10 Biofortification of Sorghum (*Sorghum bicolor*)

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Abstract

Malnutrition due to intake of nutritionally poor food is a serious problem among the developing nation. It affects the lives of around two billion people globally, of which most are children and women of reproductive age. Biofortification serves as an excellent, feasible, and cost-effective tool to meet the micronutrient requirement of the populations with limited access to nutrient-rich diets. This strategy not only increases the concentration of essential micronutrients but also enhanced their bioavailability. Sorghum is an important crop of arid and semiarid regions of the world and feeds the poor population of underprivileged countries. Its C_4 nature with intrinsic high photosynthetic rate and biomass potential makes it more tolerant to adverse environmental stresses like heat and drought. Being the cheapest source of micronutrients, it is the most preferred crop for biofortification. Current chapter reviews the nutritional importance of sorghum along with various techniques including agronomic, breeding, transgenic, and genome editing approaches to augment the desired micronutrient in the crop. The

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limitations and the areas that needed intervention are also discussed along with the challenges that biofortified sorghum holds to address the malnutrition.

Keywords

Agronomic · Breeding · Biofortification · Hidden hunger · Micronutrients · Sorghum

10.1 Introduction

Micronutrient deficiency leading to malnutrition is a worldwide problem but more rampant in developing nations (Ruel-Bergeron et al. 2015). It is estimated that around two billion people across the globe suffer from malnutrition (Hodge 2016; Sumithra et al. 2013). Women along with children below 5 years are most affected (Bailey et al. 2015). This situation will be worsened with the addition of 83–132 million people because of the COVID-19 pandemic, which illustrates the sensitivity of fragile food and agriculture systems. Until now the agriculture system focused only on increasing yield and productivity which currently needs a shift in producing crops having adequate amounts of micronutrients. This will help in countering the effect of micronutrient malnutrition among the population (Khush et al. 2012). Therefore, fortification of crops with essential micronutrients is prerequisite to curb malnutrition among the target population. Biofortification is the nutritional enrichment of food crops with increased bioavailability to humans and can be developed by conventional plant breeding, modern biotechnological techniques, and agronomic practices. It offers a sustainable and long-term solution for human consumption as well as fodder crop for animals (Meenakshi et al. 2010; Hefferon 2016).

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in relation to area and production. It is a crucial and staple food crop for habitants living in semiarid areas of the world, particularly in West Africa, sub-Saharan Africa, and semiarid zones of South Asia (Kumar et al. 2013a, 2013b), while in developed countries, it is used as livestock feed along with several industrial uses. It is cultivated in more than 100 countries with the USA, Mexico, Sudan, India, Nigeria, Niger, Ethiopia, Australia, China, and Brazil together contributing 77% of the world's total production (Aruna and Cheruku 2019). In 2019, about 59 million tonnes of sorghum were produced in the world, with an average yield of ~1.49 MT/ha (FAOSTAT 2020). Table 10.1 presents the details of economic status, hunger index, child mortality, human population, and sorghum production of major sorghum-growing countries worldwide. The child mortality status (deaths per 1000 live births) of top sorghum-growing countries is in the range of 3–13% and the hunger index in the range of moderate (10–19.9) to serious (20–34.9). This necessitates it to be biofortified to curb malnutrition and ultimately lower child mortality and hunger index rate.

Table 10.1 Details of economic status and hunger index in major sorghum-growing countries worldwide

Country	Population (thousands)[a]	Sorghum production (tonne)[b]	GDP per capita (current USD)[c]	Hunger index[d]	Child mortality[e]
Nigeria	206,140	6,665,000	2208.50	29.20	129.13
Ethiopia	114,964	5,265,580	917.88	26.20	55.00
Sudan	43,849	3,714,000	713.79	27.20	66.92
China	1,439,324	3,602,268	11712.85	<5	8.40
India	1,380,004	3,475,410	2030.62	27.20	36.00
Brazil	212,559	2,672,245	6728.17	<5	12.26
Niger	24,207	1,896,638	567.40	–	125.98
Burkina Faso	20,903	1,871,791	850.79	25.80	106.35
Argentina	45,196	1,601,435	9095.10	5.30	9.68
Mali	20,251	1,511,110	992.31	22.90	106.16
Cameroon	26,546	1,216,926	1657.08	19.10	75.70
Chad	16,426	972,516	710.18	44.70	131.68
Bolivia	11,673	949,039	3618.18	14.00	28.58
United Republic of Tanzania	59,734	731,877	1132.13	25.00	66.28
Uganda	45,741	400,000	971.28	–	72.54
Yemen	29,826	230,766	572.56	–	47.48

