Breeding pearl millet cultivars for high iron density with zinc density as an associated trait

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Abstract

Pearl millet, as a species, has higher levels of iron (Fe) and zinc (Zn) densities than other major cereal crops. However, this study showed the existence of about twofold variability for Fe density (31–61 ppm) and zinc density (32–54 ppm) among 122 commercial and pipeline hybrids developed in India. Thus, there is a need to increase the cultivation of hybrids having higher Fe and Zn levels and enhance their consumption to better address various health problems associated with the deficiencies of these micronutrients. High-yielding open-pollinated varieties (OPVs) and hybrids with higher levels of Fe and Zn densities than those found in most of the commercial cultivars otherwise not bred for these micronutrients as target traits have been developed and are available for commercialization. Breeding lines and germplasm with still higher levels of Fe and Zn densities have been identified. Their utilization in breeding has the potential to enable development of hybrids with >75 ppm Fe density and >55 ppm Zn density. The primary focus of pearl millet biofortification is on improving Fe density with Zn density as an associated trait. Depending on the genotypic composition of the trials, moderate to high correlations between Fe and Zn densities have been observed, indicating good prospects of simultaneous genetic improvement for both traits, but perhaps also the need to make conscious selection for Zn density along with Fe density. Lack of association of Fe and Zn densities with grain size showed that both micronutrients can be improved without compromising on seed size. The association of Fe and Zn densities with grain yield was weak and negative, but not always significant, indicating that both micronutrients can be improved without significantly compromising grain yield by using large segregating populations. This, however, is one area that merits further research.

Introduction

Micronutrient malnutrition is increasingly being recognized as a serious public health problem affecting more than half of the population worldwide, with preschool children and women in the developing countries being most vulnerable (UN SCN 2004). Most widespread of these are dietary deficiencies in iron (Fe), zinc (Zn) and vitamin A. Among the 20 major risk factors of the global burden of disease estimates, Fe deficiency ranks 9th and Zn deficiency ranks 11th, while in the high mortality countries, including India, they rank 6th and 5th, respectively (WHO 2002). Iron deficiency is the principal cause of anemia that leads to morbidity and mortality of mother and child at childbirth, and impairs cognitive skills and physical activity (Scrimshaw 1984, Hercberg et al. 1987, Welch 2002), while Zn deficiency leads to immune system dysfunctions and high susceptibility to infectious diseases, retardation of mental development, altered reproductive biology, gastrointestinal problems and stunted growth of children (Prasad 1996, Solomons 2003, Black et al. 2008). These nutritional deficiency problems have enormous socioeconomic impacts at the individual, community and national levels (Darnton-Hill et al. 2005, Stein 2010). India annually loses about 4 million disability-adjusted life years (DALYs) due to Fe deficiency and another 2.8

million DALYs due to Zn deficiency (Qaim et al. 2007). Pharmaceutical supplementation, industrial food fortification, and agricultural approaches of dietary diversification and biofortification have been suggested to address these problems. Biofortification of stable crops is most cost-effective, sustainable, consumer-acceptable, pro-rural and pro-poor intervention.

Pearl millet (*Pennisetum glaucum*), grown as a major warm-season cereal crop on about 28 million ha in the arid and semi-arid regions of Asia and Africa (Yadav et al. 2012), is highly nutritious with high protein content and dietary fiber, more balanced amino acid profile and high mineral content (Andrews and Anand Kumar 1992). A recent study showed that pearl millet accounts for 19–63% of the total Fe intake and 16–56% of the total Zn intake from all food sources in some parts 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 as compared to other cereals and vegetables. Large variability for Fe and Zn density has been reported in a diverse range of breeding lines, improved populations and germplasm (Velu et al. 2007, 2008, Rai et al. 2012), indicating that cultivars with higher levels of these micronutrients can be developed in pearl millet. Pearl millet biofortification program at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), supported by HarvestPlus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR), has initiated a major effort towards the development of high-yielding cultivars with high Fe and Zn density. In this paper, we report on pearl millet biofortification strategy, variability for Fe and Zn density in commercial and pipeline cultivars as well as in breeding lines, association between micronutrient densities and productivity, and prospects of breeding high-yielding cultivars with high levels of these micronutrients.

