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Effect of grain colour on iron and zinc density in pearl millet

M. Govindaraj*, A. S. Rao, H. Shivade and K. N. Rai

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Telangana, India

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Abstract

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a climate resilient crop with higher nutrition and serve as staple food for several million populations in semi-arid regions of India and Africa. To utilize the nutritional variability of this crop, biofortification research has been initiated to combat micronutrient malnutrition, chiefly iron (Fe) and zinc (Zn) deficiency. Large variability for grain Fe and Zn density has been reported in pearl millet and mostly the high-Fe lines had relatively dark grey grain colour. Therefore, this study was designed to investigate the effect of grain colour on grain Fe and Zn density in pearl millet. Two dark grey lines were crossed with five white grain colour lines to produce 10 hybrids. These hybrids were evaluated along with their parental lines for Fe and Zn density in two seasons. Highly significant Fe density differences observed for both parents and hybrids while significant Zn density differences observed only for hybrids. The significant genotype × environment (G × E) interaction observed for Fe and Zn density in hybrid trial. Interestingly, grain colour× environment variance was not significant for both micronutrients. Results indicate both micronutrients were not differed from white to grey grain lots among hybrids (70-103 mg kg^{-1} Fe density and 64-80 mg kg^{-1}Zn density), implying the genetic improvement of grain Fe and Zn density in pearl millet is highly feasible without compromising the grain colour preference of the farmers and consumers. Further, highly positive and significant correlation between these two micronutrient density irrespective of the grain colour recommended increase in Zn density as an associated trait while breeding for high Fe density in pearl millet.

Key words: Biofortification; grain color; iron; zinc; pearl millet.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the staple food crops feeding several million populations in arid and semi-arid tropical regions of Asia (largely India) and sub-Saharan Africa, having climate change adaptation traits (drought, heat and salinity etc.) with higher nutrition. Crop biofortification is a cost-effective and sustainable agricultural approach to address micronutrient malnutrition, which is a widespread public health problem, mostly in the populations of developing countries dependent predominantly on cereal-based diets for their energy and nutritional requirements (Bouis et al. 2011). Large variability observed for iron (Fe) and zinc (Zn) density in commercial cultivars, breeding lines and germplasm of pearl millet (Rai et al. 2013, 2015a) provides opportunity to reduce Fe and Zn deficiencies in populations dependent on this crop for their daily energy and nutritional requirements. HarvestPlus- a challenge program of CGIAR, realized the importance of pearl millet and its nutritional variability, and continuously supporting pearl millet biofortification and delivered conventionally bred high iron and zinc pearl millet cultivars in India to tackle these two micronutrient deficiencies. Almost all the cultivars and breeding lines with high levels of Fe density, and generally high Zn density, have been found to be largely or entirely based on *iniadi* germplasm (Velu et al. 2007, 2008; Rai et al. 2013). This germplasm has grains that are typically large in size and light grey to dark grey in colour (Andrews and Anand Kumar 1996). Currently, *iniadi* germplasms are being exploited for hybridization with elite genetic backroads that are adopted to India at ICRISAT to breed for high Fe and Zn density in breeding lines and hybrid parents. Therefore, the question arises if the grain colour has any association with grain Fe density, and due to high positive association, with grain Zn density as well. This question is of particular relevance because in the major

*Corresponding author's e-mail: m.govindaraj@cgiar.org
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parts of India (except Maharashtra) and elsewhere in the world, dark grey colour grain is not preferred for food purposes. Besides increasing awareness on biofortified food products and their health benefits among the households, it is important to know some of the visible factors such as grain color are expected to be affect grain nutritional quality and consumer preference. All the commercial hybrids and varieties bred so far having creamy to grey grain colour. This perhaps correlate with rural household consumption pattern across the country with negligible intake of food industries where fine white color is more preferred to attract urban markets. However, in future, besides the improvement in yield, consumer acceptance and industry preferences need to be addressed by breeding white grain millets without loss of important minerals and vitamins. This will make pearl millet a competitive food grain in 21st century. Till date, there is no information available in the literature relating Fe and Zn density with the grain colour in pearl millet. The objective of this research, therefore, was to investigate the effect of white and grey grain colour on grain Fe and Zn density.

