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Biofortification for Combating Micronutrient Malnutrition: Identification of Commercial Sorghum Cultivars with high Grain Iron and Zinc Concentrations

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ABSTRACT: Sorghum is the dietary staple of more than 500 million people over 30 countries in Africa and Asia. Per capita consumption is 75 kg/year in major sorghum areas in India. It is one of the cheapest sources of energy, protein, Fe and Zn and contributes to >50% of the Fe and Zn. ICRISAT is working on sorghum biofortification to develop micronutrient-dense (Fe and Zn) sorghum cultivars with increased nutrient bioavailability. A total of 63 commercial sorghum cultivars developed in India were used to assess the variability in the commercial sorghum cultivars that are currently being cultivated by sorghum farmers in India. The grain Fe and Zn concentrations were determined in the grain samples by using Atomic Absorption Spectrometer (AAS) and the X-ray fluorescence spectrometer (XRF). The mean grain Fe concentration in genotypes ranged from 22 to 44 mg /kg and grain Zn concentrations from 15 to 33 mg /kg. This variability was significant considering the base levels of grain Fe (30 mg /kg) and Zn (20 mg /kg) concentrations in sorghum. Highly significant positive correlation was found between the grain Fe and Zn concentration (r = 0.853; P <0.01). Compared to post-rainy sorghums predominantly grown for food use in India, the rainy season grown commercial hybrids possessed up to 50% higher Fe and Zn concentrations.

Key words: Sorghum, biofortification, iron, zinc, hybrids, post-rainy sorghum, AAS, XRF

Sorghum, a heat and drought tolerant C_4 plant is a widely consumed cereal staple 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 *et al*., 2006; Ashok Kumar *et al*., 2011). Limited studies indicate 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 anaemia (even death) in more than half of the world's population (Underwood, 2000; Sharma, 2003; Welch and Graham, 2004) and efforts are being made to provide fortified foods to the vulnerable groups of the society. Biofortification where possible, is the most 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 in the arid-tropics and sub-tropics of developing countries. Biofortification of sorghum by increasing mineral micronutrients (especially iron and zinc) in the grains is of widespread interest (Pfeiffer and McClafferty, 2007; Zhao, 2008; Ashok Kumar *et al*., 2012).

ICRISAT is working on sorghum biofortification for enhancing the grain Fe and Zn concentrations (Reddy *et al*., 2005 and Ashok Kumar *et al*., 2013). This work is an attempt to assess the variability in the commercial sorghum cultivars that are currently being cultivated by the farmers in India. Identification of commercial cultivars with high grain Fe and Zn would help in expanded dissemination of the cultivars to complement the on-going efforts for combating the micronutrient malnutrition.

Materials and Methods

We collected 63 commercial sorghum cultivars developed in India for this study. As all the material was not received in time, we divided the material into two sets to start the evaluation of Set I first followed by Set II. The Set I included a total of 20 commercial sorghum cultivars developed in India by the Indian (National Agricultural Research System) 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). These 20 cultivars were evaluated along with two controls (CSH 16 and PVK 801) during the 2008 and 2009 post-rainy seasons at the ICRISAT farm, 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 were evaluated along with two controls (CSH 16 and PVK 801) as Set II during the 2009 and 2010 post-rainy seasons at ICRISAT-Patancheru. In both the trials, the cultivars along with controls were evaluated in a randomized block design (RBD) with three replications under high fertility conditions (80 N: 40 P) on Vertisols at ICRISAT farm in Patancheru, located at an altitude of 545 m above mean sea level, latitude of 17.53^0 N and longitude of 78.27^0 E. The soils at the experimental site had pH ranging from 6.8 to 7.9. The soils were moderate in organic carbon (C), and adequate in extractable phosphorus (P), zinc (Zn) and iron (Fe); they were high in extractable potassium (K). The experimental site soils had a very low electrical conductivity, indicating no salt problem. Each cultivar was grown in four-row plots of two m length with 75 cm spacing between the rows. Irrigations (4-5) were applied as required during the cropping season. Utmost care was taken to raise a healthy crop and to get clean grain for lab analysis for grain Fe and Zn concentrations. The data were recorded for the agronomic traits - days to 50% flowering, plant height (m), grain yield (t/ha) and grain size (g /100) following the standard procedure. The panicles (4-5) were bagged with Kraft paper bags prior to flowering in each replication to avoid pollen contamination and to harvest pure seed for grain Fe and Zn analysis.

