Short Communication

Maternal inheritance for grain iron and zinc densities in pearl millet

Anand Kanatti, Mahalingam Govindaraj*, Kedar Nath Rai and Aluri S. Rao

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana 502 324

Abstract

Genetic variation and inheritance of micronutrients in pearl millet has largely been studied in recent years as part of biofortification initiatives. In this study, maternal (reciprocal) effect on inheritance of grain Fe and Zn was studied using a set of diverse breeding material. Entries were paired for low and high for Fe density to produce direct and reciprocal crosses. Over two-seasons, Fe density among parents varied 31-64 mg kg^{-1} and Zn density varied 28-43 mg kg^{-1}. Difference between each direct and reciprocal crosses for Fe (1 to 4 mg kg^{-1}) and Zn (0 to 2 mg kg^{-1}) were negligible and non-significant, hence cytoplasmic or maternal genes are not likely to modify inheritance of these traits. These results indicate that high Fe/Zn inbred can be used either as female or male parent in hybrid-parent breeding program.

Key words: Maternal effect, iron, zinc, biofortification

Globally, anemia affects more than 25% of the population and the highest prevalence of anemia is related to low dietary iron (Fe) intake in pre-school children (47%) and pregnant women (42%) (WHO 2008). It was estimated that nearly 17% of the global population is at a risk of inadequate zinc (Zn) intake (Wessells and Brown 2012) and nearly 26% children under age 5 are stunted (UNICEF 2013). Pearl millet is highly cross-pollinated crop thus both hybrids and open pollinated varieties (OPVs) are cultivar options. Pearl millet is rich in minerals than other cereals, but not all commonly grown and consumed cultivars has higher level of iron and zinc. Recent study by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) revealed the average grain iron and zinc content among 120 released commercial hybrids so far in India is 42 mg kg^{-1} and 32 mg kg^{-1},

respectively (Rai et al. 2016). In fact, in India, nearly 58% of children and 50% of pregnant women are anaemic, while 38% of children under five are stunted (NFHS 2017). To address the issue of malnutrition, HarvestPlus Challenge program of the CGIAR has initiated resource mobilization through global biofortification research coordination of several staple crops including pearl millet.

To breed or combine the Fe and Zn traits with elite and high yielding genetic backgrounds, it is necessary to understand the underlying genetics of these traits. Recent studies at ICRISAT in pearl millet had been reported large variability for both Fe (31-125 ppm) and Zn (35-82 ppm) densities (Govindaraj et al. 2016; Rai et al. 2012), and these traits were largely under additive genetic control beside no or negligible better-parent heterosis (Velu et al. 2011; Govindaraj et al. 2013; Kanatti et al. 2014a). Maternal effect (ME) or reciprocal effects (RE) are important components, especially when traits are maternally determined. However, no information is available on the maternal attributes related to grain Fe and Zn densities. Thus, information on reciprocal cross differences and maternal effects is of immense value in determining the selection response and breeding behavior of the hybrid-parents breeding program. This study was conducted with set of diverse breeding material such as inbreds, populations and hybrids, in view to understand the maternal effect on accumulation of grain Fe and Zn densities in pearl millet.

Fourteen genotypes (4-populations, 6-hybrids and

4-inbred lines) with diverse range for iron and zinc densities were paired in contrast for high and low Fe density, i.e. two pairs for populations, three pairs for hybrids and two pairs for inbreds. All these were crossed direct and reciprocal way to produce F_1 seeds. Total 14 hybrid combinations were produced from seven pairs during 2011 summer season. Trial was evaluated in Randomized Complete Block Design (RCBD) with three replications during 2012 summer and rainy season in alfisols at ICRISAT, Patancheru. Paired-plot randomization was used so that hybrid pairs (direct and reciprocal) could be in side-by-side-paired-plots to avoid any soil heterogeneity effect on grain micronutrient densities. While parents were planted separately in close adjacent to hybrid block. Each entry was planted in two rows of 2 m spaced at 0.60 m and 0.75 m between the rows in summer and rainy season, respectively. Overplanted plots were thinned after 15 d to a single plant spaced 0.15 m apart within each row. A basal dose of 100 kg ha^{-1} diammonium phosphate (18% N and 20% P) was applied at the time of field preparation and 100 kg ha^{-1} of urea (46% N) was applied as top dress within 2 to 4 d after thinning. Trials were irrigated 7-10 d interval during summer crop season, and twice in rainy crop season to ensure no moisture stress throughout the crop season.

