Published January 9, 2015

RESEARCH

Seed Set and Xenia Effects on Grain Iron and Zinc Density in Pearl Millet

Kedar Nath Rai,* Mahalingam Govindaraj, Wolfgang Helmut Pfeiffer, and Aluri Sambasiva Rao

ABSTRACT

Three types of seed samples (selfed, sibbed and open pollinated) can be used for mineral analysis in pearl millet [*Pennisetum glaucum* (L.) R. Br.] and other cross-pollinated crops. Cost effectiveness and reliability of mineral estimates in these types of seed samples have a direct bearing on breeding efficiency. Three sets of experiments using a diverse range of materials were conducted to examine the potential use of selfed and open pollinated (OP) seed samples for the analysis of grain Fe and Zn density in pearl millet. The results of this study showed that reduction in seed set under selfing, a genotype-dependent typical trait of this crop, led to significant and large overestimates of Fe and Zn density, indicating that selfed seeds cannot be used for reliable estimation of grain Fe and Zn density. There was no significant difference between the sibbed and crossed seeds, indicating that there was no xenia effect. Differences among the sibbed and OP seeds for Fe, Zn, and Al density were small in magnitude and not always significant, indicating that dust contamination was not a significant factor determining Fe and Zn density. Since production of OP seed is most cost effective, it can be used for reliable estimation of Fe and Zn density when dealing with a large number of breeding lines, thereby enhancing the breeding efficiency for these micronutrients in pearl millet.

K.N. Rai, M. Govindaraj, and A.S. Rao, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Telangana, India; W.H. Pfeiffer, HarvestPlus, International Center for Tropical Agriculture (CIAT), A.A. 6713, Cali, Colombia. Received 16 Apr. 2014. *Corresponding author (k.rai@cgiar.org).

Abbreviations: CGIAR, Consultative Group of International Agricultural Research; G × E, genotype × environment, ICRISAT, International Crops Research Institute for the Semi-Arid Tropics; ML/GP, differences between the high and low genotypes at the micronutrient level within the genotype pairs; ML/GP × E, differences between the high and low genotypes at the micronutrient level within the genotype pairs × environment; OP, open pollinated; OPV, open-pollinated variety; SSC, seed set class; SSC × E, SSC × environment; SSG, differences among the seed sources within genotypes; SS/G × E, SS/G × environment.

Micronutrient malnutrition arising from dietary deficiency of vitamin A and mineral micronutrients such as Fe and Zn has been recognized as a major public health problem, affecting more than two billion people worldwide (WHO, 2002). This problem is particularly serious in the populations of developing countries, relying predominantly on staple cereals for their daily energy and nutritional requirements. Addressing this problem through food supplements and food fortification, especially in the rural areas, is not a practical solution due to poor purchasing power of the consumers and unsatisfactory delivery infrastructure. Diversified food uses and biofortified crop cultivars provide cost-effective and sustainable options to reduce micronutrient malnutrition in these areas. Biofortified crop cultivars offer a rural-based intervention that, by design, initially reach more remote populations, which comprise a majority of the malnourished, and then penetrate

Published in Crop Sci. 55:1–7 (2015).
doi: 10.2135/cropsci2014.04.0305
Freely available online through the author-supported open-access option.

to urban populations as production surpluses are marketed (Bouis et al., 2011). The HarvestPlus Challenge Program of the Consultative Group of International Agricultural Research (CGIAR) has undertaken to support the development of biofortified cultivars of several crops. With this support, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has undertaken to develop improved breeding lines and hybrid parents of pearl millet with high levels of Fe and Zn density.

Pearl millet is globally cultivated on about 30 million ha, mostly in the arid and semiarid tropical regions of Asia and Africa, with India having the largest area of about 9 million ha (Yadav et al., 2012). It is a significant source of grain Fe and Zn density both in India and sub-Saharan Africa. For instance, it accounts for 20 to 63% of the total cereal consumption in some of the major pearl millet growing states of India such as Maharashtra, Gujarat, and Rajasthan, and it accounts for 19 to 63% of the Fe and 16 to 56% of the Zn intake from all food sources (Parthasarathy Rao et al., 2006). It is also the cheapest source of these micronutrients as compared with other cereals and vegetables. Earlier studies in pearl millet have shown large variability both for Fe and Zn density (Bashir et al., 2014; Jambunathan and Subramanian, 1988; Rai et al., 2012; Velu et al., 2007, 2008;).

