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Variability in seed mineral composition of foxtail millet (*Setaria italica* L.) landraces and released cultivars

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Abstract

Foxtail millet (*Setaria italica* L.) belongs to poaceae and an important research model plant to explore nutritional pathways. The present study represents a comprehensive micronutrient report of twenty landraces, four released cultivars, and their genetic variability in micronutrient content. FT-IR analysis recorded various absorption peaks at different wavelenths coressponding to certain chemical compounds and functional groups such as carbohydrates, alkenes, proteins, sulfur compounds, amines and lipids, etc, indicate that all the studied genotypes endowed with carbohydrates, proteins and lipids. The ICP-OES analysis revealed a wide range of variation in micronutrient concentrations across the studied genotypes *i.e* Iron (3.69 to 7.51mg/100g), Zinc (4.54 to 5.71 mg/100g), Calcium (13.13 to 39.58 mg/100g), Potassium (219.43 to 349.47 mg/100g), Copper (0.60 to1.09 mg/100g), Manganese (1.05 to 1.64 mg/100g). The PCA and cluster analaysis highlight a wide range of genetic variability among the genotypes. Further, these genotypes were clustered into six variables based on the micronutrient content. In overall performance of landraces better than released cultivars in terms of micronutrient content. Landraces like S1G4, S1G2; and relesed varity Narasimharaya recorded higher quatities of micronutrient compared to other genotypes studied. These genotypes would be useful to fish out the genes responsible for higher micronutrient occumulation and also as parental lines in breeding progrmmes to develop enhanced micronutrient genotypes.

Introduction

Plant-based foods contribute an array of nutrients that are essential for the day-to-day needs of human beings and they endorse good health. Humans require at least twenty-two micro and macro elements for their proper health, growth, and development (50). However, global estimates suggest that, over 60% of the people suffering from iron (Fe), 30% zinc (Zn), 30% iodine (I) and 15% selenium (Se) deficiencies. In addition to them, calcium (Ca), manganese (Mn), and copper (Cu) deficiencies are common in many of the developed and developing countries (40). Malnourishment is a global issue; especially developing countries from Asia and Africa facing severe micronutrient deficiencies in their dietary food (19, 42). The health and diet are co-dependent; the physiological functions of the human body are influenced by food components (29). Even though the requirements of micronutrients are minimal, they play a crucial role in proper growth and development. The deficiencies of micronutrients cause severe health complications such as physical and mental

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retardation, blindness, gastrointestinal health complications, reduced immunity, etc. (9). Newborn babies and pregnant women of India severely affected by micronutrient deficiency and most of the infants are born underweight. It is estimated that nearly 7.4 million children remain undernourished (19). Thus, functional foods are gaining importance in the prevention and/or treatment of diseases.

Among the plant-based foods, cereals alone play a key role as a staple food and provide ~50% of the dietary requirements of humans. Globally, among the cereals rice alone provides 50-60% of required calories to 2.7 billion people. However, the principle drawback of rice-based food products are being low in iron, zinc, proteins, vitamins, and other essential nutrients along with high water requirements for its cultivation (18, 50). On contrary small millets consists of diverse micronutrients, rich in essential amino acids and high water use efficient, grow in harsh environmental conditions, resistant to abiotic and biotic stress conditions. Hence, recently small millets gaining more importance and they might play a crucial role as functional foods.

The small millet, foxtail millet (*Setaria italica* L.) is an important nutritious crop belongs to the family Poaceae, known for its origin from China. Due to its drought tolerance capacity, it is very well grown in semi-arid regions such as South Asia and Sub-Saharan Africa as nutritional food. It is also cultivated in South Korea, North Korea, Japan, Russia, Australia, France and the United States as a forage crops, feed for birds and cattle (12). Foxtail millet endowed with high amounts of protein, vitamins, minerals, starch, and fat content (39). It has twice the content of protein and fat as compared to rice (35). Nutrient analysis of core collection of foxtail millet seeds revealed that it had a wide range of nutrients such as calcium (171.2–288.7 mg/kg), iron (58.2–68.0 mg/kg), zinc (54.5–74.2 mg/kg) and protein (15.6–18.5%) (43). However, due to the presence of anti-nutrients made them less bioavailable (1, 2, 15, 23, 30).

Bio-fortification of millets is an emerging approach to overcome the problem of anti-nutrients and to add more nutritional content to the crop plants. Conventional breeding, agronomical practices, and biotechnological strategies are the key approaches to improve the bio-fortification of crop plants. Agronomical practices such as supplementing the deficit soil with inorganic fertilizers were successfully practiced in Finland and Turkey for the high accumulation of Se and Zn in the seeds (50).

Genetic variation for the trait of interest is a prerequisite for plant breeding (13). Foxtail millet genotypes exhibited variation in seed protein, fat, starch, and amino acids (51); Fe content (31); vitamin E (25); cooking quality traits (38). Thippeswamy et al (2017) screened 25 genotypes and identified two genotypes namely GS78 and GS71 as superior for grain micronutrients (Zn, Fe, and Ca) and protein content (41). Four genotypes of foxtail millet genotype 00002, 0011 (red colour bran) and Slovenský, Friderica (yellow colour bran) displayed varied amounts of nutritive components, fatty acids, phenolic compounds and antioxidants (28). A greater amount of variability was observed in 78 elite genotypes for nutritional parameters such as moisture, protein, fat, crude fibre, carbohydrate, total minerals, total energy, and micronutrients (Cu, Mn, Zn, and Fe) (7, 21).

