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Multivariate Analysis of Okra [*Abelmoschus esculentus* (L.) Moench] Genotypes and Hybrids based on Mineral Content

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ABSTRACT

Background: In 2021, the world production of okra [*Abelmoschus esculentus* (L.) Moench] was over 10.8 million tonnes which is led by India (about 60%). The objective of this study was to determine mineral content of genotypes and hybrids of okra identified by our in-house heterosis breeding programme.

Methods: The samples were digested in a heating block using nitric acid and hydrogen peroxide and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) for mineral profiling of okra samples.

Result: The results were evaluated using multivariate analysis. The genotype EC169474 was with the highest concentration of minerals Na (184.91 µg/g), K (14613.52 µg/g) and Fe (268.76 µg/g), while, EC169477 was found to have highest concentration of Mg (6530.76 µg/g) and Ca (273.58 µg/g). Comparatively, in hybrids, EC 169470 × EC169474 contains highest concentration of minerals Na (177.27 µg/g) and Ca (253.01 µg/g), EC169474 × EC169477 contains highest Mg (6301.74 µg/g) and hybrid EC169468 × EC169477 contains highest K (14412.99 µg/g) and Fe (246.51 µg/g). Principal component analysis (PCA) reveals that the element that contributed most for the variability between the genotypes and hybrids was K, moderate contribution by Ca, Fe and Na, while, Mg being the least. Score plot of principal components reveals EC169470 and EC169477 among the most variable genotypes and EC169470 × EC169477 was the most variable hybrid. Hierarchical cluster analysis (HCA) tends to separate all analyzed samples in two major clusters. However, the genotypes EC169474 and EC169477 were grouped in hybrid cluster, while, hybrid EC169470 × EC169477 grouped with genotype cluster. The multivariate analysis revealed a systematic difference in the mineral content of okra. The results presented in this study are of great value for nutritionally dense breeding programmes for okra that provides important nutritional diversity by hybrids.

Key words: *Abelmoschus esculentus*, HCA, ICP-MS, PCA.

INTRODUCTION

Rational nourishment and nutrition of food are closely connected to the health of humans. In recent decades, science has made great efforts to understand how nutrients and functional ingredients regulate human physiology and responses. Growing consumer interest in the role of nutrition in achieving good health reflects some of the findings that have already been made. As a result, several conventional and non-conventional vegetables have been critically examined for their nutritional content (Pinela *et al*., 2017). They have been shown to reduce the risk of chronic diseases such as cancer and diabetes (Martins *et al*., 2016). Among them is and annual herb [*Abelmoschus esculentus* (L.) Moench] of the Malvaceae family. Native to Africa, this economically important vegetable crop is now widely cultivated in Southern Europe, the Middle East, Asia and the Americas (Durazzo *et al*., 2019). Okra was produced worldwide in 2021 in excess of 10.8 million tonnes, with India producing the majority (60%) followed by Nigeria (18%) and Mali (over 6%) (FAOSTAT, 2021).

Since ancient times, infusions and decoctions of the fruits (pods) of *A. esculentus* have been used in folk medicine as a diuretic and for diarrhea, acute inflammation, gastric and intestinal inflammation, catarrhal infections, gonorrhea and dysuria, dental disease, It has been used to treat bronchitis and pneumonia (Habtemariam *et al*., 2019). Okra can be eaten whole; however, unripe pods have a unique flavor and are traditionally used in preparing soups, salads and stew (Liu *et al*., 2021). Over the past five years, various *in vitro* and *in vivo* studies have shown that *A. esculentus* extracts have cardiac, renal, gastric and neuroprotective effects (Habtemariam *et al*., 2019), as well as antioxidant, antidiabetic and antihyperlipidemic effects (Durazzo *et al*., 2019), anti-fatigue, antibacterial (Petropoulos *et al*., 2018), anti-inflammatory and analgesic (Alves *et al*., 2018) properties.

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It is known that the superior phenolic and pectin content of *A. esculentus* fruit is highly dependent on the percentage of seeds (Petropoulos *et al*., 2018; Xu *et al*., 2020). Okra is mucilaginous and low in calories, yet nutritious and a great source of fiber. Studies have shown that pods are an excellent source of minerals (Na, Mg, K, Ca, Fe) and are very low in cholesterol and saturated fat (Habtemariam *et al*., 2019). In component to direct ingestion, the fruit of okra is also exploited in the pharmaceutical and food industries as an emulsifier additive, blood volume expander and in drug tablets due to its high content of biopolymers and bioactive molecules such as polysaccharides and flavonoids used in prescription (Liu *et al*., 2021). However, mineral content investigations of Indian okra are still in very early stage, especially in hybrids.

