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Increasing grain Fe and Zn concentration in sorghum: progress and way forward

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Introduction

Sorghum (*Sorghum bicolor*) is among the top ten crops that feed the world (Goldschein 2011). It is one of the cheapest sources of energy and micronutrients; and a vast majority of population in Africa and central India depend on sorghum for their dietary energy and micronutrient requirement (Parthasarathy Rao et al.). 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 vulnerable groups of the society. Biofortification, where possible, is the most cost-effective and sustainable solution for tackling micronutrient deficiencies as intake of micronutrients is on a continuing basis with no additional cost to the consumer in developing countries of arid-tropical and subtropical regions. Biofortification of sorghum by increasing mineral micronutrients [especially iron (Fe) and zinc (Zn)] in grain is of widespread interest (Pfeiffer and McClafferty 2007, Zhao 2008, Ashok Kumar et al. 2009). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has been working for a decade on sorghum biofortification for enhancing grain Fe and Zn concentration. This paper briefly reviews the progress made thus far and way forward for biofortifying sorghum.

Initial work on grain Fe and Zn, β-carotene and phytates concentration

In total, 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 were assessed for grain Fe and Zn, β-carotene and phytates content. Significant genetic differences were observed for Fe, Zn and phytates content and for agronomic and grain traits (Reddy et al. 2005). The grain Fe content in these lines ranged from 20.1 ppm (ICSR 93031) to 37 ppm (ICSB 472 and 296 B) with an average of 28 ppm, while grain Zn content ranged from 13.4 ppm (JJ 1041) to 31 ppm (IS 1199) with an average of 19 ppm. However, the variability for β-carotene content was quite low in all the lines including the yellow endosperm lines with a maximum of 1.13 ppm in IS 26886 (Reddy et al. 2005). Considering narrow differences between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), and high heritabilities for micronutrients, it was concluded that Fe and Zn concentration can be improved by genetic means without altering grain phytates content. It was also concluded that it is feasible to breed for high Fe and Zn simultaneously with high grain yield.

Diversity in core collection of sorghum germplasm accessions

ICRISAT has one of the largest sorghum collections (about 39000 accessions) in its gene bank which represents 85% of the global sorghum diversity (Ashok Kumar et al. 2011). As it is difficult to evaluate all these lines for grain Fe and Zn concentration, the core collection of sorghum germplasm accessions that represents nearly entire diversity in sorghum gene bank was assessed for grain Fe and Zn. From these studies large variability was found for both Fe and Zn concentration (Ashok Kumar et al. 2009, 2012). A good number of germplasm accessions with higher Fe concentration (>60 mg kg^{-1}) were identified for use in the breeding programs (Table 1).

Phenotyping tools for assessing grain Fe and Zn

Having a reliable phenotyping method is critical for any crop improvement program. The grain Fe and Zn concentrations in sorghum can be estimated using Inductively Coupled Plasma Spectrometry (ICPS) (Houk 1986) and Atomic Absorption Spectrometry (AAS). Both

Table 1. Mean performance of selected sorghum landraces in 2010 and 2011 postrainy season at ICRISAT, Patancheru, India.

IS No.	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Time to 50% flowering (days)	Plant height (m)	Grain yield (t ha^{-1})	Grain size (g 100^{-1})	Agronomic score[1]
12750	76	39	76	2.5	1.9	2.43	2.3
27054	73	29	75	2.4	3.6	2.53	2.0
12858	73	32	68	2.2	1.8	2.13	2.8
12785	70	27	77	2.4	1.7	2.28	2.3
1563	70	38	73	1.8	1.3	2.29	3.0
34	69	27	82	2.5	2.4	2.10	2.3
13	64	33	74	2.1	2.9	2.52	2.3
30	62	32	82	2.5	2.1	2.54	1.8
20962	62	24	77	2.3	4.7	2.35	1.8
9150	62	28	80	2.3	2.9	2.28	2.0
Controls							
PVK 801	40	22	75	1.5	3.6	3.40	1.0
ICSB 52	30	16	71	1.3	2.9	3.68	1.2
Mean	43.62	26.57	74	1.89	2.38	2.71	2.42
SE+	2.71	1.70	1.73	0.09	0.32	0.14	0.27
CD (5%)	7.54	4.72	4.81	0.24	0.89	0.39	0.76

1. Agronomic score taken on a scale of 1 to 5, where 1 = more desirable and 5 = least desirable.

these methods are precise in estimating Fe and Zn but are destructive and laborious. In addition they are expensive and low-throughput. Therefore there is a need to develop efficient and high-throughput micronutrients assessment methods. One of the rapid and low-cost methods for assessing grain Fe and Zn concentrations is by using X-Ray Fluorescence Spectrometry (XRF), which is non-destructive and can be used routinely to screen the breeding materials. There is high correspondence between Fe and Zn values obtained by XRF and ICPS methods (Table 2) indicating that XRF can be used for assessing grain Fe and Zn concentrations particularly for discarding poor lines in the breeding program and finally confirming Fe and Zn using ICPS/AAS method.

