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Grain mineral nutrient profiling and iron bioavailability of an ancient crop tef (*Eragrostis tef*)

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Abstract

Tef (*Eragrostis tef*) is an underutilized food crop rich in minerals, vitamins, and amino acids. However, mineral profiling of diverse tef accessions, and estimation of bioavailable iron from tef has been lacking. In this study, we analyzed the mineral content of 41 tef accessions along with major cereals. Our analysis revealed that tef seeds contain significantly more minerals than maize, rice, and the wheat varieties used in this study. A significant variation in mineral content was also observed across the tef accessions. We also performed a relative estimation of Fe bioavailability from selected tef accessions and reference crops using an established *Caco-2* cell bioassay. This bioassay measures human intestinal cell Fe uptake via intracellular ferritin formation, a storage protein that is a validated marker of Fe uptake. Higher levels of Fe uptake were observed in the PI-149307, PI-494425, and PI-195937 accessions, than those recorded in cells fed wheat, rice, or tef accessions PI-329681, PI-494408 PI-494293. There was no marked difference in phytic acid (PA) content between tef and wheat, while the PA level in rice was lower than tef and wheat. Enhanced Fe uptake evident in tef accession PI494425 could not be explained by seed Fe content. The Fe content of PI-494425 was lower than the other tef accessions, suggesting that other factors control the amount of bioavailable Fe from tef. Considerable variation in mineral content and bioavailable Fe between tef and other cereals indicate a potential for improving mineral nutrition from this vital food crop.

Keywords: *Eragrostis tef*, mineral content, *Caco-2* cells, iron bioavailability, phytic acid.

Introduction

Micronutrient deficiency remains a global challenge affecting over two billion people (Goedecke et al., 2018, Nunn et al., 2019). The burden of malnutrition across the world remains unacceptably high (Webb et al., 2018), being directly or indirectly linked to death and disability worldwide (Ahmed et al., 2012). Malnutrition is predominant in low- and middle-income countries, which are deficient in one or more vitamins and minerals, such as iron (Fe), zinc (Zn), and vitamin A (Nunn et al., 2019). It primarily affects children between zero and five year of age, women of childbearing age, and pregnant women are most at risk of Fe deficiency due to their elevated Fe requirements (Webb et al., 2018; Lopez et al., 2015). Insufficient intake of bioavailable Fe is the primary cause of their deficiencies (Nguyen et al., 2014).

Poor bioavailability of minerals is one of the factors that leads to their deficiency. Plant products such as cereals, legumes, nuts, and fruits contain antinutrients such as oxalic acid, phytic acid (PA), polyphenols, and dietary fibers which inhibit minerals and reduce their bioavailability (Gibson et al., 2010; Tamilmani and Pandey, 2016). PA, *myo*-inositol hexakisphosphate, is the main phosphate storage in most cereals (Fisher et al., 2010; Perera et al., 2018). Tef grains contain high levels of PA ((Fisher et al., 2010; Zhu et al., 2018), which has a strong chelating potential for divalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} in the human digestive tract (Schlemmer et al., 2009). The PA-mineral chelates are insoluble and prevent the absorption of essential minerals and trace elements from plant-based foods (Hurrell et al., 2004) in the absence of a modulator. Several approaches are reported to reduce the effect of PA including the addition of ascorbic acid (AA), ethylenediamine tetraacetic acid (EDTA) complexes, and dephytinization by milling or exogenous application of phytase enzyme (Gibson et al. 2010; Engle-Stone et al., 2005) and incubation with microorganisms such as yeast and lactic acid bacteria (Fischer et al., 2014; Hollstroem et al., 2012). Polyphenols are other constituents of plant-based foods that render Fe unavailable (Tamilmani and Pandey, 2016) [8].

Although there are more than 250,000 known edible plants in the world, only three main cereals; maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) provide two-third of global plant-derived foods (Cheng, 2018, Sarrocco et al. 2019). These starch-rich cereals are typically poor in nutrients, thus leaving populations that rely on them in a non-diverse diet that is deficient in essential nutrients (Ricachenevsky et al., 2019; Bekkering and Tian, 2019). Moreover, these crops are not well suited to thrive in hostile weather patterns and low-input conditions (FAO, 2017). Whereas underutilized cereals such as tef (*Eragrostis tef*), finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), fonio (*Digitaria exilis*) and pseudocereals including quinoa (*Chenopodium quinoa*), amaranth (Amaranthus spp.) and buckwheat (*Fagopyrum esculentum*) have both outstanding nutritional values and a high tolerance to biotic and abiotic stresses (Cheng, 2018; Bikkering and Tian, 2019; Shankher et al., 2018; Schauberger et al., 2017). However, these crops have not attracted significant research interests and global awareness until recently. Although still considered underutilized, 'orphan' or 'lost' crops, some are staple crops in many countries (Bikkering and Tian, 2019; Gupta et al., 2017). For example, millets are staple in the semi-arid and arid regions of the world (Gupta et al., 2017), whereas tef is a staple food in East Africa.

Tef is the most important cereal crop in the Horn of Africa, especially in Ethiopia and Eritrea (Gebremariam et al., 2014), where it has been used as a staple crop for thousands of years (Ketema et al., 1997). In Ethiopia alone, tef is cultivated on over three million hectares of land, which is equivalent to 30% of the total area allocated to cereals, producing close to five million tons of grain (Minten et al., 2016; Cannarozzi et al., 2018). Because of its growing popularity as a healthy grain, tef production is expanding in several countries, including Australia, China, India, South Africa, and U.S. Tef is one of the most nutrient-rich crops containing high amounts of macro and trace minerals (e.g., Ca, Fe and Zn) and water-soluble vitamins (Spaenij-Dekking et al., 2005; Abebe et al., 2007). Because tef is a gluten-free grain (Spaenij-Dekking et al., 2005), it is an alternative food for people who have celiac disease, the immune reaction to consuming gluten-containing foods such as wheat, barley, and rye, which affects approximately 1.0 % of the world population (Gujral et al., 2012).

