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Prospects of breeding biofortified pearl millet with high grain iron and zinc content

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Abstract

Development of crop cultivars with elevated levels of micronutrients is being increasingly recognized as one of the approaches to provide sustainable solutions to various health problems associated with micronutrient malnutrition, especially in developing countries. To assess the prospects of this approach in pearl millet (*Pennisetum glaucum*), a diverse range of genetic materials, consisting of 40 hybrid parents, 30 each of population progenies and improved populations, and 20 germplasm accessions, was analysed for grain iron (Fe) and zinc (Zn) content, deficiencies of which adversely affect human health. Based on the mean performance in two seasons at ICRISAT, Patancheru, India, large variability among the entries was found, both for Fe (30.1–75.7 mg/kg on dry weight basis) and Zn (24.5–64.8 mg/kg). The highest levels of grain Fe and Zn were observed in well-adapted commercial varieties and their progenies, and in the parental lines of hybrids, which were either entirely based on *iniari* germplasm, or had large components of it in their parentage. There were indications of large within-population genetic variability for both Fe and Zn. The correlation between Fe and Zn content was positive and highly significant ($r = 0.84$; $P < 0.01$). These results indicate that there are good prospects of simultaneous selection for both micronutrients, and that selection within populations, especially those with the predominantly *iniari* germplasm, is likely to provide good opportunities for developing pearl millet varieties and hybrid parents with significantly improved grain Fe and Zn content in pearl millet.

Key words: *Pennisetum glaucum* — grain iron and zinc — genetic resources — correlation — biofortification

Micronutrient malnutrition, resulting from the dietary deficiency of some of the important minerals such as iron (Fe) and zinc (Zn), and vitamin A, has been reported to be a widespread food-related health problem, affecting more than two billion people worldwide (WHO 2002). This problem is particularly serious in developing countries where most of the population has limited access to meat, fruits and vegetables, which are generally rich sources of these micronutrients. The HarvestPlus initiative of the Consultative Group on International Agricultural Research (CGIAR) has recently embarked on addressing this issue through the development of improved crop cultivars with elevated levels of these micronutrients. Pearl millet [*Pennisetum glaucum* (L.) R. Br.], grown on more than 26 million ha in the arid and semi-arid regions of Asia and Africa, and serving as a major source of dietary energy for a vast population in these regions, is one of the crops included in this initiative. A pearl millet breeding line with yellow grain colour derived from a germplasm accession originating from Burkina Faso, with β-carotene (precursor of vitamin A) levels as high as 137 µg/100 g has been identified at ICRISAT, Patancheru, India (Hash et al. 1996). Attempts are underway to identify molecular markers of the gene(s) associated with this trait and use molecular marker technology to transfer it into commercial open-pollinated varieties and hybrid parents. Pearl millet has high grain Fe and Zn content. There are reports of up to 580 mg/kg Fe and 70 mg/kg Zn in pearl millet germplasm accessions (Jambunathan and Subramanian 1988), but neither the identity of these accessions is available, nor have the micronutrient analyses protocols been reported. The initial objective of the HarvestPlus project on pearl millet is to initiate an intensive search to identify sources of higher grain Fe and Zn content in this crop. This research reports on the extent of variability for Fe and Zn content in grains, the consistency of their expression across environments, and the relationship between Fe and Zn in a diverse range of materials, to assess the prospects of their genetic enhancement in cultivar development.

Materials and Methods

The experimental material consisted of 120 entries of pearl millet, which included 30 each of improved populations and population progenies (partial inbreds), and 20 each of germplasm accessions, seed parents and pollinators. This was inclusive of the three controls, consisting of the first ICRISAT-bred and the most widely cultivated open-pollinated variety 'WC-C75', the seed parent (81B) and the pollen parent (ICMP 451) of the first ICRISAT-bred and the most widely cultivated hybrid 'ICMH 451'. The trial was laid out in a randomized complete block design with two replications during the 2004 summer and rainy seasons (hereafter referred to as environments) at ICRISAT, Patancheru, India. Each entry was grown in four rows, 4 m long with 75 cm spacing between the rows and 15 cm spacing between plants within the rows. The trials were conducted in Alfisols with applied fertilizer levels of 75 kg/ha N (50% basal and 50% top dressing) and 35 kg/ha P during both the summer and the rainy season. The trials were irrigated eight times during the summer season and five times during the rainy season to ensure there was no moisture stress. The micronutrient levels of soil measured in six samples (three each at 0–15 cm and 15–30 cm depth) at the time of planting varied from 6.2 ± 0.45 mg/kg Fe and 1.6 ± 0.52 mg/kg Zn for the field used during the summer season to 15.8 ± 3.11 mg/kg Fe and 2.2 ± 0.63 mg/kg Zn for the field used during the rainy season.

