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Efficiency of Single Plant Selection for Grain Iron and Zinc Density in Pearl Millet

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ABSTRACT

Single plant selection, if effective, can make significant contributions to enhance breeding efficiency. This hypothesis was tested for grain iron (Fe) and zinc (Zn) density in four populations of pearl millet (*Pennisetum glaucum* (L.) R. Br.). Inbreeding and selection in advancing generations is normally practiced by evaluating progenies in unreplicated nurseries or at most in 2-replication trials. In each population in this study, grain samples of 40 random individual plants (hereafter referred to as S_0 plants) and their S_1 progenies grown in 2-replication trials for two seasons (called as environments) were analyzed for Fe and Zn density using ICP analytical method. In each population, correlation coefficients between S_0 plants and their respective S_1 progenies (whether individual environment or the mean of both environments) both for Fe and Zn density were positive, highly significant, and of the similar order as the correlation coefficients between the two environments for the S_1 progeny performance. Also, the patterns of correlation coefficients between the S_0 plants and either of the two replications of the S_1 progenies in each environment were similar to those between the two replications for S_1 progeny performance in both environments and in all four populations. While the Fe and Zn density were positively and highly significantly correlated, these were not correlated with grain mass. The patterns of these associations were similar both at the S_0 plant level as well as at the S_1 progeny level in each population. These results suggest that individual plant selection can be effectively used for simultaneous genetic improvement of both grain Fe and Zn density without compromising on grain size.

Keywords: correlation, micronutrients, *Pennisetum glaucum*, selection

INTRODUCTION

Micronutrient malnutrition arising from dietary deficiency of vitamin A and mineral micronutrients such as iron (Fe) and zinc (Zn) has been recognized as a major public health problem. About three billion people worldwide suffer from micronutrient malnutrition (Welch and Graham 2004). Micronutrient malnutrition increases mortality and morbidity rates and health-care costs, reduces labor productivity, and thus impacts on national developmental efforts. The effect of malnutrition is particularly serious in high risk groups such as pregnant women, infants and adolescent children. For instance, more than 2 million children die every year globally due to Fe, Zn and vitamin A deficiency (WHO 2002). About 80% of the pregnant women, 52% of the non-pregnant women, and 74% of the children (6-35 months) in India suffer from iron deficiency-induced anemia (Chakravarty and Ghosh 2000). In sub-Saharan Africa, 92% of the children in Burkina Faso and 85% of the children in Mali in 6-59 months age group have been reported to be affected by Fe-deficiency induced anemia. Zinc deficiency is assumed to be as widespread, but there are few reliable data to confirm it. Addressing this problem through food supplements and food fortification in these areas is not a practical solution due to poor purchasing power of the consumers and unsatisfactory delivery infrastructure, especially in the rural areas. Diversified food uses and biofortified crop cultivars provide cost-effective and sustainable options to reduce micronutrient malnutrition in these areas. A socioeconomic study covering 12 countries in Asia, Africa and Latin America showed that costs of biofortification to avert Disability Adjusted Life Year (DALY) losses often fall in the highly cost-effective category (Meenakshi *et al.* 2010). Biofortified crop cultivars offer a rural-based intervention that, by design, initially reach those more remote populations, which comprise a majority of the malnourished, and then penetrate to urban populations as production surpluses are marketed (Bouis *et al.* 2011).

