RESEARCH

Combining Ability and Heterosis for Grain Iron and Zinc Densities in Pearl Millet

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ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important staple food crop in the semiarid tropical regions of Asia and Africa. As part of a major initiative to improve its grain Fe and Zn densities, two sets of line × tester studies were conducted. Results showed that the underlying physiological processes determining the grain Fe and Zn densities were largely under additive genetic control, and Fe and Zn densities of the inbred lines per se and their general combining ability (GCA) were positively and highly significantly correlated. This would imply that recurrent selection can be effectively used to improve the breeding populations for grain Fe and Zn densities and that breeding lines selected for high Fe and Zn densities per se are more likely to include those with high GCA for these micronutrients. Lack of better-parent heterosis indicated that to breed hybrids with high Fe and Zn densities would require high levels of these micronutrients in both parental lines. Highly significant and positive correlations between the Fe and Zn densities, between the GCA of Fe and Zn densities, and between the specific combining ability (SCA) of the Fe and Zn densities showed that simultaneous selection for both micronutrients is likely to be effective with respect to all these performance parameters. Consistency in the patterns of results across both sets of trials and across the environments for all the parameters implies that these results could be of wider application to the genetic improvement of Fe and Zn densities in pearl millet.

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Abbreviations: σ^2_{GCA}, variance attributed to general combining ability; σ^2_{SCA}, variance attributed to specific combining ability; AAS, atomic absorption spectrophotometer; B-line, maintainer line; BPH, better-parent heterosis; DALY, disability-adjusted life year; GCA, general combining ability; MPH, midparent heterosis; QTL, quantitative trait loci; R-line, restorer line; RCBD, randomized complete block design; SCA, specific combining ability.

Micronutrient malnutrition, the so-called hidden hunger, affects more than one-half of the world's population, especially women and preschool children in developing countries (UNSCN, 2004) and results in an enormous negative socioeconomic impact at the individual, community, and national levels (Darnton-Hill et al., 2005; Stein, 2010). Among the 26 major risk factors of the global burden of disease estimates, Fe deficiency ranks 9th and Zn deficiency ranks 11th (Ezzati et al., 2002). India loses about 4 million disability-adjusted life years (DALYs) annually because of Fe deficiency (Qaim et al., 2007), with another 2.8 million DALYs lost because of Zn deficiency (Stein et al., 2007). Several approaches, including supplementation, food fortification, dietary diversification, and biofortification, have been advocated to address this problem, which is challenging both in scale and intensity. Crop biofortification is of particular significance from the view point of its cost effectiveness and sustainability,

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considering that micronutrient malnutrition is especially a serious problem in developing countries. An ex ante research on a variety of staple crops biofortified with Fe, Zn, and pro-vitamin A in 12 countries in Africa, Asia, and Latin America showed that biofortification could be highly cost effective, especially in Asia and Africa (Meenakshi et al., 2010). Furthermore, crop biofortification offers a rural-based intervention that, by design, initially reaches those more remote populations that make up a majority of the malnourished and who can ill afford adopting other interventions and then it penetrates to urban populations as production surpluses are marketed (Bouis et al., 2011).

Pearl millet is a significant source of dietary energy for millions of people in India and sub-Saharan Africa. For instance, pearl millet accounts for 20 to 63% of the total cereal consumption in major pearl millet-growing states of India, such as Maharashtra, Gujarat, and Rajasthan (Parthasarathy Rao et al., 2006). In parts of these states, contribution of pearl millet to Fe and Zn intake is higher than other cereals (19–63% of the total Fe intake and 16–56% of the total Zn intake). In India, pearl millet is grown on 9.4 million ha, of which 50% of the area is under improved cultivars, mostly hybrids. Pearl millet cultivars have been found to have higher Fe and Zn densities than other major cereals, such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and sorghum [*Sorghum bicolor* (L.) Moench] (Dwivedi et al., 2012). Furthermore, large variability has been reported in pearl millet, with some of the lines having more than 100 mg kg^{-1} grain Fe density and more than 80 mg kg^{-1} Zn density, which indicates good prospects of their genetic enhancement (Rai et al., 2012). An understanding of the nature of inheritance and heterosis of Fe and Zn densities can significantly contribute to the breeding efficiency for these micronutrients. There are limited studies on genetics of grain Fe and Zn densities and on exploitation of heterosis for Fe and Zn densities in pearl millet. One study reported predominantly additive genetic variance with no better-parent heterosis (Velu et al., 2011b) while another study reported predominance of nonadditive genetic variance, with 20 to 25% of the hybrids having >80% better-parent heterosis for these micronutrients (Arulselvi et al., 2006). In this paper, we report results on combining ability, nature of genetic variability, and heterosis for grain Fe and Zn densities based on a diverse range of inbred lines and their hybrids in two sets of line × tester trials to assess if the results are specific to the genotypes used in these two sets of materials or they present some general patterns for wider application to the genetic enhancement of grain Fe and Zn densities in pearl millet.

MATERIALS AND METHODS

Experimental Materials

Two sets of line × tester hybrids and their parental lines were involved in this study. Set I consisted of eight maintainer lines (B-lines), hereafter referred to as "lines," nine restorer lines (R-lines), hereafter referred to as "testers" (Table 1), and 72 line × tester hybrids. Set II consisted of 16 B-lines and 12 R-lines (Table 2) and 192 line × tester hybrids. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) had developed more than 100 male-sterile lines and their B-lines, which had been evaluated for agronomic traits and screened for grain Fe and Zn densities. ICRISAT had also developed more than 1700 R-lines (including sister lines of several R-lines), of which about 100 in active collection had been evaluated for agronomic traits. Some of these R-lines had also been screened for grain Fe and Zn densities. Based on medium maturity (75–80 d) and a wide range for grain Fe and Zn densities, B-lines and R-lines were selected for use as lines and testers in both sets of trials. These parental lines of diverse parentage represent large variability for grain Fe (34 to 102 mg kg^{-1}) and Zn (34 to 84 mg kg^{-1}) densities as well as for several agronomic traits, such as plant height, tillering, panicle size, and 1000-grain weight. Hybrids of set I were produced during the 2008 summer season and set II hybrids were produced during the 2009 summer season.

