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Iniadi pearl millet germplasm as a valuable genetic resource for high grain iron and zinc densities

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Abstract

Crop biofortification is increasingly being recognized as a cost-effective and sustainable approach to address the widespread micronutrient malnutrition arising from Fe and Zn deficiencies. Pearl millet as a cereal crop species has higher Fe density than all other major cereals. Earlier studies in pearl millet have shown that breeding lines, hybrid parents, improved populations and composites having high Fe and Zn densities were often based largely or entirely on *iniadi* pearl millet germplasm. In an attempt to identify additional sources of high Fe density in this group of germplasm, 297 accessions were screened using Perl's Prussian Blue staining, of which 191 accessions (118 from Togo, 62 from Ghana and 11 from Burkina Faso) were re-evaluated during the 2010 rainy and 2012 summer seasons using the inductively coupled plasma atomic emission spectroscopy method. On the basis of the mean performance over the two seasons (environments), large variability was observed for both Fe (51–121 mg/kg) and Zn (46–87 mg/kg) densities. There was a highly significant and positive correlation between the two micronutrients ($r = 0.77$, $P < 0.01$). Of these re-evaluated accessions, 49% had higher Fe density than the high-Fe control commercial cultivar ICTP 8203 (81 mg/kg), and most of these accessions also had Zn density ≥61 mg/kg (59 mg/kg for ICTP 8203). A total of 27 accessions (20 from Togo and seven from Ghana) having a Fe density of 95–121 mg/kg (1 standard error of difference above that for ICTP 8203) and a Zn density of 59–87 mg/kg were selected as a valuable germplasm resource for genetic improvement of these two micronutrients in pearl millet.

Keywords: germplasm; *iniadi*; iron; pearl millet; *Pennisetum glaucum*; zinc

Introduction

Malnutrition arising from dietary deficiency of mineral micronutrients such as iron (Fe) and zinc (Zn) has widely been recognized as a serious public health problem worldwide, affecting more than 2 billion people (WHO, 2002). Fe deficiency ranks 9th and Zn deficiency ranks 11th among the 26 major risk factors for the global burden of disease estimates (Ezzati *et al.*, 2002). This problem is particularly serious in developing countries, especially in high-risk groups such as pregnant women, infants and adolescent children. For instance, in India, about 80% of pregnant women, 52% of non-pregnant women and 74% of children in the age group of 6–35 months suffer from Fe deficiency-induced anaemia (Chakravarty and Ghose,

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2004). In sub-Saharan Africa, 88% of pre-school children in Burkina Faso and 77% in Mali suffer from Fe deficiency-induced anaemia (Birner *et al.*, 2007). Numerous health problems arising from Zn deficiency could be as serious as those arising from Fe deficiency; however, there are no well-documented studies to establish this fact. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a highly nutritious major warm-season cereal grown primarily for grain production on more than 28 million ha in some of the most marginal arid and semi-arid tropical environments of Asia and Africa (Yadav *et al.*, 2012). It is a major source of dietary energy and nutritional security for vast rural communities in these regions. A recent study has shown that pearl millet accounts for 19 to 63% of the total Fe intake and 16 to 56% of the total Zn intake from all food sources in some of the major pearl millet-growing states of India such as Maharashtra, Gujarat and Rajasthan (Parthasarathy Rao *et al.*, 2006). It is also the cheapest source of Fe and Zn when compared with other cereals and vegetables.

Considering the magnitude of Fe and Zn deficiency problem as well as the potential of pearl millet in addressing this problem, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), supported by the HarvestPlus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR) (a global research partnership for a food secure future), has initiated research-for-development efforts to breed improved genetic materials (breeding lines, populations, hybrid parents and hybrids) with high levels of Fe and Zn densities. Results of preliminary studies conducted at ICRISAT showed that most of the potential hybrid parents, composites, improved populations and population progenies with high levels of Fe density were entirely or largely based on the so-called *iniadi* germplasm in their parentage (Velu et al., 2007, 2008). *Iniadi*, also known by various local names such as *nara*, *nata*, *ignati*, *ignate*, *ignie*, *misse* and *likoun*, refers to an early-maturing and large-seeded landrace found in the adjoining parts of Togo, Ghana, Benin and Burkina Faso (Andrews and Anand Kumar, 1996). The objective of this study was to conduct a wider search in this group of germplasm to examine the magnitude of variability and to identify accessions as additional sources of high levels of grain Fe and Zn densities.

