Association of Grain Fe and Zn Contents with Agronomic Traits in Sorghum

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Genetic variability and the association of grain Fe and Zn-contents with other agronomic traits were studied in 1,394 accessions from core germplasm maintained at the ICRISAT gene bank. A large variability for grain Fe and Zn contents were observed. The grain Fe and Zn contents of the accessions with white grains were marginally higher than those with colored grains and these white grain sorghums originated mostly from India and Zimbabwe. The Fe and Zn contents of the accessions with testa and without testa were comparable. However, endosperm texture and grain size appeared to influence grain Fe and Zn contents. Strong positive correlation between grain Fe and Zn contents and their weak association with the agronomic and grain traits indicated possibility to breed simultaneously for high grain Fe and Zn traits in varied plant agronomic backgrounds that might be suitable for different agro-climatic regions across the world.

Key Words: Iron, Micronutrient, Variability, Zinc

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a staple food grain in many semi-arid tropical (SAT) and tropical areas of the world because of its good adaptation to harsh environments and its good yield (Dicko *et al*., 2006). More than 35% of sorghum is grown for direct human consumption. The rest is used primarily for animal feed, alcohol production and industrial products (FAO, 1995). Micronutrient malnutrition is primarily the result of diets poor in bio-available vitamins and minerals, mostly iron (Fe) and zinc (Zn) (Dar *et al*., 2006). Crop varieties selected and/or bred for this purpose through a plant breeding approach will complement the existing approaches (such as other fortified foods and medical pills), and at a fraction of the cost of existing approaches. Current research focuses on identifying varieties with high Fe and Zn meeting specific agricultural and food requirements from the greater biodiversity of sorghums for their direct use and/or introgression of the traits into breeding lines to ensure health. In a set of 84 breeding lines evaluated at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, the Fe content varied from 20.1 to 37.0 mg kg^{-1} grain and Zn content varied from 13.4 to 31.0 mg kg^{-1} grain, indicating limited variability (Reddy *et al*., 2005). Hence, a need was felt to explore the germplasm to identify lines with high grain Fe and Zn contents and study their associations with agronomic traits before utilization in the breeding programs.

Materials and Methods

A total of 2,974 accessions from the core collection maintained at the ICRISAT genebank representing the variability of the entire germplasm collection (about 36,774 accessions) were evaluated on Vertisols in an Augmented Design at the ICRISAT center farm, Patancheru along with four checks *viz.*, ICSV 745, IRAT 204, Ladde Samrudhi and Paccha Jonna known for their high grain Fe and Zn contents. The material was sown on 30th November 2005. The experimental site is located at an altitude of 545 m above mean sea level with latitude of 17.53º N and longitude of 78.27º E. The site receives an average annual rainfall of 887.56 mm (average of 31 years from 1974 to 2004).

For the sake of convenience, while collecting data on plant growth and grain yield and its component traits, harvesting and processing of grain samples for Fe and Zn contents estimation, the accessions were evaluated (in contiguous blocks) as three separate groups classified based on days to 50% flowering (early: d” 65 days; medium: 66 to 80 days; late: > 80 days) in Augmented Design. The early group consisted of 1,095 accessions along with 4 checks, each repeated 11 times; the medium group consisted of 1,128 accessions along with the 4 checks, each repeated 12 times; the late group consisted of 751 accessions along with 4 checks, each repeated 8 times. Each accession was sown in one row of 2

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m length. One week after seedling emergence, the plants were thinned to maintain 10 cm spacing between plants. A fertilizer dose of 80 Kg N (half as basal and remaining half at 30 days after sowing) and 40 Kg P_2O_5 (entire as basal) was applied. Apart from this, the recommended crop production and protection packages were followed to raise a healthy crop. In each accession, the border plants were left for open-pollination and all the middle plants were selfed.

