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Genetic variability for grain iron and zinc content in cultivars, breeding lines and selected germplasm accessions of sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

Sorghum is an important cereal grain consumed in semi-arid tropics of Asia and Africa, which is nutritionally superior with more micronutrient content compared to rice and wheat. Grains of adapted cultivars, parental lines, advance breeding lines and selected germplasm accessions of sorghum were characterized for the levels of iron (Fe) and zinc (Zn). Considerable variability for grain Fe and Zn contents was observed. In general, germplasm lines had higher values for Fe and Zn compared to cultivars and breeding lines. The mean values were found to be less repetitive upon reanalysis of selected genotypes. Hence, the entire set was reanalyzed which again established sufficient variability for grain Fe (12.1-83.4 mg/kg) and Zn (6.3-51.4 mg/kg) contents among the test genotypes as well as high heritability. Significant positive association between grain Fe and Zn content was observed (r = 0.2 – 0.5, p < 0.05) indicating possibility of simultaneous improvement of both micronutrients.The availability of high variability for grain Fe and Zn contents among the genotypes tested holds promise for grain micronutrient enrichment through breeding programmes.

Key words: Biofortification, grain iron, grain zinc, micronutrients, sorghum

Introduction

Deficiency of micronutrients and vitamins in the diet affects the human health in nearly two billion people in the developing world [1], and in India micronutrient malnutrition has been a persistent problem with alarmingly high deficit among children, women of reproductive age, and pregnant and lactating women [2]. Iron (Fe) and zinc (Zn) deficiencies are the most common micronutrient deficiencies in human beings leading to a number of health disorders including impaired growth and development, reduced work capacity, weak immune system, impaired mental development, and even mortality in infants [3-5]. Iron deficiency in particular leads to anaemia in kids of 6 to 35 months of age and in women between 15 to 49 years of age [5]. Zinc deficiency is another important micronutrient deficiency next to vitamin A and Fe, which lead to diarrhoea and pneumonia in children [6]. It effects growth and promote study. Development of micronutrient-dense staple crops using the best traditional breeding practices or modern biotechnology (“Biofortification”) and making it available to the target population can significantly improve the amount of Fe and Zn available through food crops and alleviate micronutrient malnutrition among the poor.

Sorghum grains act as principal source of energy, protein, vitamins and minerals for the poor people living in the semi-arid tropics. India ranks first in terms of area sown to sorghum (7.67 m ha) and second in terms of total grain production (6.98 m tonnes) globally [6]. In India, Maharashtra, Karnataka and Andhra Pradesh together account for nearly 80% of the all-India production [7]. The annual per capita consumption by rural consumers in the major sorghum-producing regions is up to 75.2 kg/year [8]. It contributes nearly 50% of the total cereal intake in central India, and accounts for about 35% of the total intake of calories, protein, Fe and Zn in the dominant consumption regions. Sorghum is superior to rice for contents of

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protein, minerals and Fe, while the values are on a par or marginally better than that of wheat [9]. Considering the superiority of sorghum grains for Fe and Zn density compared to most popular staples like rice and wheat, genetic enhancement of these minerals further, or in other words Biofortification holds promise to enhance Fe and Zn supply to the predominantly sorghum consuming population of central India, and thereby improving the micronutrient status of consumers in a sustainable manner. Limited reports are available on the grain Fe and Zn density in sorghum genotypes [10-12]. This paper reports the genetic variability for grain Fe and Zn density among popular sorghum cultivars and parental lines of India, some advance breeding lines and selected germplasm accessions collected from major sorghum growing states (Maharashtra, Karnataka, Madhya Pradesh and Andhra Pradesh) as well as IS (International Sorghum) lines, and prospects of breeding for micronutrient enrichment in sorghum.

Materials and methods

Plant material

A total of 222 sorghum genotypes were used for the study. Grain samples from popular cultivars and parental lines (49), high yielding advance breeding lines (34), elite low-amylose content breeding lines (26) and selected germplasm accessions (113) collected from major sorghum growing states, which had been multiplied at DSR and conserved in medium-term cold storage, were characterized for contents of Fe and Zn. Replicated grain samples (50 g each) from each of the genotype were collected in clean cloth bags and used for estimation of grain Fe and Zn.

