citrullus lanatus* var. *lanatus*	74	DWM 197	*Citrullus lanatus* var. *lanatus*
21	DWM 41	*Citrullus lanatus* var. *citroides*	48	DWM 112	*Citrullus lanatus* var. *lanatus*	75	DWM 201	*Citrullus lanatus* var. *lanatus*
22	DWM 43	*Citrullus lanatus* var. *citroides*	49	DWM 113	*Citrullus lanatus* var. *lanatus*	76	DWM 204	*Citrullus lanatus* var. *lanatus*
23	DWM 45	*Citrullus lanatus* var. *lanatus*	50	DWM 114	*Citrullus lanatus* var. *lanatus*	77	DWM 203	*Citrullus lanatus* var. *lanatus*
24	DWM 46	*Citrullus lanatus* var. *lanatus*	51	DWM 115	*Citrullus lanatus* var. *lanatus*	78	DWM 208	*Citrullus lanatus* var. *lanatus*
25	DWM 50	*Citrullus lanatus* var. *lanatus*	52	DWM 116	*Citrullus lanatus* var. *lanatus*	79	DWM210	*Citrullus colocynthis*
26	DWM 51	*Citrullus lanatus* var. *lanatus*	53	DWM 117	*Citrullus lanatus* var. *lanatus*	80	Sugar Baby	*Citrullus lanatus* var. *lanatus*
27	DWM 55	*Citrullus lanatus* var. *lanatus*	54	DWM 121	*Citrullus lanatus* var. *lanatus*			

(0.34), calcium (0.29) and zinc (0.21). PC2 showed highest positive factor loading for potassium (0.70) and sodium (0.69). Therefore, manganese, copper, magnesium and iron contents contributed largely to the variation present in the studied genotypes. On the basis of nutritional attributes, 6 genotypes *viz*; DWM 164, DWM 129, DWM 165, DWM 115, DWM 12 and DWM 45 were identified as superior. Based on higher genetic distance among clusters and higher mean value of genotypes for nutritional traits, DWM 164, DWM 129, DWM 165, DWM 115, DWM 142, DWM 117, DWM 45, DWM 196 and DWM 12 may be utilized in hybridization programme for developing nutrient rich watermelon varieties/ hybrids.

Table 2: Contribution of different mineral nutrients towards genetic diversity of watermelon.

Traits	Contribution (%)	Number of first rank
Sodium (mg/100 g)	4.3	136
Potassium (mg/100 g)	25	791
Zinc (mg/100 g)	7	222
Manganese (mg/100 g)	11	348
Copper (mg/100 g)	9	285
Iron (mg/100 g)	10	316
Magnesium (mg/100 g)	22.7	718
Calcium (mg/100 g)	10.5	348

Table 3: Clustering pattern of eighty watermelon genotypes based on eight mineral nutrients.

Clusters	Number of genotypes	Genotypes
I	68	DWM 30, DWM 51, DWM 122, DWM 27,DWM 102, DWM 25, DWM 8, DWM 43, DWM 169, DWM 164, DWM 67, DWM 9,DWM 178, DWM 65, DWM 121, DWM 61, DWM 99, DWM 184, DWM 50, DWM 10, DWM 136, DWM 13, DWM 32, DWM 56, DWM 203, DWM 114, DWM 165, DWM 35, DWM 26, DWM 129, DWM 115, DWM 68, DWM 66, DWM 55, DWM 4, DWM 16, DWM 174, DWM 98, DWM 176, DWM 162, DWM 197, DWM 171, DWM 34, DWM 134, DWM 100, DWM 70, DWM 116, DWM 40, DWM 109, DWM 7, DWM 77, DWM 59, DWM 62, DWM 113, DWM 36, DWM 28, DWM 195, DWM 204, DWM 64, DWM 189, DWM 201, DWM 152, DWM 76, Sugar Baby, DWM 210, DWM 41, DWM 63, DWM 208
II	6	DWM 15, DWM 90, DWM 46, DWM 112, DWM 108, DWM 39
III	1	DWM 142
IV	1	DWM 196
V	1	DWM 45
VI	1	DWM 117
VII	1	DWM 12
VIII	1	DWM 143

Table 4: Intra (bold face) and inter-cluster distance (D^2) among eighty watermelon genotypes.