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major warm-season cereal grown on more than 26 million ha in some of the harshest environments in the arid and semi-arid tropical regions of Africa (> 16 million ha) and Asia (>10 million ha), with India being the largest producer, cultivating this crop on about 9.4 million ha. Pearl millet is a significant source of these micronutrients both in India and sub-Saharan Africa. For instance, it accounts for 20-62% 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 20-62% of the Fe and 16-44% of the Zn intake from all food sources (Parthasarathy Rao *et al.* 2006). It is also the cheapest source of these micronutrients as compared to other cereals and vegetables. A preliminary study conducted with a limited number of 27 genotypes at ICRISAT had shown high levels of and large variability for both iron (40 to 580 mg kg^{-1}) and zinc density (10 to 66 mg kg^{-1}) in pearl millet grains (Jambunathan and Subramanian 1988). A more recent study consisting of a wider range of germplasm, improved populations, population progenies, and hybrid parents also showed large variability both for Fe (30-76 mg kg^{-1}) and Zn density (25-65 mg kg^{-1}) with significant environmental effect (Velu *et al.* 2007). Further studies have shown much wider range and higher levels of these micronutrients in the germplasm and breeding lines (KN Rai, unpub.). Efficient screening procedure will have significant effect on the exploitation of this variability for genetic improvement of these micronutrients. Cost-effective and rapid colorimetric screening procedures for the qualitative analysis of these micronutrients have

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Table 1 Mean square for grain iron (Fe) and zinc (Zn) density (mg kg^{-1}) in S_1 progenies of four diverse populations of pearl millet over two environments[1], Patancheru.

Trait	Source of variation	df	Mean Square			
			ICTP 8203	JBV 3	AIMP 92901	ICMR 312
Fe	Environments (E)	1	51396.8**	29604.6**	26046.6**	28143.0**
	Replication / E	2	14.1	0.2	77.1	76.0
	S_1 progeny (S_1)	39	1018.1**	846.7**	491.1**	265.9**
	S_1 x E	39	238.0	162.1**	167.8	129.9**
	Error	78	178.0	54.2	106.6	62.0
Zn	Environment (E)	1	21532.5**	16880.7**	12208.3**	16800.0**
	Replication / E	2	17.3	4.0	2.0	0.8
	S_1 progeny (S_1)	39	325.3**	376.8**	360.8**	89.4**
	S_1 x E	39	80.3	68.6	67.9	45.0
	Error	78	62.6	57.4	53.9	32.7

[1] ICTP 8203 and JBV 3 evaluated during the summer and rainy season of 2009; and AIMP 92901 and ICMR 312 evaluated during the 2009 rainy season and 2010 summer season.
* = Significant at $P \leq 0.05$; ** = Significant at $P \leq 0.01$.

been developed (Velu *et al.* 2006, 2008). Cost-effective and rapid screening procedures for quantitative analysis of these micronutrients have also been identified and standardized (James Stangoulis, pers. comm.). The objective of this study was to assess the efficiency of single plant selection for these micronutrients, which will have a direct bearing on the breeding efficiency in terms of handling a large number of plants and progenies, and savings on the land and material resources for experimentation.

MATERIALS AND METHODS

Experimental materials for the study were derived from three open-pollinated varieties ('ICTP 8203', 'AIMP 92901' and 'JBV 3') and a population ('ICMR 312'). 'ICTP 8203' was developed at ICRISAT by recombining five S_2 progenies selected from an *iniari* landrace originating from northern Togo. It was released in 1988 for cultivation specifically in peninsular India (Rai *et al*. 1990). 'AIMP 92901' was jointly developed by ICRISAT and Marathwada Agricultural University, National Agricultural Research Project Station, Aurangabad, Maharashtra, by random mating 272 S_1 progenies from the C_5 cycle bulk of a Bold-Seeded Early Composite (BSEC) selected for agronomic traits at Aurangabad and the C_5 S_1 bulk of these selected progenies of BSEC that were found to be resistant to downy mildew (*Sclerospora graminicola* (Sacc. Schroet.) disease in screening at ICRISAT. 'AIMP 92901' was released in 2001 for cultivation in peninsular India. 'JBV 3' was jointly developed by ICRISAT and Jawaharlal Nehru Krishi Vishwa Vidyalaya, College of Agriculture, Gwalior, Madhya Pradesh by recombining 15 full-sib progenies from the third cycle bulk of Smut Resistant Composite II. 'JBV 3' was released in 2001 for cultivation in northern India. 'ICMR 312' was developed at ICRISAT by mass selection in BSEC with further progeny testing to improve its male fertility restoration ability and resistance to downy mildew. 'ICMR 312' is population pollen parent of a topcross hybrid 'ICMH 312' which was developed at ICRISAT and it was released in 1993 for cultivation in peninsular India.

