Research Articles

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Grain iron and zinc densities in released and commercial cultivars of pearl millet *(Pennisetum glaucum)*

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ABSTRACT

Crop biofortification is a cost-effective and sustainable agricultural strategy to reduce micronutrient malnutrition arising from iron (Fe) and zinc (Zn) deficiencies. A large number of hybrids and open-pollinated varieties (OPVs) of pearl millet *[Pennisetum glaucum* (L.) R. Br.] have been released and/or commercialized in India. Eighteen OPVs and 15 high-Fe candidate hybrids were evaluated in multi-location trials for Fe and Zn density to identify those with high density of these micronutrients. The Fe density in OPVs varied from 42 mg/kg to 67 mg/kg, and Zn density from 37 mg/kg to 52 mg/kg with ICTP 8203 having the highest Fe density (67 mg/kg) followed by ICMV 221 (61 mg/kg) and AIMP 92901 (56 mg/kg). While ICTP 8203 had also the highest level of Zn density (52 mg/kg), ICMV 221 and AIMP 92901 had 45-46 mg/kg Zn density. The Fe density in hybrids varied from 46 mg/kg to 56 mg/kg and Zn density from 37 mg/kg to 44 mg/kg. Four hybrids, viz. Ajeet 38, Proagro XL 51, PAC 903 and 86M86 had the highest Fe density of 55-56 mg/kg and 39-41 mg/kg Zn density. The six commercial cultivars (2 OPVs and 4 hybrids) identified in this study with high Fe and Zn densities can be undertaken for expanded cultivation in their recommended ecologies to specifically address the Fe and Zn deficiencies in India. This study also enabled to re-define base line for Fe density at 42 mg/kg for hybrids, the most dominant cultivar type grown in India.

Key words: Base line, Cultivar, Iron, Pearl millet, Stability, Variability, Zinc

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major warm-season cereal, grown primarily for grain production on more about 28 million ha in the arid and semi-arid tropical environments of Asia and Africa with India being the largest producer, both in terms of area (>9 m ha) and production (10 million tonnes) (Yadav *et al.* 2012). It is a significant source of Fe and Zn in the major pearl millet growing states of India. For instance, it has been shown to account for 19-63% of the total Fe and 16-56% of total Zn intake from all food sources in pearl millet growing areas of Maharashtra, Gujarat and Rajasthan (Parthasarathy Rao *et al.* 2006). It is also the cheapest source of these micronutrients as compared to other cereals and vegetables. Cultivated pearl millet has higher levels of both micronutrients, especially Fe content, than other major cereals such as rice, wheat, maize and sorghum (Dwivedi *et al.* 2012). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), in alliance with the HarvestPlus Biofortification Challenge Program of the CGIAR and in partnerships with public and private sector breeding programs in India, has undertaken a major initiative to examine the prospects of breeding pearl millet cultivars with higher Fe and Zn levels. Large variability for these micronutrients has been observed in pearl millet germplasm and breeding materials (Rai *et al.* 2012, 2015). Considering the larger variability for Fe density, greater seriousness of Fe deficiency than Zn deficiency in certain populations, and highly significant and positive association between these two micronutrients, major emphasis is on Fe density, with Zn density being improved as an associated trait (Rai *et al.* 2013).

ICRISAT has developed and disseminated a large number and diverse range of improved breeding lines and hybrid parents to pearl millet breeding programs in India, which have been used along with the locally bred materials to develop open-pollinated varieties (OPVs) by various research programs in the public sector, and hybrids in both public and private sector (Gowda *et al.* 2004, Mula *et al.* 2007). Considering the large variability for Fe and Zn densities in ICRISAT-bred lines (Rai *et al.* 2012, 2014a) and their extensive utilization in cultivar development, large variability for these micronutrients can be expected among

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these hybrids and OPVs. The objective of this research was to assess the magnitude of variation for these micronutrients among the released OPVs and hybrids (both released and commercialized as truthfully labeled seed), and to identify the ones with high Fe and Zn density, which may be undertaken for expanded cultivation and preferentially included in development programs targeting crop-based nutritional security.

