

Intra-population Variability for Grain Iron and Zinc Densities in Commercial Open-pollinated Varieties of Pearl Millet

A. Kanatti^{1&2*}, K. Radhika¹, V. Padma¹, P. Janila², M. Govindaraj² and K. N. Rai²

¹Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana (500 030), India

²International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Telangana (502 324), India

Corresponding Author

A. Kanatti

e-mail: k.anand@cgiar.org

Article History

Article ID: IJEP78

Received in 14th August, 2015

Received in revised form 4th September, 2015

Accepted in final form 26th September, 2015

Abstract

Intra-population variability using S_1 progenies was studied for grain iron (Fe) and zinc (Zn) densities and 1000-grain weight in two released and commercial open-pollinated varieties (ICTP 8203 and ICMV 221) of pearl millet. Analysis of variance showed highly significant variability for all the traits in both populations. In ICTP 8203, Fe density varied from 44 to 76 mg kg⁻¹ and Zn density from 40 to 60 mg kg⁻¹; while in ICMV 221, Fe density varied from 40 to 78 mg kg⁻¹ and Zn density from 31 to 52 mg kg⁻¹. Higher broad-sense heritability was observed for both micronutrients in both populations (77% in ICTP 8203 and 86% in ICMV 221 for Fe; and 71% in ICTP 8203 and 72% in ICMV 221 for Zn). This substantial genetic variability coupled with high heritability implies good prospects for improvement of both populations for these micronutrients. The highly significant and high positive correlation observed between Fe and Zn densities ($r=0.66$ in ICTP 8203 and $r=0.72$ in ICMV 221, $p<0.01$), suggested that both micronutrients can be effectively improved simultaneously. Both micronutrients had non-significant or small positive correlation with 1000-grain weight, implying these can be improved in large-seeded genetic backgrounds.

Keywords: Pearl millet, iron, zinc, open-pollinated varieties, variability

1. Introduction

Micronutrient malnutrition is wide spread worldwide, and more striking of these are iron and zinc deficiencies. Deficiency of iron and zinc in the diet results in poor growth, reduced immunity, fatigue, irritability, weakness, hair loss, wasting of muscles, sterility, morbidity and even death in acute cases (Stein, 2010). Several interventions have been in vogue to alleviate micronutrient malnutrition. Of these, biofortification is more efficient, cost-effective and sustainable. It relies on conventional plant breeding and modern biotechnology to increase the micronutrient density in edible portion of staple crops, and holds great promise for improving the nutritional status and health of poor populations in both rural and urban areas of developing countries (Bouis, 2003).

Pearl millet (*Pennisetum glaucum* (L.) R.Br.), is a highly cross-pollinated crop with 75-80% outcrossing (Burton, 1974). This breeding behavior provides for the development of open-pollinated varieties (OPVs) and hybrids as the two broad cultivar options. Since OPVs are highly heterogeneous and heterozygous populations, it is possible to improve them further for traits for which substantial variability may exist in these populations. Earlier studies identified ICTP 8203 and ICMV 221 having high levels of Fe and Zn densities (Velu, 2006;

Gupta et al., 2009). Both are released OPVs (Rai et al., 1990; Witcombe et al., 1997) and are under commercial production. The objective of this study was to assess the extent of variability for these two micronutrients and 1000-grain weight in these two populations.

2. Materials and Methods

Thirty-five S_1 progenies derived from ICTP 8203 and 37 S_1 progenies derived from ICMV 221 were evaluated in randomized complete block design with three replications as separate trials but in adjacent blocks in 2012 rainy season and 2013 summer season at ICRISAT, Patancheru. The plot size was one row of 2 m length spaced at 60 cm in summer season and 75 cm in rainy season with plant-to-plant spacing of 15 cm within the rows. All the recommended agronomic practices were followed for good crop stand and plant growth.

Open-pollinated grain samples were collected and analyzed for grain Fe and Zn 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). Random samples of 200 grains were used to estimate 1000-grain weight.



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, 2009). Associations among the traits were determined by the Pearson correlation coefficient.