Initially, 'ICTP 8203' and 'JBV 3' were planted in 20 rows each during the 2008 rainy season and main panicles of more than 100 plants were bagged with parchment paper bag at the time of panicle emergence. Of these, selfed panicles of more than 60 plants (S_0 plants) in each population with >90% seed set were harvested and threshed to produce the S_1 seed and those 40 plants in each population that produced large amount of seed for 2-season replicated S_1 progeny trials were finally selected for the study. In each population, 40 S_1 progenies were planted in 1-row plots of 2 m length replicated twice in a randomized complete block design during the 2009 summer and rainy seasons. Two additional populations ('AIMP 92901' and 'ICMR 312') were planted during the 2009 summer season and S_1 seeds were produced following the same procedure as mentioned above for 'ICTP 8203' and 'JBV 3'. Forty-entry S_1 progeny trials of these two populations were conducted during the 2009 rainy season and 2010 summer season, following the same procedure as for 'ICTP 8203' and 'JBV 3'. In all three years, the sowing was done on ridges spaced at 60 cm during the summer season and 75 cm during the rainy season. Spacing between the plants was 10 cm during both seasons. Diammonium phosphate (DAP) at 100 kg ha^{-1} was applied as a basal dose, and 100 kg ha^{-1} urea was applied as topdressing about 15 days after the sowing in all the trials.

In all the trials, 5-8 main panicles per plot were selfed and bulk harvested to produce grain samples for analysis of Fe and Zn density following the Inductively Coupled Plasma Spectroscopy (ICP) method at the Waite Analytical Services Laboratory at the University of Adelaide, Australia. Random 100 grains from each plot were used to determine 1000-grain mass. The analysis of variance and the correlations among the traits were done following Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The difference among the S_1 progenies was highly significant ($P < 0.01$) both for grain iron (Fe) and zinc (Zn) density in all four populations (**Table 1**). The progeny × environment interaction for Zn density was non-significant in all four populations. For the Fe density, the interaction term was significant in only two populations ('JBV 3' and 'ICMR 312'), but its contribution to the total viability was about half to one-fifth of that contributed by the difference among the progenies. These results showed that though both micronutrients were stable, Zn density was slightly more stable than the Fe density over these two contrasting environments. Stability of these micronutrients, however, need to be tested over the large number and more diverse environments, including different soil Fe and Zn content levels. Several studies in wheat have evaluated the stability of Fe and Zn density through more extensive multilocation trials, but the results are not always consistent. For instance, while Morgounov *et al.* (2007) and Joshi *et al*. (2010) found no significant genotype × environment interaction both for Fe and Zn density, highly significant genotype × environment interactions for both micronutrients were found in other studies, with the Fe being relatively less stable than Zn (Zhang *et al.* 2010) or just the reverse (Gomez-Becerra *et al*. 2010).

Since in our study, genotype × environment interaction

Table 2 Mean and range for grain Fe and Zn density in S_0 plants and S_1 progenies of four diverse populations of pearl millet, Patancheru.

Population	Progeny[1]	Fe (mg kg^{-1})		Zn (mg kg^{-1})	
		Mean	Range	Mean	Range
ICTP 8203	S_0	77	53 - 103	65	46 - 85
	S_1	91	61 - 133	74	50 - 91
JBV 3	S_0	50	30 - 75	47	34 - 73
	S_1	57	37 - 76	53	38 - 68
AIMP 92901	S_0	69	36 - 122	61	32 - 92
	S_1	64	40 - 96	56	40 - 79
ICMR 312	S_0	71	47 - 98	63	51 - 79
	S_1	63	49 - 82	52	44 - 63

[1] S_0 plants evaluation of ICTP 8203 and JBV 3 done in 2008 rainy season; and those of AIMP 92901 and ICMR 312 in summer 2009; ICTP 8203 and JBV 3 S_1s were evaluated during the summer and rainy season of 2009; and AIMP 92901 and ICMR 312 S_1s were evaluated during the 2009 rainy season and 2010 summer season; S_1 represents the mean of two environments.

