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# Genetic Enhancement Perspectives and Prospects for Grain Nutrients Density

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# Contents

1	Introduction	792			
2	Standardization of Phenotyping Methods and Establishing the Baselines				
3	Variability for Grain Fe and Zn in Sorghum				
4	Gene Action and Heterosis for Fe and Zn 7				
5	5 Trait Associationship 79				
6	6 Grain Size Play Key Role in Improving Fe and Zn				
7	7 Multi-environment Testing to Tackle Significant $G \times E$ Interaction				
8	QTL Identification and Synteny Study for Fe and Zn in Sorghum	798			
9	Other Quality Parameters				
	9.1 Carbohydrates	800			
	9.2 Protein	800			
	9.3 Vitamins	802			
	9.4 Fat and Fiber	802			
	9.5 Minerals	803			
	9.6 Phytates	803			
	9.7 Antioxidants, Phenolic Acids	804			
10	Conclusion	804			
Refe	eferences				

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#### Abstract

Diet-induced micronutrient malnutrition continues to be a major challenge globally, especially in the developing world. With the ever-increasing population, it becomes a daunting task to feed millions of mouths with nutritious food. It is time to reorient agricultural systems to produce quality food to supply the calorie and nutrient requirements needed by the human body. Biofortification is the process of improving micronutrients density by genetic means. It is cheaper and sustainable and complements well with the nutrient supplementation and fortification the short-term strategies that are currently deployed to address the micronutrient malnutrition. Sorghum is one of the important food crops globally, adapted to semi-arid tropics, and there is increased awareness on its nutritional importance. Further, there is great opportunity to improve sorghum for nutritional quality. This chapter deals about the genetic enhancement perspectives and prospects for improving the nutritional quality with main emphasis on grain micronutrient density in sorghum.

#### **Keywords**

Biofortification · Micronutrients · Grain Fe · Grain Zn · Anti-nutritional factors

# 1 Introduction

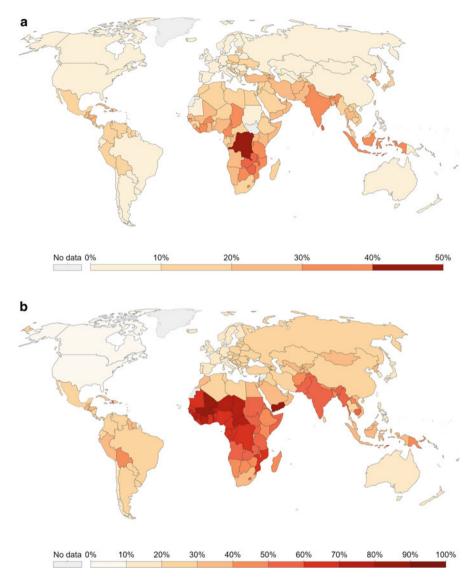
Micronutrient malnutrition is one of the greatest global challenges of our times. Micronutrients are essential for living organisms, which are limiting in many diets, particularly in the low-income populations. Micronutrients although required by the body in very small amounts, are vital for development, disease prevention, and wellbeing. Micronutrients are not produced in the body and must be derived from the diet. Deficiencies in micronutrients such as iron, iodine, vitamin A, folate, and zinc can have devastating consequences. At least half of the children worldwide aged 6 months to 5 years suffer from one or more micronutrient deficiency, and globally more than two billion people in the developing world are affected (Phuke et al. 2017). Iron (Fe) is an essential mineral critical for motor and cognitive development. Children and pregnant women are especially vulnerable to the consequences of iron deficiency. Low hemoglobin concentration (anemia) affects 43% of children below 5 years of age and 38% of pregnant women globally (Stevens et al. 2013). Iron deficiency adversely affects cognitive development, resistance to infection, work capacity, productivity, and pregnancy. Flour fortification with Fe and folic acid is globally recognized as one of the most effective and low-cost micronutrient interventions (Engle-Stone et al. 2017). Zinc (Zn) is a mineral that promotes immunity, resistance to infection, and proper growth and development of the nervous system and is integral to healthy pregnancy outcomes. Nearly 17.3% of the global population is at risk for zinc deficiency due to dietary inadequacy, though up to 30% of people are at risk in some regions of the world (Wessels et al. 2013). Zinc deficiency leads to impaired growth, immune dysfunction, increased morbidity