3. Results and Discussion

There were highly significant differences among the progenies

of both ICTP 8203 and ICMV 221 for Fe and Zn densities and 1000-grain weight in individual environments as well as over the environments (Table 1). In ICTP 8203, genotype by environment (G×E) interaction was significant only for Fe and Zn densities, with the contribution of G×E interaction to variability relative to those due to the genotypic differences being 29% for Fe density and 40% for Zn density. In ICMV 221, G×E interaction was significant for all three traits, with the contribution of this interaction to variability relative to those due to the genotypic differences being 19% for Fe density, 40%

Table 1: Mean square for grain iron (Fe) and zinc (Zn) densities and 1000-grain weight (GW) among S₁ progenies of ICTP 8203 and ICMV 221, Patancheru

Source	df†	Mean square								
		Fe (mg kg ⁻¹)			Zn (mg kg ⁻¹)			GW (g)		
		P	R12	S13	P	R12	S13	P	R12	S13
ICTP 8203										
Environment (E)	1		5789.0**			47323.1**			85.0**	
Replication /E	4 (2)	240.0**	76.6	403.5**	94.6**	76.1*	113.0**	2.0	0.2	3.8*
ICTP 8203 S1 (G)	34	282.2**	170.3**	193.1**	151.8**	49.9**	162.8**	9.2**	6.4**	4.2**
G×E	34	80.7**			60.8**			1.4		
Error	136 (68)	41.4	35.4	47.3	21.4	19.4	23.3	1.3	1.5	1.0
ICMV 221										
Environment (E)	1	19291.1**			44836.9**			134.7**		
Replication /E	4 (2)	264.5**	65.9	463.1**	32.3	19.8	44.8	2.9	0.3	5.4*
ICMV 221 S1 (G)	36	566.9**	201.7**	476.4**	157.1**	42.5**	176.8**	9.7**	7.8**	4.4**
G × E	36	108.3**			62.9**			2.4**		
Error	144 (72)	33.4	27.1	39.7	20.0	7.7	32.4	1.3	1.3	1.2

†: Values in parentheses indicate individual environment degrees of freedom; P: Pooled across environments; R12: 2012 rainy season; S13: 2013 summer season; *, **: Significant at $p = 0.05$, $p = 0.01$, respectively

for Zn density, and 25% for 1000-grain weight. This indicated that Fe density was more stable than Zn density across these two specific environments in both populations. Similar results of greater G×E interaction for Zn density than Fe density have been reported in pearl millet (Kanatti et al., 2014b) and maize (Prasanna et al., 2011). This may apparently imply greater sensitivity of Zn content to changes in environmental conditions, but could also be due to larger differences among the progenies for Fe density than for Zn density.

Based on mean performance over the environments, Fe density in progenies of ICTP 8203 varied from 44 to 76 mg kg⁻¹ with an average of 67 mg kg⁻¹ (Figure 1), with 19 progenies exceeding the Fe density of control variety ICTP 8203 (66 mg kg⁻¹ Fe), which has been found having highest level of Fe density among OPVs released so far (Velu et al., 2008). Twenty-four of these progenies had 50-60 mg kg⁻¹ Zn density (49 mg kg⁻¹ in ICTP 8203), with range among all progenies under test varying from 40 to 60 mg kg⁻¹ with an average of 52

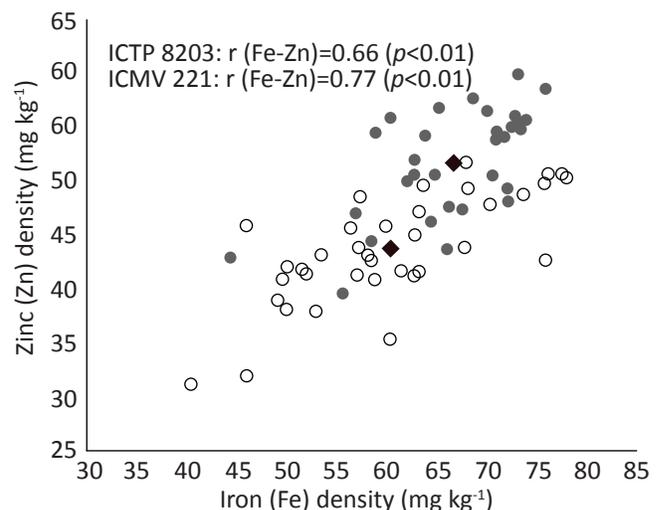


Figure 1: Variability for and relationship between grain iron (Fe) and zinc (Zn) densities in ICTP 8203 (●) and ICMV 221 (○), progeny means for ICTP 8203 (◆) and ICMV 221(◇)

mg kg⁻¹. In ICMV 221, Fe density varied from 40 to 78 mg kg⁻¹ with an average of 60 mg kg⁻¹ and Zn density varied from 31 to 52 mg kg⁻¹ with an average of 44 mg kg⁻¹. While 10 progenies with 68-78 mg kg⁻¹ Fe density exceeded the Fe density of ICTP 8203, and five of these with 50-52 mg kg⁻¹ Zn density exceeded the Zn density of ICTP 8203. The relatively greater range of variability for Fe density in ICMV 221 population could be due to its genetic constitution, which was bred by random mating 124 S₁ progenies selected from Bold-Seeded Early Composite (BSEC) (Witcombe et al., 1997). ICTP 8203 has much narrower genetic base as it was developed by random mating five S₂ progenies selected from an *Injadi* landrace, originating from northern Togo (Rai et al., 1990). In ICTP 8203, variability for 1000-grain weight, based on mean performance over the environments varied from 13 to 17 g with an average of 15 g; and in ICMV 221, it varied from 11 to 17 g with an average of 14 g.