Tef seeds are reported to contain higher and variable mineral levels than those found in other cereals, including maize, wheat, and rice (Zhu, 2018; Bekkering et al., 2019; Spaenij-Dekking et al., 2005; Mengesha, 2016). There are over 5,000 tef collections maintained at the Ethiopian Biodiversity Institute, which provides ample opportunities for genetic improvement for various traits (Asefa et al., 2015). Information on the genotypic diversity in mineral content in tef and bioavailability of the minerals is still lacking, and a considerable variation in grain mineral content has been reported previously in the scarce literature.

In this study, we profiled mineral content in 41 tef accessions along with a variety of maize, wheat, and rice. We also assayed Fe bioavailability in representative tef accessions. Our findings revealed that the tef panel displayed significant variation in both macro- and trace minerals. Our findings also showed that tef accessions contain more minerals than dominant cereals maize, wheat, and rice. Using a physiologically relevant cell model, we showed that tef seed Fe was more bioavailable than wheat and rice. To our knowledge, this is the first report on mineral profiling of diverse tef accession and Fe bioavailability using a human cell model.

Results and Discussion

Variation in mineral content

Tef is one of the underutilized and nutrient-dense crops originated in Ethiopia, where it has been used as a staple crop for thousands of years (Ketema, 1997; Mottaleb, 2018). Higher levels of macronutrient such as Ca, and micronutrients such as Fe and Zn have been reported in tef grains (Zhu, 2018, Spaenij-Dekking, 2005). However, a comprehensive grain ion profiling is lacking, as a considerable variation in grain content has been reported previously in the scarce literature. Moreover, genotypic variation in mineral content among diverse tef accessions has never been reported before. In this study, we determined mineral content in 40 tef accessions and the variety Dessie, the latter being widely grown in the U.S., along with maize, wheat, and rice varieties. Our findings revealed a great variation among the cereals and between tef accessions. Statistically significant differences ($P < 0.01$) were observed in seed Ca content among the accessions (Table 1). The Ca content ranged from 981-1,811 mg Kg^{-1} seed, with the highest and lowest Ca detected in accession PI-494307 and PI-494276, respectively. The average seed Ca content was 1,310 mg Kg^{-1}, which was higher than that found in the other cereals, wheat (540 mg kg^{-1}), maize (33 mg kg^{-1}), and rice (153 mg kg^{-1}). Significant differences ($P < 0.01$) in K content were also observed among the tef accessions. The average K content (kg^{-1} seeds) was 4,440 mg kg^{-1} seeds, which was significantly higher than the K content of wheat, corn, and rice. Among the tef accessions, the highest K content was detected in accession PI-193514 (5,608 mg kg^{-1}), and the lowest was measured in PI-494425 (3,050 mg kg^{-1}) and the common cultivar Dessie (3,162 mg kg^{-1}).

Tef accessions also showed a small but statistically significant difference in Mg, P and S content ($P<0.01$). The Mg content (mg per kg^{-1} seed) ranged from 1,520-1,967 mg kg^{-1}. The highest Mg content was measured in PI-329681, whereas the lowest was measured in PI-494425 (Table 1). Seed P content (mg per kg^{-1}) among the accessions ranged from 3,747 mg in PI494425 to 4,423 mg in PI-494408 whereas the highest S content (1,545 mg per kg^{-1}) and the lowest (1,176 mg per kg^{-1}) were measured in PI-494408 and PI-194475, respectively. Seed Mg and P content in all the tef accessions were significantly higher ($P<0.01$) than those found in maize, rice, and wheat. Similarly, significant difference in S content were observed among the cereals.

The content of micronutrients Fe, Mn, Zn, Na, and Ba in seeds of tef and other cereal is shown in Table 2. These results showed a significant variation ($P<0.01$) in micronutrient content among tef accessions. In addition, tef seeds had a higher micronutrient content than that found in wheat, rice, and maize varieties analyzed in this study. The Fe content of all the tef accession was significantly higher (*P<0.01*) than that found in maize and rice, while only two accessions PI-195937 and PI-494282 contained significantly more Fe than wheat (Table 1). The Fe content of PI-494293 was lower than all the other tef accessions. The Fe content in rice and maize was 12 mg and 20 mg per kg^{-1}, respectively. The Mn content in all the tef accessions was higher than maize and rice (Table 2), while three tef accessions (Dessie, PI-494324, and PI-494307) had higher Mn content than wheat. Higher levels of Na and Ba were also measured in seeds of tef accessions as compared to wheat, rice, and maize. The highest Na was measured in PI-195937 (193 mg per kg^{-1}), and the lowest (25 mg kg^{-1}) was measured in PI-494307. The Na content in maize, rice, and wheat was 5, 7, and 10 mg per kg^{-1}, respectively. The Ba content ranged from 16-59 mg per kg^{-1} in tef accessions whereas it was negligible in wheat, rice, and maize. These slightly high levels of Ba could be due to contamination from the soil and require future investigation, since at high levels Ba could pose significant health risks (Krishna et al. 2020). Overall, these data highlight a significant genotypic variation in grain mineral concentration among tef accessions and between tef and other cereals analyzed in this study.