Field Trials

Field trials of both sets were conducted in Alfisols at ICRISAT, Patancheru. The set I trial of 89 entries (72 hybrids and 17 parental lines) was conducted in a randomized complete block design (RCBD) with two replications during the 2009 summer season and rainy season, which represented two contrasting environments. For instance, during the 2009 rainy crop season (16 July to 22 October), the mean maximum temperature was 31.1°C, mean relative humidity was 88% at 0700 h and 58% at 1400 h, and total evaporation was 498 mm. The 2009 summer crop season (14 February to 19 May) had higher mean maximum temperature (37.1°C), lower relative humidity (68% at 0700 h and 28% at 1400 h), and higher total evaporation (856 mm). The set II trial of 220 entries (192 F_1 hybrids and 28 parental lines) was also conducted in a RCBD with two replications during the 2009 rainy season and 2010 summer season, which again represented two contrasting environments. In comparison to the 2009 rainy season mentioned above, the 2010 summer crop season (8 February to 18 May) had higher mean maximum temperature (37.1°C), lower relative humidity (72.6% at 0700 h and 27.8% at 1400 h), and higher total evaporation (855 mm). In both sets of trials, hybrids and parents were randomized separately in adjacent strips. The parents and the hybrids were planted in a single row of 2 m length on ridges spaced at 75 cm in the 2009 rainy season and 60 cm in the 2009 and 2010 summer seasons. Overplanted plots were thinned 15 d after planting to single plants spaced 15 cm apart. A basal dose of 100 kg ha^{-1} of $(NH_4)_2HPO_4$ containing 18% N and 46% P was applied at the time of field preparation before planting, with 100 kg ha^{-1} of urea (46% N) top dressed within 2 to 4 d of thinning. The rainy season trials were grown under rainfed conditions with <750 mm rainfall during the crop season while

Table 1. Parentage of inbred lines used in line × tester set I trial of pearl millet.

Parental line	Parentage
	Line†
ICMB 89111	[843B × (GNS × SS-48-40-4)-1-9-8]-30-B-B-1
ICMB 93222	(26B × 834B)-11-2-B-B
ICMB 93333	(843B × ICMPS 900-9-3-8-2)-21-8-4
ICMB 94111	{(ICMB 89111 × ICMB 88002) × [(81B × SRL53-1) × 843B]-3+ × IP9402-2+}-31
ICMB 97111	HTBC-48-B-1-1-1-1
ICMB 98222	ARD-288-1-10-1-2 (RM)-5
ICMB 00888	(843B × ICTP8202-161-5)-20-3-B-B-3
ICMB 04777	(SRC II C3 S1-19-3-2 × HHVBC)-17-3-1-3
	Tester
IPC 390	(F4FC 1498-1-1-3 × J 104)-11-2-1-1
IPC 616	(J 260-1 × 700557-1-4-10-5-1)-1-2-1-3
IPC 774	[{(J934 × 700544+) × (J1644 × 700490+)} × {G75-FS+ × (J1623 × 700544+)}]-4+
IPC 828	(B 282 × S10B-38)-2-1-5-1-1
IPC 843	[(J834 × 700516)]-1-4-4-2-4
IPC 1178	(A 836 × J 1798-32-2-2)-5-1-1
IPC 1307	(LCSN 72-1-2-2 × S10B-106)-2-2-4
IPC 1354	EICP 8103-5
IPC 1650	[(J1623 × 700544+) × 700651+} × {G75-FS-171 × (J1623 × 700544+)]

†GNS, Gero New Strain; HHVBC, High Head Volume B-Composite.

the summer season crop was irrigated at 7 to 10 d intervals until full crop maturity was achieved. In both sets of trials, main panicles of 5 to 8 plants in each plot were selfed with parchment paper bags at the initiation of panicle emergence to protect from foreign pollen. These were harvested at physiological maturity, sun dried for 10 to 15 d, and bulk threshed using a single-head thresher (ID780ST4; Wintersteiger) to produce grain samples for Fe and Zn analysis. The grains were manually cleaned and free from glumes and panicle chaff. About 20-g grain samples were taken from the grain lot of each plot and transferred to new nonmetal fold envelops for grain Fe and Zn analysis. Care was taken at each step to avoid any contamination of the grains with dust particles and any other extraneous matter.

Grain Micronutrient Analysis

The Fe and Zn estimations were done using an atomic absorption spectrophotometer (AAS) based on tri-acid mixture method (Sahrawat et al., 2002) at the Central Analytical Services Laboratory, ICRISAT, Patancheru. The grain samples were finely ground (<60 mesh for grain samples) using cyclone mill and oven dried at 60°C for 48 h before conducting the Fe and Zn assays. The ground and dried grain samples of 0.5 g were transferred to 125-mL conical flasks. Twelve milliliters of tri-acid mixture of HNO_3, H_2SO_4, and $HClO_4$ (9:2:1 [v/v]) were added to the flasks. The flour samples were digested at room temperature for 3 h followed by digestion for 2 to 3 h on a hot plate until the digest was clear or colorless. The digests were used for Fe and Zn determination using an AAS.

Table 2. Parentage of inbred lines used in line × tester set II trial of pearl millet.

