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Characterization of finger millet germplasm for mineral contents: Prospects for breeding

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Knowledge of existing genetic variability is essential for initiating a successful breeding program. A set of 628 finger millet accessions comprising accessions from the core collection, farmer preferred and improved varieties released in Kenya, Tanzania and Uganda were profiled for nutrient content. Accessions showed very high variability for the different nutrient contents. Local cultivars and varieties released in the ESA region had significantly lower levels of the main essential nutrients (Ca, Fe, Zn) found in finger millet. Country of origin was highly significant for all the nutrients, with accessions from eastern and southern Africa having significantly lower nutrient contents. Grain color was associated with nutrient content with darker grains having higher compared to white colored. All nutrients were positively correlated (P<0.001) to each other. Grain yield was not significantly correlated to any nutrient content. The substantial variability for the grain nutrients observed in the finger millet core collection and local germplasm indicates the possibility for the selection of nutrient-rich accessions for use in the breeding programs.

Key words: Finger millet, micro and macro nutrients, diversity, grain color, glume cover, malnutrition.

INTRODUCTION

Finger millet (*Eleusine coracana* L. Gaertn.) indigenous to eastern Africa is a staple crop upon which millions of people depend on for food and income in rural households. It is adapted to adverse agro-ecological conditions and require minimal input (Adekunle, 2012). This African native crop probably originated in the highlands of Uganda and Ethiopia, where farmers have been growing it for thousands of years (Hilu and de Wet, 1976b). It ranks fourth in importance among millets in the world after sorghum (Sorghum bicolor), pearl millet (*Pennisetum glaucum*) and fox tail millet (*Setaria italica*) (Upadhyaya et al., 2007a). Finger millet is widely cultivated in Africa and south Asia under varied agro-climatic conditions (Dida et al., 2008). In Africa, it is extensively cultivated in Uganda, Kenya, Tanzania, Ethiopia, Rwanda, Burundi, Zambia and Malawi (Mnyenyembe and Gupta, 1998; Obilana et al., 2002). In south Asia, finger millet is widely cultivated in India and Nepal (Upadhyaya et al., 2007b). Wide adaptability (Upadhyaya et al., 2007b), higher nutritional quality

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(Gopalan et al., 2002), higher multiplication rate and longer shelf life (Iyengar et al., 1945), make finger millet an ideal crop for use as a staple food and for famine reserve. The crop has dual importance as source of food grain as well as straw for fodder. Finger millet is a rich source of calcium (Ca) (344 mg/100 g) which is 5 to 30 times more than in most cereals, making it the richest plant source (Gopalan et al., 2002; Gupta et al., 2017, Ceasar et al., 2018; Kumar et al., 2018). The grain has a fair amount of protein (7.3 g/100 g) (Malleshi and Klopfenstein, 1998; Sharma et al., 2017) and dietary fibre (15-20%) (Chethan and Malleshi, 2007). Finger millet carbohydrate has unique property of slower digestibility making it a food for long sustenance. All these, deficient in most cereals are crucial to human health and growth, qualify finger millet as an important crop against malnutrition.

Intake of diet poor in iron (Fe), zinc (Zn) and protein is the major cause for micronutrient and protein malnutrition. Fe deficiency leads to anemia. About 79% of the pre-school children between 6 and 35 months of age and 56% of women between 15 and 49 years of age are anaemic in poor countries (Krishnaswamy, 2009). Protein deficiency causes retarded physical and mental growth. Zinc deficiency leads to diarrhea, pneumonia and reduced immunity to diseases, and increased infant mortality (Gibson et al., 2008). Deficiencies of Fe and Zn are widespread worldwide (FAO/WHO, 2001; Cakmak, 2008) especially in sub-Saharan Africa and south and southeast Asia (Reddy Belum et al., 2005). In its report, FAO (2008) singled out sub-Sahara Africa as having the highest prevalence of under nutrition in the world, with one in three people being chronically hungry. A large proportion of people in this part of Africa especially in the rural communities are poor and live on a diet composed primarily of staple foods prepared from cereals (Oniang'o et al., 2003).

Finger millet being a promising source of micronutrients and protein (Malleshi and Klopfenstein, 1998) besides energy, can make a contribution to alleviating micronutrient and protein malnutrition (Underwood, 2000). Because of its high nutrient contents, finger millet is gaining importance in east and southern Africa for its potential use in the preparation of a variety of foods such as porridge, bread, biscuits, pastas, instant baby food, and composite flour (Dendy, 1993; Senthil et al., 2005). The high proportion of carbohydrates in form of non-starchy polysaccharides and dietary fibers in finger millet grain helps in reducing cholesterol. Slow release of glucose during digestion makes it suitable for diabetic patients. The nutritional quality of finger millet grain makes it an ideal food for expectant women, breast-feeding mothers, children, the sick, and diabetics (National Research Council, 1996). This in addition to the high quality protein content makes finger millet a “super crop” in nutritional terms.

The most cost effective approach for mitigating micronutrient and protein malnutrition is to introduce finger millet varieties selected and/or bred for increased Ca, Fe, Zn and protein contents. Plant breeding approach scores over others (such as food fortification, micronutrient supplements, dietary strategies and medical interventions) because it complements the existing approaches to combat micronutrient deficiency. It does not require any special program to change the behavior of farmers/consumers. Cultivars rich in Ca, Fe, Zn and protein with farmer preferred grain quality and adaptation traits are readily accepted (Welch and Graham, 2004; Graham et al., 2007; Pfeiffer and McClafferty, 2007; Prasad, 2010). Furthermore consumption of biofortified foods does not have side effects such as change in taste, bioavailability and risk of developing disease usually associated with inorganic fortification and taking of supplements (Bolland et al., 2010; Institute of Medicine, 2011).