The content of major and trace minerals observed in some of the tef accessions is comparable with those reported previously by USDA (USDA, 2019) for Ca (2,000 mg per kg^{-1}), K (4,890 mg per kg^{-1}), Mg (2,000 mg per kg^{-1}), Fe (72 mg per kg^{-1}) and Zn (30 mg per kg^{-1}). Our findings also showed that the concentrations of most of the minerals in the tef accessions were higher than that of wheat, maize, and rice used in this study (Table 1). This finding is consistent with that previously reported by Mengesha (2016), where tef grains were shown to have higher Ca content than wheat, barley and sorghum, as well as those reported in Forsido et al. (2013), indicated higher Mg, P, Zn, and Cu in tef grains than maize and wheat.

Correlation and principal component analysis

We performed Spearman correlation analysis of mineral concentration between the tef accessions. The correlation heatmap in Figure 1 shows that the tef genotypes are grouped into three major clades based on mineral contents. Seventeen tef accessions were grouped in Clade I, including PI-195937 and PI-494282, which contained the highest Fe, and PI-193514 which contained the highest K. Clade II includes PI-494307, PI-494408, Dessie and PI-494324 which were high in Ca content. The wheat variety tested in this study was also grouped in Clade II. Clade III includes PI-494293 (low Fe), PI-494425 (low Ca and K) and PI-494409 (high P and S), and also the rice and maize varities. To determine which mineral explained most of the variation observed between the accessions, we conducted principal component analysis (PCA). The result in PCA score biplot is shown in Figure 2 shows that PCA1 explained 42% of the variation in mineral content among the accessions while PCA2 explained 20% of the variation. PCA1 showed a high association with Na content and differentiated it from the other minerals, while it had a low association with Mn content, and had no association with the other minerals. Most variation in PC1 is attributed to the variation in Na content. Whereas PCA2 differentiated the minerals into four groups, K (group 1), Ca, Mg, Cu, P and S (Group 2), Mn (Group 3), and Fe and Zn (Group 4). PCA2 had a strong and positive association with K content and a negative association with Fe and Zn contents but had a mild association with the other ions. We also analyzed the correlation between the different minerals. A significantly high correlation was observed between Mg and P, Mg and Ca, Ca and P, Mg and K, Mg and Cu, and P, suggesting a potential application of some of these accession in biofortification strategies.

Factors affecting mineral content

The current study showed a significant variation in the content of all minerals among the tef accessions. Aside from genetic variation, grain mineral content is dictated by environmental factors, including soil physical and chemical properties such as fertilizer level, pH, organic matter, and moisture and the seed preparation and analysis method used. However, because the tef accessions used in this study were grown under the same environmental condition and agronomic practice, the observed difference in mineral concentration can be attributed to genetic variability among the tested tef accessions. There is a large tef germplasm collection globally, including over 5,000 collections maintained at the Ethiopian Institute of Biodiversity (Assefa et al., 2015), providing a great potential to improve the nutritional quality of tef through traditional breeding or biotechnology.

Phytic acid content mineral bioavailability

The observed grain mineral nutrient content may not correlate well with the amount of bioavailable ion due to the presence of antinutrient seed constituents such as PA, which is the main phosphate storage for most cereals, legumes, and nuts (Fischer et al., 2014). PA in the seeds is considered an antinutrient because it chelates minerals such as Zn, Fe, Ca, and Mg (Magallanes-Lopez et al., 2017; Akond et al., 2011). Fe uptake by the cell is more influenced by food phytate level than total Fe content (Forsido et al., 2013). Tef is known to accumulate high levels of PA. For example, Baye et al. (Baye et al. 2014) reported up to 15.44 g PA per kg^{-1} seeds. The PA content has been shown to decrease with fermentation, which is an essential step in the preparation of tef bread. PA contents of 34 –10.0 mg per kg^{-1} (Fischer et al., 2014) and 2.34-3.0 mg per kg^{-1} (Mihrete et al., 2019) have been reported in tef bread. Other studies have shown a significant reduction (68%) in PA content in tef bread after fermentation (Fischer et al., 2014; Shumoy and Raes et al., 2017).

Because PA is known to limit the bioavailability of minerals, including Fe, in this study, we determined the level of PA in representative tef accessions, wheat, and rice, before the Fe bioavailability assay in *Caco-2* cells. Ferritin level was used as a marker for Fe bioavailability because *Caco-2* cells synthesize ferritin in response to increased intracellular Fe. As shown in Figure 3, there was no marked difference in PA contents within the representative tef accessions tested, with the content ranging between 9.8 g per kg^{-1} to 11.3 g per kg^{-1}. The PA content of the analysed tef accessions was also not markedly different from that found in wheat, while that of rice (6.7 mg per kg^{-1}) was lower than both wheat and tef accessions.

Fe bioavailability

Tef grains contain a higher level of Fe compared to other cereals (Table 1; Baye et al., 2014). However, the bioavailability of Fe

Table 1. Seed mineral ion content (mg Kg^{-1}) in tef, wheat, maize, and rice. Means differing at least by MSD are statistically significant ($P < 0.01$). Highlighted are accessions which were tested for Fe bioavailability.

