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Genetic Variability and Character Association for Grain Iron and Zinc Contents in Sorghum Germplasm Accessions and Commercial Cultivars

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ABSTRACT

Sorghum a widely consumed cereal stapled in sub-tropical and semi-arid regions of Africa and Asia. Sorghum is the second cheapest source of energy and micronutrients (after pearl millet). Micronutrient malnutrition, primarily the result of diets poor in bio-available vitamins and minerals, causes blindness and anemia (even death) in more than half of the world's population, especially among women and children. Biofortification wherever possible, is a cost effective and sustainable solution for tackling the micronutrient deficiencies as the intake of micronutrients is on a continuing basis with no additional costs to the consumer. ICRISAT is working on sorghum biofortification for enhancing Fe and Zn contents of the grain. Large variability for grain Fe and Zn contents was found from assessment of core germplasm collections (>2200), and from these, promising donors were identified for further improvement. Significant positive association observed between grain Fe and Zn contents indicated that it is feasible to develop high Fe and Zn containing cultivars with high yielding and different maturity backgrounds. The commercial sorghum cultivars (66) currently being cultivated by the farmers in India, were evaluated to identify high Fe and Zn cultivars in adapted backgrounds. Identification of commercial cultivars with high grain Fe and Zn content would help in wider dissemination of the cultivars to complement the on-going efforts for combating the micronutrient malnutrition.

Keywords: hybrids, landraces, micronutrients, *Sorghum bicolor*, varieties, yield and correlation

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop grown on 40 million ha in 105 countries in Africa, Asia, Oceania, and the Americas. The USA, India, Mexico, Nigeria, Sudan, and Ethiopia are the major sorghum producers globally (http://faostat.fao.org/site/567/default.aspx#ancor). Sorghum, a heat and drought tolerant C_4 plant, is a widely consumed cereal stapled in sub-tropical and semi-arid regions of Africa and Asia (Kresovich *et al.* 2005; Reddy *et al.* 2009). Sorghum is the second cheapest source of energy and micronutrients (after pearl millet); and a vast majority of the population in Africa and central India depend on sorghum for their dietary energy and micro-nutrient requirement (Parthasarathy Rao 2006). Limited studies indicated that mineral concentrations and bioavailability are limited in cooked sorghum grain (Kayode *et al.* 2006); but this needs to be further validated.

Micronutrient malnutrition, primarily the result of diets poor in bio-available vitamins and minerals, causes blindness and anemia (even death) in more than half of the world's population, especially among women of reproductive age, pregnant and lactating women and pre-school children (Underwood 2000; Sharma 2003; Welch and Graham 2004) and efforts are being made to provide fortified foods to the vulnerable groups of the society. The results of a socio-economic study covering 12 countries in Asia, Africa and Latin America showed that the costs of biofortification to avert Disability Adjusted Life Years (DALY) losses often fall in the highly cost-effective category (Meenakshi *et al.* 2010). Biofortified crop cultivars offer a rural-based intervention that, by design, initially reach those more remote populations, which comprise a majority of the malnourished, and then penetrate to urban populations as production surpluses are marketed (Bouis *et al*. 2011). Biofortification of sorghum by increasing mineral micronutrients (especially iron and zinc) in the grains is of widespread interest (Pfeiffer and McClafferty 2007; Zhao 2008).

ICRISAT is working on sorghum biofortification for enhancing the grain Fe and Zn contents. Preliminary studies by Reddy *et al.* (2005) indicated a limited variability for grain Fe and Zn contents in a study on sorghum hybrid parents, advanced breeding lines and a few selected germplasm accessions. Field study made at the ICRISAT farm in Patancheru to enhance the grain Fe and Zn contents under balanced nutrient application (nitrogen, phosphorus, potassium along with sulfur, boron, iron and zinc were applied to the soil) did not increase the grain Fe and Zn contents probably because the inherent availability of these nutrients in the soil was not limiting. In this study, the genetic enhancement route is attempted for increasing grain Fe and Zn contents in sorghum through identification of promising donors from the core collection of sorghum germplasm accessions for use in the crossing program. The other important aspect of this study was identification of high Fe and Zn containing cultivars in the popular commercial sorghum cultivars that are currently being cultivated by the farmers in India that can be disseminated to complement the on-going efforts for combating the micronutrient malnutrition. We describe here briefly the methodologies followed and the results obtained in these two areas.