Landraces are a heterogeneous population, well adapted to local climatic conditions, and are extremely nutritious. They also serve as genetic material to breed high nutritional and stress adapted genotypes (11). Maxican maize landraces were successfully used to breed high-quality protein maize lines and cultivars (32). Similarly, Sorghum popular Indian landrace Maldandi (M35-1) was used in the breeding programme to develop several restorer lines (ICSR#) and cultivars (ICSV#) (34). In pearl millets West African drought tolerant landrace “Iniadi” was used to develop several cultivars e.g.: ICTP 8203 (33). As many as 245 foxtail millet traditional varieties from different regions of Shanxi, China was evaluated for seed folic acid variability and

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found a wide variability ranging (0.37–2.37 mg/g) of which 24 varieties with higher folic acid content were identified, among them, Jingu 21, a major leading cultivar, recorded folic acid content of 2 mg/g (36). Similarly, a panel of 92 foxtail millet landraces preserved by Taiwan indigenous peoples were assessed for seed amylase content (AC) using a rapid viscosity analyzer (RVA). A huge range of diversity (0.7% to 16.9%) in physiochemical properties was observed among the studied genotypes (52).

Thus, identification of elite genotypes for micronutrient content is very important towards the development of improved varieties through classical as well as modern tools. To identify the elite nutritious genotypes in the present study, twenty local landraces collected from various locations of Andhra Pradesh and four released cultivars were selected, nutrient content was analysed using modern analytical tools such as ICP-OES (Inductively coupled plasma atomic emission spectroscopy) and FT-IR (Fourier Transform Infrared Spectrophotometer) and data were subjected to multivariate statistical analysis.

Materials and Methods

Plant material : The seeds of foxtail millet landraces collected from the farmer fields of Rayalaseema region, Andhra Pradesh, and twenty pure lines were developed by single seed descent method (SSD). The details of their development and molecular characterization were described elsewhere (Ramesh et al., manuscript Unpublished). Twenty pure landraces along with four released cultivars (Table.1) were surfaced sterilized with 0.01% $HgCl_2$ followed by rinsing with distilled water. Seeds were sowed in the well-prepared seedbeds of natural field soil in a completely random blocked design with three replicates per sample. Each genotype was grown in three rows in net house at Yogi Vemana University, Kadapa, Andhra Pradesh under natural environmental conditions (30±1ºC/37±1ºC and relative humidity varied from 50-80%), by following standard agriculture practices. Seeds were harvested from panicles after maturation and stored in a cool dry place until further use. The seeds were de-husked and milled into flours by using a clean and sterilized mortar and pestle. The flours were kept at 55° C for 4-5 hours in a hot-air-oven to remove the moisture content if any. Dehydrated flours were subjected to nutrient analysis for macro, micronutrients, and essential biochemical groups.

Sample preparation and FTIR Analysis : Dehydrated flour of all twenty-four genotypes was used for the preparation of KBr (potassium bromide) pellets to analyze functional groups of flour. Ten mg of dehydrated seed flour was mixed with 100mg of KBr and vigorously ground into a fine powder with mortar and pestle. This mixture was compressed into diaphanous pellet discs using a hydraulic pressure pump. These diaphanous pellets were subjected to Fourier transformed infrared spectroscopy analysis using Perkin Elmer Spectrum Two (Perkin Elmer Inc., USA). Chemical and functional groups of the samples were obtained with 16 scans at a resolution of $2^{cm-1}$with the wavenumber range from 400^{cm-1} to 4000 $^{cm-1}$ at room temperature. The spectrometer has an auto-correction system to eliminate water vapor without purging with nitrogen.

Sample preparation and ICP-OES analysis : Five hundred mg of dehydrated flour of all twenty-four genotypes in three replications was taken into a clean acid prewashed polypropylene tube. The samples were digested with 2 ml of HNO_3 (65%V/V) and 0.5 ml of H_2O_2 (30% H_2O_2) in a closed vessel microwave digestion system and allowed the vessel to stand overnight at room temperature (cold digestion). The vessel is closed with a screw lid; sample-acid mixture was heated for 30 min at 125ºC for digestion. The samples were allowed to cool and make up to 10 ml with distilled water. The mixture was subjected to orbital shaker for 1 min for proper mixing. Finally, samples were filtered using Whatman No 1 filter paper. The supernatant was subjected to ICP-OES (ICPOES HD Prodigy –Lemans) for macro and micronutrient analysis. The ICP-OES operating conditions as

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followed: RF power 1.1kw; coolant flow 18 L/min; Auxiliary flow 0.0L/m; Nebulizer type Hildebrand Nebulizer; Nebulizer pressure 34 psi; Sample uptake rate 1.4 ml/min; Spray chamber cyclonic; Torch: Dual view; Pure gas Nitrogen; Pure gas flow- 0.7 L/min; Axial time-10 seconds; Radial time- 5 sec and Wash time – 40 seconds. All the samples analyzed in triplicates. The data was read by the inbuilt software of the instrument. The elemental parameters of studied macro and micronutrients of ICP-OES analysis are described in Table 2.

Statistical analysis: The ICP-OES data was subjected to XLSTAT 2019.3.1.60379 software for principal component analysis (PCA) followed by the "R" program for multivariate hierarchical cluster analysis. The data of mineral content of 24 Foxtail millet genotypes are expressed as mean± Standard deviation of at least three replicates. The PCA analysis was used to determinate correlation among seed residues from wild and released cultivars of Foxtail millet. The analyzed data were statistically subjected to analysis of variance technique using STATISTIX 8.1 Software and the significance was evaluated by least significance test (LSD) at 5% probability level (Table 3).

Table 1 : 20 Landraces and 4 released cultivars of Foxtail millet

S No	Name of Foxtail millet genotype	Variety type	Origin
1	S1G1	Landrace	Eipperu, Anantapur
2	S1G2	Landrace	Eipperu, Anantapur
3	S1G4	Landrace	Eipperu, Anantapur
4	S1G5	Landrace	Eipperu, Anantapur
5	S1C1	Landrace	Eipperu, Anantapur
6	S1C2	Landrace	Rakatla, Anantapur
7	S2G1	Landrace	Korrapadu, Kadapa
8	S2G2	Landrace	Korrapadu, Kadapa
9	S2C1	Landrace	Korrapadu, Kadapa
10	S2C2	Landrace	Korrapadu, Kadapa
11	S3G1	Landrace	Dudyala, Kadapa
12	S3G2	Landrace	Dudyala, Kadapa
13	S3G3	Landrace	Dudyala, Kadapa
14	S3G4	Landrace	Dudyala, Kadapa
15	S3G5	Landrace	Dudyala, Kadapa
16	RED	Landrace	Punganur, Chittoor
17	BLACK	Landrace	Basavanapalli, Anantapur
18	Srilakshmi	Released Cultivar	RARS, Nandyal, AP, India
19	Prasad	Released Cultivar	RARS, Nandyal, AP, India
20	Krishnadevaraya	Released Cultivar	RARS, Nandyal, AP, India
21	Narasimharaya	Released Cultivar	RARS, Nandyal, AP, India
22	S4G4	Landrace	Maddikera, Kurnool
23	S4C4-G	Landrace	Maddikera, Kurnool
24	S4C4	Landrace	Maddikera, Kurnool