Sib-mated grains were produced for laboratory analysis of Fe and Zn content. Plants were selfed with parchment paper bags at the initiation of panicle emergence. Bulk pollen collected from 50–60 plants in a plot was used to cross 20 plants of the same plot. The sib-mated panicles were harvested at physiological maturity, machine-threshed (Wintersteiger – ID780ST4 – Single head thresher, Ried, Austria), and the grains cleaned of any glumes before being transferred to paper envelopes with metal seals. Precautions were taken in each step to avoid any contamination of grains with dust particles and any other extraneous matter. The grain samples from the trials were analysed using an atomic absorption spectrophotometry (Thermo Electron Corporation, Cambridge, UK) fitted with a GFS97 auto-sampler, a system equivalent to the Inductively Coupled Plasma Atomic Absorption Spectrophotometry at the National Institute of Nutrition, Hyderabad, India. Pearl millet samples were powdered to pass through a 10-mesh sieve using a cyclone sample mill (Udy Corporation, Fort Collins, CO, USA). Aliquots of the powdered sample were taken as two subsamples from each entry and ashed. Dry ashing and mineral solution preparation were carried out according to the method described by Jorhem (1993). The analytical method used was validated using NIST standard certified reference material (1584A). Every day an in-house quality control sample was ashed and quantified, together with a blank, to expose any systematic errors. For blanks, no major interference was found. Discrepancies between in-house quality control samples and concentrations quantified were below 5% and the coefficient of variation for single measurements was below 10%. Grain Fe and Zn content data (expressed in mg/kg on a dry weight basis) were analysed for individual environments, as well as across the two environments following a fixed model analysis of variance (Gomez and Gomez 1984).

Results

The average grain Fe content in the trial was comparable to the average Zn content both in the summer season (41.5 mg/kg Fe and 40.1 mg/kg Zn) and rainy season (49.5 mg/kg Fe and 47.7 mg/kg Zn) (Table 1). It was also observed that the average grain Fe and Zn contents in the rainy season were 19–20 % higher than those in the summer season. This may be due to the high Fe and Zn levels in the soil, as the field used during the rainy season had 155% more Fe and 38% more Zn than the field used during the summer season. Analysis of variance (data not presented) showed highly significant differences among the entries for grain Fe and Zn content in both seasons ($P < 0.001$). There were also significant entry × environment interactions for both micronutrients, but the correlation of micronutrient content of genotypes between the two seasons was highly significant, both for Fe ($r = 0.66$; $P < 0.01$) and Zn ($r = 0.69$; $P < 0.01$), indicating high levels of consistency of the rankings of entries across the two seasons for both Fe and Zn content.

Based on the mean performance across the two environments, a large variability among the entries was observed for both Fe (30.1–75.7 mg/kg) and Zn content (24.5–64.8 mg/kg) (Table 1). A majority of the entries had grain Fe content in the range of 35–55 mg/kg and Zn content in the range of 35–50 mg/kg (Fig. 1). Although the differences among the three controls (81B, 'ICMP 451' and 'WC-C75') were not statistically significant, the open-pollinated variety 'WC-C75' had the highest levels of both Fe (41.9 mg/kg) and Zn (39.5 mg/kg) content. Twenty-one entries had 28–81% higher grain Fe than

Table 1: Selected pearl millet inbred lines and populations with high grain Fe and Zn content, 2004 summer and rainy seasons, ICRISAT, Patancheru

