RESEARCH

Tester Effect on Combining Ability and Its Relationship with Line Performance per se for Grain Iron and Zinc Densities in Pearl Millet

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ABSTRACT

Development of inbred lines with high general combining ability (GCA) is an important aspect of hybrid breeding research. Although broad-based open-pollinated populations (varieties and composites) are widely used to assess GCA of inbred lines and population progenies, identification of the most effective testers has remained a continuing challenge. Two broad-based and diverse populations of pearl millet [*Pennisetum glaucum* (L.) R. Br.] were crossed with 14 diverse inbred lines in each of two experiments to produce 56 topcross hybrids, which were evaluated along with their parental lines for grain Fe and Zn densities. Results showed that there was highly significant and moderately high positive correlation between the topcross hybrid performance per se (a measure of GCA) produced with the two testers, both for Fe and Zn densities in both experiments. However, the correlation between performance per se of lines and their topcross hybrids averaged over both testers (giving more reliable estimates of GCA than individual testers) was even higher for both micronutrients and in both experiments. Thus, while each tester was effective in identifying 30 to 35% of the top-ranking high general combiners as reflected in topcross hybrid performance, line performance per se was even more effective in identifying these top-ranking combiners for both micronutrients in both experiments, indicating that based on performance per se lines can be selected for high GCA of Fe and Zn densities in pearl millet.

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Abbreviations: AAS, atomic absorption spectroscopy; B-line, maintainer line; GCA, general combining ability; ICMB, ICRISAT millet B-line; ICP–OES, inductively coupled plasma optical emission spectrometry; IPC, ICRISAT pollinator collection; OPV, open-pollinated variety; PRP, potential restorer parents; R-line, restorer line; TC, topcross.

Inadequate intake of Fe is the most common inducer for anemia, which affects more than 25% of the population globally, and the highest prevalence of anemia related to low Fe intake is in preschool-age children (47%) and pregnant women (42%) (WHO, 2008). An estimated 17.3% of the global population is at risk of inadequate Zn intake (Wessells and Brown, 2012). Deficiency of Fe and Zn results in poor growth, reduced immunity, fatigue, irritability, weakness, hair loss, wasting of muscles, sterility, morbidity and even death in acute cases (Haas and Brownlie, 2001; Pfeiffer and McClafferty, 2007; Stein, 2010). The HarvestPlus Challenge Program of the CGIAR has initiated resource mobilization and research coordination to address this malnutrition issue through biofortification of several staple crops including pearl millet. Pearl millet is an important staple food crop in the arid and semiarid tropical regions of Asia and Africa. In India, for instance, pearl millet accounts for 20 to 63% of the total cereal consumption in parts of major pearl millet growing states, such as Maharashtra, Gujarat, and Rajasthan (Parthasarathy Rao et al., 2006). Recent studies at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) conducted in alliance with the HarvestPlus Program have shown the presence of large heritable genetic variability for grain Fe and Zn densities in pearl

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millet (Velu et al., 2008a, 2008b; Rai et al., 2012, 2015b; Govindaraj et al., 2013), indicating good prospects for genetic enhancement of these micronutrients.

In cultivar development programs of cross-pollinated crops, performance per se of lines and population progenies for yield, and traits related to biotic stress resistance and abiotic stress tolerance, is as important as their GCA. For quality traits, however, it is the combining ability which is utmost important, especially in hybrid development programs. Development of simple and cost-effective methods to assess the GCA of new inbred lines has been a major challenge in the development of new hybrids (Matzinger, 1953). One way to study the combining ability of a set of lines is to cross them in all possible combinations or with another set of inbred lines as testers. The number of hybrids to be produced and evaluated, following these methods, becomes increasingly expensive as the number of lines involved increases. Thus, Davis (1927) suggested the use of open-pollinated populations (varieties and composites) as testers whereby inbred lines and population progenies crossed with them enable evaluation of topcross hybrid performance, which gives a measure of GCA of lines. However, much of the research conducted on maize (*Zea mays* L.) on an appropriate population tester remains inconclusive (Hallauer et al., 1988). In the pearl millet biofortification program targeted for genetic improvement of Fe and Zn densities, population progenies are advanced and parental lines of potential hybrids are developed based on their performance per se for these micronutrients. There is no information on broad-based tester effect on GCA, if the program were to undertake selection for GCA for Fe and Zn densities. The objectives of this research were to examine the effect of two open-pollinated varieties (OPVs) as testers on topcross performance (a measure of GCA), and also the relationship between performance per se of lines and their topcross performance for Fe and Zn densities in pearl millet.