Attempts to breed finger millet for enhanced grain micronutrient and protein contents are still in its infancy stage. Exploitation of existing variability among germplasm accessions is the first step and short-term strategy for developing and delivering micronutrient and protein-dense finger millet cultivars to address the micronutrient and protein malnutrition in the target population (Upadhyaya et al., 2010). International Crop Research Institute for Semi-Arid Tropics (ICRISAT) has the world mandate for finger millet research, with some of its core activities being the collection, characterization, preservation and distribution of germplasm. The ICRISAT genebank at Patancheru (India) holds 5940 accessions of finger millet from 23 countries. Using 14 quantitative traits data on these accessions, Upadhyaya et al. (2006) established a core collection in finger millet, which consists of 622 accessions representing geographical regions and biological races from the entire collection. Accessions from Africa (58.7%) and Asia (35.8%) were predominant in the core, while those from America and Europe were represented by 0.8 to 1.1% only. The subsp. coracona accessions were represented by 97.4%, while those from subsp. africana were 2.6% only (Upadhyaya et al., 2010). A set of 590 accessions from the core collection, 30 farmer preferred varieties and 8 released varieties from the east and southern Africa region were used in the study.

The objective of this present research study was to assess the finger millet core collection, farmer preferred varieties and improved varieties released in the east and southern Africa region for grain mineral content and prospects for breeding for increased Ca, Fe, Zn and protein grain content.

MATERIALS AND METHODS

The material for the study consisted of 590 accessions from the finger millet core collection (Upadhyaya et al., 2006), 30 farmer preferred cultivars and 8 released varieties. Of the released varieties, four are released in Kenya (Okhale 1, Nakuru FM 1, Ikhulule and P224), five in Uganda (Ending, Engeny, Gulu E, U15 and P224) and two in Tanzania (U15 and P224). The material was planted in Kenya at the ICRISAT-Kiboko, field station during the 2013 long rains for evaluation and generation of samples for analysis. ICRISAT-Kiboko is located at altitude 960 m above sea level, Latitude 2° 20' S and Latitude 37° 45' E. The accessions were planted in an augmented design (Federer, 1961) with released varieties P224 and U15 and farmer preferred varieties, Nakuru FM 1, Engeny and Okhale 1 used as checks. Each test entry was planted in a single row of 4 m length, with inter-row spacing of 0.4 m and intra-row of 0.1 m with two replications. The experimental plots were maintained weed and insect pest free. Fertilization was done at the rate of 20 kg N/ha and 50 kg P/ha at planting and 50 kg N/ha was applied at top dressing 30 days after planting.

In the field, the accessions were evaluated for the following agronomic traits-plant vigor, days to flowering (DAF), plant height, and number of productive tillers. After harvesting they were evaluated for grain related traits-grain color, glume cover and grain yield. Grain from the two replicates of each accession was homogenously mixed and a representative sample taken for analysis. Freshly harvested grain samples were sent to the Central Analytical Services laboratory in ICRISAT, Patancheru, India for the nutrient analysis using atomic absorption spectrophotometer (Sahrawat et al., 2002). Great care was taken to avoid contamination of grain samples during preparation, handling and shipping. Nitrogen content was determined, and converted to protein percentage weight (% wt) by a factor of 6.25 (Jones, 1941).

Analysis of variance was performed using PROC MIXED (sas V 9.4, SAS Institute Inc. 2017), considering both region and country as fixed. In order to pool the data across the countries, individual country variances were modelled to error distribution using restricted maximum likehood (REML) procedure. Pairwise comparison of means was performed for significant region effect. To analyze for association between grain nutrient contents and grain color, the accessions were classified into five categories as Purple Brown, Dark Brown, Copper Brown, Light Brown, and White. For association with geographical origin, the accessions were classified into seven regions of collection as those from Asia, East Africa, Europe, South Africa, USA, West Africa and Unknown. Pearson correlations were estimated among various micronutrients and macronutrients and between the nutrients and different agronomic traits. Based on 10 nutrients (Ca, Cu, Fe, K, Mg, Mn, P, S, Zn and Protein) and 23 country origins, accessions were grouped into different clusters. This was done following Ward's method based on Euclidean distance matrix using SAS cluster procedure (SAS V9.4) (Spark, 1973; Fundora et al., 2004).

RESULTS AND DISCUSSION

Nutrient content

Very high variability was observed in all the quality traits determined, iron ranged from 1.37 to 30.04 mg/100 g, potassium from 45.0 to 1427. 0 mg/100 g. Magnesium ranged from 53.0 to 217.8 mg/100 g, manganese from 4.40 to 22.07 mg/100 g, phosphorous from 7.10 to 380.3 mg/100 g, sulphur from 54.8 to 191.7 mg/100 g and zinc from 0.04 to 3.73 mg/100 g. Protein content ranged from 0.50 to 10.10% weight with a mean of 5.86% wt. The highest diversity was observed in potassium ($\sigma 2 = 10721.0$), followed by calcium ($\sigma 2 = 3633.0$), phosphorous ($\sigma 2 = 2111.0$); while the least diversity was observed in Copper ($\sigma 2 = 0.08$) and Zinc ($\sigma 2 = 0.20$).