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Table 2: Elemental parameters of ICPOES analysis

Element	View	Wave length
Potassium	Radial	766.490
Calcium	Radial	317.993
Iron	Axial	259.940
Zinc	Axial	213.856
Manganese	Axial	257.610
Copper	Axial	327.393

Results

In the present study, twenty-four foxtail millet genotypes were analyzed for their micro and macronutrient analysis using FTIR and ICP-OES analysis. FTIR analysis was recorded at 400^{cm-1} to 4000^{cm-1}region to identify different functional groups such as amino acids, carbohydrates, alkenes, proteins, Sulphur compounds, amines, and lipids present the flour. IR spectrum data was presented in Fig. 1.

The absorbance regions below 800 $^{cm-1}$, between 800 $^{cm-1}$-1500 $^{cm-1}$ (fingerprint region); 2800-3000 $^{cm-1}$ (C-H stretch region) and 3000-3600 $^{cm-1}$ (O-H stretch region) indicates the presence of starch. All of the genotypes exhibited similar kinds of peaks below 800$^{cm-1,}$ C-H stretch region, and O-H stretch region. However, the genotypes S4C4-G and S4C4 displayed very weak infrared peaks at 1015.11 $^{cm-1}$ and 1017.45^{cm-1} respectively, in contrast to other genotypes. The IR peaks at 1022 $^{cm-1}$ indicates the amorphous structure of flour and the peak at 1041^{cm-1} sensitive to the crystalline structure of flour. The genotype S1G4, S3G2, S3G5 shows absorption peaks at 1020.73^{cm-1}, 1021.88^{cm-1}, 1023.09^{cm-1} respectively, and could be highly amorphous. The genotype S2G1 displays a peak at 1041$^{cm-1,}$ indicates the crystalline structure of flour.

The absorption peaks at 1660^{cm-1}and 1550^{cm-1}detects the two forms of proteins amide I and amide II. The peak at 1660^{cm-1}detects C=O of the Amide I, the peak at 1550 $^{cm-1}$ detects NH bending of amide II in the crude protein. The strong absorption peak of the NH bond in the FTIR spectrum indicates that genotype protein richness. In the present study, the strong absorbance peak of the NH group was observed at 1543.69 $^{cm-1}$, 1543.09^{cm-1} in the genotypes S1G2, S4G4 respectively, which are considered to be as protein rich genotypes compared to other genotypes. The released cultivars Prasad and Narasimharaya have absorption peaks at 1542.45 $^{cm-1}$ and 1542 $^{cm-1}$ respectively. The genotype S4C4-G has a maximum absorbance at 1664.45 $^{cm-1}$which is a strong amide I bond and might contain very low protein content. The IR spectral peaks in the region 1600^{cm-1}- 1700^{cm-1} and 1550^{cm-1}-1570^{cm-1}indicates the presence of crude fat. In the present study, all the analyzed genotypes recorded peaks at 1645^{cm-1} and 1664.45^{cm-1}. However, the genotype S4C4-G and S4C4 did not record peaks in the region 1550 $^{cm-1}$ -1570 $^{cm-1}$. The moisture content of the flour can be determined by measuring OH stretching and H bending vibrations at IR spectral peaks from 1640 $^{cm-1}$ to 3300 $^{cm-1}$. All the genotypes in the present study recorded strong peaks in the prescribed spectral region. The IR spectral peaks at 1648.28^{cm-1}, 1658.84^{cm-1} indicates the occurrence of carbonyl group and amines 1 bond, IR peak at 2923.12 $^{cm-1}$ specifies presence of C-H compound (lipids). All the genotypes of present study displayed peak at 2923.12^{cm-1} and 1648.28^{cm-1} indicating the presence of lipids and aromatic compounds in their flour. The presence of thin bands at 2923.12^{cm-1} is associated with symmetric stretching vibrational modes of the C-H bonds in alkylic CH2 and CH3 groups, which are mainly due to the presence of lipids in flour.

The ICP-OES data of twenty-four genotypes seed flour indicates that there was a high genotypic variation for Fe, Zn, Mn, Cu, Ca, and K as showed in Table 3. Analysis of variance indicates that foxtail millet genotypes displayed non-significance variation ($P< 0.05$) for Fe content. Most of the studied genotypes recorded high Fe content, which is ranging from 3.69±0.38 to

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7.51±0.68 mg/100gm. Especially, the genotype S1G4 recorded high Fe content in its seed flour and followed by the released cultivars Krishnadevaraya, Prasad, and Narasimharaya. The low concentration of Fe noticed in landrace S2C2 (3.69±0.38 mg/100g). Almost all the studied genotypes recorded a greater amount of Zn ranging from 4.54± 0.34 to 5.71± 0.33 mg/100gm. The maximum content of Zn (5.71± 0.33) found in the landrace Black and followed by the genotypes S2C1, RED, S2C2, and S2G2. The genotype S1G1 contains a minute amount of Zn. However, genotypes recorded non- significant variation in Zn content (P< 0.05). The calcium content in foxtail millet germplasm under study varied from 13.13±0.57 mg/100g to 39.58±0.83 mg/100g. The genotypes displayed huge variation for Ca content (P< 0.05). Highest concentration of Ca was observed in the landrace S4C4 (39.58±0.83), followed by S1G2 (32.91±0.64), S3G1 (31.03±0.45) and S1C1 (28.12±0.83mg/100g). The low Ca content was observed in the landrace S4C4-