MATERIALS AND METHODS

Genetic Materials

Two experiments were conducted that included 28 inbred lines and 56 topcross hybrids. Exp. 1 consisted of 14 maintainer lines (hereafter referred to as B-lines) and their 28 topcross hybrids (referred to as hybrids). Hybrids were produced by crossing each B-line with tester population Raj 171 (TC1) and tester population ICMR 312 (TC2). Similarly, Exp. 2 consisted of 14 restorer lines (hereafter referred to as R-lines) and their 28 topcross hybrids. Hybrids were produced by crossing each R-line with Raj 171 (TC3) and ICMR 312 (TC4). The B- and R-lines included in this study represented inbred lines having twofold differences both for Fe (30–82 mg kg^{-1}) and Zn (27–56 mg kg^{-1}) densities and had been previously used in a line × tester study of combining ability for Fe and Zn densities (Kanatti et al., 2014). Raj 171 is a released commercial open-pollinated variety (Christinck et al., 1999) and ICMR 312 is a population used as a pollinator of a released topcross hybrid ICMH 312 (Witcombe et al., 1996). These two populations represent diverse genetic backgrounds. Raj 171 was developed by recombining eight S_1 progenies derived from inter-varietal composite. ICMR 312 was developed by recombining 200 S_1 progenies derived from Bold-Seeded Early Composite (BSEC TCP2 C3), which is entirely an *Iniadi* germplasm-based early-maturing population.

At the panicle emergence stage, panicles of 12 to 15 plants of each inbred line and about 400 plants of each tester population were covered with parchment paper bags to avoid the pollination of stigmas from any foreign pollen (full stigma emergence takes 3–5 d from boot leaf stage). Pollen collected from 50 to 60 plants of each tester were bulked and crossed onto five to six plants of each inbred line at full stigma stage, to produce topcross hybrids. This process continued until crosses were completed on all inbred lines. Cross-pollinated panicles of each inbred line were harvested at or after physiological maturity, sundried for 10 to 12 d, and bulk threshed to produce the topcross hybrid seed for a hybrid trial. The bagged panicles of six to eight plants of each line left covered till maturity were harvested, sundried and bulk threshed to produce the selfed seed of B- and R-lines for parental trial.

Field Trials

The hybrid and parent trials were evaluated during 2012 summer season (January–April) and 2013 summer season (January–May) in Alfisols at ICRISAT, Patancheru. The area receives 800 mm average annual rainfall; the average minimum temperature is 19°C and maximum temperature 32°C. Monthly mean temperatures varied from 15 to 38°C and precipitation from 0 to 17 mm during the 2012 summer season. In the 2013 summer season, monthly mean temperatures varied from 15 to 40°C and precipitation from 0 to 68 mm. Topcross hybrids in both experiments were planted in three replications in split-plot design with genetic backgrounds of the two testers populations as main plots and hybrids of each tester population randomized as subplots. The parental lines were planted in three replications in randomized complete block design by the side of the hybrid trial. Planting was done by tractor-mounted 4-cone planter (7100 US model) having base units from John Deer, Moline, IA, and metering units from WinterSteiger AG, Dimmelstraβe 9, 4910 Ried/Innkreis, Austria. Each entry was planted in one row of 4 m in parental trials and two rows of 4 m in topcross hybrid trials, spaced at 60 cm between the rows. Overplanted plots were thinned and manually weeded 15 d after planting to single plants spaced 15 cm apart within each row. Another manual weeding was also done 30 d after planting. A basal dose of 100 kg of diammonium phosphate (18% N and 46% P) was applied at the time of field preparation and 100 kg of urea (46% N) was applied as top dressing within 2 to 4 d after thinning. Trials were irrigated at 7 to 10 d intervals to ensure no moisture stress throughout the crop season.