Parental line	Parentage
	Line†
841 B	Downy mildew resistant selection from 5141B
843 B	Selection from KSU line BKM 2068
863 B	Togo-13-4-1
ICMB 88006	[(81B × SRL 53-1) × 843B]-30-2-B
ICMB 91222	(26B × 81B)-4-1-2
ICMB 92111	(81B × 843B)-11-1-1-B
ICMB 95333	[{(B282 × S10B-38)-35 × Togo-29-2-2}-53 × {843B × {843B × (B 282 × 3/4 EB-100)-11-9-2}}]-60-29-1
ICMB 96333	[{{843B × (843B × 700651)-11-1-2-B} × 1163B} × (ICMB 89111 × ICMB 88005)]-5-2-2
ICMB 99444	(SPF3/S91-327 × SPF3/S91-5)-6-2-2
ICMB 00999	(ICMB 89111 × 863B)-65-8-B-B
ICMB 02444	(BSECBPT/91-38 × SPF3/S91-529)-2-1-B-2
ICMB 03111	[{{843B × (843B × 700651)-11-1-2-B} × 1163B} × (ICMB 89111 × ICMB 88005)]-5-3-B
ICMB 04222	(843B × EEBC S1-407)-12-3-B
ICMB 04555	{D2BLN/95-214 × (ICMB 96333 × HHVBC)}-11-B-2
ICMB 04888	[(843B × ICTP 8202-161-5)-20-3-B-B-3 × B-lines bulk]-2-B-1-3
ICMB 04999	(EBC-Gen-S1-40-2-2-1 × B-line bulk)-25-B-B
	Tester
IPC 338	(LCSN 439-5-3-2 × Gulisitha)-6-1-1-1
IPC 404	Togo 17-4-1-18
IPC 536	(J 260-1 × 700557-1-4-10-5-1)-1-2-2-2
IPC 689	R-294-1-2-8-2
IPC 735	(J 1399-1 × B 282)-6-1-2-1-2
IPC 811	(B 282 × S10B-38)-6-1-1-1
IPC 1254	(700619 × 700599)-3-2-13-7-3-4
IPC 1268	8082-1-3-4-1
IPC 1642	(23DBE-19-2 × S10B-106)-2-1-2-4
ICMR 356	(B 282 × J 104)-12-B-B-B-B
ICMR 06333	SDMV 90031-S1-93-3-1-1-3-2-2-1-1-B
ICMR 06888	MRC HS-219-2-1-2-B-B-B-B

†KSU, Kansas State University; BSECBPT, Bold-Seeded Early Composite Best Population Progeny Trial; HHVBC, High Head Volume B-Composite.

Statistical Analyses

Analysis of variance for the individual as well as combined environments was done following Steel and Torrie (1980), using a fixed-effects model. The line × tester analysis of combining ability was done assuming a fixed-effects model and following Kempthorne (1957). Both analyses were done using SAS version 9.2 (SAS Institute Inc., 2008). From the analysis of combining ability ANOVA, variances attributed to general combining ability (GCA) (σ^2_{GCA}) and specific combining ability (SCA) (σ^2_{SCA}) were derived to estimate the predictability ratio $2\sigma^2_{GCA}/(2\sigma^2_{GCA} + \sigma^2_{SCA})$ following Baker (1978). The estimates of midparent heterosis (MPH) and better-parent heterosis (BPH) were derived for individual environments as well as for the mean of two environments following Hallauer and Miranda (1981). The MPH of a hybrid was estimated as percent deviation of the performance of F_1 from the midparental value. The BPH for a hybrid was estimated as the percent deviation of the performance of F_1 from the parent having higher value of the micronutrient in question. The tests of significance for MPH and BPH were done via "Z" test.

Table 3. Mean squares and genetic components for grain Fe and Zn densities in line × tester (Set I) trial of pearl millet in the 2009 summer season (E1) and rainy season (E2) at Patancheru.

		Mean square					
		Fe (mg kg^{-1})			Zn (mg kg^{-1})		
Source of variation	df	Combined	E1	E2	Combined	E1	E2
Environments (E)	1	29,241.7**	–	–	34,826.0**	–	–
Replication/E	2 (1)†	165.1	0.1	330.0**	98.2	2.8	193.7**
Hybrids (H)	71 (71)	322.8**	288.4**	95.4**	267.9**	218.8**	89.7**
Lines (L)	7 (7)	1,067.7**	990.9**	241.9**	811.0**	771.3**	183.8**
Testers (T)	8 (8)	1,294.6**	1,132.8**	336.6**	1,353.7**	938.0**	481.9**
Line × tester (L × T)	56 (56)	90.9*	80.0	42.7*	44.9*	47.0*	21.9
H × E	71	61.0*	–	–	40.6*	–	–
L × E	7	165.0**	–	–	144.1**	–	–
T × E	8	174.8**	–	–	66.2**	–	–
L × T × E	56	31.8	–	–	24.0	–	–
Error	142 (71)	39.8	55.6	24.0	25.2	26.9	23.6
				Genetic components			
σ^2_{GCA}‡		34.2	60.2	16.1	32.3	49.7	19.4
σ^2_{SCA}‡		12.8	12.2	9.4	4.9	10.0	–0.8
Predictability ratio [$2\sigma^2_{GCA}/(2\sigma^2_{GCA} + \sigma^2_{SCA})$]		0.84	0.91	0.77	0.93	0.91	1.00
% contribution of σ^2L§		35.5	39.5	29.5	32.0	38.2	24.2
% contribution of σ^2T§		48.7	51.2	47.7	60.8	52.6	78.0
% contribution of σ^2L × T§		15.9	9.3	22.8	7.2	9.3	–2.3

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

†Figures in parentheses indicate individual environment degrees of freedom.

‡σ^2_{GCA}, variance attributed to general combining ability; σ^2_{SCA}, variance attributed to specific combining ability.

§σ^2L, line variance; σ^2T, tester variance; σ^2L × T, line × tester variance.

RESULTS

Genetic Variability

Analysis of variance showed that differences among the parental lines (data not presented) and among the hybrids in the set I trial (Table 3) and in the set II trial (Table 4) were highly significant ($p < 0.01$) both for Fe and Zn densities in individual environments and across environments. Environmental effect on these two micronutrients in both trials was also highly significant, with its relative contribution to the total variability being up to twice that attributable to hybrids. Further partitioning of the variability among hybrid showed that the effects of lines and testers were highly significant for both micronutrients in both trials. The line × tester interaction was significant ($p < 0.05$) only in the rainy season for Fe density and in the summer season for Zn density in the set I trial while it was highly significant ($p < 0.01$) for both micronutrients in the set II trial. The relative contribution of line × tester interaction, however, was much smaller than those of lines and testers for both micronutrients in both trials. The hybrid × environment interaction was significant ($p < 0.05$) in the set I trial, but its magnitude was only 19% of that attributable to hybrids for Fe density and 15% of that attributable to hybrids for Zn density. In the set II trial, this interaction effect was highly significant ($p < 0.01$), with its magnitude being 19% of that attributable to hybrids for Fe density and 30% of that attributable to hybrids for Zn density. The interaction of lines and testers with environments was highly significant for both micronutrients in both trials. The line × tester × environment interaction for these micronutrients was significant ($p < 0.05$) for Fe density and highly significant ($p < 0.01$) for Zn density but only in the set II trial. The magnitude of σ^2_{GCA} was higher than the σ^2_{SCA} for both Fe and Zn densities. The predictability ratio, a measure of the relative importance of GCA and SCA, was generally ≥0.84 for both micronutrients in the set I and set II trial in individual environments and across environments.

