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VARIABILITY FOR GRAIN IRON AND ZINC CONTENT IN A DIVERSE RANGE OF PEARL MILLET POPULATIONS

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ABSRACT

Micronutrient malnutrition, resulting from dietary deficiency of iron (Fe) and zinc (Zn), is a widespread food-related health problem, especially in developing countries. Diversified food and crop cultivars with elevated levels of these micronutrients provide the most cost-effective solution to this problem. In an effort to address this problem through genetic improvement, field trials of pearl millet (*Pennisetum glaucum* (L) R. Br.) were conducted at Patancheru during 2004-2006 to examine the magnitude of variability for these micronutrients. Mean Fe content among 68 diverse populations ranged from 42.0 to 79.9 mg/kg and Zn content ranged from 42.0 to 79.9 mg/kg and Zn content ranged from 27.2 to 50.2 mg/kg. Intra-population variability in four improved populations was also studied. Over two-fold range among the progenies of four *iniadi* based populations was found for Fe (51-150 mg/kg) as well as Zn contents (39-107 mg/kg) with some of the progenies having > 120 mg/kg Fe and > 80 mg/kg Zn content. The correlation co-efficient between Fe and Zn content was positive and highly significant (r=0.66 to 0.85; P<0.01), indicating the likely effectiveness of simultaneous improvement for both micronutrients.

Index words: Pearl millet, *Pennisetum glaucum,* Grain iron and zinc content, Correlation, Plant breeding.

Micronutrient malnutrition has been recognized as a massive health problem, afflicting over 2 billion people, mostly women, children and infants worldwide (WHO 2002). Agricultural approaches of diversified crop-based food products and biofortified crop cultivars have been suggested as the most cost-effective and sustainable approaches to address this problem, particularly at the levels of rural households in the developing world (Bouis 2000). Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a major warm-season cereal grown on 26 million ha in some of the most marginal arid and semi-arid tropical environments of Asia and Africa. It is a major source of dietary energy and nutritional security for a vast population in these areas. A recent study showed that pearl millet accounts for 50% of the cereal consumption in some of the pearl millet growing areas of India, and it is the cheapest source of grain iron (Fe) and (Zn) as compared to other cereals and vegetables (Parthasarathy Rao *et al.* 2006).

A recent pearl millet study involving inbred lines, improved open-pollinated varieties and germplasm detected large variability for grain Fe and Zn contents among the test entries (Velu *et. al.* 2007). It also showed large variability among a small sample of progenies from a few populations. The objective of the present study involving a series of experiments was to examine the magnitude of variability for grain Fe and Zn content among a larger set of populations of diverse origin and also intra-population variability using a larger set of progenies derived from four improved populations that had been earlier identified having relatively higher levels of Fe and Zn.

MATERIAL AND METHODS

Two experiments were conducted, each with different types of materials. Experiment 1, designed to study the magnitude of variability among populations, consisted of 68 improved populations, both composites and open-pollinated varieties (OPVs), developed by ICRISAT and/or its partners in the National Agricultural Research System (NARS) in India (Asia region), and the Africa regions. It included WC-C75 (the first ICRISAT-bred widely cultivated OPV in India) as a control. Experiment 2 consisted of 50 S_1 progenies from a popular OPV (ICTP 8203) released in India and 47 S_1 progenies each from three improved populations (CGP, GGP and PVGGP 6) developed by ICRISAT for the Western and Central Africa. These four populations had been identified having high Fe and Zn levels in experiment 1 of present study.

Both experiments were laid out in a randomized complete block design (RCBD) with two replications at ICRISAT, Patancheru, India (17° N; 78° E). The trials were conducted in Alfisols with the applied fertilizer levels of 75 kg/ha N (50% basal in the form of urea) and 35 kg/ha P during both the rainy and summer seasons. The trials were irrigated at the interval of 7-9 days in the summer season and as needed in the rainy season, to ensure no moisture stress.

In experiement 1, each population was grown in 2 rows of 4 m length at 75 cm spacing between the rows in 2004 rainy season, and 60 cm spacing in 2005 summer season, with 15 cm spacing between plants within the row during both the seasons (hereafter referred to as environments). Six soil samples from each of the top layer (0-15 cm depth) and the subsurface layer (15-30 cm depth) were taken at the time of planting. The mean soil Fe and Zn content extracted by diethylene triamine pentaacetic acid (DTPA) varied from 15.8 mg/kg Fe and 2.2 mg/kg Zn for the field in which the experiment was conducted during the 2005 summer season (February to May). The sib-mated panicles were harvested at physiological maturity, machine threshed (Wintersteiger - ID780ST4 - Single head thresher, Ried, Austria) and the grains produced for laboratory analysis of Fe and Zn contents. Crop maturity and grain size are the two important traits for which strong farmers preference have been found. Thus, to examine if the Fe and Zn contents were associated with these traits, data were recorded for days to 50% flower on plot basis (i.e. 50% of the plants having fully exserted stigmas on the panicles) and 1000-gain weight (based on a sample of 200 random grains) for each plot.