[a]World Population Prospects 2019
[b]FAOSTAT 2021
[c]World Economic Outlook Database 2021
[d]Global Hunger Index 2020
[e]UN IGME Database 2020

Sorghum is remarkably unique and vital for poor farmers because of its acclimation under drought and heat-prone environments. Being a C_4 plant with the ability to adapt in soils with low phosphorus availability, it is more attractive among farmers of arid and semiarid regions (Leiser et al. 2012; Haussmann et al. 2012). Apart from the food source, sorghum has several other uses such as feed, forage, fuel, and beverage and has phytoremediation potential (Liu et al. 2020) and therefore can be categorized as grain, forage, sweet, and broom type (Batey 2017). Sorghum grain is enriched with starch, protein, micronutrients, and crude fibers (Chavan and Patil 2010) and thus has the potential to provide more than half of the dietary micronutrients to families with low income (Rao et al. 2006, 2010).

Sorghum crop has been utilized for biofortification with various micronutrient concentration strategies, like provitamin A (beta-carotene) by expressing *Homo188-A* gene (Lipkie et al. 2013); enhanced protein content by expressing high lysine protein (Zhao et al. 2003); and digestibility improvement by silencing the *γ-kafirin* using RNAi (Grootboom et al. 2014; Elkonin et al. 2016). There is great interest in sorghum biofortification for Fe and Zn (Pfeiffer and McClafferty 2007; Zhao. 2008), and in line with this in 2018, India released its first biofortified sorghum variety ICSR 14001 also called ‘Parbhani Shakti’ via conventional breeding technique, having iron (Fe) and zinc (Zn) concentration of 45 ppm and 32 ppm, respectively. Besides this, it has higher protein (11.9%) and low phytate content (4.14 mg/100 g) (“http://www.icrisat.org/india-gets-its-first-biofortified-sorghum”). This chapter reviews the role of various micronutrients in the human diet along with their augmentation in sorghum using various biological activities, such as classical plant breeding, agronomic biofortification, and genetic engineering, or with the latest genome editing tools.

10.2 Top Priorities for Sorghum Biofortification

Nearly 0.5 billion people in 30 nations consume sorghum as a cereal crop (Kumar et al. 2013a, 2013b). It is a highly heat- and drought-tolerant crop with good nitrogen use efficiency. In addition, it is one of the low-cost sources of energy, protein, fat, carbohydrates, Fe, and Zn (Kumar et al. 2015). Further, its gluten-free nature (Ciacci et al. 2007), low glycemic index, and antioxidant properties make it a favorable diabetic cereal (Serna-Saldivar and Espinosa-Ramírez 2019). It supplies more than 50 percent of the dietary micronutrients to rural peoples with low income (Rao et al. 2006; Rao et al. 2010). However, some studies reported limited mineral content and bioavailability in cooked grains of sorghum (Kayodé et al. 2006). Human needs micronutrients for their proper metabolic needs and to stay healthy; however, their deficiency causes malnutrition or hidden hunger. These deficiencies can be overcome by various ways, viz., genetic ways to improve nutrient content, by taking supplements, dietary diversification, and biofortification. Among all these, biofortification is the only cost-efficient and sustainable method to eradicate this malnutrition.

