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Relationship of grain iron and zinc content with grain yield in pearl millet hybrids

Anand Kanatti • KN Rai • K Radhika • M Govindaraj • KL Sahrawat • K Srinivasu • H Shivade

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Abstract Development of pearl millet cultivars with high levels of grain iron (Fe) and zinc (Zn) content can make significant contribution to reducing widespread deficiencies of these micronutrients in populations heavily dependent on staple cereals for their dietary energy and nutritional requirements. It is imperative that breeding of such cultivars must not compromise on grain yield and farmer-preferred traits. Multi-location evaluation of two sets of hybrids with differing genetic composition showed that Fe and Zn contents had highly significant and high positive correlations in both sets of hybrids and in all environments, and they were not correlated with grain weight, implying simultaneous genetic improvement of both micronutrients in large-seeded background is likely to be effective. Both micronutrients had moderate to low negative correlations with grain yield in both sets of hybrids, although not always significant. Such associations might have resulted due to the involvement of *iniadi* germplasm as a common source of high Fe and Zn content in both male and female parents, thereby reducing the genetic diversity between the parental lines for traits associated with heterosis for grain yield. Whether this could also be due to natural negative association between genetic factors for these micronutrients on one hand and grain yield on the other merits further studies through selection experiments using genomic tools as the resolution of this issue has a direct bearing on breeding high-yielding hybrids with high levels of Fe and Zn content in pearl millet.

Anand Kanatti • K Radhika
Department of Genetics and Plant Breeding
Acharya NG Ranga Agricultural University
Rajendranagar, Andhra Pradesh

KN Rai (✉) • M. Govindaraj • KL Sahrawat • K Srinivasu • H Shivade
International Crops Research Institute for the Semi-Arid Tropics, Patancheru
e-mail: k.rai@cgiar.org

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Introduction

Micronutrient malnutrition arising from dietary deficiencies of iron (Fe) and zinc (Zn) is now increasingly being recognized as a serious public health problem, affecting more than two billion people worldwide (WHO 2002). This problem is particularly alarming in populations of developing countries, heavily dependent on staple cereals as a major source of dietary energy and nutritional requirements. India is among the countries most affected by deficiencies of Fe and Zn as about 80% of the pregnant women, 52% of the non-pregnant women, and 74% of the children in 6-35 months age group suffer from iron deficiency (Chakravarty and Ghose 2000). About 52% of the children below 5 years are Zn deficient. Addressing this problem requires a multi-pronged approach involving medical supplementation, industrial food fortification, dietary diversification and biofortification. Crop biofortification is of special significance for predominantly agriculture-based societies such as India since cultivars developed with elevated levels of Fe and Zn content first reach the rural households, and then the production surpluses penetrate the urban markets (Bouis et al. 2011). Further, cultivars bred for high levels of these micronutrients have also ready consumer acceptance as such cultivars are no different in taste and appearance from other improved cultivars traditionally grown. Crop biofortification is also a cost–effective and sustainable approach.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) as such is a highly nutritious crop with higher levels of Fe and Zn content than other major cereals (Dwivedi et al. 2012) Recent research has shown large variability for these micronutrients in improved cultivars, breeding lines and germplasm (Velu et al. 2007, 2008a, 2008b; Rai et al. 2012, 2014). Considering the genetic variability and the significant dietary nutritional values of Fe and Zn content, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), in collaboration with partners from the public and private sector, has undertaken a major initiative towards the development of high-iron cultivars. Such efforts, however, can only be successful and sustainable if grain Fe and Zn improvement does not compromise on grain yield improvement. Limited studies in pearl millet (Gupta et al. 2009, Rai et al. 2012) have shown that grain Fe and Zn content are either not correlated with grain yield, or there are moderate to low negative correlations. In this paper, we report on the correlation of Fe and Zn content with grain yield and other agronomic traits in two sets of pearl millet hybrids evaluated in multilocational trials.