Since pearl millet is a highly cross-pollinated crop, three types of seed samples can be used for the analysis of seed quality traits. These include selfed seeds, sibbed seeds, and OP seeds. When dealing with a large number of breeding lines, production of sibbed seeds is time consuming and expensive, and hence it is not a practical solution. Burton (1952) and Burton et al. (1980) observed immediate effect of pollen source (i.e., xenia effect) on seed size, nitrogen content, and proline content in pearl millet and suggested that selfed seeds should be used for accurate determination of grain characteristics in cross-pollinated crops such as pearl millet and maize (*Zea mays* L.). Also, it is much less time consuming and more cost effective to produce selfed seeds than sibbed seeds. However, selfing in pearl millet leads to variable seed set, which is genotype and environment dependent. The effect of seed set on the Fe and Zn density in pearl millet is not known. Open-pollinated panicles generally have >90% seed set in pearl millet, but OP seeds in breeding fields are produced with random pollen from other genotypes and pollen source effect (i.e., xenia effect) on grain Fe and Zn density has not been studied in pearl millet and maize. However, a genetics study on Fe and Zn density in maize, concerned with possible xenia effect, used controlled pollination to produce the seed samples for Fe and Zn analysis (Long et al., 2004). The main objectives of this research were to study the effects of seed set and pollen source (or xenia effect) on the Fe and Zn density and also to examine if dust contamination of OP seeds could be a significant factor determining the Fe and Zn density in pearl millet.

MATERIALS AND METHODS

Experimental Materials

Three sets of trials were conducted during 2010 to 2011. A seed set trial consisted of nine maintainer lines and seven restorer lines of diverse parentage. On the basis of previous results, a wide range of Fe density among these lines was an additional criterion for including them in this trial. A xenia trial consisted of two each of high-Fe and low-Fe OP varieties (OPVs), two each of high-Fe and low-Fe hybrids, and two each of high-Fe and low-Fe inbred lines of diverse genetic backgrounds. Since Fe and Zn density are generally highly significantly and positively correlated (Rai et al., 2012; Velu et al., 2007, 2008), the high-Fe genotypes generally had high Zn density, and low-Fe genotypes had low Zn density. A dust contamination trial was conducted that consisted of four OPVs, five hybrids, and five inbred lines of diverse origin and with varying Fe and Zn density. All three trials were planted in Alfisols at ICRISAT, Patancheru, India.

Seed Set Trial

The seed set trial was planted in four-row plots of 4-m length replicated three times in a randomized completed block design during the 2010 summer and rainy season. The rows spaced at 75 cm during the rainy season and 60 cm during the summer season were overplanted and thinned 10 to 12 d after planting to single plants spaced at 10 cm. A basal dose of diammonium phosphate at 100 kg ha^{-1} (i.e., 18 kg N and 46 kg P) was applied before planting with 100 kg ha^{-1} of urea (i.e., 46 kg N) side-dressed 15 d after planting. The field was manually weeded just after thinning and irrigated at about a week interval during the summer season and twice during the rainy season to protect from any moisture stress. At the time of panicle emergence, main panicles of 90 to 100 plants in each plot were covered with parchment paper bags. At full stigma emergence stage, the parchment paper bags of 70 to 80 bagged panicles in each plot were gently pushed up and down for 2 to 5 s to induce variable stigma shedding and consequent reduction in seed set. The other bagged panicles were left as such till maturity. All the bagged panicles were harvested at physiological maturity and sundried for 10 to 15 d. These were then classified into three different seed set classes (<10%, 40–50%, and >90%) following the standard scoring scale developed for ergot scoring (Thakur and Williams, 1980) and used in earlier seed set studies (Rai and Hash, 1990; Rai et al., 1996). Those panicles which did not fall clearly into these three seed set classes were discarded. The panicles in each seed set class were bulk threshed plotwise and seed samples produced for mineral analysis. In one maintainer line and four restorer lines, there were not adequate panicles falling in the <10% seed set class, thus leading to eight maintainer lines and three restorer lines which were used for Fe and Zn density analysis.

Xenia Trial

The xenia trial was planted in four-row plots of 4-m length during the 2010 rainy season and 2011 summer season. The genotypes in this trial were paired such that each pair consisted of a high-Fe and low-Fe OPV or a high-Fe and low-Fe hybrid or a high-Fe and low-Fe inbred line, hereafter referred to as high–low genotypes. Thus, there were two pairs each of OPVs, hybrids, and inbred lines. The genotypes were randomized in

a split-plot design and replicated three times. The high–low pairs made the main plots and genotypes within the pair made the subplots. Twenty rows of these genotypes were planted in a separate side block to serve as pollen source. The planting, spacing, and crop-management practices were similar to those described for the respective seasons for the seed set trial. At panicle emergence, main panicles of 40 to 50 plants in each plot and about 200 plants of each genotype in the pollen source block were covered with parchment paper bags. About 12 to 15 plants of each plot of OPVs and 6 to 8 plants in each plot of hybrids and inbred lines were crossed with the bulk pollen from the same plot to produce the sibbed seeds. Also, 12 to 15 plants in each plot of OPVs and 6 to 8 plants in each plot of hybrids and inbred lines within each pair were crossed reciprocally using bulk pollen from the respective genotypes in the pollen source block to produce crossed seed samples. The sibbed panicles and crossed panicles were harvested at maturity as separate bulks and sundried following the same method as described for the seed set trial. These were threshed as sibbed bulks and crossed bulks in each plot to produce seed samples for Fe and Zn analysis.