Parental Performance Per Se and Combining Ability

Based on the mean performance across the two environments, the Fe density varied from 50 to 102 mg kg^{-1} among the lines and from 34 to 101 mg kg^{-1} among the testers in the set I trial whereas it varied from 41 to 96 mg kg^{-1} among the lines and from 36 to 100 mg kg^{-1} among the testers in the set II trial, indicating large variability for Fe density in all four groups of parental lines (Table 5). Three of the five lines with >63 mg kg^{-1} Fe density in the set I trial had highly significant ($p < 0.01$) and positive GCA whereas all three low-Fe lines (<54 mg kg^{-1}) had highly significant and negative GCA. A similar pattern was

Table 4. Mean squares and genetic components for grain Fe and Zn densities in line × tester (Set II) trial of pearl millet in the 2009 rainy season (E1) and 2010 summer season (E2) at Patancheru.

		Mean square					
		Fe (mg kg^{-1})			Zn (mg kg^{-1})		
Source of variation	df	Combined	E1	E2	Combined	E1	E2
Environments (E)	1	42,502.3**	–	–	27,050.1**	–	–
Replication/E	2 (1)†	240.3	73.3	7.3	153.8	38.4	99.2
Hybrids (H)	191 (191)	350.5**	124.9**	292.6**	112.9**	56.1**	91.0**
Lines (L)	15 (15)	2,301.3**	802.0**	1,700.9**	404.2**	197.4**	248.1**
Testers (T)	11 (11)	1,867.1**	432.1**	1,695.1**	887.1**	304.3**	659.6**
Line × tester (L × T)	165 (165)	72.1**	42.9**	71.1**	34.8**	26.7**	38.8**
H × E	191	67.1**	–	–	34.2**	–	–
L × E	15	201.6**	–	–	41.3**	–	–
T × E	11	260.1**	–	–	76.8**	–	–
L × T × E	165	42.0*	–	–	30.7**	–	–
Error	382 (191)	30.4	22.7	38.0	19.1	16.6	21.6
				Genetic components			
σ^2_{GCA}‡		39.4	492.5	1,061.2	10.4	116.8	150.0
σ^2_{SCA}‡		10.4	10.1	16.5	3.9	5.1	8.6
Predictability ratio [$2\sigma^2_{GCA}/(2\sigma^2_{GCA} + \sigma^2_{SCA})$]		0.88	0.99	0.99	0.84	0.98	0.97
% contribution of σ^2L§		54.7	58.7	50.4	31.4	34.9	24.9
% contribution of σ^2T§		33.2	23.1	37.6	53.2	41.6	52.5
% contribution of σ^2L × T§		12.1	18.2	12.0	15.4	23.5	22.6

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

†Figures in parentheses indicate individual environment degrees of freedom.

‡σ^2_{GCA}, variance attributed to general combining ability; σ^2_{SCA}, variance attributed to specific combining ability.

§σ^2L, line variance; σ^2T, tester variance; σ^2L × T, line × tester variance.

observed for testers, where three of the five testers with >73 mg kg^{-1} Fe density had highly significant and positive GCA whereas three of the four low-Fe testers (<46 mg kg^{-1}) had highly significant and negative GCA. Four of the six high-Fe lines (>73 mg kg^{-1}) in the set II trial had highly significant and positive GCA whereas seven of the 10 low-Fe lines (<58 mg kg^{-1}) had highly significant and negative GCA. In this trial also, four of the six high-Fe testers (>50 mg kg^{-1}) had highly significant and positive GCA whereas five of the remaining six low-Fe testers (<50 mg kg^{-1}) had highly significant and negative GCA. Considering lines and testers together, there were highly significant ($p < 0.01$) and positive correlations between performance per se of the parental lines and their GCA for Fe density ($r = 0.75$ in the set I trial and $r = 0.68$ in the set II trial).

Large variability was also observed for Zn density both among lines (40–73 mg kg^{-1}) and among testers (34–84 mg kg^{-1}) in the set I trial and among lines (38–72 mg kg^{-1}) and among testers (40–77 mg kg^{-1}) in the set II trial (Table 5). Barring a few exceptions, the patterns of relationships between performance per se of the parental lines and their GCA for Zn density were similar to those observed for Fe density in both trials, leading to highly significant ($p < 0.01$) and positive correlations between performance per se of parental lines and their GCA ($r = 0.78$ in the set I trial and $r = 0.69$ in the set II trial). Highly significant ($p < 0.01$) and positive correlations were also observed between the Fe and Zn densities in parental lines ($r = 0.90$ in the set I trial and $r = 0.86$ in the set II trial). Similarly, there were highly significant ($p < 0.01$) and positive correlations between the GCA of Fe and Zn densities in parental lines ($r = 0.89$ in the set I trial and $r = 0.78$ in the set II trial). In both groups of parents (lines and testers) in the set I and the set II trials, inbred lines with highest and significantly positive GCA for Fe density also had the highest and significantly positive GCA for Zn density.

Heterosis and Combining Ability

The Fe density, averaged across the two environments, varied from 33 to 76 mg kg^{-1} among the hybrids in the set I trial (Fig. 1A) and from 30 to 80 mg kg^{-1} in the set II trial (Fig. 1B). The Zn density among these hybrids varied from 34 to 70 mg kg^{-1} in the set I trial and from 31 to 64 mg kg^{-1} in the set II trial. There was not a single hybrid in these trials that transgressed the Fe density or Zn density levels of the parents having the higher levels of these micronutrients, implying there was no indication of BPH (data not presented). In the set I trial, 55 of the 72 hybrids had significant (mostly $p < 0.01$) MPH, all in the negative direction, whereas 93 hybrids had significant (mostly $p < 0.01$) MPH in the set II trial, of which 91 were in the negative direction. For Zn density, 37 hybrids in the set I trial and 82 hybrids in the set II trial had significant (mostly $p < 0.01$) MPH, and all were in the negative direction.