MATERIALS AND METHODS

Two experiments were conducted for multi-location evaluation of cultivars to assess variability for Fe and Zn densities. In one trial, 18 released OPVs developed at nine public sector research organizations, including ICRISAT, were evaluated at seven locations (three in peninsular India and four in northern India) in 2011. One-hundred and twenty two hybrids (21 released hybrids from nine public sector research organizations, including ICRISAT; and 101 released and/or commercialized as truthfully labeled seed from 33 seed companies) had been evaluated in a preliminary trial at Mandor, Jodhpur, Rajasthan; and Patancheru, Telangana in 2011 rainy season. Fifteen hybrids having high-Fe density (50 to 61 mg/kg) in this trial were re-evaluated at 10 locations (five each in the northern and peninsular India) in 2012 rainy season. Both trials were conducted in two-row plots of 4 m in a randomized complete block design, replicated three times (except one location in each trial had 2 replications). Standard cultural practices, in terms of planting time, plant spacing, fertilizer application, thinning and irrigation (required, if any) recommended for the regions were followed for crop management. At maturity, open-pollinated main panicles of 12 plants from each plot in OPV trial and 10 plants in hybrid trial were harvested, put in gunny bags, sundried for 10-12 days and bulk threshed to collect 30 g seed samples from each plot for Fe and Zn analysis.

Ground flour from seed (whole grain) samples from both trials were analyzed for Fe and Zn density using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method at the Waite Analytical Services, University of Adelaide, Australia, which is a reference laboratory of the HarvestPlus Program for Fe and Zn analysis. Best Linear Unbiased Predictors (BLUPs) were calculated and combined analysis of variance was carried out using SAS MIXED procedure (SAS Inst 2013), assuming genotype and environment as fixed effects. Least Square means were estimated for main and interaction effects. Additive Main Effects and Multiplicative Interaction (AMMI) model (Zobel *et al.* 1988) was used for the analysis of genotype × environment (G×E) interaction that partitions G×E interaction sum of square into various Interaction Principal Components Axes (IPCAs). Following Purchase *et al.* (2000), these IPCAs were used to derive a single stability measure called AMMI Stability Value (ASV) for each genotype.

From among the 122 hybrids evaluated at two locations in 2011, the data from 15 common hybrids included in the same 2-location trial in 2011 and tested in 10-location trial in 2012 were used to estimate the probable mean of the remaining 107 hybrids for 2012 based on their mean in 2011. These values were used to derive the base line of Fe density as given below:

$$\text{Fe base level} = \left[\frac{m_3 m_1 n_1}{m_2} + m_3 n_2\right] / N$$

where, N, Total number of hybrids evaluated in 2011 (=122); n_1, number of hybrids only evaluated in 2011 (=107); n_2, number of hybrids evaluated in both years (=15); m_1, mean Fe density of n_1 hybrids at two locations in 2011 (=43); m_2, mean Fe density of n_2 hybrids at two locations in 2011 (=55); m_3, mean Fe density of n_2 hybrids at 10 locations in 2012 (=52).

$$\text{So, the base Fe level} = \left[\left(\frac{52}{55} \times 43 \times 107\right) + (52 \times 15)\right] / 122$$

RESULTS AND DISCUSSION

Variability in OPVs

Genotypic (G) effects were highly significant (P<0.01) both for grain Fe and Zn densities (Table 1) and contributed 20-30% of total variation. Based on the mean performance over seven environments, the mean Fe density ranged from 42 to 67 mg/kg and Zn density from 37 to 52 mg/kg (Table 2). ICTP 8203 had the highest Fe density among all the OPVs. The other two high-Fe OPVs were ICMV 221 (61 mg/kg) and AIMP 92901 (56 mg/kg). There was a highly

Table 1 Mean square for additive main effects and multiplicative interaction model for iron (Fe) and zinc (Zn) densities in open-pollinated varieties and commercial hybrids of pearl millet.

Source of variation	df	Mean square			
		Open-pollinated varieties trial		Commercial hybrids trial	
		Fe density	Zn density	Fe density	Zn density
Rep (Environment)	13 (19)†	103*	45.3*	21.4	28.1**
Environments	6 (9)	4896**	1959.6**	4383**	4769**
Genotypes	17 (14)	1222.4**	326.8**	365.2**	180.6**
Interactions	99 (124)	92.4**	57.2**	68.3**	27.7**
IPCA1	22 (22)	226.7**	115.9**	152.3**	45.3**
IPCA2	20 (20)	72	65.3**	72.8**	37.0**
IPCA3	18 (18)		39.5*	74.8**	31.1**
IPCA4	(16)			57.4**	29.2**
IPCA5	(14)			41.8**	21.2**
IPCA6	(12)			29.7*	11.5
Residuals	57 (22)	47.7	28.1	20.8	10.9
Error	210 (260)	49.3	21.1	14.8	7.6
Total	377 (449)	188.3	74.3	127.6	114.5