The plots of all the entries were harvested at or after physiological maturity (85–90 d after planting). An earlier study had shown that open-pollinated grain samples in pearl millet provide more reliable and cost-effective sampling for Fe and Zn densities analysis as there is neither xenia effect nor any significant dust contamination effect in pearl millet (Rai et al., 2015a). Therefore, open-pollinated main panicles of 10 random plants from each plot in hybrid and parental trials were harvested in a cloth bag and sundried for 10 to 15 d. These were hand threshed and approximately 20 g of grains were collected for Fe and Zn densities analysis.

Micronutrient Analysis

Grain Fe and Zn densities were analyzed at the Charles Renard Analytical Laboratory, ICRISAT, Patancheru, India following the method described by Wheal et al. (2011). The ground samples were digested in closed tubes; and Fe and Zn in the digests were analyzed using inductively coupled plasma optical emission spectrometry (ICP–OES). Briefly, grain samples were finely ground and oven dried at 60°C for 48 h before analyzing them for Fe and Zn. Ground sample (0.2 g) was transferred to 25 mL polyprophelene PPT tubes; digestion was initiated by adding 2.0 mL of concentrated nitric acid (HNO_3) and 0.5 mL of 30% hydrogen peroxide (H_2O_2). Tubes were vortexed to ensure the entire sample was wetted, and then predigested overnight at room temperature. Tubes were vortexed again before placing them into the digestion block and initially heated at 80°C for 1 h, followed by digesting at 120°C for 2 h. After digestion, the volume of the digest was made to 25 mL using distilled water; and the content was agitated for 1 min by vortex mixer. The digests were filtered and Fe and Zn densities were determined using ICP–OES. Care was taken at each step to avoid any contamination of the grains with dust particles and any other extraneous matter following Stangoulis and Sison (2008). The soil samples collected from top 30 cm layer were analyzed for extractable Fe and Zn content by atomic absorption spectroscopy (AAS) as described by Lindsay and Norvell (1978). The mean soil Fe and Zn contents extractable with diethylene triamine pentaacetic acid (DTPA) were respectively, 12.1 and 4.5 mg kg^{-1} in the 2012 summer season, and 3.6 and 3.5 mg kg^{-1} in the 2013 summer season. These Fe and Zn contents in the soil were well above the critical levels of 2.6 to 4.5 mg kg^{-1} Fe content, and 0.6 to 1.0 mg kg^{-1} Zn content required by plants (Tisdale et al., 1993; Sahrawat and Wani, 2013).

Statistical Analysis

Data were analyzed using Statistical Analysis System (SAS) version 9.2 (SAS Institute, 2009). Analysis of variance pooled over the two environments were performed using Generalized Linear Model procedures following fixed-effects model (Hallauer et al., 1988; McIntosh, 1983). The Pearson correlation coefficient among the traits was calculated using the PROC CORR procedure.

RESULTS AND DISCUSSION

Variability Among Inbred Parents

There were highly significant differences ($P < 0.01$) among inbred lines in both experiments (B-lines and R-lines) for grain Fe and Zn densities (Table 1). Inbred line × environment interactions were also highly significant ($P < 0.01$), except for Fe density in R-lines. However, the contribution of this interaction to the variability relative to that due to genotypic differences among the B-lines was 15% for Fe density and 32% for Zn density. The contribution of R-line × environment interaction to the variability relative to that due to genotypic differences among R-lines was very low (8%) for Zn density.

Table 1. Mean squares for grain Fe and Zn densities across 2 yr (environment) among B-lines and R-lines of pearl millet in parental trial, Patancheru.

		B-line		R-line	
		Fe density	Zn density	Fe density	Zn density
Environment (E)	1	506.3	2106.7**	1411.5**	2030.5**
Replication/E	4	96.9**	62.1	99.3*	17.7
Inbred (P)	13	1416.8**	398.1*	1208.9**	750.5**
P × E	13	208.4**	128.8**	34.2	60.2**
Error	52	26.3	44.0	33.0	15.7

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

Table 2. Mean squares for grain Fe and Zn densities across 2 yr (environment) among B-line topcross hybrids of pearl millet in Exp. 1, Patancheru.

