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Gene effects and heterosis for grain iron and zinc density in pearl millet (*Pennisetum glaucum* (L.) R. Br)

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Abstract Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major warm-season cereal, grown primarily for grain production in the arid and semi-arid tropical regions of Asia and Africa. Iron (Fe) and zinc (Zn) deficiencies have been reported to be a food-related primary health problem affecting nearly two billion people worldwide. Improving Fe and Zn densities of staple crops by breeding offers a cost-effective and sustainable solution to reducing micronutrient malnutrition in resource poor communities. An understanding of the genetics of these micronutrients can help to accelerate the breeding process, but little is known about the genetics and heterosis pattern of Fe and Zn densities in pearl millet. In the present study, ten inbred lines and their full diallel crosses were used to study the nature of gene action and heterosis for these micronutrients. The general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of hybrids showed significant differences for both of the micronutrients. However, the predictability ratio ($2\sigma^2$gca/($2\sigma^2$gca + σ^2sca)) was around unity both for Fe and Zn densities, implying preponderance of additive gene action. Further, highly significant positive correlation between mid-parent values and hybrid performance, and no correlation between mid-parent values and mid-parent heterosis confirmed again the predominant role of additive gene action for these micronutrients. Barring a few exceptions with one parent, hybrids did not outperform the parents having high Fe and Zn levels. This showed that there would be little opportunity, if any, to exploit heterosis for these mineral micronutrients in pearl millet. In general, high Fe and Zn levels in both of the parental lines would be required to increase the probability of breeding high Fe and Zn hybrids.

Keywords Combining ability · Grain iron and zinc density · Gene action · Heterosis · Pearl millet · *Pennisetum glaucum*

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Introduction

Pearl millet is a highly cross-pollinated crop with an out-crossing rate in excess of 85% (Burton 1974).

This breeding system allows for the development of open-pollinated varieties (OPVs) as the most natural cultivar state of this species. However, the heterogeneous and heterozygous nature of OPVs allows for partial exploitation of heterosis. On the other hand, single cross hybrids provide opportunity for maximization of heterozygosity and thus allow for the maximum exploitation of heterosis. Commercially viable cytoplasmic-nuclear male-sterility (CMS) systems have made hybrid cultivar development feasible, resulting in a strong pearl millet hybrid seed industry in India, with more than 80 single-cross hybrids (by name) reportedly cultivated on about 5 million ha of the total 10 million ha pearl millet area in India, which is the largest pearl millet growing country in the world (Mula et al. 2007). Pearl millet hybrids are also grown on a limited scale in the United States of America. Africa as a whole has 15–16 million ha under this crop, where OPVs (mostly landraces) are cultivated. In this region, hybrids are being experimented and are likely to be adopted in the near future.

Micronutrient malnutrition resulting from the dietary deficiency of minerals such as iron (Fe) and zinc (Zn) has recently been recognized as a serious human health problem, especially in the developing countries (WHO 2002). The HarvestPlus challenge program of the Consultative Group on International Agricultural Research (CGIAR) has undertaken to support the development of biofortified crop cultivars with elevated levels of micronutrients in several crops, including pearl millet to address this problem. A recent study conducted under this project has shown that pearl millet accounts for a major share of Fe and Zn intake in some of the region of pearl millet growing areas in India and it is the cheapest source of Fe and Zn as compared to other cereals (Parthasarathy Rao et al. 2006). It is a major source of mineral micronutrients in several African countries as well. Results of a preliminary study have shown large variability for grain Fe and Zn among breeding lines and populations as well as within the populations (Velu et al. 2007). An understanding of the nature of gene action and heterosis would be a significant input into designing effective breeding strategies for the development of OPVs and hybrids. There is no information available on the nature of gene action and heterosis for grain Fe and Zn densities in pearl millet. The objective of the research reported in this paper was to fill this gap and examine its implications in breeding pearl millet cultivars with high levels of grain Fe and Zn densities.

Materials and methods

Experimental material and field trial

An earlier study consisting of 120 entries had shown large variability among the inbred lines for Fe and Zn densities, which could be classified into high, medium and low micronutrient groups (Velu et al. 2007). The present study used random lines from each of these groups (Table 1). These lines were crossed in a diallel fashion to generate 90 F_1s (including reciprocals). The crossed panicles were harvested for each of the cross combination, and few selfed panicles from parental lines were also harvested to reproduce the parental lines.

