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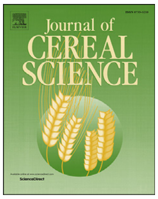

Terminal drought and a d_2 dwarfing gene affecting grain iron and zinc density in pearl millet

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ABSTRACT

Pearl millet, predominantly a rainfed dryland crop, often encounters terminal drought in crop season. Grain hybrids grown in India are of medium to tall height, and majority of them are based on d_2 dwarf seed-parents. Eight pairs of tall and d_2 dwarf isogenic-lines, developed from two diverse composites, were evaluated under irrigated control and imposed terminal drought for two-years to examine the effect of d_2 dwarfing gene and terminal drought on grain iron (Fe) and zinc (Zn) density. In general, terminal drought had a significant effect on increasing the Fe and Zn density, the d_2 dwarfing gene or the linked gene block significantly decreased both micronutrients, with the magnitude of increase or decrease, respectively, dependent on the environment and genetic background of the isolines. Terminal drought has severe adverse impact on grain yield, but grains produced from such environments are likely to be more nutritious with respect to Fe and Zn density. The d_2 dwarf hybrids are likely to be less nutritious than non-d_2 hybrids with respect to Fe and Zn density. Whether the d_2 dwarf seed parents with reduced Fe and Zn density presumably may adversely affect grain yield and micronutrient levels of even non-d_2 hybrids developed on them, and this aspect merits further investigation.

1. Introduction

Micronutrient malnutrition arising from dietary deficiency of mineral micronutrients such as iron (Fe) and zinc (Zn) has been recognized as a serious public health problem, affecting more than two billion people worldwide. This problem is particularly serious in the developing countries, especially in high-risk groups such as pregnant women, infants and adolescent children. For instance, about 80% of the pregnant women, 52% of the non-pregnant women, and 74% of the children in 6–35 months age group in India suffer from iron deficiency-induced anemia (Chakravarty and Ghose, 2000). About 52% of the children below 5 years age in India suffer from Zn deficiency. The numerous health problems arising from Zn deficiency could be as serious as those from Fe deficiency, but are not so well documented. Crop biofortification is now globally being recognized as a cost-effective and sustainable agricultural approach to reduce and, in some cases, even overcome micronutrient malnutrition. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a highly nutritious cereal with Fe and Zn contents much higher than those found in fine cereal grains like rice and wheat. It is a significant and cheaper source of Fe and Zn, accounting for 19–63% of the total Fe intake 16–56% of the total Zn intake from all food sources in parts of the pearl millet growing areas of Maharashtra, Gujarat and Rajasthan (Parthasarathy Rao et al., 2006). Further, studies at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have shown that there is large variability in the germplasm for Fe and Zn density (Govindaraj et al., 2013; Rai et al., 2013), thus increasing the prospects of breeding biofortified cultivars with elevated levels of these micronutrients.

Pearl millet is cultivated on more than 28 million ha, mostly in the arid and semi-arid tropical environments of Asia and Africa. In these regions, it is cultivated primarily for grain production, but is also valued for its stover used mostly for fodder purposes. Therefore, farmers prefer medium and tall height pearl millet, mostly in the range of 150–300 cm, depending on the relative value of grain and stover, and crop growing environments. Of the five dwarfing genes (d_1 to d_5) reported in pearl millet, a d_2 dwarfing gene has been most widely used in hybrid parents development. Dwarfness in pearl millet is controlled by recessive gene and the precise origin of the d_2 dwarf mutation is unknown. Pearl millet is being experimented as a grain crop in the USA and Brazil where cultivars need to be dwarf for mechanized harvesting. Although dwarf

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hybrids are not cultivated in India, most of the seed parents of hybrids are d_2 dwarf, and they are preferred for seed production on account of being less prone to lodging under high management conditions. High zinc content in seed has been shown having positive effect on plant growth and development and grain yield in wheat and rice (Cakmak, 2008; Slaton et al., 2001; Yilmaz et al., 1998). There is no such study in pearl millet. Also, there is no information on whether d_2 dwarfing gene has any effect on Fe and Zn density in pearl millet. Pearl millet is mostly cultivated as a rainfed crop, and often it encounters end-of –the-season or terminal drought. There is no information on the effect of terminal drought on grain Fe and Zn density in pearl millet. Therefore, the objective of this research was to examine the effect of a d_2 dwarfing gene and terminal drought on grain Fe and Zn density in pearl millet.