High broad-sense heritability estimates for Fe density (77% and 86%) and Zn density (71% and 72%), respectively, in ICTP 8203 and ICMV 221 were closer to those for 1000-grain weight (86% in ICTP 8203 and 80% in ICMV 221). Earlier studies on pearl millet (Velu, 2006; Gupta et al., 2009) also reported high broad-sense heritability for both Fe and Zn densities. Availability of large genetic variability for grain Fe and Zn coupled with high heritability observed in this study and predominantly additive gene action (Velu et al., 2011; Govindaraj et al., 2013; Kanatti et al., 2014a) for Fe and Zn density implies good prospects of improving these populations for both micronutrients.

Based on mean performance over the environments, highly significant and high positive correlations were observed between Fe and Zn densities among S₁ progenies of ICTP 8203 ($r=0.66$), varying from 0.59 to 0.73 ($p<0.01$) across the

two environments (Table 2). In ICMV 221, the correlation coefficient varied from 0.66 to 0.72 ($p<0.01$) across the environments. Similar relationships between these micronutrients have been reported in earlier studies on pearl millet (Velu et al., 2008; Gupta et al., 2009; Rai et al., 2012, 2013, 2014; Govindaraj et al., 2013; Kanatti et al., 2014a, 2014b, 2016). These positive associations between Fe and Zn densities 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) and pearl millet (Kumar et al., 2016), implying that simultaneous selection for both micronutrients is likely to be highly effective.

Both Fe and Zn densities did not show any significant association with 1000-grain weight in ICTP 8203. In ICMV 221, significant positive but low correlation was observed only between Zn density and 1000-grain weight ($r=0.33$, $p<0.05$). Earlier studies in pearl millet also observed non-significant association for grain Fe and Zn densities with grain weight (Gupta et al., 2009; Rai et al., 2012). This suggests that selection for both micronutrients can effectively be made without compromising on grain size.

4. Conclusion

The wide range of variability coupled with high heritability implies good prospects for improvement of both populations for Fe and Zn densities. The highly significant and high positive correlation between both Fe and Zn densities, suggesting that both micronutrients can be effectively improved simultaneously. Both micronutrients had non-significant or small positive correlation with 1000-grain weight, implying these can be improved in large-seeded genetic backgrounds.

5. Acknowledgement

The research reported here forms part of a Ph.D. thesis of Anand Kanatti, submitted to Professor Jayashankar Telangana State Agriculture University, Hyderabad 500 030, Telangana, India. It was supported by a grant from the Harvest Plus Challenge Program of the CGIAR, and conducted under the CGIAR Research Program on Agriculture for Nutrition and Health.

6. References

- Blair, M.W., Astudillo, C., Grusak, M., Graham, R., Beebe, S., 2009. Inheritance of seed iron and zinc content in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding* 23, 197–207.
- Bouis, H.E., 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? *Proceedings of the Nutrition Society* 62, 403–411.
- Burton, G.W., 1974. Factor affecting pollen movement and natural crossing in pearl millet. *Crop Science* 14, 802–805.

Table 2: Correlation among grain iron (Fe) and zinc (Zn) densities and 1000-grain weight (GW) in S₁ progenies of ICTP 8203 (below diagonal) and ICMV 221 (above diagonal), Patancheru

Environment	Trait	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	GW (g)
P	Fe	1	0.72**	0.22
	Zn	0.66**	1	0.33*
	GW	0.05	-0.01	1
R12	Fe	1	0.60**	0.10
	Zn	0.59**	1	0.36*
	GW	0.04	0.08	1
S13	Fe	1	0.72**	0.22
	Zn	0.73**	1	0.33*
	GW	0.00	-0.06	1

P: Pooled across environments; R12: 2012 rainy season; S13: 2013 summer season; *, **: Significant at $p=0.05$, $p=0.01$, respectively