Materials and methods

The experimental materials included in this study consisted of two sets of hybrids, hereafter referred to as Set-A and Set-B. Set-A consisted of 32 hybrids developed from crosses between 18 male-sterile lines and 31 restorer lines, and 3 controls. Set-B consisted of 28 hybrids developed from crosses between 10 male-sterile lines and 28 restorer lines, and 3 controls. Set-A hybrids were evaluated at Patancheru and two additional locations (Ahmedabad and Aligarh) in northern India. Set-B hybrids were evaluated at Patancheru and two additional locations (Aurangabad and Dhule) in peninsular India. Both sets of hybrids were evaluated in randomized complete block design with three replications in 2012 rainy season. The plot size was two rows of 4 m length spaced at 75 cm apart at Patancheru and 50 cm at other locations, with plant-to-plant spacing of 15 cm within the rows at all the locations. All the recommended agronomic practices were followed for good crop stand.

Days to 50% flower, grain yield and effective tillers per plant were recorded on plot basis. Five random plants were used to determine plant height and panicle length, and random samples of 200 grains were used to estimate 1000-grain weight. Grain samples were collected from open-pollinated panicles harvested at physiological maturity, sundried for more than 15 days and carefully threshed to ensure no dust contamination. These were analyzed for grain Fe and Zn content using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) at the Charles Renard Analytical Laboratory, ICRISAT, Patancheru, following the closed-tube method as described by Wheal et al. (2011). Analysis of variance (ANOVA) for individual environments and across the environments was performed assuming random model (Steel and Torrie 1980) and using PROC GLM (Generalized Linear Model) procedure in Statistical Analysis Systems (SAS) version 9.2 (SAS Institute 2004).

Results and discussion

Analysis of variance showed highly significant differences among the hybrids for all traits in both sets of hybrids, in individual environments (data not presented) as well across the environments (Table 1). Hybrid (H) × environment (E) interactions were also highly significant for all traits in both sets of hybrids. However, variability attributable to H × E interaction relative to those due to differences among the hybrids was 31% and 34% for Fe content and 70% and 65% for Zn content in set-A and set-B, respectively. Similar results of greater G × E interaction for Zn content than Fe content have been reported in maize (Prasanna et al. 2011). This may apparently imply greater sensitivity and differential response of hybrids for Zn than Fe content to changes in the soil and climatic conditions. This could also be due to proportionately larger differences among the hybrids for Fe content than for Zn content. For instance, about two-fold differences among the hybrids were observed for Fe content in all the environments in both sets of hybrids, with a range of 40-84 mg kg^{-1} in set-A hybrids and 33-74 mg kg^{-1} in set-B hybrids (Table 2). In comparison, the differences among the hybrids were much smaller for Zn content in each environment in both sets of hybrids, with a range of 24-46 mg kg^{-1} in set-A hybrids and 32-52 mg kg^{-1} in set-B hybrids.

The contribution of H x E interaction to variability for grain yield relative to those due to differences among the hybrids was 29% in set-A, and much higher (73%) in set-B. While grain yield differences among the hybrids were two-to-four-fold with a range of 1.11- 4.75 t ha^{-1} in set-A, these were less than two-fold with a range of 2.35 - 6.29 t ha^{-1} in set-B. The contribution of H × E interaction to variability relative to those due to differences among hybrids for grain weight was 33% in set-A and 24% in set-B. The grain weight differences among the hybrids were generally 1.5 to 1.8-fold in both sets of hybrids. The contribution of H × E interaction to variability relative to those due to differences among the hybrids was lowest (14-16%) for days to 50% flower. For plant height, these were similar to those observed for Fe content in both sets of hybrids, while these varied from 50% in set-A to 71% in set-B for effective tillers; and from 44% in set-A to 8% in set-B for panicle length. The range among the hybrids for days to 50% flower was 40-58 days in set-A and 38-48 days in set-B. For plant height, the range was 160-252 cm in set-A and 182-251 cm in set-B, while for panicle length the range was 15-25 cm in set-A and 16-29 cm in set-B.