10.2.1 Essential Micronutrients/Metals: Zn and Fe

Soil micronutrient deficiencies affect crop productivity more prominently due to the higher use of chemical fertilizers (Sanchez and Swaminathan 2005). Micronutrients like Fe and Zn are important for the human body but are found to be deficient in human diets these days. More than two billion people are affected due to micronutrient deficiencies, mostly from low-income families in developing nations in which Fe, Zn, vitamin A, and B9 deficiencies are most common (Kennedy et al. 2003; Bailey et al. 2015). It has been reported that during 2008, more than 4.5 lakh children below 5 years died because of diarrhea caused by Zn deficiency (Black et al. 2008). Its deficiency also leads to pneumonia and dwarfism in children (Cakmak et al. 1999; Walker et al. 2009). Zinc is an important micronutrient for growth and development having a role in plant vital functions (photosynthesis and respiration) and is also important for the nutritional value of feed and food-based plant products (Epstein and Bloom 2005).

Fe deficiency is the most prominent and prevalent nutrient deficiency. As per WHO, Fe deficiency affected 38%, 29%, and 43% of pregnant women, non-pregnant women, and preschool children, respectively (WHO 2017). It is assessed that approximately 50% cases of anemia are caused due to inadequate Fe intake and are responsible for poor immunity and lower pregnancy outcomes (Stevens et al. 2013; WHO 2017). It resulted in impaired cognitive development, low productivity, and growth retardation, with complications in health and economic systems (Bailey 2015). Anemia disease is becoming a serious issue these days worldwide. Both zinc and iron deficiency among children create a threat to the physical and mental health of human beings (Bains et al. 2015).

10.2.2 Basic Micronutrients: Selenium and Iodine

Selenium (Se) and iodine (I) are basic micronutrients not essential for plant metabolism. Both of these micronutrients are basically required for humans and animals and that's why these should be present in the diet. Se is important for human health as it has a vital role in the brain, thyroid, gonads, and heart. It has antioxidant, antibacterial, anticancer, and antiviral activities (Lyons et al. 2009), which thus helps in fighting cancer, reducing asthma symptoms, improving immunity, and reducing skin disorders. Its deficiency causes chronic diseases. Selenium also provides stimulating effect on plant growth and development (Abbas 2012). Minimal concentrations of selenium give a favorable effect on growth and development and also increase antioxidative capacity by increasing stress tolerance (Kong et al. 2005). In addition, selenium also helps in protecting DNA against damage and slows down aging of cells.

Iodine is also very important for human life; it helps in preventing chronic diseases. Iodine deficiency among human beings is a big problem nowadays. It is highly common among people from both developing and developed nations (Cakmak et al. 1999). The recommended dietary allowance (RDA) of I for adults is 150–200 μg per day and for lactating or pregnant females is 230 to 260 μg per day (Lawson et al. 2015). However, due to its insufficiency, it causes different health-related problems like goiter, mental disability, growth retardation, and increased miscarriage and infant mortality (Pearce et al. 2013; Lazarus 2015). Earlier reports showed that even mild iodine deficiency affects pregnant women and is associated with cognitive impairment in their children (Pearce et al. 2013). There are so many methods to overcome these deficiency problems. Biofortification of plants is one of the best methods to overcome I and Se deficiency in humans and animals (Smoleń et al. 2016).

10.2.3 Provitamins

Micronutrient malnutrition, mainly the consequence of poor bioavailability of vitamins and minerals in diets, causes blindness, anemia, beriberi, pellagra, scurvy,

Table 10.2 Comparative profiling of sorghum grains with other cereal grains

	Sorghum	Rice	Wheat	Maize	Barley	RDA
Minerals						
Iron (mg)	3.36	0.20	3.71	1.74	2.68	7–18
Zinc (mg)	1.67	0.49	2.96	2.24	2.0	3–11
Calcium (mg)	13.0	10.0	33.0	5.0	32.0	700–1300
Selenium (μg)	12.2	7.5	12.7	2.2	37.7	20–55
Iodine (μg)	0	0	0	0	0	90–250
Provitamins						
Vitamin A (IU)	0	0	0	214	0	300–900
Thiamin; B1 (mg)	0.332	0.02	0.297	0.16	0.37	0.5–1.2
Riboflavin; B2 (mg)	0.096	0.013	0.188	0.23	0.114	0.5–1.3
Niacin; B3 (mg)	3.69	0.4	5.35	2.6	6.27	6.0–16
Pantothenic acid; B5 (mg)	0.367	0.39	1.01	0.55	0.145	6.0–16
Pyridoxine; B6 (mg)	0.443	0.093	0.191	0.47	0.396	0.5–1.7
Folate; B9 (μg)	20.0	3.0	28.0	19.0	8.0	150–400
Vitamin B12	0	0	0	0	0	0.9–2.4
Vitamin C	0	0	0	0	0	15–90
Vitamin D	0	0	0	0	0	15–20
Alpha-tocopherol; E (mg)	0.5	0.04	0.53	0.49	0.57	6.0–15
Vitamin K1 (μg)	0	0	1.9	0.3	2.2	30–120
Beta-carotene (μg)	0	0	5	97	0	–
Lutein + zeaxanthin (μg)	0	0	220	1355	160	–
Protein						
Protein (g)	10.6	2.69	9.61	8.75	10.5	13–56