- Govindaraj, M., Rai, K.N., Shanmugasundaram, P., Dwivedi, S.L., Sahrawat, K.L., Muthaiah, A.R., Rao, A.S., 2013. Combining ability and heterosis for grain iron and zinc densities in pearl millet. *Crop Science* 53, 507–517.
- Gupta, S.K., Velu, G., Rai, K.N., Sumalini, K., 2009. Association of grain iron and zinc content with grain yield and other traits in pearl millet (*Pennisetum glaucum* (L.) R.Br.). *Crop Improvement* 36(2), 4–7.
- Kanatti, A., Rai, K.N., Radhika, K., Govindaraj, M., Sahrawat, K.L., Rao, A.S., 2014a. Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. *SpringerPlus* 3, 763.
- Kanatti, A., Rai, K.N., Radhika, K., Govindaraj, M., Sahrawat, K.L., Srinivasu, K., Shivade, H., 2014b. Relationship of grain iron and zinc content with grain yield in pearl millet hybrids. *Crop Improvement* 41(1), 91–96.
- Kanatti, A., Rai, K.N., Radhika, K., Govindaraj, M., 2016. Tester effect on combining ability and its relationship with line performance per se for grain iron and zinc densities in pearl millet. *Crop Science* 56, 689–696.
- Kumar, S., Hash, C.T., Thirunavukkarasu, N., Singh, G., Rajaram, V., Rathore, A., Senapathy, S., Mahendrakar, M.D., Yadav R.S., Srivastava, R.K., 2016. Mapping quantitative trait loci controlling high iron and zinc content in self and open pollinated grains of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Frontiers in Plant Sciences* 7, 1636.
- Peleg, Z., Cakmak, I., Ozturk, L., Yazici, A., Jun, Y., Budak, H., Korol, A.B., Fahima, T., Saranga, Y., 2009. Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. *Theoretical and Applied Genetics* 119, 353–369.
- Prasanna, B.M., Mazumdar, S., Chakraborti, M., Hossain, F., Manjaiah, K.M., Agrawal, P.K., Guleria, S.K., Gupta, H.S., 2011. Genetic variability and genotype × environment interactions for kernel iron and zinc concentrations in maize (*Zea mays*) genotypes. *Indian Journal of Agricultural Sciences* 81(8), 704–711.
- Rai, K.N., Anand Kumar, K., Andrews, D.J., Rao, A.S., Raj, A.G.B., Witcombe, J.R., 1990. Registration of ICTP 8203 pearl millet. *Crop Science* 30, 959.
- Rai, K.N., Govindaraj, M., Rao, A.S., 2012. Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance and Safety of Crops & Foods* 4, 119–125.
- Rai, K.N., Velu, G., Govindaraj, M., Upadhyaya, H.D., Rao, A.S., Shivade, H., Reddy, K.N., 2014. Iniadi pearl millet germplasm as a valuable genetic resource for high grain iron and zinc densities. *Plant Genetic Resources* (Published online) doi:10.1017/S14792621140006655.
- Rai, K.N., Yadav, O.P., Rajpurohit, B.S., Patil, H.T., Govindaraj, M., Khairwal, I.S., Rao, A.S., Shivade, H., Pawar, V., Kulkarni, M.P., 2013. Breeding pearl millet cultivars for high iron density with zinc density as an associated trait. *Journal of SAT Agricultural Research* 11, 1–7.
- Stangoulis, J.C.R., Huynh, B.L., Welch, R.M., Choi, E.Y., Graham, R.D., 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* 154, 289–294.
- Statistical Analysis Systems Institute Inc., 2009. SAS/STAT® 9.2 User's Guide (2nd Edn.). Cary, NC: SAS Institute Inc.
- Steel, R.D.G., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Edn.). McGraw-Hill, Inc, New York, NY.
- Stein, A.J., 2010. Global impacts of human malnutrition. *Plant and Soil* 335, 133–154.
- Velu, G., 2006. Genetic variability, stability and inheritance of grain iron and zinc content in pearl millet (*Pennisetum glaucum* (L.) R.Br.). Ph.D Thesis. Tamil Nadu Agricultural University, Coimbatore, India.
- Velu, G., Rai, K.N., Muralidharan, V., Longvah, T., 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.
- Velu, G., Rai, K.N., Sahrawat, K.L., 2008. Variability for grain iron and zinc content in a diverse range of pearl millet populations. *Crop improvement* 35(2), 186–91.
- Witcombe, J.R., Rao, M.N.V.R., Raj, A.G.B., Hash, C.T., 1997. Registration of 'ICMV 88904' pearl millet. *Crop Science* 37, 1022–1023.
- Wheal, M.S., Fowles, T.O., 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. *Analytical Methods* 3, 2854–2863.