and mortality, adverse pregnancy outcomes, and abnormal neurobehavioural development. Zinc supplementation reduces the incidence of premature birth, decreases childhood diarrhoea and respiratory infections, lowers all-cause mortality, and increases growth and weight gain among infants and young children (Zimmermann 2011). Globally, efforts are underway to eliminate deficiencies in Fe and Zn along with vitamin A, iodine, and folate. However, there are constraints in terms of access, affordability, and sustainability of these interventions. Therefore, biofortification (increasing the minerals/vitamins in edible plant parts by genetic means) is critical to improve the grain Fe and Zn concentration in staple crops. Here the intake of minerals will be regular with no additional costs to the consumers. It is cheaper and sustainable and complements well with the nutrient supplementation and fortification that are currently deployed to address the micronutrient malnutrition.

Sorghum is a major food crop globally, and it forms principal staple for more than 500 million people in sub-Saharan Africa and South Africa, which incidentally are the major food insecure, and micronutrient malnutrition prone areas (Fig. 1a, b). The low-income group populations in these countries depend upon sorghum for more than 50% of their micronutrient requirement as they do not have affordability or access to nutrient-rich foods. Therefore, biofortifying staples including sorghum are of great importance. The major objective of biofortification is to significantly increase the grain Fe and Zn concentration in the high-yielding, farmer-preferred sorghum cultivars and pumping them into the food chain by increasing their adoption by the farmers though innovative seed chain. In this chapter, we summated the genetic approaches for biofortifying sorghum and improving its nutritional quality.

# 2 Standardization of Phenotyping Methods and Establishing the Baselines

Sorghum is blessed with large genetic diversity, and by now more than 42,000 germplasm accessions conserved in global gene banks (e.g. ICRISAT) are available for sharing. It provides great opportunity to improve the nutritional quality along with other traits in sorghum. However, the germplasm is not characterized for all nutritional traits for, e.g. micronutrients. Precision phenotyping is of critical importance for improvement of traits, particularly, the grain mineral nutrient concentration. In the process of biofortifying sorghum, to start with, we first standardized the phenotyping methods for assessing the grain Fe and Zn in sorghum. After trying the Perls' Prussian blue for assessing Fe and zero echo-time (ZTE) methods for Zn quickly moved to using atomic absorption spectrometry (AAS) and the inductively coupled plasma (ICP)-optical emission spectrometry (OES) that give very precise results on Fe and Zn along with other nutrients. Using the AAS and ICP-OES methods, a large number of sorghum advanced breeding lines, hybrid parents and germplasm accessions were assessed for Fe and Zn in sorghum (Reddy et al. 2005; Kumar et al. 2009, 2012). While AAS and ICP-OES give reliable results, they are expensive and destructive methods and do not have high throughput. So it is not



**Fig. 1** (a) Global prevalence of zinc deficiency, 2005 (source: Wessells and Brown 2012). (b) Global prevalence of anemia in children, 2016. (Source: World Bank; downloaded from https://ourworldindata.org/micronutrient-deficiency)

feasible to deploy them in assessing the breeding populations. To overcome this problem, we standardized the X-ray fluorescence spectrometer (XRF) for assessing the grain Fe and Zn, which is a low-cost, robust, and non-destructive method. There is good correspondence between ICP and XRF methods for assessing the grain Fe and Zn, but ICP is more accurate and reliable (Table 1) (Kumar et al. 2013a, 2015).

Table 1 Correlation   between Fe and Zn Estimated by ICP and XRF   methods Estimated by ICP and XRF	Trait Restorers trial	Fe_ICP	Fe_XRF	Zn_ICP
	Fe_XRF	0.465**		
	Zn_ICP	0.671**	0.332*	
	Zn_XRF	0.582**	0.514**	0.792**
	r (n - 2 = 50  d.f.) = 0.273  at  5%  and  0.354  at  1%			
	F <sub>1</sub> s and parents trial			
	Fe_XRF	$0.768^{**}$		
	Zn_ICP	0.907**	0.655**	
	Zn_XRF	0.775**	0.676**	0.900**
	r (n - 2 = 33  d.f.) = 0.335  at  5%  and  0.430  at  1%			

\* Significant at 5%

\*\* Significant at 1%

So we used XRF method for discarding the lines with low Fe and Zn in the segregating populations of breeding programme and validated all high Fe and Zn lines with ICP-OES method.