Micronutrient estimation

Grains free from soil or metallic contamination were subjected to the estimation of contents of Fe and Zn following Atomic Absorbance Spectroscopy (AAS) based method. Dried grain sample was accurately weighed and digested on a heating mantle using the 3 acid mixture (nitric acid, perchloric acid and sulphuric acid in 3:2:1 proportion). The digested sample was diluted with 10% solution of 3 acid mixture and readings were taken on AAS (Perkin Elmer 5000AA, USA). Standard solutions of Fe and Zn were prepared as stock of 1000 ppm and were suitably diluted to get a series of working solutions in the range of 0.1-10 ppm using metal free water (Millipore Q). Certified reference rice flour samples from Center for Environmental Measurement and Analysis, National Institute for Environmental Studies (NIES), Tsukuba-City, Ibaraki, Japan were used as standards to check the precision and accuracy of the method. The measured concentrations of Fe and Zn were considered appropriate when they agreed well with the certified values.

Statistical analysis

The replication-wise data on grain Fe and Zn content were analyzed statistically for analyses of variance (ANOVA) and comparison of means using WINDOSTAT Ver. 7.5 statistical package (www.windostat.org), and results were tabulated. The variability in each category of genotypes was represented as box plots using Statistix Ver. 8.1. Twenty genotypes which showed either extreme values or parents of available mapping populations (RILs) developed at this Directorate for different agronomic traits of interest were re-analyzed for confirmation. Results of both the sets were compared for grain Fe and Zn content to confirm the repeatability of the values. A two-sample student's *t*-test was performed to test the equality of the population means that underlie each sample using MS Excel 2007. The *t*-values were plotted against the genotypes to represent the repeatability of the results for grain Fe and Zn. The Pearson correlation was used to determine relationship between the two sets of data as well as between grain Fe and Zn content. As the mean values for these 20 genotypes were found to be less repetitive, the entire set of 222 genotypes was re-analyzed for contents of grain Fe and Zn following the above mentioned method and data were analyzed category-wise.