Calcium content

Calcium values ranged from 155.5 to 554.0 mg/100 g with a mean of 321.5 mg/100 g. A number of accessions recorded higher Ca content than levels cited in literature of 350 mg/100 g (United States National Research Council/National Academy of Sciences (1982). Accessions with the highest Ca content included: IE 2008 with 544 mg/100 g, IE 6541 with 483 mg/100 g, IE 3038 with 475 mg/100 g, IE 4491 with 471 mg/100 g, IE 2644 with 468 mg/100 g, KNE 392 with 466 mg/100 g, IE 595 with 466 mg/100 g, KNE 1149 with 466 mg/100 g, IE 3489 with 460 mg/100 g and IE 6013 with 460 mg/100 g (Table 1). The farmer preferred and released varieties Okhale 1 Ending, Nakuru FM 1, P224 and U15 had low Ca values of 230, 248, 293, 311 and 319 mg/100 g, respectively. They all fall below the conventionally accepted Ca values for finger millet and below the accessions mean of 321. 5 mg/100 g. Okhale 1 the variety with the lowest estimated Ca content is a popular variety in western Kenya. Ending variety with the second lowest Ca content is a farmer local variety preferred by many farmers in eastern and northern Uganda.

The high Ca content accessions also compared well with the adapted varieties in Zn and Protein content, maturity period (days to flowering (DAF)) and grain yield. These accessions are being tested further for adaptation for possible release and have also been incorporated into the Ca biofortification breeding programs in the region. The use of these accessions as donors is also likely to increase the amounts of Fe and Zn in the progeny. Upadhyaya et al. (2010), working on the same core collection in India found accessions to have Ca range of 386 to 489 mg/100 g with a mean of 430 mg/100 g, which is narrower than was found in this study but falls within the range. The higher range in the present study was mainly due to inclusion of the locally cultivated and released varieties which had low mineral content.

In several studies on estimation of calcium content in different genotypes of finger millet, high calcium values have been reported (Sharma et al., 2017). Calcium content was found to vary from 162 to 487 mg/100 g with a mean value of 320.8 mg/100 g grain in 36 genotypes of finger millet (Vadivoo et al., 1998), 293 to 390 mg/100 g in six varieties of finger millet (Babu et al., 1987), and 50 to 300 mg/100 g in another set of six varieties (Admassu et al., 2009). Furthermore, very high calcium content, 450 mg/100 g (Panwar et al., 2010) and 489 mg/100 g (Upadhyaya et al., 2011) has been reported in few finger millet genotypes. These studies are in agreement with the current study that high variability in Ca exists in finger millet germplasm.

Iron content

Very high Fe values were estimated for the accessions as compared to the often cited value of 3.9 mg/100 g

Table 1. Performance of the ten (10) calcium most dense accessions for grain yield, grain quality and nutrient content.

Accession	Origin[a]	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Protein (% wt)	Grain yield (t/ha)	DAF[b]	Glume cover[c]	Grain color[d]
IE 2008	India	544	8.81	2.12	7.4	2.18	68	3	5
IE 6541	Nigeria	483	5.23	1.71	5.1	2.79	84	2	5
IE 3038	India	475	8.28	1.31	7.1	2.86	67	3	5
IE 4491	Zimbabwe	471	5.53	2.02	9.1	3.13	62	3	4
IE 2644	Malawi	468	9.17	1.60	7.5	2.21	67	3	5
KNE 392	Kenya	466	7.79	2.50	8.3	3.09	72	3	4
IE 595	India	466	7.84	1.41	7.3	1.87	58	3	4
KNE 1149	Kenya	466	10.18	2.55	10.0	2.15	72	3	4
IE 3489	Kenya	461	5.11	1.46	4.5	2.14	69	2	3
IE 6013	Nepal	460	4.33	1.10	6.8	1.63	50	3	5
P224	Uganda	311	4.63	0.73	5.8	3.49	69	2	3
U15	Uganda	319	4.42	0.86	5.4	3.34	65	2	3
Ending	Uganda	248	5.24	1.48	7.3	3.22	64	2	3
Okhale-1	Nepal	230	4.02	1.45	5.8	1.19	76	2	4
Nakuru FM 1	Kenya	293	8.54	0.83	4.8	3.56	77	2	4

[a]Origin, Country of origin; [b]DAF, days from planting to flowering; [c]Glume cover, glume covering scored on a scale of 1-5 where 1=Exposed, 2= Intermediate, 3=Enclosed; [d]Grain color, assessed on a scale of 1-5 where 1=White, 2=Light Brown, 3=Copper Brown, 4=Dark Brown, 5=Purple Brown.

Table 2. Performance of the ten (10) iron most dense accessions for grain yield, grain quality and nutrient quality.