Pearl millet biofortification strategy

The thrust of ICRISAT's pearl millet improvement program at Patancheru, India is on the development and dissemination of a wide range of improved breeding lines, populations and hybrid parents for utilization by public and private sector pearl millet programs, largely targeted for India, which has the largest pearl millet area (>9 million ha) in the world. As a consequence of this strategy, a large number and diverse range of germplasm (mostly from the western African regions) has been used, and diversified breeding products have been developed and disseminated. These have been extensively used both in the public and private sector to develop a large number of hybrids. In view of the potential large diversity among the hybrids, it was considered to examine hybrids under cultivation and those in the pipeline for release with respect to variability for Fe and Zn density, and identify those with high levels of these micronutrients to enhance their adoption. Open-pollinated varieties (OPVs) were also evaluated for Fe and Zn density to identify those with high levels of these micronutrients for use in further biofortification research and possible development of their improved versions. Parental lines of potential hybrids and improved breeding lines were also evaluated for variability for Fe and Zn density to identify those for use in biofortified hybrid and hybrid parent development. For the longer term, these micronutrient-dense breeding lines, hybrid parents and lines derived from populations identified for high Fe and Zn densities will be used in hybridization programs and population breeding to develop a diverse range of high-yielding and disease resistant breeding lines and hybrid parents with higher levels of Fe and Zn densities in the context of Fe and Zn density being used as core traits and integral part of pearl millet improvement. Thus, in search for additional sources of high Fe and Zn density for sustained long-term genetic improvement, germplasm evaluation has been undertaken.

Since almost all the high-Fe breeding lines and populations were reported to be entirely or largely based on *iniadi* germplasm (Velu et al. 2007, 2008), which are specifically adapted to peninsular India, the initial thrust in pearl millet biofortification research has been targeted to this region. These studies also showed that there is larger variability for Fe density than for Zn density, and there is high positive association between these two traits. Also, Fe deficiency is a more serious problem (both in extent and intensity) than Zn deficiency. Thus, higher priority in the program was given to genetic improvement of Fe density, with Zn density as an associated trait. In terms of breeding approach, the total efforts have been through conventional breeding than genomic approaches due to former's greater cost-effectiveness and short time frame for biofortified cultivar development and delivery. Considering the quantitative inheritance of Fe and Zn density and hence the resultant genotype × environment interaction, a broad-based partnership with the public and private sector breeding programs was developed for multilocation testing to accelerate the biofortification breeding process.

Variability in commercial and pipeline hybrids

A trial of 122 hybrids (21 hybrids from 9 public sector research organizations, including ICRISAT; and 101 hybrids from 33 seed companies) that had been released or were either marketed as truthfully labeled seed by the private sector, or were in the pipeline, were evaluated in a

3-replication trial of 1-row plot of 4 m length in a randomized complete block design at AICPMIP (All India Coordinated Pearl Millet Improvement Project) Center, Mandor, Rajasthan in northwestern India and at Patancheru, Andhra Pradesh in peninsular India during the 20l1 rainy season. ICTP 8203 was used as a high-Fe control, which is an OPV developed at ICRISAT by direct selection in an *iniadi* landrace from northern Togo (Rai et al. 1990) and had been found having the highest level of Fe density among several populations in a preliminary test (Velu et al. 2008) and in several trials that had followed over the years.