Statistical techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) are used for the analytical evaluation of data to correlate several variables and to identify information relevant to characterization of samples. Because of their easy interpretation, these techniques are frequently used for data processing in several areas. PCA creates graphs with the samples represented in Cartesian coordinates and the PCs represented by the axes by using a linear combination of variables. These techniques have recently been employed in the characterization of nutritional composition variability in foods (dos Santos *et al*., 2013; Saha *et al*., 2009; Ryan *et al*., 2008).

Thus, present study is designed as an attempt to identify information relevant to genotypes and hybrids of okra based on their mineral content using inductively coupled plasma mass spectrometry (ICP-MS). The results were assessed using the multivariate analysis techniques PCA and HCA.

MATERIALS AND METHODS

Sample collection, storage and preparation

Seeds of okra genotypes (*Abelmoschus esculentus* L.) were collected from National Bureau of Plant Genetic Resources, India (EC169462, EC169468, EC169477, EC169474, EC169470) and superior hybrids (EC169470 × EC169477, EC169474 × EC169477, EC169453 × EC169474, EC169470 × EC169474 and EC169468 × EC169477) selected based on their morphological and productive attributes during *in-house* heterosis breeding in field trials conducted at Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, during the spring seasons of 2020 and 2023 were collected and used in this study.

After collection, samples were stored separately in closed, refrigerated bags to prevent the microbial proliferation. Vegetable was washed and rinsed with de-ionized water and dried on paper towels and then weighed. A Ceramic Bead Tube containing 1.4 mm ceramic beads (MolBio Laboratories Catalogue number 13113-50, available from Qiagen) and ice was used with 20 to 200 mg of weighed tissue. Four volumes of cold, pure and distilled water is added (assuming 1 mg = 1 uL). The tissue is homogenized with vigorous shaking using an Omni bead-ruptor bead mill. The homogenate stored frozen after removal from bead tube. ICP-MS analysis was done at Central research facility, Indian Institute of Technology, New Delhi, India.

Sample digestion and analysis

For each ICP-MS replicate, 30 to 50 uL of homogenate or cell lysate are digested with a 5:1 mixture of ultrapure hydrogen peroxide (ULTREX II, 30%, Fisher Scientific) and nitric acid (OPTIMA grade, 70%). 2 mL polypropylene tubes (VWR 10011-742, Axygen REF MCT-200-C clear, homo-polymer, boil-proof) are filled with 30-50 uL of lysate/ homogenate, 500 uL of nitric acid and 100 uL of hydrogen peroxide. The following day, 0.8 mL of additional 2% nitric acid and 0.2 mL of a 10X internal standard (made in 2% nitric acid) are added to the mixture after pipetting it up and down to guarantee resuspension. All measurements are performed using an Agilent 7900 ICP-MS in the helium (He) collision cell gas mode. 2% OPTIMA Grade Nitric Acid is used to prepare calibration standards and samples. An eight-point calibration curve is created by preparing solutions to Agilent Multi-element Calibration Standard 2A. Commonly used as an impartial standard is the Agilent Environmental Calibration Standard. According to recommendations from IUPAC, the detection and quantification limits were established (Thompson *et al*., 2002). Relative standard deviation, expressed as a percentage, was used to gauge precision.

Statistical analysis

To identify the relationship between the samples studied and the mineral content, exploratory analysis using principal component analysis (PCA) and Hierarchical cluster analysis (HCA) were performed using Scilab (Campbell, 2010).

RESULTS AND DISCUSSION

Mineral content of Okra genotypes and hybrids

Concentrations of calcium, magnesium, sodium, potassium and iron were determined in genotypes and hybrids. The concentrations of these macro- and micronutrients, expressed as ìg of analyte per g of sample (ìg /g) is shown in Table 1 and RSD values in Table 2. Fig 1 show regression curves for the concentrations obtained by obtained by ICP-MS for K, Ca, Fe, Na and Mg. The concentration of Na ranged from 80.29 to 184.91; Mg ranged from 5702.15 to 6530.76; K ranged from 2340.58 to 14613.52; Ca ranged from 103.74 to 273.58 and Fe ranged from 157.74 to 268.76. The genotype EC169474 was with the highest concentration of minerals Na (184.91 µg/g), K (14613.52 µg/g) and Fe (268.76 µg/g), while, EC169477 was found to have highest concentration of Mg (6530.76 µg/g) and Ca (273.58 µg/g). Comparatively, in hybrids, EC 169470 × EC169474 contains highest concentration of minerals Na (177.27 ìg/g) and Ca (253.01 µg/g), EC169474 × EC169477 contains highest Mg (6301.74 µg/g) and hybrid EC169468 × EC169477 contains highest K (14412.99 µg/g) and Fe (246.51 µg/g). The genotype EC169470 was found to have the lowest concentration of the minerals Na, Mg, K and Fe, while, the hybrid EC 169474 × EC169477 has the lowest concentration of Ca.