Accession #	Fe	Ca	K	Mg	P	S
PI-195937	48 ± 0	1322 ± 6	4976 + 4	1781 ± 2	3996 ± 10	1477 ± 13
PI-494282	43 ± 0	1291 ± 5	5176 ± 18	1846 ± 3	4009 ± 10	1418 ± 1
PI-494283	43 ± 0	1381 ± 3	5325 ± 9	1706 ± 3	3809 ± 2	1360 ± 12
PI-494409	42 ± 0	1187 ± 16	3372 ± 270	1759 ± 14	4336 ± 22	1479 ± 4
PI-494475	41 ± 0	1131 ± 1	3610 ± 40	1590 ± 1	3847 ± 5	1176 ± 3
PI-193513	39 ± 0	1414 ± 8	5319 ± 40	1717 ± 2	3984 ± 21	1364 ± 4
Wheat	39 ± 0	540 ± 11	2636 ± 120	1168 ± 42	3084 ± 131	1384 ± 3
PI-494287	37 ± 0	1322 ± 2	4591 ± 101	1655 ± 2	4160 ± 22	1344 ± 2
PI-193511	37 ± 0	1213± 3	4849 ± 258	1684 ± 3	4054 ± 26	1381 ± 10
PI-494306	37 ± 0	1648 ± 5	5022 ± 15	1811 ± 1	4403 ± 57	1448 ± 2
PI-494299	36 ± 0	1464 ± 8	4545 ± 83	1840± 11	4048 ± 34	1369 ± 1
PI-494467	35 ± 0	1158 ± 14	3248 ± 224	1684 ± 14	3994 ± 55	1393 ± 19
PI-494324	34 ± 0	1638 ± 4	3795 ± 97	1885 ± 3	4271 ± 17	1317 ± 6
PI-494453	33 ± 0	1250 ± 9	3473 ± 289	1775 ± 8	4183 ± 9	1370 ± 2
PI-494292	33 ± 0	1290 ± 2	4879 ± 16	1817 ± 4	4065 ± 29	1237 ± 11
PI-494307	33 ± 0	1811 ± 7	3655 ± 23	1797 ± 2	3850 ± 3	1370 ± 14
PI-193510	33 ± 0	1234 ± 26	4855 ± 151	1804 ± 25	3789 ± 70	1191 ± 44
PI-494345	33 ± 0	1188 ± 2	3312 ± 165	1770 ± 7	3858 ± 8	1235 ± 5
PI-494344	32 ± 0	1408 ± 11	3677 ± 201	1809 ± 21	4139 ± 28	1310 ± 4
PI-193514	32 ± 0	1185 ± 2	5608 ± 50	1869 ± 0	3990 ± 14	1255 ± 11
PI-494393	32 ± 0	1263 ± 6	3573 ± 48	1736 ± 19	4086 ± 61	1325 ± 2
PI-494296	32 ± 0	1277± 7	4239 ± 14	1784 ± 30	4129 ± 18	1236 ± 2
PI-494272	32 ± 0	1446 ± 24	4859 ± 282	1864 ± 13	4310 ± 25	1432 ± 8
PI-494277	31 ± 0	1295 ± 18	5278 ± 148	1806 ± 0	4253 ± 15	1269 ± 0
PI-494408	31 ± 0	1667 ± 0	4713 ± 12	1848 ± 3	4423 ± 24	1545 ± 4
PI-49445	31 ± 0	1249 ± 8	3596 ± 227	1651 ± 5	4120 ± 53	1297 ± 2
PI-494276	31 ± 0	981 ± 1	4823 ± 106	1740 ± 6	3851 ± 31	1418 ± 14
PI-494280	31 ± 0	1252 ± 0	5020 ± 76	1727 ± 7	4031 ± 8	1305 ± 3
PI-494273	31 ± 1	1456 ± 11	5051 ± 50	1867 ± 9	4392 ± 36	1316 ± 3
PI-243912	30 ± 0	1321 ± 14	4796 + 22	1706 ± 8	3883 ± 18	1255 ± 9
PI-54442	30± 0	1119 ± 31	3849 ± 32	1641 ± 15	3868 ± 18	1367 ± 26
PI-494208	30 ± 0	1164 ± 15	468 ± 317	1869± 12	3987 ± 2	1199 ± 5
Dessie	30 ± 0	1669 ± 6	3162 ± 86	1774 ± 9	3995 ± 12	1519 ± 18
PI-494295	29 ± 0	1121 ± 8	4845 ± 48	1667 ± 4	3964 ± 17	1234 ± 4
PI-195934	28 ± 1	1054 ± 5	5396 ± 14	1830 ± 15	4120 ± 182	1385 ± 31
PI-494301	28 ± 0	1374 ± 3	4422 ± 29	1844 ± 3	4377 ± 50	1400 ± 2
PI-494285	28 ± 0	1364 ± 19	4593 ± 134	1713± 9	4056 ± 40	1250 ± 9
PI-494425	26 ± 0	1007 ± 15	3050 ± 14	1520 ± 5	3747 ± 2	1198 ± 1
PI-494216	25 ± 0	1203 ± 1	4572 ± 142	1770 ± 6	4050 ± 7	1264 ± 1
PI-329681	24 ± 0	1220 ± 3	4772 ± 166	1967± 4	4386 ± 32	1349 ± 2
PI-494293	22 ± 0	1263 ± 6	5029 ± 304	1634 ± 1	4029 ± 7	1239 ± 2
Maize	20 ± 1	33 ± 3	2509 ± 13	1207 ± 20	3336 ± 81	1251_22
Rice	12 ± 0	153 ± 9	2379 ± 50	1283 ± 3	3277± 36	1242 ± 8
MSD	**3.7**	**7.83**	**92.3**	**73.3**	**234**	**78**
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Table 2. Seed trace mineral ion content (mg Kg^{-1}) in tef, wheat, maize, and rice. MSD = minimum significance Difference. Means differing at least by MSD are statistically significant (*P < 0.01*).