In experiment 2, progenies derived from all four populations were grown during the summer and rainy seasons of 2006 in single row plots of 4 m length at 75 cm spacing between the rows during the rainy season and 60 cm spacing during the summer season (hereafter referred to as environments), with 15 cm spacing between plants in both the seasons. The micronutrient levels (DTPA-extractable) in the soil samples collected the same way as for experiment 1, varied from 5.8 mg/kg Fe and 1.5 mg/kg Zn in the field in which experiment was conducted during the summer season; and 10.5 mg/kg Fe and 2.9 mg/kg Zn in the field in which the experiment was conducted during the rainy season. Selfed seeds were produced for laboratory analysis of Fe and Zn contents. Data on days to 50% flower and 1000-grain weight were recorded the same way as for experiement 1.

In experiement 1, the grain samples were analyzed using an Atomic Absorption Spectrophotometry (Thermo Electron Corporation, Cambridge, UK) fitted with a GFS97 autosampler, a system equivalent to the Inductively Coupled Plasma Atomic Absorption Spectrophotometry (Jorhem 1993) at the National Institute of Nutrition (NIN), Hyderabad, India. In Experiment 2, the grain samples were analyzed for Fe and Zn in ICRISAT laboratory using the method described by Sahrawat *et al* (2002).

Grain Fe and Zn content, days to 50% flower and 1000-grain weight data were analyzed for individual environments as well as across the two environments following a fixed model ANOVA of randomized complete block design (Gomez and Gomez 1984) using Gen Stat 8th version computer program.

RESULTS AND DISCUSSION

The differences among the populations both for grain Fe and Zn content were highly significant ($P<0.01$) (Table 1). The population x environment interaction was significant only for Zn, and its contribution to the mean square was 50% of that contributed by difference among the populations. Based on the mean performance across the two environments, large variability among the populations was observed both for Fe and Zn contents. The largest proportion of population had 30-40mg/kg Zn and 45-55 mg/kg Fe content. The Fe content among the populations ranged from 42.0 to 79.9 mg/kg and Zn content ranged from 27.2 to 50.2 mg/kg (Table 2). Eleven populations (one-sixth of the total populations in the trial) had more than 65 mg/kg Fe (well over trial the mean + SE_d), which was also more than the level observed in control WC-C-75 (57.6 mg/kg). Seven of these populations (i.e. 64%) also had more than 45 mg/kg Zn, well over the trial mean + SE_d and for higher than the level observed in WC-C-75 (38.8 mg/kg). The group of populations with high Fe content having proportionately higher percentage of those with high Zn content as well was not unexpected as there was a highly significant and positive correlation between Fe and Zn content (r=0.84; P<0.01). Similar high correlation between Fe and Zn has been found in an earlier pearl millet study involving a wide range of populations and inbred lines (Velu *et al.* 2007). Significant positive correlations between Fe and Zn have been found in an earlier pearl millet study involving a wide range of populations and inbred lines (Velu *et al.* 2007). Significant positive correlations between Fe and Zn have also been reported in wheat, maize, lentil, potato and yam (Pfeiffer and McClafferty 2007). Thus, it would appear that while selecting for high Fe content, there would be a correlated selection response for high Zn content, and perhaps the vice-versa.

Table 1: Analysis of variance for grain Fe and Zn content (mg/kg), days to 50% flower (DF) and 1000-grain weight (GW) (g) in experiment 1 (population trial) and experiment 2 (progeny trials) in pearl millet

Source of Variation	df	Mean square			
		Fe	Zn	DF	GW
		Populations			
Environment	1	214	585	524	6.4
Rep/Environment	2	1336	300	13	3.8
Population	67	264**	87**	70**	9.6**
PopulationxEnvironment	67	93	44*	36*	15.5**
Error	134	97	32	12	0.3
		ICTP 8203 S_1 progenies			
Environment	1	1087	112	3312	12.3
Rep/Environment	2	545	168	218	5.9
Progeny	49	1253**	429**	369**	18.6**
ProgenyxEnvironment	49	239**	160**	115**	20.2**
Error	98	75	41	36	1.2
		CGP S_1 progenies			
Environment	1	1259	1840	1154	9.9
Rep/Environment	2	649	973	119	6.3
Progeny	46	1016**	223**	215**	15.2**
ProgenyxEnvironment	46	289**	86**	99**	5.2
Error	92	74	45	28	0.6
		GGP S_1 progenies			
Environment	1	759	325	985	22.5
Rep/Environment	2	402	200	411	11.2
Progeny	46	1615**	283**	333**	19.6**
ProgenyxEnvironment	46	323*	95*	98**	6.9
Error	92	105	59	25	1.0
		PVGGP 6 S_1 progenies			
Environment	1	1772	3896	2958	11.0
Rep/Environment	2	888	2123	425	6.9
Progeny	46	1053**	324**	522**	16.2**
ProgenyxEnvironment	46	257**	135**	89**	10.5
Error	92	46	39	28	0.6