Results and discussions

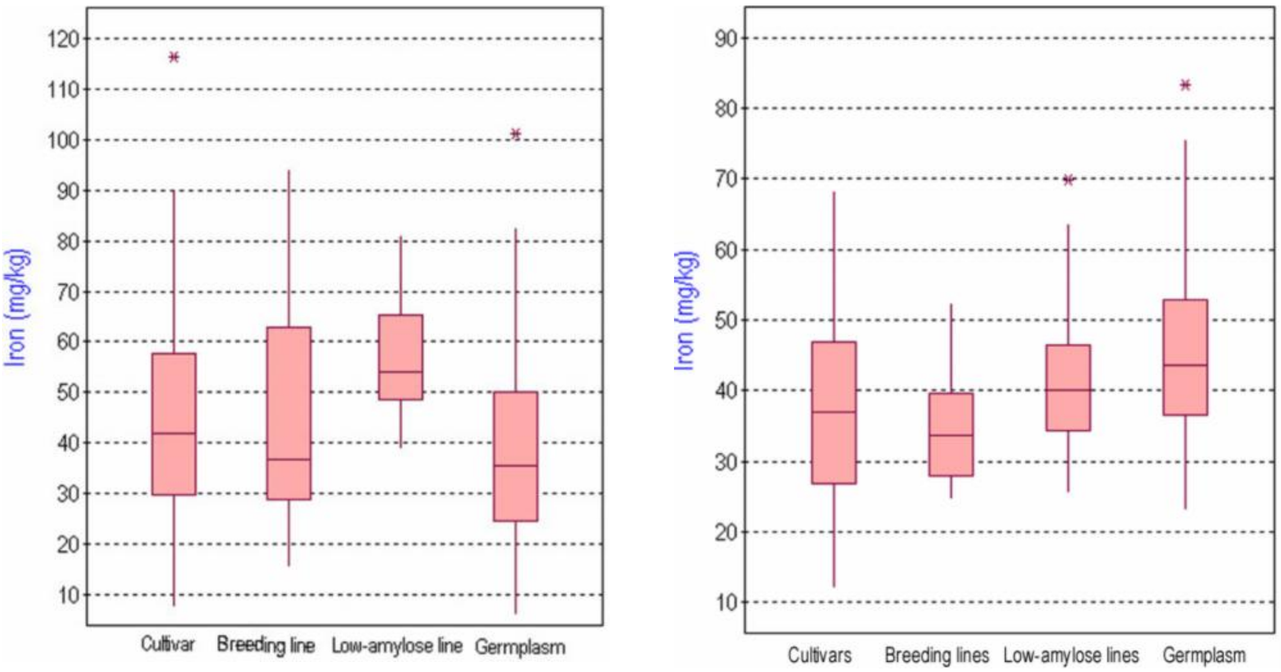
Variability for grain Fe

Availability of sufficient variability in the working collection of sorghum can assist the breeding programmes for Fe enrichment. The ANOVA for all four categories of materials demonstrated significant variation for grain Fe due to genotypes as indicated by highly significant mean squares (Table 1). Good range was observed for grain Fe among the four categories of the materials (Fig. 1a). The grain Fe content in the cultivars and parental lines ranged from 7.9 to 116.4 mg/kg with a mean of 43.8 mg/kg (Table 1). In case of advance breeding lines and low-amylose elite lines the range was 15.8-94.0 mg/kg and 39.1-80.9 mg/kg, respectively (Table 1). The range observed in the germplasm accessions was wider with a mean

Table 1. ANOVA and variability for grain iron (mg/kg) and zinc (mg/kg) among 222 sorghum genotypes (1st analysis)

Items	Cultivars/parents		Adv. breeding lines		Low-amylose lines		Germplasm lines	
	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
Mean Squares	927.8***	119.5***	1094.0***	79.6***	236.5***	137.4***	1193.1***	191.8***
Mean ± SEm	43.8±5.8	14.7±2.4	45.6±7.4	15.3±3.1	56.2±5.2	30.6±4.4	40.7±6.9	19.2±2.5
Range	7.9-116.4	3.6-37.9	15.8-94.0	5.3-30.3	39.1-80.9	17.4-44.7	6.3-168.1	4.2-87.0
CD (5%)	16.6	6.9	21.2	9.1	15.2	12.9	19.2	7.0
ECV (%)	18.8	23.2	22.8	29.1	13.1	20.5	23.8	18.3
GCV (%)	47.3	49.9	48.7	35.8	17.0	22.9	57.6	49.2
PCV (%)	50.9	54.9	53.8	46.1	21.5	30.7	62.3	52.5
h^2 (bs)	0.86	0.82	0.82	0.60	0.63	0.56	0.85	0.88
Gen. Adv. (GA)	39.7	13.7	41.4	8.7	15.6	10.7	44.6	18.3
GA as % of mean	90.5	93.1	90.9	57.2	27.7	35.1	109.6	95.0

ECV – Environmental Coefficient of Variation, GCV – Genotypic Coefficient of Variation, PCV – Phenotypic Coefficient of Variation, h^2(bs) – Heritability (broad sense), GA – Genetic advancement at 5% selection intensity, *-significant



(a) (b)

Fig. 1. Variability for grain iron content (a) 1st analysis, (b) 2nd analysis

of 40.7 mg/kg. The coefficient of variation (CV or Environmental CV) for Fe content was high in germplasm accessions (23.8%) followed by breeding lines (22.8%). Though preliminary studies [10] have indicated limited variability for grain Fe content in sorghum, other recent studies at ICRISAT, Patancheru, Andhra Pradesh have reported significant variability for grain Fe content among parental lines, varieties and germplasm accessions from core collection [11-14].

