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Combining ability and heterosis for grain iron and zinc content 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 ($2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$) 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, Heterosis, Pearl millet, *Pennisetum glaucum*

Introduction

Micronutrient malnutrition resulting from dietary deficiency of one or more micronutrients has been recognized as a serious human health problem worldwide. The most striking of these are iron (Fe) and zinc (Zn) deficiencies that rank 9^{th} and 11^{th}, respectively, among the top 20 risk factors contributing to global burden of disease (WHO 2002) [12]. Pharmaceutical supplementation, industrial fortification and dietary diversification are some of the interventions that have been used to address this problem. Notwithstanding the recurring cost, the impact of supplementation and fortification in the developing countries remains limited because of poor infrastructure and delivery system. Dietary diversification raises an issue of diverse food affordability since a sharp increase in food prices will have a large impact on poor households. It also has problem of consumer acceptance in case dietary diversification calls for including foods which are not a part of conventional diets. Biofortification of staple crops, especially for mineral micronutrients, is a sustainable and cost-effective approach. It has great promise for improving the mineral nutritional status and health of poor populations in both rural and urban areas of the developing world. Biofortified cultivars of staple crops improved for mineral micronutrients are also readily acceptable to consumers as their adoption does not call for change in dietary habits.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a major warm-season cereal grown on 28 million ha for grain and fodder production in some of the most marginal areas of the arid and semi-arid tropical regions of Asia and Africa. In these regions, pearl millet is a major source of dietary energy and mineral micronutrients. India is the largest producer of this crop with >9 million ha area and 8.5 million tons of grain production. The contribution of pearl millet to the total Fe and Zn intake from all food sources has been reported to very widely vary across rural India.

For instance, it was observed to be contributing 19-63% of the total Fe intake and 16-56% of the total Zn intake in parts of Rajasthan, Maharashtra and Gujarat states (Parthasarathy Rao *et al*. 2006) [10]. Large genetic variability for Fe and Zn density observed in the breeding lines, improved populations and germplasm (Velu *et al*. 2007) [11] provides for good prospects to breed improved pearl millet cultivars with elevated levels of these micronutrients. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), supported by Harvest Plus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR), and in partnership with the public and private sector breeding program in India, has undertaken a major initiative to develop high-yielding hybrids with high levels of Fe and Zn density in pearl millet.

Pearl millet is a highly cross-pollinated crop with open-pollinated varieties (OPVs) and hybrids as the two broad cultivar options. Hybrids are the most dominant cultivars in India, occupying >70% of area under improved pearl millet cultivars, with OPVs cultivated on limited scales. A preliminary study showed about two-fold differences for Fe and Zn densities among pearl millet hybrids under cultivation in India, with Fe density varying from 31 to 61 mg kg^{-1} and Zn density varying from 32 to 54 mg kg^{-1} . An understanding of the nature of genetic variability and heterosis will have a direct bearing on devising effective hybrid breeding strategies for Fe and Zn density. There is limited information on genetic variability and heterosis for Fe and Zn density in pearl millet. While improving the Fe and Zn densities, it is important that genetic gains for these micronutrients are not made at the expense of grain yield and grain size. The main objective of this research was to examine the nature of genetic variability in relation to heterosis for Fe and Zn density. Since, there exists a wealth of literature on genetic variability and heterosis for grain yield and grain size, genetic variability and heterosis for these two traits were studied in the specific context of their associations with Fe and Zn densities.

Material and methods

Ten genetically diverse inbreds of pearl millet viz., J-2340, MIR-525-2, RIB-192, RIB-494, RIB-3135-18, RIB 57, RIB-335/74, HBL-11, H-77/833-2-202 and G-77/107 were crossed in all possible combinations excluding reciprocals during summer 2015 to generate a diallel set. Ten parents' along with their 45 F1s were evaluated for grain yield and 13 yield components in a Randomized Block Design with three replications at Rajasthan Agricultural Research Institute, Durgarura, Jaipur (Rajasthan), India, during Kharif-2015. Each entry was sown in two rows of 4.0 m length having 50 x 15 cm crop geometry. All the recommended cultural practices were adopted to raise good crop of pearl millet. Observations were recorded on ten randomly selected competitive plants for each entry, in each replication for 13 characters (Table 1). Days to flowering and days to maturity were noted on the basis of whole plot.

a) **Iron and Zinc content analysis**
b) The Iron and Zinc will be analyzed at ICRISAT using an energy-dispersive X-ray fluorescence Spectrometry (EDXRF) method that had been standardized at the Flinders University, Australia. For the analysis of Zinc and Iron the ear heads of the selected plants are harvested separately and then sun dried for seven days and stored for one to two months before analysis of iron and Zinc content.

