

Prospects of Breeding for Micronutrients and β -Carotene-Dense Sorghums

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Introduction

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 pre-school children (Underwood 2000). Two micronutrients, iron (Fe) and zinc (Zn) and pro-vitamin A (β -carotene) are recognized by the World Health Organization (WHO) of the United Nations as limiting. Deficiency for Fe, Zn and β -carotene is highest in South and Southeast Asia and sub-Saharan Africa (SSA). These are also the regions [typified as semi-arid tropics (SAT)] where sorghum (*Sorghum bicolor*) is cultivated and consumed as a staple food by millions of people. The introduction of crop varieties selected and/or bred for increased Fe, Zn and pro-vitamin A contents through plant breeding approach will complement the existing approaches (such as fortified foods and food supplementation while processing) to combat micronutrient deficiency. The plant breeding approach would avoid dependency on behavioral changes in farmers or consumers unlike other programs.

In this paper, we report and discuss the results of pre-breeding research carried out at ICRISAT, Patancheru, as a part of the short-term strategy of HarvestPlus, [the Consultative Group on International Agricultural Research's (CGIAR's) challenge program seeking to reduce micronutrient malnutrition by developing micronutrient-rich crop varieties in high-yielding background] and their implications on the prospects of breeding for micronutrients and β -carotene-dense sorghums.

Materials and Methods

The material for the study consisted of a set of 84 diverse sorghum lines involving parental lines of popular hybrids, varieties, yellow endosperm lines, germplasm lines, high protein digestible lines, high lysine lines and waxy lines. The lines were evaluated at ICRISAT, Patancheru, during 2003–04 postrainy season following Randomized Complete Block Design (RCBD) with three replications.

Each entry was grown in 4 rows of 4 m length with a row-to-row spacing of 0.75 m and 0.1 m between plants within a row. All the recommended production practices were followed to raise a healthy crop with protective irrigation. The randomly selected five plants from the middle two rows of each entry were used for recording data on agronomic traits, such as days to flowering, plant height, grain yield, stover yield; and grain traits, such as grain size (g 100⁻¹ seeds) and grain hardness. The grain hardness (breaking strength) was determined as force (in kg) required to break the grain, using Kiya grain hardness tester. The panicles from five selfed plants of each entry from only two replications were hand-threshed and utmost care was exercised to avoid contact of any metal particles with grains while cleaning them. The grain samples were collected in clean cloth bags and sent to National Institute of Nutrition, Hyderabad, India, for estimation of micronutrients (grain Fe and Zn) and β -carotene contents and anti-nutritional constituents (phytates). The Fe, Zn and phytate contents were estimated using Inductively Coupled Plasma Spectrometry (Houk 1986). The β -carotene content was estimated spectrophotometrically and was confirmed by High-Performance Liquid Chromatography (HPLC) in selected samples.

Statistical analyses. The computed mean values of data recorded on sample plants in three replications for agronomic traits and mean values of estimates of micronutrients and anti-nutritional contents (phytates) from grain samples collected from two field replications were used for statistical analysis. Analyses of variance were carried out to assess the genetic variability (Steel and Torrie 1980). The phenotypic and genotypic variances were estimated and were standardized as phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV), respectively, to compare the extent of variability for grain Fe, Zn and phytates, which were expressed in different units of measurements. The broad-sense heritability was estimated as the ratio of genotypic variance to phenotypic variance. The correlation coefficients of micronutrients and phytate contents with agronomic and grain traits and among themselves were estimated.

Results and Discussion

Genetic variability. The analysis of variance revealed significant genetic differences for Fe, Zn and phytate contents (Table 1), and for agronomic and grain traits. While the grain Fe content ranged from 20.1 ppm (ICSR

93031) to 37.0 ppm (ICSB 472 and 296 B) with an average of 28 ppm, grain Zn content ranged from 13.4 ppm (JJ 1041) to 31.0 ppm (IS 1199) with an average of 19 ppm (Table 1). Wehmeyer (1969) reported a much larger range of grain Fe (25 to 115 ppm) and Zn contents (15 to 65 ppm) among the 79 sorghum cultivars, which might be partially due to native and managed soil fertility and laboratory protocol used. Nevertheless, it is worth re-testing the lines used by Wehmeyer (1969) to confirm Fe and Zn contents, and subsequently using those having high Fe and Zn contents in breeding programs. Thus, it is evident that substantial genetic variability exists for grain Fe and Zn, and phytate contents and this variation does not appear to be significantly influenced by environment as reflected from narrow differences between PCV and GCV, and high heritabilities (Table 1). The substantial variability coupled with higher heritability offers good prospects of breeding Fe- and Zn-dense sorghum cultivars under low phytate background.

Micronutrients contents and the kind of genetic material. When the grain Fe and Zn contents were compared between different categories of genetic material [maintainer (B-) lines, varieties/restorer (R-) lines and germplasm lines], the mean grain Fe and Zn contents in germplasm lines were significantly higher than those in other categories of genetic material (B-lines and varieties/R-lines), although the differences were not large (Table 2). While mean grain Fe content was slightly higher in B-lines compared to that in varieties/R-lines,

there were no significant differences in grain Zn contents between B-lines and varieties/R-lines.