Trait relationships

Knowledge on trait relationships helps in formulating appropriate breeding strategy. In sorghum, a highly significant and positive correlation was observed between grain Fe and Zn content (r = 0.853; $P < 0.01$) (Table 3), 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 (Reddy et al. 2010, Ashok Kumar et al. 2012). These results indicated potential for simultaneous genetic improvement for both micronutrients. The weak association of grain Fe and Zn with other traits studied – time to 50% flowering, plant height, grain yield and grain size – indicated that there is no penalty for enhancing grain Fe and Zn concentration in sorghum and it is possible to develop high Fe and Zn lines with large grain size, higher grain yield and required maturity duration. In order to realize the potential impact of micronutrient-dense cultivars, micronutrients must be delivered in high-yielding backgrounds with farmers' preferred traits such as early maturity, acceptable seed color and large seed size.

Genetic control and breeding approaches for enhancing grain Fe and Zn

Understanding inheritance of the trait is critical for its improvement. Studies on a number of crosses showed

Table 2. Correlation coefficients of iron (Fe) and zinc (Zn) traits estimated by ICP and XRF spectrometry methods[1].

Trait	Fe-ICP	Fe-XRF	Zn-ICP
Restorers Trial			
Fe-XRF	0.465**	1.000	
Zn-ICP	0.671**	0.332*	1.000
Zn-XRF	0.582**	0.514**	0.792**
df (N–2 = 50) = 0.273 at 5% and 0.354 at 1%			
F_1s and Parents Trial			
Fe-XRF	0.768**	1.000	
Zn-ICP	0.907**	0.655**	1.000
Zn-XRF	0.775**	0.676**	0.900**
df (N–2 = 33) = 0.335 at 5% and 0.430 at 1%			

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

Table 3. Correlation coefficients of sorghum landraces evaluated for iron (Fe) and zinc (Zn) concentrations in 2007 and 2008 postrainy season at ICRISAT, Patancheru, India[1].

Trait	Fe content	Zn content	Time to 50% flowering	Plant height	Glume coverage	Grain yield
Fe content	1.00					
Zn content	0.75**	1.00				
Time 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*

1. df (n–2) = 29 and r = 0.36 at 5% and 0.46 at 1%.
* = Significant at 5% level; ** = Significant at 1% level.

that grain Fe and Zn in sorghum like in other crops are quantitatively inherited with continuous variation. Analysis of the F$_2$ populations derived from crosses involving high Fe and Zn and low Fe and Zn parents show a normal distribution for grain Fe and Zn. As genetic variability for grain Fe and Zn contents and trait relationships were well established in sorghum in a range of materials, a study was made to understand the gene action and heterosis for Fe and Zn to help enhance the breeding program for improving the grain Fe and Zn (Ashok Kumar et al. 2012). High predictability ratio [$2\sigma^2$gca/($2\sigma^2$gca + σ^2sca); gca denotes general combining ability and sca denotes specific combining ability] for Zn in the study indicated that the expression of grain Zn content in sorghum is governed predominantly by additive gene effects, and suggested high effectiveness of progeny selection in pedigree selection or population breeding to develop lines with increased levels of grain Zn contents. Low predictability ratio for Fe indicated that the expression of grain Fe content in sorghum is governed predominantly by non-additive gene effects in combination with additive gene effects, suggesting a scope for heterosis breeding to develop lines with increased levels of grain Fe content in addition to progeny selection (Ashok Kumar et al. 2013a). Highly significant and positive correlation between gca and performance per se of lines suggests that the performance per se of the genotypes could be a good indicator of its ability to transmit grain Fe and Zn contents to its hybrids and progenies; and genetically superior parents could be identified by evaluation of their Fe and Zn contents. Some of the hybrids significantly outperformed the parents having high levels of Fe, indicating that it is possible to exploit heterosis for grain Fe in sorghum, whereas it has limited value in improving for Zn. From these studies, it was suggested that to develop heterotic hybrids for high Fe levels in sorghum, at least one of the parents should possess high Fe concentration, and for Zn, both parents should possess high mean values for grain Zn (Ashok Kumar et al. 2013a). It is possible to predict the hybrid performance based on parental performance as there is good correspondence between mid-parent values and hybrid performance for Fe and Zn (Figs. 1 and 2).