Accession	Origin[a]	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Protein (% wt)	Grain yield (t/ha)	DAF[b]	Glume cover[c]	Grain color[d]
KNE 628	Kenya	249	30.04	1.71	7.9	3.19	77	3	2
Acc 32	Unknown	267	26.83	1.15	5.1	3.30	65	3	4
IE 6321	Zimbabwe	273	24.54	0.92	5.8	2.90	69	2	4
IE 4245	Zimbabwe	292	23.84	1.06	5.4	2.88	69	3	4
IE 2818	Nepal	331	23.57	1.11	7.2	2.28	79	3	5
IE 5831	Nepal	367	21.78	1.75	7.6	1.96	63	3	4
IE 2014	India	413	21.33	1.91	9.1	3.03	79	3	4
IE 5485	India	424	21.31	1.39	7.9	2.74	70	3	4
IE 3704	Uganda	299	21.29	1.29	4.5	2.56	73	2	2
IE 3780	Uganda	345	20.54	1.10	4.0	2.74	75	2	3
P224	Uganda	311	4.63	0.73	5.8	3.49	69	2	3
U15	Uganda	319	4.42	0.86	5.4	3.34	65	2	3
Ending	Uganda	248	5.24	1.48	7.3	3.22	64	2	3
Okhale-1	Nepal	230	4.02	1.45	5.8	1.19	76	2	4
Nakuru FM 1	Kenya	293	8.54	0.83	4.8	3.56	77	2	4

[a]Origin, Country of origin; [b]DAF, days from planting to flowering; [c]Glume cover, glume covering scored on a scale of 1-5 where 1=Exposed, 2= Intermediate, 3=Enclosed; [d]Grain color, assessed on a scale of 1-5 where 1=White, 2=Light Brown, 3=Copper Brown, 4=Dark Brown, 5=Purple Brown.

(United States National Research Council/National Academy of Sciences, 1982). Ten of the best accessions for Fe content had values five times higher than the cited Fe content (Table 2). They include KNE 628 (30.04 mg/100 g), Acc 32 (26.83 mg/100 g), IE 6321 (24.54 mg/100 g), IE 4245 (23.84 mg/100 g), IE 2818 (23.57 mg/100 g), IE 5831 (21.78 mg/100 g), IE 2014 (21.33 mg/100 g), IE 5485 (21.31 mg/100 g), IE 3704 (21.29 mg/100 g) and IE 3780 (20.54 mg/100 g). Released and farmer preferred varieties had values higher than the accepted, but four times lower than those of the high Fe content accessions. Three of the high Fe content accessions Acc 32, KNE 628 and IE 2014 compared favorably well in agronomic traits (yield, DAP, plant height) with the released and farmer preferred varieties and are now being fast tracked for release. Ten high Fe

Table 3. Performance of the ten (10) zinc most dense accessions for grain yield, grain quality and nutrient quality.

Accession	Origin[a]	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Protein (% wt)	Grain yield (t/ha)	DAF[b]	Glume cover[c]	Grain color[d]
IE 6952	Zambia	293	18.43	3.73	5.5	2.48	68	3	3
IE 6705	Zaire	377	9.73	3.60	5.5	0.63	79	3	4
IE 3663	Uganda	293	9.80	3.28	3.9	2.14	70	2	4
Acc 32 FMB/01 WK	Unknown	287	6.86	2.93	9.4	3.30	65	3	4
IE 4181	Uganda	255	8.78	2.68	4.8	1.85	71	3	4
KNE 1149	Kenya	466	10.18	2.55	10.0	2.15	72	3	4
IE 5806	Nepal	456	13.67	2.51	8.3	2.61	63	3	4
KNE 392	Kenya	466	7.79	2.50	8.3	3.09	72	3	4
KNE 741	Kenya	356	9.39	2.44	9.8	2.64	55	3	5
IE 6033	Nepal	454	14.84	2.35	7.2	2.88	74	3	4
P224	Uganda	311	4.63	0.73	5.8	3.49	69	2	3
U15	Uganda	319	4.42	0.86	5.4	3.34	65	2	4
Ending	Uganda	248	5.24	1.48	7.3	3.22	64	2	3
Okhale-1	Nepal	230	4.02	1.45	5.8	1.19	76	2	4
Nakuru FM 1	Kenya	293	8.54	0.83	4.8	3.56	77	2	4

[a]Origin, Country of origin; [b]DAF, Days from planting to flowering; [c]Glume cover, glume covering scored on a scale of 1-5 where 1=Exposed 2= Intermediate 3=Enclosed; [d]Grain color, assessed on a scale of 1-5 where 1=White, 2=Light Brown, 3=Copper Brown, 4=Dark Brown; 5=Purple Brown.

content accessions have been incorporated into the breeding program.

Zinc content

Highest ten Zn content accessions had values ranging from 2.35 to 3.73 mg/100 g with the highest being IE 6952, IE 6705, IE 3663 Acc 32 FMB/01 WK and IE 4181 with concentrations of 3.73, 3.60, 3.28, 2.93 and 2.68 mg/100 g, respectively (Table 3). As with previous nutrients, released and farmer preferred varieties had lower nutrient levels with P224 having the lowest concentration of 0.73 mg/100 g.

The aforementioned results for the micro-nutrients compare well with other crops. CIMMYT Scientists assessed 132 wheat accessions in Mexico and found grain Fe concentrations to range from 2.88 to 5.65 mg/100 g with a mean of 3.72 mg/100 g (Graham et al., 1999; Monasterio and Graham, 2000). Zinc concentrations ranged from 2.52 to 5.33 mg/100 g with a mean of 3.50 mg/100 g. They observed that there was enough genetic variation existing within the wheat germplasm to increase Fe and Zn concentrations substantially in wheat grain. Scientists at IRRI working on 939 rice accessions found Fe concentration to range from 0.75 to 2.44 mg/100 g. Zinc on the other hand ranged from 1.35 to 5.8 mg/100 g (Graham et al., 1999). Within the genotypes tested, there was about a 4-fold difference in Fe and Zn concentrations suggesting genetic potential to increase the concentrations of these micronutrients in rice grain. They also found that the varieties with high Fe concentrations also contained the highest grain-Zn. Additional data demonstrated that high-Fe and high-Zn grain traits are expressed in all rice environments tested and imply the two can be increased concurrently (Welch and Graham, 2004). Researchers at CIAT (Beebe et al., 2000) evaluated a core collection of over 1000 common bean (*Phaseolus vulgaris* L.) accessions in the field in one season. They found Fe concentrations to range from 3.4 to 8.9 mg/100 g with average of 5.5 mg/100 g (Graham et al., 1999). They observed that there was sufficient genetic variability to increase Fe concentrations significantly (by about 80%) and Zn (by about 50%) in common beans.