Table 3: Analysis of Variance (one way-Least significance test) showing the results of different macro and micro nutrient concentrations (mean value and SD) of 24 genotypes of Foxtail millet (At 5% probability level)

	Fe	Zn	Ca	K	Mn	CU
S1G1	4.92±0.34^{bcde}	4.54±0.34^{a}	23.72±0.29^{d}	258.03±0.69^{ji}	1.49±0.16^{abc}	0.63±0.16^{de}
S1G2	6.3±0.42^{abcd}	5.33±0.42^{a}	32.9173±0.64^{b}	251.057±0.49^{j}	1.38±0.16^{abc}	0.64±0.30^{de}
S1G4	7.51±0.68^{a}	4.65±0.38^{a}	21.09±0.72^{defgh}	267.63±0.41^{h}	1.49±0.20^{abc}	1.02±0.13^{abc}
S1G5	5.07±0.44^{bcde}	5.16±0.50^{a}	18.27±0.59^{hijk}	219.433±0.32^{n}	1.16±0.14^{bc}	1.09±0.17^{a}
S1C1	5.39±0.65^{abcde}	5.2±0.56^{a}	28.12±0.83^{c}	250.48±0.61^{l}	1.13±0.13^{c}	0.92±0.27^{abcd}
S1C2	5.63±0.60^{abcde}	4.69±0.48^{a}	20.83±0.43^{defgh}	349.54±0.81^{a}	1.59±0.20^{ab}	1.05±0.21ab
S2G1	5.28±0.76^{abcde}	5.4±0.37^{a}	21.53±0.38^{defgh}	238.75±0.54^{m}	1.28±0.12^{abc}	0.85±0.18^{abcde}
S2G2	4.81±0.73^{cde}	5.45±0.29^{a}	18.52±0.51^{ghij}	238.70±0.73^{m}	1.38±0.24^{abc}	0.73±0.07^{cde}
S2C1	5.83±0.86^{abcde}	5.67±0.35^{a}	15.11±0.75^{jkl}	257.41±0.42^{ijk}	1.31±0.10^{abc}	0.71±0.08^{de}
S2C2	3.69±0.38^{e}	5.59±0.40^{a}	19.29±0.50^{ghi}	255.16±0.83^{jkl}	1.19±0.10^{bc}	0.8±0.09^{abcde}
S3G1	5.06±0.54^{bcde}	4.64±0.24^{a}	31.03±0.45^{bc}	300.65±0.69^{f}	1.47±0.19^{abc}	0.67±0.07^{de}
S3G2	5.08±0.45^{bcde}	4.65±0.31^{a}	18.05±0.46^{hijk}	253.08±0.63^{kl}	1.49±0.15^{abc}	0.81±0.11^{abcde}
S3G3	4.48±0.56^{cde}	4.87±0.36^{a}	18.27±0.48^{hijk}	290.05±0.64^{g}	1.31±0.17^{abc}	0.6±0.10^{e}
S3G4	5.54±0.06^{abcde}	4.96±0.28^{a}	22.87±0.69^{def}	317.54±0.53^{cd}	1.64±0.21^{a}	0.77±0.09^{bcde}
S3G5	4.16±0.21^{de}	5.11±0.39^{a}	15.79±0.56^{ijkl}	313.51±0.32^{de}	1.2±0.14^{abc}	0.69±0.07^{de}
Red	4.76±0.52^{cde}	5.65±0.33^{a}	14.79±0.49^{kl}	271.4±0.57^{h}	1.12±0.10^{c}	0.83±0.11^{abcde}
Black	4.45±0.66^{cde}	5.71±0.33^{a}	23.70±0.28^{d}	261.08±0.60^{i}	1.33±0.15^{abc}	0.63±0.06^{de}
S4G4	5.58±0.93^{abcde}	5.56±0.25^{a}	15.06±0.55^{jkl}	316.98±0.53^{cd}	1.38±0.21^{abc}	0.79±0.10^{bcde}
S4C4-G	3.87±0.27^{e}	4.65±0.19^{a}	13.13±0.57^{l}	309±0.41^{e}	1.05±0.16^{c}	0.71±0.04^{de}
S4C4	3.8±0.42^{e}	4.66±0.26^{a}	39.58±0.83^{a}	317.58±0.35^{cd}	1.08±0.13^{c}	0.79±0.08^{bcde}
Srilakshmi	3.99±0.31^{e}	5.26±0.23^{a}	19.95±0.49^{fgh}	320.92±0.64^{c}	1.33±0.12^{abc}	0.74±0.05^{cde}
Prasad	6.63±0.76^{abc}	5.45±0.37^{a}	20.011±0.39^{efgh}	321.29±0.42^{c}	1.12±0.10^{c}	0.83±0.11^{abcde}
Krishnadevaraya	7.15±0.72^{ab}	5.04±0.35^{a}	22.06±0.70^{defg}	303.67±0.55^{f}	1.15±0.14^{bc}	0.69±0.07^{de}
Narasimharaya	6.48±0.69^{abc}	5.31±0.40^{a}	23.25±0.79^{de}	333.58±0.54^{b}	1.09±0.19^{c}	0.66±0.03^{de}
Mean	5.23	5.13	21.55	284.07	1.30	0.78
LSD<0.05	1.929	1.103	11.263	48.331	0.422	0.282
CV	26.78	16.34	10.04	1.05	20.91	22.91