Table 3 Correlation coefficient among the S_0 plants and S_1 progenies for grain Fe and Zn density in four diverse populations of pearl millet, Patancheru.

Population	Correlation between	Fe			Zn		
		S 2009	**R 2009**	**Pooled**	**S 2009**	**R 2009**	**Pooled**
ICTP 8203	S_0 and S_1 (mean)	0.69**	0.50**	0.66**	0.61**	0.63**	0.69**
	S_1 (E1) and S_1 (E2)	-	-	0.64**	-	-	0.61**
	S_0 and S_1 (R_1)	0.66**	0.46**	-	0.57**	0.51**	-
	S_0 and S_1 (R_2)	0.60**	0.39*	-	0.51**	0.57**	-
	S_1 (R_1) and S_1 (R_2)	0.69**	0.41**	-	0.57**	0.48**	-
JBV 3	S_0 and S_1 (mean)	0.54**	0.62**	0.58**	0.64**	0.57**	0.65**
	S_1 (E1) and S_1 (E2)	-	-	0.87**	-	-	0.74**
	S_0 and S_1 (R_1)	0.46**	0.62**	-	0.51**	0.61**	-
	S_0 and S_1 (R_2)	0.59**	0.50**	-	0.68**	0.35*	-
	S_1 (R_1) and S_1 (R_2)	0.86**	0.60**	-	0.71**	0.39*	-
		R 2009	**S 2010**	**Pooled**	**R 2009**	**S 2010**	**Pooled**
AIMP 92901	S_0 and S_1 (mean)	0.58**	0.59**	0.66**	0.65**	0.69**	0.73**
	S_1 (E1) and S_1 (E2)	-	-	0.54**	-	-	0.71**
	S_0 and S_1 (R_1)	0.37*	0.42**	-	0.48**	0.58**	-
	S_0 and S_1 (R_2)	0.60**	0.60**	-	0.69**	0.66**	-
	S_1 (R_1) and S_1 (R_2)	0.42**	0.55**	-	0.58**	0.64**	-
ICMR 312	S_0 and S_1 (mean)	0.53**	0.69**	0.75**	0.29^{ns}	0.69**	0.61**
	S_1 (E1) and S_1 (E2)	-	-	0.36*	-	-	0.33*
	S_0 and S_1 (R_1)	0.45**	0.63**	-	0.27^{ns}	0.54**	-
	S_0 and S_1 (R_2)	0.47**	0.59**	-	0.18^{ns}	0.61**	-
	S_1 (R_1) and S_1 (R_2)	0.48**	0.55**	-	0.28^{ns}	0.41**	-

*= Significant at $P \leq 0.05$; ** = Significant at $P \leq 0.01$.

was not significant, data from both environments were pooled. Results showed large variability among the individual (S_0) plants and among the corresponding S_1 progenies for both micronutrients in all four populations (**Table 2**). The highest levels with wide range of variability both for Fe and Zn density in the S_0 plants and the S_1 progenies were found in 'ICTP 8203', and the lowest levels and the smallest range in 'JBV 3'. The higher levels of Fe and Zn in 'ICTP 8203' compared to those in 'JBV 3' is not unexpected since 'ICTP 8203' is derived from an *iniari* genetic background which is generally found to have higher levels of both micronutrients than the non-*iniari* genetic background which 'JBV 3' represents (Velu *et al.* 2007). In both populations, the Fe and Zn densities in the S_1 progenies were generally 6-14 mg kg^{-1} higher than in the S_0 plants. In the other two populations ('AIMP 92901' and 'ICMR 312'), the Fe and Zn densities in the S_1 progenies were generally 5-11 mg kg^{-1} less than in the S_0 plants. These S_0 vs S_1 differences could be partly due to environmental effects since the grain samples from the S_0 plants came from environments that were different from those in which S_1 progenies were evaluated and grains were produced for micronutrient analysis. For instance, during the 2009 rainy season and 2010 summer season when the S_1 progeny derived from 'AIMP 92901' and 'ICMR 312' were tested, the extractable soil Fe content in the top 30 cm layers were 5.8 and 6.1 mg kg^{-1} and Zn contents were 2.3 and 4.3 mg kg^{-1}, while during the 2009 summer season (S_0 test environment) the soil Fe content was 15.4 mg kg^{-1} and soil Zn content was 5.2 mg kg^{-1}.