2. Materials and methods

2.1. Experimental materials

Nine pairs of tall and dwarf near-isogenic lines (hereafter referred to as isolines), derived from two diverse composites were used in this study. Four pairs of isolines were derived from Early composite (EC) and five pairs from Medium composite (MC) (Table 1). A d_2 dwarfing gene from GAM 73 (a synthetic bred in Senegal) was introduced in EC and MC by three backcrosses. The detail procedure for deriving these isolines has been described elsewhere (Rai and Rao, 1991). Briefly, starting at BC_3F_3 and onwards, it involved seven generations of selfing 7–8 tall plants (that would include at least one plant heterozygous at the D_2/d_2 locus) at successive selfing generations following head –to-row generation advance. This would lead to complete homozygosity at all the loci, except the D_2/d_2 locus, in different genetic backgrounds. At F_8 stage, tall and dwarf plants were identified and F_9 progeny pairs uniform for tall and dwarf height were selected and seed produced for field trials.

2.2. Field trials

The isolines were planted in a split-plot design with three replications during the 2010 and 2012 summer season under irrigated control as well as under imposed terminal drought in Alfisols at ICRISAT, Patancheru. Earlier studies (Govindaraj et al., 2013; Kanatti et al., 2016) have shown these soils having far higher Fe and Zn content than critical levels of Fe (2.6–4.5 mg kg^{-1}) and Zn (0.6–1.0 mg kg^{-1}) required by plants for normal growth and dvelopment (Sahrawat and Wani, 2013). The two treatments planted in strips were separated by an 8-row buffer planting. Weekly irrigation was provided in both treatments, which was continued until physiological maturity in irrigated control, and terminated after flowering in terminal drought treatment. Isoline pairs were randomized as main plots and isolines within the pairs were randomized as subplots. Each plot consisted of 1 row of 4 m length, spaced 60 cm apart. Overplanted plots were thinned to single plants spaced 10 cm apart 12–14 days after planting. A basal dose of 100 kg ha^{-1} of DAP (Diammonium phosphate, contains 18%N: 46%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 weeding. Main panicles of five random plants with good seed set from each plot were harvested at or after physiological maturity, stored in cloth bags, sun dried on tarpouline sheet for 12–15 days, and hand threshed to produce grain samples for laboratory analysis.

Table 1
Pedigree of isogenic lines (genotypes) of pearl millet evaluated under terminal drought and irrigated control in 2010 and 2012 summer season, Patancheru.

Composite	Isogenic pair	Pedigree
Early composite (EC)	EC1	ECIL-F9-6-2
	EC2	ECIL-F9-119-1-2
	EC3	ECIL-F9-159-4-4
	EC4	ECIL-F9-203-1
Medium Composite (MC)	MC5	MCIL-F9-4-3-1
	MC6	MCIL-F9-4-4-5
	MC7	MCIL-F9-4-7-4
	MC8	MCIL-F9-31-5-2
	MC9	MCIL-F9-191-5-3

2.3. Micronutrient analysis

The grain samples were analyzed for Fe and Zn density at the Waite Analytical Services Laboratory, University of Adelaide, Australia, using Inductively Coupled Plasma Optical Emission Spectroscopy (Spectro Analytical Instruments, Kleve, Germany) as described by Wheal et al. (2011). Briefly, grain samples were oven-dried overnight at 85 °C prior to digestion, grounded enough to pass through a 1 mm stainless steel sieve using Christie and Norris hammer mill, and stored in screw-top polycarbonate vials. The samples were digested with di-acid (nitric/perchloric acid) mixture. After digestion, the volume of the digest was made to 25 mL using distilled water, and the content was agitated for 1 min by a vortex mixer. The digests were filtered and the Fe concentration was read at 259.94 nm and the 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.

2.4. Statistical analysis

Statistical test showed isoline MC9 Tall as an outlier, hence isoline pair MC9 was not included in the analysis. Data from 8 isoline pairs were analyzed using Statistical Analysis System (SAS V 9.3) (SAS Institute, 2009). Analysis of variance was done following fixed model (Gomez and Gomez, 1984). Statistical significance between various comparisons was tested using least significance difference (LSD) at 0.05 level of significance (https://egret.psychol.cam.ac.uk/statistics/local_copies_of_sources_Cardinal_and_Aitken_ANOVA/spspplot.pdf). The Pearson correlation coefficient among the traits was calculated using PROC CORR procedure.