When the 20 selected genotypes were re-analyzed, the mean grain Fe values obtained were found to be different in most of the genotypes compared to the previous results. Student's *t*-test indicated significant difference between the mean values in genotypes EP 95, 27 B, 2219 B, N 13, IS 18551, CSV 216R and HC 308 (Fig. 2) with significant *t*-values. In other genotypes also the mean values were highly different though the *t*-values were non-significant because of large replication differences. The correlation between the two sets of values was non-significant (r = 0.04, p = 0.86) indicating the non-repeatability of the results. Following these results, the entire set of 222 genotypes was characterized again for grain Fe content. Significant variability was again observed (Fig. 1b) among all four categories of genotypes (Table 2). Grain Fe ranged from 12.1 to 68.1 mg/kg with a mean of 37.5 mg/kg in case of cultivars and parental lines. The CV was only marginally less from the previous result, but the range had narrowed down significantly, and appears to be within the range already reported by Ashok Kumar [11] for commercial sorghum cultivars. There was no significant correlation between the grain Fe contents recorded previously and at present (r = 0.12, p = 0.41), thereby indicating the necessity of repeated analysis before identifying suitable donor parents. Considering the extent of genetic variability observed among landraces and level of sorghum consumption, the target level for grain Fe through genetic enhancement has been put at 60 mg/kg from the base level of 30 mg/kg at ICRISAT. Some of the parental lines which had much higher grain Fe (> 45 mg/kg) than the above mentioned base level in 1st as well as 2nd analyses were 104B (female parent of hybrids CSH 15R and CSH 19R), HC 308 (single-cut forage sorghum variety), N 13 (Striga resistant sorghum line), DJ 6514 (cultivar from Dharwad), CS 3541 (male parent of CSH 5, CSH 6 and CSH 9, and one of the contributing parents in CSV 4, CSV 7, CSV 10 and CSV 11), B 58586 (grain mold tolerant line), Pant Chari 5 (single-cut forage sorghum variety), AKR 150 (male parent of CSH 14) and CSV 23 (*kharif* sorghum variety) (Table 3). Very high heritability (broad-sense) (proportion of phenotypic variance that is due to all genetic effects) was observed for grain Fe (> 0.8) indicating higher gains through selection or genetic advance in the breeding programmes.

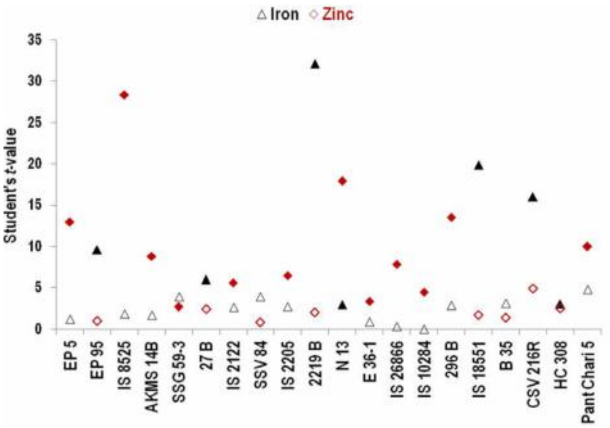


Fig. 2. Plotting of student's *t*-value against the genotypes (solid fills indicate significant *t*-values)

In case of advance breeding lines, the range observed during re-analysis was narrower (24.7-52.2

Table 2. ANOVA and variability for grain iron (mg/kg) and zinc (mg/kg) among 222 sorghum genotypes (2nd analysis)

Items	Cultivars/parents		Adv. breeding lines		Low-amylose lines		Germplasm lines	
	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
Mean Squares	387.1***	171.8***	115.2***	86.9***	245.6***	80.2***	300.8***	153.7***
Mean ± SEm	37.5±4.6	22.1±2.8	34.5±3.2	24.0±3.0	41.9±3.0	21.6±2.6	46.2±3.5	29.5±3.5
Range	12.1-68.1	10.8-43.8	24.7-52.2	12.7-45.1	25.7-69.8	12.7-40.3	23.3-83.4	6.3-51.4
CD (5%)	13.1	7.9	9.1	8.7	8.7	7.7	9.8	9.9
ECV (%)	17.3	17.7	12.9	17.9	10.0	17.3	10.7	16.8
GCV (%)	35.1	39.9	20.0	24.3	25.5	26.6	25.5	27.2
PCV (%)	39.1	43.7	23.8	30.2	27.4	31.7	27.6	31.9
h^2 (bs)	0.80	0.84	0.71	0.65	0.87	0.70	0.85	0.72
Gen. Adv. (GA)	24.3	16.7	11.9	9.7	20.5	9.9	22.3	14.1
GA as % of mean	64.7	75.4	34.6	40.4	48.9	45.9	48.4	47.7

ECV – Environmental Coefficient of Variation, GCV – Genotypic Coefficient of Variation, PCV – Phenotypic Coefficient of Variation, h^2(bs) – Heritability (broad sense), GA – Genetic advancement at 5% selection intensity, *-significant

Table 3. Cultivars/parental lines with high grain iron content

S.No.	Cultivars/parents	Fe (mg/kg)
1	104B	68.1
2	HC 308	63.5
3	N 13	60.6
4	DJ 6514	60.5
5	CS 3541	52.2
6	B 58586	50.9
7	Pant Chari 5	49.0
8	AKR 150	47.6
9	CSV 23	47.5