Grain samples were collected from open-pollinated panicles, harvested at or after physiological maturity, sundried for more than 15 days and carefully threshed using stainless single-head thresher to ensure no dust or rust contamination. These were analyzed for grain iron and zinc densities 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). Soil samples were collected at the time of planting from 0 to 30 cm depth top-layer. The mean soil iron and zinc content extractable with Diethylene Triamine Penta Acetic acid (DTPA) varied from 12.1 and 4.5 mg kg^{-1} in 2012 summer season and 11.9 and 1.9 mg kg^{-1} in 2012 rainy season, respectively. Analysis of variance and paired t-test was performed in Statistical Analysis System (SAS) version 9.3 (SAS Institute 2009).

In parental trial, the analysis of two seasons (hereafter referred to as environment) data showed highly significant variability among the genotypes for all studied traits (Table 1). Parents × environment interaction was significant for all traits except for Fe density. The Fe density among parents averaged over the environments varied from 31 to 64 mg kg^{-1} and Zn density varied from 28 to 43 mg kg^{-1}. Highly significant mean squares observed for all crosses, direct crosses and reciprocal crosses shown large variations among hybrids for Fe and Zn density, 50% flowering and grain

Table 1. Mean square for grain iron (Fe), zinc (Zn), days to 50% flowering (DF) and grain yield (GY), across 2012 rainy and summer seasons in pearl millet at Patancheru

Source	Degrees of freedom	Mean square							
		Fe		Zn		DF		GY	
Environments (E)	1	2555.4	**	4187.0	**	312.4	**	68.7	**
Replication/E	4	58.9	ns	8.3	ns	3.1	ns	0.7	**
Parents (P)	13	629.0	**	130.6	**	139.9	**	2.4	**
P × E	13	36.7	ns	38.7	**	18.5	**	0.7	**
Error (a)	50	38.5		12.5		1.8		0.2	
Environments (E)	1	3194.8	**	3904.3	**	180.1	**	45.6	**
Replication/E	4	236.8	**	20.1	**	3.3	ns	2.3	**
All crosses (C)	13	115.2	**	55.9	**	24.3	**	0.4	*
Direct crosses (DC)	**6**	166.9	**	49.9	**	29.5	**	0.3	*
Reciprocal crosses (RC)	**6**	83.4	**	70.2	**	23.2	**	0.5	*
DC vs RC	**1**	0.5	ns	6.7	ns	0.3	ns	0.1	ns
C × E	13	60.2	**	28.9	**	11.2	**	0.4	**
DC × E	6	42.1	*	25.7	**	10.9	**	0.5	**
RC × E	6	93.6	**	35.2	**	13.0	**	0.4	ns
DC vs RC × E	**1**	6.9	ns	10.3	ns	2.0	ns	0.1	ns
Error (b)	50	18.5		5.7		2.4		0.2	

*,** Significant at the 0.05,0.01 probability levels, respectively; ns- non-significant

yield. Although in both direct and reciprocal crosses, genotype × environment interaction was significant for Fe and Zn density and 50% flowering, the contribution of G×E interaction was very low relative to that due to genotypic variation. For instance, the D×E interaction variation relative to that due to genotypic variation relative to direct crosses was 25% for Fe and 52% for Zn density. R×E interaction variation relative to reciprocal crosses was 50% for Zn and while it was 12% higher for Fe density. Further, the correlation of micronutrient content of entries between the two environments was highly significant both for Fe and Zn, indicating high levels of consistency of the rankings of entries across the environments both for Fe and Zn content. Fe density among direct crosses varied from 38 to 51 mg kg^{-1} with an average of 45 mg kg^{-1} and Zn density varied from 31 to 38 mg kg^{-1}. Reciprocal crosses Fe density varied from 38 to 48 mg kg^{-1} with an average of 45 mg kg^{-1}, and Zn density varied from 31 to 40 mg kg^{-1} with an average of 35 mg kg^{-1}. Non-significant mean-square from DC vs. RC for micronutrients indicate no maternal effect for grain micronutrients. DC vs. RC × E was also non-significant, this further confirming consistency in differences for DC vs. RC across the environments. Earlier studies in pearl millet (Kanatti et al. 2014b) and maize (Prasanna et al. 2011) reported lower G×E for Fe density than Zn density. Maternal effect is presumed to be a result of non-additive gene effects, thus presence of maternal effect is expected in grain yield traits. Present study did not show any significant difference for days to 50% flower as well as grain yield. Reciprocal cross differences have been reported in pearl millet mostly for head traits (Gupta and Nanda 1968), total callus and E callus volumes (Mythili et al. 1997) and in maize for early maturity (Khehra and Bhalla 1976).