The data were collected on the traits such as days to 50% flowering, plant height (m), grain yield (t ha^{-1}), 100-grain weight (g), grain color (LB= light brown, Br= brown, DB= dark brown, Cr= cream, CW= chalky white, G= gray, R= red, W= white, Y= yellow), grain shape (C= circular, E= elliptic, NE= narrow elliptic), Grain endosperm texture (0 = 0% corneous or 100% farinaceous to 100%= 100% corneous or vitreous), Germ size (S= small, M= medium, L= large), plant agronomic aspect (taken on a 1 to 5 scale, where 1= most desirable and 5= least desirable), panicle shape (taken on a 1 to 5 scale, where 1= reverse pyramid, 2= panicle broader in the upper part, 3= symmetric, 4= panicle broader in the lower part and 5= lower part pyramidal), panicle compactness (C= compact, SC= semi-compact, SL= semi-loose, L= loose and VL= very loose), glume color (Bl= black, Br= brown, G= green, Lr= light red, P= purple, R= red, S= straw), glume coverage (0% to 100%) and presence/absence of seed sub-coat. The grain samples from some of the accessions could not be collected due to severe bird damage and in few accessions the available grains were not sufficient for estimation of Fe and Zn contents. The grain samples harvested from selfed panicles of 698 accessions of early maturity, 459 accessions of medium maturity, and 237 accessions of late maturity (making a total of 1,394 accessions), and the grain samples harvested from open-pollinated panicles of 118 accessions of early maturity, 66 accessions of medium maturity, and 30 accessions of late maturity (making a total of 214 accessions) where sufficient quantities were available. The contents of Fe and Zn in the ground grain samples (one replication per sample, random samples were duplicated) were determined by using the triacid digestion method and the Fe and Zn contents in the aliquot was determined by atomic absorption spectrometer (Sahrawat *et al*., 2002).

Results and Discussion

Genetic variability

The Fe and Zn contents of the checks although varied, the variability was minimal as the CV% was below 15. Assuming that the variability of the check varieties was same as that of the test varieties, the trait-values of the accessions were adjusted and reported here under. A large variability for grain Fe (7.7 to 132.6 mg kg^{-1}) and Zn (15.1 to 91.3 mg kg^{-1}) contents was observed among the 1394 core accessions. The variability observed in core germplasm collection was much higher than that reported earlier, based on screening of 86 genotypes consisting of germplasm lines, hybrid parents of released/marketed hybrids and popular varieties. In general, sorghums used as human food have white pericarp, tan plant color, straw color glumes and produce grain with medium to hard endosperms. Sorghums with brown or red color, including those with pigmented testa, are often used to produce malt and local alcoholic beverages and porridges (Rooney and Waniska, 2000). Interestingly, grain Fe and Zn contents of accessions with white grains (43.6 mg kg^{-1} Fe and 35.1 mg kg^{-1} Zn), which are used for human consumption in India, were marginally higher than those with colored grains (Fe content ranged from 40.4 to 42.6 ppm and Zn content ranged from 30.9 to 34.0 ppm) (Table 1). The Fe and Zn rich, white or cream colored grain sorghums originated mostly from India, Zimbabwe, Sudan and Yemen apart from other countries while those with low Fe and Zn in similar grain color background had India, Algeria, Cameroon and Sudan origin. The Fe and Zn rich, brown colored grain sorghums originated from USA, India, Spain, China, Angola and Tanzania while those with low Fe and Zn in similar grain color background had Kenya, Uganda, Mexico, Australia, Mali and Korea origin (Table 2). The Fe and Zn contents of accessions with testa (42.5 ppm Fe and 34.2 ppm Zn) and without testa (42.9 ppm Fe and 33.2 ppm Zn) were comparable (Table 1). However, endosperm texture and grain size appeared to influence grain Fe and Zn contents. While the accessions with 100% corneous endosperm had 56.2 ppm Fe and 44.3 ppm Zn contents, those with 0% to 75% corneous endosperm had less than 44.8 ppm Fe and 35.0 ppm Zn (Table 1). However, further studies are needed to confirm this. The texture of endosperm has significance in food preparations. The grains with 50% corneous endosperm are useful for

Table 1. Relationship of grain traits (colour, testa presence/absence and endosperm texture) with grain Fe and Zn contents in sorghum core germplasm collection, 2005 post-rainy season, Patancheru