Data was obtained from USDA database; Dietary Reference Intakes 2011, 2019 [children (1–8 yrs), male and female (8 to >70)]

and rickets in more than 50% of the global population, particularly common among pregnant and lactating women and preschool children (Underwood 2000; Welch and Graham 2004; Asensi-Fabado and Munné-Bosch 2010). It has been reported that vitamin A deficiency leads to the blindness of up to 5 lakh children and the death of 6 lakh women because of pregnancy complications, which can be reduced by the consumption of vitamin A-enriched diets.

Sorghum grain contains several vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), and folate (vitamin B9) (Table 10.2). But in the RDA, these vitamins are insufficient to supply the nutritional requirement for children and adults; thus these need to be augmented using various biofortification approaches, viz., conventional or classic plant breeding and agronomic and genetic engineering. Further, some important vitamins such vitamin A, vitamin B12, and vitamins C, D, and E are deficient in sorghum grains but can be enhanced using genetic engineering or transgenic approaches.

10.2.4 Proteins

Sorghum grain lacks gluten content; thus, it serves as a good source of protein for gluten-sensitive individuals. As per data obtained from the USDA database, sorghum grains contain 10.6 g of proteins. However, RDA for children (1–8 yr) is 13–19 g, for males (9 to >70 yr), it is 34–56 g, for females (9 to >70 yr), it is 34–56 g, and during pregnancy 71 g per day is recommended. To combat this malnutrition, protein biofortification is an important and sustainable measure to enhance its bioavailability in staple plant foods (Taylor and Taylor 2011). Lower digestibility of seed storage proteins (SSP) and starch and lower nutritional grain value are some important factors to be addressed for sorghum biofortification along with other micronutrients. These seed storage proteins have low lysine and threonine content which are among the essential amino acids (Mudge et al. 2016). Further, most sorghum food is cooked or heated during preparation; this heat treatment resulted in up to 50% reduced digestibility compared to other cereal grains.

In recent years, biofortification of sorghum grains with proteins has been accomplished with the help of genomic tools, chemically induced mutations, and genetic engineering. These include impaired synthesis of kafirins (Mehlo et al. 2013); identification of natural allelic variants of kafirins (Mudge et al. 2016; Laidlaw et al. 2010; Cremer et al. 2014); increasing the lysine content (Zhao et al. 2003); silencing of γ- and/or α-kafirins genes (Kumar et al. 2012, Grootboom et al. 2014, Elkonin et al. 2016); and knockout strategy (Li et al. 2018).