In developing countries, deficiencies of minerals have become a major health concern, especially Fe, Zn and Iodine malnutrition impacts more (Lockyer *et al*., 2018). Low intakes of minerals can have several negative health effects including stunting, anemia and cognitive impairments in children (Gernand *et al*., 2016). Orphan crops like Okra are researched scarcely in relation to their nutritional biodiversity and hold a huge potential in terms of food and nutrition security. Also, they are reported to adapt in changing climatic conditions (Baldermann *et al*., 2016; Van Jaarsweld *et al*., 2014). Therefore, advances in science

Table 1: Determination of the element concentration in okra genotypes and hybrids.

Sample	Na^+ [He] µg/g	Mg^{2+} [He] µg/g	K^+ [No Gas] µg /g	Ca^+ [He] µg/g	Fe^{2+} [He] µg/g
EC169462	174.20	6064.38	12621.19	190.04	232.78
EC169468	138.40	5955.63	12571.58	216.54	236.60
EC169477	100.47	6530.76	13889.21	273.58	230.54
EC169474	184.91	6338.89	14613.52	173.27	268.76
EC169470	80.29	5793.40	12340.58	254.63	157.74
EC 169470 × EC169477	138.06	5702.15	12561.45	155.90	214.90
EC 169474 × EC169477	157.44	6301.74	14106.09	103.74	212.40
EC 169453 × EC169474	143.39	6062.88	14152.30	186.69	229.37
EC 169470 × EC169474	177.27	6277.09	14217.99	253.01	210.79
EC 169468 × EC169477	158.15	6278.25	14412.99	165.91	246.51