MATERIALS AND METHODS

To assess the variability in sorghum germplasm, a total of 29 accessions selected earlier for high Fe and Zn contents (based on

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previously assessed >2200 accessions) from core collection along with two controls ICSR 40 (an ICRISAT-developed restorer line) and 296B [Directorate of Sorghum Research (DSR)-Hyderabad, India developed hybrid seed parent] were evaluated. The accessions used in the study belonged to different races with different geographical origins and were selected from the core collections of sorghum germplasm accessions. The material was grown in the field during 2007 and 2008 postrainy seasons under high soil fertility conditions (N80: P40: K0) in a replicated trial with randomized complete block design (RCBD) with three replications (4 rows 2 m length plots) at the ICRISAT farm Patancheru (located at an altitude of 545 m above mean sea level, latitude of 17.53° N and longitude of 78.27° E). Care was taken to raise a healthy crop. The data were recorded for the agronomic traits; days to 50% flowering, plant height (m), glume coverage (%), grain yield (t ha^{-1}) and grain size (g 100^{-1}) as per the standard procedure. Three to five panicles were bagged with Kraft paper bags prior to flowering in each replication to avoid pollen contamination and to harvest pure seed for grain analysis. The panicles were harvested at maturity and the grain was threshed carefully without any contact with metal or dust to avoid contamination. The cleaned seeds were collected into cloth bags and used for micronutrient analysis in the ICRISAT Central Analytical Services Laboratory (CASL) at Patancheru. The grain Fe and Zn contents were determined in the ground grain samples by using the triacid digestion method (Sahrawat *et al.* 2002). The data on agronomic traits along with grain Fe (mg kg^{-1}) and Zn (mg kg^{-1}) were statistically analyzed using the GENSTAT 9.1 package.

To assess the variability in commercial sorghum cultivars, a total of 20 commercial sorghum cultivars (Set 1) developed in India by the Indian NARS in partnership with the ICRISAT or the NARS alone (17 hybrids contributed by seven private sector seed companies, one hybrid by the Marathwada Agricultural University, Parbhani and two varieties by the Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Palem) were evaluated along with two controls (CSH 16 and PVK 801) in a replicated RBD (n = 3) trial during the 2008 and 2009 postrainy seasons at the ICRISAT farm in Patancheru. Similarly, 43 commercial cultivars (24 hybrids and 19 varieties), four landraces developed in India by Indian NARS either in partnership with ICRISAT or NARS per se as Set II were evaluated along with two controls (CSH 16 and PVK 801) in a replicated RBD (n = 3) trial during the 2009 postrainy season at ICRISAT-Patancheru. Each cultivar was grown in four-row plots of two m length with 75 cm spacing between the rows. Irrigations (4-5 times) were applied as required during the cropping season. Utmost care was taken to raise a healthy crop in both the studies and to get clean grain for lab analysis for grain Fe and Zn contents. The agronomic data were recorded and analyzed as indicated above.

RESULTS AND DISCUSSION

Variability in core germplasm accessions

The ANOVA showed significant differences among the genotypes and years for all the traits studied except for grain Fe contents among years (data not shown). Significant genotypes × year interactions existed for the days to 50% flowering, plant height, grain yield, grain size and grain Fe and Zn contents. Over the two years, mean grain Fe content ranged from 26 to 60 mg kg^{-1} and Zn content ranged from 21 to 57 mg kg^{-1} (**Table 1**). Both controls 296 B and ICSR 40 had grain Fe content of 40 and Zn content of 24 mg kg^{-1}. Twelve lines were significantly superior to both control lines for grain Fe content ranging from 48 to 61 mg kg^{-1}, while 27 accessions were superior for grain Zn content ran-

Table 1 Mean performance of selected sorghum germplasm lines evaluated for grain Fe and Zn contents at ICRISAT-Patancheru during the 2007 and 2008 postrainy seasons.