A critical examination of grain Fe, Zn and β -carotene contents in individual lines in different categories of materials (data not shown), revealed encouraging results. Several ICRISAT, Patancheru-bred high-yielding *milo* (A_1) cytoplasm-nuclear male sterility (CMS)-based maintainer (B-) lines such as ICSB 37, ICSB 38, ICSB 39, ICSB 52, ICSB 74 and ICSB 101, shoot fly resistant line ICSB 418, and stem borer resistant line ICSB 472 had grain Fe contents more than 30 ppm. The varieties/R-lines bred at ICRISAT, Patancheru, such as ICSV 745 (high-yielding, midge-resistant variety released in midge endemic areas in northern Karnataka state in India), PVK 801 (high-yielding, grain mold resistant variety released in Maharashtra state in India), IRAT 204 (high-yielding variety released in Burkina Faso in western Africa), ICSR 89058 (male parent of high-yielding hybrid released in Maharashtra, India) and ICSV 21005 (high-yielding, stay-green restorer line) also had grain Fe contents more than 30 ppm. Among germplasm/landraces, which had more than 30 ppm Fe content, paccha Jonna is a highly popular variety in Andhra Pradesh state in India and IS 7776 is a yellow-endosperm line with high β -carotene content.

Similarly, the hybrids parents with higher Zn content (more than 20 ppm) include ICSB 472 (also high Fe content) and ICSB 484 among the CMS lines and ICSR 90017 and IRAT 204 (also high Fe content) among the R-lines/varieties. B-lines and R-lines/varieties with higher

Table 1. Estimates of mean, and variability parameters and heritability for grain Fe, Zn, phytates contents in sorghum, 2003 postrainy season, ICRISAT-Patancheru, India.

Micronutrient	F-test	Mean \pm SE	Range	PCV	GCV	Heritability (%)
Fe (ppm)	**	28.0 \pm 0.9	20.1–37.0	0.12	0.11	84.99
Zn (ppm)	**	19.0 \pm 0.8	13.4–31.0	0.15	0.14	85.73
Phytates (mg g ⁻¹)	**	7.6 \pm 0.1	3.8–13.5	0.21	0.20	99.22

** – Significant at P=0.01; PCV: Phenotypic coefficient of variability; GCV: Genotypic coefficient of variability

Table 2. Category-wise performance of the sorghum lines for mean grain Fe and Zn, and phytates, 2003 postrainy season, ICRISAT-Patancheru, India.

Material	No. of lines	Iron (ppm)	Zinc (ppm)	Phytates (mg g ⁻¹)
Maintainer (B-)Lines	19	29.5	18.9	7.6
Varieties/restorer (R-) lines	47	26.8	18.1	7.8
Germplasm lines	18	30.0	21.9	7.2
F-test	-	**	**	NS
LSD (5%)	-	0.15	0.18	-

** – Significant at P=0.01; NS = Non-significant.

Table 3. Estimates of correlation coefficients of micronutrients (Fe and Zn) and phytates with agronomic traits in sorghum, 2003 postrainy season, ICRISAT-Patancheru, India.

Grain micronutrient/ agronomic trait	Phytate	Iron	Zinc	Days to 50% flowering	Plant height	Stover yield	Grain yield	Grain size	Grain hardness
Phytate	1.00								
Iron	0.02	1.00							
Zinc	0.12	0.55**	1.00						
Days to 50% flowering	-0.06	0.18	0.12	1.00					
Plant height	-0.28*	-0.02	0.30**	0.18	1.00				
Stover yield	0.02	-0.29**	-0.54**	0.13	-0.19	1.00			
Grain yield	0.04	-0.32**	-0.54**	0.06	-0.22*	0.98**	1.00		
Grain size	-0.16	-0.18	-0.11	-0.15	0.32**	-0.12	-0.22*	1.00	
Grain hardness	0.23*	-0.10	-0.09	-0.14	-0.27*	0.03	0.05	0.02	1.00
Grain lustre	0.28*	0.32**	0.15	-0.18	-0.31	-0.20	-0.18	-0.19	0.26*

N-2=82 degrees of freedom

* – Significant at 5% level; ** – Significant at 1% level.

Fe and Zn contents along with several R-lines such as ICSR 93031, ICSR 91027, ICSR 94489, ICSR 94035 and ICSR 89001, and varieties such as JJ 1041, PVK 801 and ICSV 93046 with lower Fe and Zn contents are good for inheritance studies as well as developing mapping populations for identification of quantitative trait loci (QTL) for high Fe and Zn contents.

Association between grain Fe and Zn contents.

Significant and fairly higher positive correlation ($r = 0.55$) between grain Fe and Zn contents (Table 3) suggested the possibilities of combining both the micronutrients in single agronomic background. It is interesting to note that seeds rich in Fe and Zn contents show several agronomic advantages such as higher seedling vigor, especially in low-fertile soils, higher levels of resistance to diseases, and empowering plants with higher water-use efficiency, all of which are decisive and critical advantages in SAT (Graham and Welch 1996).