Dust Contamination Trial

This trial was planted in four-row plots of 4-m length, replicated three times in a randomized complete block design during the 2010 rainy season and 2011 summer season. The planting, spacing, and crop-management practices were similar to those described for the respective seasons for the seed set trial. At full anthesis, main panicles of 15 random plants in each plot were covered with parchment paper bags to avoid dust contamination, if any, of the developing grains. Bulk seeds produced from these panicles were referred to as "OP-bagged" seeds. Bulk seeds were also produced from the OP main panicles of 15 random plants left unbagged till maturity, which would otherwise be prone to dust contamination, if any. These were referred to as "OP-unbagged" seeds. About 40 plants in each plot were bagged with parchment paper bags at the panicle emergence. Of these, 10 to 12 plants were crossed using the bulk pollen from respective plots to produce sibbed seeds. The sibbed, OP-bagged, and OP-unbagged panicles were harvested at maturity, sundried, and threshed as three separate bulks in each plot to produce grain samples for Fe and Zn analysis.

Micronutrient and Statistical Analyses

Micronutrient (Fe and Zn) analysis of seed samples of all three trials and analysis of Al as an index element for dust contamination in the dust contamination trial, were analyzed 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).

The seed set trial was analyzed as a two-factor nested experiment, with the three seed set classes nested within the genotypes. The dust contamination trial was also analyzed as a two-factor nested experiment, with OP-bagged, OP-unbagged, and sibbbed seeds nested within the genotypes. The xenia trial was analyzed as a 3-factor nested experiment with a high-Fe and a low-Fe genotype nested within each pair of OPVs or hybrids or inbred lines, and sibbed and crossed seeds nested within each genotype. All these trials were analyzed assuming fixed model following Gomez and Gomez (1984) and using GenStat statistical package (Payne et al., 2011). Duncan's Multiple Range Test (DMRT) was used for multiple comparisons of treatments means in the seed set trial to determine which ones were significantly different from each other (Gomez and Gomez, 1984). In the dust contamination trial, sibbed seeds were used as reference for comparing OP-bagged and OP-unbagged seeds.

Table 1. Pertinent mean squares for grain Fe, Zn, and Al density and 1000-seed weight across two environments in pearl millet, Patancheru, India.

Source of variation	df	Mean square			
		Fe density	Zn density	1000-seed weight	Al density
		—— mg kg^{-1} ——		g	mg kg^{-1}
Seed set trial					
Environment (E)	1	57,481	10,223	10.6	–
Genotype (G)	10	5,055**	1,866**	18.8**	–
G × E	10	1,950**	685**	7.5**	–
Seed set class (SSC)/E	22	2,219**	730**	2.5**	–
SSC/G × E	22	213**	68**	2.0**	–
Xenia trial					
Environment (E)	1	875	1,390	174.6	–
Genotype pair (GP)	5	958**	221**	135.1*	–
GP × E	5	174**	92**	1.9	–
Micronutrient level (ML)/GP	6	3,756**	628**	35.2**	–
ML/GP × E	6	107**	46*	2.8**	–
Seed source (SS)/ML/GP	12	33	19	2.2*	–
SS/ML/GP × E	12	26	11	3.2**	–
Dust contamination trial					
Environment (E)	1	2,623	3,233	222	4.44
Genotype (G)	13	3,336**	596**	63**	1.24*
G × E	13	89**	62**	5**	1.74**
Seed source (SS)/G	28	82**	55**	3.5**	1.86**
SS/G × E	28	34**	15	2.6*	1.11

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

RESULTS

Seed Set Effect

The mean Fe density, averaged across all the 11 genotypes, varied from 89 mg kg^{-1} in 2009 rainy season to 122 mg kg^{-1} in 2010 summer season, while the mean Zn density varied from 73 to 89 mg kg^{-1} (data not presented). There were highly significant differences among the genotypes ($p < 0.01$), both for Fe and Zn density and 1000-seed weight (Table 1). The genotype × environment (G × E) interactions were also highly significant for both micronutrients and for 1000-seed weight, but their magnitudes were 37 to 40% of those due to genotypic differences. On the basis of the mean performance in the two environments, large differences were observed among the genotypes with 59 to 113 mg kg^{-1} Fe density and 50 to 84 mg kg^{-1} Zn density in >90% seed set class that approximately resembles the natural

Table 2. Grain Fe and Zn density and 1000-seed weight in three diverse selfed seed set classes across two environments in pearl millet, Patancheru, India.