The parents and their 90 F_1 hybrids were evaluated in a randomized complete block design (RCBD) with three replications as two separate experiments laid side by side (one for hybrids and the other one for inbred lines) at ICRISAT, Patancheru. The trials were conducted in Alfisols with the applied fertilizer levels of 75 kg/ha nitrogen (50% basal in the form of diammonium phosphate (DAP) and 50% top dressed at 20 days after sowing in the form of urea) and 35 kg/ha phosphorous during both the seasons.

Each hybrid/parent was grown in 2 rows of 4 m length at 75 cm spacing between the rows in 2005 rainy season and 60 cm spacing during 2006 summer season, with 15 cm spacing between plants within the row during both the 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. All recommended agronomic practices were followed for raising a good pearl millet crop. Six soil samples from each of the top layer (0–15 cm depth) and the sub-surface layer (15–30 cm depth) were taken at the time of planting. The mean soil Fe and Zn contents extractable with diethylene triamine pentaacetic acid (DTPA), varied from 13.4 to 5.8 mg/kg for Fe and 3.0 to 2.4 mg/kg for Zn for the field used during the 2005 rainy season and 2006 summer season, respectively. Sib-mated grains were produced by crossing 15 plants of a line/hybrid with bulk pollen collected from 25 to 30 plants of the same

Table 1 The parents of diallel crosses in pearl millet

Line	Micronutrient class	Identity	Pedigree	Fe density (mg/kg)	Zn density (mg/kg)
1	High	863B	Togo-13-4-1	72.7	55.8
2		ICMB 94111	{(ICMB 89111 × ICMB 88002) × [(81B × SRL53-1) × 843B]-3+ × IP9402-2+}-31	63.6	56.8
3		ICMB 00888	(843B × ICTP8202-161-5)-20-3-B-B-3	60.4	56.7
4		AIMP 92901 S_4	AIMP 92901 S1-15-1-2-B	75.7	64.8
5	Medium	ICMB 95222	{[843B × (GNS × SS-48-40-4)-29-7-4-B] × (843B × ICMPES-29)-23-2-3}-16	41.8	47.1
6		ICMV 93074 S_5	ICMV 93074 S1-9-1-1-1-B	43.5	45.6
7		MC 94 C_2 S_4	MC 94 C2-S1-46-1-1-B	44.8	40.9
8	Low	81B	Induced downy mildew resistant selection from Tift $23D_2B$	34.4	31.7
9		ICMS 8511 S_5	ICMS 8511 S1-17-2-1-1-B	30.1	24.5
10		ICMV 91059 S_6	ICMV 91059 S1-14-2-4-2-2-B	36.3	33.5

ICMB ICRISAT millet B-line, *AIMP* Aurangabad-ICRISAT millet population, *MC* medium composite, *ICMS* ICRISAT millet synthetic, *ICMV* ICRISAT millet variety

line/hybrid. 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. Care was taken to avoid any contamination of grains with dust particles.

Laboratory analysis

The grain samples were analyzed using an Atomic Absorption Spectrophotometer (Thermo Electron Corporation, Cambridge, UK) fitted with a GFS97 autosampler, a system equivalent to the Inductively Coupled Plasma (ICP), at the National Institute of Nutrition (NIN), 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 sub-samples from each entry ashed. Dry ashing and mineral solution preparation were carried out according to the method described by Jorhem (1993). The analytical method used was validated with 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%.

Statistical analysis

The estimates of general combining ability (GCA), specific combining ability (SCA) and reciprocals effects were obtained following Griffing's method 3 model 1 (fixed model) (Griffing 1956), which included one set of F_1s and reciprocals, leading to $[p\ (p-1)]$ hybrids. Data were analyzed with the DIALLEL-SAS05 program of Zhang et al. (2005). Significance of GCA, SCA and reciprocal effects was determined by a t test (Griffing 1956) Estimate of variances due to general combining ability (σ^2gca) and specific combining ability (σ^2sca) were derived to get estimates of predictability ratio (PR): $2\sigma^2$gca/($2\sigma^2$gca + σ^2sca) (Baker 1978).