	Fe content (mg/kg)			Zn content (mg/kg)		
Inbred line/population	Summer	Rainy	Mean	Summer	Rainy	Mean
Hybrid parent (n = 40)						
863 B	69.0	76.5	72.7	54.0	57.7	55.8
ICMB 94111	51.7	75.4	63.6	43.7	69.9	56.8
ICMB 98222	51.3	74.6	62.9	46.6	59.1	52.9
ICMB 00888	56.7	64.1	60.4	49.3	64.1	56.7
ICMB 88004	48.9	71.8	60.4	43.6	62.8	53.2
ICMB 00999	44.3	72.7	58.5	49.5	68.9	59.2
843 B	46.0	70.9	58.4	47.4	71.9	59.6
ICMB 94555	47.5	59.9	53.7	44.0	57.1	50.5
MIR 97171	50.9	60.4	55.7	42.8	50.7	46.7
Population progenies (n = 30)						
AIMP 92901 S1-15-1-2-B	74.7	76.6	75.7	61.6	68.0	64.8
AIMP 92901 S1-183-2-2-B	49.2	102.0	75.6	46.5	80.8	63.7
SDMV 90031-S1-84-1-1-2-B	53.2	84.8	69.0	50.8	45.4	48.1
RCB-2 S1-33-1-3-3-2-B	45.7	65.7	55.7	41.1	57.1	49.1
ICMR 312 S1-25-1-1-1-3-B	47.8	62.1	55.0	43.8	52.8	48.3
AIMP 92901 S1-421-2-3-B	46.0	63.1	54.6	45.6	56.8	51.2
ICMS 7704-S1-51-4-1-1-B	45.1	62.9	54.0	45.6	69.8	57.7
Improved population (n = 30)						
GB 8735	52.3	73.1	62.7	45.2	61.0	53.1
EEBC	51.0	64.1	57.5	45.5	59.2	52.4
ICMV 221	47.5	67.0	57.3	43.4	56.7	50.1
HHVDBC	48.7	61.2	55.0	43.2	51.9	47.6
Germplasm accession (n = 20)						
IP 8964	47.7	61.1	54.4	43.0	54.7	48.8
Control						
81 B	32.4	36.4	34.4	31.3	32.2	31.7
ICMP 451	39.6	38.4	39.0	36.0	34.9	35.4
WC-C 75	42.0	41.9	41.9	38.2	40.9	39.5
Trial (n = 120)						
Minimum	29.3	29.4	30.1	25.0	24.0	24.5
Maximum	74.7	102.0	75.7	61.6	80.8	64.8
Mean	41.5	49.5	45.5	40.1	47.7	43.9
LSD (P = 0.05)	10.5	16.8		6.4	11.1	
CV (%)	12.8	17.2		8.0	11.8	

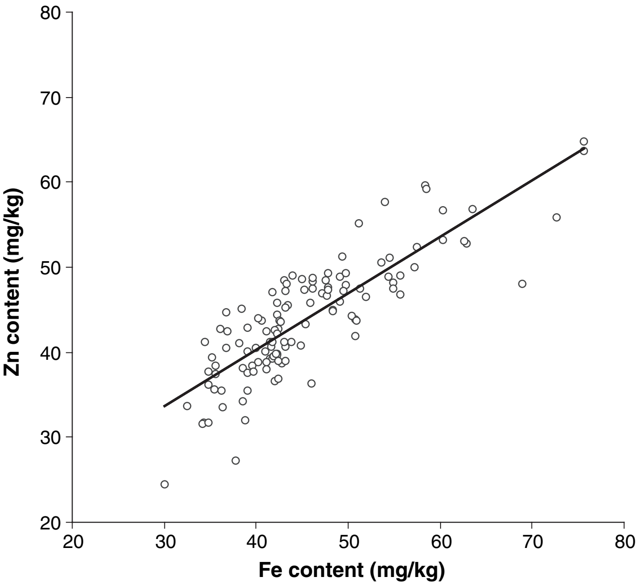

Fig. 1: Relationship between grain Fe and Zn content in pearl millet

that of 'WC-C75' (Table 1). Of these, 15 entries also had 25–62% higher Zn content than that of 'WC-C75'. Two S_4 progenies derived from an open-pollinated variety ('AIMP 92901') had the highest levels of both Fe (about 76 mg/kg) and Zn (about 65 mg/kg), which exceeded the grain Fe and Zn of 'AIMP 92901' (50.8 mg/kg Fe and 42.0 mg/kg Zn) by 46% and 52%, respectively. 'AIMP 92901' is a high-yielding open-pollinated variety, collaboratively developed by ICRISAT and the National Agricultural Research Project Centre of Marathwada Agricultural University, Aurangabad, Maharashtra, India. 'AIMP 92901' was released in 1998 as 'Samrudhi' for cultivation in pearl millet zone B (central and southern states) of India. This variety was developed from a Bold-seeded Early Composite that was constituted from open-pollinated varieties and several other progenies produced from the *iniari* germplasm. The *iniari* landrace has been observed as a promising germplasm with several positive attributes such as early maturity, large seed size and compact panicles (Andrews and Anand Kumar 1996). An S_5 progeny derived from another variety 'SDMV 90031' also had a high Fe content (69.0 mg/kg), which was 44% more than that in the original population (Fe 47.8 mg/kg). 'SDMV 90031' was developed largely based on the *iniari* germplasm by ICRISAT in partnership with the national research programmes in the southern African region. Evaluation of just two or three progenies from each of these two populations revealed large intra-population variability, with an Fe content in the range of 46–76 mg/kg in AIMP 92901 progenies and 43–69 mg/kg in the SDMV 90031 progenies, while the Zn content was in the range of 51–65 mg/kg in the AIMP 92901 progenies and 32–48 mg/kg in the SDMV 90031 progenies. This showed that evaluation of a larger number of progenies from these varieties might lead to identification of progenies with still higher grain Fe and Zn content.