Accession	Mn	Zn	Na	Ba
PI-494307	44 ± 0	11 ± 12	25 ± 2	57 ± 0
PI-193510	23 ± 1	27 ± 1	49 ± 1	33 ± 1
PI-193511	22 ± 0	38 ± 0	77 ± 03	31 ± 1
PI-193513	25 ± 0	36 ± 0	111 ± 1	30 ± 0
PI-193514	23 ± 0	35 ± 0	88 ± 0	35 ± 1
PI-195934	21 ± 0	36 ± 0	62 ± 1	27 ± 0
PI-195937	31 ± 0	40 ± 0	193 ± 0	20 ± 0
PI-243912	25 ± 0	30 ± 0	117 ± 2	46 ± 1
PI-329681	25 ± 0	35 ± 1	119 ± 4	38 ± 1
PI-494208	28 ± 0	35 ± 1	67 ± 2	45 ± 2
PI-494216	22 ± 0	30 ± 0	72 ± 2	37 ± 0
PI-494272	32 ± 0	31 ± 0	78 ± 2	28 ± 1
PI-494273	27 ± 0	37 ± 0	86 ± 1	48 ± 1
PI-494276	23 ± 0	30 ± 0	98 ± 5	16 ± 0
PI-494277	25 ± 0	34 ± 0	102 ± 1	36 ± 1
PI-494280	24 ± 0	30 ± 0	45 ± 2	46 ± 0
PI-494282	37 ± 0	41 ± 0	82 ± 3	23 ± 1
PI-494283	28 ± 0	31 ± 1	41 ± 3	31 ± 0
PI-494285	26 ± 0	30 ± 0	59 ± 01	45 ± 1
PI-494287	27 ± 0	41 ± 0	121 ± 3	32 ± 0
PI-494292	30 ± 0	35 ± 0	71 ± 0	26 ± 3
PI-494293	30 ± 0	30 ± 0	46 ± 2	31 ± 1
PI-494295	38 ± 0	37 ± 0	38 ± 1	33 ± 0
PI-494296	36 ± 0	39 ± 0	33 ± 1	38 ± 0
PI-494299	31 ± 1	33 ± 0	46 ± 0	42 ± 1
PI-494301	31 ± 0	31 ± 0	43 ± 2	35 ± 0
PI-494306	29 ± 1	39 ± 1	81 ± 3	34 ± 0
PI-494324	52 ± 0	37 ± 0	52 ± 1	59 ± 1
PI-494344	31 ± 0	31 ± 1	40 ± 4	40 ± 1
PI-494345	30 ± 0	38 ± 0	28 ± 1	28 ± 1
PI-494393	21 ± 0	41 ± 0	38 ± 0	33 ± 1
PI-494408	35 ± 0	34 ± 0	56 ± 1	48 ± 0
PI-494409	27 ± 0	38 ± 0	60 ± 1	25 ± 1
PI-494425	27 ± 0	28 ± 0	60 ± 2	30 ± 1
PI-49445	39 ± 0	35 ± 0	111 ± 3	30 ± 0
PI-494453	35 ± 0	39 ± 0	35 ± 0	34 ± 1
PI-494467	36 ± 0	44 ± 0	72 ± 0	31 ± 1
PI-494475	35 ± 0	40 ± 0	89 ± 4	26 ± 1
PI-54442	29 ± 0	40 ± 1	69 ± 2	26 ± 3
Dessie	75 ± 1	30 ± 1	30 ± 1	16 ± 1
Wheat	40 ± 1	27 ± 1	10 ± 0	5 ± 1
Maize	12 ± 0	28 ± 1	5 ± 1	0 ± 0
Rice	8 ± 0	30 ± 1	7 ± 1	0 ± 0
MSD	2.1	11	13.8	0
P-value	<0.0001	<0.0001	<0.0001	<0.0001