Large variability was observed both for Fe and Zn density among the hybrids (Table 1). Ninety-five (78%) hybrids had 36–50 ppm Fe density and 92 (75%) hybrids had 36–45 ppm Zn density. Nine hybrids had high Fe density (56–65 ppm) and Zn density (46–55 ppm), which were far higher than densities observed in improved wheat (*Triticum aestivum*) varieties (25–35 ppm Fe and 24–31 ppm Zn) (G Velu, CIMMYT, personal communication). ICTP 8203 had the highest Fe density (71 ppm) as well as Zn density (58 ppm). Shradha, developed from a cross between RHRBH1A and RHRBI 138 at Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, is a large-seeded and dark gray color hybrid, typical of *iniadi* landrace (Andrews and Anand Kumar 1996). The remaining eight hybrids are from private seed companies. The hybrid 86 M 86 has been developed by Pioneer Seed Company and it has *iniadi* germplasm in both its male and female parents (personal communication, RS Mahala, Pioneer Overseas Corporation, India). While the broad nature of germplasm involved in the parental lines of other hybrids is not known, hybrids PAC 903 and PAC 982 also have *iniadi* germplasm in their parentage. Shradha is specifically adapted to Peninsular India while the other three hybrids, 86 M 86, PAC 903 and PAC 982, have wider adaptation with 86 M 86 among these being cultivated most widely.

Character association

There was a highly significant and high positive correlation (r=0.78; P <0.01) between Fe and Zn density in hybrids tested in the hybrid trial (Fig. 1) although these hybrids had not been bred for these micronutrients as target traits. Similar high positive correlations between Fe and Zn density in pearl millet have been reported in other studies (Velu et al. 2007, 2011, Govindaraj et al. 2013). This would indicate that while breeding for high Fe density as the priority trait in pearl millet, Zn density would improve as an associated trait. Further investigations in six pearl millet biofortification hybrid trials have shown highly significant but moderate to high positive correlations between Fe and Zn density, ranging from 0.49 to 0.71 (P <0.01) (Table 2). Thus, it would appear that for significant genetic gains to be made for Zn density, conscious selection for it along with Fe density will also have to be made, and simultaneous selection for both micronutrients should be effective.

Breeding for high Fe and Zn density must not compromise with grain yield and grain size. Any adverse association of Fe and Zn density with grain yield (dilution effect) and grain size (concentration effect) would impact the efficiency of breeding for these micronutrients in high-yielding and large grain size backgrounds. Results of the six hybrid trials mentioned above showed weak to moderate negative correlations between Fe density and grain yield, although not always significant, and mostly negative and weak but statistically non-significant correlations between Zn density and grain yield (Table 2). It would appear that the effect of such weak and variable associations can be circumvented, especially for Zn density, using large segregating populations. Both Fe and Zn densities had non-significant weak and often positive correlations with grain size, indicating that breeding for these micronutrients in large seed size background would require no additional resources. Further research using random sets of lines derived from random-mated populations constituted from crosses between high-Fe/Zn and low-Fe/Zn lines is required to examine the magnitude and direction of association of these micronutrients with grain yield and seed size.

Prospects of breeding high-yielding cultivars with high Fe and Zn density

Large intra-population variability for Fe and Zn densities had been reported in several populations, including ICTP 8203 (Velu et al. 2008). ICTP 8203 is a popular OPV in peninsular India, grown on about 800,000 ha in Maharashtra alone at the peak of its adoption in 1995 (Bantilan et al. 1998). It is still cultivated on about 150,000 ha in this region and parts of Rajasthan and Uttar Pradesh in northern India. It has been observed that Fe and Zn densities are largely under additive genetic control (Velu et al. 2011, Govindaraj et al. 2013), implying effective intra-population improvement for Fe and Zn density. Thus, four improved versions of ICTP 8203 based on S_2 progeny testing were developed in 2009 and further three versions of it, based on S_3 progeny testing, were developed in 2010. Four of these (two from each year) had been evaluated in 42 common trials (10 trials in 2010 and 32 trials in 2011) across India. One version, designated as ICTP 8203 Fe 10-2, which had been developed by recombining 11 S_3 progenies, had on

Table 1. Distribution of released, commercial and pipeline pearl millet hybrids in various grain iron (Fe) and zinc (Zn) density classes, 2011 rainy season[1].