IS No. Pedigree	Race	Origin	Days to 50% flowering	Plant height (m)	Grain yield (t ha^{-1})	Grain size (g 100^{-1})	Iron (mg kg^{-1})	Zinc (mg kg^{-1})
5427	Durra	India	65	2.0	2.0	2.8	61	57
5514	Guinea-bicolor	India	68	1.7	1.4	3.0	56	45
55	Durra-caudatum	US	71	1.0	1.3	2.6	54	38
3760	Caudatum-bicolor	USSR	68	1.9	2.2	2.2	53	37
3283	Bicolor	US	66	1.8	1.9	2.7	50	42
17580	Caudatum	Nigeria	66	1.9	1.6	2.1	50	41
15952	Guinea	Cameroon	81	2.4	2.5	3.4	49	41
3813	Durra	India	79	2.2	1.4	1.7	49	38
15266	Caudatum	Cameroon	70	1.4	2.7	2.7	49	44
2939	Kafir	US	69	2.0	3.6	3.9	48	37
4159	Durra	India	65	1.9	1.5	3.3	48	38
3929	Kafir-durra	US	75	2.0	2.2	2.1	48	40
3443	Guinea-caudatum	Sudan	68	1.7	3.3	3.5	47	39
3925	Durra-caudatum	US	78	2.0	2.4	2.0	47	39
5460	Durra-bicolor	India	66	1.5	1.4	2.7	47	46
12452	Caudatum-bicolor	Sudan	64	1.9	3.2	4.3	47	33
2801	Caudatum	Zimbabwe	71	1.8	2.3	3.1	46	45
2536	Kafir-caudatum	US	72	1.8	2.3	2.4	45	37
5429	Durra	India	66	1.7	2.8	3.0	44	30
356	Durra	US	84	1.1	2.2	2.7	44	33
2265	Durra-bicolor	Sudan	75	2.2	1.8	1.7	44	41
12695	Bicolor	South Africa	68	1.9	2.8	2.6	44	39
5538	Durra	India	65	1.4	1.7	2.2	44	37
5476	Durra	India	69	1.5	2.1	2.7	41	36
16337	Caudatum	Cameroon	80	1.7	2.4	3.0	41	34
5853	Guinea-durra	India	65	1.7	2.4	5.9	41	32
14318	Bicolor	Swaziland	79	2.1	2.2	2.3	39	38
10674	Durra-caudatum	China	65	2.1	1.9	4.0	39	38
22215	Durra-bicolor	USSR	80	2.1	2.9	2.4	26	21
Controls								
ICSR 40			70	1.2	3.3	3.2	40	24
296B			86	1.1	2.7	2.8	40	24
Mean			71	1.76	2.26	2.9	46	37
SE +			0.96	0.12	0.26	0.2	3	3
CV (%)			2.32	11.51	20.12	10.4	10	13

Table 2 Correlation coefficients of sorghum landraces evaluated for iron and zinc estimations in 2007 and 2008 postrainy seasons at the ICRISAT, Patancheru.

	Iron	Zinc	Days to 50% flowering	Plant height	Glume coverage	Grain yield
Iron	1.00					
Zinc	0.75**	1.00				
Days to 50% flowering	-0.35	-0.35	1.00			
Plant height	0.02	0.28	-0.01	1.00		
Glume coverage	0.19	0.27	-0.17	0.31	1.00	
Grain yield	-0.36*	-0.46**	0.10	0.01	-0.14	1.00
Grain size	-0.12	-0.17	-0.37*	-0.06	-0.43*	0.37*

df (n-2)=29 and r=0.36 at 5% and 0.46 at 1%

Table 3 Mean performance of the commercial sorghum cultivars (Set I) for grain Fe and Zn contents at ICRISAT- Patancheru, India during 2008 and 2009 postrainy seasons.

Hybrid name	Seed source	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})		
		2008	2009	Mean	2008	2009	Mean
NSH 703	Nuziveedu Seeds, Hyderabad	50	38	44	36	28	32
GK 4035	Ganga Kaveri Seeds, Hyderabad	57	31	44	46	19	33
Mahabeej 703	MSSCL, Akola	53	33	43	36	22	29
NSH 702	Nuziveedu Seeds, Hyderabad	49	37	43	37	28	32
8562	Bayer Bio Sc., Hyderabad	51	31	41	37	23	30
Mahabeej 704	MSSCL, Akola	48	31	40	34	19	26
KDSH 1179	Krishidhan Seeds, Jalna	48	30	39	31	22	27
BSH 45	Biostadt Mh Seeds, Aurangabad	48	29	39	32	22	27
Madhura -SS hybrid	Nimbkar Seeds, Paltan	52	25	39	43	21	32
Mahabeej 7	MSSCL, Akola	52	25	39	33	18	26
GK 4009	Ganga Kaveri Seeds, Hyderabad	46	30	38	36	17	27
Hi-jowar 52	Biostadt Mh Seeds, Aurangabad	42	33	38	28	22	25
PSV 2 (variety)	ARS, Palem	47	28	38	36	16	26
CSH 25	MAU, Parbhani	53	27	37	35	19	25
8340	Bayer Bio Sc., Hyderabad	47	27	37	29	20	25
KDSH 209	Krishidhan Seeds, Jalna	48	28	36	31	22	26
PSV 1 (variety)	ARS, Palem	47	26	36	31	17	24
BSH 47	Biostadt Mh Seeds, Aurangabad	42	28	35	26	19	23
GK 4044	Ganga Kaveri Seeds, Hyderabad	43	26	33	32	16	22
8568	Bayer Bio Sc., Hyderabad	37	23	30	29	17	23
Controls							
PVK 801 (variety)		55	30	43	41	20	30
CSH 16 (hybrid)		50	32	41	34	22	28
Mean		48	29	39	34	20	27
SE ±		2.86	1.85	2.76	2.09	1.44	2.00
CD (5%)		8.39	5.27	7.84	6.13	4.10	5.68