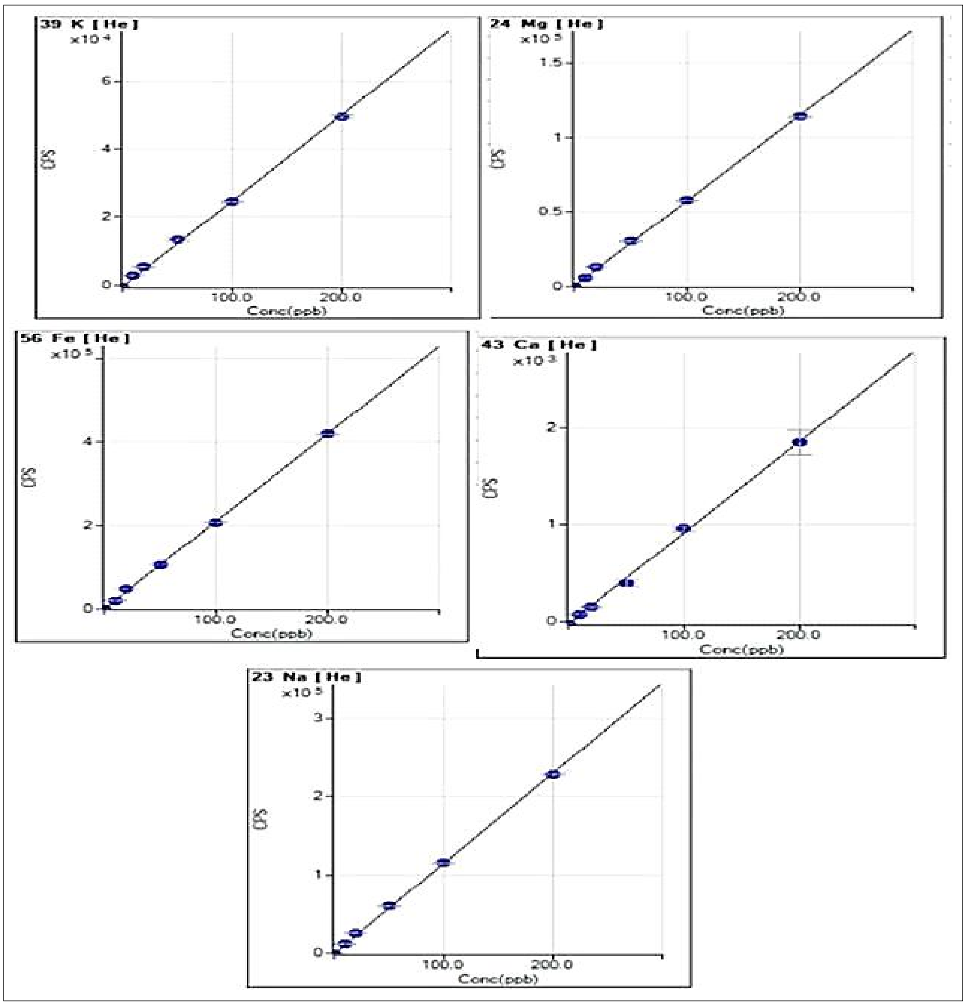

Fig 1: Regression curves the ICP-MS measurements for K, Mg, Ca, Fe and Na.

and technology that are related to the nutritional status of culturally significant orphan crops could significantly inform current and predicted future nutrition responses. In this study, the ICP-MS results differed significantly between the genotypes and hybrids for a range of minerals except K. Gemede *et al.* (2016) revealed considerable differences for Ca, P, Mg, Fe and Zn among local accessions of Okra. In fact, genotypic differences along with environment and farming conditions have significant influence on elemental composition of okra in the Mediterranean region (Petropoulos *et al*., 2018). In this study, the abundance of minerals follows the order: K>Mg> Fe>Ca>Na in both genotypes and hybrids. Similar order of abundance was observed by Kehinde *et al.* (2015) except a drop in Mg and raise in Ca. Similar results have been reported by several studies (Ferguson *et al*., 1989; Mitchikpe, 2007; Kamga *et al*., 2013). However, in a nigerian study (Kehinde *et al*., 2015), lower K content was reported.

Several authors have reported moderate amounts of Ca (Kamga *et al*., 2013; Ponka *et al*., 2014; Del valle *et al*., 2011) which is in accordance with our study. However, studies have also reported higher amount (Petropoulos *et al*., 2018; El-Nahry *et al*., 1978) of Ca in okra pods and seeds and lower amount (Kehinde *et al*., 2015, Ferguson *et al*., 1989) than what has been found in the present study. The elevation in Ca in the present study might be ascribed to the inclusion of seeds (Alake, 2020; Petropoulos *et al*., 2017; El-Nahry *et al*., 1978). As revealed in this study, okra is a good source of Magnesium (Mg). The same observation was made by Mitchikpe (2007) in dried okra pod.

Green leaves are usually considered as the plant source of Fe (Orech *et al*., 2007; Ayaz *et al*., 2006)). In our study, we found good amount of Fe in hybrids. Authors have reported levels of Fe in okra accessions (Gemede *et al*., 2016; Avallone *et al*., 2007) similar to our study. Zinc is a co-factor for many enzymes and helps maintain structural integrity of proteins and reduce infections (Salgueiro *et al*., 2000). Previous studies reported similar ranges for Zn in dried okra pods and okra based sauce (Avallone *et al*., 2007; Avallone *et al*., 2008) as found in our study. Okra has really been found to be a reliable source of digestible zinc. Variable reported outcomes may be explained by different potentials for absorption and accumulation among varieties and genotypes (Alake, 2020; Ahiakpa *et al*., 2014). Soil, climatic conditions (geographic origin), seasonal fluctuations, physiological state and maturity and the use of agricultural pesticides are the main reasons of variation in nutritional composition. In addition to the role played by varietal and environmental variations, the inclusion of okra seeds, which make up about 13.5% of dried okra (Gemede *et al*., 2016; Xu *et al*., 2020), which are a rich source of minerals (El-Nahry *et al*., 1978; Alake, 2020), may be responsible for the difference in mineral profiles.

Cluster analysis

The mineral concentrations in okra samples were assessed using principal component and hierarchical

Table 2: ICP-MS-Results obtained for the quantification limit and relative standard deviation (RSD).