Genotype	Fe density in seed set class			Zn density in seed set class			1000-seed weight in seed set class		
	>90%	40–50%	<10%	>90%	40–50%	<10%	>90%	40–50%	<10%
	mg kg^{-1}						g		
ICMB 91222	112c†	128b	144a	84b	90b	100a	7.6a	6.9ab	6.3b
ICMB 95333	92c	113b	131a	66c	73b	82a	8.6a	8.7a	6.8b
ICMB 99444	91c	107b	125a	77c	91b	103a	9.3a	8.7ab	8.0b
ICMB 03999	113b	119b	146a	74b	77b	93a	8.7a	6.8a	7.4a
ICMB 93222	84c	104b	119a	63c	77b	83a	10.3a	9.3ab	9.0b
ICMB 00999	59b	69b	89a	62b	69b	84a	8.3a	7.9ab	8.2b
ICMB 02444	105b	113b	139a	73b	78b	93a	8.9a	7.4ab	6.7b
ICMB 03111	74c	92b	129a	63c	73b	95a	8.1a	8.4a	7.9a
IPC 1356	94c	111b	125a	62b	71a	75a	6.6a	5.9a	5.7a
IPC 715	70c	79b	104a	50c	59b	70a	6.5a	7.1a	6.8a
IPC 1650	75c	99b	126a	79c	93b	106a	6.9a	6.8a	5.9b
Mean	89	103	124	71	80	92	8.1	7.6	7.1

† Values between seed set classes for each entry followed by the same letter are not significantly different according to LSD (0.05).

seed set of most of the inbred lines under open pollination in pearl millet (Table 2). There were also large differences among genotypes for seed size, with 6.5 to 10.3 g of 1000-seed weight in this seed set class (SSC). The differences among the three SSCs were highly significant as were the SSC × environment (SSC × E) interactions for both micronutrients and 1000-seed weight. However, the magnitudes of variability due to these interaction effects were about 10% of those due to SSC differences for the Fe and Zn density. In case of 1000-seed weight, variances due to SSC differences and SSC × E interaction were of similar order.

Averaged across the 11 genotypes and the two environments, the Fe density was 14 mg kg^{-1} (16%) higher in 40 to 50% SSC and it was 35 mg kg^{-1} (39%) higher in <10% SSC as compared with the reference >90% SSC (Table 2). Large differences were observed among the genotypes for increase in Fe density due to reduction in seed set, with 6 to 24 mg kg^{-1} (5–32%) increase in Fe density in 40 to 50% SSC and 30 to 55 mg kg^{-1} (29–74%) increase in <10% SSC. While the reduction in seed set to 40 to 50% level led to significant increase in Fe density in eight genotypes, further increase in the Fe density in <10% SSC was significant in all the genotypes. As compared with >90% SSC, the mean Zn density averaged across all the 11 genotypes and two environments increased by 9 mg kg^{-1} (13%) in 40 to 50% SSC and by 21 mg kg^{-1} (30%) in <10% SSC. Large differences were found among the genotypes, with 3 to 14 mg kg^{-1} (4–22%) increase in Zn density in 40 to 50% SSC and 13 to 32 mg kg^{-1} (21–51%) increase in Zn density in < 0% SSC. While the reduction in seed set to 40 to 50% SSC led to significant increase in Zn density in six genotypes, further increase in the Zn density in <10% SSC was significant for all except one genotype. Reduction in seed set can be expected to produce larger seeds due to partitioning of assimilates to fewer seeds. However, contrary to this expectation, reduction in seed set either in 40 to 50% SSC or <10% SSC led to reduction in seed weight in several genotypes. It was observed that varying proportion of seeds in these two SSCs, especially in <10% SSC, were shriveled as compared with almost all seeds being well filled and plump in >90% SSC.

The Fe density in the >90% SSC was positively and highly significantly correlated with those in the 40 to 50% SSC ($r = 0.94$, $p < 0.01$) and in <10% SSC ($r = 0.87$, $p < 0.01$). An identical pattern was observed for the Zn density between >90% SSC and the other two SSCs, with $r = 0.94$ and 0.87, respectively. However, there was no correlation between the Fe density in >90% SSC and magnitude of Fe increase either in the 40 to 50% SSC ($r = 0.26$) or in <10% SSC ($r = 0.06$). Similarly, there was no correlation between the Zn density in >90% SSC and the magnitude of Zn increase either in 40 to 50% SSC ($r = 0.06$) or in <10% SSC ($r = 0.02$).