ging from 32 to 57 mg kg^{-1}.

In earlier studies also, the superiority of landraces over controls for grain Fe and Zn contents in sorghum were reported (Reddy *et al.* 2005; Sreeramaiah *et al.* 2007). In the top five genotypes for grain Fe contents, the two accessions (IS 5427 and IS 5514) were from India, two accessions (IS 55 and IS 3283) from the USA and one accession (IS 3760) was from the USSR. They recorded grain Fe content > 50 mg kg^{-1}, which is 25% higher than that recorded in the best control, ICSR 40 (grain Fe 40 mg kg^{-1}). All these lines are early to medium for the days to 50% flowering (65-71 days) with good plant height (1.0 to 2.0 m). Grain yield in these lines ranged from 1.3 to 2.2 t ha^{-1} and the grain size varied from 2.23 to 2.97 g 100^{-1}. The grain Zn contents in these lines ranged from 37 to 57 mg kg^{-1} compared to the best control, 296 B with grain Zn content of 24.0 mg kg^{-1}.

The correlations of grain iron and zinc with grain yield and plant traits are presented in **Table 2**. Significant positive correlation existed between grain Fe and Zn contents (r = 0.75) of the lines under evaluation. Both grain Fe (-0.36) and Zn (-0.46) contents showed small but significant negative association with grain yield but numerically low indicating that genetic enhancement for grain Fe and Zn content does not have yield penalty. However, there was no significant negative association of grain iron and zinc content with the other traits studied. This is in conformity with the earlier study (Reddy *et al.* 2010).

Variability in commercial cultivars

The mean grain Fe content among the set I genotypes ranged from 30 to 44 mg kg^{-1} and grain Zn content from 22 to 33 mg kg^{-1} (**Table 3**). Among the 20 hybrids tested in set I, six hybrids showed higher than 39 mg kg^{-1} grain Fe, and six hybrids had higher than 27 mg kg^{-1} grain Zn, which were well above the trial means for grain Fe and Zn contents. The controls - PVK 801 and CSH 16 showed 43 and 41 mg kg^{-1} grain Fe and 30 and 28 mg kg^{-1} grain Zn. Four hybrids (NSH 703, GK 4035, Mahabeej 703 and NSH 702) were superior to the control hybrid, CSH 16 for grain Fe content that ranged from 43 to 44 mg kg^{-1}, while six genotypes had grain Zn content ranging from 29 to 33 mg kg^{-1} and were superior to the control (CSH 16 Zn content 28 mg kg^{-1}). The hybrids GK 4035 and Mahabeej 703 showed higher mean Fe contents over two years (**Table 3**) indicating their stability for this trait. Among the varieties, PVK 801 showed higher grain Fe (43 mg kg^{-1}) and Zn contents (30 mg kg^{-1}) than the other varieties. It is interesting to note that PVK 801 a white grained high yielding and grain mold resistant variety also had high grain Fe and Zn contents. The range for Fe and Zn contents in the commercial cultivars in the present study was numerically higher than those of hybrid seed parents or advanced breeding lines assessed earlier (Reddy *et al.* 2005); but this comparison may have limited value considering significant G × E interaction. A significant positive association was observed between the grain Fe and Zn contents (r = 0.85).

Evaluation of 46 commercial cultivars in set II showed

Table 4 Mean performance of the commercial sorghum cultivars (Set II) for grain Fe and Zn contents at ICRISAT- Patancheru, India during 2009 postrainy season, Patancheru.