There was large micronutrient difference between the parental pairs of each crosses. For Fe, the difference between parental pairs was 11-16 mg kg^{-1} in population, 25-33 mg kg^{-1} in inbred and 6-15 mg kg^{-1} in hybrid. While for Zn, the difference between parental pairs was 2-7 mg kg^{-1} in populations, 8-9 mg kg^{-1} in inbreds and 6-10 mg kg^{-1} in hybrids. The difference between direct and reciprocal crosses were negligible or very low, when averaged over the environments and replications, it varied from 0 to 4 mg kg^{-1} for Fe and 0 to 2 mg kg^{-1} for Zn (Fig. 1). This low/negligible difference resulted in non-significant differences in paired t-test between direct and reciprocal crosses, thus implying that inheritance of both the
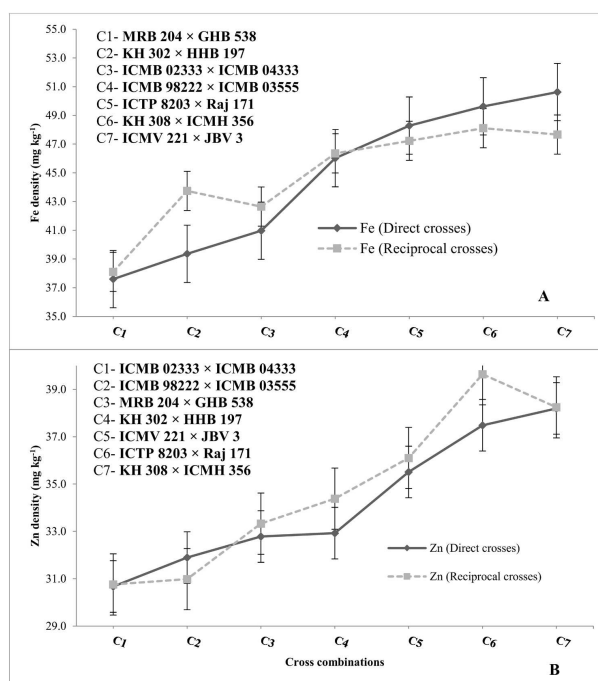

Fig. 1. Difference between direct and reciprocal crosses for grain iron (A) and zinc (B) densities (mg kg^{-1}) across two seasons in pearl millet

micronutrients is controlled by nuclear determinants and can be exploited through a common breeding program. Also, high Fe/Zn inbreds/genotypes can be used either male or female parent in breeding for biofortified hybrid cultivars as well as in parental breeding program. Earlier study in pearl millet by Velu et al. (2011) reported non-significant difference between direct and reciprocal hybrid performance in a set of inbred lines for Fe and Zn densities.

None of the hybrids showed better performance over better parents for both the micronutrients which could be due to largely under additive genetic control. Similar findings were reported in earlier studies in pearl millet (Velu et al. 2011; Govindaraj et al. 2013; Kanatti et al. 2014a, 2016). Significant positive correlation observed Fe and Zn densities among parents (r=0.73, p<0.01), direct crosses (r=0.73, p<0.01) and reciprocal crosses (0.54, p<0.05). Similar relationships between these micronutrients have been reported in earlier studies on pearl millet (Rai et al. 2012; Govindaraj et al. 2013; Kanatti et al. 2014a). This implying that both the micronutrients can be improved simultaneously, that is, direct selection for high-Fe could also indirectly select for higher value for Zn. Correlations between the micronutrient traits in reciprocal and direct crosses

were highly positively significant. From our results, we could conclude that high-Fe trait can be incorporated into elite genetic background through crossing program using high-Fe lines either as a female or male parent (considering within seed and restorer gene pool) and select for elite agronomic performance with high-Fe trait in the segregating populations.