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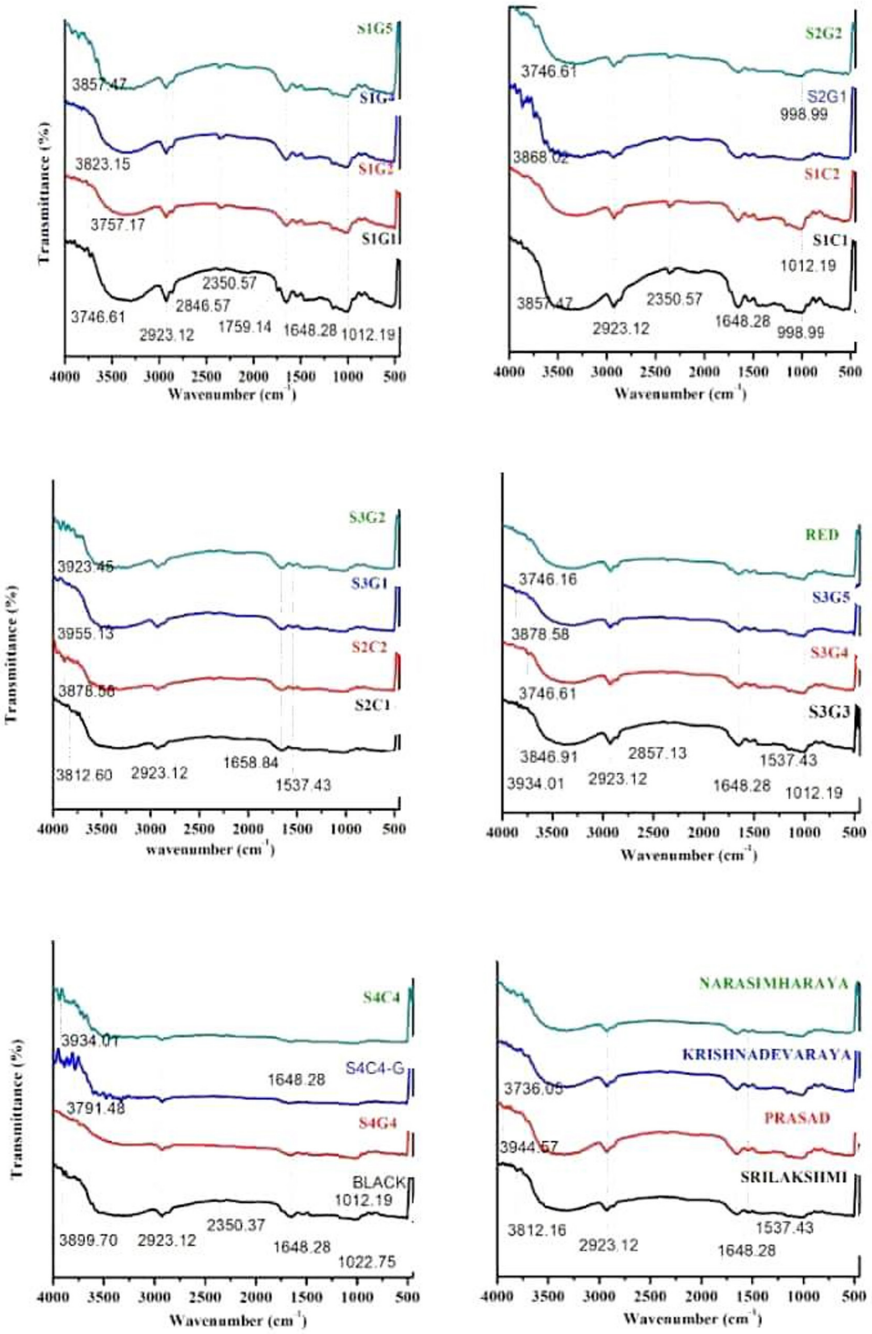

Fig 1: FTIR Spectra showing the functional groups of the organic and inorganic compounds of 20 Landraces and 4 released cultivars

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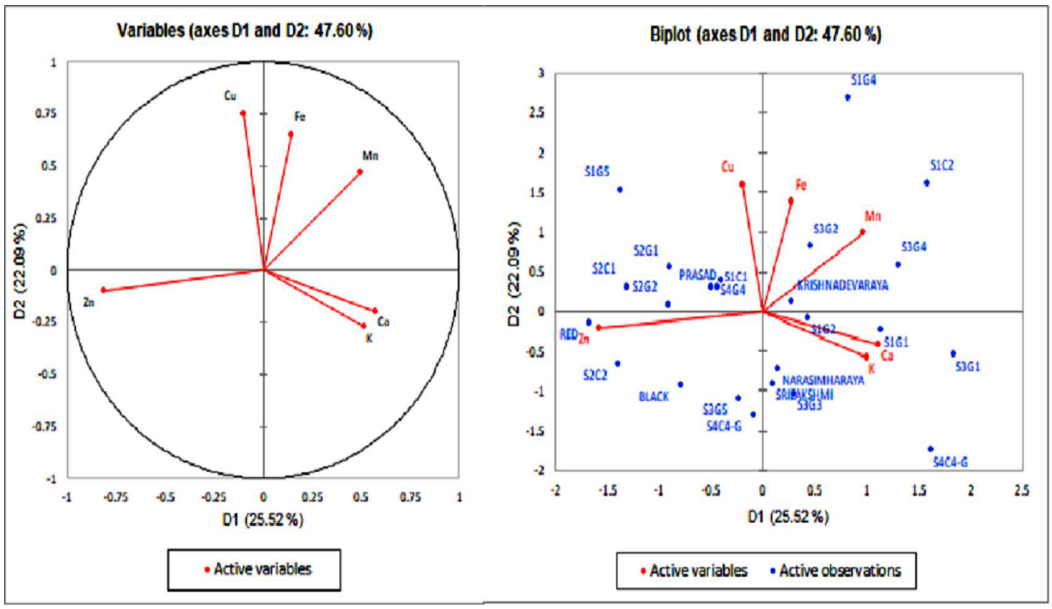

Fig 2: Scatter plot of variables and Biplot of 24 foxtail millet genotypes for first two Principal components contributing 47.60 per cent to total variability