Statistical analysis

c) Analysis of variance for combining ability will be done environment wise following Griffing's (1956) [4] Method II and Model I. The combining ability analysis of data pooled over the environments will be performed using the method elaborated by. Heterosis expressed as percent increase or decrease in the mean value of F_1 hybrid over mid parent, and heterobeltiosis as increase or decrease in the mean value of F_1 hybrid over better parent, will be calculated according to method suggested by.

Results

d) **Combining ability**
e) analysis was done for individual environment. Highly significant mean sum of square due to GCA and SCA in all the three environments indicate that all the characters were controlled by both additive and non-additive gene effects. variance of GCA/variance of SCA ratio was less than unity for all the characters which showed preponderance of non-additive gene actions in all the three environments. (Table 1, 2)

Parent RIB-3135 followed by RIB-335/74, MIR-525-2 and RIB-192 were found to be uniformly best parent across the environments for grain yield per plant with Fe and Zn content, while parent G77/107 was found to be uniformly undesirable parent across the environment with high negative effects. Parent HBL-11, RIB-57 and H77/833-2-202 was found to be a better general combiner across the environments for Fe content, parent G77/107 was found to be a better general combiner for both Fe and Zn content.

In the present study, crosses with high SCA effects involving good x good general combiners were P1 x P2, P2 x P5,P2 x P6, P2 x P7, P4 x P7, P5 x P9, P6 x P7 and P7 x P9 (E_1, E_2 and E_3 environments), P5 x P10 and P3 x P8 (E_1 environment), P5 x P10 (E_2 environment) and P3 x P8 (E_3 environment) for grain yield per plant and related traits (Table: 3). These crosses offer good promise for improvement of respective component trait and ultimately grain yield in respective environment. The transgressive segregants could be isolated in higher frequency from these crosses and utilize to generate inbred lines using conventional breeding method for further crop improvement programmes.

Heterosis

Fe content

In the present study, the magnitude of heterosis over mid parent value ranged from – 50.75 (P2 x P4) to 24.72 (P2 x P6) in E_1, from -44.54 (P7 x P9) to 66.08 (P4 x P6) in E_2 and from -30.07 (P3 x P6) to 40.68 (P4 x P6) in E_3. Similarly, heterobeltiosis ranged from -56.18 (P2 x P4) to 17.46 (P6 x P8) in E_1, from -53.87 (P7 x P9) to 59.15 (P4 x P6) in E_2 and from – 35.43 (P3 x P6) to 25.34 (P4 x P6) in E_3 (Table 4.5.12).Out of forty five crosses, fourteen crosses in E_1, ninteen crosses in E_2 and twenty one crosses in E_3 showed positive significant heterosis over mid parental value. Similarly, six crosses in each in E_1, twelve in E_2 and fourteen crosses in E_3 showed positive significant heterosis over their respective superior parent. The crosses P6 x P8 showed positive significant heterosis and heterobeltiosis in the entire three environments. Hence, these crosses were desirable for higher Fe content. (Table 4).

Zn content
In the present study, the magnitude of heterosis over mid parent value ranged from – 63.41 (P2 x P4) to 32.59 (P2 x P6) in E_1, from -37.72 (P7 x P9) to 123.37 (P4 x P6) in E_2 and from -59.40 (P3 x P6) to 112.92 (P4 x P6) in E_3. Similarly, heterobeltiosis ranged from -63.41 (P2 x P4) to 28.81 (P6 x P8) in E_1, from -46.89 (P7 x P9) to 94.18 (P4 x P6) in E_2 and from – 63.68 (P3 x P6) to 91.69 (P4 x P6) in E_3.Out of forty five crosses, eleven crosses in E_1, twenty eight crosses in E_2 and thirty crosses in E_3 showed positive significant heterosis over mid parental value. Similarly, seven crosses in $E_{1,}$ twenty two in E_2 and fourteen crosses in E_3 showed positive significant heterosis over their respective superior parent. The crosses P1 x P10 and P2 x P4 showed positive significant heterosis and heterobeltiosis in the entire three environments. Hence, these crosses were desirable for higher Zn content. (Table 4).

Conclusions
In present investigation, there was no consistency over environment for the ranks of crosses with high SCA effects. Furthermore, a number of crosses exhibited changes in the magnitude and direction of SCA effects in different environments, which might be consequence of high SCA x Environment interaction. Crosses offer good promise for improvement of respective component trait and ultimately grain yield in respective environment. The transgressive segregants could be isolated in higher frequency from these crosses and utilize to generate inbred lines using conventional breeding method for further crop improvement programmes.