Sample	^{23}Na		^{24}Mg		^{39}K		^{43}Ca		^{56}Fe	
name	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD
Blank	0	N/A	0	N/A	0	N/A	0	N/A	0	N/A
10 ppb	11.055	4.8	10.395	2.3	-	-	10.248	27.4	10.04	2.2
20 ppb	23.402	2.2	23.09	7.5	24.354	1.3	19.083	11.6	23.206	0.1
50 ppb	53.119	1.2	53.466	2.2	57.49	1.7	45.234	18.6	50.85	1.6
100 ppb	100.534	1.5	100.577	0.9	104.84	2.4	104.605	5.4	98.926	1.1
200 ppb	198.56	0.7	198.516	0.3	197.244	0.7	198.968	13.8	200.002	0.4
EC169462	9.394	4.8	327.022	0.2	680.598	0.9	10.248	41.4	12.553	3.7
EC169468	7.453	6.4	320.711	1.3	676.98	0.9	11.661	5.2	12.741	1.8
EC169477	5.451	5.3	354.294	0.9	753.49	1.2	14.842	46.5	12.507	4.2
EC169474	9.805	11.1	336.12	2.4	774.882	1.6	9.188	52.9	14.251	0.7
EC169470	4.346	7.6	313.568	0.2	667.934	1	13.782	23.5	8.538	3
EC 169470 × EC169477	7.511	22.1	310.197	1.1	683.343	1.3	8.481	19.1	11.691	4.1
EC 169474 × EC169477	8.581	16.1	343.445	2.8	768.782	1	5.654	88.6	11.576	3.2
EC 169453 × EC169474	7.6	12.2	321.333	1.1	750.072	0.8	9.895	53.9	12.157	4.2
EC 169470 × EC169474	9.409	1.9	333.157	0.7	754.62	2.8	13.429	41.8	11.188	0.8
EC 169468 × EC169477	8.422	8.7	334.317	1.1	767.492	0.8	8.835	56.7	13.127	1.5

cluster analysis. Scilab (Campbell *et al*., 2010) was used to calculate scores and loadings. The loadings of the original variables in the first two principal components and the variance explained by each component, are given in Table 3. The first two principal components had substantial loadings for all five variables. They accounted for 99.99 % of total variance. The dominant variables for the first principal component (PC1) were K, Na, Ca and Fe, representing 98.95% of total variance. These four elements contributed most of the variability among the samples and were negatively correlated with PC1 (except K which is positively correlated). Examining loadings, K, Ca, Na and Fe were the dominant variables, with smaller contributions from Mg. The second principal component (PC2) accounted for 0.03% of total variance and included Mg as the dominant variable. Fig 2 shows a projection of the first two PCs on the genotypes and hybrids. The score plot depicts the major negative cluster with three most variable samples (EC169477, EC169470 and EC169470 × EC169474). As such, we can infer that the hybrids had the highest concentrations of all five elements when compared to the genotypes. This inference is corroborated by Table 1, which presents the concentration of each mineral in genotypes and hybrids.

Table 3: Eigen vectors and total percentage variation for the principal component axes of Okra genotypes and hybrids.

Minerals	PC1	PC2
Na	-2.093	-0.027
Mg	1.1199	0.0979
K	5.0879	-0.044
Ca	-2.064	-0.006
Fe	-2.051	-0.022
% of variance	99.966	0.0317
Cumulative (%)	99.966	99.998

As shown in Table 3, PC1 had large negative loadings to Na, Ca and Fe. Elements with high negative loadings showed the declines in hybrids generally with exceptions, while elements with positive loadings had the higher concentrations in hybrids. Thus, it was possible to differentiate the elements according to the variations observed in genotypes and hybrids. It is possible that distinct interactions within the okra macromolecules account for the various decreases seen in these minerals. These macromolecules might be found in the fibres, protein, or crude fat that make up 31.4%, 27% and 21.72%, respectively, of the structure of okra (Jarret *et al*., 2011).

The samples EC169477, EC169470 and EC169470 × EC169474 separated from the main cluster in score plot (Fig 2). The high concentrations of Ca, Fe and Na meant the loadings for these elements were highly negative for this PC. This was confirmed, as these elements are shown in average concentrations in Table 3. The score plot reveals Ca, Fe and Mg concentrations higher in genotypes, while, Na and K concentrations higher in hybrids. Further, no tendency towards separation of genotypes and hybrids samples was observed in PC1 or PC2.