Materials and methods

Germplasm materials

A set of 297 *iniadi* germplasm accessions originating from northern Togo (146) and adjoining eastern Ghana (83), southern Burkina Faso (63) and western Benin (5) regions of Western Africa (Fig. 1) were selected from genetic resource collections in the genebank at ICRISAT, Patancheru. These accessions were evaluated along with a control commercial open-pollinated variety, ICTP 8203, in an unreplicated nursery during the 2006 summer season at ICRISAT, Patancheru, and selfed grains were produced. These grain samples were initially screened for Fe density using Perl's Prussian Blue staining technique that provides a qualitative classification of Fe density based on the intensity of blue stain absorbed by the powdered grain samples (Velu *et al.*, 2006). Of these screened accessions, 191 staining dark blue to medium blue and presumed to have high Fe density were selected for this study (Fig. 1).

Field trials

The experiment was conducted in a randomized complete block design with two replications in Alfisols at ICRISAT, Patancheru, India (17° N; 78° E). Each accession was grown in one row of 4 m at a spacing of 75 cm between the rows in the 2010 rainy season, and at a spacing of 60 cm in the 2012 summer season, with a spacing of 15 cm between the plants within the row during the two seasons. The trials involved the application of 75 kg

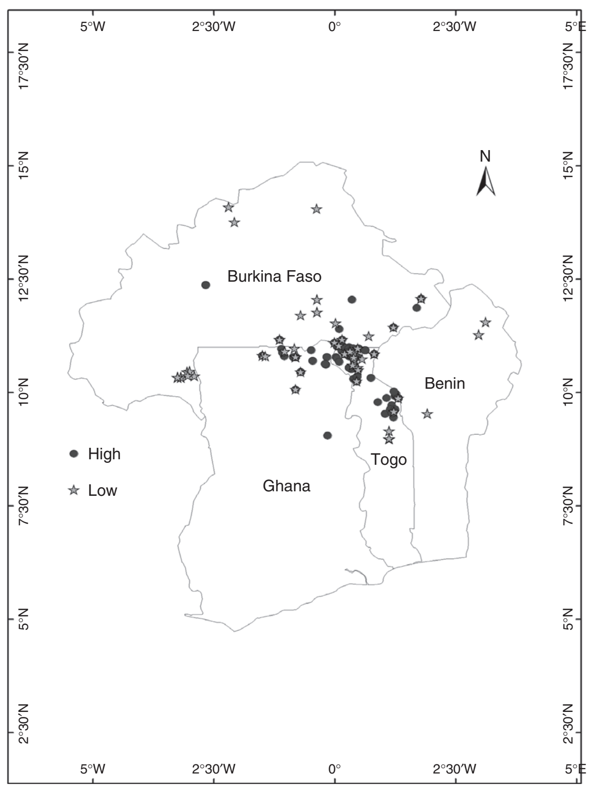

Fig. 1. Geographic distribution of 295 (190 high-Fe and 105 low-Fe) geo-referenced accessions of *iniadi* pearl millet germplasm for grain iron and zinc densities.

Table 1. Mean squares for grain iron and zinc densities, time to 50% flowering and 1000-grain mass for the accessions of *iniadi* pearl millet germplasm across two environments at Patancheru

Source of variation	df	Mean square: Fe (mg/kg)	Zn (mg/kg)	Time to 50% flowering (d)	1000-grain mass (g)
E	1	66 132.6	1525.6	4415.6	–
Rep/E	2	458.7	776.2	160.6	–
A	190	569.5**	206.6**	38.6**	5.6**
A × E	190	224.9	113.2**	15.4**	–
Error	378 (190)[a]	164.7	73.3	5.3	1.9

df, degree of freedom; E, environment; Rep/E, replication within the environment; A, accession.
[a] df for 1000-grain mass. ** Significant at the $P < 0.01$ level.