	Zn density class (ppm)					No. of hybrids (Fe)
Fe density class (ppm)	31–35	36–40	41–45	46–50	51–55	
31–35	HHB 197, ABH 999, Bio 70	GHB 719, Mahodaya 325, RBH 173, RBH 121, GHB 538	HHB 223			9
36–40	N 61, RHB 154, Hi Pearl 51, Bio 8494, 86 M 66	Shanti, GHB 744, Mukhia, GK 1135, Mahodaya 318, PHB 2168, JKBH 1103, NPH 1651, GHB 732, NPH 2798, Bio 8141, HHB 94, MPMH 17, RBH 177, Proagro 9444	NBH 1188, KBH 261			22
41–45	HTBH 4201, VBBH 3028, Mahodaya 331	ABH 1, NBH 1717, Bisco 5151, NBBH 411, X 7, KBH 9119, Bio 13, NBH 5464, JKBH 1096, TEJAS, Biogene 33+, Navbharat Gavari, HTBH 4202, RBTH 10-007, VBH 444, VBH 456, M 64, Nu 306, Kaveri Boss 65	KBH 11, NPH 3685, Ajeet 37, S 368, VBBH 3087, GHB 558, Mahodaya 330, Gangotry 67, NBBH 908, KH 302, Bisco 5141, B 2301, 86 M 88, Solid 78, GK 1104, Euro 1133, MRB 204, NPH 2475, Kaveri Super Boss	86 M 01, Hi Pearl 138		43
46–50		Sujlam 68, Hi Pearl 91, VIRAT, Kaneri × 563, GK 1116, B 2195, S 361, KBH 7206, N 68	MRB 2210, TNBH 0642, S 362, Ajeet 35, RBH 1818, Proagro 9450, Nu 310, VBBH 3115, PAC 909, RBTH 10-005, NBH 1024, GK 1090, Hi Pearl 130, KBH 2360, JKBH 1097	KBH 287-36, Sanjivani 333, VBBH 3125, JKBH 676	NBH 4903, Dhamal	30
51–55		HTBH 4203	Navbharat Banas express, PAC 931, KBH 7201, NBH 1134, ICMH 356	Saburi, Sanjivani 2312, RBH 9		9
56–60			HTBH 4204	PAC 982, Ajeet 39, XL 51, 86 M 86, PAC 903	Shradha	7
61–65				Ajeet 38	Sanjivani 222	2
No. of hybrids (Zn)	11	49	43	15	4	122

1. Mean of two locations.
Open-pollinated variety ICTP 8203, used as a control, had 71 ppm Fe density and 58 ppm Zn density.

an average, 71 ppm Fe density (9% more than ICTP 8203), 38 ppm Zn density (comparable to ICTP 8203), and 2.2 t ha^{-1} grain yield (11% more than ICTP 8203) (Table 3). It was also the most farmer-preferred variety in on-farm trials conducted by Nirmal Seeds Pvt. Ltd. in Maharashtra. A similar intra-population selection for Fe density was carried out in another commercial OPV (ICMV 221). Based on X-Ray Fluorescence Spectrometry (XRF) results of limited 4-environment trials at Patancheru, a high-Fe version of it, designated as ICMV 221 Fe-11-1, had 87 ppm Fe density (22% more than ICMV 221), 62 ppm Zn density (16% more than ICMV 221) and 4.02 t ha^{-1} grain yield (2% higher than ICMV 221. Multilocational evaluation of these two varieties is underway. The two most popular and widely cultivated hybrids are 86 M 86 and 9444. While both are equally high-yielding, 9444 was among those having the lowest levels of Fe and Zn density and 86 M 86 was among those few with the highest levels of Fe and Zn densities (Fig. 1). All these results further indicate that it is possible to combine high grain yield with high levels of Fe and Zn densities.