Source of variation	df	Fe density	Zn density
Environment (E)	1	0.1	2118.3**
Replication (R)/E	4	13.9	43.1
Tester (T)	1	733.2**	101.6
T × E	1	29.9	34.3
T × R/E	4	8.2	16.5
B-line hybrid (BLH)	13	359.2**	148.0**
BLH × E	13	55.0	46.4**
BLH × T	13	75.8**	39.7**
BLH × T × E	13	31.3	33.2**
Pooled error	104	25.7	14.8

** Significant at the 0.01 probability level.

Tester Effect on Topcross Hybrids

In Exp. 1, the difference between the two population testers as well as among the B-lines for their effect on topcross hybrids was highly significant for Fe density (Table 2). Hybrid × tester interaction was also highly significant for both micronutrients, accounting for 21 to 27% of the variation relative to those due to differences among the hybrids. These low order of hybrid × tester interactions were reflected in significant and moderate positive correlations between the topcross hybrids of Raj 171 (TC1) and those of ICMR 312 (TC2), both for Fe density ($r = 0.64$, $P < 0.05$) and Zn density ($r = 0.62$, $P < 0.05$) (Table 3). Topcross hybrid of ICMB 08222 in TC2 had the highest Fe density of 62 mg kg^{-1}. The other four hybrids that closely followed it with 53 to 60 mg kg^{-1} Fe density were based on ICMB 93222, ICMB 98222, ICMB 05555, and ICMB 88004, in that order. Hybrids of four of these

Table 3. Performance per se of B-lines (B) of pearl millet and their topcross hybrids with testers Raj 171 (TC1) and ICMR 312 (TC2) for grain Fe and Zn densities, averaged over a 2 yr study, Patancheru.

	Fe density				Zn density			
		Topcross hybrid				Topcross hybrid		
B-line	B	TC1	TC2	Mean	B	TC1	TC2	Mean
	mg kg^{-1}							
ICMB 88004	59	56	53	55	50	54	51	53
ICMB 92111	40	38	42	40	40	41	48	44
ICMB 92888	44	37	52	45	43	40	48	44
ICMB 93222	81	59	60	60	58	56	53	54
ICMB 97111	46	42	43	43	39	44	42	43
ICMB 98222	76	47	55	51	53	48	50	49
ICMB 02555	58	46	52	49	54	50	48	49
ICMB 04888	54	46	48	47	43	43	47	45
ICMB 05555	77	52	54	53	60	50	52	51
ICMB 07555	54	49	47	48	48	50	48	49
ICMB 07777	41	45	46	46	37	45	45	45
ICMB 07999	37	42	47	45	37	43	46	44
ICMB 08222	78	51	62	56	57	49	54	52
ICMB 08333	45	39	50	44	43	44	49	46
Mean	56	46	51	49	47	47	49	48
LSD (0.05)	5.9	5.8	5.9	5.8	7.7	4.5	4.4	4.4
	Correlation coefficient†							
r1 (B, TCs)		0.78**	0.84**	0.89**		0.77**	0.83**	0.88**
r2 (TC1, TC2)		0.64*				0.62*		
r3 (Fe, Zn)	0.93**	0.95**	0.87**	0.95**				

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† r1, correlations of B with TC1, TC2, and mean of TC1 and TC2; r2, correlation between TC1 and TC2; r3, correlations between Fe and Zn densities.

Table 4. Mean squares for grain Fe and Zn densities across 2 yr (environment) among R-line topcross hybrids of pearl millet in Exp. 2, Patancheru.

Source of variation	df	Fe density	Zn density
Environment (E)	1	153.0	3216.5**
Replication (R)/E	4	43.7	39.1
Tester (T)	1	1392.1**	351.4**
T × E	1	<0.1	34.7
T × R(E)	4	16.5	8.1
R-line hybrid (RLH)	13	546.5**	336.6**
RLH × E	13	27.0	9.4
RLH × T	13	72.7**	40.7**
RLH × T × E	13	40.6*	10.5
Pooled error	104	22.4	11.3