The correlation coefficients between the performance of S_0 plants and the mean performance of their S_1 progenies were positive and highly significant for both micronutrients in all four populations with the correlation coefficient (r) for Fe ranging from 0.58 to 0.75 and for Zn density ranging from 0.61 to 0.73 (**Table 3**). The correlation coefficients between the performance of the S_1 progenies in the two environments were also positive and highly significant for both micronutrients (r ranging from 0.54 to 0.87 in three populations and 0.36 in one populations for Fe, and r ranging from 0.61 to 0.74 in three populations and 0.33 in one populations), which is not unexpected considering the absence of progeny × environment interactions, or relatively lesser contribution of this interaction to the total variability in comparison to that due to differences among the progenies. These results showed that the S_1 progeny performance for these micronutrients can be as well predicted from the S_0 plants from which these progenies were derived

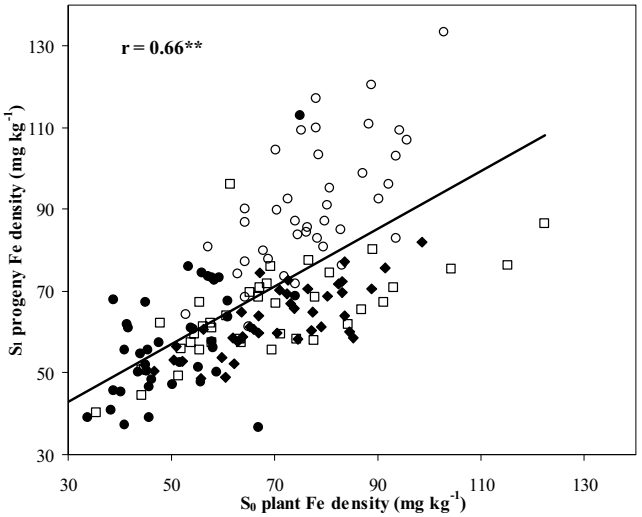

Fig. 1 Relationship between S_0 plants and S_1 progenies for grain Fe density in four pearl millet populations. ICTP 8203 (open circle); JBV 3 (closed circle); AIMP 92901 (open square); ICMR 312 (closed diamond).

as it can be done from the performance of the same progenies grown in another environment. The correlation between the S_0 plants and the S_1 progenies were of similar order (r = 0.66 for Fe and 0.58 for Zn) when all four populations were considered together as they were for the individual populations. However, with the larger spread of the values, as depicted for Fe density (**Fig. 1**), the low Fe lines could easily be discarded using S_0 plant performance.

Progeny evaluation in breeding for Fe and Zn density, as for any other trait, is generally conducted in unreplicated nurseries. Thus, we examined the effectiveness of unreplicated S_1 progeny selection with that of the S_0 plant selection by comparing the correlation of the S_1 progeny performance between two replicates with correlations of the S_0 performance with either of the two replicates (**Table 3**). It was observed that in all cases the correlation coefficients were positive and highly significant for both micronutrients in all four populations, and there was no systematic pattern to indicate that the S_0 plant selection was any less effective than the S_1 progeny selection. For instance, in 8 cases represented by 2-seaon S_1 trials of four populations, top ranking 20 S_0 plants (i.e., 50% of the plants used in this study) for high Fe density corresponded to 70-90% of the top ran-

Table 4 Correlation among grain Fe and Zn density (mg kg^{-1}) and grain mass (GMS) in four diverse populations of pearl millet, Patancheru.