Protein content

Protein content ranged from 2.80 to 10.10% weight with a mean of 5.86% wt. Finger millet is reported to have on average 7% protein but large

Table 4. Comparison of mean grain micro and macro nutrient content (mg/100 g) and protein (% wt) content in finger millet accessions based on region of origin.

Region	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorous	Potassium	Protein	Sulphur	Zinc
Asia	293.33	0.52^{ab}	6.07	117.29	11.60^{a}	224.41^{a}	554.69	5.51	89.05	0.97
East Africa	300.34	0.29^{bc}	7.15	115.73	11.08^{ab}	213.24^{ab}	527.26	6.04	93.71	1.01
Europe	350.33	0.41^{abc}	6.00	140.67	9.49^{b}	236.33^{a}	497.00	6.68	116.33	1.20
Southern Africa	303.93	0.28^{c}	6.01	115.86	10.46^{b}	192.99^{b}	524.66	5.48	94.54	0.84
USA	310.40	0.64^{a}	5.28	125.00	11.52^{ab}	235.40^{a}	595.00	6.72	105.20	1.11
Unknown	319.39	0.66^{a}	6.97	118.90	11.10^{ab}	214.97^{ab}	502.51	5.93	98.41	1.04
WA	401.40	0.52^{ab}	7.73	131.00	13.04^{a}	215.40^{ab}	507.20	5.49	99.00	1.15

variations in protein content from 5.6 to 12.70% have been noted (Ravindran, 1991; Rao, 1994; Marimurthu and Rajagopalan, 1995; Antony et al., 1996; Vadivoo et al., 1998; Mushtari, 1998; Gautam, 2000; Bhatt et al., 2003). Singh and Srivastava (2006) analyzed 16 finger millet varieties and found out that protein content ranged from 4.88 to 15.58% wt with a mean value of 9.73% wt. Vadivoo et al. (1998) analyzed 36 genotypes of finger millet and reported their protein content in the range of 6.7 to 12.3 mg/100 g with the mean of 9.7 mg/100 g. These show that the protein content in the test material was lower than that reported earlier and need to be enhanced. However, a number of accessions possessed high protein content and are being used as donor parents.

The first step in breeding crops for better nutrition is to evaluate the genetic diversity of available germplasm for target nutritional traits. This study revealed very high variability in the main micro nutrients available in finger millet namely Ca, Fe and Zn. It also revealed that the commonly cultivated varieties are low in these nutrients, which seems to be the trend with other staple crops, like the case of pearl millet in India (Rai et al., 2013). Identified accessions with high nutrients have been used as donors to improve nutrient levels in adapted low nutrient farmer preferred varieties. Accessions IE 2008, IE 6541, IE 3038, IE 4491, IE 2644 and KNE 392 have been incorporated into the biofortification program as donors for Ca. Accessions KNE 628, Acc. 32, IE 6321, IE 4245 and IE 2014 for Fe; and IE 6952, IE 3663, Acc 32 FMB/01 WK, KNE 1149 and IE 5806 for Zn. Three accessions IE 2034, KNE 1149 and KNE 741 are being used as donors for protein.

Nutrient composition by region of collection (origin)

Nutrient concentrations differed with region of collection (Region), though no particular region had accessions superior in all the nutrients (Table 4). Of the main micro nutrient of interest in finger millet (Ca), accessions from West Africa had the highest mean concentration of 401.4 mg/100 g followed by Europe (350.3 mg/100 g) and Unknown (319.4 mg/100 g), while east and southern Africa regions had about the lowest of 300.0 and 303.9 mg/100 g, respectively. Accessions collected from West Africa had the highest mean Fe concentration of 7.73 mg/100 g with east Africa faring well with 7.15 mg/100 g. Not much diversity was expressed in the Zn concentration. Accessions collected from USA had the highest average protein content (6.72% wt) while those from West Africa (5.79% wt) and southern Africa (5.48% wt) had the lowest content (Table 4). Upadhyaya et al. (2010) working on similar material in India found that comparison of mean grain nutrients contents of the accessions classified by geographic origin indicated poor evidence for the relationship of grain nutrient contents with geographical origin. The highest diversity for grain nutrient contents is likely to be present in Africa and Asia, the primary and secondary centers of origin of finger millet, respectively (Hilu and de Wet, 1976a; Dida et al., 2008). Barbeau and Hilu (1993) found significantly higher Ca content (515 mg/100 g) in finger millet accessions from Ethiopia compared to those from Kenya (401 mg/100 g) and India (375 mg/100 g).