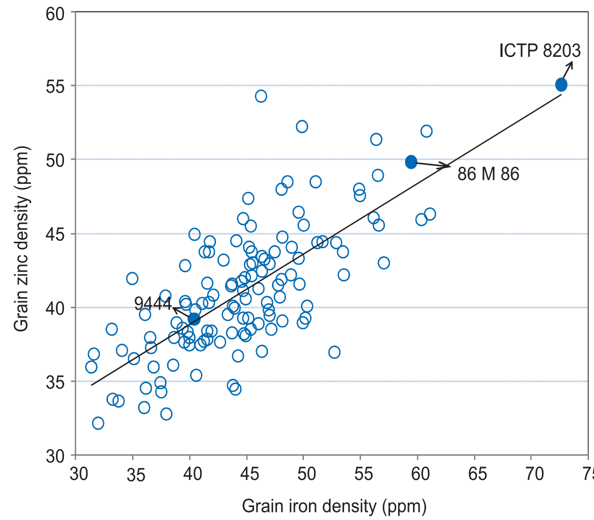

Figure 1. Variability and relationship between grain iron (Fe) and zinc (Zn) density in commercial hybrids of pearl millet (Note: Mean of two locations. Popular cultivars denoted by solid circles).

Table 2. Correlation among grain iron (Fe) and zinc (Zn) density, grain yield (GY) and 1000-seed weight (SW) in four initial hybrid trials (IHTs) and two advanced hybrid trials (AHTs) of pearl millet, 2011 rainy season, Patancheru, India[1].

		Correlation coefficient (r)[2]				
Trial	No. of entries	Fe vs. Zn	Fe vs. GY	Zn vs. GY	Fe vs. SW	Zn vs. SW
AHT-A	24	0.53**	–0.13	0.17	–0.01	0.05
AHT-B	28	0.49**	–0.24	–0.32	0.21	0.02
IHT-A1	30	0.67**	–0.58**	–0.24	0.32	0.22
IHT-A2	26	0.67**	–0.39*	–0.04	0.12	–0.05
IHT-B1	36	0.71**	–0.27	–0.18	0.04	0.29
IHT-B2	34	0.58**	–0.48**	–0.28	–0.15	0.07

1. Source: Rai et al. (2012).
2. * = Significant at 5% level; ** = Significant at 1% level.

Table 3. Grain iron (Fe) and zinc (Zn) density, grain yield and time to 50% flower of biofortified pearl millet cultivars.

	Fe (ppm)		Zn (ppm)		Grain yield (t ha^{-1})		Time to 50% flower[1] (days)	
Cultivar	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Variety trial[2]								
ICTP 8203	65	31–94	39	19–71	1.97	0.63–4.30	45	38–56
ICTP 8203 Fe 10-2	71	49–106	38	18–71	2.20	0.65–5.65	45	40–55
Hybrid trial[3]								
ICMH 1201	76	47–97	40	21–58	3.70	2.28–6.20	47	41–56
86 M 86	57	42–71	38	22–61	4.51	2.73–6.47	54	47–60
ICTP 8203	74	50–102	42	23–75	2.69	1.33–5.06	44	38–55

1. Mean of 25 trials for variety trial.
2. Conducted in 42 locations × year environments during 2010 and 2011.
3. Conducted in 31 locations × year environments during 2011 and 2012.

As mentioned before, the thrust of pearl millet biofortification program at ICRISAT, Patancheru is towards hybrid development. The most extensive multilocation field trials of biofortified hybrids, for the first time, were initiated in 2011. Based on the mean performance in 31 trials conducted in 2011 and 2012, an experimental hybrid, designated as ICMH 1201, was identified that had 76 ppm Fe density and 40 ppm Zn density (both comparable to those in ICTP 8203), but its yield was 3.7 t ha^{-1} of grain (38% higher than ICTP 8203). This hybrid flowered only 3 days later than ICTP 8203, so it fits well in the same early maturity group. ICTP 8203 is particularly adapted to marginal environments associated with low productivity. Since the productivity levels of the environments in this trial had a wide range (2.28–2.83 t ha^{-1}), we examined if there existed any relationship between the productivity levels of the environments and relative yield advantage of ICMH 1201 over ICTP 8203. At each productivity level within this range, ICMH 1201 had higher grain yield than ICTP 8203, and there was no relationship between the productivity level of the environments and grain yield advantage of ICMH 1201 over ICTP 8203 (Fig. 2). This experimental hybrid and ICTP 8203 are being evaluated in on-farm trials in Maharashtra. Further, both Fe and Zn densities in this hybrid were not associated with grain yield levels as observed in various trials (Fig. 3).