Based on mean performance across the environments, there were highly significant and high positive correlations between Fe and Zn content in both sets of hybrids (r=0.69 in set-A and 0.65 in set-B; p<0.01), and this trend was consistent across the environments, with the correlation coefficient varying from 0.52 to 0.70 in set-A and from 0.55 to 0.64 in set-B (Table 3). Similar relationships between these micronutrients have been reported in earlier studies on pearl millet studies (Velu et al. 2007, 2008b; Gupta et al. 2009, Rai et al. 2012, 2013, 2014; Govindaraj et al. 2013), and in other cereals, such as sorghum (Ashok-Kumar et al. 2010, 2013), maize (Oikeh et al. 2003, 2004), rice (Stangoulis et al. 2007, Anandan et al. 2011), wheat (Garvin et al. 2006; Peleg et al. 2009; Zhang et al. 2010; Velu et al. 2011a), and finger millet (Upadhyaya et al. 2011). These positive associations between Fe and Zn content may likely result from common and overlapping quantitative trait loci (QTL) as reported in wheat (Peleg et al. 2009), rice (Stangoulis et al. 2007), common bean (Blair et al. 2009, Cichy et al. 2009) and pearl millet (Kumar 2011), implying that simultaneous selection for both micronutrients is likely to be highly effective.

Based on the mean performance over the environments, Fe content had highly significant and moderate negative correlations with grain yield in both sets of hybrids (r= -0.55 in set-A and -0.52 in set-B; P<0.01). However, it varied from -0.33 to -0.56 across the environment in set-A, and from -0.21 to -0.56 in set-B, and was not always significant (Table 3). The correlation between Zn content and grain yield was also negative, except in one environment in set-A, but was smaller in magnitude compared to the correlation between Fe content and grain yield, and it was also not always significant. Such patterns of relationships of Fe and Zn content with grain yield are not unexpected considering the high positive correlation between Fe and Zn content and larger G ´ E interaction effect relative to genotypic effect for Zn content than for Fe content.

Almost all the ICRISAT-bred breeding lines and hybrids parents having high Fe and Zn content are largely or entirely based on *iniadi* germplasm in their parentage (Velu et al. 2007, 2008b, Rai et al. 2012). Further, it has been found that both these micronutrients are predominantly under additive genetic control with no better-parent heterosis (Velu et al. 2011b, Govindaraj et al. 2013). This would imply that hybrids with high Fe and Zn content would have both parents high in these micronutrients and such parents are likely to have largely *iniadi* germplasm in their parentage. Therefore, though high in Fe and Zn content, there is likely to be reduction in genetic diversity between parents for traits and physiological functions related to heterosis for grain yield, which is predominantly under non-additive genetic control (Khairwal et al. 1999). Conversely, hybrids with average and low Fe and Zn content would have less of the *iniadi* germplasm in the percentage of parental lines and hence probability of greater diversity and consequently greater heterosis for traits related to high grain yield. This would call for application of genomic tools for selective introgression of only those genes and genomic regions from *iniadi* germplasm into the parental lines which are associated with high Fe and Zn content to ensure that diversity for traits related to heterosis for grain yield is not reduced. Such an approach would enhance the probability of combining high grain yield with high Fe and Zn content in hybrids.

Table 1 Mean squares for the analysis of variance of experimental design pooled over environments for grain iron (Fe) and zinc (Zn) densities, 1000-grain weight (GW), grain yield (GY), days to 50% flower (DF), plant height (PH), panicle length (PL) and effective tillers (ET) in two sets of pearl millet hybrids, 2012 rainy season