Baselines in sorghum were established to act as reference points for further improvement and to measure the progress. To set up the baselines, the entire spectrum of commercial sorghum cultivars (66) grown in India were assessed. The Fe and Zn concentration in the most preferred cultivars (post-rainy landraces) for food use in India was found to be low (30 ppm Fe and 20 ppm Zn), which were frozen as baselines for sorghum for increasing the grain Fe and Zn (Table 2) (Kumar et al. 2012). We targeted to improve the Fe and Zn by atleast 50% higher than the baseline without compromising the grain yield, stover yiels and other preferred traits and further to increase the levels to 60 ppm Fe and 40 ppm Zn, so that they meet the mjor nutrient requirement by the consumers.

### 3 Variability for Grain Fe and Zn in Sorghum

In one of the oldest studies, Wehmeyer (1969) had reported a range of 25–115 ppm for grain Fe and 15–65 ppm for Zn contents among the 79 sorghum cultivars. In a first major effort to assess the variability for grain Fe and Zn, as well as  $\beta$ -carotene and phytate concentration in sorghum, a total of 84 diverse sorghum lines involving parental lines of popular hybrids, cultivars, yellow endosperm lines, germplasm accessions, high-protein digestible lines, high-lysine lines, and waxy lines were assessed. Significant genetic differences were observed for Fe, Zn, and phytate concentration in these lines ranged between 20.1 (ICSR 93031) and 37 ppm (ICSB 472 and 296 B) with an average of 28 ppm, while grain Zn concentration ranged between 13.4 (JJ 1041) and 31 ppm (IS 1199) with an average of 19 ppm. However, the variability for  $\beta$ -carotene concentration was low including in yellow endosperm lines, whose maximum was 1.13 ppm in IS 26886 (Reddy et al. 2005). Given the

<b>able 2</b> Assessing the Fe	Cultivar	Fe (ppm)	Zn (ppm)
and Zn in sorghum cultivars preferred for food use in	Phule Chitra	32.2	22.0
ndia	Phule Anuradha	31.1	19.9
	Parbhani Jyoti	30.9	25.3
	Giddi Maldandi	30.8	21.5
	NTJ 2	30.7	22.0
	M 35-1	30.4	21.0
	Solapur Dagdi	29.8	20.6
	Parbhani Moti (SPV 1411)	28.5	22.3
	Barsizoot	28.0	20.6
	Phule Vasudha	27.6	19.5
	PVK 801 (Control)	44.0	24.0

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 concentrations can be improved by plant breeding without altering the concentration of grain phytates. It was also concluded that it is feasible to breed for high Fe and Zn simultaneously with high grain yield. However, there is limited scope to enhance grain  $\beta$ -carotene concentration through breeding efforts. Since then the emphasis was given for improving Fe and Zn only.

# 4 Gene Action and Heterosis for Fe and Zn

Grain Fe and Zn are quantitatively inherited showing continuous variation. To establish the gene action, a full diallel study involving three sets of crosses, one set using five parents contrasting for both grain Fe and Zn, the second set using six parents contrasting only for Fe, and the third set with four parents contrasting only for Zn showed that both additive and non-additive gene action play a role in conditioning grain Fe and Zn concentration in sorghum (Kumar et al. 2013b). However, non-additive gene action is predominant in conditioning grain Fe; and additive gene action in conditioning grain Zn. Some of the crosses showed significant heterosis for grain Fe concentration without yield penalty and some crosses showed higher grain Zn concentration (40 ppm) coupled with higher grain yields. The results also showed that it is possible to improve grain Fe concentration through exploiting heterosis, but there would be little opportunity, if any, for improving grain Zn through heterosis breeding. To develop hybrids with high grain Fe and Zn concentration in sorghum, both parents need to be improved for these micronutrients. Combining higher grain Fe and Zn with high grain yield is feasible (Kumar et al. 2013b). In another study using half-diallel analysis, significant correlation between mean parental performance and GCA effects (r = 0.86 for Zn, r = 0.67 for Fe) was reported indicating that selection of genotypes with high mean Zn and/or Fe levels would be highly effective in selecting for high GCA (Hariprasanna et al. 2014a).