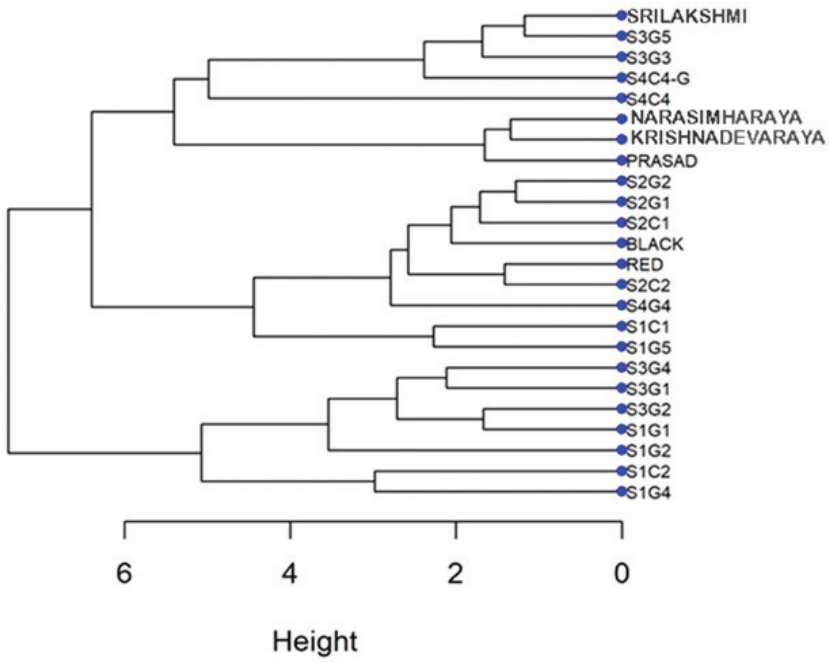

Fig 3: Hierarchical cluster dendrogram showing clusters in 24 Foxtail millet genotypes under various micro and macronutrient concentration

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G (13.13±0.57mg/100g). The grain Cu content of genotypes ranged from 0.60±0.10 mg/100g to 1.09±0.17 mg/100g. However, genotypes displayed non significance variation (P< 0.05) in Cu content. The greater amount of Cu observed in S1G5 (1.09±0.17 mg/100g), while the genotypes S1C2 and S1G4 had the medium range 1.05±0.21, 1.02±0.13 mg/100g respectively. The lowest concentration of Cu was recorded in the landrace S3G3 (0.60±0.10 mg/100g). Genotypes exhibited non-significance variation (P< 0.05) for manganese. The Mn concentration in genotypes ranged from 1.05±0.16 to 1.64±0.14 mg/100g. The genotype S3G4 has the highest Mn concentration, followed by S1C2, S3G2, S1G1, and S1G4. The genotype S4C4-G recorded the lowest concentration of Mn (1.05±0.16 mg/100g). Foxtail millet genotypes displayed a high significance of variation for potassium content (P< 0.05). The K content in foxtail millet germplasm was ranging from 219.43±0.32 mg/100g to 349.47±0.81 mg/100g. The landrace S1C2 accumulated the highest concentration (349.47±0.81), followed by Narasimharaya, Prasad, Srilakshmi, and S4C4. The genotype S1G5 accumulated a low concentration of K among all studied genotypes. Overall, the landraces S1C2, S3G4, S1G5 have the highest concentration of K, Mn, and Cu respectively.

Principal component analysis: The PCA analysis was applied to the determinate correlation of mean values of six micronutrients of the genotypes. The principal component analysis grouped the genotypes based on the variables of Iron, Zinc, Calcium, Potassium, Copper, and Manganese. PCA demonstrated that out of six; the first two principle components elucidated superiority of total variation. These two principal components have eigenvalue >1 and contributed 47.601% percent of total variability and were plotted to examine the relationships between different clusters with PC1 on X-axis and PC II on Y-axis (Fig. 2). Out of six PC's, PC I and PC II have eigenvalues of 1.557, and 1.299 respectively. PC III had the eigenvalue 0.964 and PC IV had the eigen value of 0.907 (Table 4). The PC I contributed maximum regards variability (25.953%) followed by PC II (21.648%), PC III (16.074%) and PC IV (15.112%). The first four components contributed for 78.78% the variability of all the data and the remaining two components contributed 21.22% of variability among the 24 genotypes for micronutrient and macronutrient concentration. In the present study, the PC I had the highest positive loading for Zinc (0.803) and higher negative loading for Manganese (-0.617), Calcium (-0.480). In PC II, Copper (0.745), Iron (0.569) has positive loadings and Potassium having a negative loading (-0.419). PC III attributed to Potassium (0.764), Iron (0.228), and Calcium (-0.559) with positive and negative loadings respectively. The PC IV consists of variables regards to Iron (0.622), Calcium (0.502) and Zinc (0.285) with positive loadings and Manganese (-0.414) negative loadings (Table 4).

The score values of each principal component for 24 foxtail millet genotypes were represented in Table 5. Based on the factor scores, the PC1 component explains that Zn concentrations are higher in RED followed by S2C2, S2C1, BLACK, and S2G2 and lower in S1C2, S1G4, S3G1, S3G4, S1G1, and S4C4. The PC II reveals that Cu, Fe, Mn and Zinc concentrations are higher in S1G4, S1G5, S1C2, S2G1, and S2C1 and lower in S4C4, S4C4-G, S3G1, and S3G3. The PC III loadings elucidated that concentrations of K, Fe, Zn and Cu are higher in Prasad, S4G4, S1C2, Narasimharaya, and S4C4-G, lower in S1G2, S4C4, S2G1 and S1C1. The PC IV loadings represent Fe, Ca, Zn and K concentrations are higher in Narasimharaya, Krishnadevaraya, S1G2, Prasad, S1C1 and S4C4-G, lower in S3G2, S4C4-G and S1G1.

In the present investigation, the principal component score plot of PC I and PC II differentiated the genotypes into four quadrants depending on the concentrations of six trace metals. Out of four quadrants based on the compositions of macro and micronutrients,

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genotypes were scattered into three quadrants in PC analysis. The genotypes on the top right quadrant represent Zinc concentration. The genotypes that were scattered in the top left quadrant were associated to Mn, Fe, Cu concentrations. The genotypes on the left bottom quadrant were corresponding to their Ca and K concentrations. However, the genotypes in the right bottom quadrant did not exhibit any associations in the measured traits.