At maturity, the panicles were harvested and the grain was threshed carefully without any contact with metal containers to avoid contamination. The cleaned seeds were collected into cloth bags and used for micronutrient analysis. The grain Fe and Zn concentrations were determined in the ground grain samples by using the triacid digestion method (Sahrawat *et al*., 2002) for Set I using the Atomic Absorption Spectrometer (AAS) and the X-ray fluorescence spectrometer (XRF) for Set II. Significant positive association was observed between the grain Fe (r^2=0.6) and Zn concentrations (r^2=0.9) estimated using the AAS and XRF methods. The data on agronomic traits along with grain Fe (mg /kg) and Zn (mg /kg) were statistically analyzed using the GENSTAT 9.1 package to assess the significant differences among the cultivars for mean performance for grain Fe and Zn concentrations and agronomic traits.

Results and Discussion

The mean grain Fe concentration among the Set I genotypes ranged from 30 to 44 mg /kg and grain Zn concentration from 22 to 33 mg /kg$^-$ (Table 1). Among the 20 hybrids tested in Set I, six hybrids showed > 39 mg Fe /kg grain, and six hybrids showed > 27 mg Zn/ kg grain Zn well above the trial means for grain Fe and Zn concentrations. The controls - PVK 801 and CSH 16 showed 43 and 41 mg Fe /kg grain and 30 and 28 mg Zn /kg grain. Four hybrids (NSH 703, GK 4035, Mahabeej 703 and NSH 702) were superior to the control hybrid, CSH 16 for grain Fe concentration that ranged from 43 to 44 mg /kg, while six genotypes had grain Zn concentration ranging from 29 to 33 mg /kg and were superior to the control (CSH 16 with Zn concentration 28 mg /kg). The hybrids GK 4035 and Mahabeej 703 showed higher Fe concentrations in both the years indicating their stability for this trait. Among the varieties, PVK 801 showed higher grain Fe (43 mg /kg) and Zn concentrations (30 mg /kg) than the other varieties. It is interesting to note that PVK 801, a white grained high yielding, grain mold resistant variety also possessed high grain Fe and Zn concentrations. The range for Fe and Zn concentrations in the commercial cultivars in the present study was numerically higher than that of hybrid seed parents or advanced breeding lines assessed earlier (Reddy *et al*., 2005); but this comparison may have limited value considering significant GxE interaction. Significant positive association was observed between the grain Fe and Zn concentrations (r=0.85). However compared to the grain Fe and Zn concentration observed in postrainy sorghums (on an average 30 mg Fe /kg and 20 mg Zn / kg$^)$ which are predominantly grown for food, the rainy season grown commercial hybrids possessed up to 50% higher Fe and Zn concentrations. This implies that it is important to promote these hybrids in food basket to contribute for reducing the micronutrient malnutrition.

Evaluation of 46 commercial cultivars in Set II showed Fe concentrations ranging from 25 to 41 mg/ kg and Zn concentrations from 17 to 28 mg /kg with an average Fe concentration of 33 mg/kg and average Zn concentration of 22 mg/kg (Table 2). Four cultivars showed higher grain Fe and one cultivar showed higher

Table 1 : Mean performance of the commercial sorghum cultivars (Set I) for grain Fe and Zn concentration during 2008 and 2009 post-rainy seasons

Hybrid/variety	Seed source	Fe (mg/kg)	Zn (mg/kg)
NSH 703	Nuziveedu Seeds, Hyderabad	44	32
GK 4035	Ganga Kaveri Seeds, Hyderabad	44	33
Mahabeej 703	MSSCL, Akola	43	29
NSH 702	Nuziveedu Seeds, Hyderabad	43	32
8562	Bayer Bio Sc., Hyderabad	41	30
Mahabeej 704	MSSCL, Akola	40	26
KDSH 1179	Krishidhan Seeds, Jalna	39	27
BSH 45	Biostadt MH seeds,, Aurangabad	39	27
Madhura -SS hybrid	Nimbkar Seeds, Paltan	39	32
Mahabeej 7	MSSCL, Akola	39	26
GK 4009	Ganga Kaveri Seeds, Hyderabad	38	27
Hi-jowar 52	Biostadt MH seeds, Aurangabad	38	25
PSV 2 (variety)	ARS, Palem	38	26
CSH 25	MAU, Parbhani	37	25
8340	Bayer Bio Sc., Hyd	37	25
KDSH 209	Krishidhan Seeds, Jalna	36	26
PSV 1 (variety)	ARS, Palem	36	24
BSH 47	Biostadt MH seeds,, Aurangabad	35	23
GK 4044	Ganga Kaveri Seeds, Hyderabad	33	22
8568	Bayer Bio Sc., Hyderabad	30	23
Controls			
PVK 801 (variety)		43	30
CSH 16 (hybrid)		41	28
Mean		39	27
SE $\pm$		2.76	2
CD (P=0.05)		7.84	5.68

Zn concentrations than the trial mean. The controls PVK 801 and CSH 16 showed grain Fe concentration 37 and 34 mg/kg and Zn concentration 22 and 21 mg/kg, respectively. Four commercial hybrids (BSH 53, Mahabeej 707, HTJH 6290 and Mahabeej 706) were superior for Fe (40 to 41 mg/kg) and Zn concentrations (23 to 27 mg/kg) than controls. None of the popular post-rainy season sorghum cultivars which are 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 concentrations.