10.3 Agronomic Biofortification of Sorghum

Biofortification mitigates the hidden hunger by increasing micronutrient concentrations and bioavailability in the food grain crops (Wakeel et al. 2018). Enhancement of Fe and Zn concentration in the grains of particular crops through application of Fe- and Zn-containing fertilizers is known as agronomic biofortification. Zuo and Zhang (2009) reported that any method that could intensify the root growth can result in higher uptake from the soil and can play a vital role in biofortification. Agronomic practices are also known as pre-harvest practices which enhance the nutrient content in crops. Food is categorized as biofortified if practices are pre-harvest and otherwise categorized as fortified if practices are postharvest. Some important agronomic biofortification approaches are the soil or foliar application of organic fertilizer, inorganic fertilizers, and biofertilizer and nutrient priming. For different mineral micronutrients, soil and foliar application of micronutrient fertilizer were found to be effective. Foliar application is found to be highly effective for zinc and selenium as Zn responds best and quick by agronomic biofortification methods mainly for cereal crops (Cakmak 2014). Organic manures, those that contain animal or plant sources such as vermicompost, farmyard and poultry manure, etc., are considered as a cost-efficient, environment-friendly alternative approach for inorganic synthetic fertilizers. Organic manures help in maintaining soil fertility. For instance, vermicompost increases Zn and Fe content by 4% and 7%, respectively, in barley crops (Maleki et al. 2011); poultry manure increases Fe content in rice and wheat by 10 and 15%, respectively (Ramzani et al. 2016, 2017). In addition to these, biofertilizers consisting of microorganisms enhance the productivity and growth of plants by increasing the supply or availability of nutrients (Barbosa et al. 2015; Bhardwaj et al. 2014). These include mycorrhizal fungi, blue-green algae, and cyanobacteria. Some biofertilizers like cyanobacteria were used in the Zn biofortification of wheat (Prasanna et al. 2015). Apart from these, nutri-priming is another approach whereby seeds were treated in micro- and macronutrient solutions before sowing (Farooq et al. 2011, 2019). With nutri-priming only grain zinc content of chickpea was increased by 29% (Farooq et al. 2019), thus considered to be low-cost method for nutrient enrichment (Poblaciones and Rengel 2016).

Only few studies use agronomic approaches to biofortify sorghum (Table 10.3). These include work of Mishra et al. (2015) who recommended the production of micronutrients (Fe and Zn) in post-rainy sorghum cultivar. Further, when soil application of $ZnSO_4$ + $FeSO_4$ (50 kg/ha of each) followed by foliar application (0.50% + 1.0%) was applied to Phule Maulee cultivar of sorghum at 45 DAS, Zn content of plant was increased up to 37.79–37.85 mg/kg along with increased green fodder yield and quality of fodder (Ahmad et al. 2018). More recently, application of a recommended dose of fertilizer with enriched vermicompost [(50 kg vermicompost/ha + 15 kg ZnSO4/ha) + (50 kg vermicompost/ha + 15 kg FeSO4/ha)] was also shown to increase the contents of Zn and Fe (Kumar and Kubsad 2017; Maganur and Kubsad 2020). In addition to these, high Zn content was obtained in CSV-31 genotype of sorghum using soil application of $ZnSO_4$ @ 50 kg/ha + foliar spray @ 0.2% at the knee-high stage and flowering stage (Markole et al. 2020).

10.4 Breeding Efforts for Sorghum Biofortification

Previous trait inheritance studies indicated that Fe and Zn are multigenic traits, hence controlled by many genes (Gregorio et al. (2000) in rice; Distelfeld et al. (2007) in wheat; Lonergan et al. (2009) in barley; Lung'aho et al. (2011) in maize). Thus, to map such genes, quantitative trait loci (QTL) mapping strategy is employed. In this strategy, contrasting parents for the trait of interest say lines with high and low Fe or Zn content will be crossed to generate F_1 hybrid. Then mapping population segregating for these traits will be developed after continuous selfing for over seven to eight generations. After this, phenotyping and genotyping of this population lead to the mapping of traits of interest. Using this strategy, Kotla et al. (2019) have recently mapped Fe and Zn QTLs in F_6 recombinant inbred line (RIL) population of sorghum developed by the crossing of 296B x PVK 801 contrasting parents. The details of QTLs, their marker interval, LOD score, and percent phenotypic variance explained have been summarized in Table 10.4.