Table 4. Germplasm accessions with high grain iron content

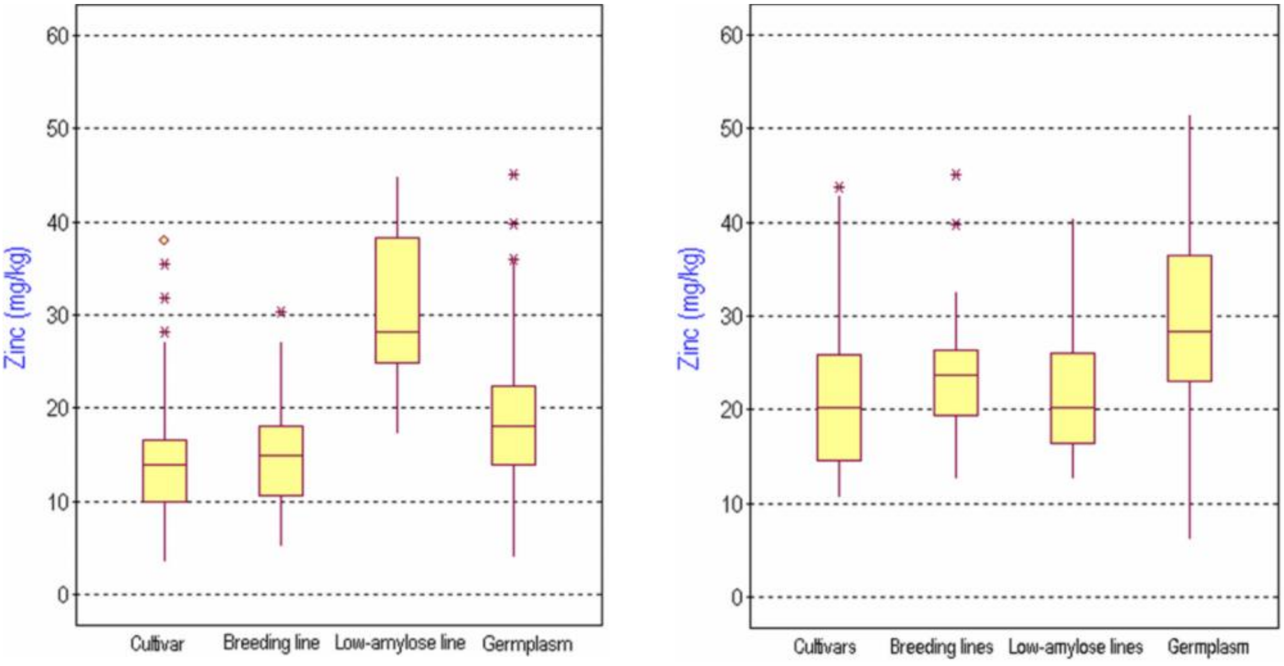
S.No.	Germplasm accession	Source	Fe (mg/kg)
1	EP 92	Maharashtra, Karnataka	75.5
2	EP 117	-do-	75.2
3	EC 33	Andhra Pradesh	66.9
4	EC 22	-do-	61.6
5	EP 33	Maharashtra, Karnataka	55.3
6	EA 6	Tamil Nadu	51.8
7	EP 11	Maharashtra, Karnataka	51.6
8	EP 68	-do-	50.8
9	EP 41	-do-	50.6

mg/kg) compared to the previous values, and the CV was much lower than earlier (Table 2). Similar were the results for phenotypic (PCV) and genotypic coefficient of variations (GCV). The mean grain Fe content in the low-amylose lines also was lower with marginally lesser CV and higher heritability (broad-sense) compared to previous results. The correlation between old and new values for grain Fe was significant (r = 0.38, p = 0.03) in case of advance breeding lines while in case of low-amylose lines it was non-significant (r = 0.14, p = 0.49). In case of germplasm lines the grain Fe content ranged from 23.3 to 83.4 mg/kg with an overall mean of 46.2 mg/kg. The CV was much lower at 10.7% compared to previous value of 23.8% indicating a better estimation of grain Fe. Though there was no correlation between old and new values, the range observed in the selected germplasm accessions appears more realistic and within or near to the range already reported [12-14]. Some of the germplasm accessions like EP 92 (IC 343591), EP 117 (IC 345206), EC 33 (IC 345735), EC 22 (IC 345724), EP 33 (IC 305914), EA 6 (IC 345248), EP 11 (IC 305892), EP 68 (IC 343567) and EP 41 (IC 305922) had above 50 mg/kg of grain Fe in 1st as well as 2nd analyses (Table 4). Very high heritability (> 0.8) was observed in case of germplasm accessions for grain Fe content indicating the possibility of improving the trait through conventional breeding approaches. High heritability estimates indicate the presence of large number of fixable additive factors and hence the trait may be improved by selection. The effectiveness of selection depends upon genetic advance of the character selected along with heritability.