Table 5. Performance per se of the lines and testers and their general combining ability (GCA) effects for grain Fe and Zn densities in pearl millet across two environments at Patancheru.

	Line					Tester			
	Fe (mg kg^{-1})		Zn (mg kg^{-1})			Fe (mg kg^{-1})		Zn (mg kg^{-1})	
Identity	Mean	GCA	Mean	GCA	Identity	Mean	GCA	Mean	GCA
					Set I trial				
ICMB 89111	56.8	−2.6*	58.5	−1.2	IPC 390	45.7	−8.7**	38.8	−8.1**
ICMB 93222	90.1	6.6**	65.1	2.7**	IPC 616	91.8	1.3	77.5	−0.4
ICMB 93333	53.7	−5.9**	55.4	0.9	IPC 774	83.5	3.0**	84.1	3.8**
ICMB 94111	85.8	0.9	61.6	−2.4**	IPC 828	54.6	−4.7**	47.3	−7.3**
ICMB 97111	50.1	−8.7**	40.1	−9.9**	IPC 843	73.1	3.8**	66.0	5.7**
ICMB 98222	101.5	5.9**	73.1	4.4**	IPC 1178	80.6	12.2**	73.6	10.8**
ICMB 00888	63.7	0.7	56.1	0.5	IPC 1307	46.7	−0.5	39.9	−2.0*
ICMB 04777	76.1	3.1**	63.4	5.0**	IPC 1354	40.5	−7.1**	34.4	−6.2**
					IPC 1650	100.7	0.7	82.8	3.7**
					Set II trial				
841 B	47.9	−4.9**	52.9	3.5**	IPC 338	52.4	2.4**	45.2	−1.1*
843 B	52.7	−4.3**	55.6	−0.3	IPC 404	48.8	2.7**	49.8	2.5**
863 B	73.4	10.5**	61.4	4.6**	IPC 536	60.2	−2.2**	54.8	0.6
ICMB 88006	42.2	−9.1**	38.2	−4.1**	IPC 689	58.5	8.2**	53.8	4.9**
ICMB 91222	88.7	14.4**	60.3	3.1**	IPC 735	99.8	5.7**	76.7	5.3**
ICMB 92111	33.5	−10.9**	42.0	−5.4**	IPC 811	44.9	−6.4**	39.5	−6.8**
ICMB 95333	95.9	5.8**	61.4	1.2	IPC 1254	45.8	−2.4**	40.8	−1.3*
ICMB 96333	81.8	0.7	72.1	1.0	IPC 1268	36.3	−8.5**	47.5	−2.9**
ICMB 99444	82.3	1.3	63.1	1.2	IPC 1642	50.2	8.2**	46.1	4.6**
ICMB 00999	53.5	−1.8*	59.5	1.1	ICMR 356	54.4	−3.0**	50.9	−0.7
ICMB 02444	79.5	2.1**	57.5	−0.1	ICMR 06333	44.8	−2.1**	41.7	−3.4**
ICMB 03111	52.9	0.9	50.7	0.8	ICMR 06888	49.9	−2.6**	44.9	−1.7**
ICMB 04222	55.4	4.9**	50.5	−0.9					
ICMB 04555	45.5	−8.8**	46.2	−4.0**					
ICMB 04888	57.9	2.1**	55.4	1.8**					
ICMB 04999	41.1	−2.7**	43.0	−3.4**					

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

The 10% top-ranking hybrids (7 out of 72) in the set I trial had 62 to 76 mg kg^{-1} Fe density (Table 6). All the parental lines of these hybrids had 64 to 102 mg kg^{-1} Fe and at least one of the parents of each hybrid had >80 mg kg^{-1} Fe density. Mostly both and in the other cases at least one of the parents of these hybrids had highly significant ($p < 0.01$) and positive GCA. Similarly, 10% top-ranking hybrids had 62 to 70 mg kg^{-1} Zn density. All the parental lines of these hybrids had >58 mg kg^{-1} Zn density and at least one of the parents of each hybrid had >73 mg kg^{-1} Zn density. Mostly both and in other cases at least one parent of these hybrids had highly significant ($p < 0.01$) and positive GCA. Four of these hybrids were common for Fe and Zn densities. In the set II trial, 10% top-ranking hybrids (19 out of 192) had 61 to 80 mg kg^{-1} Fe density (Table 7). Except for three testers (found in a total of 19 hybrids) that had 46 to 50 mg kg^{-1} Fe, all the other parental lines (both lines and testers) of these hybrids had 51 to 96 mg kg^{-1} Fe density, and except for two hybrids, at least one of the parents of the other hybrids had >73 mg kg^{-1} Fe density. Mostly both and in other cases at least one of the parental lines of these hybrids had highly significant ($p < 0.01$) and positive GCA. Similarly, 10% top-ranking hybrids had 53 to 64 mg kg^{-1} Zn density. Except for two parental lines (found in a total of 19 hybrids) that had 46 to 50 mg kg^{-1} Zn density, all the other parental lines had 51 to 72 mg kg^{-1} Zn density, and at least one of the parents of each hybrid had highly significant ($p < 0.01$) and positive GCA. Nine of these hybrids were common for Fe and Zn densities. Parental lines (lines as well as testers) of the bottom 10% hybrids in both sets of trials had mostly low Fe and Zn densities and highly significant ($p < 0.01$) and negative GCA for both micronutrients (data not presented).