Among the inbred lines, produced as parental lines of hybrids, the maintainer line 863B had the highest level of Fe (72.7 mg/kg), and it was amongst the top five hybrid parents for high Zn content (55.8 mg/kg) as well. It is interesting that, except for MIR 97171, all the other hybrid parents with high Fe and Zn content were either directly selected from *iniari* germplasm such as 863B, ICMB 98222 and ICMB 88004, or involved a large proportion of *iniari* germplasm in their parentage, such as ICMB 94111, ICMB 00888, ICMB 00999, 843B and ICMB 94555.

Among the open-pollinated varieties, 'GB 8735', also developed largely from the *iniari* germplasm, had the highest grain Fe (62.7 mg/kg) and Zn (53.1 mg/kg) content. This early-maturing and large-seeded variety, developed by ICRISAT in partnerships with the national programmes in the western and central African region, has been released in Chad, Mauritania, Nigeria and Benin. Amongst the other populations with high Fe and Zn level, they were either derived entirely from the *iniari* germplasm, such as EEBC and ICMV 221 (=ICMV 88904), or had a substantial proportion of the *iniari* germplasm in their parentage such as HHVDBC. ICMV 221, having performed better than the popular commercial variety ICTP 8203 (15% higher grain yield than ICTP 8203), was first released in India and subsequently in Kenya and Eritrea in eastern Africa. EEBC is a photoperiod-insensitive, extra-early maturing population (matures in < 65 days) with large seed size (13–14 g/1000) and a high level of resistance to downy mildew. High Head Volume Dwarf B-Composite (HHVDBC) is a dwarf population (110 cm plant height) of mid-late maturity (90 days to mature), large seed size (13.5 g/1000) and thick panicles (40 mm diameter). Both EEBC and HHVDBC have been extensively used at ICRISAT for breeding seed parents.

Discussion

These results showed that the high levels of grain Fe and Zn content in pearl millet are almost twice those in sorghum (Reddy et al. 2005) and 50% more than those in maize (Gregorio 2002). Furthermore, these entries with high Fe and Zn content are some of the released and widely-cultivated varieties and hybrid parents of popular high-yielding hybrids, which can now be used for bioavailability evaluation.

There was a highly significant and positive correlation between the Fe and Zn content in both the summer season ($r = 0.78$; $P < 0.01$) and the rainy season ($r = 0.82$; $P < 0.01$). Based on the mean performance across the two seasons, this correlation was still higher ($r = 0.84$; $P < 0.01$) (Fig. 1), indicating that simultaneous genetic improvement for the elevated levels of both micronutrients should be highly effective. Highly significant and positive correlation between grain Fe and Zn content has also been observed in maize (Maziya-Dixon et al. 2000), wheat (Graham et al. 1999) and sorghum (Reddy et al. 2005). Highly significant positive correlations of 1000-grain weight with Fe ($r = 0.80$; $P < 0.01$) and Zn ($r = 0.85$; $P < 0.01$) content per grain indicated that breeding for the higher levels of these micronutrients could be achieved without compromising the large grain size, which is a preferred grain trait in several African countries (Chintu et al. 1994, Ipinge et al. 1994) and in central and southern parts of India (especially in Maharashtra).

Thus, the results of this study indicate that prospects of genetic enhancement for increased levels of both Fe and Zn content in pearl millet are high, and that intensive exploitation of *iniari* germplasm is likely to lead to the identification of lines with still higher Fe and Zn content in relatively elite agronomic backgrounds. This may enable rapid development of high-yielding open-pollinated varieties and hybrid parents with high grain Fe and Zn content. Much higher grain Fe and Zn levels have been reported in some of the earlier studies (Jambunathan and Subramanian 1988, Abdalla et al. 1998, Malik 1999). The higher grain Fe and Zn densities reported in these studies could be due either to environmental influence or to different germplasm, indicating the need to screen even non-*iniari* germplasm for identifying additional sources of high grain Fe and Zn content.

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