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

In order to realize maximum impact of micronutrient-dense cultivars, the micronutrients must be delivered in top-yielding cultivars with farmer-preferred grain quality evident traits, such as pearly white, lustrous and bold grains. Under this premise, correlation of grain Fe and Zn contents with desirable agronomic and grain quality traits were estimated. Though statistically significant (negative) a rather weaker correlation of grain Fe content with grain (-0.32) and stover yields (-0.29) (Table 3) indicated the possibility of breeding for high Fe content in high yielding background, significant negative and relatively strong correlation of grain Zn content with grain (-0.54)

and stover yields (-0.54) suggested the need for compromising optimization of Zn content and grain and stover yields. The poor correlation of agronomic traits such as days to 50% flowering and plant height with grain Fe and Zn contents indicated the possibility of developing micronutrient-dense lines in desired maturity and height background, with little compromise in grain and/or stover yields. While grain luster, one of the most important farmer-preferred attributes, had significant positive association with grain Fe content, it had a very weak relationship with grain Zn content. However, other farmer-preferred grain traits such as grain size and grain hardness appeared to have poor correlation with grain Fe and Zn contents. These results suggest that it is possible to deliver high Fe and Zn contents in cultivars with farmer's preferred traits such as early maturity, high yield potential, bold grain and lustrous grain.

Variability for β -carotene content. The grains of non-yellow endosperm lines had only traces of β -carotene content. However, in 11 yellow endosperm germplasm lines the grain β -carotene content ranged from 0.56 (IS 24724) to 1.132 ppm (IS 26886) with six lines (IS 7684, IS 7776, IS 24703, IS 24868, IS 24883 and IS 26886) having higher β -carotene contents than the average of 0.85 ppm. The grain samples analyses of 20 yellow endosperm sorghum germplasm lines by Kapoor and Naik (1970) also revealed similar range of β -carotene contents (0.2 to 1.4 ppm).

Association of grain β -carotene content with Fe and Zn contents and grain yield: It appeared that the genes controlling grain Fe and Zn contents are independent of those controlling β -carotene content as indicated by the poor and negative association of β -carotene content with grain Fe ($r = -0.24$) and Zn ($r = -0.31$). However, it may be noted that these correlation coefficients are only indicative, as the number of genotypes on which the correlations are estimated are rather fewer for arriving any conclusions on selection scheme. Fairly higher grain Fe and Zn and β -carotene contents in IS 26886 provide strong evidence to support breeding for all the three vital nutrients. Enriching sorghum cultivars with all the three nutrients – Fe, Zn and β -carotene – is highly desirable as there are potential synergistic interaction among these for their absorption, transport, and functioning and hence results in increased bioavailability in the human body (Graham and Rosser 2000).

Micronutrients vs. phytates. Sorghum grains contain phytic acid or the phytates, which are recognized as anti-nutritional factors as they form complexes with micronutrients such as Fe, Zn and β -carotene, thus interfering with their bioavailability. In the present study, the absence of significant differences between improved genetic materials (B- and R-lines) and un-improved germplasm lines for phytates (Table 2) indicated that genetic enhancement for agronomic traits did not result in concomitant variation in phytates contents providing a clue that they are under independent genetic control.

The narrow differences between PCV and GCV for phytates contents, which are amply reflected in high heritability (Table 1), suggest that selection for low levels of phytates contents would be highly effective. The weak correlations of phytates contents with grain Fe (0.02), and Zinc contents (0.12) (Table 3) indicate that it is possible to breed Fe and Zn-dense cultivars with low phytate contents.

Conclusions

Significant genetic variability was evident for grain Fe and Zn contents and anti-nutrients (phytates). While grains of non-yellow endosperm lines had only traces of grain β -carotene content, those of yellow endosperm germplasm lines had β -carotene content ranging from 0.56 to 1.13 ppm with six lines having higher β -carotene contents than the experimental average of 0.85 ppm. Several trait-based hybrid parents bred at ICRISAT had grain Fe (> 30 ppm) and Zn contents (> 22 ppm), fairly higher than the trial average levels (Fe=28 ppm; Zn=19

ppm). Substantial genetic variability coupled with high heritability and weak association of Fe and Zn contents with β -carotene and phytate contents suggest that it is possible to breed Fe and Zn and β -carotene-dense cultivars with low phytate contents. Further, significant and fairly higher positive correlation between grain Fe and Zn contents and their poor correlation with agronomic traits such as days to 50% flowering and plant height and with farmer-preferred grain traits such as grain size and grain hardness indicated the possibility of delivering high Fe and Zn contents in cultivars with farmer's preferred traits such as early maturity, high yield potential, bold grain and lustrous grains.

Considering that the grain sorghum is grown in different soil types with varying levels of native soil fertility with/without farmer's managed fertility in India, it is necessary to examine the stability of micronutrient-dense cultivars across different soil types and soil fertility levels typical to the areas for which these cultivars are targeted.

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