	Trait	# of lines	Fe (mg kg^{-1}) Range	Fe Mean	SD	Zn (mg kg^{-1}) Range	Zn Mean	SD
1.	**Colour of the grain**							
	Light Brown	29	28.8 to 63.6	41.2	8.6	21.6 to 44.4	31.8	5.7
	Brown	604	7.7 to 132.6	42.6	13.4	15.8 to 91.3	34.0	8.5
	Dark Brown	30	27.7 to 51.7	40.4	6.0	21.7 to 55.8	32.2	7.5
	Cream	244	12.9 to 96.0	42.0	11.3	15.9 to 66.0	33.7	8.9
	Chalky White	139	21.8 to 90.5	42.1	9.1	15.1 to 56.8	31.2	7.8
	Gray	8	33.1 to 53.8	42.4	7.6	27.9 to 41.0	33.1	4.4
	Light Red	11	31.5 to 48.1	41.6	4.8	19.8 to 29.5	30.9	9.4
	Red	133	12.4 to 84.4	41.9	9.1	20.3 to 64.9	33.8	7.7
	White	138	18.9 to 71.0	43.6	10.4	17.6 to 59.9	35.1	9.1
	Yellow	58	22.1 to 65.6	40.9	10.6	15.7 to 52.7	30.9	8.3
2.	**Testa**							
	Present	728	7.7 to 132.6	42.5	11.7	15.7 to 66.0	34.2	8.3
	Absent	666	17.6 to 128.9	42.9	11.7	15.1 to 91.3	33.2	8.7
3.	**Texture of the endosperm**							
	0 % corneous	299	20.8 to 112.4	43.4	13.2	15.8 to 91.3	34.9	9.1
	25% corneous	703	7.7 to 132.6	41.7	10.3	16.0 to 63.7	33.2	7.7
	50% corneous	329	15.0 to 96.0	43.2	11.3	15.1 to 66.0	33.8	9.4
	75% corneous	53	24.8 to 88.6	44.8	12.4	20.0 to 55.1	35.0	8.8
	100% corneous	10	48.1 to 73.9	56.2	7.8	35.1 to 53.0	44.3	5.5
4.	**Grain size**							
	Bold grain (>3.5 g 100^{-1})	384	12.4 to 83.1	40.8	13.9	15.1 to 58.3	32.2	9.1
	Medium grain (>2.5 g to 3.5g 100^{-1})	564	19.0 to 108.4	42.6	10.2	15.9 to 66.0	33.5	8.3
	Small grain (<2.5 g 100^{-1})	446	7.7 to 132.6	44.4	9.3	15.8 to 91.3	35.9	7.7
	Overall	1394	7.7 to 132.6	42.3	11.6	15.1 to 91.3	33.5	8.5

Table 2. Origin of germplasm lines with high and low grain Fe and Zn contents in varied grain color backgrounds

Important grain color	Micronutrient	Line* High	Line* Low
Cream	Fe	IS 8078 (Japan); IS 6413 (India), IS4622 (India), IS 2263 (Sudan)	IS 30655 (Cameroon); IS 916 (Algeria), IS 4116 (India), IS 31697 (Algeria)
	Zn	IS 2248 (India); IS 26012 (Mali), IS 28084 (Yemen); IS 27108 (Zimbabwe)	IS 5273 (India); IS 916 (Algeria); IS 23453 (India), IS 20607 (Sudan)
White	Fe	IS 25386 (Kenya); IS 30056 (Zimbabwe); IS 6975 (Sudan); IS27906 (S. Africa)	IS 16054 (Cameroon); IS 29012 (Yemen); IS 9918 (Sudan); IS 20537 (Sudan)
	Zn	IS 3790 (Taiwan); IS 24895 (Zimbabwe); IS 30056 (Zimbabwe); IS 23879 (Yemen)	IS 20537 (Sudan); IS 20552 (Sudan); IS 22381 (Sudan); IS 20502 (Sudan)
Light Red	Fe	IS 19053 (Sudan); IS 26280 (Togo); IS 22356 (Botswana); IS 20435 (Niger)	IS 22231 (Australia); IS 14433 (Lesotho); IS 20298 (Niger); IS 14869 (Cameroon)
	Zn	IS 26280 (Togo); IS 22356 (Botswana); IS 22756 (Somalia); IS 20435 (Niger)	IS 14869 (Cameroon); IS 22231(Australia); IS 3158 (S. Africa); IS 20298 (Niger)
Red	Fe	IS 31906 (Yemen); IS 2885 (Italy); IS 28376 (Yemen); IS 26200 (Togo)	IS 16546 (Cameroon); IS 26513(Benin); IS 13885 (S. Africa); IS 25713 (Mali)
	Zn	IS 31906 (Yemen); IS 18088(Lebanon); IS 31984 (Yemen); IS 26200 (Togo)	IS 13585 (Uganda); IS 18745(USA); IS 9571 (S. Africa); IS 271 (Unknown)
Yellow	Fe	IS 7881 (Nigeria); IS 22022 (India); IS 22311 (Botswana); IS 25331 (Ethiopia)	IS 21761 (Sudan); IS 24632 (Lebanon); IS 18333 (India); IS 15697 (Cameroon)
	Zn	IS 5522 (India), IS 7881 (Nigeria); IS 10973 (USA); IS 22311 (Botswana)	IS 21761 (Sudan); IS 24632 (Lebanon); IS 15697 (Cameroon); IS 24561 (Lebanon)
Brown	Fe	IS 28 (USA), IS 613 (USA); IS 6335 (India); IS 13211 (Spain)	IS 9058 (Kenya), IS 902 (Mexico); IS 22227 (Australia); IS 30520 (Korea)
	Zn	IS 6335 (India), IS 20888 (Angola); IS 30310 (China); IS 12701 (Tanzania)	IS 8891(Uganda), IS 9058(Kenya); IS 8084 (Uganda); IS 12380 (Mali)