Effect of color on nutrient composition

Mean values of the different nutrient contents showed color to be associated with their concentration, with dark colored grains having highest concentration (Table 5). White colored grains had a mean of 296.6 mg/100 g Ca compared to 333.8 mg/100 g in purple colored grains, 0.33 mg/100 g Cu in white and 0.49 mg/100 g in purple colored while Fe was 5.17

Table 5. Comparison of mean grain micro and macro nutrient content (mg/100 g) and protein content (% wt) in finger millet accessions based on grain color.

Color	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorous	Potassium	Protein	Sulphur	Zinc
White	296.6	0.33	5.17	112.0	9.9	198.3	561.2	5.74	94.3	0.74
Copper brown	317.7	0.40	6.30	121.6	10.6	204.2	503.1	5.59	94.2	1.04
Light brown	306.5	0.65	7.65	123.7	10.7	220.8	526.8	6.11	97.7	1.23
Dark brown	323.4	0.45	6.88	126.5	11.0	216.3	533.8	5.93	97.7	1.10
Purple brown	333.8	0.49	7.50	136.9	12.4	244.6	566.3	6.33	102.0	1.22

Table 6. Analysis of variance (ANOVA) for different minerals by region.

Effect	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorous	Potassium	Protein	Sulphur	Zinc
	Fixed Effect (F-value)									
Region	2.72	8.66**[a]	0.93	0.39	3.72*[b]	5.04*	1.58	1.39	2.09	1.24
Origin(Region)	11.74**	29.41**	2.12	9.41**	7.38**	2.39	1.46	5.37*	11.25**	22.66**
Error	2867.02	0.06	10.83	621.10	3.94	1345.42	8849.51	1.60	314.25	0.15

[a]Significant at Prob<0.01; [b]Significant at Prob<0.05.

mg/100 g in white and 7.50 mg/100 g in purple brown colored grains. Magnesium was 112.0 mg/100 g and 136.9 mg/100 g, Mn 9.9 mg/100g and 12.4 mg/100 g, P 198.3 mg/100 g and 244.6 mg/100 g in white and purple brown colored grains respectively. On the other hand K had 561.2 and 566.3 mg/100 g, S 94.3 and 102.0 mg/100 g and Zn 0.74 and 1.22 mg/100 g for the white colored and the purple brown colored grains, respectively. Protein content was estimated at 5.74% wt for the white colored grains and 6.33% wt for the purple brown colored grains. In all the nutrients there was a gradual increase in grain nutrient content from white to purple brown colored grains indicating that color can be a good indicator for grain micro and macro nutrient and can be used for selecting for all the nutrient traits. Vadivoo et al. (1998) working on 36 finger millet genotypes reported that the protein content of brown seeded types was higher than white seeded type. Similar findings were reported by Samantaray and Samantaray (1997).

Glume covering

Glume covering was found to be associated with nutrient content. Accessions with enclosed grains had higher nutrient content (Ca=336 mg/100 g, Fe=6.80 mg/100 g, Zn=1.13 mg/100 g and protein=6.00% wt) compared to intermediate covered (Ca=313 mg/100 g, Fe=6.32 mg/100 g, Zn=1.03 mg/100 g and protein=5.60% wt).

Analysis of variance (ANOVA)

ANOVA revealed country of origin (Country) to be highly significant (P≤0.01) for calcium, copper, magnesium, manganese, sulphur and zinc. It was significant (P≤0.05) for protein and not significant for Manganese (Table 6). Region was highly significant (P<0.01) for copper and was significant at P<0.05 mangenese and phosphorous. Lee et al. (2016), working on variability of nutrient composition of cereal grains from different origin found that country of origin was significant (P<0.05) for all the nutrients and concluded that, source of material was important when considering nutrient composition.

Trait correlation

Correlation analysis was performed among the nutrients and between the nutrients and grain related traits. Highly significant (P<0.001) values

Table 7. Estimates of correlation coefficients among Calcium, Iron and Zinc (main micro nutrient present in finger millet), protein content, grain characteristics and yield.

Correlation	Calcium (mg/100 g)	Iron (mg/100 g)	Zinc (mg/100 g)	Protein (% wt)	DAF[a]	Glume cover[b]	Grain color[c]	1000-seed weight (g)
Calcium								
Iron	0.22***							
Zinc	0.56***	0.38***						
Protein	0.32***	0.24***	0.48***					
DAF	-0.33***	-0.05	-0.19***	-0.09*				
Glume covering	0.23***	0.06	0.12**	0.15***	-0.23***			
Grain color	0.07	0.14***	0.14**	0.15***	0.10*	0.17***		
1000 seed weight	-0.05	-0.13**	-0.09	-0.09	-0.02	-0.05	-0.01	
Grain yield	0.01	-0.06	-0.06	-0.06	-0.03	-0.08	-0.07	0.09*

[a]DAF, Days from planting to flowering; [b]Glume cover, glume covering scored on a scale of 1-5 where 1=Exposed, 2= Intermediate, 3=Enclosed; [c]Grain color, assessed on a scale of 1-5 where 1=White, 2=Light Brown, 3=Copper Brown, 4=Dark Brown, 5=Purple Brown.

were observed among the nutrients calcium, copper, iron, potassium, magnesium, manganese, potassium, sulphur, zinc, and protein (Table 7). Ngu'ni et al. (2011) working on southern African sorghum observed significant positive correlations between grain minerals. Quoting Kumar et al. (2010) who concluded that it indicated that either genetic factors for each pair of minerals are associated, or physiological mechanisms were interconnected for their uptake/ translocation in the grains. Positive correlation between Fe and Zn grain content have also been established in pearl millet (Velu et al., 2007, 2011; Gupta et al., 2009; Govindaraj et al., 2013; Rai et al., 2015; Upadhyaya et al., 2016). This implies that there is potential of simultaneous genetic improvement for two or more grain minerals. Grain color was highly correlated (P≤0.01) to iron, zinc and protein, with no significant association to calcium, indicating that color can be used as morphological marker for the nutrients. The low nutrient content in the farmer preferred varieties and the new released varieties is likely a result of selection against the dark colored varieties as most farmers prefer light and copper brown colored grains. Glume covering was highly correlated to calcium, zinc and protein but not correlated to iron. It was also highly correlated to grain color, an indication that grain color and glume covering can both or interchangeably be used for selecting.