3. Results

The Fe density, averaged over all the 8 pairs of isolines, varied from 53 mg kg^{-1} in 2012 irrigated treatment (hereafter referred to as control) to 64 mg kg^{-1} in 2010 terminal drought, and Zn density varied from 50 mg kg^{-1} in 2010 control to 65 mg kg^{-1} in 2012 terminal drought (data not presented). The differences among the isoline pairs (hereafter referred to as genotypes) were highly significant both for Fe and Zn densities (Table 2). Moisture effect (control vs terminal drought) was highly significant for Fe density ($P < 0.01$) and significant for Zn density ($P < 0.05$). Genotype × moisture (G × M) interaction was non-significant for Zn density, and while significant for Fe density, its contribution to variability relative to that due to genotype was only 3%. Genotype × year (G × Y) interaction was also highly significant for both micronutrients, but its contribution to variability relative to that accounted for by differences among the genotypes was only 13% for the Fe density and 22% for Zn density. Averaged over the two years and over the two treatments, Fe density among the genotypes varied from 43 mg kg^{-1} to 74 mg kg^{-1}, and Zn density varied from 48 mg kg^{-1} to 74 mg kg^{-1} (Table 3). Averaged over all the 8 genotypes and two years, the Fe density was 61 mg kg^{-1}

under terminal drought (7 mg kg^{-1} higher than control), and Zn density was 60 mg kg^{-1} under terminal drought (5 mg kg^{-1} higher than control). The Fe density was always higher under terminal drought than control in all genotypes, with the difference varying from 1 mg kg^{-1} to 16 mg kg$^{-1,}$ but it was statistically significant only in two genotypes of EC and four genotypes of MC. Broadly, similar pattern was observed for Zn density. Except for one genotypes (EC4), the Zn density in seven genotypes was higher under terminal drought than control, with the difference between the two treatments varying from 2 mg kg^{-1} to 11 mg kg^{-1}, and being statistically significant in one corresponding genotypes of EC and four corresponding genotypes of MC. There was highly significant effect of drought stress on seed weight with 1000-seed weight under terminal drought, averaged over all genotypes and two years, being 0.65 g (10%) less than under the control. The seed weight difference between the two treatments was significant in four genotypes of EC and one genotypes of MC.

The effect of plant height associated with the d_2 dwarfing gene was highly significant ($P < 0.01$) for Fe density (Table 2), with the dwarf isolines, averaged over all the genotypes and two years, having 9–10 mg kg^{-1} less Fe than the counterpart tall isolines under control and terminal drought. However, there was highly significant height × genotype interaction, with the contribution of latter to variability being 71% of that accounted for by height differences. Although dwarf isolines under control had less Fe than tall isolines in all genotypes, this difference was significant only in two genotypes of EC, where dwarf isolines had 15–19 mg kg^{-1} less Fe and three genotypes of MC where dwarf isolines had 6–16 mg kg^{-1} less Fe than tall isolines (Table 3). Broadly similar pattern was observed under terminal drought where in two genotypes each of EC and MC the dwarf isolines had significantly less Fe than the tall isolines, with the difference being 19–20 mg kg^{-1} in two genotypes of EC and 7–16 mg kg^{-1} in the two genotypes of MC. These similarities in the magnitude and direction of differences between the tall and dwarf isolines resulted in highly significant and very high positive correlation ($r = 0.96$, $P < 0.01$) between control and terminal drought for the difference in Fe density of tall and dwarf isolines (Table 4). This was also reflected in non-significant height/G × M interaction (Table 2). The magnitude of difference between the tall and dwarf isolines was not influenced by the Fe density levels of genotypes as reflected in non-significant correlation between the two, both under control and terminal drought (Table 5).

Table 2
Mean square for isogenic lines of pearl millet evaluated under terminal drought and control in 2010 and 2012 summer, Patancheru.