Nine of the 72 hybrids in the set I trial had significant SCA for Fe density, of which three were positive and six were negative, whereas 29 of the 192 hybrids in the set II trial had significant SCA, of which 14 were positive and 15 were negative. For Zn density, seven hybrids in the set I trial had significant SCA, of which three were positive and four were negative, whereas 18 hybrids in the set II trial had significant SCA, of which eight were positive and 10 were negative. Among all these hybrids with significant SCA, those significant at the 0.01 probability level were as common as those

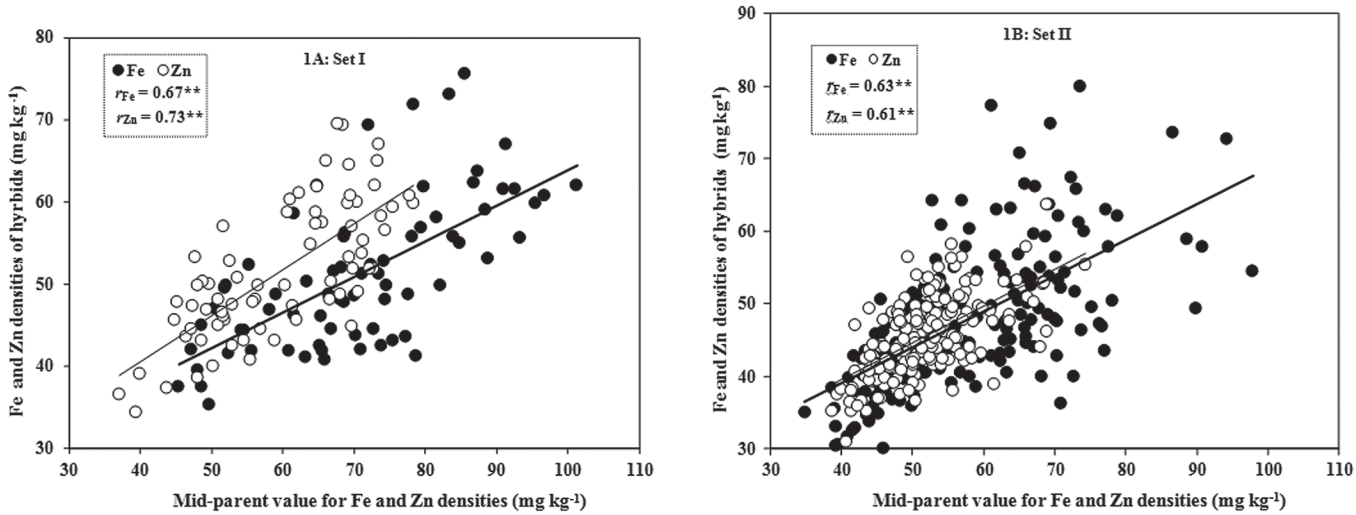

Figure 1. Relationship between grain Fe and Zn densities of hybrids and midparent values in line × tester set I (1A) and set II (1B) trials across two environments. **Significant at the 0.01 probability level.

Table 6. Performance per se, general combining ability (GCA), specific combining ability (SCA), and midparent heterosis (MPH) of top ranking 10% hybrids for high Fe and Zn densities in the set I trial of pearl millet across two environments at Patancheru.

Hybrid		Mean micronutrient densities (mg kg^{-1})			GCA			
Line (P1)	Tester (P2)	F_1	P1	P2	P1	P2	SCA	MPH (%)
				High Fe density hybrids				
ICMB 93222 ×	IPC 1178	75.5	90.1	80.6	6.6**	12.2**	5.63	–11.70
ICMB 94111 ×	IPC 1178	73.0	85.8	80.6	0.9	12.2**	8.85**	–12.44*
ICMB 04777 ×	IPC 1178	71.8	76.1	80.6	3.1**	12.2**	5.38	–8.45
ICMB 00888 ×	IPC 1178	69.3	63.7	80.6	0.7	12.2**	5.27	–3.99
ICMB 98222 ×	IPC 1178	67.0	101.5	80.6	5.9**	12.2**	–2.15	–26.58**
ICMB 98222 ×	IPC 843	63.8	101.5	73.1	5.9**	3.8**	3.01	–27.04**
ICMB 93222 ×	IPC 774	62.3	90.1	83.5	6.6**	3.0**	1.57	–28.35**
				High Zn density hybrids				
ICMB 94111×	IPC 1178	69.5	61.6	73.6	–2.4**	10.8**	9.64**	2.58
ICMB 04777×	IPC 1178	69.3	63.4	73.6	5.0**	10.8**	2.00	1.09
ICMB 04777×	IPC 774	67.0	63.4	84.1	5.0**	3.8**	6.66**	–8.84
ICMB 98222×	IPC 1178	65.0	73.1	73.6	4.4**	10.8**	–1.69	–11.41*
ICMB 89111×	IPC 1178	65.0	58.5	73.6	–1.2	10.8**	3.89	–1.70
ICMB 93222×	IPC 1178	64.5	65.1	73.6	2.7**	10.8**	–0.50	–7.03
ICMB 04777×	IPC 1650	62.0	63.4	82.8	5.0**	3.7**	1.78	–15.07**

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

significant at the 0.05 probability level. There were highly significant ($p < 0.01$) and positive correlations between performance per se of hybrids and midparental values for both micronutrients ($r = 0.67$ for Fe and $r = 0.73$ for Zn) in the set I trial (Fig. 1A). Similarly, there were highly significant ($p < 0.01$) and positive correlations between performance per se of hybrids and midparental values for both micronutrients ($r = 0.63$ for Fe and $r = 0.61$ for Zn) in the set II trial (Fig. 1B). Highly significant ($p < 0.01$) and positive correlations between performance per se of the hybrids and their SCA were observed for both Fe density ($r = 0.47$) and Zn density ($r = 0.36$) in the set I trial (Fig. 2A) and in the set II trial ($r = 0.42$ for Fe and $r = 0.52$ for Zn) (Fig. 2B). There were highly significant correlations ($p < 0.01$) between Fe and Zn densities in the hybrids in the set I trial ($r = 0.84$) and in the set II trial ($r = 0.75$). The correlations between the SCA of Fe density and Zn density in hybrids were also highly significant ($p < 0.01$) and positive in the set I trial ($r = 0.64$) as well as in the set II trial ($r = 0.65$).

DISCUSSION

Results of the two sets of line × tester studies conducted across two contrasting seasons (environments) showed fairly consistent patterns of combining ability and genetic

Table 7. Performance per se, general combining ability (GCA), specific combining ability (SCA), and midparent heterosis (MPH) of top ranking 10% hybrids for high Fe and Zn densities in the set II trial of pearl millet across two environments in Patancheru.