Material and methods

The experimental material consisted of five white grain colour lines, two grey grain colour lines, and 10 hybrids produced by crossing white grain colour lines onto grey grain colour lines (Table 1). The 10 hybrids and 7 parental lines were planted in single row plots of 4 m length replicated two times in a randomized complete block design (RBD) during the summer and rainy season of 2009 in Alfisols at ICRISAT, Patancheru. Hybrids and parental lines were planted in separate strips side-by-side. All these plots were planted with a spacing of 60 cm and 75 cm between rows in summer and rainy season, respectively. The seedlings were thinned at 15 days after sowing to maintain one seedling per hill at a spacing of approximately 10 cm in both summer and rainy season. Basal dose of 100 kg of DAP (Diammonium phosphate, contains 18%N: 20%P) was applied at the time of field preparation and 100 kg ha^{-1} of urea (46%N) was applied as side-dressing after the thinning. Ten random plants in each plot were selfed at the panicle emergence stage. These were harvested at maturity, sun dried for 15 days, and hand threshed to produce bulk grain samples from each plot. Since white grain colour is dominant over grey grain colour and expressed as xenia effect, after crossing grey female lines with the white male lines, all the crossed seeds (F_0) in female panicles were completely white. Therefore, the grains on the F_1 plants segregated for white and grey grain colour in the same panicle (Fig. 1), which was manually separated into two grain colour classes for Fe and Zn analysis.

Table 1. Grain iron and zinc density in parental lines of pearl millet. Mean of two seasons, Patancheru

L.No.	Parents	Grain colour	Micronutrient (mg kg^{-1}) Fe	Zn
SP-1	863B	Grey	83.0	67.0
SP-2	AIMP 92901	Grey	77.0	64.0
PP-1	(D2BLN95-262 x EEBC C1-3)-12-B-1-B-B-1-1	White	88.0	71.0
PP-2	(ICMB 96555 x ICMB 99111)-9-1-1	White	77.0	65.0
PP-3	(ICMB 96555 x ICMB 99111)-10-2-3	White	77.0	67.0
PP-4	ICMB 96555-3	White	74.0	59.0
PP-5	HHVBC-II D2 HS-161-1-5-5-1xIP 6140-1-1-1-B-1-B-1-1	White	45.0	58.0
CV (%)			12.9	12.2
LSD (5%)			15.0	12.0

SP-seed parent (grey), PP-pollen parent (white)

Fig. 1. Representative panicles showing the segregation for grain colour in F_1 hybrid (center) derived from a cross between a grey colour grain female parent (P1) and a white colour grain male parent (P2)

Grain samples were analyzed for Fe and Zn density using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) at the Waite analytical laboratory, Adelaide, Australia, following the method described by Wheal et al. (2011). Briefly, after di-acid digestion, the volume of the digest was made to 25 mL using distilled water; and the density was agitated for 1 minute by vortex mixer. The digests were filtered and the Fe concentration was read at 259.94 nm and Zn concentration at 213.86 nm using ICP-OES and these micronutrients were expressed as mg kg^{-1}. Care was taken at each step to avoid any contamination of the grains with dust particles and any other extraneous matter (Stangoulis and Sison 2008). The data from F_1 hybrids were analyzed as a nested design using fixed model analysis of variance (Gomez and Gomez 1984) and data on parental lines were analyzed following RBD using the PROC GLM procedure of the Statistical Analysis System (SAS) version 9.2 (SAS Institute 2009).

Results and discussion

The mean Fe density, averaged over the seven parental lines, varied from 39 mg kg^{-1} in the rainy season to 110 mg kg^{-1} in the summer season, while the mean Zn density varied from 49 mg kg^{-1} in the rainy season to 82 mg kg^{-1} in the summer season (data not presented). Averaged over the two environments, the two grey colour lines (863B and AIMP 92901) had high Fe density of 83 mg kg^{-1} and 77 mg kg^{-1} Fe density, respectively (Table 1). These two lines also had high Zn density of 67 mg $kg^{-1}$and 64 mg kg^{-1}, respectively. Amongst the five white grain colour lines, four lines had high Fe density of 74-88 mg kg^{-1} and one line had low Fe density of 45 mg kg^{-1}. While three of these lines had high Zn density of 65-71 mg kg^{-1}, two lines had relatively low Zn density of 58-59 mg kg^{-1}. All these five white seeded lines comes from three different genetic backgrounds. The four white seeded high-Fe lines come from improved EEBC and ICMB 96555 derivatives with early and medium maturity, respectively, while low-Fe line come from high head volume derivatives.