Source	Degrees of freedom	Mean squares					
		Iron		Zinc		1000-seed weight	
Year (Y)	1	558.5	**	3860.4	**	44.9	**
Replication/Y	4	271.3	**	176.3	**	2.2	**
Moisture (M)	1	2465.9	**	762.2	*	15.9	**
M × Y	1	155.7		0.1		14.3	*
Residual-1	4	52.3		51.6		0.5	
Genotypes (G)	7	3210.5	**	1627.7	**	21.9	**
G × Y	7	429.4	**	351.5	**	2.6	**
G × M	7	101.5	*	49.5		0.9	
G × Y × M	7	64.8		25.1		0.7	
Residual-2	56	35.0		35.1		0.5	
Height class/G	8	668.1	**	343.9	**	2.2	**
Height class (H)	1	3187.6	**	960.8	**	0.0	
H × G	7	325.0	**	229.1	**	2.5	**
H/G × Y	8	112.9	**	78.9	**	0.9	
H × Y	1	172.9	*	102.4	*	1.5	
H × Y × G	7	109.4	**	73.4	*	0.8	
H/G × M	8	20.3		40.9		0.4	
H × M	1	30.6		38.9		0.0	
H × M × G	7	18.4		40.3		0.5	
H/G × M ×Y	8	13.7		20.6		0.4	
H × Y × M	1	2.2		38.6		1.1	
H × Y × M × G	7	15.5		19.3		0.3	
Residual-3	64	26.7		25.2		0.56	

*Significant at 0.05 probability level; ** significant at 0.01 probability level.

Effect of plant height on Zn density was also highly significant ($P < 0.01$) (Table 2), with the dwarf isolines, averaged over all the eight genotypes and two years, having 8 mg kg^{-1} less Zn than tall isolines under control and 3 mg kg^{-1} less Zn under terminal drought (Table 3). There was highly significant height x genotype interaction, which contributed 67% more to the variability than accounted for by height differences. The difference between the tall and dwarf isolines was significant in two genotypes of EC and three genotypes of MC under control where a dwarf isoline of EC1 had 6 mg kg^{-1} higher Zn than the counterpart tall isoline, while in other four genotypes dwarf isolines had 8–19 mg kg^{-1} less Zn than their counterpart tall isolines. Broadly, similar pattern was observed under terminal drought where dwarf isoline of EC1 had 14 mg kg^{-1} higher Zn density than its tall counterpart isoline, while in another two genotypes of EC and one genotypes of MC dwarf isolines had 8–15 mg kg^{-1} less Zn density than their counterpart tall isolines. These similarities in the magnitude and direction of differences between the tall and dwarf isolines resulted in highly significant and very high positive correlation ($r = 0.83$, $P < 0.01$) between the control and terminal drought (Table 4). The magnitude of difference between the tall and dwarf isolines was not influenced by the Zn density levels of the genotype as reflected in the non-significant correlation between the two, both under control and terminal drought (Table 5).

There was highly significant and high positive correlation between the Fe and Zn density in tall isolines ($r = 0.81$, $P < 0.01$) and dwarf isolines ($r = 0.88$, $P < 0.01$) under control as well as under terminal drought ($r = 0.72$ in tall isolines and 0.80 in dwarf isolines) (Table 6). Interestingly, correlations between Fe and Zn density for the difference between the tall and dwarf isolines were significant, both under control ($r = 0.68$, $P < 0.01$) and terminal drought ($r = 0.72$) (Table 4). The difference between the tall and dwarf isolines for seed weight was not correlated either with the difference between the isolines for Fe density or for Zn density (Table 5).

4. Discussion

Terminal drought, in general, led to significant increase in Fe and Zn density, and the magnitude of this increase varied across genotypes. Drought also led to significant reduction in seed weight, and the magnitude of reduction varied across genotypes. Earlier studies have also reported on terminal drought reducing seed size in pearl millet (Bieler et al., 1993). Reduction in seed size under the drought results largely from reduction in the endosperm component of the seed, thus outer grain layers constituting relatively larger proportion of the seed size under drought than under the irrigated control. It has been observed that both Fe and Zn in pearl millet grain are mostly concentrated in the germ (consisting of scutellum and embryo) and the outer seed layers (pericarp and aleurone) of pearl millet (Minnis-Ndimba et al., 2015). Thus, reduction in seed size under drought may partly account for higher grain Fe and Zn densities. Drought effect on increasing Fe and Zn density, the more of the latter, has been reported in wheat; and this has been largely ascribed to reduction in seed size (Velu et al., 2016). It has also been observed, though not systematically documented, that terminal drought leads to genotype-dependent variable reduction in seed set in pearl millet. Studies in wheat (*Triticum aestivum* L.) (Weldearegay et al., 2012) and maize (*Zea mays* L.)