† Value within parenthesis is df for commercial hybrid trial.
*,** significant at 0.05 and 0.01 probability level, respectively

Table 2 Mean performance and AMMI stability value (ASV) of released open-pollinated varieties (OPVs) for grain iron (Fe) and zinc (Zn) density in pearl millet, 2011 rainy season

OPV	Breeding organization	Fe density (mg/kg)		Zn density (mg/kg)	
		Mean	ASV	Mean	ASV
MBC 2	AICPMIP, Mandor	43	1.9	38	2.1
CZP 9802	CAZRI, Jodhpur	42	3.9	37	0.9
HC 10	CCSHAU, Hisar	49	1.1	45	7.3
HC 20	CCSHAU, Hisar	50	1.7	45	0.3
Pusa Composite 334	IARI, New Delhi	49	0.9	44	1.3
Pusa Composite 383	IARI, New Delhi	46	1.5	44	2.1
Pusa Composite 443	IARI, New Delhi	46	6.4	40	2.2
Raj 171	ICRISAT, Patancheru	49	0.8	45	1.9
WC - C75	ICRISAT, Patancheru	47	4.8	41	1.3
ICMS 7703	ICRISAT, Patancheru	50	1.7	44	1.1
ICTP 8203	ICRISAT, Patancheru	67	19.3	52	6.0
ICMV 155	ICRISAT, Patancheru	48	1.1	43	2.0
ICMV 221	ICRISAT, Patancheru	61	2.2	45	2.2
JBV 2	JNKVV, Gwalior	47	0.4	44	0.4
JBV 3	JNKVV, Gwalior	44	1.4	40	0.6
PPC 6	NARP, Aurangabad	53	4.3	46	1.9
AIMP 92901	NARP, Aurangabad	56	4.0	46	1.5
Co (Cu) 9	TNAU, Coimbatore	52	1.6	46	2.1
LSD (P<0.05)		3.9		2.7	

†AICPMIP, All India Coordinated Pearl Millet Improvement Porject; CAZRI, Central Arid Zone Research Institute; CCSHAU, ChowdharyCharan Singh Haryana Agricultural University; IARI, Indian Agricultural Research Institute; ICRISAT, International Crops Research for the Semi-Arid Tropics; JNKVV, Jawaharlal Nehru Krishi Vishwa Vidyalay; NARP, National Agricultural Research Project; TNAU, Tamil Nadu Agricultural University.

significant and high positive correlation between the Fe and Zn density (r = 0.86). Similar high and positive relationships between these two micronutrients have been reported in earlier pearl millet studies (Velu *et al.* 2007, Gupta *et al.* 2009, Govindaraj *et al.* 2013, Rai *et al*. 2012, 2013, 2015, Kanatti *et al.* 2014) . Thus, it is not unexpected that ICTP 8203 also had the highest level of Zn density (52 mg/kg) and ICMV 221 and AIMP 92901 were among those with relatively high Zn density (45-46 mg/kg). ICTP 8203, developed at ICRISAT, and released and notified in 1988 for cultivation in Maharashtra and Andhra Pradesh (Rai *et al*. 1990), is still cultivated in these states and in Karnataka, all in the same geographical domain of peninsular India. It is also grown on limited scales in parts of Uttar Pradesh and Rajasthan (Mula *et al.* 2010). ICMV 221, also developed at ICRISAT, and released and notified in 1993 (Witcombe *et al*. 1997) at All India level, has been under cultivation, specifically in Maharashtra and Tamil Nadu. AIMP 92901, jointly developed by Marathwada Agricultural University and ICRISAT, was released in 2001 as Samrudhi for cultivation in peninsular India (Yadav *et al.* 2012). Its adoption has been very limited and short lived due to lack of seed production. It was interesting to note that all these three high-Fe OPVs are based on *iniadi* germplasm, which has been found to be the best source of both Fe and Zn densities (Rai *et al.* 2015).