*,** Significant at 5% and 1% probability levels, respectively

Fourteen populations that had Fe and Zn levels higher than the trial mean + SEd are given in Table 2. Except for three populations (HiGrop, Lubasi and IAC-ISC-TCP 4), all the remaining populations having high Fe and Zn are based entirely or largely on iniadi germplasm. ICTP 8203 a commercial open-pollinated variety with highest Fe content (79.9 mg/kg) and ranking among the top five for high Zn level (47.1 mg/kg), was developed from direct selection within an *iniadi* landrace originating from northern Togo in Western Africa (Rai *et al.* 1990). Released in India in 1988, this variety has been under cultivation since 1989, occupying 0.6-0.7 million ha at the peak of its adoption in 1995 in Maharashtra state alone (Bantilan *et al.* 1998). This variety is still cultivated on about 0.3 million ha (Rosana Mula, ICRISAT, Unpub). GGP (C_0) an *iniadi*-based composite, ranked close second to ICTP 8203 for Fe content (78.5 mg/kg) and had the highest level of Zn content (50.2 mg/kg) among all the populations in the trial.

Large grain size (> 10 g/1000 seed) is a farmer-preferred trait in pearl millet in parts of India and Africa. Higher micronutrient levels have frequently been observed in wild relatives and landraces than in improved cultivars of wheat (Cakmak *et al.* 2000) and rice (Zeng *et al.* 2004), implying the possible negative relationship of Fe and Zn with grain size in these two cereals. Early maturity is another farmer-preferred trait that enables the crop to escape terminal drought in short season environments and permits double cropping. But shorter crop duration may also lead to reduced total micronutrient uptake from the soil, and consequently might affect their levels in the grains. Thus, this study examined the relationship of Fe and Zn with these two farmer-preferred traits. The difference among the populations was highly significant for both traits (Table 1), with the 1000 - grain weight ranging from 7.1 to 13.6 g and days to 50% flower ranging from 41 to 57 days (Table 2). Except for HiGrop, all populations selected for high Fe content had > 10 g of 1000-grain weight. This was not unexpected considering a highly significant positive correlation between grain weight and Fe content (r= 0.56; P< 0.01) (Table 3). Since there was a high positive correlation between Fe and Zn, the correlation between Zn and grain weight was also positive and highly significant (r=0.46; P<0.01). Seven of the 14 selected populations were early-maturing (<45 days to flower) and none of these took more than 51 days to flower. This was again not unexpected as there was a negative, though non-significant, correlation between Fe and days to flower (r= -0.29) and Zn and days to flower (r= -0.28). Interestingly, the two populations ICTP 8203 and GGP (C_0) having the highest levels of Fe and Zn were among those that were earliest to flower (42-43 days). These also had the largest seed size (13.1 and 13.6 g/1000) among all the populations in the trial.

Intra-population variability : An earlier pearl millet study found that a progeny from AIMP 92901 a commercial open-pollinated variety had the highest Fe level among its few progenies that had been tested (Velu 2007; Velu *et al.* 2007), indicating that pearl millet populations, highly heterozygous and heterogeneous as they are, could have large exploitable variability for these micronutrients. This hypothesis was tested using S1 progenies derived from four other populations (ICTP 8203, CGP, GGP and PVGGP 6) that had been found in experiment 1 having high levels of Fe and Zn contents. Results showed that the differences among the prognies for Fe and Zn were highly significant in all the four populations (P<0.01) (Table 1). The progeny x environment interaction was also highly significant for both micronutrients in all four populations, but its contribution to the mean square was about one-third of that contributed by the differences among the population progenies. Averaged over the environments, over two-fold range among the progenies was observed for Fe and Zn in all the four populations (Table 4). In total, 59 progenies (i.e. 10%) had > 100 mg/kg Fe, and of these 23 progenies (i.e. 40%) had > 80 mg/kg Zn content. Highly significant and large differences among the progenies derived from all the four populations indicated that there was large genetic variation for Fe and Zn content within the populations, which could be exploited by effective selection.