Clusters	I	II	III	IV	V	VI	VII	VIII
I	**279.07**	1499.25	714.06	563.20	628.04	3689.33	590.60	568.29
II		**342.14**	2191.77	2418.20	1154.74	777.63	2027.47	1347.37
III			**0.00**	949.59	1305.37	4745.00	1242.98	983.62
IV				**0.00**	1072.47	5052.36	237.27	718.01
V					**0.00**	2831.07	756.74	405.93
VI						**0.00**	4249.16	3234.85
VII							**0.00**	542.37
VIII								**0.00**

Table 5: Cluster means of eight mineral nutrients in watermelon genotypes.

Clusters	Sodium (mg/100 g)	Potassium (mg/100 g)	Zinc (mg/100 g)	Manganese (mg/100 g)	Copper (mg/100 g)	Iron (mg/100 g)	Magnesium (mg/100 g)	Calcium (mg/100 g)
I	195.90	2625.63	2.69	0.02	0.02	0.88	1.61	10.19
II	258.79	3139.50	11.65	0.03	0.02	1.49	1.55	10.80
III	728.17	8326.20	0.54	0.01	0.01	1.15	1.23	10.46
IV	199.13	1878.77	0.04	0.03	0.02	2.34	3.63	10.43
V	162.80	1212.07	4.85	0.03	0.01	1.01	1.68	19.92
VI	220.47	2528.07	18.11	0.04	0.03	1.49	1.94	11.02
VII	108.10	1159.80	1.83	0.03	0.03	1.05	4.47	12.75
VIII	370.07	2760.00	3.53	0.15	0.07	1.13	2.47	14.78

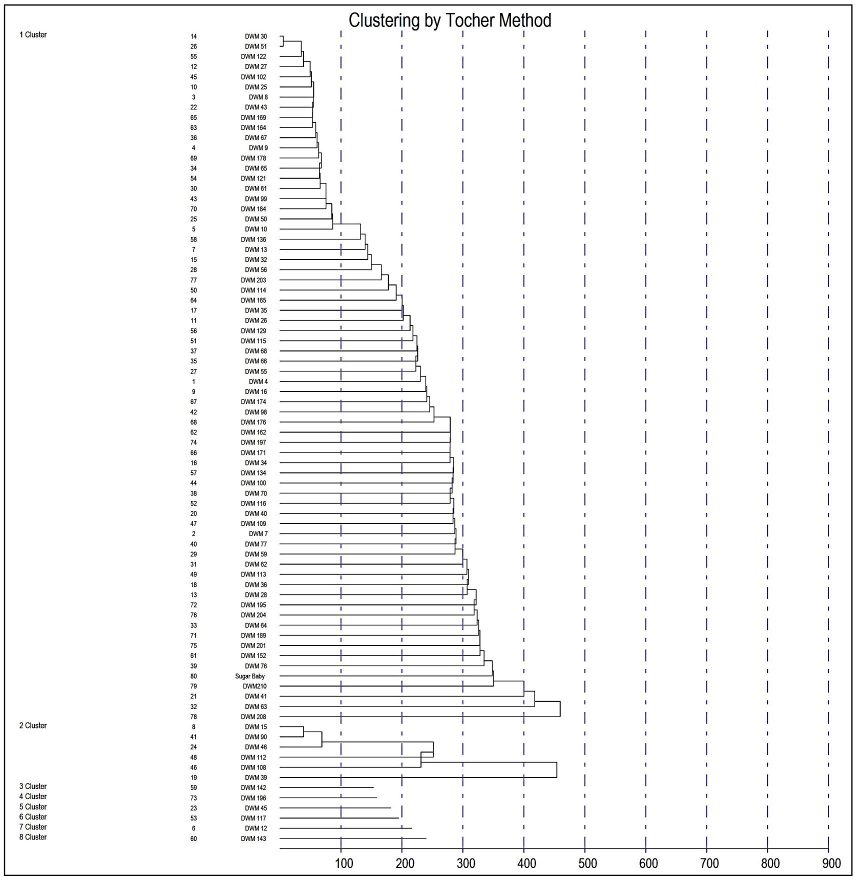

Fig 1: Dendrogram of eighty different genotypes of watermelon into 8 clusters.