Hierarchical cluster analysis: Further, to identify the genetic variability more accurately in the genotypes, the hierarchical cluster analysis was performed based on the concentrations of their macro and micronutrients by using R Program software package. The analysis in the form of dendrogram revealed that the investigated genotypes were separated with a euclidean distance ranging from 0 to 6; which indicates a high genetic variation between the genotypes. The dendrogram classified the genotypes into four main clusters as shown in Fig. 3. The first main cluster (I) consists of only two genotypes *i.e.*, S1G4, and S1C2. This cluster was characterized by genotypes with high Fe and K contents, moderate levels of Mn, Cu and Zn and lowest concentration of Calcium. Cluster II consists of genotypes S3G4, S3G1, S3G2, S1G1, and S1G2 with the moderate levels of all macro and micronutrients. However, the genotype S3G2 recorded lowest concentration of Ca, K and S3G4 has the highest Manganese content compared to other genotypes of Cluster II. Cluster III consists of eight genotypes namely, S1C1, S1G5, S4G4, S2C2, RED, BLACK, S2C1, S2G1, and S2G2. This cluster consists of genotypes with high levels of Fe, Zn, Cu, K, and Mn. The lowest calcium content genotypes were also identified in this group. The genotype S2C2 consists of a low level of Fe concentrations. The fourth cluster consists of eight genotypes namely PRASAD, KRISHNADEVARAYA, NARASIMHARAYA, S4C4, S4C4-G, S3G3, S3G5, and SRILAKSHMI. The three genotypes PRASAD, KRISHNA DEVARAYA, and NARASIMHARAYA were mainly separated from this cluster due to high levels of Fe, Zn, K, Mn and lowest contents of Ca and Cu. The genotype S4C4 was separated from this cluster due to highest concentration of Ca.

Discussion

Recently, millets are gaining importance due to their survivability under extreme environments, low glycemic index, gluten-free rich protein, and affluence in minerals, vitamins, and antioxidants (24). The seed mineral composition depends on the nature of the genotype, uptake, and acquisition, allocation capacity of minerals into seeds. Due to their cost effectiveness, the research community more focused on the enrichment of mineral nutrients in the millets through genetic improvement programs. Understanding the natural genetic variants for mineral nutrition and identification of genotypes with greater mineral content is an important step in this direction and landraces might serve as great primary resources. Until recently, the scientific community reluctant to use exotic germplasm due to the fear of loss of co-adapted gene complexes, linkage drag, and lengthened pre-breeding time (11). However, recent research community started focusing ion exotic germplasm for improvement of abiotic stress resistance, and improved nutritional capacity due to the advantage of improved plant phenotyping facilities, genomics resources and discovery, exploration of allelic diversity (11).

FTIR technique in combination with multivariate statistical analysis; has been successfully applied for chemical mapping of minerals in several agriculture products (17, 49). In a previous study, Fourier transform near infrared spectroscopy (FT-NIRS) was used to evaluating 259 foxtail millet genotypes of China, based on the analysis of Protein, Fat, Starch and Amino acids (51). Similarly, in the present study, FTIR analysis revealed the presence of organic and inorganic components in foxtail millet genotypes. The IR spectra as depicted in Fig.1, displayed the presence of biochemical constituents such as amino acids, carbohydrates, alkenes, proteins,

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Sulphur compounds, amines, and lipids in all of the genotypes. The absorption spectra 800-1500^{cm-1} is a highly overlapping region, which displays various peaks with complex spectra, and identification of specific peaks is very difficult (46). The location and glycosidic linkage bands also affect the presence of peaks in the fingerprint region. The infrared spectra of polysaccharides (amylose, amylopectin, cellulose, and starch) which are present in the fingerprint region are majorly evaluated, from the vibrational bands of monomeric unit glucose (8, 47, 47). The infrared bands 1022 $^{cm-1}$ and 1041 $^{cm-1}$ are attributed to amorphous structure and crystalline structure respectively, as reported previously in flours of raw foxtail millet and rice (48) (20). The genotypes S1G4, S3G2 and S3G5 displayed amorphous starch structure, which might contain more amylopectin, whereas genotype S2G1 displayed the characteristic features of crystalline starch. The IR peaks at 1022^{cm-1}and 1048^{cm-1}are associated to the C-O bond stretching of the C-O-C group in the anhydrous glucose ring (45). The IR region between1640-3300^{cm-1} indicates the moisture content; the presence of strong peaks in that region indicates the presence of moisture in flour (27). Moisture content can be detected by its OH stretching and H bending vibrations (10). However, it is very difficult to quantify moisture content based on IR spectral peaks as the other OH group- containing compounds such as alcohols, phenols, hydro -peroxides also interfere spectral bonding of H and OH groups (10). We did not observe any genotypic variation for moisture content in the present investigation. A strong absorption peak of the NH bond in the FTIR spectrum indicates the protein richness of the sample (3). We have observed a high genetic variation for the NH group among the studied genotypes. The presence of a strong absorbance peak of the NH group in S1G2, S4G4, Prasad and Narasimharaya signifies their protein richness in the flour. The presence of amide II spectral bond at 1550 $^{cm-1}$ in the landraces S1G2 and S4G4 mostly from the NH bending secondarily from the CN stretch effect indicates these landraces are rich in protein (3). The released cultivars Prasad and Narasimharaya have been considered as second-ranking genotypes with regards to protein content having an absorption peak at 1542.45 $^{cm-1}$ and 1542 $^{cm-1}$ respectively. The landrace S4C4-G, considered as low protein rich genotype having a very weak NH bond in its spectrum of flour. These results are in agreement with the wheat genotypes (3). FTIR spectroscopy has been widely used as a quality control method to detect fat and moisture in high-fat products (44). The absorption spectra in the range of 1600 $^{cm-1}$ to 1700 $^{cm-1}$ and 1550 $^{cm-1}$ to 1570 $^{cm-1}$ indicate the presence of fat. In the present investigation, we have not observed genotypic variation for fat content among the studied genotypes. The IR peak 1648.28^{cm-1}, 1658.84^{cm-1}and 2923.12 $^{cm-1}$indicates the presence of carbonyl group, amines 1 bond, and C-H compound (regards lipids) respectively. All the investigated genotypes exhibited similar kinds of peaks for carbonyl groups, amines 1 and lipids. These results are in agreement with the data obtained for finger millet flour and pearl millet flour (14).