Xenia Effect

The mean Fe density, averaged across all the 12 genotypes, varied from 57 mg kg^{-1} during the 2010 rainy season to 62 mg kg^{-1} during the 2011 summer season, while the Zn density varied from 44 to 50 mg kg^{-1} (data not presented). The differences among the pairs of genotypes (GP) as well as between the high and low genotypes within the pairs at the micronutrient level (ML/GP) were highly significant ($P < 0.01$), as were their interactions with the environments (ML/GP × E), both for the Fe and Zn density and 1000-seed weight (Table 1). However, the contribution of ML/GP × E interaction to the total variability was 3% of that due to ML/GP for Fe density and 7% of that due to ML/GP for the Zn density. Averaged across the two environments, the difference between the high-Fe and low-Fe genotypes in the sibbed seeds varied from 15 to 41 mg kg^{-1} in five pairs and its was 6 mg kg^{-1} in one pair (Table 3). Relatively smaller differences were observed between the genotypes within the pairs for Zn density, varying from 5 to 15 mg

Table 3. Grain Fe and Zn density and 1000-seed weight in sibbed and crossed seed samples across two environments in pearl millet, Patancheru, India.

Genotype pair†	Fe density		Zn density		1000-seed weight	
	Sibbed	Crossed	Sibbed	Crossed	Sibbed	Crossed
	mg kg^{-1}				g	
Open-pollinated varieties						
ICTP 8203 (P1)	73	76	55	56	13.7	13.8
Raj 171 (P2)	53	48	47	45	9.4	10.3
ICMV 221 (P1)	62	63	46	48	11.9	12.2
JBV 3 (P2)	47	45	39	39	9.2	10.1
Hybrids						
KH 302 (P1)	69	62	52	50	13.8	13.5
HHB 197 (P2)	45	44	37	35	11.0a‡	9.2b
MRB 204 (P1)	56	57	49	50	12.4	13.4
GHB 538 (P2)	50	49	45	43	11.6	11.9
Inbred lines						
ICMB 98222 (P1)	86	81	57	53	7.1	7.5
ICMB 03555 (P2)	57	52	45	42	6.2a	7.7b
ICMB 02333 (P1)	82	80	53	50	6.9	7.2
ICMB 04333 (P2)	41	42	45	41	7.5	7.4
LSD (0.05)	6.0		4.0		1.2	

† P1, high-Fe and high-Zn parents; P2, low-Fe and low-Zn parents.

‡ The values between sibbed and crossed seeds for each genotype and for all three traits are statistically not significant at 0.05 probability level except for 1000-seed weight of two genotypes indicated with different letters.

kg^{-1}. The differences between the sibbed seeds and those produced from reciprocal crosses were nonsignificant both for the Fe and Zn density in all 12 genotypes in high as well as low density genetic backgrounds. The difference between the sibbed and crossed seeds were significant for 1000-seed weight ($p < 0.05$), but this largely resulted from the sibbed seeds being significantly larger than crossed seeds in hybrid HHB 197 and crossed seeds being significantly larger than sibbed seeds in inbred line ICMB 03555.

Dust Contamination Effect

The mean Fe density, averaged across all the 14 genotypes, varied from 54 mg kg^{-1} in the 2010 rainy season to 61 mg kg^{-1} in the 2011 summer season, and Zn density varied from 43 mg kg^{-1} in the rainy season to 50 mg kg^{-1} in the summer season (data not presented). The differences among the genotypes as well as their interactions with the environment (G × E) were highly significant ($p < 0.01$) for Fe, Zn, and Al density and for 1000-seed weight (Table 1). However, the contributions of G × E interaction to variability relative to those due to genotypic differences were <3% for Fe density, 10% for Zn density, and 8% for 1000-seed weight, while it was 40% higher for Al density. The differences among the three seed sources (SS/G) as well as their interactions with the environment (SS/G × E) were also highly significant for all three traits, except SS/G × E for Zn and Al density. The contribution of SS/G × E interaction to variability relative to those due to seed sources (SS/G) was 41% for Fe density and 74% for 1000-seed weight.

Averaged across the two environments, the mean Fe density in the sibbed seeds varied from 43 to 90 mg kg^{-1} among the genotypes (Table 4). As compared with sibbed seed, the mean Fe density in the OP-bagged seeds was significantly less by 6 to 7 mg kg^{-1} in two genotypes, while it was significantly higher by 9 mg kg^{-1} in one genotype. In case of OP-unbagged seeds, the Fe density was significantly lower by 6 to 10 mg kg^{-1} in 6 genotypes. A similar pattern was found for the Zn density. There was no significant difference in the Zn density of sibbed and OP-bagged seeds except in one genotype, while OP-unbagged seeds had significantly less Zn density by 5 to 8 mg kg^{-1} in eight genotypes. As compared with sibbed seeds, significantly larger seeds were found in OP seed samples (either or both bagged and unbagged) only in two genotypes. The Al density in the sibbed seeds varied from 0.75 to 1.37 mg kg^{-1} across the genotypes. The OP-unbagged seeds had 1.43 to 2.84 mg kg^{-1} Al density, being significantly higher but by <1.5 mg kg^{-1} than those in the sibbed seeds in seven genotypes. Except for one genotype, there was no significant difference for Al density between sibbed and OP-bagged seeds, and except for three genotypes (JBV 3, KH 302, and ICMB 98222), there was no significant difference for Al density between OP-bagged and OP-unbagged seeds.