Variability for grain Zn

Similar to grain Fe content, ANOVA indicated significant variation for grain Zn content in all four categories of genotypes tested (Table 1). Wide range was also noticed among the genotypes (Fig. 3a). The grain Zn content in the cultivars and parental lines ranged from 3.6 to 37.9 mg/kg with a mean of 14.7 mg/kg (Table 1). In case of advance breeding lines and low-amylose elite lines the range was 5.3-30.3 mg/kg and 17.4-44.7 mg/kg, respectively (Table 1). The very wide range was observed for grain Zn content in case of germplasm accessions also (4.2-87.0 mg/kg) with a mean of 19.2 mg/kg. Very high CV (>20%) was observed in case of advance breeding lines, cultivars and parental lines and low-amylose lines. The heritability (broad sense) ranged from 0.56 to 0.88 indicating the high genotypic contribution in the inheritance of the trait analogous to grain Fe.

Upon re-analysis of 20 genotypes selected to confirm the results, it was observed that in 12 out of 20 genotypes the values were significantly different as indicated by the Student's *t*-test (Fig. 2). The mean values varied widely in case of EP 5, IS 8525, AKMS 14B, IS 2122, N 13, E 36-1, IS 26866, IS 10284 and Pant Chari 5. In other genotypes also like SSV 84, IS 18551 and CSV 216R, the mean values were highly different though the *t*-values were non-significant due to large replication differences. The correlation coefficient between the two sets of values was higher in magnitude compared to that for Fe content, though non-significant (r = 0.33, p = 0.15). Presence of considerable variability for grain Zn content among all four categories of material (Fig. 3b, Table 2) was



(a) (b)

Fig. 3. Variability for grain zinc content (a) 1st analysis, (b) 2nd analysis

confirmed through reanalysis of the entire set of 222 genotypes. Grain Zn ranged from 10.8 to 43.8 mg/kg with a mean of 22.1 mg/kg in case of cultivars and parental lines. This range was slightly higher compared to 15-33 mg/kg reported by Ashok Kumar [14] for commercial sorghum cultivars. The CV was significantly less from the previous result indicating a better estimation. There was no significant correlation between the previous and present result ($r = 0.19$, $p = 0.20$). Some of the parental lines with above 20 mg/kg grain Zn (20 mg/kg is considered to be base level and target level for grain Zn is 40 mg/kg) in 1st as well as 2nd analyses were CS 3541, HC 308, SSG 59-3 (popular multi-cut forage sorghum variety), Surat 1 (grain sorghum cultivar) and PB 15881-1 (germplasm line) (Table 5). Very high heritability (broad-sense) was observed for grain Zn (> 0.82) indicating that the grain Zn content can be improved through selection.

Among the advance breeding lines the variation for grain Zn content was found to be 12.7 to 45.1 mg/kg with a mean of 24 mg/kg, which was slightly higher than that observed previously (Table 2). The CV was much lower at 17.9% compared to previous value of 29.1%. The heritability value (0.65) obtained was higher compared to previous result. Contrary to advance breeding lines, the mean grain Zn content in the low-amylose lines was lower compared to previous range with marginally lesser CV (17.3%) and higher heritability (0.7). Correlation between old and new values was non-significant ($r = 0.1$, $p = 0.59$) in case of breeding lines, whereas in case of low-amylose lines it was highly significant ($r = 0.59$, $p < 0.01$), thereby indicating inconsistency of the trends depending on the material. The grain Zn content in case of germplasm lines ranged from 6.3 to 51.4 mg/kg with an overall mean of 29.5 mg/kg (Table 2). The range obtained was narrower compared to previous result and CV was also lower at 16.8%. The values of PCV and GCV were also lower compared to previous analysis. The heritability value was low at 0.72 compared to earlier value. The lower level of grain Zn observed in this study is much less than the variability reported earlier in case of core germplasm accessions (15.1-91.3 mg/kg [12]; 21-57 mg/kg [14]). Some of the germplasm accessions like EP 41 (IC 305922), GGUB 31 (IC 319874), GGUB 34 (IC 319877), EP 115 (IC 345204), EP5 (IC 305886), GGUB 37 (IC 319880), EP 97 (IC 345186), EA 6 (IC 345248), IS 2122, EA 10 (IC 345252), GGUB 32 (IC 319875), GGUB 40 (IC 319883) and PYPS 19 had above 25 mg/kg of grain Zn in both analyses (Table 6). The mean Zn contents in the 1st and 2nd analyses were found to be related with a significant correlation ($r = 0.21$, $p = 0.03$). Therefore, compared to grain Fe content, the Zn