Effects due to environment (E) as well as those due to genotype × environment (G × E) interaction were all highly significant (P<0.01) both for grain Fe and Zn densities (Table 1). Environmental effects made the largest contribution to the variability for both Fe and Zn density, accounting for 40-45% of the total sum of square. G×E interaction accounted for 10-20% of the variation for Fe and Zn densities. AMMI analysis showed that first principal component accounted for 53% of the G×E sum of square, while the first two principal components cumulatively accounted for 71% of the G×E sum of square for Fe density (Table 1). While ICTP 8203 had the highest mean Fe density, about four-fold differences were observed across the environments, and with AMMI Stability Value (ASV) of 19.3, it was the most unstable OPV for Fe density. Yet, it was the top ranking OPV in four environments and had only 1-3 mg/kg less Fe density than ICMV 221 in three environments where ICMV 221 was the top ranking OPV. ICMV 221 with 2.2 ASV was among the relatively more stable OPVs and AIMP 92901 with ASV of 4.0 was moderately stable. All of the most stable OPVs with <1.5 of ASV had <50 mg/kg of average Fe density. For Zn density, the first principal component accounted for 45% of the G×E sum of square, and the first two principal components cumulatively accounted for 68% of the G×E sum of square. ICTP 8203 with ASV of 6.0 was again among the two most unstable OPVs. However, it was the top ranking OPV in four environments, and second ranking in one environment.

Table 3 Mean performance and AMMI stability value (ASV) of released and commercial hybrids for grain iron (Fe) and zinc (Zn) density in pearl millet, 2012 rainy season

Hybrids	Breeding organization	Fe density (mg/kg)		Zn density (mg/kg)	
		Mean	ASV	Mean	ASV
PAC 903	Advanta Seeds	55	1.7	39	2.1
PAC 931	Advanta Seeds	54	4.6	44	0.7
PAC 982	Advanta Seeds	49	1.6	37	1.0
Ajeet 38	Ajeet Seeds	55	2.7	41	1.2
XL 51	Bayer Biosciences Pvt Ltd	55	5.7	40	0.5
ICMH 356	ICRISAT	46	2.8	39	2.0
JKBH 676	JK Agri Genetics Ltd	48	4.2	38	2.4
Krishna 7201	Krishna Agri. Res. & Dev	47	5.8	36	2.8
Saburi	MPKV, Rahuri	52	3.0	39	1.3
Shradha	MPKV, Rahuri	54	5.3	44	1.3
Navbharat Banas Express	Navbharat Seeds Pvt Ltd	50	2.2	40	2.3
NBH 1134	Nuziveedu Seeds Pvt Ltd	54	2.0	39	1.2
86M86	Pioneer Overseas Corporation	56	3.3	40	1.1
RBH 9	Rasi Seeds	53	0.8	38	2.3
VBBH 3125	Vibha Agrotech Ltd.	49	4.0	40	2.7
LSD ($P<0.05$)		5.5		3.9	

ICMV 221 had moderate ASV of 2.2, and yet it ranked second and third only in one environment each. HC 20 and JBV 2 with ASV <0.5 were among the most stable OPVs and had, respectively, 46 and 44 mg/kg Zn density over the environments.

Utilizing the intra-population variability for Fe density, an improved version of ICTP 8203, designated as ICTP 8203 Fe10-2 and released as Dhanashakti, has been developed that has 71 mg/kg Fe density (9% more than ICTP 8203), and 2.2 tonnes/ha of grain yield (11% more than ICTP 8203), with no changes in Zn density, seed size, flowering time and other traits (Rai *et al*. 2014b, 2014c). This indicates that high-Fe OPVs identified in this study can be further improved for Fe as well as for the Zn density, and the same applies to other OPVs provided there is significant variability within the populations. Variability for Fe and Zn density has been observed in ICMV 221 and AIMP 92901 (M Govindaraj, unpublished), indicating that effective selection in these OPVs can also be made to develop their improved versions with higher levels of these micronutrients. Further, these improved versions can also be used for progeny-based selection to develop parental lines of hybrids with high levels of these micronutrients. Such breeding work in these high-Fe OPVs has been initiated at ICRISAT.