Results and discussion

Performance per se of inbred lines

The combined analysis of variance across the two environments showed highly significant differences among the parents for grain Fe and Zn densities ($P < 0.01$) (Table 2), indicating that there were significant differences among the parents for these

Table 2 Pooled analysis of variance of parents and hybrids for grain Fe and Zn densities across two seasons (2005 rainy season and 2006 summer season) in pearl millet, Patancheru

Sources of variation	df	Mean square					
		Combined		Rainy		Summer	
		Fe	Zn	Fe	Zn	Fe	Zn
Parents							
Environment (E)	1	7.9	1884**	–	–	–	–
Reps within E	4 (2)	106**	180**	99	25	47	12
Parents	9	1275**	456**	582**	156**	743**	352**
Parents × E	9	51**	53**	–	–	–	–
Error	36 (18)[a]	19	9	17	9	21	9
Hybrids (H)							
Environment (E)	1	236*	11767**	–	–	–	–
Reps within E	4 (2)[a]	73	215**	27	116**	119**	314**
Hybrids (H)	89	664**	254**	238**	84**	511**	199**
GCA	9	5666**	2193**	1924**	662**	4219**	1682**
SCA	35	146**	54**	59**	23**	141**	47**
Reciprocal	45	66	21	40	16*	58	20
H × E	89	86**	29**	–	–	–	–
GCA × E	9	477**	150**	–	–	–	–
SCA × E	35	54	16	–	–	–	–
Reciprocal × E	45	32	15	–	–	–	–
Error	356 (178)[1]	44	16	31	11	56	21
σ^2g		277.2	108.9	95.7	32.5	201.4	83.1
σ^2s		101.9	38.2	27.3	12.5	84	25.8
$2\sigma^2g/(2\sigma^2g + \sigma^2s)$		0.84	0.85	0.88	0.84	0.83	0.87

*, ** Significant at 0. 05 and 0.01 probability levels, respectively

[a] Values in *parentheses* indicate individual environment degree of freedom

micronutrients. Averaged over the two environments, the Fe density varied from 35.5 to 71.8 mg/kg and Zn density from 28.8 to 51.4 mg/kg (Tables 3, 4). Though the parents × environment interaction was significant, the correlation of parental values between the two environments was highly significant both for Fe ($r = 0.93$; $P < 0.01$) and Zn ($r = 0.86$; $P < 0.01$), indicating high levels of consistency of the rankings of parents across the two environments.

Combining ability analysis

The statistical analysis over the environments revealed highly significant differences ($P < 0.01$) among hybrids (Table 2). Significant differences were also observed between environments for both Fe and Zn densities. The reciprocal effects among the hybrids were not significant for both Fe and Zn densities. The GCA and SCA effects were highly significant for both Fe and Zn in the individual environments as well as when pooled over the two environments, indicating both additive and non-additive gene effects controlling these two micronutrients. However, the magnitude of GCA mean squares was much higher than SCA mean squares for both Fe and Zn densities, implying the predominance of additive gene action for these traits.

The hybrids × environment (H × E) interaction was significant, thus total sum of squares was partitioned into GCA × E, SCA × E and reciprocals × E interaction effects. The GCA × E was found to be significant, indicating that Fe and Zn densities were sensitive to environmental conditions and data from additional seasons or environments would provide more precise estimates of GCA effects. The SCA and reciprocal effects, on the other

Table 3 Average grain Fe density (mg/kg) of parents and hybrids, and general combing ability (GCA) and specific combining ability (SCA) of diallel crosses across two seasons (2005 rainy season and 2006 summer season) in pearl millet, Patancheru