Table 3
Grain iron (Fe) and zinc (Zn) densities, 1000-grain weight (SW) of isogenic lines of pearl millet under terminal drought and control, mean of two years, Patancheru.

Isogenic pair	Height class	Fe (mg kg^{-1})[†]				Zn (mg kg^{-1})[†]				SW (g)[†]			
		Control		Drought	Mean	Control		Drought	Mean	Control		Drought	Mean
EC1	Tall	63^{a}	c	78^{a}	71^{a}	61^{a}	c	68^{a}	65^{a}	7.11^{a}		6.78^{a}	6.94^{a}
	Dwarf	58^{a}	c	73^{a}	66^{b}	67^{b}	c	82^{b}	75^{b}	8.01^{b}	c	6.95^{a}	7.48^{a}
	Mean	60	c	76	68	64	c	75	70	7.56	c	6.86	7.21
EC2	Tall	80^{a}	c	86^{a}	83^{a}	73^{a}	c	81^{a}	77^{a}	7.66^{a}	c	6.74^{a}	7.20^{a}
	Dwarf	65^{b}		67^{b}	66^{b}	72^{a}		69^{b}	70^{b}	7.63^{a}		7.20^{a}	7.42^{a}
	Mean	72		76	74	72		75	74	7.65	c	6.97	7.31
EC3	Tall	53^{a}		54^{a}	53^{a}	56^{a}		55^{a}	55^{a}	7.76^{a}	c	6.87^{a}	7.31^{a}
	Dwarf	52^{a}		55^{a}	53^{a}	54^{a}		59^{a}	57^{a}	7.41^{a}		6.81^{a}	7.11^{a}
	Mean	53		54	53	55		57	56	7.59	c	6.84	7.21
EC4	Tall	78^{a}	c	87^{a}	83^{a}	60^{a}		60^{a}	60^{a}	7.05^{a}		6.38^{a}	6.71^{a}
	Dwarf	59^{b}	c	67^{b}	63^{b}	52^{b}		52^{b}	52^{b}	6.48^{a}		5.68^{a}	6.08^{b}
	Mean	68	c	77	73	56		56	56	6.76	c	6.03	6.4
MC5	Tall	40^{a}	c	46^{a}	43^{a}	48^{a}		51^{a}	50^{a}	9.03^{a}		8.43^{a}	8.73^{a}
	Dwarf	39^{a}	c	47^{a}	43^{a}	43^{a}	c	51^{a}	47^{a}	8.13^{b}		8.48^{a}	8.30^{a}
	Mean	40	c	46	43	46	c	51	48	8.58		8.45	8.51
MC6	Tall	53^{a}	c	63^{a}	58^{a}	60^{a}		64^{a}	62^{a}	7.63^{a}		7.30^{a}	7.47^{a}
	Dwarf	37^{b}	c	47^{b}	42^{b}	41^{b}	c	49^{b}	45^{b}	9.56^{b}		8.82^{b}	9.19^{b}
	Mean	45	c	55	50	51	c	56	53	8.6		8.06	8.33
MC7	Tall	46^{a}		51^{a}	49^{a}	55^{a}		60^{a}	58^{a}	7.75^{a}	c	6.42^{a}	7.08^{a}
	Dwarf	40^{b}	c	52^{a}	46^{a}	47^{b}	c	55^{a}	51^{b}	7.50^{a}	c	5.91^{a}	6.70^{a}
	Mean	43	c	52	47	51	c	58	54	7.62	c	6.16	6.89
MC8	Tall	56^{a}		58^{a}	57^{a}	51^{a}		52^{a}	52^{a}	5.80^{a}		5.49^{a}	5.64^{a}
	Dwarf	43^{b}	c	51^{b}	47^{b}	41^{b}	c	47^{a}	44^{b}	4.93^{b}		4.83^{a}	4.88^{b}
	Mean	50	c	54	52	46	c	50	48	5.37		5.16	5.26
Mean	**Tall**	**59^{a}**	**c**	**66^{a}**	**62^{a}**	**58^{a}**	**c**	**61^{a}**	**60^{a}**	**7.47^{a}**	**c**	**6.80^{a}**	**7.14^{a}**
	Dwarf	**49^{b}**	**c**	**57^{b}**	**53^{b}**	**52^{b}**	**c**	**58^{b}**	**55^{b}**	**7.46^{a}**	**c**	**6.83^{a}**	**7.15^{a}**
	Mean	**54**	**c**	**61**	**58**	**55**	**c**	**60**	**57**	**7.47**	**c**	**6.82**	**7.14**
LSD (5%) [\$]		5.95				5.78				0.86			
LSD (5%) [\$\$]		2.11				2.05				0.41			
LSD (5%) [\$\$\$]		4.22				4.1				0.61			
LSD (5%) [\$\$\$\$]		1.49				1.45				0.22			