Hybrids	Source	Degrees of freedom	Fe (mg kg^{-1})	Zn (mg kg^{-1})	GW (g 1000^{-1})	GY (t ha^{-1})	DF (d)	PH (cm)	PL (cm)	ET (no.)
Set-A	Environment (E)	2	4364**	4539**	767.8**	28.4**	942**	31983**	426.6**	2.29**
	Replication / E	6	146**	33*	1.5	0.7**	3.6**	166	8.7**	0.11**
	Hybrids (H)	34	460**	69**	21.5**	3.5**	88.5**	2366**	23.7**	0.24**
	H × E	68	72**	24**	3.5**	0.5**	6.2**	409**	5.2**	0.06**
	Error	204	25	13	1.3	0.19	1.2	118	2.3	0.04
Set-B	Environment (E)	2	5559.7**	2555.1**	96.1**	30.6**	1607**	48583**	313.9**	1.9**
	Replication / E	6	62.8	74.3**	7*	0.38	3.1	62	1.6	0.13*
	Hybrids (H)	30	404.5**	89.3**	32.8**	2.41**	45.2**	1407**	83.3**	0.22**
	H × E	60	68.9**	29.0**	3.9*	0.88**	2.6**	250**	3.3**	0.08**
	Error	180	40.2	16.3	2.6	0.25	1.5	94	3	0.04

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

Table 2 Range for grain iron (Fe) and zinc (Zn) densities, 1000-grain weight (GW), grain yield (GY), days to 50% flower (DF), plant height (PH), panicle length (PL) and effective tillers (ET) in two sets of pearl millet hybrids, 2012 rainy season

	Set-A				Set-B			
Trait	E1	E2	E3	Mean	E1	E4	E5	Mean
Fe (mg kg^{-1})	38-75	31-66	40-84	36-67	46-74	49-89	33-74	44-76
Zn (mg kg^{-1})	24-37	24-46	18-28	22-34	25-39	33-48	32-52	32-44
GW (g 1000^{-1})	13-20	9-16	10-15	11-17	11-20	11-19	11-17	12-18
GY (t ha^{-1})	2.64-5.03	1.11-4.75	1.76-5.08	2.01-4.95	2.62-4.95	2.35-6.29	2.64-4.37	2.70-5.03
DF (d)	37-49	40-58	39-54	40-53	38-48	48-55	44-53	43-52
PH (cm)	155-197	160-252	163-238	163-226	172-207	182-251	205-260	186-234
PL (cm)	16-21	15-25	19-26	17-24	16-29	20-32	22-33	19-31
ET (no.)	1.13-2.07	0.91-1.63	1.02-1.77	1.04-1.70	0.83-1.83	1.04-2.35	1.08-1.47	1.11-1.87

E1, Patancheru; E2, Ahmedabad; E3, Aligarh; E4, Aurangabad; E5, Dhule.

Table 3 Correlation coefficients among the traits in individual environment and pooled across the environments in hybrid set-A (below diagonal) and set-B (above diagonal) for iron (Fe) and zinc (Zn) densities, 1000-grain weight (GW), grain yield (GY), days to 50% flower (DF), plant height (PH), panicle length (PL) and effective tillers (ET), 2012 rainy season