Parents/ hybrids	1	2	3	4	5	6	7	8	9	10
1	69.2 (15.6**)	74.8	67.3	72.2†	58.4	75.8‡	53.2‡	61.5‡	49.8	58.7†
2	(5.9**)	71.8 (3.6**)	56.4‡	54.7†	48.6†	55.5‡	49.6‡	41.3‡	36.3‡	48.3‡
3	1.3	0.8	70.2 (4.2**)	65.8	47.6‡	54.6‡	50.9‡	46.1‡	45.5‡	48.9‡
4	−2.8	−4.6*	6.0**	65.8 (7.4**)	54.6	59.6	51.8	53.3	55.0	51.3
5	−3.7*	2.7	−0.9	1.1	44.5 (−5.8**)	38.3‡	46.1	36.7	37.5	35.7‡
6	7.3**	−0.6	−1.1	−0.9	−4.3*	51.4 (−0.5)	53.2	41.5	42.3	44.1
7	−8.2**	1.0	−0.7	−2.0	3.6*	3.0	49.1 (−3.4**)	38.2†	37.5†	46.9
8	3.3	−1.4	−2.7	2.6	0.4	−2.0	−1.3	35.5 (−8.0**)	36.9	34.1
9	−4.7*	−2.3	0.0	3.0	1.5	−0.2	−1.0	2.0	35.9 (−8.2**)	35.9
10	1.8	−1.6	−2.7	−2.3	−0.5	−1.2	5.7**	−0.8	1.8	40.9 (−5.1**)

Diagonal Fe density of parents, *diagonal parentheses* GCA effects of parents, *above diagonal* Fe densities of crosses, *below diagonal* SCA effects of crosses

*, ** Significant at 0. 05 and 0.01 probability levels, respectively

† , ‡ Hybrids with significant mid-parent heterosis at 0.05 and 0.01 probability levels, respectively

hand, were stable across the two environments as indicated by the non-significant SCA × E and Reciprocals × E interaction.

Baker (1978) suggested that the ratio of combining ability variance components $(2\sigma^2_{gca}/(2\sigma^2_{gca} + \sigma^2_{sca}))$ termed as predictability ratio provides a measure of the predictability of the performance of hybrids and its progenies. The closer this ratio to unity, the greater the predictability based on GCA alone. Based on combined analysis over the two environments, predictability ratio was 0.84 for Fe and 0.85 for Zn, and it was of the similar order for individual environments, implying preponderance of additive gene action and indicating that hybrid performance can be predicted based on GCA alone. Assessing the contribution of individual lines to hybrid performance was accomplished by comparing the GCA effects among the parents (Tables 3, 4). GCA effects were highly significant ($P < 0.01$) for all the parents for both Fe and Zn densities except for the medium Fe/Zn parent 'ICMV 93074 S_5'. The high Fe and Zn parents were the best general combiners having positive significant GCA effects and the parents with medium and low grain Fe and Zn densities had significant negative GCA effects. The correlation coefficient between mean performance per se of parents and GCA effects was highly significant and positive for both Fe ($r = 0.89$; $P < 0.01$) and Zn densities ($r = 0.92$; $P < 0.01$), indicating that selection of lines with high Fe and/or Zn levels would be highly effective in selecting for high GCA. Considering the performance per se and GCA effects together, the high Fe/Zn parent '863B' was identified as the best combiner for further breeding programs. The SCA effects in each parental combination are shown in Tables 3 and 4 for Fe and Zn, respectively. Ten hybrids for Fe and four hybrids for Zn had significant SCA effects, indicating presence of non-additive effects. Significant positive SCA effects were observed in 5 hybrids for Fe and 3 hybrids for Zn, of which two hybrids for Fe and a hybrid for Zn had '863B' in their parentage. '863B' is a large-seeded

Table 4 Average grain Zn density (mg/kg) of parents and hybrids, and general combing ability (GCA) and specific combining ability (SCA) of diallel crosses across two seasons (2005 rainy season and 2006 summer season) in pearl millet, Patancheru

Parents/ hybrids	1	2	3	4	5	6	7	8	9	10
1	45.9 (9.2**)	50.2	51.1	52.4	43.7	52.9†	39.9	45.7‡	41.1†	47.6‡
2	0.7	49.2 (1.5*)	42.9‡	41.7‡	41.3	40.3‡	38.2	36.7	31.9‡	35.6‡
3	0.2	−0.3	50.2 (3.4**)	50.9	40.8†	44.1	39.7	39.6	38.0	38.7†
4	−2.5	−2.3	3.4*	51.4 (4.8**)	43.1	41.7	40.8	41.5	38.6	39.6
5	−2.6	3.9*	−1.1	0.6	36.9 (−2.3**)	35.5†	40.0	30.6	32.8	30.7‡
6	1.9	−0.9	−0.3	−1.1	−1.8	39.9 (0.7)	40.4	34.8	36.5	37.6
7	−4.1*	1.7	0.2	−0.5	3.9	1.2	36.7 (−3.1**)	30.2‡	28.9	31.6
8	3.0	0.4	0.0	2.5	−1.6	−1.2	−3.1	29.6 (−4.1**)	30.3	29.3
9	−0.3	−1.9	−0.9	0.9	1.0	1.2	−0.4	0.3	28.8 (−5.4**)	26.4
10	3.7*	−1.2	−1.3	−0.9	−2.4	1.0	1.2	−0.2	0.2	31.9 (−4.7**)