The differences among the parents were highly significant for Fe density and no significant differences were observed for Zn density. The differences among the F_1 hybrids were highly significant (p<0.01), both for Fe and Zn density, but there was no significant difference between the grey and white colour grains for either of these micronutrients (Table 2). Parent × environment interaction for both Fe and Zn density

Table 2. Mean square for grain iron and zinc density in parents and grain colour lots of pearl millet hybrids

Source	DF	Mean square	
		Fe density	Zn density
Parents			
Environments (E)	1	6522.3**	1884.4**
Replication/E	2	16.3	12.0
Parents	6	516.4**	63.0
Parents × E	6	171.2	32.2
Error	11	94.2	61.9
Hybrids			
Environments (E)	1	23679.0**	4614.1**
Replication/E	2	226.8*	56.9
Grain lot	19	519.7**	126.8**
F1 hybrid (F_1)	9	1082.2**	254.1**
Grain colour/F_1	10	13.5	12.1
Grain lot × E	19	111.0	53.2
F_1 × E	9	200.3*	91.2**
Grain colour/F_1 × E	10	30.7	19.1
Error	38	71.4	29.1

*,** significant at 0.05 and 0.01 probability level, respectively

was not significant, while the F_1 hybrid × environment interaction was significant for both micronutrients. The grain colour × environment interaction was non-significant for both micronutrients indicating seed colour trait as highly heritable across environment like any other qualitative trait. Averaged over the two environments, the Fe density among the F_1 hybrids varied from 69 mg kg^{-1} to 103 mg kg^{-1} in grey colour grain lots, and from 70 mg kg^{-1} to 103 mg kg^{-1} in white colour grain lots (Table 3). The Zn density among these hybrids varies from 64 mg kg^{-1} to 80 mg kg^{-1} in grey colour grain lots and Zn density from 63 mg $kg^{-1}$to 80 mg kg^{-1} in white colour grain lots. There was highly significant and very high positive correlation between the grey colour and white colour grain lots, both for Fe density (r = 0.97, P<0.01) and Zn density (r = 0.96, P<0.01) (Fig. 2). This highly positive and highly significant correlation between these two micronutrient density also reflected within grey seed lot (r = 0.79, P<0.01) and white seed lot (r = 0.84, P<0.01) (Fig. 3). These results clearly showed that grain colour had no effect on Fe and Zn density. Similar positive and significantly higher magnitude of correlation coefficient between grey and white seed

Table 3. Grain iron and zinc density in white and grey colour grains of F_1 hybrids in pearl millet, mean of two seasons, Patancheru

Hybrid		Fe density (mg kg^{-1})		Zn density (mg kg^{-1})	
Female (P1)	Male (P2)	Grey	white	Grey	white
SP-1	× PP-1	77	75	71	70
SP-1	× PP-2	80	73	74	68
SP-1	× PP-3	93	94	75	73
SP-1	× PP-4	95	98	78	78
SP-1	× PP-5	103	103	80	80
SP-2	× PP-1	81	75	69	68
SP-2	× PP-2	76	77	66	65
SP-2	× PP-3	80	81	64	63
SP-2	× PP-4	70	70	65	63
SP-2	× PP-5	69	70	72	69
C V (%)		10.4		7.7	
LSD (5%)		9		6	

SP-seed parent (grey), PP-pollen parent (white)

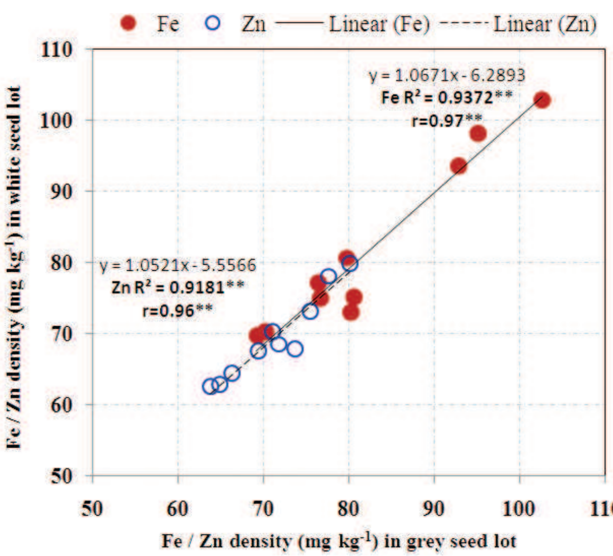

Fig. 2. Relationship between white and grey colour grain lot in F_1 hybrids for Fe (solid circle) and Zn (open circle) density in pearl millet (, significant at 0.01 probability level)**

lot for Fe (r=0.85, P<0.01) as well as for Zn (r = 0.73, P<0.01) density. These suggested that increase simultaneous genetic improvement is feasible and is concurrent with earlier studies (Velu et al. 2011; Govindaraj et al. 2013; Kanatti et al. 2014). This present