#### 5 Trait Associationship

The association of grain Fe and Zn contents with other agronomic traits was studied in 1394 accessions received from core germplasm maintained at the ICRISAT genebank. The grain Fe and Zn contents of the accessions with white grains were marginally higher than those with coloured grains, and these white grain sorghums originated mostly from India and Zimbabwe. The Fe and Zn contents of the accessions with testa and without testa were comparable. However, endosperm texture and grain size appeared to influence grain Fe and Zn contents. Strong positive correlation between grain Fe and Zn contents and their weak association with the agronomic and grain traits indicated possibility to breed simultaneously for high grain Fe and Zn traits in varied plant agronomic backgrounds that might be suitable for different agro-climatic regions across the world (Sanjana Reddy et al. 2010). Similarly, significant positive association between grain Fe and Zn and little or no strong negative associationships with other agronomic traits have been reported (Kumar et al. 2012, 2013a; Hariprasanna et al. 2014b), thus indicating possibility of simultaneous improvement of both Fe and Zn. Strong correlation between protein content and Fe and Zn in the grains apart from strong association between Fe and Zn (Venkateswarlu et al. 2018) has also indicated that protein content improvement may increase Fe and Zn in sorghum grains as minerals are associated with proteins for biochemical activity. The positive associationship between Fe and Zn can be explained by the co-localization of OTLs controlling Fe and Zn (Anuradha et al. 2019).

# 6 Grain Size Play Key Role in Improving Fe and Zn

In sorghum improvement, increasing the grain size (100 grain weight) and grain number is the major selection criteria for improving the grain yields. In most of the recent hybrids, the grain size goes up to 3.5 g per 100 grains. However, from a nutritional point of view, it is not a desirable feature. The localization studies on Fe and Zn in sorghum indicates that most of the Fe is in the germ portion along with other micronutrients, while Zn is mostly concentrated in germ but also distributed in aleurone layer. When the grain size is increased, there is more accumulation of starch with no concomitant increase in Fe and Zn. As the Fe and Zn are expressed in ppm, with increase in grain size, their proportion appears to be less compared to rest of the grain contents. It was more clearly observed in multi-location, multi-year analysis of two parents (296 B and PVK 801), and a set of recombinant inbred line (RIL) population (342) derived from it. In both the parents and RILs, the Fe and Zn increased with decrease in grain size (Phuke et al. 2017). Though significant positive association was found between grain size and hardness, significant correlation was not found between grain hardness and Fe and Zn. Significant negative association was found between grain size and protein, grain hardness and protein, and grain size and Fe and Zn (Venkateswarlu et al. 2018). So optimizing grain size and increasing grain number is critical to simultaneously improve both yield and grain micronutrient concentration.

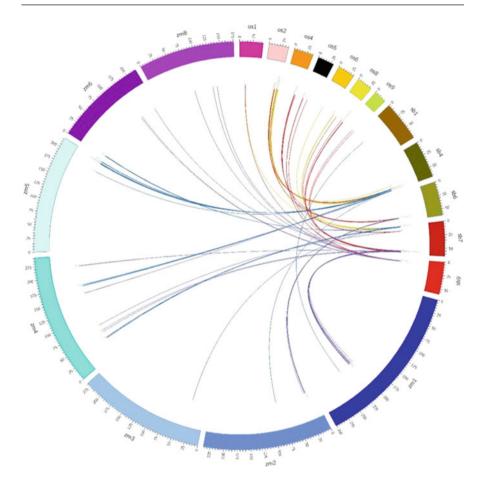
# 7 Multi-environment Testing to Tackle Significant G × E Interaction

Various studies in sorghum showed that the heritability is high for Fe and Zn indicating the extent of genetic variation vis-à-vis phenotypic variation in various populations (Reddy et al. 2005; Kumar et al. 2013a; Hariprasanna et al. 2014b). However, the  $G \times E$  interaction was significant in all the experiments indicating the role of environment in influencing trait expression (Feil et al. 2005; Hariprasanna et al. 2012; Phuke et al. 2017). The Fe and Zn concentration in sorghum is influenced by soil micronutrient content, uptake by the roots, translocation in the vascular system, and loading in to grains. Large number of QTLs controlling grain Fe and Zn in sorghum vindicates this (Anuradha et al. 2019). Therefore, it is important to test the advanced breeding lines, varieties and hybrids in multi-location trials over years by assessing soil nutrient status and by ensuring balanced fertilization. It enables to identify genotypes with stable performance. This approach was successfully used in developing and commercializing first biofortified sorghum variety 'Parbhani Shakti' in India (ICRISAT 2019).