Hybrid		Mean micronutrient densities (mg kg^{-1})			GCA			
Line (P1)	Tester (P2)	F1	P1	P2	P1	P2	SCA	MPH (%)
				High Fe density hybrids				
ICMB 91222 ×	IPC 689	80.0	88.7	58.5	14.4**	8.2**	9.69**	8.67
863 B ×	IPC 404	77.2	73.4	48.8	10.5**	2.7**	18.72**	26.36**
ICMB 91222 ×	IPC 1642	74.7	88.7	50.2	14.4**	8.2**	4.49	7.61
863 B ×	IPC 735	73.5	73.4	99.8	10.5**	5.7**	9.54**	−15.14**
ICMB 91222 ×	IPC 735	72.7	88.7	99.8	14.4**	5.7**	4.93	−22.85**
ICMB 02444 ×	IPC 1642	70.7	79.5	50.2	2.1**	8.2**	12.68**	8.53
ICMB 95333 ×	IPC 404	67.3	95.9	48.8	5.8**	2.7**	11.06**	−7.05
863 B ×	IPC 689	66.5	73.4	58.5	10.5**	8.2**	0.03	0.83
ICMB 91222 ×	IPC 1254	66.0	88.7	45.8	14.4**	−2.4**	6.35*	−1.83
ICMB 95333 ×	IPC 1642	65.8	95.9	50.2	5.8**	8.2**	4.09	−9.98
ICMB 04222 ×	IPC 689	64.2	55.4	58.5	4.9**	8.2**	3.43	12.72
ICMB 04222 ×	IPC 1642	64.2	55.4	50.2	4.9**	8.2**	3.66	21.45*
ICMB 02444 ×	IPC 689	63.6	79.5	58.5	2.1**	8.2**	5.55*	−8.19
863 B ×	ICMR 356	63.2	73.4	54.4	10.5**	−3.0**	7.91**	−1.07
ICMB 95333 ×	IPC 689	62.9	95.9	58.5	5.8**	8.2**	1.16	−18.52**
863 B ×	IPC 1642	62.9	73.4	50.2	10.5**	8.2**	−3.57	1.72
ICMB 91222 ×	IPC 338	62.2	88.7	52.4	14.4**	2.4**	−2.32	−11.92
ICMB 04888 ×	IPC 735	62.1	57.9	99.8	2.1**	5.7**	6.64*	−21.18**
ICMB 91222 ×	IPC 536	61.1	88.7	60.2	14.4**	−2.2**	1.25	−16.81**
				High Zn density hybrids				
863 B ×	IPC 735	63.6	61.4	76.7	4.6**	5.3**	8.29**	−7.88
863 B ×	IPC 404	58.2	61.4	49.8	4.6**	2.5**	5.71**	4.71
ICMB 04888 ×	IPC 735	57.7	55.4	76.7	1.8**	5.3**	5.15*	−12.53*
ICMB 91222 ×	IPC 689	56.5	60.3	53.8	3.1**	4.9**	3.03	−1.00
841 B ×	IPC 1642	56.4	52.9	46.1	3.5**	4.6**	3.03	14.10*
ICMB 95333 ×	IPC 404	56.3	61.4	49.8	1.2	2.5**	7.17**	1.29
ICMB 96333 ×	IPC 735	55.4	72.1	76.7	1.0	5.3**	3.68	−25.51**
863 B ×	ICMR 356	55.2	61.4	50.9	4.6**	−0.7	6.01**	−1.62
ICMB 95333 ×	IPC 1642	55.0	61.4	46.1	1.2	4.6**	3.86	2.37
ICMB 04888 ×	IPC 1642	54.0	55.4	46.1	1.8**	4.6**	2.23	6.54
ICMB 03111 ×	IPC 689	53.6	50.7	53.8	0.8	4.9**	2.48	2.42
ICMB 02444 ×	IPC 1642	53.5	57.5	46.1	−0.1	4.6**	3.62	3.28
863 B ×	IPC 536	53.3	61.4	54.8	4.6**	0.6	2.81	−8.18
ICMB 96333 ×	IPC 1642	53.2	72.1	46.1	1.0	4.6**	2.26	−9.93
841 B ×	IPC 735	53.2	52.9	76.7	3.5**	5.3**	−1.03	−17.90**
841 B ×	IPC 689	53.1	52.9	53.8	3.5**	4.9**	−0.70	−0.50
ICMB 96333 ×	IPC 689	53.1	72.1	53.8	1.0	4.9**	1.73	−15.68**
ICMB 95333 ×	IPC 689	53.0	61.4	53.8	1.2	4.9**	1.51	−8.02
ICMB 99444 ×	IPC 735	52.8	63.1	76.7	1.2	5.3**	0.93	−23.02**

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

variability both for the Fe density and Zn density. Thus, although the variances were significant for both GCA and SCA, the former was predominant with predictability ratio of ≥0.84 for Fe density and ≥0.91 for the Zn density in both sets of trials. This showed that the underlying physiological processes determining both micronutrients were largely under additive genetic control. Highly significant ($p < 0.01$) and positive correlations between the midparental values and hybrid performance for Fe and Zn densities provided further support to the argument of largely additive genetic control of the physiological processes determining these two micronutrients. Earlier studies in pearl millet (Velu et al., 2011b) and maize (Arnold and Bauman, 1976; Long et al., 2004; Chen et al., 2007) also observed these micronutrients to be largely under additive genetic control. This would imply that recurrent selection can be effectively