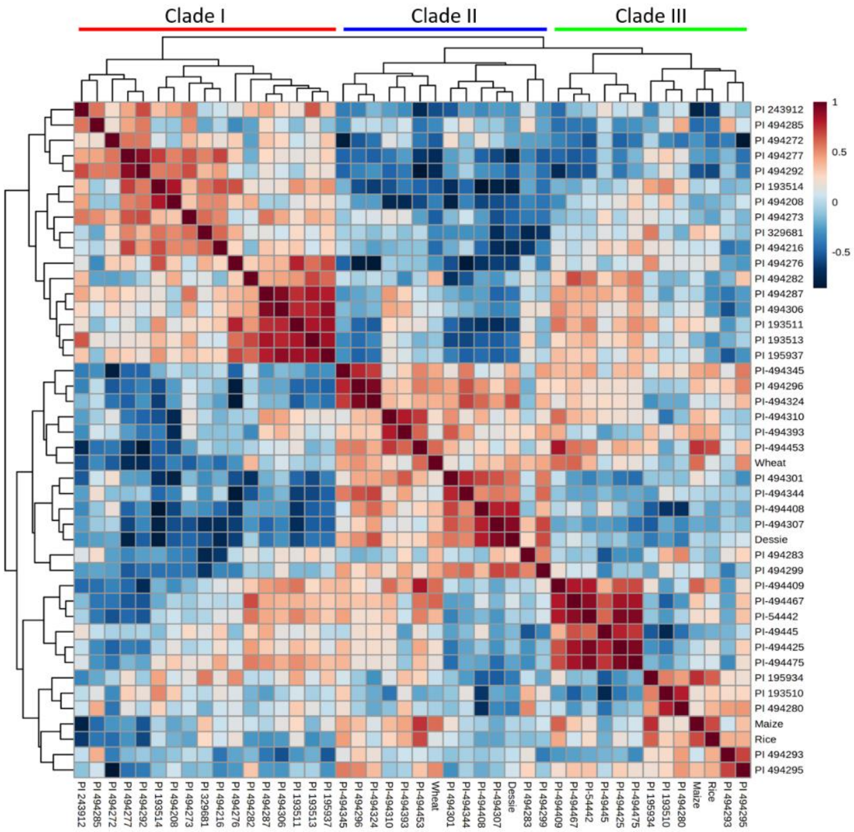

Figure 1. Spearman correlations heatmap of mineral contents in all the tef accessions along with wheat, rice and maize. Samples with dark red indicate strong correlations while dark blue indicate negative correlations.

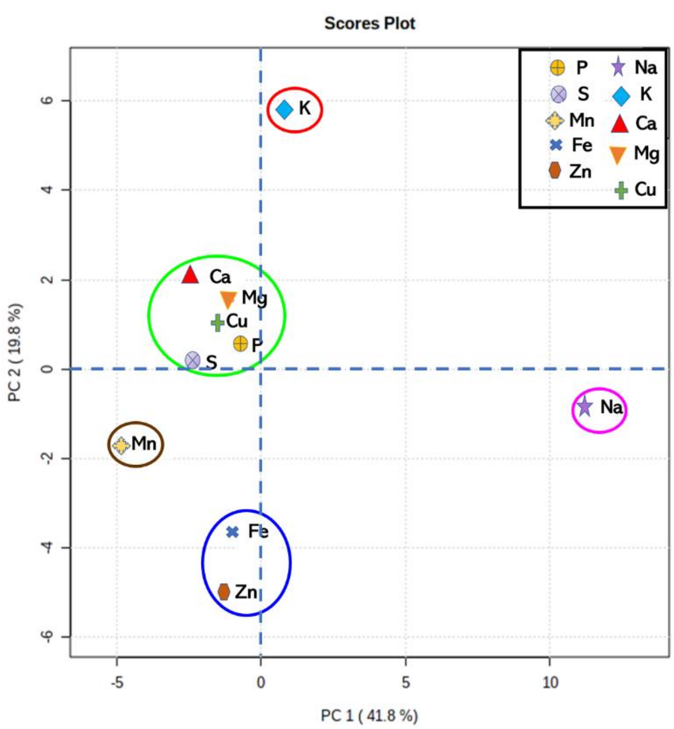

Figure 2. Score plot of PCAs of mineral contents in 41 tef accessions generated using generated using MetaboAnalyst (Xia et al., 2012).

Table 3. Correlation coefficient of different minerals in tef accessions. ***significant (p<0.05) ****highly significant (p<0.01) correlations

	Fe	Ca	K	Mg	P	S	Mn	Zn	Cu	Na
Fe	1									
Ca	0.36*	1								
K	0.21	0.42*	1							
Mg	0.17	**0.80****	**0.62****	1						
P	0.19	**0.74****	0.51*	**0.83****	1					
S	0.40*	0.35*	0.017	0.24	0.34*	1				
Mn	0.23	0.52*	-0.17	0.24	0.22	0.36*	1			
Zn	0.38*	0.05	0.043	0.15	0.30*	0.14	0.065	1		
Cu	0.07	0.63*	0.42*	**0.69****	**0.56****	0.004	0.11	0.17	1	
Na	0.39*	0.25	0.49*	0.37*	0.39*	0.15	0.066	0.28	0.33*	1

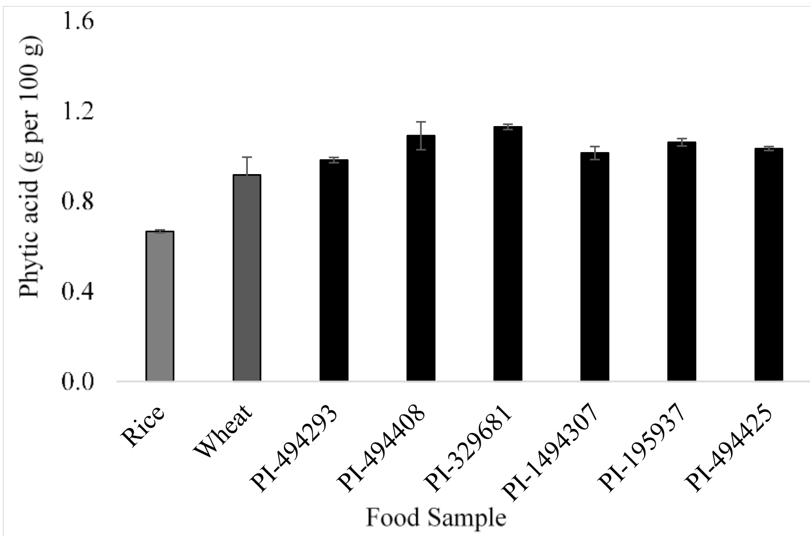

Figure 3. PA content of tef, wheat and rice samples.