N/ha (50% basal in the form of diammonium phosphate and 50% top dressed at 20 d after sowing in the form of urea) and 35 kg P/ha during the two seasons. While the rainy season trial was conducted under rain-fed conditions, the summer season trial involved irrigation at an interval of 7 to 10 d until physiological maturity to avoid any moisture stress. Briefly, two soil samples were taken from the top layer (30 cm deep) at the time of planting to determine the available levels of Fe and Zn densities in the soil. In each plot, open-pollinated main panicles of eight to ten plants were harvested, sun-dried for 10 to 15 d and machine threshed (Wintersteiger – ID780ST4 – Single head thresher, Ried, Austria), and then clean grain samples (about 50 g), free of any debris, were collected for analysis of Fe and Zn density levels at the laboratory. Time to 50% flowering was recorded when the stigma in the main panicles of 50% of the plants in each plot had fully emerged. Random samples of 100 grains were used to determine 1000-grain mass in the 2012 summer season trial.

Laboratory and statistical analyses

Soil samples were analysed at the Central Analytical Services Laboratory at ICRISAT, Patancheru by extraction of Fe and Zn with the diethylenetriamine penta-acetic acid (DTPA) method. Fe and Zn contents in the extract were determined using an atomic absorption spectrophotometer (Model Savant AA; GBC Scientific Equipment, Victoria, Australia), as described by Lindsay and Norvell (1978). Grain samples were analysed using the inductively coupled plasma optical emission spectroscopy method developed by Wheal *et al.* (2011) at the Waite Analytical Services, Adelaide, Australia. A fixed model analysis of variance for testing the significance and determining the correlation between the levels of Fe and Zn was done following Gomez and Gomez (1984).

Results

Grain Fe and Zn density levels, time to 50% flowering and 1000-grain mass were found to be highly significantly different among the accessions ($P < 0.01$; Table 1). Also, there was a high significant effect of the accession × environment interaction, accounting for 40% of the variability due to differences in Fe density and time to flowering and 58% of the variability due to differences in Zn density among the accessions. The mean performance over the two seasons (environments) revealed that Fe density varied from 51 to 121 mg/kg and Zn density from 46 to 87 mg/kg among the accessions (Table 3; Fig. 2). There was a highly significant and high positive correlation ($r = 0.77$, $P < 0.01$) between the two micronutrients (Fig. 2). Fe density in 101 accessions (53%) was equal to or higher than that in the early-maturing, large-seeded and high-Fe control commercial

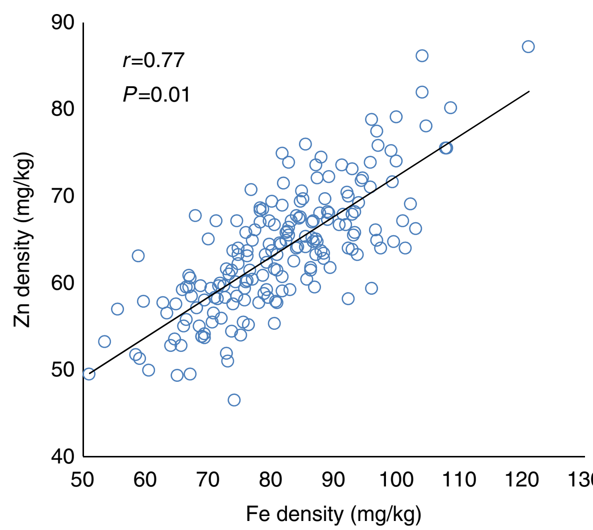

Fig. 2. Correlation between grain Fe and Zn densities in the accessions of *iniadi* pearl millet germplasm (mean performance over the two environments).

open-pollinated variety ICTP 8203 (81 mg/kg; Table 2). Of these high-Fe density accessions, ninety-two also had Zn density $\geq$61 mg/kg (59 mg/kg for ICTP 8203; Table 3). The Fe density of 27 accessions exceeded that of ICTP 8203 by $>$1 SED (standard error of difference). The Zn density of all these accessions exceeded that of ICTP 8203, with 20 accessions exceeding by $>$1 SED.