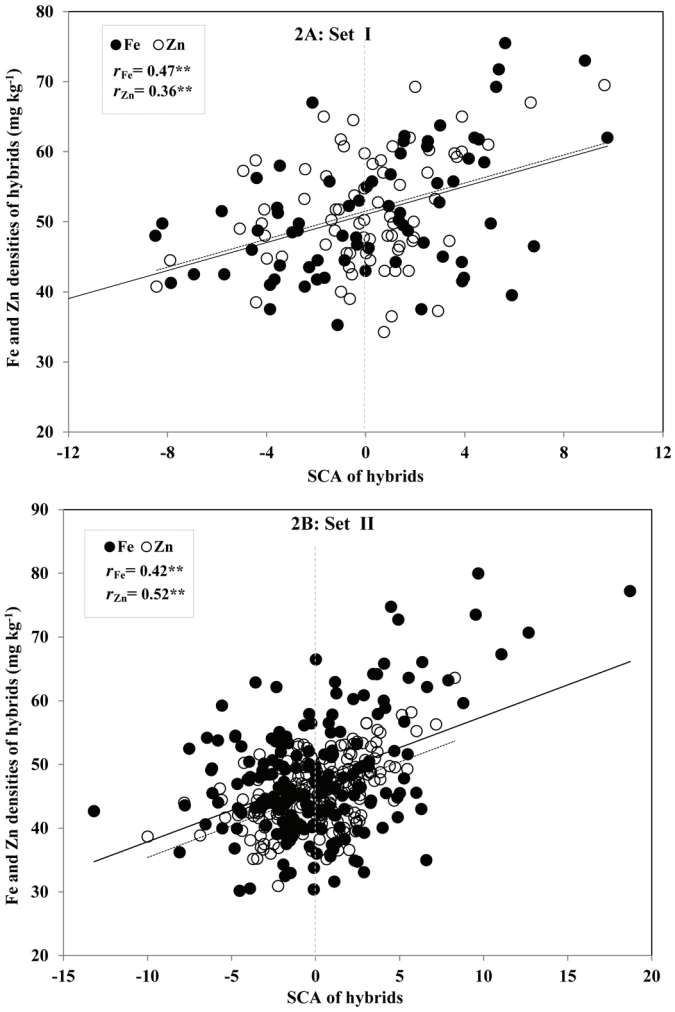

Figure 2. Relationship between grain Fe and Zn densities of hybrids and their specific combining ability (SCA) effects in line × tester set I (2A) and set II (2B) trials across two environments. **Significant at the 0.01 probability level.

used for intrapopulation improvement of Fe and Zn densities. There were highly significant ($p < 0.01$) and positive correlations between the Fe and Zn densities of the parental lines per se and their GCA in both sets of trials. An earlier pearl millet study also reported a highly significant and positive correlation between performance per se of the inbred lines and their GCA for both micronutrients (Velu et al., 2011b). This would imply that, as compared to breeding lines with low Fe and Zn densities, lines selected for high Fe and Zn densities are more likely to include those with high GCA for these micronutrients.

There was not a single hybrid in either trial that transgressed the parental lines that had higher Fe density or Zn density levels, implying there was no better-parent heterosis. The midparent heterosis for Fe and Zn densities, statistically significant in about 50% of the hybrids, was almost always in the negative direction for both micronutrients in both sets of trials. In an earlier pearl millet study, Velu et al. (2011b) also reported similar results. This further supports the fact that the physiological processes determining Fe and Zn densities in grains were largely under additive genetic control, but it also indicates some degree of partial dominance of genes responsible for low Fe and Zn densities over those responsible for high Fe and Zn densities. Hybrids with high Fe density or Zn density levels involved mostly both parental lines having high levels of these micronutrients, implying that breeding hybrids with high Fe and Zn densities would entail genetic improvement of both parental lines for high Fe and Zn densities. Hybrids with high Fe and Zn densities often had both parental lines with significant and positive GCA, which is not unexpected considering the highly significant and positive correlation between the performance per se of the parental lines and their GCA.

There were highly significant ($p < 0.01$) and positive correlations between the Fe and Zn densities in the parental lines as well as in the hybrids. Several studies in pearl millet (Velu et al., 2007, 2008; Gupta et al., 2009; Govindaraj et al., 2009; Rai et al., 2012) and other cereals, such as sorghum (Ashok Kumar et al., 2009), maize (Oikeh et al., 2003, 2004), rice (Stangoulis et al., 2007; Anandan et al., 2011), and wheat (Peleg et al., 2009; Zhang et al., 2010; Velu et al., 2011a), have also reported highly significant and positive correlations between these two micronutrients. This would imply likely effectiveness of simultaneous selection for both micronutrients. Interestingly, results of this study showed highly significant and positive correlations between the GCA of Fe density and Zn density ($r = 0.78$ to 0.89) as well as the SCA of Fe density and Zn density ($r = 0.64$ to 0.65), indicating that effective selection for simultaneous genetic improvement of performance per se and GCA of Fe and Zn densities in the parental lines as well as SCA effect in the hybrids would be possible in pearl millet.

This study also identified B-lines and R-lines that had high Fe and Zn densities and were also high general combiners for these micronutrients. Because environments had a significant effect on Fe and Zn densities, there is a need to further evaluate these parental lines for their performance per se and GCA in additional diverse environments. Furthermore, to be commercially viable, genetic improvement of Fe and Zn densities must not compromise on grain yield. An earlier study in pearl millet did not find any adverse association of these micronutrients with grain yield (Gupta et al., 2009). In a later study involving four different hybrid trials, Fe density was significantly negatively correlated with grain yield in two trials and uncorrelated in two trials. Zinc density was not correlated with grain yield in any of the four trials (Rai et al., 2012). Obviously, there is a need to pursue such character association studies using a diverse range of materials and diverse environments.

Similar patterns of genetic variability for Fe and Zn densities and positive relationships between these two micronutrients with respect to performance per se, the GCA and SCA observed in this study, indicated that some of the genes responsible for these two micronutrients were either tightly linked or were involved in common physiological processes. Molecular marker studies in wheat (Peleg et al., 2009; Singh et al., 2010), rice (Stangoulis et al., 2007), common bean (*Phaseolus vulgaris* L.) (Cichy et al., 2009; Blair et al., 2011), and pearl millet (Kumar, 2011) have identified common and overlapping quantitative trait loci (QTL) for Fe and Zn densities. Diverse sources with high levels of Fe and Zn densities have been identified in pearl millet (Rai et al., 2012), which provide useful experimental materials for developing mapping populations and for identifying those with different physiological mechanisms with respect to efficient uptake of Fe and Zn from the soils, their translocation through the vegetative parts, and loading in the grains. Identification of high-Fe and high-Zn QTL for these mechanisms and pyramiding them in the parental lines using marker-assisted selection may be the most effective approach to breeding hybrids with high Fe and Zn densities in pearl millet.

Acknowledgments

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