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

B-lines, except for ICMB 98222, had 51 to 59 mg kg^{-1} Fe density and were among the four top-ranking entries in TC1. Hybrids of the above B-lines, with 50 to 54 mg kg^{-1} Zn density were also among the five top-ranking entries in TC2, and hybrids with 50 to 56 mg kg^{-1} Zn were among the five top-ranking entries in TC1. Based on mean performance over both testers that give more reliable estimates of GCA than individual testers, B-lines of four top-ranking hybrids for Fe density were also among the four top-ranking hybrids for Zn density. This is not unexpected considering that there was highly significant and high positive correlation ($r = 0.95$, $P < 0.01$) between the Fe and Zn densities (Table 3). Similar order of high positive correlations between these micronutrients have been reported in previous pearl millet studies (Velu et al., 2007; Gupta et al., 2009; Rai et al., 2012, 2013, 2015b; Govindaraj et al., 2013; Kanatti et al., 2014). These results are in conformity with the findings of an earlier study (Kanatti et al., 2014) in which all these above B-lines had been found among the five top-ranking general combiners, both for Fe and Zn densities.

In Exp. 2 also, the difference between the two testers as well as among the R-lines for their effects on topcross hybrids was highly significant, both for Fe and Zn densities (Table 4). Hybrid × tester interactions were also highly significant for both micronutrients, but these accounted for only 12 to 13% of variability relative to those

Table 5. Performance per se of R-lines (R) of pearl millet and their topcross hybrids with testers Raj 171 (TC3) and ICMR 312 (TC4) for grain Fe and Zn densities, averaged over a 2 yr study, Patancheru.

	Fe density				Zn density			
		Topcross hybrid				Topcross hybrid		
R-line	R	TC3	TC4	Mean	R	TC3	TC4	Mean
	mg kg^{-1}							
PRP 1	47	41	48	45	50	47	52	50
PRP 2	89	57	57	57	69	55	54	54
PRP 3	73	53	56	54	57	53	48	50
PRP 4	61	56	65	60	54	54	58	56
PRP 5	66	43	59	51	60	48	56	52
PRP 6	42	42	47	44	41	44	47	46
PRP 7	52	43	54	48	46	46	50	48
PRP 8	60	45	57	51	55	45	55	50
PRP 9	50	36	39	37	39	39	38	39
IPC 390	48	42	40	41	38	38	42	40
IPC 616	85	52	51	52	71	52	51	52
IPC 843	67	47	51	49	63	52	54	53
IPC 1178	57	44	51	47	65	50	54	52
IPC 1354	37	36	41	38	38	40	43	42
Mean	60	46	51	48	53	47	50	49
LSD (0.05)	6.7	5.2	5.8	5.4	4.6	4.1	3.7	3.9
	Correlation coefficient†							
r4 (R, TCs)		0.83**	0.58*	0.75**		0.87**	0.72**	0.84**
r5 (TC3, TC4)		0.75**				0.78**		
r6 (Fe, Zn)	0.88**	0.88**	0.87**	0.89**				

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† r4, correlations of R with TC3, TC4, and mean of TC3 and TC4; r5, correlation between TC3 and TC4; r6, correlations between Fe and Zn densities.

due to genetic differences among the hybrids. There was highly significant and high positive correlation between the hybrids of Raj 171 (TC3) and those of ICMR 312 (TC4) for Fe density ($r = 0.75$, $P < 0.01$) and Zn density ($r = 0.78$, $P < 0.01$) (Table 5). Hybrid of PRP 4 in TC4 had the highest Fe density of 65 mg kg^{-1}. It was followed by hybrids on PRP 5, PRP 8, PRP 2, and PRP 3 in that order, which had 56 to 59 mg kg^{-1}of Fe density that were not significantly different from one another. Hybrids of three of these R-lines with 53 to 57 mg kg^{-1} Fe were among the three top ranking entries in TC3. Hybrid of PRP 4 in TC4 also had highest Zn density of 58 mg kg^{-1}. It was closely followed by hybrids of PRP 5, PRP 8, IPC 1178, and IPC 843, which had 54 to 56 mg kg^{-1} of Zn density. Hybrids of these were among the five top-ranking entries in TC3, although not significantly different from one another. Based on the mean performance of hybrids averaged over both testers, PRP 4, PRP 2, PRP 3, IPC 616, and PRP 8, in that order, were the five top-ranking entries with 51 to 60 mg kg^{-1} Fe density. While hybrid of PRP 4 was also the top-ranking entry with 56 mg kg^{-1} of mean Zn density, hybrids of other R-lines with 50–54 mg kg^{-1} Zn density closely followed it and were not significantly different from one another. An earlier study (Kanatti et al., 2014) also found PRP3, PRP2, PRP5, and PRP4, in that order, as the four highest general combiners for Fe density, and these were also among the five top-ranking general combiners (except PRP 4) for Zn density.