Variability in hybrids

Genotypic effects among hybrids were highly significant ($P<0.01$) for grain Fe and Zn densities (Table 1). Based on the mean performance of hybrids over 10 environments, the mean Fe density among the hybrids ranged from 46 to 56 mg/kg and Zn density from 37 to 44 mg/kg (Table 3). Hybrid 86M86, the most widely grown and a highly productive cultivar, had the highest level of Fe density (56 mg/kg). It was followed by PAC 903, Ajeet 38 and Proagro XL 51, all of which had 55 mg/kg Fe density. There were five other hybrids which had >50 mg/kg of Fe density. Shradha and PAC 931 had the highest and similar Zn density (44 mg/kg), and these two hybrids also had similar and high Fe density (54 mg/kg). Hybrids 86M86, Ajeet 38, PAC 903 and Proagro XL 51 had 39-41 mg/kg Zn density. The correlation between Fe and Zn density in hybrids was much less ($r = 0.57$) than that observed in OPVs, which resulted from relatively less variability for these micronutrients in hybrids that had been selected for high Fe density from a previous trial in which the Fe density varied from 31 to 61 mg/kg and Zn density varied from 32 to 52 mg/kg (Rai *et al*. 2013). Both parental lines of 86M86 have in their parentage some component of *iniadi* germplasm (R S Mahala, Pioneer Overseas Corporation, pers. Comm.). Judging by the large seed size and dark grey seed color of high-Fe hybrids, typical of *iniadi* germplasm (Andrews and Anand Kumar 1996), parental lines of other high-Fe hybrids also are likely to have some component of *iniadi* germplasm in their parentage.

Environmental effects as well as those due to G×E interaction were all highly significant ($P<0.01$) for grain Fe and Zn densities (Table 1). Environmental effects made the largest contribution to variability for Fe and Zn density, accounting for 70-85% of the total sum of square, followed by G×E interaction (7-15%). First principal component accounted for 40% of the G×E sum of square, while the first two principal components cumulatively accounted for 57% of the G×E sum of square for Fe density (Table 1). Amongst the four high-Fe hybrids, PAC 903 with 1.7 ASV was most stable and Proagro XL 51 with 5.7 ASV was the least stable, while Ajeet 38 and 86M86 with 2.7-3.3 ASV were between the two. Although none of these hybrids top ranked in more than two environments, Ajeet 38 and Proagro XL 51 were among the five top ranking hybrids in seven environments and 86M86 was among the five top ranking hybrids in six environments. For Zn density, the first principal component accounted for 29% of the G×E sum of square and the first two principal components cumulatively accounted for 50% of the G×E sum of square. Of the two highest-Zn hybrids, PAC 931 with 0.7 ASV was most stable and Shradha with 1.3 ASV was less stable. Proagro XL 51 with 0.5 ASV was most stable among all the hybrids, while Ajeet 38 and 86M86 with 1.1-1.2 ASV were relatively less stable among the high-Zn group of hybrids. Based on their continued cultivation and significant coverage, and high-Fe density, 86M86, Ajeet 38 and Proagro XL 51 were included in the Nutri-Farm pilot program, initiated by the Government of India.

Base line for iron (Fe) density

WC-C75 is the first ICRISAT-bred pearl millet variety, which was widely grown for a long time in 1980s and 1990s. In the initial years of biofortification research at ICRISAT, a 2-year study showed WC-C75 having 42 mg/kg of Fe density (Velu *et al*. 2007). Based on the Fe assessment of a representative sample of diversity spectrum, HarvestPlus set an ambitious working base line of 47 mg/kg (5 mg/kg above WC-C75) and worked out a target level of 77 mg/kg Fe assuming 300g/d of consumption, 90% retention and 5% bioavailability. This gives an incremental target of 30 mg/kg. Pearl millet OPVs in India are the cultivars of the past, grown on <500,000 ha, while hybrids are cultivated on more than 4.5 million ha. Thus, the base line of Fe was re-examined using more extensive hybrid trial data of this study to derive a base line value for hybrids which occupy more than 90% of pearl millet area under improved cultivars in India. The relative performance of cultivars across the environments changes due to G×E interaction. However, relative means of various groups of cultivars, if the numbers in these groups are sufficiently large, are unlikely to be significantly affected by G×E interaction. The data from 15 common hybrids evaluated at two locations in 2011 and 10 locations in 2012 were used to estimate the probable mean of the remaining 107 hybrids evaluated in 2011 at the same two locations, which enabled to derive a base line value of 42 mg/kg for Fe density.

With this revised base line, the new target level would be 72 mg/kg, and with recent results on bioavailability at 7.0-7.5% (Kodkany *et al.* 2013, Cercamondi *et al.* 2013), the target level would be still lower. Assuming an incremental target of 30 mg/kg HarvestPlus defines a hybrid as biofortified if it exceeds base line + 50% incremental target, which works out as 57 mg/kg. In this context, it is significant to note that all the high-Fe cultivars (both OPVs, and hybrids) identified in this study have ≥ 39 mg/kg of Zn density, exceeding 33 mg/kg of target level initially determined for biofortified high-Zn wheat varieties (Velu *et al.* 2012), which was later revised to 37 mg/kg.

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