The other strategy for mapping QTLs is through genome-wide association studies (GWAS). In this, breeders can directly use a natural structured population or sorghum germplasm for mapping of these genes (Fe, Zn, Se, I, etc.) using an association mapping panel. Association mapping panel or core collection can be

Table 10.3 List of significant studies performed for the sorghum biofortification

Biofortification trait	Agronomic practices	Success/level of improvement	Other note	Reference
High zinc	Fertilization	Soil application of $ZnSO_4$ @ 50 kg ha-1 + foliar spray of $ZnSO_4$ @ 0.2% at the knee-high and flowering stage significantly increase Zn concentration in grain and stover	Genotypes of sorghum, CSV-31, recorded higher stover zinc concentration and uptake (19.00 ppm and 103.67 kg/ha, respectively)	Markole et al. 2020
High zinc and Fe	Fertilization	Application of $ZnSO_4$ + $FeSO_4$ @ 50 kg ha^{-1} *fb.* and foliar application (0.50% + 1.0%) at 45 DAS with RDF (80: 40:40, N/P/K) resulted in Zn- and Fe-rich rainy sorghum	The genotype, Phule Maulee, recorded highest Fe (41.59 mg/kg) and Zn (20.80 and 26.42 mg/kg) over CSH 15R, M 35–1, Phule Chitra, Phule Yashoda	Mishra et al. 2015
High zinc and N	Fertilization	Application of Zn and N at rate of 10 and 120 kg/ha increased the Zn content of fodder sorghum	Zn content of plant also increased up to 37.79 to 37.85 mg kg^{-1} in 2 years	Ahmad et al. 2018
High zinc and iron	Fertilization	Application of recommended dose of fertilizer with enriched vermicompost [(50 kg vermicompost/ ha + 15 kg ZnSO4/ ha) + (50 kg vermicompost/ ha + 15 kg FeSO4/ha)] increased the contents of Zn and Fe	Increase in Fe and zinc contents of grain up to 39.52 and 28.44 mg/kg, respectively	Kumar and Kubsad 2017
High zinc and iron	Fertilization	Application of $ZnSO_4$ + $FeSO_4$ @ 15 kg ha_{-1} each enriched with FYM to kharif sorghum enhanced Zn and Fe content in grain	Increase in Zn and Fe contents of grain up to 23.43 and 33.89 mg/kg, respectively	Maganur and Kubsad 2020

developed after studying genetic diversity, population structure, and removing relative kinship between individuals. This will not only save time but also help in the identification of strongly linked markers to the targeted trait. More recently, with this GWAS strategy, Cruet-Burgos et al. (2020) has mapped provitamin QTLs, the

Table 10.4 List of significant studies performed for the identification of QTL that can be used for sorghum biofortification

Biofortification trait	Mapping population	QTL ID	Marker interval (cM)	LOD	PVE (%)	Reference
High Fe	F_6 RILs (296B x PVK 801)	qfe6.1(E1)	Sn2647940-Sn2657501 (90.3–91.5)	4.6	5.44	Kotla et al. (2019)
		qfe7.1(E1)	Dt3625344-Sn2644846 (36.8–49.6)	3.6	5.82	
		qfe7.2(E1)	Sn2653248-Xtxp525 (123.4–126.7)	4.3	5.09	
		qfe7.1(E3)	Dt3627294-Dt2648007 (65.4–65.6)	3.9	5.19	
		qfe1.1(E4)	Sn1933402-Sn2650907 (107.3–107.6)	5.5	6.80	
		qfe7.1(E6)	Sn2645682-Sn2653275 (60.9–61.4)	4.4	5.66	
		qfe7.2	Xtxp525-Sn2653248 (123.4–126.5)	5.6	6.7	
High Zn	F_6 RILs (296B x PVK 801)	qzn7.1 (E1)	Dt3625344-Sn2644846 (36.8–49.4)	5.6	9.42	Kotla et al. (2019)
		qzn7.2 (E1)	Dt2646020-Sn1925068 (53.0–54.7)	6.8	8.80	
		qzn7.3 (E1)	Sn1895281-Sn2650637 (57.0–57.9)	5.9	6.96	
		qzn7.1 (E3)	Sn1875097-Xiabtp360 (63.5–64.2)	4.9	6.29	
		qzn7.2 (E3)	Dt3628977-Dt2649259 (67.1–67.6)	4.3	5.83	
		qzn7.4	Dt2649259-Dt3628977 (67.1–67.6)	4.5	5.7	
		qzn7.1	Dt2645576-Sn2033434 (55.4–56.2)	4.5	5.7	
Provitamin A	Diverse panel of 403 accessions	*ZEP*	Sobic.006G097500-Sobic.004G281900	–	–	Cruet-Burgos et al. (2020)

details of which are summarized in Table 10.4. Once identified, these QTLs or genes can be used for pyramiding or introgression studies using marker-assisted breeding and for elucidation of their biochemical pathways.