The two OPV testers used this study were of diverse genetic backgrounds, and had large difference for Fe density (45 mg kg^{-1} for Raj 171 and 56 mg kg^{-1} for ICMR 312). However, these had similar levels of Zn density (45–46 mg kg^{-1}). Therefore, a natural question arises whether use of testers with much larger difference for Fe and Zn densities could have led to much greater hybrid × tester interactions, and consequently to reduction in correlation coefficients between the hybrids of these testers. This would then raise a further question on whether low or high micronutrient population would be considered a more appropriate tester for GCA assessment. Since, all the B- and R-lines used in this study had been included in an earlier line × tester study of combining ability (Kanatti et al., 2014), we re-analyzed the data of that study by constituting three different sets of B-lines

Table 6. Means and ranges of grain Fe and Zn densities for three different tester sets of B- and R-line in pearl millet (data from Kanatti et al., 2014).

Inbred line	Testers set	Fe Mean	Fe Range	Zn Mean	Zn Range
		mg kg^{-1}			
B-line	High	65	59–77	44	41–45
	Medium	49	44–53	39	37–40
	Low	35	30–39	30	27–33
R-line	High	65	56–82	50	46–55
	Medium	46	43–52	41	38–44
	Low	38	32–42	32	29–36

Table 7. Correlation coefficients among general combining ability estimates of pearl millet B- and R-lines with tester sets at three levels of grain Fe and Zn densities (data from Kanatti et al., 2014).

Tester sets	B-line Fe	B-line Zn	R-line Fe	R-line Zn
High vs. Medium	0.95**	0.87**	0.91**	0.98**
High vs. Low	0.84**	0.83**	0.88**	0.95**
Medium vs. Low	0.79**	0.75**	0.87**	0.96**

** Significant at the 0.01 probability level.

as well as three different sets of R-lines, each of which included four inbred lines with high, medium, and low levels of Fe and Zn densities. The mean and range of Fe and Zn densities for these three sets of both B- and R-lines are given in Table 6. The three sets of B-lines were used as testers to determine the combining ability of 14 R-lines. Similarly, the three sets of R-lines were used as testers to determine the combining ability of 14 B-lines. Results showed that even with most divergent sets of high vs. low testers, there were highly significant and high positive correlations between the general combining ability estimates in B-lines as well as in R-lines, both for Fe and Zn densities ($r \geq 0.83$, $P < 0.01$) (Table 7). In fact, these correlation coefficients were higher than those observed in the present topcross tester study, indicating that Fe and Zn densities levels of testers would not have any effect on the topcross hybrid performance for these micronutrients. Such results are expected when the characters are largely under additive genetic control, as reported for Fe and Zn densities in pearl millet (Velu et al., 2011; Govindaraj et al., 2013; Kanatti et al., 2014).

Relationship between Line Performance per se and Topcross Hybrids

In Exp. 1, there was highly significant and high positive correlation between performance per se of B-lines and their hybrids averaged over the two testers for Fe density ($r = 0.89$, $P < 0.01$) as well as for Zn density ($r = 0.88$, $P < 0.01$) (Table 3). ICMB 93222, ICMB 08222, ICMB 05555, and ICMB 98222 were, in that order, the four top-ranking lines with highest Fe density of 76 to 81 mg kg^{-1}, although not significantly different from one another. Hybrids of these B-lines were among the five top-ranking entries with 51 to 60 mg kg^{-1} of Fe density. The above four B-lines were also among the five top-ranking genotypes for Zn density with respect to performance per se (53–60 mg kg^{-1}) as well for hybrid performance (49–54 mg kg^{-1}). In an earlier study also, these four lines had been identified among the four top-ranking entries with respect to general combining ability of both micronutrients and for performance per se of Fe density; and among the five top-ranking entries for performance per se of Zn density (Kanatti et al., 2014).