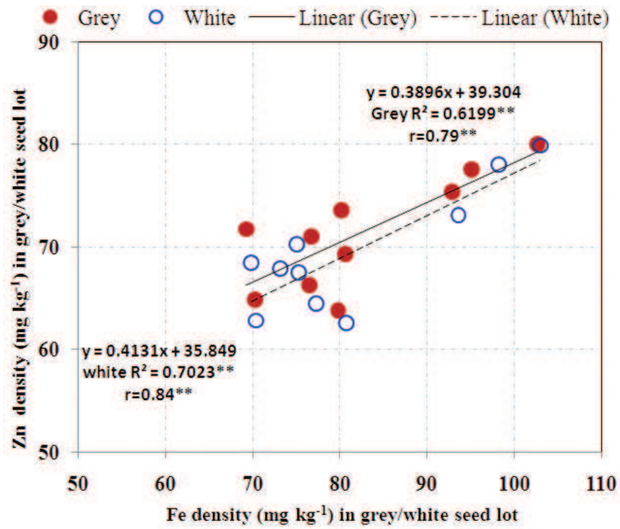

Fig. 3. Relationship between Fe and Zn density in F_1 hybrids for grey (solid circle) and white (open circle) seed lot in pearl millet (, significant at 0.01 probability level)**

finding with a recent study that demonstrated there was no xenia (pollen source) effect on grain Fe and Zn density in pearl millet (Rai et al. 2015b) will enhance the breeding efficiency for biofortification.

Except for three hybrids based on 863B where the Fe density in F_1 hybrids (both grey and white colour lots) showed better-parent heterosis for Fe density (8-24%), Fe density in other hybrids did not exceed the Fe levels of their high-Fe parental lines. In five hybrids, including one based on AIMP 92901, the F_1 hybrids had better parent heterosis (8-19%) for Zn density. In earlier studies based on a large number of hybrids that did not involve white grain colour lines, there was no indication of any better-parent heterosis for Fe and Zn density in pearl millet (Velu et al. 2011; Govindaraj et al. 2013; Kanatti et al. 2014). Selfing in pearl millet leads to reduction in seed set, which is influenced by genotype and the environment. Further, it has been observed that reduction in seed set leads to overestimates of Fe and Zn density in earl millet with the magnitude of overestimate dependent on the genotypes and the degree of reduction in seed set (Rai et al. 2015b). No seed set data were recorded in the present study that used selfed seeds for Fe and Zn density analysis. Therefore, it is likely that the heterotic expressions in some of the hybrids are artifacts of variable seed set reductions in the selfed panicles. This finding suggesting there is a potential prospect for breeding high-iron and zinc pearl millet cultivars with white grain color for industrial food products preparations. Therefore, study result implying the genetic improvement of grain Fe and Zn density in pearl millet is highly feasible without compromising the grain colour preference of the farmers and consumers.

In conclusion, biofortification is a food-based approach to overcome the nutritional insecurity by delivering nutrient-dense cultivars at the door steps of poor populations. unlike orange-seed-maize and orange-flesh-cassava biofortification for pro-Vit A, biofortification of pearl millet like other cereal crops with grain iron and zinc much more difficulties to discriminate from the non-biofortified for their mineral dense appearance. However, the association of biofortification traits with seed color has merits and demerits. Merit is to discrimination/certify easily from other non-biofortified seeds whereas change of color in the staple food grains is not always accepted by consumers and has product uptake implications. On the other hands, in pearl millet, all the high-Fe/Zn sources so far had grey to dark grey grain colour would be a future constraint that capable of overwhelming the grain quality breeding including biofortification. In this context, present study indicates (i) there was no significant correlation between seed color and grain micronutrients in pearl millet, (ii) increase in Zn density as an associated trait while breeding for high Fe density in pearl millet, will enhance the biofortification breeding efficiency without compromising grain color preferences. However, further studies to validate with the large and diverse set of isogeneic materials are required for large scale breeding applications.

Authors' contribution

Conceptualization of research (KNR, MG); Designing of the experiments (KNR, MG); Contribution of experimental materials (KNR, ASR); Execution of field/ lab experiments and data collection (ASR, HS); Analysis of data and interpretation (MG, HS); Preparation of manuscript (MG, KNR, ASR, HS).

Declaration

The authors declare no conflict of interest.

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