In addition to the breeding approach, chemical mutagenesis (Taylor and Taylor 2011; Teferra et al. 2019) and transgenics (Zhao et al. 2003; Lipkie et al. 2013; Grootboom et al. 2014; Che et al. 2016; Elkonin et al. 2016) approach has been used to generate biofortified sorghum enriched with high protein, vitamin A, Fe, and Zn. The details of biofortified sorghum varieties developed to date are summarized in Table 10.5. In addition to this, Parbhani Shakti (ICSR 14001) biofortified sorghum variety has been developed in India using conventional breeding approaches whereby the sorghum line was enriched with high Fe and Zn content (Kumar et al. 2018).

10.5 Challenges, Limitations, and Success of Breeding Approaches for Sorghum Biofortification

Genetic variation is critical to any plant breeding program, as well as for sorghum biofortification. Conventional plant breeding can only be useful if an ample amount of genetic variability exists in the germplasm. In sorghum, significant genetic variability exists in nature for Fe, Zn, and phytate concentration, and the same has been extensively studied and improved by various workers (Reddy et al. 2005; Kumar et al. 2009, 2012); however for β-carotene low variability exists. Thus, only Fe and Zn can be enhanced using conventional plant breeding approaches. This is the major limitation of conventional breeding approaches.

Further, sorghum also contains some anti-nutritional factors, which makes it inferior to the other major cereals; these include lower digestibility and nutritional value of kafirins (a type of seed storage protein having a low content of lysine and threonine) and lower digestibility of starch. In addition to this, sorghum grain contains phosphorus in the form of phytic acid. The negative charge of this has a strong affinity to micronutrients especially Fe and Zn, thus making them inaccessible to humans and animals even in high concentrations. However, this can be overcome by using transgenics and the latest genome editing tools. So, with the availability of recent genomics tools, any trait can be bred in any crop, and the same has been successfully done in sorghum also.

Table 10.5 List of biofortified sorghum varieties developed by different institutions worldwide

Biofortified sorghum varieties	Method of variety development	Improved traits	Other note	Reference
Protein biofortified sorghum	Chemically induced mutation and genetic engineering	Grain protein quality	Achieved twice protein digestibility-corrected amino acid score than the null controls	Taylor and Taylor (2011)
Protein biofortified sorghum	Agrobacterium co-transformation	Protein quality; enriched lysine	Transformed with lysine-rich *HT12* gene; hemizygous seeds showed 40–60% increase in lysine	Zhao et al. (2003)
Provitamin A biofortified sorghum	Genetic modification	β-carotene	Genetically modified sorghum event Homo188-A shows largest bio-accessibility of β-carotene content, with a four- to eightfold increase from non-transgenic	Lipkie et al. (2013)
Protein biofortified sorghum	Genetic transformation	Protein digestibility	Co-suppression of three genes (γ kafirin-1, γ-kafirin-2, α-kafirin A1) significantly increases digestibility	Grootboom et al. 2014
Biofortified sorghum	Genetic engineering	β-carotene	Co-expression of *homogentisate geranylgeranyl transferase* (*HGGT*) extended half-life of β-carotene from less than 4 week. to 10 week. on average	Che et al. (2016)
Protein biofortified sorghum	*Agrobacterium*-mediated genetic transformation	High protein digestibility	RNAi silencing of the γ-kafirin gene resulted in enhanced digestibility index up to 85–88% compared with 59% in the control line	Elkonin et al. (2016)
Parbhani Shakti (ICSR 14001)	Conventional breeding	Fe, Zn, protein	Higher Fe (45 ppm) with Zn (32 ppm) and increased protein content (11.9%) and decrease in phytates (4.1 mg/100 g)	Kumar et al. (2018)
Protein biofortified sorghum	Mutation breeding	Protein digestibility	Mutant lines showed more protein digestibility (69.4% raw, 57.6% cooked) compared to wild-type lines (61.7% raw, 45.6% cooked)	Teferra et al. (2019)