Weak positive and negative correlation exists between grain yield and calcium; and grain yield and iron, zinc and protein, respectively, implying that breeding for improved nutrient content of the micro and macro nutrients will not affect yield and vice versa. Significant negative correlation was established between DAF and Ca (-0.33), Zn (-0.19), and protein (-0.09) and not with Fe (-0.05). Seed size (1000-seed weight) had a negative association with all the nutrients of interest in finger millet, but the association was only significant with Fe (-0.13). Earlier studies have demonstrated the weak or negative correlation between grain yield and grain nutrient traits in different crops. Upadhyaya et al., (2010) working on core finger millet collection in India found weak and non-significant correlations between grain yield and Fe (0.03), Zn (0.05), Ca (0.001) and protein (0.09) contents. Bänzinger and Long (2000) found weak relation between grain Fe and Zn contents in maize with yield. Kumar et al. (2010) and Ng'uni et al. (2011) demonstrated Fe and Zn weak negative relationships with yield in sorghum. Both crops are cereal staple crops in sub Saharan Africa. In pearl millet, a close relative of finger millet no significant association has been established between Fe and Zn grain content; and grain yield and seed size (Velu et al., 2007, 2011; Gupta et al., 2009; Govindaraj et al., 2013). This implies you can increase Fe and Zn grain contents without interfering with grain yield.

The positive correlation among the nutrients implies that several nutrients can be improved concurrently. On the other hand, the weak or lack of association between the different nutrient values and yield related traits (grain yield and seed size) allows for selection for nutrient content without necessarily affecting yield and vice versa. These findings do show that biofortification of finger millet grain can be done easily without comprising yield. According to FAO/WHO (2013), conventional breeding research has demonstrated

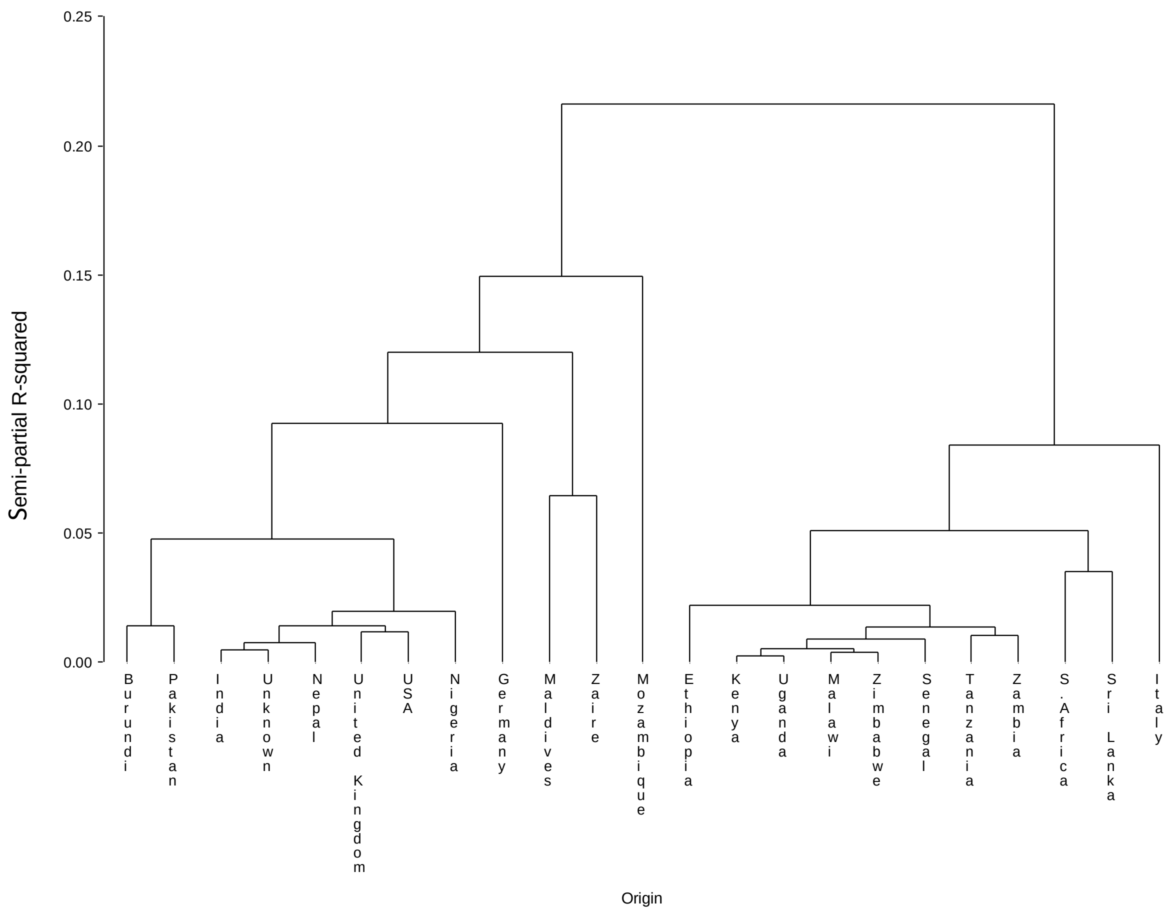

Figure 1. Dendogram showing clustering pattern of 638 finger millet accessions based on the different micro and macro nutrients.