Averaged over all accessions, the mean Fe density level during the 2012 summer season was found to be 91 mg/kg, which was 26% higher than that in the 2010 rainy season (Table 3). Grain Zn density was comparable in both seasons (65 mg/kg in summer and 62 mg/kg in the rainy season). The DTPA-extractable Fe content in the soil was found to be 11.1 mg/kg in the 2012 summer season and 7.7 mg/kg in the 2010 rainy season, while the DTPA-extractable Zn content in the soil was comparable in both seasons (3.7 mg/kg in summer and 3.8 mg/kg in the rainy season). Fe density in the rainy season was found to be $<$100 mg/kg in most of the accessions selected for very high Fe levels, while it was $>$100 mg/kg in almost all of these accessions in the summer season, varying from 102 to 127 mg/kg (mostly between 15 and 50% higher than in the rainy season; Table 3). The differences in Zn density were much smaller between the rainy and summer seasons in most of the accessions, and where relatively larger differences did occur, more frequently, the levels of Zn density were found to be higher in the summer season.

On the basis of the mean performance over the two seasons, large differences were observed among the re-evaluated set of 191 accessions for time to 50% flowering (43–58 d), with 94% of the accessions flowering in 45 to 55 d (data not shown). None of the accessions selected for high levels of Fe and Zn densities flowered as early as ICTP 8203, flowering of which occurred in 40 d (Table 3). The summer season data showed large variability for 1000-grain mass (8.7–17.6 g), with 59% of the accessions selected for high levels of Fe density having 12 to 16 g of 1000-grain mass. Except for two accessions, all the others had smaller seeds than ICTP 8203 (14.3 g of 1000-grain mass), although 16 accessions had $>$12 g of 1000-grain mass, which is considered to be a large grain size in pearl millet. In the full set of 191 accessions, there was no significant correlation observed between grain mass

Table 2. Iron (Fe) and zinc (Zn) density classes for the accessions of *Iniadi* pearl millet germplasm (mean performance over the two environments) at Patancheru

Fe density class (mg/kg)	Zn density class (mg/kg)			
	51–60	61–70	71–80	81–90
81–90	IP 17 755, IP 17 843, IP 17 699, IP 17 634, IP 10 448, IP 17 621, IP 17 844	IP 17 770, IP 17 710, IP 17 550, IP 9386, IP 17 599, IP 17 597, IP 17 748, IP 17 861, IP 17 675, IP 8962, IP 17 596, IP 17 817, IP 12 852, IP 17 778, IP 8950, IP 9373, IP 9417, IP 17 701, IP 17 574, IP 17 542, IP 9298, IP 9396, IP 9466, IP 17 552, IP 9445, IP 8949, IP 17 605, IP 9406, IP 17 600, IP 17 648, IP 17 746, IP 17 521, IP 17 821, IP 9378, IP 9446, IP 17 612, IP 17 806, IP 17 814, IP 9506, IP 17 614, IP 17 607, IP 9438	IP 9362, IP 17 765, IP 11 535, IP 17 637, IP 17 659, IP 9371, IP 9503, IP 9410, IP 9268	
91–100	IP 17 752, IP 9372	IP 17 768, IP 17 650, IP 17 633, IP 17 846, IP 17 766, IP 17 747, IP 17 573, IP 17 832, IP 17 635, IP 17 616, IP 17 611, IP 17 741, IP 17 797, IP 17 608, IP 17 620, IP 12 926, IP 17 834	IP 12 937, IP 17 548, IP 17 591, IP 17 580, IP 17 685, IP 9571, IP 9447, IP 17 581, IP 17 816, IP 17 549, IP 17 672, IP 8960, IP 9414	
101–110		IP 12 933, IP 17 613, IP 17 609, IP 17 773	IP 17 556, IP 9259, IP 9404, IP 17 673	IP 17 836, IP 17 561, IP 17 602

Table 3. Iron (Fe) and zinc (Zn) densities and 1000-grain mass for the selected accessions of *iniadi* pearl millet germplasm, and trial means with standard errors of difference (± SED)