# 8 QTL Identification and Synteny Study for Fe and Zn in Sorghum

Identification of quantitative trait loci (QTL) plays an important role in trait improvement. However the genetic control of complex traits such as grain Fe and Zn, which are controlled by many genes, is critical for developing an appropriate breeding strategy for its improvement. To identify genomic regions associated with grain Fe and Zn, a sorghum recombinant inbred line (RIL) population in F<sub>6</sub> (342 lines derived from cross 296B × PVK 801) was phenotyped at three different locations for 2 years and genotyped using simple sequence repeat (SSR), DArT and DArTSeq (Diversity Array Technology) markers. Forty-seven QTLs (individual) and seven QTLs (across) environments with small main-effect and 21 co-localized QTLs for Fe and Zn were identified (Anuradha et al. 2019). Highly significant genotype × environment interactions were observed for both micronutrients; grain Fe showed greater variation than Zn. Genomic resources produced on grain Fe and Zn in sorghum can help in developing high Fe and Zn lines in a cost-effective and efficient manner in the future.

Putative candidate genes associated with sorghum grain Fe and Zn content were identified through a synteny study among different cereals (Fig. 2). The study identified 91 candidate genes which are involved in governing grain Fe and Zn concentrations in rice, wheat, maize, and barley. These genes were blast searched on sorghum genome database and found 77 hits on sorghum genomic regions that are



**Fig. 2** Candidate genes underlying the region associated with grain Fe/Zn QTLs on chromosome SBI-01, SBI-04, SBI-06, SBI-07, and SBI-09 in sorghum showing gene syntenic relationship with *Zea mays* on chromosomes 1, 2, 3, 4, 6, 8, and 10 and *Oryza sativa* on chromosomes 1, 2, 4, 5, 6, 8, 9, 10, and 11. Note: Homologues genes are connected by lines; *Sb* Sorghum bicolor, *Zm* Zea mays, *Os* Oryza sativa

involved in Fe and Zn homeostasis. Majority (24 genes) of the genes were found on chromosome SBI-01 and were in homology with the genes from wheat, barley, rice, and maize pertaining to *NAS*, *Zn transporter protein* (*Zip*), and *yellow stripe like* (*YSL*). Genes associated with grain Fe and Zn concentration from maize and wheat showed 100% homology on sorghum genome. Based on the homology, it was assumed that these genes might be associated with grain Fe and Zn concentrations in sorghum. Candidate genes (homologs) identified in this study can be used for the development of functional markers for improving grain Fe and Zn concentrations in sorghum (Anuradha et al. 2013). These putative genes can be validated using the

published genomic sequence (Paterson et al. 2009) and can further be used in marker-assisted breeding. Identified QTL and genes once validated can also be used for the development of functional markers for improving grain Fe and Zn concentration in sorghum.