*The origin of the germplasm line is given in the parentheses

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preparation of '*roti*' or '*chapati*' (unleavened bread), the most popular food forms of sorghum grains in India, and for '*ingera*' (leavened bread), the most popular food forms of sorghum grains in some parts of Africa. Grains with more than 75% corneous endosperm are useful for preparation of '*to*' the most popular food forms of sorghum grains in some parts of Africa (Anglani, 1998). The accessions with small grains (< 2.5 g 100-grain weight) had higher grain Fe (44.4 ppm) and Zn (35.9 ppm) contents than those with medium grain weight of 2.5 g to 3.5 g 100-grains (42.6 ppm Fe and 33.5 ppm Zn) and those with large grain weight of >3.5 g 100-grains (40.8 ppm Fe and 32.2 ppm Zn) (Table 1).

Association of grain Fe and Zn contents with other grain traits and agronomic traits

Correlation of grain Fe and Zn contents with agronomic and grain traits in 1394 core germplasm lines were estimated (Table 3). Fairly high correlation (r=0.6) between grain Fe and Zn contents suggests ample scope for simultaneous improvement of both the micronutrients. Though correlation of grain Fe and Zn contents with days to 50% flowering (0.1 for Fe and 0.2 for Zn), plant height (0.2 for Fe and 0.4 for Zn) was significant and positive, the lower magnitude of the correlation suggests near-independence of the crop growth traits and grain micronutrients contents. The results indicated that sorghum grain Fe and Zn contents can be improved in different maturity and plant stature backgrounds. Similarly, significant negative but lower magnitude of correlation of grain Fe and Zn contents with grain yield (-0.2 Fe and -0.2 Zn) and grain size (-0.1 Fe and -0.1 Zn) suggested that it is possible to enhance grain Fe and Zn contents in high yielding backgrounds with large grain. Significantly high and positive correlations were observed for grain Fe (0.9) and Zn (0.8) contents estimated from grain obtained from open-pollinated and selfed panicles.

A large variability for grain Fe and Zn contents was observed among 1394 core collection accessions. Strong positive correlation between grain Fe and Zn contents and their weak association with the agronomic and grain traits indicated possible positive outcomes from breeding simultaneously for high grain Fe and Zn traits in varied plant agronomic backgrounds that may be suitable for different regions across the world.

Table 3. Estimates of correlation of agronomic and grain traits with grain Fe and Zn contents in sorghum core germplasm collection, 2005 postrainy season, Patancheru

Trait	Fe (mg kg^{-1})	Zn (mg kg^{-1})
Days to 50% flowering	0.088**	0.207**
Plant height (m)	0.220**	0.380**
100-grain weight (g)	-0.130**	-0.143**
Grain yield (t ha^{-1})	-0.161**	-0.200**
Panicle shape[1]	0.063*	0.083**
Panicle compactness[2]	0.214**	0.125**
Glume color[3]	-0.038	-0.173**
Glume coverage%[4]	0.278**	0.292**
Grain color[5]	-0.016	0.002
Grain shape[6]	-0.156**	-0.179**
Grain endosperm texture[7]	0.071*	0.032
Germ size[8]	0.009	0.032
Zn (ppm)	0.604**	-

n-2 = 1392; r at 5% = 0.062; r at 1% = 0.081 [1]Panicle shape (taken on a 1 to 5 scale, where 1= reverse pyramid, 2= panicle broader in the upper part, 3= symmetric, 4= panicle broader in the lower part and 5= lower part pyramidal), [2]panicle compactness (taken on a 1 to 5 scale, where 1= compact, 2= semi-compact, 3= semi-loose, 4= loose and 5= very loose), [3]glume color (taken on a 1 to 7 scale, where 1= green, 2= straw, 3= brown, 4= light red, 5= red, 6= purple, 7= black), [4]glume coverage (taken on a 1 to 4 scale, where 1= 25%, 2= 50%, 3= 75% and 4= 100%), [5]grain color (taken on a 1 to 9 scale, where 1= white, 2= chalky white, 3= cream, 4= yellow, 5= gray, 6= red, 7= light brown, 8= brown and 9= dark brown), [6]grain shape (taken on a 1 to 3 scale, where 1= narrow elliptic, 2= elliptic and 3= circular), [7]Grain endosperm texture (taken on a 1 to 5 scale, where 1= 0% corneous, 2= 25%, 3= 50%, 4= 75% and 5= 100%) and [8]Germ size (taken on a 1 to 3 scale, where 1 = small, 2= medium, 3= large).

The accessions with high grain Fe and Zn contents are needed to be evaluated again for confirmation and simultaneously involved in the development of micronutrient-enriched cultivars with farmer-preferred traits.

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