		Fe density (mg/kg)			Zn density (mg/kg)			Time to 50% flowering (d)			1000-grain mass (g)
Accession	Country of origin	Rainy 2010	Summer 2012	Mean	Rainy 2010	Summer 2012	Mean	Rainy 2010	Summer 2012	Mean	Summer 2012
IP 9372	Ghana	67	125	96	48	71	59	43	52	48	11.8
IP 9404	Ghana	89	127	108	75	76	76	49	49	49	13.9
IP 9414	Ghana	96	105	100	75	73	74	50	51	50	12.4
IP 9447	Ghana	74	118	96	61	96	79	48	50	49	11.5
IP 9571	Ghana	88	104	96	81	67	74	48	51	49	14.4
IP 12 926	Ghana	80	116	98	62	65	64	44	52	48	11.6
IP 12 933	Ghana	94	108	101	67	67	67	48	53	50	14.3
IP 8960	Togo	93	108	100	89	69	79	48	50	49	11.7
IP 9259	Togo	96	120	108	76	74	75	48	51	49	14.6
IP 17 549	Togo	84	115	99	69	81	75	45	55	50	13.2
IP 17 556	Togo	93	117	105	78	78	78	48	55	52	15.1
IP 17 561	Togo	95	114	104	83	89	86	44	50	47	12.7
IP 17 580	Togo	87	102	95	71	74	72	48	51	49	12.1
IP 17 581	Togo	84	110	97	71	84	77	49	52	50	13.5
IP 17 591	Togo	75	114	95	74	70	72	45	53	49	10.7
IP 17 602	Togo	119	124	121	96	78	87	45	47	46	11.7
IP 17 608	Togo	95	98	97	73	59	66	42	50	46	16.0
IP 17 609	Togo	90	115	102	65	73	69	45	55	50	11.6
IP 17 613	Togo	82	121	101	52	76	64	55	61	58	10.7
IP 17 620	Togo	90	104	97	65	64	65	53	56	54	13.2
IP 17 672	Togo	91	108	99	68	76	72	53	54	54	13.3
IP 17 673	Togo	100	117	109	85	75	80	45	53	49	13.4
IP 17 685	Togo	83	109	96	61	81	71	57	57	57	11.2
IP 17 773	Togo	88	119	103	64	68	66	51	48	49	12.2
IP 17 816	Togo	91	104	97	78	74	76	41	49	45	11.2
IP 17 834	Togo	84	116	100	58	71	65	48	53	51	12.3
IP 17 836	Togo	93	115	104	80	84	82	45	52	48	11.7
Control ICTP 8203		79	83	81	62	55	59	40	40	40	14.3
Trial mean		72	91	81	62	65	64	47	51	49	12.8
Trial minimum		44	50	51	40	41	46	40	44	43	8.7
Trial maximum		119	127	121	96	96	87	58	61	58	17.6
SED		12.6	13.5	12.9	8.3	8.8	8.8	2.6	2.0	2.5	1.4

and Fe and Zn densities ($r = 0.12$ and 0.04, respectively). Similarly, there was no significant correlation observed between time to flowering and Fe and Zn densities ($r = 0.08$ and 0.04, respectively). Also, there was no correlation observed between grain mass and time to flowering ($r = 0.05$).

Discussion

Among a large number of pearl millet cultivars produced in India, an early-maturing and large-seeded open-pollinated variety, ICTP 8203, derived from selection within an *iniadi* landrace from Togo has been found to have the highest level of Fe density (Rai *et al.*, 2013). This variety, released in India in 1988 (Rai *et al.*, 1990), was cultivated on more than 0.8 million ha at the peak of its adoption in 1995 (Bantilan *et al.*, 1998), and it is still cultivated on about 0.2 million ha, mostly in the Maharashtra state of India. While ICTP 8203 serves as a good source of high levels of Fe density for breeding high-Fe cultivars, as are some of the population progenies of *iniadi* origin, this study identified several *iniadi* accessions having far higher levels of Fe density than those observed in ICTP 8203. Most of these high-Fe accessions also had far higher levels of Zn density than ICTP 8203, which resulted from a highly significant and high positive correlation between these two micronutrients. Earlier studies in pearl millet have also shown a highly significant and high positive correlation between the levels of Fe and Zn densities (Velu et al., 2007, 2008; Gupta *et al.*, 2009; Rai *et al.*, 2012; Govindaraj *et al.*, 2013). Similar relationships between Fe and Zn densities have been reported in other cereals such as finger millet (Upadhyaya *et al.*, 2011), sorghum (Ashok Kumar *et al.*, 2009, 2013), maize (Oikeh et al., 2003, 2004), rice (Anandan *et al.*, 2011) and wheat (Garvin *et al.*, 2006; Velu *et al.*, 2011). These positive correlations could be due to common and overlapping quantitative trait loci (QTL) for grain Fe and Zn densities as reported in pearl millet (Kumar, 2011), wheat (Peleg *et al.*, 2009; Singh *et al.*, 2010), rice (Stangoulis *et al.*, 2007) and common bean (Blair *et al.*, 2009; Cichy *et al.*, 2009). Thus, it would appear that these selected *iniadi* accessions can be effectively used to introgress high levels of Fe and Zn densities into elite breeding lines as selection for any one of these micronutrients is likely to lead to a correlated selection response for the other micronutrient.