### 9 Other Quality Parameters

# 9.1 Carbohydrates

The carbohydrate content in sorghum is composed of starch, soluble sugar, and fiber (pentasons, cellulose, and hemicellulose). The average starch content in sorghum lies between 67.7% and 70.7% (Hulse et al. 1980; Jambunathan and Subramanian 1987: Longvah et al. 2017). Starch is the most abundant component, while soluble sugars are low. Variants of sorghum such as sweet sorghums accumulate up to 25% sugar, 1.4-2.7 times more whole-plant nonstructural carbohydrates than grain sorghums, in the parenchyma of juicy stalks (Vietor and Miller 1990; Ming et al. 2001). In sorghum, we can find high variability in major fractions such as carbohydrate, protein, and fat across diverse germplasm, and this could be due to adaptation to different environmental factors (Shewayrga et al. 2012; Sukumaran et al. 2012). The waxy and normal starch consists of 1.2% of free sugars. The waxy sorghum has lesser amylose proportion and higher amylopectin proportion. Molecular studies identified a significant marker-trait association in which six genes were found to be involved in starch synthase pathways (Sh2, Bt2, SssI, Ae1, and Wx) or grain storage proteins (o2) (Figueiredo et al. 2010). In another study, eight significant marker trait associations for kernel weight, kernel diameter, and acid detergent fiber were identified which are being further used for marker assisted breeding (Sukumaran et al. 2012). A SNP in the starch synthase IIa (SSIIa) gene which is associated with kernel hardness and a SNP in the starch synthase (SSIIb) gene which is associated with starch content were of high importance. Genome-wide association for sorghum grain protein, fat, and starch identified a putative alpha-amylase-3 gene to be strongly associated with protein and fat variation, but no significant SNPs were identified for starch, suggesting high heritability and starch variation are likely controlled by many small effect genes (Rhodes et al. 2017). In a previous study, the alpha-amylase-3 gene was also shown to be associated with sorghum grain composition traits (Rhodes et al. 2017). Also, recently, Boyles et al. (2017) found a strong QTL associated with crude fat on chromosome number SBI-10, in which four SNPs were in tight linkage at 50 Mb. This QTL explained up to 28.1% of the genetic variance in the test environment.

# 9.2 Protein

Proteins form the second major component of sorghum grains. Compositional analysis of diverse sorghum genotypes showed that the protein content ranged

from 8.1% to 18.8% (Rhodes et al. 2017). The amount of protein present in sorghum is comparable with that of wheat and maize. The nutritional value of sorghum is far better than rice and wheat. The protein quality in sorghum is compromised due to the presence of high leucine and tannins (Saleh et al. 2013). Along with amino acids, genetic and environmental factors are responsible for the variation in protein content in sorghum (Singh et al. 2016). Sorghum cultivars have been proven to have reduced amounts of lysine, threonine, and total sulphur amino acids (Shewayrga et al. 2012; Sukumaran et al. 2012). Sorghum storage proteins contain a higher proportion of cross-linked fractions and are hydrophobic, explaining their greater propensity to form intermolecular disulfide cross-linkages and possibly additional protein aggregates that could facilitate the formation of more covalent bonds (Belton et al. 2006; Hamaker and Bugusu 2003).

The protein content showed very wide variation (3.5–12.6%) among 112 local post-rainy sorghum landraces and varieties cultivated in southern and central Indian provinces (Badigannavar et al. 2016). Significant association of protein content with copper and zinc was also observed. Among the sorghum races, the *durra* and bicolor-durra sorghum races had the highest protein content, i.e. 12.6%, while *kafir* sorghum had the lowest protein content of 10.9%. The major fraction of the protein is kafiring inside protein bodies accompanied by a small amount of gluteling and minute amounts of albumins and globulins (Taylor et al. 1984). It was shown that  $\beta$ - and  $\gamma$ -kafirins are located on the core and the periphery of the protein bodies, whereas the  $\alpha$ -kafirin is 80% and is found in interior portion of protein bodies (Shull et al. 1992). The kafirin protein bodies together with glutelin-type protein form a tight matrix with starch granules in the corneous (hard) starchy endosperm which reduces protein and starch digestibility (Taylor and Emmambux 2010). This aspect is currently exploited for developing functional foods with nutraceutical properties for prevention and alleviation of type 2 diabetes. Studies are in progress in sorghum to improve the protein content, quality, and digestibility. Two genes floury-2 and opaque-2 (o2) from maize regulates kafirin levels and protein digestibility; these genes can be used as target in sorghum to increase sorghum nutrition value for animal feed industry (Singh and Axtell 1973; Kriegshauser et al. 2006).