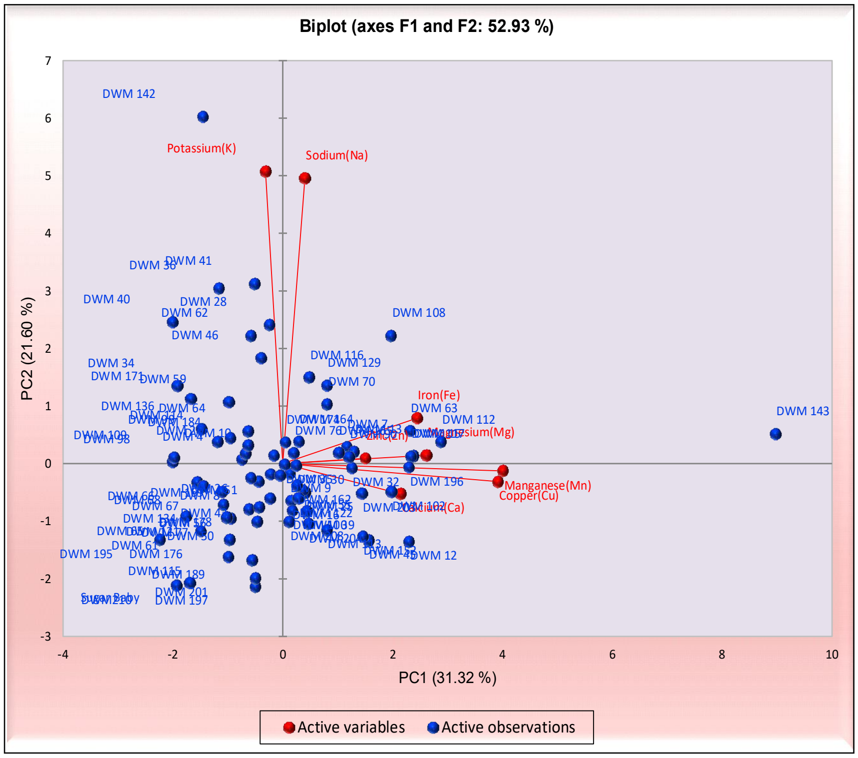

Fig 2: PCA Plot of PC1 and PC2 for eight mineral nutrients of watermelon.

Table 6: Principal component analysis for eighty watermelon genotypes based on eight mineral nutrients.

Parameter	PC 1	PC 2	PC 3	PC 4	PC 5
Cumulative eigen value	2.506	1.728	1.136	0.928	0.804
Explained variation (%)	31.325	21.601	14.197	11.605	10.050
Cumulative explained variation (%)	31.325	52.926	67.123	78.728	88.778
Traits	Eigen value				
Sodium (mg/100 g)	0.056	0.692	-0.108	-0.194	0.056
Potassium (mg/100 g)	-0.044	0.708	0.002	0.037	-0.078
Zinc (mg/100 g)	0.210	0.013	0.688	-0.305	0.460
Manganese (mg/100 g)	0.559	-0.018	-0.060	-0.269	-0.323
Copper (mg/100 g)	0.547	-0.043	0.106	-0.126	-0.455
Iron (mg/100 g)	0.341	0.110	0.345	0.581	0.251
Magnesium (mg/100 g)	0.365	0.020	-0.351	0.560	0.153
Calcium (mg/100 g)	0.299	-0.073	-0.508	-0.358	0.618

CONCLUSION

The results of this study showed the presence of significant variations in mineral nutrient composition of watermelon genotypes. Variations were relatively large for Zn, K and Na contents indicating the concentrations of these minerals may be further improved. D^2 analysis placed the eighty genotypes in eight distinct clusters indicating divergence among the studied genotypes. Among minerals K, Mg and Mn contents contributed largely towards diversity. Principal component analysis showed more than 50% of cumulative variance was controlled by PC1 and PC2. The selected genotypes in this study may be used to develop nutrient rich watermelon varieties including hybrids.

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