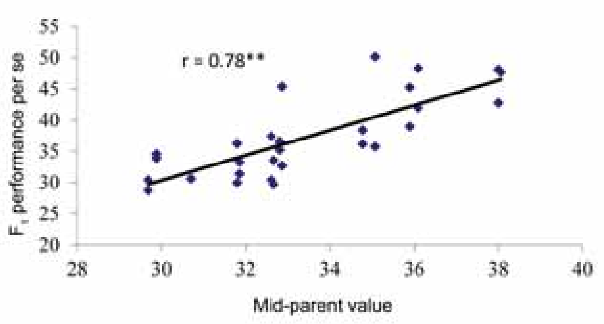

Figure 1. Relationship between mid-parent values and F$_1$ performance per se for grain Fe content (mg kg^{-1}) in sorghum crosses.

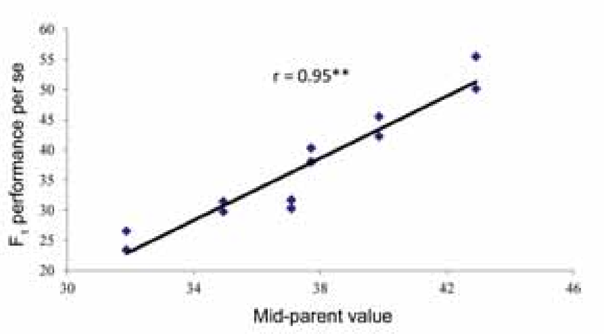

Figure 2. Relationship between mid-parent values and F$_1$ performance per se for grain Zn content (mg kg^{-1}) in sorghum crosses.

Agronomic biofortification

In crops like wheat (*Triticum aestivum*), grain Fe and Zn concentration can be increased by application of micronutrient fertilizers (Cakmak 2008), which is termed as agronomic fortification or fertifortification. We attempted this in sorghum by using different genotypes, different concentrations of micronutrient fertilizers, different soil types under on-station (no-deficiency in soil for micronutrients) and on-farm (deficiency in soil for some micronutrients) conditions. Micronutrient fertilization increased grain yield marginally but did not improve Fe and Zn concentration in grains, implying that there is limited scope for agronomic biofortification in sorghum (Ashok Kumar et al. 2007). Recently we observed an increase of 5–12% for grain Fe and 5–8% for grain Zn concentration with foliar application of micronutrient fertilizers under on-farm conditions (soils deficient in micronutrients). This difference is not statistically significant but has implications on nutrition as any incremental gain in grain micronutrient concentration contributes to combating the micronutrient malnutrition.

Breeding pipeline

The popular postrainy sorghum cultivars, viz, M 35-1, Dagadi Sholapur, Parbhani Moti, Phule Vasudha, Phule Anuradha and Phule Chitra used as food in India possess low Fe and Zn concentration (30 ppm Fe and 20 ppm Zn). To harvest low hanging fruits, first we identified promising rainy season adapted hybrids possessing >50% higher grain Fe and Zn than the postrainy sorghum cultivars (Ashok Kumar et al. 2010, 2013b). These hybrids can be multiplied in postrainy season; for example, in rice (*Oryza sativa*) fallows in Andhra Pradesh where temperatures do not go to such low levels to affect the restoration in hybrids and can be supplied to low income group predominantly sorghum eating populations. Further we established the grain Fe and Zn concentration of large number of sorghum hybrid parents (Ashok Kumar et al. 2012) and kept it in public domain (http://hdl.handle.net/11038/10081).

New hybrids possessing increased Fe and Zn coupled with high yield were developed and are currently under multilocation testing for commercialization. The high Fe and Zn germplasm lines were used in the crossing program and large number of transgressive segregants possessing high Fe and Zn coupled with agronomic desirability are being identified. The maintainers identified in the breeding program are being converted into male-sterile lines.

The way forward

Use of new tools enhances the efficiency of breeding program. We are working towards this on identification of quantitative trait loci (QTL) for grain Fe and Zn concentration which can be used in marker-assisted backcrossing. Similarly, efforts are underway for association mapping of Fe and Zn. On commercialization front, the superior hybrids identified can be seed multiplied and distributed. New hybrid combinations will be developed using the high Fe and Zn parental lines identified and tested in multilocation trials for commercialization. Superior donors from germplasm will be used in making large number of crosses to identify the transgressive segregants. To combine the three essential but limiting micronutrients, Fe, Zn and vitamin A, sorghum transgenics with higher β-carotene content in the background of high Fe and Zn need to be developed. Awareness among consumers needs to be developed on dietary diversification and use of biofortified products to address the micronutrient malnutrition.

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