Diagonal Zn density of parents, *diagonal parentheses* GCA effects of parents, *above diagonal* Zn density of crosses, *below diagonal* SCA effects of crosses

*, ** Significant at 0. 05 and 0.01 probability levels, respectively

†, ‡ Hybrids with significant mid-parent heterosis at 0.05 and 0.01 probability levels, respectively

and drought-tolerant seed parent of three commercial hybrids in India, and it has remained highly resistant to multiple pathotypes of the most prevalent disease, downy mildew, caused by *Sclerospora graminicola* (Sacc.) Schroet.

Estimates of heterosis

Combined analysis of variance showed that reciprocal effects and its interaction with the environment was non-significant. Thus the means of reciprocal and direct crosses were used for calculating mid-parent heterosis. Highly significant positive correlation between the mid-parental values and hybrid performance per se ($r = 0.84$; $P < 0.01$ for Fe and $r = 0.78$; $P < 0.01$ for Zn) (Fig. 1), and no correlation between mid-parent values and mid-parent heterosis ($r = -0.01$ for Fe and $r = -0.05$ for Zn) (Figs. 2, 3), provided additional indications of the predominant role of additive gene action for these traits. Some of the earlier studies have also reported the greater importance of additive gene action (GCA effects) for grain Fe and Zn densities in maize (Gorsline et al. 1964; Arnold and Bauman 1976; Long et al. 2004) and rice (Gregorio 2002).

The level of heterosis varied widely among hybrids both for Fe (−21 to 19%) (Fig. 2) and for Zn (−18 to 20%) (Fig. 3). Overall, average heterosis of the hybrids was negative for both Fe (−6.1%) and Zn (−2.8%). Among the hybrids, 22 hybrids showed significant mid-parent heterosis for Fe and 14 hybrids for Zn, of which 4 were in positive direction for both Fe (11.5–19.3%) and Zn (11.8–19.6%). Highest positive significant mid-parent heterosis was observed in a cross '863B × ICMV 93074 S_5' for Fe, and '863B × 81B' for Zn. Hybrids with significant mid-parent heterosis are shown by solid circles in Figs. 2 and 3 for Fe and Zn, respectively, and the significant heterotic hybrids were clearly distinct from others as they were away from regression line, suggesting parents of the hybrids with significant positive mid-parent heterosis could be used as