	Correlation between	Correlation coefficient			
Materials		**ICTP 8203**	**JBV 3**	**AIMP 92901**	**ICMR 312**
S_0 plant	Fe and Zn	0.70**	0.77**	0.87**	0.63**
	Fe and GMS	0.19	-0.11	0.07	-0.17
	Zn and GMS	0.29	-0.02	-0.06	-0.24
S_1 progeny[1]	Fe and Zn	0.80**	0.82**	0.78**	0.43**
	Fe and GMS	0.19	-0.06	0.15	-0.04
	Zn and GMS	0.17	0.00	0.24	-0.13

[1]ICTP 8203 and JBV 3 S_1s were evaluated during the summer and rainy season of 2009; and AIMP 92901 and ICMR 312 S_1s were evaluated during the 2009 rainy season and 2010 summer season; S_1 represents the mean of two environments.
* = Significant at $P \leq 0.05$; ** = Significant at $P \leq 0.01$.

king 10 S_1 progenies (i.e., 25% of the top ranking S_1 progenies). Single plant selection, if carried at low population density, has been found effective even for the variability within the cultivars for phosphorus content in alfalfa (*Medicago sativa* L.; Miller *et al*. 1987) and protein content in wheat (*T. aestivum* L.; Tokatlidis *et al.* 2004). Single plant selection at low plant density has also been shown to be effective in soybean (*Glycine max* (L.) Merr.) both for oil and protein content (Fasoula and Boerma 2005). Effectiveness of low plant density on selection for Fe and Zn density in pearl millet remains to be evaluated.

In an applied breeding program, genetic improvement for Fe and Zn density would seek to combine these micronutrients with high grain yield potential and other agronomic traits such as early maturity and large grain size. An earlier study has shown that Fe and Zn density are not correlated with grain yield, grain size and time to flowering (Gupta *et al*. 2009). Progeny-based selection is more effective than single plant selection for grain yield, and it is routinely practiced in all crop breeding programs, including pearl millet. This implies that individual plant selection backed by S_1 selection will allow for two-stage selection for Fe and Zn density, which is likely to be more effective than the single-stage S_1 selection. However, exercising this approach would have a cost implication with respect to grain samples analysis for Fe and Zn density. An X-ray Fluorescence Spectrometer (XRF) analytical tool has now been standardized that runs 300 samples a day, does non-destructive analysis, and costs USD <1/samples compared to USD >17/sample for the ICP analysis at Adelaide. Very high and significantly positive correlation (r > 0.98) between the XRF and ICP values have been found both for Fe and Zn density in pearl millet (James Stangoulis, pers. comm.).

Based on the mean performance over the two seasons, there was positive and highly significant correlation between Fe and Zn density with the correlation coefficients ranging from 0.78 to 0.82 in three populations and 0.43 in one population (**Table 4**). This supports earlier results of pearl millet studies (Velu *et al*. 2007; Gupta *et al*. 2009). None of these two micronutrients were correlated with grain mass, which also supports the results of an earlier pearl millet study (Gupta *et al*. 2009). A similar pattern was found in the S_0 plants, with the correlation coefficients between Fe and Zn density ranging from 0.63 to 0.87 in all four populations, and none of the two micronutrients correlated with grain mass. These results indicate that simultaneous selection both for Fe and Zn density will be equally effective whether based on the individual plants or on the S_1 progeny performance and that these two micronutrients can be improved without compromising on the grain size.