DISCUSSION

The protogynous flowering of pearl millet makes it a highly cross-pollinated crop. Hence, if the seeds representing a true genotype are to be used for any seed quality analysis, these ought to be produced either by selfing or by sibbing. Production of seed by sibbing is neither cost effective nor practical in breeding programs dealing with very large numbers of breeding lines. Production of seed by selfing is more cost effective compared with sibbing. Selfing, however, leads to variable reduction in seed set, which is genotype and environment dependent. This study showed that reduction in seed set under controlled selfing increased both grain Fe and Zn density, with an increase in these micronutrients corresponding to the extent of the reduction in seed set. This is not unexpected assuming that with reduced seed set, the Fe and Zn pool in vegetative plant parts will be translocated and loaded into fewer grains as compared with a situation where more grains are produced under good seed set conditions of natural open pollination; typically above >90% seed set in most of the inbred lines in pearl millet. Seed set reduction can be expected to increase seed size due to assimilate

Table 4. Grain Fe, Zn, and Al density and 1000-seed weight in seeds produced from open-pollinated (OP) panicles and sibbing in pearl millet. Mean of two environments, Patancheru, India.

Geno-type	Fe density			Zn density			1000-seed weight			Al density		
	OP-unbagged	OP-bagged	Sibbed	OP-unbagged	OP-bagged	Sibbed	OP-unbagged	OP-bagged	Sibbed	OP-unbagged	OP-bagged	Sibbed
	mg kg^{-1}						g			mg kg^{-1}		
ICTP 8203	69a†	73a	70a	48a	54a	52a	13.5a	14.3a	12.8a	1.92b	1.12a	0.89a
ICMV 221	60a	71b	62a	47a	52a	48a	13.5b	12.9b	10.0a	1.95b	1.04a	0.84a
Raj 171	46a	51a	50a	43a	47a	43a	10.2a	10.2a	10.9a	1.86a	1.51a	1.02a
JBV-3	41a	45a	46a	37a	39a	40a	10.8a	11.0a	10.5a	1.82a	0.82a	0.98a
KH 302	60b	70a	70a	45b	52a	53a	13.7a	13.7a	12.4a	2.07b	0.85a	0.75a
MRB 204	51b	56a	58a	44b	48a	50a	15.0a	15.2a	13.9a	1.85a	1.25a	1.13a
HHB 197	48a	43a	48a	40a	35a	39a	12.2a	10.6a	10.7a	1.86b	0.95a	0.83a
GHB 538	43b	46a	49a	39b	40a	44a	13.1a	13.4a	12.7a	1.43a	1.38a	1.15a
ICMB 98222	80b	83b	90a	55a	55a	59a	9.9a	10.0a	9.0a	1.92a	0.88a	1.08a
ICMB 02333	78a	77a	82a	48b	51a	54a	8.7a	9.0a	8.5a	2.84b	2.44b	1.37a
ICMB 03555	48a	48a	52a	36b	41a	44a	9.2a	9.3a	8.3a	2.13a	1.19a	1.19a
ICMB 04333	37b	37b	43a	37b	39b	45a	8.9a	9.4a	9.4a	2.37b	1.64a	0.93a
9333	51a	55a	55a	47b	49a	52a	11.5a	11.9a	11.1a	2.04b	1.11a	0.78a
JKBH-26	53b	57a	62a	47b	50a	53a	13.0b	11.9a	11.3a	1.79a	1.51a	1.17a
Mean	55	58	60	44	47	48	11.7	11.6	10.8	1.99	1.26	1.01
LSD (0.05)	5.0			4.0			1.5			0.95		

† The values of OP-unbagged and OP-bagged in each genotype followed by a letter different from the sibbed seed source (considered as reference) are significantly different at 0.05 probability level.

partitioning to fewer seeds compared with normal seed set conditions. However, in this study, reduction in seed set led to significant decrease in seed size on account of mostly variable seed size that included even shriveled in most of the inbred lines. Thus, besides the direct effect of reduced seed set, reduction in seed size could have had additional effect in increasing the Fe and Zn density in 40 to 50% and < 0% SSCs. The Fe and Zn density in >90% SSC was highly significantly correlated with those in the 40 to 50% SSC and 10 to 20% SSCs, which apparently may imply that selfed seed samples can be used for selection of genotypes with high Fe and Zn density in a breeding program. However, the magnitude of increase in Fe and Zn density under enforced selfing either in 40 to 50% SSC or in <10% SSC was variable across the genotypes and it was not correlated with the Fe and Zn density levels of lines in >90% SSC. Further, selfing caused variable seed set, depending on the genotypes and the environments. Therefore, selfed seed samples cannot be reliably used for precise estimation of the Fe and Zn density.