[†] Significant differences (at 0.05 probability level) between the tall and dwarf isolines have been shown with different letters (a, b), while those between the drought and control have been shown by letter c.
[\$] Least significant difference (LSD) to compare tall vs dwarf in each genotype for each moisture level; control vs drought in each genotype for each height class.
[\$\$] LSD to compare means of tall vs dwarf averaged over all genotypes for each moisture level; control vs drought averaged over all genotype for each height class.
[\$\$\$] LSD to compare tall vs dwarf averaged over moisture level in each genotype; control vs drought averaged over height class in each genotype.
[\$\$\$\$] LSD to compare tall vs dwarf averaged over all genotype and both moisture level; control vs drought averaged over all genotype and both height class.

Table 4
Correlation coefficient among traits (based on difference between tall and dwarf of isogenic lines) under terminal drought and control in pearl millet, Patancheru.

Trait	Moisture	Fe		Zn		SW	
		Control	Drought	Control	Drought	Control	Drought
Fe	Control	1.00					
	Drought	0.96**	1.00				
Zn	Control	0.68*	0.52	1.00			
	Drought	0.81**	0.72*	0.83**	1.00		
SW	Control	0.14	0.06	0.26	0.20	1.00	
	Drought	0.22	0.09	0.24	0.09	0.89**	1.00

*Significant at 0.05 probability; ** significant at 0.01 probability.

Table 5
Correlation coefficient between isogenic lines mean with difference between tall and dwarf for Fe and Zn density, and seed weight in pearl millet, Patancheru.

	Control	Drought	Mean
Fe	0.50^{ns}	0.60^{ns}	0.57^{ns}
Zn	-0.27^{ns}	-0.19^{ns}	-0.23^{ns}
SW	-0.37^{ns}	-0.62^{ns}	-0.48^{ns}

ns- Non-significant at 0.05 probability.

Table 6
Correlation coefficient among traits for isogenic lines of pearl millet under terminal drought and control, Patancheru.

		Fe	Zn	SW
Control	Fe	1	0.81**	−0.39
	Zn	0.88**	1	−0.08
	SW	−0.20	0.11	1
Drought	Fe	1	0.72*	−0.31
	Zn	0.80**	1	−0.08
	SW	−0.25	0.07	1

Correlation coefficients in tall (above the diagonal), and dwarf (below diagonal).
*Significant at 0.05 probability; ** significant at 0.01 probability.

(Herrero and Johnson, 1981) have shown reduction in seed set under terminal drought. It has been found that reduction in seed set results in genotype-dependent increases in the density of Fe and Zn in pearl millet (Rai et al., 2015). Thus, possible reduction in seed set may also have accounted, in part, for higher density of these micronutrients under terminal drought. These results indicate that grains produced in drought environments are likely to be more nutritious due to higher levels of Fe and Zn densities, assuming that such grains will not have disproportionately higher levels of phytates on account of reduced grain size.