that micronutrient density can be increased in food staples without negative effects on other farmer-preferred traits. HarvestPlus has developed high Fe pearl millet by conventional breeding (HarvestPlus, 2009). In India, Fe content of a commercially cultivated pearl millet variety was increased by more than 9% and had 11% more grain yield than the parental control (Rai et al., 2013). Rai et al. (2014) screened seed parent progenies and restorer parent progenies in a biofortification program and found that mean Fe content in the progenies increased by 5 to 66% compared to the control cultivars.

The present work is in agreement with previous studies in the different crops that (1) there is high variability with germplasm for increased nutrient content, (2) selecting for one of the nutrients will most likely lead to increase of the other micro nutrients of interest (Ca, Fe and Zn), and (3) selecting for high yield will not necessary lead to reduction of the micro nutrients and vice versa.

Cluster analysis

To establish the level of diversity among the accessions, cluster analysis was done and five clusters were established (Figure 1). Cluster one consisted of varieties from Burundi and Pakistan; cluster two from India, Unknown, Nepal, United Kingdom, USA and Nigeria; cluster three from Maldives and Zaire; Cluster four had accessions from Ethiopia, Kenya, Uganda, Malawi, Zimbabwe, Senegal, Tanzania and Zambia; and cluster five accessions from South Africa and Sir Lanka. There were however two main clusters; the largest, cluster four consisted of varieties from countries of finger millet origin, Uganda and Ethiopia (Hilu and de Wet, 1976a; Dida et al., 2008) and the main finger millet growing countries in East and Southern Africa region-Tanzania, Kenya, Malawi, and Zimbabwe. The second largest cluster contains accessions from countries from the secondary diversity region and which are among the largest finger millet producers, India and Nepal (Hilu and de Wet, 1976a; Dida et al., 2008). These two clusters represent the largest diversity for the different nutrient traits for future improvement of finger millet. The results are in agreement with accepted phenomena that, the highest diversity usually exists in centers of origin.

The value of biofortified cultivars has been demonstrated by various past researches. The fractional absorption of iron in biofortified pearl millet was found to be similar to a low iron variety when fed to young children in India (Hambidge et al., 2013) and adult women in Benin (Cercamondi et al., 2013). Therefore, the biofortified pearl millet provided significantly more total iron to the individuals who consumed it. Consistent with these results, an efficacy trial completed recently with school children in rural India demonstrated that biofortified pearl millet is efficacious in improving iron status with 64% of the iron deficiency at baseline being resolved in the intervention group after 3 months of daily pearl millet consumption with respect to the low iron pearl millet group. The recommended daily nutrient intake of an adult is 800 g of Ca; 10 mg Fe for males and 15 mg Fe for females; and 15 mg Zn for male and 12 mg Zn for females (FAO/WHO, 2000). With the current accepted finger millet nutrient values of Ca (350 mg/100 g), Fe (3.9 mg/100 g) and Zn (65.9 mg/100 g), Ca and Fe requirements can completely be met and a large portion of Zn by consuming 230 g of finger millet grain daily. With biofortification to the level of the high nutrient accessions found in the germplasm, the amount of grain required will be reduced to 150 g. This represents 35% reduction in the amount of grain required to meet the daily requirement. This can have a huge positive effect in areas with an agriculture-based economy where large segments of the population typically dependent on what they produce.

As biofortification efforts move forward, developing cultivars that have multiple micronutrients should be pursued (Nuss, 2010). For example, common beans and pearl millet already display simultaneous increases in zinc when bred for higher iron concentrations; and quality protein maize often has increased levels of zinc (Nuss, 2011). The synergistic effects between vitamin A and zinc lead to enhanced overall nutrient metabolism (Tanumihardjo et al., 2010). Therefore, a variety that has quality protein, enhanced zinc, and increased provitamin A carotenoids may supply better nutrition than any single nutrient approach for populations that have high intake of the crop. Simulations have demonstrated that adoption of zinc-biofortified rice could readily increase zinc intakes of women and children in Bangladesh (Arsenault et al., 2010). The same held true in adults following a traditional eating pattern in China (Qin et al., 2012). Manipulating both iron and zinc is feasible (Sperotto el al., 2010).

Conclusion

A number of conclusions can be made. Accessions showed very high variability for the different nutrient contents. The local cultivars and varieties released in the ESA region had significantly lower levels of the main essential nutrients (Ca, Fe, Zn) found in finger millet. Country of origin was highly significant for all the nutrients, with accessions from eastern and southern Africa having significantly lower nutrient contents. Grain color was associated with nutrient content with darker grains having higher compared to white colored. All nutrients were positively correlated to each other and grain yield was not significantly correlated to any nutrient content. The substantial variability for the grain nutrients observed in the finger millet core collection and local germplasm indicate the possibility for the selection of nutrient-rich accessions for use in the breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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