So far, biofortified hybrids for high Fe and Zn densities had been developed using parental lines identified having higher levels of these micronutrients from amongst those that had not been actually bred for Fe and Zn density as target traits in the mainstream breeding. Use of such lines would continue until parental lines with higher Fe and Zn densities are developed through targeted breeding for these micronutrients in high-yielding backgrounds. Large variability for both Fe and Zn density in advanced breeding lines not specifically bred for these micronutrients has been observed (Table 4). Progenies derived from some of the OPVs identified for high Fe density have been found having >100 ppm Fe density and >60 ppm Zn density. Further evaluation and utilization of these materials and those being identified in the germplasm will provide additional genetic resources to breed parental lines that should enable breeding high-

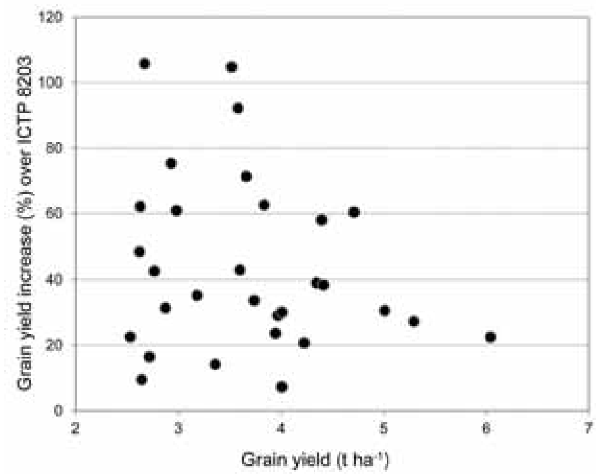

Figure 2. Grain yield superiority of high-Fe pearl millet hybrid (ICMH 1201) over ICTP 8203 at various productivity levels.

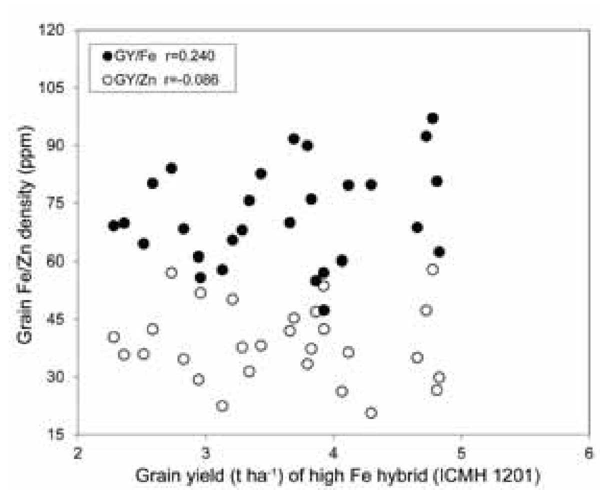

Figure 3. Grain iron (Fe) or zinc (Zn) density (ppm) and grain yield of high Fe hybrid (ICMH 1201) (Note: Means of 28 locations).

Table 4. Variability for grain iron (Fe) and zinc (Zn) density in pearl millet, 2011 rainy season, Patancheru, India[1].

Material	No. of entries	Distribution (%) of entries in micronutrient (ppm) class					
		<40	41–60	61–80	81–100	101–120	>121
Fe density							
Advanced breeding lines	386	25	47	23	5	0	0
Population progenies	232	0	2	28	50	17	3
Zn density							
Advanced breeding lines	386	48	49	3	0	0	0
Population progenies	232	0	50	46	4	0	0

1. Source: Rai et al. (2012).

yielding hybrids with >75 ppm Fe density and >55 ppm Zn density.

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