The dwarf isolines used in this study have been reported to reduce plant height by 37–47% (Rai and Rao, 1991). Dwarf isolines, in general, had significantly lower levels of both Fe and Zn density than their counterpart tall isolines, with large variation for this difference across genotypes. Several studies have shown major gene effects influenced by genetic background (Chapman et al., 2007; Rai and Rao, 1991). Unlike terminal drought, the observed patterns of differences between the tall and dwarf isolines both for Fe and Zn density under control as well under drought cannot be explained by differences between them for seed weight. Although in one genotype of EC and three genotypes of MC the differences between the tall and dwarf isolines were significant under control, in two of these genotypes dwarf isolines had 0.90 g–1.93 g (15–25%) higher 1000-seed weight, while in the other two genotypes, dwarf isolines had 0.87 g–0.90 g (10–15%) less 1000- seed weight. Under terminal drought, the difference between the isolines was significant only in one genotype where dwarf isoline had 1.52 g (21%) higher 1000-seed weight than its counterpart tall isoline. Height × genotype interaction was so high that it rendered overall height effect, and apparently the dwarfing gene effect non-significant. The difference between the tall and dwarf isoline for seed weight was not correlated with the tall-dwarf difference either for Fe or Zn density, both under control and terminal drought. However, the patterns of differences between the tall and dwarf isolines under control were similar to those under terminal drought, resulting in highly significant and very high positive correlation ($r = 0.89$, $P < 0.01$) between the two treatments for seed weight differences between the isolines. Dwarf isolines have more condensed internodes with overlapping leaf sheaths than their counterpart tall isolines. Whether dwarf isolines also have relatively less extensive root system than tall isolines is not known, nor is known the effect of these two factors on Fe and Zn intake and translocation to seeds. The implication of dwarfing gene or the gene block linked to it in reducing the nutritional value of grains from dwarf cultivars due to significant reduction in both Fe and Zn densities appeared a more likely outcome. Although none of the hybrids grown in India are dwarf, most of them are based on d_2 dwarf seed parents. The effect of seed produced on such lines, more likely to have lower Fe and Zn densities, on yield and Fe and Zn densities of grain from non-d_2 hybrids in pearl millet is not known. It has been suggested that seed with high Zn density may lead to better germination, seedling establishment, protection against environmental stresses (Cakmak, 2008), and thus contribute to enhanced productivity. In fact, a wheat study showed grain yield enhancing effect of high Zn in seed (Yilmaz et al., 1998).

Although major genes for Fe and Zn in pearl millet may exist, continuous variation among populations progenies, gives an indication of a large component of multigenic or polygenic inheritance. Expression of quantitative characters is usually influenced by a balance among polygenes. Allelic changes at major gene loci may affect this balance, resulting in variable expression of several quantitative traits (Tsunewaki and Koba, 1979). Thus, whether the changes in Fe and Zn density associated with plant height were due to the pleiotropic effect of the d_2 dwarfing gene or linkage drag cannot be ascertained from this study. Comparisons of isogenic lines in wheat suggest pleiotropic effects of major genes (Moriconi et al., 2012) as well as linkage drag (Brinkman and Frey, 1977; Zeven et al., 1983) causing changes in numerous quantitative traits. The isolines used in this study had been developed by seven generations of inbreeding and single seed generation advance that would result in almost complete homozygosity for the same allele at each locus and at all the loci in both isolines except for the dwarfing gene locus. However, even in such lines the possibility of linkage drag can not be ruled out as observed in wheat (Tsunewaki and Koba, 1979).

There was highly significant and high positive correlation between these two micronutrients in tall as well as dwarf isolines, both under drought and control. Similar relationships between these two micronutrients have been reported in earlier studies on pearl millet (Govindaraj et al., 2013; Kanatti et al., 2016; Rai et al., 2013), and in other cereals such as sorghum (Ashok Kumar et al., 2013), maize (Oikeh et al., 2004), rice (Anandan et al., 2011; Stangoulis et al., 2007) and wheat (Velu et al., 2016). Such correlations may result more likely from tight linkage between the loci responsible for these two micronutrients. A recent study in earl millet has identified 2 QTL each for Fe and Zn density, of which a QTL for Fe and a QTL for Zn were co-localized (Kumar et al., 2016). This study also observed minor QTL that had environment-specific expression, thus contributing to QTL x environment interaction. Studies in other crops such as wheat (Singh et al., 2010) and rice (Stangoulis et al., 2007), have also reported common and overlapping loci QTL for Fe and Zn densities. Interestingly, the difference in Fe density of tall and dwarf isolines was significantly and positively correlated with the difference between the isolines for Zn density (except for Fe under drought vs. Zn in control), which stems from highly significant and high positive correlation between the two micronutrients. Further, these differences between the tall and dwarf isolines either for Fe or Zn density were not correlated with the differences between the isolines for seed weight, indicating that seed weight was not factor associated with tall-dwarf differences for Fe and Zn density. These d_2 dwarf seed parents with reduced Fe and Zn density presumably may affect grain yield and micronutrient levels of even non-d_2 hybrids developed on them, and this aspect merits further investigation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcs.2017.11.005.

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