Table 2: Grain Fe and Zn content, days to 50% flower and 1000-grain weight of pearl ™millet populations selected for Fe content above trial mean + SE_d. Data are mean of two environments (2004 rainy season and 2005 summer season)

Population	Fe (mg/kg)	Zn (Mg/kg)	Days to 50% flower	1000-grain weight (g)
ICTP 8203	79.9	47.1	43	13.3
GGP (C_0)	78.5	50.2	42	13.6
CGP	73.8	45.6	46	11.5
IAC-ISC-TCP 4	72.9	47.2	50	10.8
PVGGT 4	71.6	41.8	47	10.0
PVGGT 5	71.4	47.7	44	12.4
Ugandi	70.1	49.8	54	10.1
Hi Grop	66.9	44.3	51	9.7
Lubasi	66.6	44.7	51	11.4
GGT (C0)	65.3	39.4	43	13.1
LaGrap	65.2	45.8	50	10.6
PVGGP 1	64.1	42.9	43	10.5
PVGGP 6	63.2	45.7	44	11.5
SSC C1 Brist	62.8	40.4	43	10.5
Control				
WC-C75	57.6	38.8	46	9.4
Trial mean	55.0	38.0	46	9.7
Minimum	42.0	27.2	41	7.1
Maximum	79.9	50.2	57	13.6
SE_d	7.0	4.0	2.4	0.4

Fe deficiency has been reported to be a more serious problem than Zn deficiency (Graham *et al.* 2001). Thus, Fe would be the primary selection criterion, from the micronutrient perspective, in population improvement and inbred line development. However, there was a high degree of positive correlation between grain Fe and Zn among the progenies in all the four populations (r=0.66 and 0.85; P<0.01) (Table 3) suggesting good prospects of simultaneous genetic improvement for both the micronutrients.

The correlation of both Fe and Zn with time to flower was non-significant in all the four populations. The correlation of these micronutrients with grain weight was also non-significant in two populations, with only lower order of significance (P<0.05) observed in CGP and GGP (Table 3). It is because of this nature of character association that the mean grain weight and time to flower of the selected progenies were similar to the respective means of all the progenies in the trial in all populations. This would imply that selection for Fe

Table 3: Correlation co-efficient among Fe and Zn, time to 50% flower (DF) and 1000-grain weight (GW) in population trial (experiment 1) and population progeny trials (experiment 2). Data are mean of two environments.

Experiment	Trial	Correlation coefficient (r) between				
		Fe&Zn	Fe&GW	Fe&DF	Zn&GW	An&DF
1.	Population	0.84**	0.56**	-0.29	0.46**	-0.28
2.	ICTP 8203 progeny	0.85**	0.26	-0.14	0.22	0.17
	CGP progeny	0.66**	0.36*	-0.18	0.31*	-0.21
	GGP progeny	0.66**	0.34*	-0.08	0.28*	-0.11
	PVGGP 6 progeny	0.76**	0.25	-0.22	0.19	-0.31

*, ** Significat at 5% and 1% probability levels, respectively

Table 4: Frequency distribution of grain Fe and Zn content among progenies derived from different populations. Data are mean of two environments (2006 rainy and summer season)

Micronutrient	Population	Total progenies	No. of progenies in micronutrient class (mg/kg)								
			<50	51-60	61-70	71-80	81-90	91-100	101-110	111-120	>121
Fe	ICTP 8203[a]	50	0	3	6	11	13	7	4	4	2
	CGP[b]	47	0	0	1	4	11	14	10	4	3
	GGP[b]	47	0	0	0	5	11	10	8	3	10
	PVGGP 6[b]	47	0	2	4	10	14	7	5	5	1
Zn	ICTP 8203[a]	50	2	8	21	14	4	1	0	0	0
	CGP[b]	47	0	3	20	19	5	0	0	0	0
	GGP[b]	47	0	4	17	18	8	0	0	0	0
	PVGGP 6[b]	47	3	4	24	11	6	0	0	0	0

a: Released open-pollinated variety; b: Improved population. Data are means of 2006 summer and rainy seasons

and Zn would have no adverse effect on grain weight and maturity in pearl millet. The pattern of character association in the progenies of all the populations was, in general, similar to those in the populations (experiment 1), except for the association of Fe and Zn with grain size. The significant positive correlation of these micronutrients with grain size in experiment 1 could have resulted most likely from the fact that majority of the *iniadi*-based populations typically have large grain size and these also had high levels of Fe and Zn contents. On the other hand, non-*iniadi*-based populations generally have smaller grain size, which also had low levels of Fe and Zn contests.

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