In Exp. 2 also, there was highly significant and high positive correlation between performance per se of R-lines and their topcross hybrids averaged over the two testers for Fe density ($r = 0.75$, $P < 0.01$) as well as for Zn density ($r = 0.84$, $P < 0.01$) (Table 5). PRP 2, IPC 616, PRP 3, IPC 843, and PRP 5, in that order, were the five top-ranking R-lines for performance per se with 66 to 89 mg kg^{-1} Fe density. Hybrids of four of these, except for IPC 843, were among the five top-ranking entries. Four of these R-lines, except for PRP 3, had 60 to 71 mg kg^{-1} Zn density and were among the five top-ranking lines for Zn density. Hybrids of these five R-lines, which were included in the five top-ranking entries, had 50 to 54 mg kg^{-1} of Zn density, which were not significantly different from one another. Kanatti et al. (2014) also identified PRP 2, PRP 3, PRP 5, IPC 843, and IPC 616 among the five top-ranking R-lines with respect to performance per se, both for Fe and Zn densities. While four of these, except for IPC 843, were also among the five top-ranking general combiners for Fe density; four of these, except for IPC 616, were among the four top-ranking general combiners for Zn density.

The nature of relationships between performance per se of lines and their hybrids in both experiments as mentioned above are not unexpected considering predominantly additive gene action for these micronutrients in pearl millet (Velu et al., 2011; Govindaraj et al., 2013; Kanatti et al., 2014). Highly significant and high positive correlations were observed between the Fe and Zn densities for line performance per se ($r = 0.93$, $P < 0.01$) and for hybrid performance ($r = 0.95$, $P < 0.01$) in Exp. 1 (Fig. 1) and for line performance per se ($r = 0.88$, $P < 0.01$) and hybrid performance ($r = 0.89$, $P < 0.01$) in Exp. 2 (Fig. 2). Thus, lines among the top-ranking entries for Fe density were generally among the top-ranking entries for Zn density, both for performance per se as well as for hybrid performance, the more so in Exp. 1 consisting of B-lines than Exp. 2 consisting of R-lines. All these top-ranking lines are entirely based on *Iniadi* germplasm (namely, ICMB 98222, ICMB 88004, PRP 2, PRP 3, PRP 5) or have largely *Iniadi* germplasm in their parentage. The *Iniadi* germplasm has been shown to have large genetic variability with highest levels of both micronutrients available in pearl millet germplasm (Rai et al., 2015b).

CONCLUSIONS

This study showed significant line × tester interactions for Fe and Zn densities. However, their magnitudes were small, leading to highly significant and moderate high positive correlations between the topcross performance (GCA) with the two testers for both micronutrients and experiments. Thus, any one of the two testers would be equally effective in identifying the top group of high general combiners. Further, about 30 to 35% lines selected on the basis of performance per se at various inbreeding stages would include all the top-ranking general combiners for Fe and Zn densities in pearl millet. Those few lines selected as high GCA candidate lines can then be further evaluated for their topcross performance with two to three broad-based testers to validate those as high general combiners, which can be used for their conversion into hybrid parents and for hybridization to initiate the next cycle of biofortified inbred line development.