While mean grain Fe density levels over all the accessions were found to be higher in the summer season than in the rainy season as observed for soil Fe content in these two seasons, the mean grain Zn density levels were found to be similar in both seasons as observed for soil Zn content. However, the patterns of Fe and Zn contents between the two seasons in the soil may not entirely be the reason for the corresponding patterns of grain Fe and Zn densities in these two seasons as the levels of both micronutrients in the soil were much above their critical levels of 2.0 mg Fe/kg and 0.8 mg Zn/kg. It is possible that with better control of soil moisture in the summer season, resulting from controlled irrigation regime, leading to better nutrient uptake from the soil, and more efficient nutrient translocation and loading under mostly clear and/or high-temperature conditions, accessions may show higher levels of Fe density in the summer season than in the rainy season. Considering the large genotype × environment interaction, further studies are needed to evaluate these accessions identified with high levels of Fe and Zn densities over a diverse range of environments to select those with stable performance.

Earliness is a farmer-preferred trait in most of the pearl millet-growing regions, as it helps escape terminal drought and permits double cropping. Large grain size is also a farmer-preferred trait in several parts of Africa and India. Lack of any adverse association of Fe and Zn densities with time to flowering and grain mass showed that accessions identified with high levels of Fe and Zn densities can be effectively used to breed improved high-Fe and high-Zn density cultivars without compromising on maturity and grain size. Since pearl millet germplasm accessions are highly variable populations due to the cross-pollinated breeding system of this crop, it is likely that progenies with much higher levels of these micronutrients can be produced by selecting within these accessions, as has been shown by developing a higher-Fe version of ICTP 8203 through intra-population selection (Rai *et al.*, 2013). Production and evaluation of progenies from the accessions of *iniadi* germplasm selected with high levels of Fe and Zn densities are under way at ICRISAT to derive stable breeding lines with much higher levels of these micronutrients. These breeding lines will be used in crosses with elite inbred lines to develop high-yielding hybrid parents and consequently high-yielding hybrids with higher levels of Fe and Zn densities. These lines will also be used to investigate the relationships of Fe and Zn densities with grain yield, and to identify those for breeding open-pollinated varieties that exceed the grain yield and Fe and Zn densities of ICTP 8203. Some of these lines can be used for developing mapping populations to identify QTL for high levels of Fe and Zn densities. Genomic studies of the accessions of *iniadi* germplasm selected for high levels of Fe and Zn densities would provide useful information on the extent of diversity for genes responsible for high levels of these two micronutrients in these accessions. It would also be interesting to examine the distribution of Fe and Zn densities in the grains of breeding lines selected with high levels of these micronutrients.

Numerous positive agronomic attributes of *iniadi* germplasm, such as relatively less photoperiod sensitivity, early flowering, large seeds of dark grey colour and globular shape, compact panicles and good combining ability, have led to its extensive utilization in the breeding programmes worldwide (Andrews and Anand Kumar, 1996). A recent research has shown that lines derived from this germplasm could be good sources of downy mildew (*Sclerospora graminicola* (Sacc.) Schroet) resistance, drought tolerance and high stover quality (Rai *et al*., 2008). Therefore, the results of this study showing this germplasm as a source of high levels of Fe and Zn densities, and also showing that these traits are not associated with time to flowering and seed size, indicate *iniadi* germplasm to be a valuable germplasm resource for genetic improvement in pearl millet.

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