Env./Trait		Fe (mg kg^{-1})	Zn (mg kg^{-1})	GW (g 1000^{-1})	GY (t ha^{-1})	DF (d)	PH (cm)	PL (cm)	ET (no.)	
E1	Fe	1	0.55**	-0.05	-0.56**	-0.64**	-0.38*	-0.66**	0.22	E1
	Zn	0.60**	1	0.46**	-0.34	-0.40*	0.06	-0.40*	0.13	
	GW	0.26	0.21	1	0.10	-0.27	0.17	-0.15	0.22	
	GY	-0.56**	-0.40*	-0.34*	1	0.46**	0.60**	0.48**	-0.03	
	DF	-0.54**	-0.35*	-0.32	0.85**	1	0.54**	0.70**	-0.55**	
	PH	-0.07	-0.03	-0.20	0.51**	0.54**	1	0.34	-0.33	
	PL	-0.33	-0.32	-0.32	0.55**	0.47**	0.47**	1	-0.24	
	ET	-0.11	-0.02	0.25	-0.28	-0.35*	-0.50**	-0.30	1	
E2	Fe	1	0.64**	-0.05	-0.34	-0.17	-0.20	-0.28	-0.23	E4
	Zn	0.70**	1	0.33	-0.26	-0.38*	-0.21	-0.26	0.32	
	GW	0.05	0.13	1	0.16	-0.23	0.25	0.03	0.33	
	GY	-0.45**	-0.35*	0.05	1	0.75**	0.68**	0.56**	0.01	
	DF	-0.50**	-0.39*	0.32	0.74**	1	0.69**	0.48**	-0.50**	
	PH	-0.40*	-0.28	0.36*	0.77**	0.83**	1	0.23	-0.20	
	PL	-0.54**	-0.35*	0.18	0.49**	0.43**	0.60**	1	-0.29	
	ET	0.38*	0.35*	-0.31	-0.45**	-0.68**	-0.64**	-0.56**	1	
E3	Fe	1	0.64**	0.10	-0.21	-0.15	-0.12	-0.41*	-0.25	E5
	Zn	0.52**	1	0.37*	-0.19	-0.16	0.02	-0.08	-0.14	
	GW	0.20	0.29	1	0.03	-0.40*	0.27	-0.20	0.18	
	GY	-0.33	0.14	-0.02	1	0.33	0.27	0.15	0.29	
	DF	-0.21	0.34*	-0.20	0.47**	1	0.45*	0.38*	-0.23	
	PH	-0.07	0.47**	-0.04	0.55**	0.83**	1	0.05	-0.13	
	PL	-0.06	0.20	-0.19	0.32	0.46**	0.60**	1	0.03	
	ET	-0.20	-0.17	-0.06	0.35*	-0.19	-0.12	-0.19	1	
AA	Fe	1	0.65**	-0.06	-0.52**	-0.34	-0.27	-0.53**	-0.04	AB
	Zn	0.69**	1	0.43*	-0.37*	-0.42*	-0.06	-0.34	0.33	
	GW	0.16	0.19	1	0.01	-0.32	0.21	-0.10	0.34	
	GY	-0.55**	-0.32	-0.14	1	0.71**	0.61**	0.56**	-0.03	
	DF	-0.47**	-0.23	-0.05	0.74**	1	0.64**	0.57**	-0.53**	
	PH	-0.32	-0.12	0.03	0.69**	0.83**	1	0.20	-0.23	
	PL	-0.37*	-0.30	-0.06	0.54**	0.49**	0.65**	1	-0.23	
	ET	0.01	-0.01	-0.09	-0.17	-0.44**	-0.45**	-0.38*	1	

E1, Patancheru; E2, Ahmedabad; E3, Aligarh; E4, Aurangabad; E5, Dhule; AA, across the environments in hybrid set-A; AB, across the environments set-B; *,**, significant at 5% and 1% levels of significance, respectively.

There was no correlation between Fe content and grain weight, and Zn content was positively correlated with grain weight, but only in set-B, being significant in two environments (Table 3). Similar results have been reported in earlier pearl millet studies (Gupta et al. 2009, Rai et al. 2012). Except for a low negative correlation ($r=-0.34, P<0.05$) in one environment in set-A, there was no correlation between grain yield and grain weight. These results show that hybrids with high Fe and Zn content can be developed without compromising on grain weight.

Based on the mean performance over the environments, there were significant negative correlations between Fe content and panicle length in both sets of hybrids, but only in set-A hybrids for days to 50% flower (Table 3). While Zn content had significant negative correlation with days to 50% flower in set-B, there was no correlation between Zn content and panicle length in both sets of hybrids. Neither Fe nor Zn content were correlated with plant height and effective tillers in both sets of hybrids. Days to 50% flower, plant height and panicle length had highly significant and positive correlations with grain yield, and this trend was consistent across the environments in both sets of hybrids. Plant height and panicle length were also highly significantly and positively correlated with days to 50% flower, and this trend was also consistent across the environments in both sets of hybrids. However, plant height and panicle length were positively correlated in only set-A. Results of this study, however, do not provide any clear cut indications if days to 50% flower, panicle length and plant height, owing to their positive relationships with grain yield, may have any bearing on the negative correlation observed between grain yield on one hand, and Fe and Zn content on the other. These issues of Fe and Zn associations with grain yield and other traits merit further investigations, and must be resolved through selection experiments in segregating populations, with the application of genomic tools providing further insights.

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