Inheritance studies in sorghum germplasm lines suggested that the increased amount of lysine in each line was controlled by a single recessive gene that could be easily transferred by standard plant breeding procedures (Dargo and Shiferaw 2017). At Purdue University, a chemically induced high-lysine mutant, P721, was developed and is being used in the sorghum breeding programmes for the development of high-protein sorghum lines. The protein content was positively correlated with copper and Zn. Interestingly the biofortified sorghum variety 'Parbhani Shakti' showed higher protein content (11.9%) along with higher Fe and Zn. Strong positive association between protein content and micronutrients has been reported in a selected set of sorghum genotypes (Venkateswarlu et al. 2018). It is feasible to increase the nutritional value of sorghum grain using genetic engineering approaches, primarily, by the introduction of genetic constructs that cause the silencing of  $\gamma$ - and/or  $\alpha$ -kafirins (Elkonin et al. 2018). In another study, using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-

associated protein 9 (Cas9) gene editing approach to target the k1C genes to create variants with reduced kafirin levels and improved protein quality and digestibility. A single-guide RNA was designed to introduce mutations in a conserved region encoding the endoplasmic reticulum signal peptide of  $\alpha$ -kafirins. Sequencing of kafirin PCR products revealed extensive edits in 25 of 26 events in one or multiple k1C family members. T1 and T2 seeds showed reduced  $\alpha$ -kafirin levels, and selected T2 events showed significantly increased grain protein digestibility and lysine content. Thus, a single consensus single-guide RNA carrying target sequence mismatch is sufficient for extensive editing of all k1C genes. The resulting quality improvements can be deployed rapidly for breeding and the generation of transgene-free improved cultivars of sorghum (Li et al. 2018).

# 9.3 Vitamins

Vitamins such as B-complex is abundant in sorghum. Sorghum is also rich in thiamin, riboflavin, niacin, pantothenate, and vitamin B6 which is helpful in proper energy metabolism in children as well as adults. For children of age group 1–9 years, sorghum can provide 47–26% of the WHO recommendations for thiamin, 28–16% for riboflavin, 49-24% for niacin, 63-31% for pantothenate, and 118-59% for vitamin B6 (Lindsay 2010). This quality of sorghum to provide highly nutritious and ready supply of B-complex vitamins makes sorghum an excellent food crop for resource-poor families. Considerable research is underway to study biosynthetic pathways for vitamins in plants and identify the genes encoding each and every enzyme involved in the pathway. In cereals, work has been done to increase the contents of precursors for vitamin A. In sorghum, the variability for  $\beta$ -carotene content is found to be low (Reddy et al. 2005). However, more recently it was demonstrated that vitamin A deficiency can be reduced by co-expression of vitamin E precursor homogentisate geranylgeranyl transferase (HGGT) which is then required to stabilize provitamin A (Ping Che et al. 2016). Further developments in this area can lead to development of biofortified sorghum enriched for provitamin A.

#### 9.4 Fat and Fiber

The fat content in sorghum (1.73%) is higher than rice and wheat but less than maize and other millets. Fat in sorghum is mainly found in germ (80%) and aleurone layers (20%) of the grain. Sorghum shows extensive variation for fat content across diverse germplasm, and this could be due to adaptation to different environmental factors (Shewayrga et al. 2012; Sukumaran et al. 2012). Sorghum is high in dietary fiber (10.2%) (Longvah et al. 2017) and wide variation was reported for fibre content (Hariprasanna et al. 2015). Unlike refined grains, whole grains contain the fiber which is necessary for digestion. Dietary fiber is the combination of lignin and polysaccharide which is not easily hydrolyzed by the enzymes present in the human digestive tract. This will slow down the supply of glucose to the blood stream thus reducing the risk of major disorders such as diabetes (Nugent 2005). High fiber foods help in maintaining healthy weight and reduces the risk of diabetes and cardiovascular ailments other than its main function of bowel stability and strength. Sorghum is devoid of hull unlike some other grains, so even its outer layers commonly are eaten. This means it supplies even more fiber, in addition to many other crucial nutrients, and has a lower glycemic index (Prasad et al. 2015). The Shiferaw physico-chemical properties of sorghum dietary fiber can undergo changes by heat treatment; therefore, studies on changes in fiber during thermal and enzymatic treatment may help in improving the fiber composition in the food products prepared out of sorghum.

#### 9.5 Minerals

Sorghum is a rich source of minerals, vitamins, proteins, and carbohydrates. Identifying the diversity for these minerals can help in sorghum improvement for quality. In a recent study on mineral, concentrations of local landraces grown in southern and central Indian regions showed highest variation for calcium content and lowest for magnesium. Grain yield was highly heritable and protein content was positively correlated with copper and zinc. Copper showed significant positive correlations with yield, protein, and all other micronutrients except zinc. The study also reported the positive correction of iron content with manganese and calcium (Badigannavar et al. 2016). Other micronutrients such as Boron also play a very important role in vital functions of the plant, including meristem, sugar, and hydrocarbon metabolism and their transfer, RNA and cytokinin production and transfer, pollen building, and seed formation (Murthy et al. 2006).