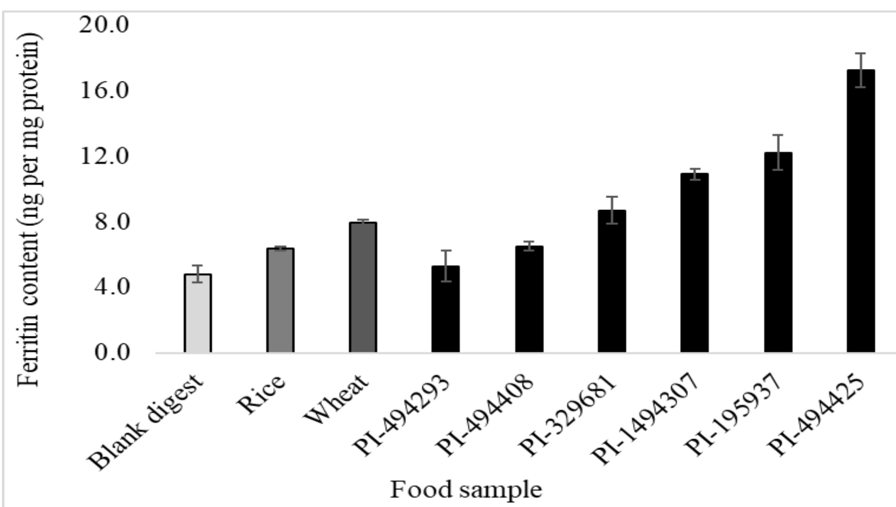

Figure 4. Iron uptake from food samples as measured via Caco-2 cell ferritin formation.

under a physiologically relevant cell model system has never been studied before. Cell-free *in vitro* Fe bioavailability studies conducted previously did not seem to provide a reasonable estimation of bioavailable Fe (Baye et al., 2014). In this study, we used the *Caco-2* cell cultures, to estimate bioavailable Fe.

Despite the lack of significant difference in phytate content, a marked difference in ferritin content was observed among the foods analyzed. The ferritin content (ng per mg protein) in all the foods tested was higher than the blank digest (cells without food digests) (Figure 4). The ferritin levels in some tef accessions PI-49425, PI-195937, and PI-49307 were higher than other tef accessions (PI-329681, PI-494408, and PI-494293) and that of wheat and rice. Ferritin level in cells fed with PI-49425 was 3.6-fold higher than control cells incubated without a food digest, and two- and three-fold higher than those found in wheat and rice, respectively, suggesting that tef may be a good source of bioavailable Fe. In the grains tested in this study, the ferritin content did not seem to have a strong correlation with PA content ($R^2 = 0.065$), suggesting that PA content is not the only factor limiting Fe bioavailability. Grain Fe content is also expected to affect Fe uptake. However, we found a positive but weak correlation ($R^2 = 0.43$) between Fe content and ferritin levels, suggesting that other factors besides PA level and seed Fe content, might be limiting the level of absorbable Fe from tef. Another factor that could affect the Fe uptake could be variation in seed constituents, for example, polyphenols such as catechins, flavonols, flavones, anthocyanins, phenolic acids.

Polyphenols are considered antinutrients because they are known to reduce Fe bioavailability by forming insoluble complexes (Hurrell et al. 2010; Tamilmani and Pandey, 2016). This effect is reversed by ascorbic acid and ethylenediaminetetraacetic acid (Hurrell et al. 2010). A previous study using biofortified pearl millet and black beans (Tako et al., 2014) showed only a slight increase in ferritin content in *Caco-2* cells treated with digested food as compared to untreated control cells due to higher levels of phenolics. High levels of flavones and phenolic acid derivatives were previously reported in tef (Salawu and Salimon, 2014; Ravisankar et al. 2018). The total phenolic content of tef was found to be higher than that found in red sorghum and cowpea (Ravisankar et al., 2018), maize and wheat (Forsido et al., 2013), and they might have played a role in limiting absorbable-Fe in some tef accessions, wheat and rice assayed in this study. This assumption is plausible given that the tef accession that resulted in higher Fe uptake (PI-49425) contained lower Fe than other tef accessions and that there was no marked difference in PA content among the accessions. However, whether there is variation in polyphenol content among the tef accessions and other cereals used in this study remains to be determined in the future. Interestingly, the Fe content in grains of accession PI-494425, which resulted in higher Fe uptake in *Caco-2* cells, contained lower grain Fe than other tef accessions PI-194307, PI-195937 and PI-494408 (Table 1). Not only Fe but also the content of all the major minerals including Ca, K, Mg, P and S (Table 1) is lower in PI-494425 than the other tef accessions analyzed in this study. Although, the relationship between Fe uptake and grain mineral content is not well understood, studies in animal cells have shown the inhibitory effect of Ca on Fe-uptake (Prather and Miller, 1992; Wauben and Atkinson, 1999; Lynch et al., 2000). Calcium has been shown to inhibit the uptake of both heme-(from meat and animal) and non-heme (from plant)-Fe in humans (Roughead et al. 2005; Candia et al., 2018). Similarly, the inhibitory effect of Ca on Fe uptake from Fe-fortified instant drink has been reported, which was reversed by the addition of AA (Walczyk et al. 2014). Whether low grain Ca in PI-494425 is the cause for increased Fe uptake in this study remains to be empirically validated.

Compared to other cereals included in this study, tef has more absorbable iron, suggesting its potential as a Fe-rich diet. Tef meal is widely consumed in Ethiopia. Thus it represents a major Fe source. As estimated by WHO, 64% of women of reproductive age and 89% of adult urban males in Ethiopia had an excessive intakes of Fe (World Health Organization, 2006). However, a more recent survey showed prevalence of Fe deficiency among children (17.8%) and non-pregnant adult women (5.8%) (Tadesse et al. 2019), and an increase of anaemia prevalence from 21.8% (postharvest) to 40.8% (preharvest) has been reported in some rural areas (Robi et al. 2013). Another study evaluating African countries including Ethiopia, Kenya, Nigeria, and South Africa, showed a relatively high (47.2–97.8mg/d) Fe intake for Ethiopia (Harika et al., 2017) due to frequent consumption of tef meals. This level of Fe is much higher than the recommended daily Fe intake by NIH, which is 8 mg (male) and 18 mg (female) for adults of age 19-50 (Rushton et al., 2009). This amount of Fe can be achieved by consuming 100g of tef bread, which contains about 18 mg Fe according to Mihrete (2019).