Acknowledgement

Authors' contribution

Conceptualization of research (KNR, MG); Designing of the experiments (KNR, MG, AK); Contribution of experimental materials (AK, ASR); Execution of field/lab experiments and data collection (AK, ASR); Analysis of data and interpretation (AK, ASR); Preparation of manuscript (MG, AK).

Declaration

The authors declare no conflict of interest.

References

Govindaraj M., Rai K. N., Shanmugasundaram P., Dwivedi S. L., Sahrawat K. L., Muthaiah A. R. and Rao A. S. 2013. Combining ability and heterosis for grain iron and zinc densities in pearl millet. Crop Sci., **53**: 507-517.

Govindaraj M., Rai K. N., Pfeiffer W. H., Kanatti A. and Shivade H. 2016. Energy-dispersive X-ray fluorescence spectrometry for cost-effective and rapid screening of pearl millet germplasm and breeding lines for grain iron and zinc density. Comm. Soil Sci. Plant Anal., **47**: 2126-2134.

Gupta V. P. and Nanda G. S. 1968. Inheritance of some plant and head characters in Pearl Millet. J. Res., **5**: 1-5.

Kanatti A., Rai K. N., Radhika K., Govindaraj M. and Rao A. S. 2016. Genetic architecture of open-pollinated varieties of pearl millet for grain iron and zinc densities. Indian J. Genet., **76**: 299-303.

Kanatti A., Rai K. N., Radhika K., Govindaraj M., Sahrawat K. L. and Rao A. S. 2014a. Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. Springer Plus, **3**: 763.

Kanatti A., Rai K. N., Radhika K., Govindaraj M., Sahrawat K. L., Srinivasu K. and Shivade H. 2014b. Relationship of grain iron and zinc content with grain yield in pearl millet hybrids. Crop Improv., **41**: 91-96.

Khehra A. S. and Bhalla S. K. 1976. Cytoplasmic effects on quantitative characters in maize (*Zea mays* L.). Theor. Appl. Genet., **47**: 271-274.

Mythili P. K., Satyavathi V., Pavankumar G., Rao M. V. S. and Manga V. 1997. Genetic analysis of short term callus culture and morphogenesis in pearl millet, *Pennisetum glaucum*. Plant Cell Tissue Organ Cult., **50**: 171-178.

NFHS. 2017. National family health survey (NFHS-4) 2015-16. International Institute for Population Sciences (IIPS), Govandi Station Road, Deonar, Mumbai 400 088. India. http://rchiips.org/nfhs/factsheet_NFHS-4.shtml.

Prasanna B. M., Mazumdar S., Chakraborti M., Hossain F., Manjaiah K. M., Agrawal P. K., Guleria S. K. and Gupta H. S. 2011. Genetic variability and genotype × environment interactions for kernel iron and zinc concentrations in maize (*Zea mays*) genotypes. Indian J. agric. Sci., **81**: 704-711.

Rai K. N., Govindaraj M. and Rao A. S. 2012. Genetic enhancement of grain iron and zinc content in pearl millet. Qual. Assur. Saf. Crop., **4**: 119-125.

Rai K. N., Patil H. T., Yadav O. P., Govindaraj M., Khairwal I. S., Cherian B., Rajpurohit B. S., Rao A. S., Shivade H. and Kulkarni M. P. 2014. Variety Dhanashakti. Indian J. Genet., **74**: 405-406.

Statistical Analysis Systems Institute Inc. 2009. SAS/STAT® 9.2 User's Guide (2nd Edn.). Cary, NC: SAS Institute Inc.

UNICEF. 2013. Improving Child Nutrition: The achievable imperative for global progress. New York, NY 10017 USA. https://www.unicef.org/gambia/Improving_Child_Nutrition_-_the_achievable_imperative_for_global_progress.pdf

Velu G., Rai K. N., Muralidharan V., Longvah T. and Crossa J. 2011. Gene effects and heterosis for grain iron and zinc density in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Euphytica, **180**: 251-259.

Wessells K. R. and Brown K. H. 2012. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS ONE, **7**: e50568.

Wheal M. S., Fowles T. O. and Palmer L. T. 2011. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Anal. Methods, **3**: 2854-2863.

WHO. 2008. Worldwide Prevalence of Anaemia 1993-2005: WHO Global Database on Anaemia (Eds. B. de Benoist, E. McLean, I. Egli and M. Cogswell). Geneva: World Health Organization Press.