Highly significant positive correlation between the grain Fe and Zn concentration ($r = 0.853$; $P < 0.01$) was observed (Table 3), indicating that either genetic factors for Fe and Zn concentrations are linked, or physiological mechanisms were interconnected for Fe and Zn uptake/translocation in the grains. These results point to the potential of simultaneous genetic improvement for both the 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 concentrations in sorghum along with other agronomic traits such as grain size and grain yield in varied maturity backgrounds. As expected, the grain size showed significant positive correlation with grain yield ($r = 0.45$).

Conclusion

Biofortification wherever feasible is cost effective and complements the on-going efforts in combating the micronutrient malnutrition. There is considerable variability for grain Fe and Zn concentrations in the elite commercial sorghum cultivars grown in India for food

Table 2 : Mean performance of the commercial sorghum cultivars (Set II) for grain Fe and Zn concentrations during 2009 and 2010 postrainy seasons

Cultivar	Source	Iron (mg/kg)	Zinc (mg/kg)	Days to 50% flowering	Plant height (m)	Grain yield (t/ha)	100-grain weight (g)	Agronomic score[1]
BSH 53	Biostadt MH seeds Ltd, Aurangabad	41	23	73	1.6	3.5	3.48	1.7
Mahabeej 707	Maharashtra State Seeds Corporation Ltd	41	26	71	1.6	2.9	3.56	2.2
HTJH 6290	Hytech Seed India Pvt Ltd	40	27	70	1.6	3.2	3.56	2.4
Mahabeej 706	Maharashtra State Seeds Corporation Ltd	40	27	69	1.5	3.3	3.62	1.7
KSH 6363	Kaveri Seed co Ltd, Hyderabad	39	27	75	1.5	2.9	3.97	1.8
KH 701	Kanchan Ganga Seed Co Pvt Ltd, Hyd	39	26	71	1.5	2.7	3.08	2.5
XSM 1556	Bayer Biosciences Pvt Ltd, Hyderabad	39	21	73	1.5	2.4	2.92	1.8
NTJ 3	ARS, Nandyal	38	21	71	2.0	3.6	2.96	2.7
JK Jyoti	JK Seeds, Hyderabad	37	26	75	1.5	3.2	3.96	1.8
GK 4009	Ganga Kaveri Seeds Pvt Ltd, Hyderabad	37	28	76	1.6	2.9	3.86	2.0
JKSH 592	JK Seeds, Hyderabad	37	21	74	1.6	4.9	3.34	1.2
DSH 4	UAS, Dharwad	36	24	75	2.0	3.2	3.81	2.5
JKSH 22	JK Seeds, Hyderabad	36	23	72	1.7	3.1	3.85	1.8
KSH 950	Kaveri Seed co Ltd, Hyderabad	36	22	72	1.7	4.2	3.12	1.5
TNSH 482	TNAU, Coimbatore	36	23	72	1.6	3.4	3.22	2.3
Hi Jowar 52	Biostadt MH seeds Ltd, Aurangabad	36	23	74	1.6	3.1	3.51	2.3
CO (S) 28	TNAU, Coimbatore	35	17	73	1.8	3.0	3.15	2.2
BSH 51	Biostadt MH seeds Ltd, Aurangabad	35	24	73	1.6	3.2	3.91	2.0
PVK 801	Marathwada Agril University, Parbhani	35	22	77	1.5	3.3	4.04	2.0
HTJH 6143	Hytech Seed India Pvt Ltd	35	21	70	1.5	4.4	3.27	1.2
TNS 598	TNAU, Coimbatore	34	18	74	1.8	3.5	3.44	2.0
Mahabeej 7	Maharashtra State Seeds Corporation Ltd	34	21	69	1.5	3.6	3.31	2.0
8340	Bayer Biosciences Pvt Ltd, Hyderabad	34	21	72	1.6	3.6	3.57	1.8
DSV 4	UAS, Dharwad	34	21	81	2.1	3.5	3.68	2.5
CSH 25	Marathwada Agril University, Parbhani	33	20	78	1.6	3.2	2.97	1.7