Materials and methods

Plant material

Seeds of 40 tef (*E. tef*) accessions and the variety Dessie which is widely grows in U.S, were obtained from the USDA-ARS National Plant Germplasm System, Plant Germplasm Introduction and Testing Research Unit (Pullman, Washington, USA), and used for this study along with maize (MO17), and commercially available white wheat and rice varieties.

Plant growth

Two-to-three seeds of each accession were planted in 7.6-liter pots filled with Metromix® 200 soil mix (Sun Gro Horticulture, Bellevue, Washington, USA). Plants were grown under greenhouse conditions of 12 h light (26 °C)/12 h dark (18 °C) photoperiod with the natural lighting limited to 1000 μmol $m^{-2}s^{-1}$ by shade cloth. After two weeks, plants were thinned to one seedling per pot. Plants were supplied with Osmocote® Smart-release® controlled-release fertilizer (Scotts Company, LLC, Marysville, OH) following the manufacturer's recommended application rates. Powdery mildew was controlled by a sulfur burner for four hours each night. Aphid infestations were controlled by applying of acephate (Orthene) (Valent BioSciences, Libertyville, Illinois, USA) in granular form to soil and aerosol forms according to the manufacturer's instructions.

Seed preparation

Five grams of seeds of each accession were pulverized for 3 min using a Waring Commercial WSG30 Medium-Duty Electric Spice Grinder (Waring Commercial, Torrington, Connecticut, USA). Half of the resulting flour was used for minerals determination using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). For representative tef accessions, the remaining half of the flour was used for determining Fe bioavailability in *Caco-2* (Caucasian colon adenocarcinoma) cells.

Determination of minerals using ICP-MS

Two hundred milligrams of tef flour were placed into 1.5 ml pre-weighed microcentrifuge tubes. The samples were digested with a cocktail of HNO_3 and perchloric acid (1:1 ratio), diluted in 10 ml of 5% HNO_3, and analyzed using Sciex Inductively coupled argon plasma (AB Sciex LLC, Framingham, Massachusetts, USA).

Measurement of phytic acid (PA) content

For PA content and Fe bioavailability assay, we selected four groups of tef accessions based on Fe and Ca content. Accessions containing high Fe (PI-195937), low Fe (PI-329681 and PI-494293), high Ca (PI-4943.7 and PI-494480) and low Ca (PI-494425) along with wheat and rice were compared. PA content, as myo-inositol hexakisphosphate, was analyzed using K-PHYT (Megazyme International, Bray, Ireland) PA (phytate)/ total phosphorus kit. Briefly, a 500 mg of flour from each cereal was extracted in 10 mL of 0.66 M hydrochloric acid while shaking at room temperature for 16 hours. One milliliter of the extract was centrifuged at 16,000 g for 10 minutes to pellet debris. A 0.5 mL sample of supernatant was recovered and neutralized with 0.5 mL 0.75 M sodium hydroxide. The K-PHYT kit was used to measure liberated phosphorous by phytase and alkaline phosphatase. Phosphorous was quantified by the molybdenum blue method (Murphy et al. 1962) using a spectrophotometer based on absorbance readings of standards of known P concentrations at 655 nm. Total phytate concentrations were calculated with Mega-Calc™ by subtracting free phosphate concentrations in the extracts from the total amount of phosphorous that is exclusively released after enzymatic PA digestion. The PA determination was performed in tef, wheat and rice grain.

Determination of Fe bioavailability

The level of absorbable Fe in tef grains was determined using an established *Caco-2* cell bioassay for Fe bioavailability (Glahn et al., 1998). This bioassay uses *Caco-2* cell ferritin formation as a marker of cell Fe uptake. *Caco-2* cells are a human intestinal cell line that in culture behave similarly to cells of the upper intestinal tract. This model has been highly validated over the past two decades as being semi-quantitatively predictive of relative amounts of Fe in humans (Tako et al., 2014). The *Caco-2* cell bioassay was performed in triplicate for each sample, and two independent assays were conducted

Statistical analysis

For grain mineral content, data were analyzed by one-way ANOVA using the PROC GLM procedure (Westfall et al. 1996). After significant *F*-tests, the Tukey multiple comparison was used to separate the means ($P < 0.01$). The data of phytate content and ferritin levels are presented as means and standard errors. The correlation between Fe and PA content with ferritin levels was estimated using a Pearson bivariate correlation function. Spearman correlation heatmap and principal components (PCs) score plot analysis was performed using an online database MetaboAnalyst (Xia et al., 2012). The correlation coefficient between different minerals in tef accessions, and significance difference between the means were determined using PSAT software (Hammer et al., 2001).

Conclusion

Our study demonstrated that tef seeds contain more macro and micronutrients than wheat, rice, and corn. Moreover, we observed a significant variation among the tef accessions in mineral content. Given the existence of an extensive tef germplasm collection, there is potential for improving the nutritional quality of tef through traditional breeding or biotechnology, thereby contributing towards addressing global macro and micronutrient deficiency.

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