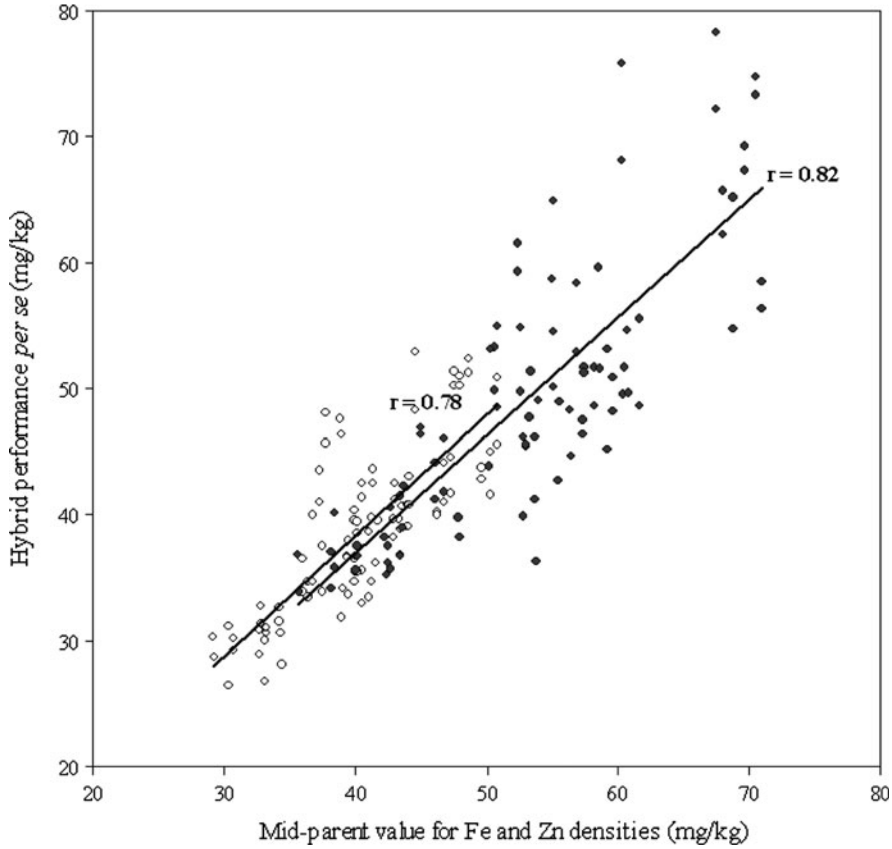

Fig. 1 Relationship between mid-parent values and hybrid performance per se for Fe (*filled circles*) and Zn (*open circles*) densities (mg/kg) in pearl millet

potential parents in breeding. All the four hybrids with positive significant mid-parent heterosis for both Fe and Zn had '863B' as one of the parents. This could result from the '863B' showing the highest positive GCA effect (15.6 $P < 0.01$ for Fe and 9.2 $P < 0.01$ for Zn), and suggesting that '863B' could be used as one of the parent to produce hybrids with high Fe and Zn densities.

Of the five hybrids having high grain Fe (>70 mg/kg), four hybrids were derived from both high × high and one hybrid from a high × medium cross combination (Table 3). Similarly for Zn, of the seven hybrids having high Zn (>50 mg/kg), six hybrids had both the high parents and one hybrid from a high × low parents cross (Table 4). None of the hybrids outperformed significantly the parents that had high levels of Fe and Zn, indicating that there would be little opportunity, if any, to exploit heterosis for these micronutrients. In general, higher micronutrient levels in both parental lines would be required to breed hybrids with elevated levels of grain Fe and Zn densities.

Conclusion

High predictability ratios $(2\sigma^2_{gca}/(2\sigma^2_{gca} + \sigma^2_{sca}))$ indicated that the expression of grain Fe and Zn densities in pearl millet is governed predominantly by additive gene effects, suggesting high effectiveness of progeny selection in pedigree selection or population breeding to develop lines and populations with increased levels of grain Fe and Zn densities. The higher additive genetic variance also prompts for recurrent selection method to improve the levels of grain Fe and Zn densities. The highly significant and positive correlation between GCA and performance per se of lines suggests that the performance per se of the genotypes could be a good indicator of its ability to transmit grain Fe and Zn densities to its hybrids and progenies; and genetically superior parents could be identified by evaluation of their Fe and Zn densities. Barring a few exceptions with one parent, none of the hybrids significantly outperformed the parents having high levels of Fe and Zn, indicating that there would be little opportunity, if any, to