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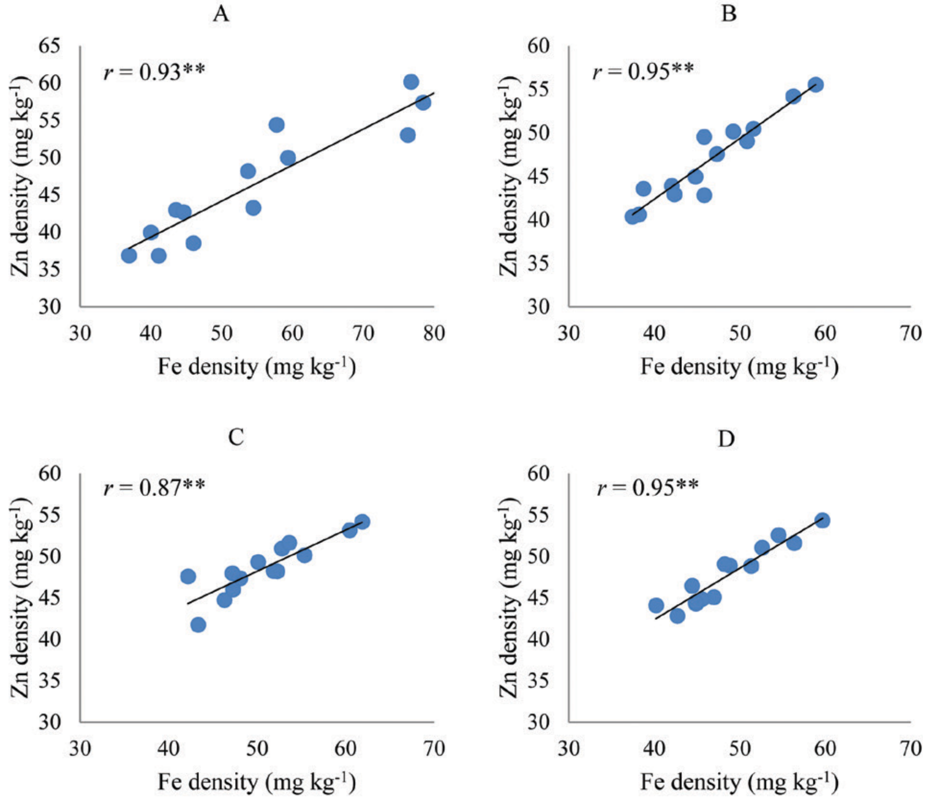

Figure 1. Relationship between grain Fe and Zn densities in (A) B-lines and their topcross hybrids, (B) tester Raj 171, (C) tester ICMR 312, and (D) the mean of both testers in Exp. 1, averaged over a 2 yr study, Patancheru. ** Significant at the 0.01 probability level.

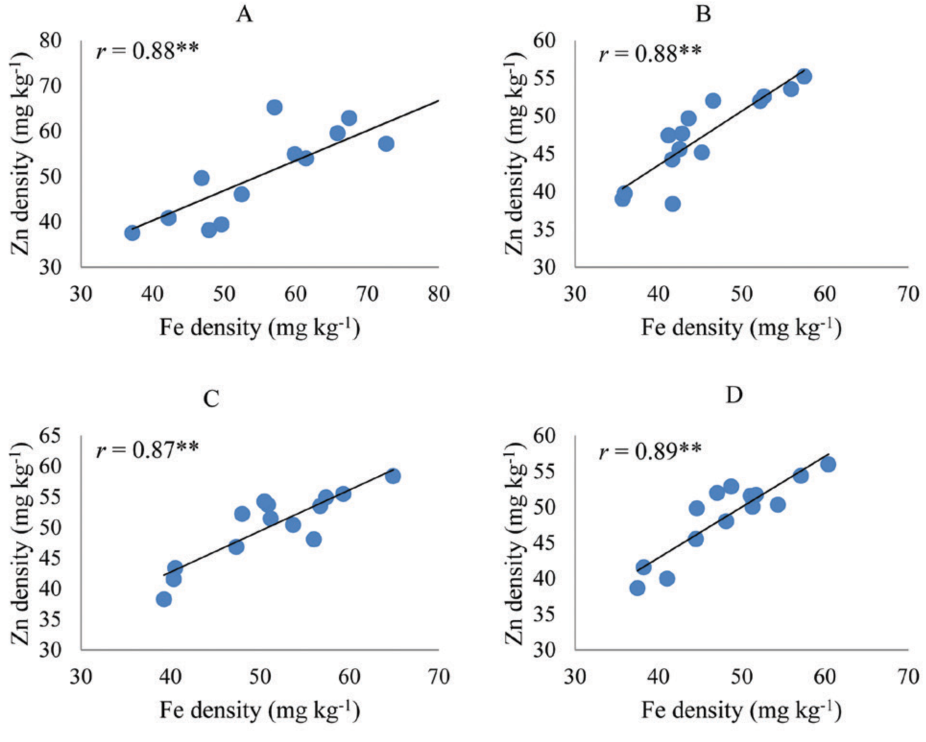

Figure 2. Relationship between grain Fe and Zn densities in (A) R-lines and their topcross hybrids, (B) tester Raj 171, (C) tester ICMR 312, and (D) the mean of both testers in Exp. 2, averaged over a 2 yr study, Patancheru. ** Significant at the 0.01 probability level.

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