### 9.6 Phytates

Phytates are the anti-nutritional factors which reduce the bioavailability of minerals by binding to the dietary minerals (Bouis et al. 2000). Soaking, sprouting, fermenting, and cooking can reduce the effect of phytic acids. During germination phytate is degraded by a specific group of enzymes, the phytases (Brinch-Pedersen et al. 2006). Phytate accumulates rapidly during seed development and can account for up to several percent of the seed dry weight (Lott 1984). Phytic acid ranged from 875.1 to 2211.9 mg/100 g among five varieties of sorghum grown in Kenya (Makokha et al. 2002). It was observed that fermentation reduces the phytic acid by 39.0% after 72 h and 64.8% after 96 h. Good variation for phytate (720–3909 mg/ 100 g) has been reported in a set of 200 Indian genotypes of sorghum comprising cultivars, parental lines, and germplasm accessions collected from major growing areas (Hariprasanna et al. 2015). In biofortification research while efforts are made to increase grain Fe and Zn, care is to be taken not to increase grain phytate content so that the increased micronutrients are bioavailable (Kumar et al. 2015).

### 9.7 Antioxidants, Phenolic Acids

Sorghum is a good source of antioxidants and has a property to reduce risks of developing cancer, diabetes, heart disease, and some neurological disorders. The free radical scavenging activity of antioxidants in sorghum helps in controlling numerous ailments. Policosanol, one among various beneficial phytochemicals present in sorghum, is very effective in controlling cholesterol. Other phytochemicals include phenolic acids, tannins, and anthocyanins, which are found in abundance in red, black and brown sorghums. The free radical scavenging effect of sorghum anthocyanins showed significant reduction in the formation of nicked DNA and increased native form of DNA (Suganya Devi et al. 2012). Phenolic compounds present in sorghum help in controlling many pest and diseases (Hahn et al. 1984) and also act as antifungal, antibacterial, and antiviral agents (Harborne and Williams 2000). Dykes et al. (2005) reported a strong correlation between total phenols and antioxidant activity indicating an association between pericarp thickness and antioxidant activity. The genotypes with thick pericarp are more susceptible to grain molds (Beta et al. 1999) due to the presence of starch granules in the mesocarp (Earp et al. 2004). Traits such as plant colour, pericarp thickness, presence of a pigmented testa, and spreader genes which are associated with increased antioxidant activity levels can be used in the breeding programmes for selection of best genotypes (Dykes et al. 2005). Considerable variability for total phenolic compounds and antioxidant activity measured as trolox equivalent antioxidant capacity (TEAC) has been observed in sorghum cultivars, parental lines and germplasm accessions (Hariprasanna et al. 2015). A recent study on phenolic composition and relative antioxidant activity using six varieties of sorghum including pigmented and non-pigmented pericarp varieties showed that there is no independent compound in sorghum that results in its high antioxidant activity, but rather it is the cumulative effect of various phenolic compounds (Rao et al. 2018). Presence of good genetic variability for other phytochemicals like cyanogens (14.2-173.6 ppm) and trypsin inhibitor (1.6-12.8 TIU) has also been reported in sorghum (Hariprasanna et al. 2016). The popular cultivars and parental lines had only moderate levels of these anti-nutritional factors and hence do not pose a serious challenge to the nutritional quality of sorghum as perceived previously in the developed world.

### 10 Conclusion

In conclusion, considering the health benefits of sorghum, it is important to focus on nutritional quality along with improving yield and adaptation. Biofortification of sorghum help enhancing the grain Fe and Zn concentration and complements well with other approaches in addressing micronutrient malnutrition. Optimizing the grain size and increasing grain numbers helps in higher accumulation of micronutrients in the germ portion which can be exploited in breeding. Excellent advances were made in understanding the genetic control, QTL identification, and genome editing technologies for improving the nutritional quality in sorghum, and it is high time to embrace them in breeding programmes.

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