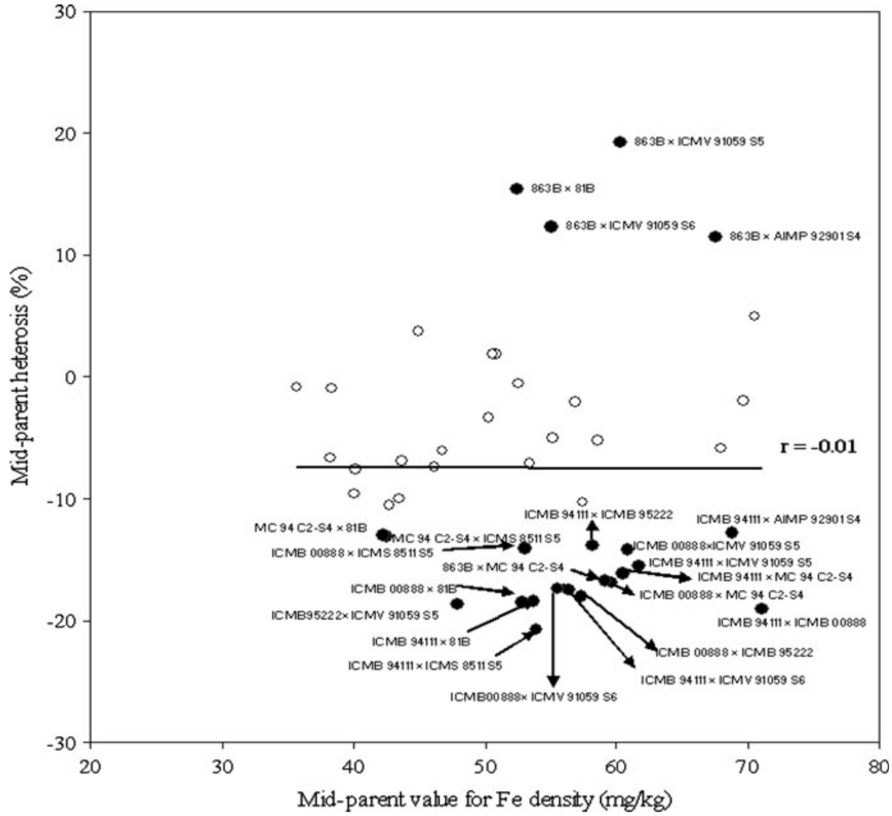

Fig. 2 Relationship between mid-parent values and mid-parent heterosis for Fe density (*filled circles* hybrids with significant positive and negative mid-parent heterosis) in pearl millet

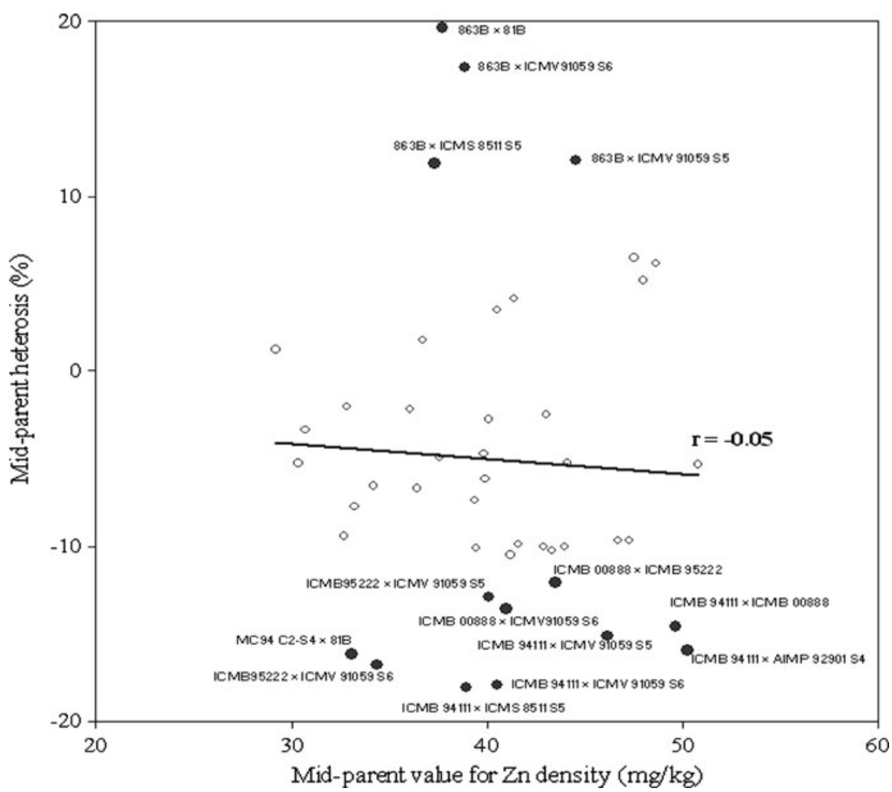

Fig. 3 Relationship between mid-parent values and mid-parent heterosis for Zn density (*filled circles* hybrids with significant positive and negative mid-parent heterosis) in pearl millet

exploit heterosis for these micronutrients in pearl millet, and to breed high Fe and Zn hybrids would require incorporating these traits into both parental lines.

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