Principle component analysis is one of the important statistical techniques, which can summarize the data from complex data sets (6). The PCA of six characteristics such as Fe, Zn, Ca, K, Mn, and Cu considered for the total variability. The eigenvalue of PCA, which is greater than 1, recognized as significant, and the PCA were greater than ±0.3 identified as meaningful (16). In the present investigation, PCA delivered a high variability among the studied foxtail millet genotypes in the range of 78.78%, which indicates a considerable level of genotypic variation among the genotypes for mineral content. PCA can analyze the genotypes through verification of studied variables such as Fe, Zn, Ca, K, Mn and Cu as explained by the first two principal components. The first principal component occupies most of the variation in the analysis as possible. The second principal component displays much longer variation and mostly incorporeal to the PC I. By analyzing PC I and PC II, one can explain the correlation between

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Table 4: Principal component analysis of six mineral elements in foxtail millet genotypes showing factor loadings, Eigen values and their percentage contribution to the total variation.

Factor loadings	F1	F2	F3	F4
Fe	-0.344	0.569	0.228	0.622
Zn	0.803	0.168	0.066	0.285
Ca	-0.480	-0.369	-0.559	0.502
K	-0.403	-0.419	0.764	0.101
Mn	-0.617	0.284	-0.105	-0.414
Cu	-0.146	0.745	0.004	-0.076
Eigen value	1.557	1.299	0.964	0.907
Variability (%)	25.95	21.64	16.07	15.11
Cumulative (%)	25.95	47.60	63.67	78.78

variables (macro and micronutrients), factor loadings, factor scores, interpretation of genotypes in the scattered plot, similarities and differences (CAMO SOFTWARE AS, 1998). In the present investigation, high loadings and high factor scores are obtained for some variables and low loadings and low factor scores are obtained for other variables (Table 4). The high loading present in particular variables involved a greater contribution to the variation among studied genotypes. The distance between the positions of any two genotypes on the score plot is directly proportional to the degree of similarity or difference between them. Therefore, the genotypes are scattered to very close proximity having similar variation, genotypes near the origin having distinctive variation and far from the central axis of score plot having an extreme variation. The extreme genotypes are useful for breeding programs due to differentiation in their biochemical concentrations compared to other genotypes. The genetic diversity of studied foxtail millet genotypes were categorized based on the mineral concentration as followed; the landraces RED and S2C2 for Zn, landraces S1G4 and S1G5 for Cu, Fe, and Mn, released cultivar Prasad and landrace S4G4 for K, released cultivars Narasimharaya, Krishnadevaraya, and S1G2 for high calcium.

Cluster analysis is a diagrammatic representation and reliable visual method to analyze the complex union of multiple traits and differentiation in the genotypes at various mineral nutrition concentrations. ICP-OES coupled with multivariate statistical analysis was used in several crop species for seed mineral content. Shergo et al., (2013) conducted a genetic diversity study for characterization of mineral, total starch, total sugar and protein contents using multivariate statistical analysis for the selection of parents for hybridization (37). Multivariate analysis was employed to study the genetic divergence among pearl millet germplasm based on their agro morphological traits and grain nutritional values (5). The genetic diversity studies were also carried out in indigenous germplasm lines of finger millet for their mineral nutrients using multivariate analysis (4). Cluster analysis measures the data based on the genetic distance among the landraces and released cultivars (26). Previously, a similar study was conducted for micro and

Table 5: The scores of the four rotated principal components.

Factor scores	F1	F2	F3	F4
S1G1	-1.249	-0.668	-1.081	-0.954
S1G2	-0.486	-0.244	-1.574	1.601
S1G4	-2.039	2.613	-0.004	0.345
S1G5	1.012	2.164	-1.057	-0.395
S1C1	0.323	0.563	-1.200	0.970
S1C2	-2.521	1.175	1.350	-0.880
S2G1	0.846	0.922	-0.929	0.109
S2G2	1.045	0.422	-0.817	-0.611
S2C1	1.419	0.818	0.235	0.178
S2C2	1.908	-0.203	-0.624	-0.626
S3G1	-1.951	-1.252	-0.761	-0.001
S3G2	-0.869	0.717	-0.599	-1.388
S3G3	0.047	-1.217	0.211	-0.861
S3G4	-1.778	0.152	0.437	-0.606
S3G5	0.708	-1.100	1.004	-0.801
RED	2.028	0.461	0.453	-0.085
BLACK	1.297	-0.710	-0.811	0.078
S4G4	0.471	0.530	1.438	-0.128
S4C4-G	0.620	-1.364	1.122	-1.227
S4C4	-1.215	-2.471	-1.212	0.952
Srilakshmi	0.244	-1.008	0.683	-0.781
Prasad	0.391	0.502	1.458	1.558
Krikrishnadevaraya	-0.374	0.030	0.897	1.697
Narasimharaya	0.123	-0.831	1.383	1.858

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macronutrient analysis using a multivariate statistical analysis of 60 pearl millet genotypes (22). In the present investigation through PCA and dendrogram of clusters, it was identified that genotypes included in this study displayed high genetic variability for seed mineral content and might serve as valuable sources for the foxtail millet improvement programs. As the studied genotypes dispersed into different clusters, they might belong to highly heterotrophic groups for breeding programs. Therefore, the present study genetic diversity analysis of foxtail millet genotypes would be crucial for foxtail millet breeders to identify genotypes to enhance their nutritional importance.

Conclusion

The present study represents a comprehensive comparison of the foxtail millet landraces and released cultivars for their micronutrient content through ICP-OES and FTIR coupled with multivariate statistical analysis. The data generated in this study would be not only helpful for the identification of elite genotypes regarding seed mineral content, but also to tap the alleles responsible for high mineral content in foxtail millet. Our findings highlighted the importance of landraces that are being underutilized earlier in the breeding programs.

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