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REFERENCES

Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011) Biofortification: A new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin* **32**, *Supplement* **1**, 31S-40S

Chakravarty I, Ghosh K (2000) Micronutrient malnutrition-present status and future remedies. *Journal of the Indian Medical Association* **98 (9)**, 539-542

Fasoula VA, Boerma HR (2005) Divergent selection at ultra-low plant density for seed protein and oil content within soybean cultivars. *Field Crops Research* **91**, 217-229

Gomez-Becerra H, Yazici A, Ozturk L, Budak H, Peleg Z, Morgounov A, Fahima T, Saranga Y, Cakmak I (2010) Genetic variation and environmental stability of grain mineral nutrient concentrations in *Triticum dicoccoides* under five environments. *Euphytica* **171**, 39-52

Gomez KA, Gomez AA (1984) *Statistical Procedures for Agricultural Research*, International Rice Research Institute Press, Philippines, pp 20-30

Gupta SK, Velu G, Rai KN, Sumalini K (2009) Association of grain iron and zinc content with grain yield and other traits in pearl millet (*Pennisetum glaucum* (L.) R. Br.). *Crop Improvement* **36 (2)**, 4-7

Jambunathan R, Subramanian V (1988) Grain quality and utilization in sorghum and pearl millet. In: de Wet JMJ, Preston TA (Eds) *Biotechnology in Tropical Crop Improvement*, ICRISAT, Patancheru, India, pp 133-139

Joshi AK, Crossa J, Arun B, Chand R, Trethowan R, Vargas M, Ortiz-Monasterio I (2010) Genotype × environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. *Field Crops Research* **116**, 268-277

Meenakshi JV, Johnson NL, Manyong VM, Degroote H, Javelosa J, Yanggen DR, Naher F, Gonzalez C, Garcia J, Meng E (2010) How cost-effective is bio-fortification in combating micronutrient malnutrition? An *exante* assessment. *World Development Report* **38**, 64-75

Miller D, Waissman N, Melton B, Currier C, McCaslin B (1987) Selection for increased phosphorus in alfalfa and effects on other characteristics. *Crop Science* **27**, 22-26

Morgounov A, Gomez-Becerra H, Abugalieva A, Dzhunusova M, Yessimbekova M, Muminjanov H, Zelensky Y Ozturk L, Cakmak I (2007) Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica* **155**, 193-203

Parthasarathy Rao P, Birthal PS, Reddy BVS, Rai KN, Ramesh S (2006) Diagnostics of sorghum and pearl millet grains-based nutrition in India. *International Sorghum and Millets Newsletter* **47**, 93-96

Rai KN, Anand Kumar K, Andrews DJ, Rao AS, Raj AGB, Witcombe JR (1990) Registration of ICTP 8203 pearl millet. *Crop Science* **30**, 959

Tokatlidis IS, Tsialtas JT, Xynias IN, Tamoutsidis E, Irakli M (2004) Variation within a bread wheat cultivar for grain yield, protein content, carbon isotope discrimination and ash content. *Field Crops Research* **86**, 33-42

Velu G, Bhattacharjee R, Rai KN, Sahrawat KL, Longvah T (2008) A simple and rapid screening method for grain zinc content in pearl millet. *Journal of SAT Agricultural Research* **6**, 1-4 http://ejournal.icrisat.org/Volume6/Sorgum_Millet/KN_Rai_A%20simple.pdf

Velu G, Kulkarni VN, Rai KN, Muralidharan V, Longvah T, Sahrawat KL, Raveendran TS (2006) A rapid screening method for grain iron content in pearl millet. *International Sorghum and Millets Newsletter* **47**, 158-161

Velu G, Rai KN, Muralidharan V, Kulkarni VN, Longvah T, Raveendran TS (2007) Prospects of breeding biofortified pearl millet with high grain iron and zinc contents. *Plant Breeding* **126 (2)**, 182-185

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**, 353-364

WHO (2002) Reducing risks and promoting healthy life. World Health Organization, Geneva, Switzerland. *The World Health Report*, pp 61-105

Zhang Y, Song Q, Yan J, Tang J, Zhao R, Zhang Y, He Z, Zou C, Ortiz-Monasterio I (2010) Mineral element concentrations in grains of Chinese wheat cultivars. *Euphytica* **174**, 303-313