Fig 3 illustrates the dendrogram for the HCA results. The results were separated into two groups, at linkage

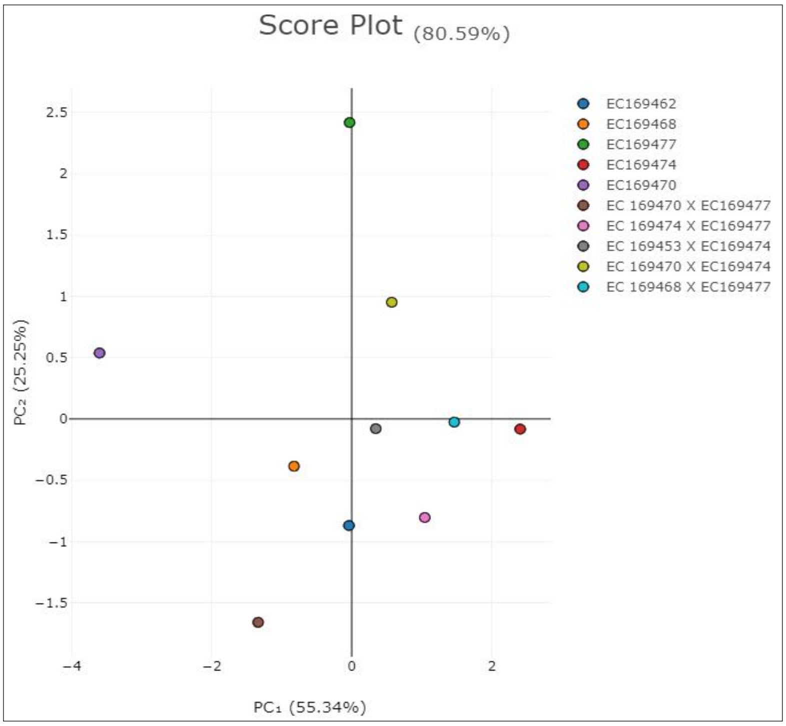

Fig 2: Score plot of PC1 and PC2 when variables are loaded in genotypes and hybrids of Okra.

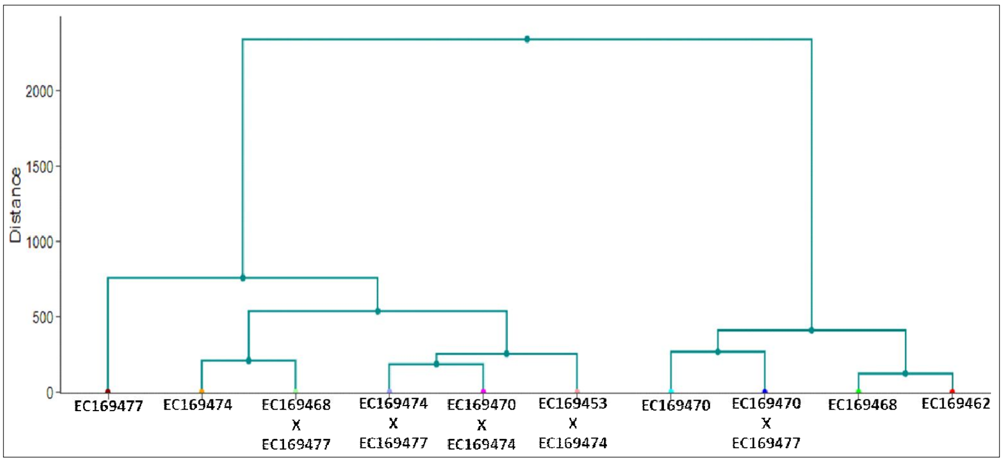

Fig 3: Dendrogram for okra samples showing ward's method with euclidean distances.

distances between 1000 and 2000, which confirmed the result obtained in PCA: three clusters of points can be observed, separating EC169477 with rest of the samples. Other two clusters largely represent genotype and hybrid group, with exceptional grouping of EC169474 in hybrid cluster and EC169470 × EC169477 in genotype cluster. This shows that the samples in the two-dimensional projection (Fig 2) are even more separate in real space once the dendrograms are based on real distances between samples; principal component analyses are only projections.

CONCLUSION

The determination of minerals in okra genotypes and superior hybrids (identified by in house heterosis breeding) by ICP-MS gave a satisfactory quantification of Ca, K, Mg, Na and Fe. PCA and HCA demonstrated that K, Ca, Fe and Na contribute most of the variability between genotypes and hybrids, with a moderate contribution from Mg. The genotypes EC169477, EC169470 and hybrid EC169470 × EC169474 were amongst the most variable samples in terms of their mineral concentration. The information herein reported suggests the importance of the Okra production and consumption in a daily-basis diet by the Indian population as a source of nutrition and because of its high mineral content especially in hybrids, they can be exploited by the pharmaceutical and food industries. Overall, efforts should be made to include nutrient-dense cultivation of crops with cultural significance, like okra.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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