Table 5. Cultivars/parental lines with high grain zinc content

S.No.	Cultivars/parents	Zn (mg/kg)
1	CS 3541	43.8
2	HC 308	42.6
3	SSG 59-3	37.6
4	Surat 1	21.9
5	PB 15881-1	21.8

Table 6. Germplasm accessions with high grain zinc content

S.No.	Germplasm accession	Source	Zn (mg/kg)
1	EP 41	Maharashtra, Karnataka	46.2
2	GGUB 31	Madhya Pradesh	45.6
3	GGUB 34	-do-	42.0
4	EP 115	Maharashtra, Karnataka	39.9
5	EP5	-do-	39.0
6	GGUB 37	Madhya Pradesh	38.7
7	EP 97	Maharashtra, Karnataka	36.7
8	EA 6	Tamil Nadu	36.6
9	IS 2122	-	34.6
10	EA 10	Tamil Nadu	33.7
11	GGUB 32	Madhya Pradesh	28.0
12	GGUB 40	-do-	27.4
13	PYPS 19	Andhra Pradesh	26.5

content was found to be less affected at least in case of germplasm accessions though significant cultivar × year [11] or genotype × environment (G × E) [15] interactions have been reported for both grain Fe and Zn content. As sorghum is grown in different types of soils with varying levels of fertility and nutrient management, it would be worthwhile to assess the stability of grain micronutrients through multi-location as well as multi-season evaluation for biofortification.

Association between grain Fe and Zn

The grain Fe and Zn content was found to be significantly and positively correlated (0.2-0.5, $p < 0.05$) among the cultivars and parental lines, breeding lines and germplasm accessions, but not in the low-amylose lines. Significant association between grain Fe and Zn contents has also been reported by other researchers [11-13, 16] which indicate that genetic control of Fe and Zn content are linked, or physiological mechanisms for uptake or accumulation of Fe and Zn in the grains are interconnected. Significant positive association between grain Fe and Zn can result in simultaneous genetic improvement for both the micronutrients.

To conclude, substantial variability for grain micronutrients has been observed among the cultivars and parental lines, and selected germplasm accessions collected from major sorghum growing regions, though the mean values were found to be less repetitive. This may be attributed to the very high sensitivity of the grain Fe and Zn to the analytical tools as well as handling procedure. Consequently, repeated analysis is necessary before identifying suitable donors for micronutrient enrichment breeding programmes. The availability of sufficient variability for grain Fe and Zn contents in the working collection can augment well the success of breeding programmes aimed at micronutrient enrichment. Thus, biofortification in sorghum appears to be a feasible strategy to alleviate the micronutrient malnutrition among the rural poor considering the high prevalence of micronutrient deficiency and high intake of sorghum in the major production/consumption regions in India. Apart from the concentration *per se*, bioavailability of grain Fe and Zn to the consumer is a major concern. Levels of tannin, phytate, fibre, etc. determine the bioaccessibility of grain Fe (4.13 ± 0.33%) and Zn (5.51 ± 0.32%), which is very low in sorghum compared to rice (8.05 and 21.4%, respectively), maize (7.83 and 7.82%, respectively) or wheat (5.06 and 8.93%, respectively) [17]. Therefore, concerted efforts aimed at development of cultivars with high grain yield potential coupled with micronutrient-dense grains and lower levels of anti-nutritional factors are necessary which in turn can support the ongoing food processing and value-addition efforts in sorghum, and promote sorghum as a health-enhancing food.

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