Since sibbed seed production in a large number of breeding lines is not practical due to the high cost and manpower resources required and selfing resulted in genotype-dependent variable seeds set and associated overestimation of Fe and Zn density, OP seed sampling may be the best option for cost-effective and reliable estimation of Fe and Zn density, provided seed micronutrient density is not affected by the pollen source (xenia effect) and dust contamination. Results of this study showed that there was no significant difference between sibbed seeds and crossed seeds either in high-density backgrounds or low-density backgrounds for both micronutrients. This clearly demonstrated that there was no xenia effect on grain Fe and Zn density in pearl millet. The difference between the sibbed and crossed seed was significant for seed weight, although it occurred in only two low-seed-weight genotypes and the changes were in opposite directions. In an inbred line, seeds produced from a cross with a relatively higher seed weight male line had significantly larger seeds than the sibbed seed, while in a hybrid, seeds produced from a cross with a relatively higher seed weight male hybrid had significantly lower seed weight than sibbed seeds. Thus, even in these two cases, there was no consistent pattern to show any xenia effect for seed size, although there are reports of xenia effects for seed size in pearl millet (Burton, 1952; Burton et al., 1980), maize (Bulant et al., 2000; Liu et al., 2010; Pletsch-Rivera and Kaeppler, 2003), faba bean (*Vicia faba* L.)(Duc et al., 2001), and cotton (*Gossypium hirsutum* L.) (Pahlavani and Abolhasani, 2006). The only study on xenia effect for a mineral content is that of Pletsch-Rivera and Kaeppler (2007) on phosphorus, where no xenia effect was observed.

Aluminum density in seeds is an indicator of possible dust contamination. Thus, any dust contamination should lead to higher Al density in the OP seeds, the more so in the OP seeds from panicles left unbagged till harvest than in the sibbed seeds. Except for one genotype, there was no significant difference between the sibbed and OP-bagged seeds, but Al density was significantly higher in the OP-unbagged seeds than in the sibbed seeds in seven genotypes. This would indicate that if the panicles are bagged soon after the open pollination, there is no risk of dust contamination. However, small though negligible

dust contamination would occur if OP grains are harvested from the panicles left unbagged till maturity. Even in these panicles, the Al density was <3 mg kg^{-1} in all the genotypes. On the basis of the analysis of Fe and Al relationship in several crops and several trials, Pfeiffer and McClafferty (2007) concluded that Al density above 5 mg kg^{-1} would indicate some degree of dust contamination and consequent overestimation of Fe density. Further, the results of this study did not show any overestimation of the Fe and Zn density in OP seeds than in the sibbed seeds. On the contrary, sibbed seeds had significantly higher Fe density than OP-bagged seeds in two genotypes and OP-unbagged seeds in six genotypes. A similar pattern was found for Zn density. This could likely result if the Fe and Zn density in some genotypes is affected by drying anthers sticking to developing grains in the sibbed panicles. There was no significant difference among the three seed sources for 1000-seed weight, except for four genotypes where OP seeds (from either or both bagged and unbagged panicles) had larger seed size than those from the sibbed panicles. This pattern of seed size differences among the three seed sources was not consistent with the patterns observed for grain Fe and Zn density differences. These results have made significant contributions to enhancing the breeding efficiency and savings on resources, as OP seeds rather than selfed or sibbed seeds (a practice followed earlier) are now being used in almost all pearl millet programs for the analysis of grain Fe and Zn density.

Acknowledgments

This study, conducted under the CGIAR research program on Agriculture for Nutrition and Health (A4NH), was undertaken with funding support from the HarvestPlus Challenge Program of the CGIAR and laboratory support from Waite Analytical Services, University of Adelaide, Australia.

References

Bashir, E.M.A., A.M. Ali, A.M. Ali, A.E. Melchinger, H.K. Parzies, and B.I.G. Haussmann. 2014. Characterization of Sudanese pearl millet germplasm for agro-morphological traits and grain nutritional value. Plant Genet. Resour. 12:35–47. doi:10.1017/S1479262113000233

Bouis, H.E., C. Hotz, B. McClafferty, J.V. Meenakshi, and W.H. Pfeiffer. 2011. Biofortification: A new tool to reduce micronutrient malnutrition. Food Nutr. Bull. 32:31S–40S.

Bulant, C., A. Gallais, E. Matthys-Rochon, and J.L. Prioul. 2000. Xenia effects in maize with normal endosperm: II. Kernel growth and enzyme activities during grain filling. Crop Sci. 40:182–189. doi:10.2135/cropsci2000.401182x

Burton, G.W. 1952. The immediate effect of gametic relationship upon seed production in pearl millet, Pennisetum glaucum. Agron. J. 44:424–427. doi:10.2134/agronj1952.00021962004400080006x

Burton, G.W., R. Rabson, and H. Axmann. 1980. Immediate effect of male and female gametes and N on five pearl millet grain characteristics. Crop Sci. 20:7–9. doi:10.2135/cropsci1980.0011183X002000010003x

Duc, G., A. Moessner, F. Moussy, and C. Mousset-Declas. 2001. A xenia effect on number and volume of cotyledon cells and on seed weight in faba bean (*Vicia faba* L.). Euphytica 117:169–174. doi:10.1023/A:1004085013735

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York. p. 680.