High predictability ratios (2rgca2/(2rgca2?rsca2)) indi-cated that the expression of grain Fe and Zn densities in pearl millet is governed predominantly by additive gene effects, suggesting high ffectiveness of prog-eny 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 out performed the parents having high levels of Fe and Zn, indicating that there would be little opportunity. if any, to exploit heterosis for these micronutrients in pearlmillet, and to breed high Fe and Zn hybrids wouldrequire incorporating these traits into all the parents.

Table 1: Pooled analysis of variance for combining ability in pearl millet evaluated in three environments.

S. No.	Source of variance	d.f.	Mean sum of square		
			Grain yield per plant (g)	Fe content (ppm)	Zn content (ppm)
1	GCA	9	48.003 **	375.786**	29.211**
2	SCA	45	77.051 **	74.596**	47.270**
4	GCA x Environments	18	2.891	31.617**	50.820**
5	SCA x Environments	90	6.391*	26.776**	33.845**
6	Error	324	2.881	0.004	0.023

*and**significant at 5% and 1% level of significance, respectively

Table 2: Analysis of variance for combining ability in individual environment (sowing dates) in pearl millet.

S.	Source of variance	d.f.	Env.	Mean sum of square		
				Grain yield per plant (g)	Fe content (ppm)	Zn content (ppm)
1	GCA	9	E_1	28.015**	113.778**	17.534**
			E_2	12.755**	97.941**	47.121**
			E_3	13.042**	227.302**	66.196**
2	SCA	45	E_1	51.921**	40.103**	34.525**
			E_2	24.406**	47.626**	44.033**
			$^E3$	13.506**	40.419**	36.402**
3	Error	105	$^E1$	5.138	0.004	0.041
			E_2	2.065	0.003	0.014
			E_3	1.441	0.004	0.015
	GCA/SCA		E_1	0.040	0.236	0.042
			E_2	0.039	0.171	0.089
			E_3	0.080	0.468	0.068

*and**significant at 5% and 1% level of significance, respectively

Table 3: Top three parents and crosses for grain yield per plant and related traits in pearl millet in E_1, E_2 and E_3 environments

	Characters	Env.	*Per se* performance		GCA	SCA	Heterobeltiosis
			Parents	F_1's			
10	Grain yield per plant	E_1	RIB-192	P5 x P9	RIB-3135-18	P5 x P9	P1 x P2
			HBL-11	P5 x P10	RIB-335/74	P4 x P6	P6XP7
			H77/833-2-202	P4 x P6	HBL-11	P1 x P2	P5XP9
		E_2	RIB-192	P5 x P9	RIB-3135-18	P5 x P9	P5XP9
			HBL-11	P5 x P10	MIR-525-2	P1 x P2	P6XP7
			H77/833-2-202	P1 x P2	RIB-192	P5 x P10	P5XP10
		E_3	H77/833-2-202	P6 x P7	RIB-3135-18	P6 x P7	P6XP7
			HBL-11	P2 x P6	RIB-335/74	P5 x P10	P2XP6
			RIB-192	P1 x P2	MIR-525-2	P7 x P9	P6XP10
12	Fe Content	E_1	H77/833-2-202	P6 x P10	G77/107	P2 x P6	P6 x P8
			G77/107	P6 x P8	H77/833-2-202	P5 x P7	P2 x P6
			RIB-3135-18	P1 x P10	HBL-11	P6 x P8	P1 X P7
		E_2	H77/833-2-202	P4 x P6	G77/107	P1 x P2	P4 x P6
			HBL-11	P1 x P10	H77/833-2-202	P1 x P10	P1 x P10
			RIB-3135-18	P8 x P9	HBL-11	P3 x P8	P1 x P2
		E_3	G77/107	P8 x P9	G77/107	P1 x P10	P4 x P6
			H77/833-2-202	P1 x P10	H77/833-2-202	P8 x P9	P8 x P9
			HBL-11	P6 x P9	HBL-11	P6 x P9	P6 x P8
13	Zn Content	E_1	RIB-335/74	P5 x P10	RIB-353-74	P5 x P10	P2 x P6
			RIB-3135-18	P1 x P10	J-2340	P1 x P3	P1 x P3
			J-2340	P3 x P4	G77/107	P3 x P7	P3 x P4
		E_2	RIB-335/74	P8 x P9	RIB-192	P4 x P6	P4 x P6
			RIB-3135-18	P4 x P6	H77/833-2-202	P8 x P9	P8 x P9
			H77/833-2-202	P6 x P9	HBL-11	P6 x P9	P1 x P4
		E_3	MIR-525-2	P4 x P9	RIB-494	P6 x P7	P4 x P8
			J-2340	P4 x P8	H77/833-2-202	P4 x P8	P4 x P9
			H77/833-2-202	P4 x P6	G77/107	P4 x P9	P6 x P7

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