Hybrid name	Seed source	Fe content (mg kg^{-1})	Zn content (mg kg^{-1})
Mahabeej 706	MSSCL, Akola	39	25
XSM 1556	Bayer Bio Sc., Hyderabad	39	19
KSH 950	Kaveri Seeds, Hyderabad	39	24
HTJH 6290	Hytech Seeds, Hyderabd	37	26
KSH 6363	Kaveri Seeds, Hyderabad	37	26
BSH 53	Biostadt Mhseeds, Aurangabad	37	23
JK Jyothi	JK Seeds, Hyderabad	36	27
GK 4009	Ganga Kaveri Seeds, Hyderabad	36	27
DSH 4	UAS, Dharwad	35	22
KH 701	Kanchan Ganga Seeds, Hyderabad	35	25
CO (S) 28 (variety)	TNAU, Coimbatore	35	17
HTJH 6143	Hytech Seeds, Hyderabad	35	21
Controls			
PVK 801 (variety)		37	21
CSH 16 (hybrid)		33	23
Mean		32	21
SE ±		2.24	1.25
CD (5%)		6.29	3.52

Table 5 Correlation coefficients of the commercial sorghum cultivars grown at the ICRISAT-Patancheru, India during 2008 and 2009 postrainy seasons.

Trait	Fe	Zn	Days to 50% flowering	Plant height (m)	Grain yield (t ha^{-1})
Zn	0.853**	1.000			
Days to 50% flowering	0.087	0.158	1.000		
Plant height (m)	-0.039	0.224	-0.017	1.000	
Grain yield (t ha^{-1})	0.020	-0.045	-0.223	0.023	1.000
Grain size (g 100^{-1})	0.279	0.221	-0.464	-0.133	0.456*

df (n-2)=20

*: significant at 5%

** : significant at 1%

Fe contents ranging from 22 to 39 mg kg^{-1} and Zn contents from 15 to 29 mg kg^{-1} with an average Fe content of 32 mg kg^{-1} and average Zn content of 21 mg kg^{-1}. Data from selected 10 entries is presented in **Table 4**. Twenty two cultivars showed higher grain Fe and Zn contents than the trial mean. The controls PVK 801 and CSH 16 showed grain Fe content 37 and 33 mg kg^{-1} and Zn content 21 and 23 mg kg^{-1}, respectively. Three commercial hybrids (Mahabeej 706, KSH 950, and HTJH 6290) were superior for both Fe (37 to 39 mg kg^{-1}) and Zn contents (24 to 26 mg kg^{-1}) than the control cultivars. The hybrid XSM 1556 was superior for grain Fe only compared to the controls. However, none of the popular postrainy season sorghum cultivars preferred for food use in India like M35-1, Dagdi Solapur, Gidda Maldandi, Parbhani Moti, Parbhani Jyoti, Phule Vasudha, Phule Chitra or Phule Anuradha showed higher grain Fe and Zn contents.

Highly significant positive correlation between the grain Fe and Zn content ($r = 0.853$; $P < 0.01$) was observed (**Table 5**), indicating that either the genetic factors for Fe and Zn contents are linked, or the physiological mechanisms were interconnected for Fe and Zn uptake and translocation in the grain. These results indicate the potential of simultaneous genetic improvement for both micronutrients. The weak association of grain Fe and Zn with the other traits studied – days to 50% flowering, plant height, grain yield and grain size – indicates that there is no penalty for enhancing the grain Fe and Zn contents in sorghum along with other agronomic traits such as grain size and grain yield in varied maturity backgrounds.

In order to realize the potential impact of the micronutrient-dense cultivars, the micronutrient-rich cultivars must be delivered in high-yielding backgrounds with farmers' preferred traits such as early maturity, acceptable seed color and large seed size.

CONCLUSIONS

Genetic enhancement of grain Fe and Zn contents helps to complement on-going efforts for combating micronutrient malnutrition. The present study led to the identification of suitable donors which is an important step in this direction. The five accessions (IS 5427, IS 5514, IS 55, IS 3760 and IS 3283) identified from this study with high grain Fe (>50 mg kg^{-1}) and Zn (>37 mg kg^{-1}) contents can be utilized to increase the diversity and micronutrient density of sorghum hybrid parents in the future.

Considerable variability exists for grain Fe and Zn contents in the elite commercial sorghum cultivars grown in India for food purposes, and the seven hybrids (NSH 703, GK 4035, Mahabeej 703, NSH 702, Mahabeej 706, KSH 950 and HTJH 6290) with high Fe and Zn contents identified in this study can be used for large scale dissemination. The correlations studies indicated that the improvement of grain Fe and Zn contents in sorghum is possible simultaneously and there is no penalty in the agronomic traits when combined with high Fe and Zn contents. Moreover, this study showed that it is possible to deliver high Fe and Zn contents in cultivars with farmers' preferred traits such as early maturity, grain color, and high yield potential and bold grain.

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