Jambunathan, R., and V. Subramanian. 1988. Grain quality and utilization in sorghum and pearl millet. In: J.M.J. de Wet and T.A. Preston, editors, Biotechnology in tropical crop improvement. ICRISAT, Patancheru, India. p. 133–139.

Liu, Y., P. Liu, S. Dong, and J. Zhang. 2010. Hormonal changes caused by the xenia effect during grain filling of normal corn and high-oil corn crosses. Crop Sci. 50:215–221. doi:10.2135/cropsci2009.04.0186

Long, J.K., M. Banziger, and M.E. Smith. 2004. Diallel analysis of grain iron and zinc density in southern African-adapted maize inbreds. Crop Sci 44:2019–2026

Pahlavani, M.H., and K. Abolhasani. 2006. Xenia effect on seed and embryo size in cotton (*Gossypium hirsutum* L.). J. Appl. Genet. 47:331–335. doi:10.1007/BF03194642

Parthasarathy Rao, P., P.S. Birthal, B.V.S. Reddy, K.N. Rai, and S. Ramesh. 2006. Diagnostics of sorghum and pearl millet grains-based nutrition in India. International Sorghum and Millets Newsletter. 44:93–96.

Payne, R.W., S.A. Harding, D.A. Murray, D.M. Soutar, D.B. Baird, A.I. Glaser, S.J. Welham, A.R. Gilmour, R. Thompson, and R. Webster. 2011. The guide to GenStat release 14, Part 2: Statistics. VSN International, Hemel Hempstead, UK.

Pfeiffer, W.H., and B. McClafferty. 2007. HarvestPlus: Breeding crops for better nutrition Crop Sci. 47:S88–S105. doi:10.2135/cropsci2007.09.0020IPBS

Pletsch-Rivera, L., and S.M. Kaeppler. 2003. Xenia effect on phosphorus concentration in outcrossed maize seed. Book of Abstracts: Arnel R. Hallauer International Symposium on Plant Breeding, Mexico City. 17–22 Aug. CIMMYT, Mexico, D.F. Mexico City.

Pletsch-Rivera, L.A., and S.M. Kaeppler. 2007. Phosphorus accumulation in maize grain is not influenced by xenia (*Zea mays* L.). Maydica 52:151–157.

Rai, K.N., and C.T. Hash. 1990. Fertility restoration in male-sterile × maintainer hybrids of pearl millet. Crop Sci. 30:889–892. doi:10.2135/cropsci1990.0011183X003000040026x

Rai, K.N., D.S. Virk, G. Harinarayana, and A.S. Rao. 1996. Stability of male-sterile sources and fertility restoration of their hybrids in pearl millet. Plant Breed. 115:494–500. doi:10.1111/j.1439-0523.1996.tb00964.x

Rai, K.N., M. Govindaraj, and A.S. Rao. 2012. Genetic enhancement of grain iron and zinc content in pearl millet. Qual. Assur. Saf. Crop. 4:119–125. doi:10.1111/j.1757- 837X.2012.00135.x

Thakur, R.P., and R.J. Williams. 1980. Pollination effects on pearl millet ergot. Phytopathology 70:80–84. doi:10.1094/Phyto-70-80

Velu, G., K.N. Rai, V. Muralidharan, V.N. Kulkarni, T. Longvah, and T.S. Raveendran. 2007. Prospects of breeding biofortified pearl millet with high grain iron and zinc contents. Plant Breed. 126:182–185. doi:10.1111/j.1439-0523.2007.01322.x

Velu, G., K.N. Rai, K.L. Sahrawat, and K. Sumalini. 2008. Variability for grain iron and zinc contents in pearl millet. SAT. J. Agric. Res. 6:1–4.

Wheal, M.S., T.O. Fowles, and L.T. Palmer. 2011. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Anal. Methods. 3:2854–2863. doi:10.1039/c1ay05430a

WHO. 2002. Reducing risks and promoting healthy life In: World Health Report 2002. World Health Organization, Geneva, Switzerland. p. 61–105.

Yadav, O.P., K.N. Rai., B.S. Rajpurohit., C.T. Hash., R.S. Mahala., S.K.Gupta., H.S. Shetty., H.R. Bishnoi., M.S. Rathore., A.Kumar., S.Sehgal, and K.L.Raghvani. 2012. Twenty-five years of pearl millet improvement in India. All India Coordinated Pearl Millet Improvement Project, Jodhpur, India.