Cultivar	Source	Iron (mg/kg)	Zinc (mg/kg)	Days to 50% flowering	Plant height (m)	Grain yield (t/ha)	100-grain weight (g)	Agronomic score[1]
HTJH 3201	Hytech Seed India Pvt Ltd	33	22	70	1.5	4.0	3.90	1.5
GK 4044	Ganga Kaveri Seeds Pvt Ltd, Hyderabad	33	20	73	1.4	2.8	2.63	1.5
NTJ 4	ARS, Nandyal	33	22	71	1.7	3.7	3.85	2.3
NTJ 1	ARS, Nandyal	33	23	73	1.3	2.2	3.25	2.7
Phule Chitra	Mahatma Phule Krishi Vidyapeeth, Rahuri	32	22	80	2.1	3.2	3.65	2.2
N 13	ARS, Nandyal	31	23	72	1.7	3.6	3.15	3.2
Phule Anuradha	Mahatma Phule Krishi Vidyapeeth, Rahuri	31	20	75	1.8	3.1	4.00	3.2
Parbhani Jyoti	Marathwada Agril University, Parbhani	31	25	80	2.3	2.4	3.75	2.2
8568	Bayer Biosciences Pvt Ltd, Hyderabad	31	21	70	1.4	3.0	3.22	1.5
Giddi Maldandi	–	31	21	86	1.4	3.6	3.06	1.8
NTJ 2	ARS, Nandyal	31	22	72	1.6	3.0	3.69	2.2
M 35-1	MPKV, Rahuri	30	21	75	2.0	3.3	3.66	2.7
Dagdi Solapur	–	30	21	79	1.9	3.4	3.50	2.5
PVK 809	Marathwada Agril University, Parbhani	29	17	76	1.6	3.7	2.74	2.2
SPV 1411	Marathwada Agril University, Parbhani	28	22	76	2.1	3.8	4.23	2.5
Barsizoot	–	28	21	80	1.9	3.8	3.55	2.0
CSV 20	DSR, Hyderabad	28	19	73	1.8	3.6	2.96	2.3
Phule Vasudha	MPKV, Rahuri	28	20	83	2.4	3.6	3.73	1.8
PKV Kranthi	PDKV, Akola	28	19	77	2.3	3.4	3.73	1.7
N 14	ARS, Nandyal	28	23	75	1.6	3.8	3.45	3.0
CSV 216R	DSR, Hyderabad	26	19	75	2.3	4.0	3.72	2.0
GK 4013	Ganga Kaveri Seeds Pvt Ltd, Hyderabad	25	21	75	1.4	3.3	3.52	1.8
Controls								
CSH 16		34	21	73	1.6	2.6	4.46	1.5
PVK 801		37	22	76	1.4	3.3	3.68	2.3
Mean		33.58	21.97	74.4	1.7	3.34	3.51	2.08
SE ±		2.09	1.40	1.05	0.07	0.4	0.17	0.32
CD (P=0.05)		5.86	3.92	2.92	0.18	1.11	0.47	0.89

[1]Agronomic score taken on a scale 1 to 5 where 1= more desirable and 5 = least desirable

Table 3 : Correlation coefficients of the commercial sorghum cultivars grown during 2008 and 2009 post-rainy seasons

Trait	Fe	Zn	Days to 50% flowering	Plant height	Grain yield
Zn	0.853**	1			
Days to 50% flowering	0.087	0.158	1		
Plant height	-0.039	0.224	-0.017	1	
Grain yield	0.02	-0.045	-0.223	0.023	1
Grain size	0.279	0.221	-0.464	-0.133	0.456*

df (n-2)=20 and *significant at 5% and **significant at 1%

purposes, and the seven hybrids NSH 703, GK 4035, Mahabeej 703, NSH 702, Mahabeej 706 and 707, BSH 53 and HTJH 6290 identified in the study can form part of the on-going efforts for combating micronutrient malnutrition through their large scale promotion. Compared to the grain Fe and Zn concentration observed in post-rainy sorghums which are predominantly grown for food in India the rainy season grown commercial hybrids possess up to 50% higher Fe and Zn concentrations. Therefore it is important to promote these hybrids in food basket to contribute for reducing the micronutrient malnutrition. Further, it is possible for simultaneous improvement of grain Fe and Zn concentrations in sorghum and there is no penalty in the agronomic traits when combined with high Fe and Zn concentrations. It is also possible to deliver high Fe and Zn concentrations in cultivars with farmers' preferred traits such as early maturity, high yield potential and bold grain.

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