10.6 Molecular Understanding of Essential Micronutrient Uptake and Deposition in Sorghum Grain

10.6.1 Iron (Fe)

10.6.1.1 Iron Uptake and Transport

Plants opt two types of strategies for Fe uptake from the soil. Strategy 1 (reduction-based) is common in dicotyledons and non-Poaceae monocotyledons. Under this strategy, the plant inaccessible Fe^{3+}, the predominant ionic form of Fe in the soil, is reduced at root surface to plant-accessible Fe^{2+} form (Zhang et al. 2019). Under iron deficiency, the uptake of chelated Fe^{3+} is facilitated by H + -ATPases (AHAs) localized in plasma membrane which aid in the reduction of rhizospheric pH and thereby increasing the solubility of Fe^{3+}. The FRO2 (ferric chelate reductase oxidase) gene catalyzes the reduction of Fe^{3+} to Fe^{2+} which is then imported into the root cells by high-affinity iron transporters, iron-regulated transporter (IRT1). Both FRO2 and IRT1 genes were first isolated and cloned from *Arabidopsis thaliana* (Eide et al. 1996; Robinson et al. 1999).

Strategy 2, also known as chelation-based, is mainly observed in graminaceous species. Plants in this category secrete phytosiderophores (PS), organic compounds belonging to the family of mugineic acids, acting as Fe^{3+} chelators (Rehman et al. 2021). The chelated Fe^{3+} is then transported to roots by yellow stripe-like (YSL) transporters (Curie et al. 2001). Sorghum is a strategy 2 plant, and recent reports suggest that arbuscular mycorrhizal fungi (AMF) can alleviate the iron deficiency symptoms via PS-mediated iron mobilization. Gene expression studies by quantitative real-time PCR revealed upregulation of *SbDMAS2* (deoxymugineic acid synthase 2), *SbNAS2* (nicotianamine synthase 2), and *SbYS1* (Fe-phytosiderophore transporter yellow stripe) in roots due to AMF in Fe-deficient sorghum (Prity et al. 2020). In another study, it was demonstrated that sorghum plants can recognize the volatile compounds released by bacteria and can induce Fe uptake mechanisms like *Arabidopsis* (Zhang et al. 2009; Hernández-Calderón et al. 2018). Of late, it has also been reported that rice plants use a combined strategy iron uptake comprising the components and strategies of both strategies 1 and 2 (Wairich et al. 2019). These iron-related genes are in turn regulated by various transcription factors, for example, basic helix-loop-helix (bHLH), FER-like iron deficiency-induced transcription factor (FIT) in *Arabidopsis*, has been found to regulate *FRO2* and *IRT1* genes for iron acquisition under iron deficiency condition (Bauer et al. 2007). Similarly, another bHLH transcription factor, POPEYE (PYE), regulates growth and development under iron deficiency (Long et al. 2010). After Fe acquisition, the ions get transported and translocated to different organs which are facilitated by two iron efflux transporters (IRON REGULATED1/Ferroportin 1 (IREG1/FPN1) and IREG2/FPN2) identified in *Arabidopsis* (Colangelo and Guerinot 2004). However, the molecular mechanism behind the long-distance iron is still under gray area. The iron ion is highly reactive and less soluble inside the plant environment, so in order to avoid precipitation and toxic effect, the ions are translocated inside the plant as complexes with citrate, mugineic acid, nicotinamine, and phenolic compounds.

Inside xylem, iron complexes with citrate at pH 5.5 and transmembrane protein ferric reductase defective 3 (*AtFRD3*) in *Arabidopsis* and the rice ortholog *OsFRDL1* (FRD-Like) help the transport of these complexes from root to shoot (Rehman et al. 2021). Iron translocation into actively growing plant sites such as shoot apex, root tips, and seeds and remobilization of iron from old parts to new ones occur via phloem. Inside phloem tissue, the iron complexes with the nicotinamine at pH 7.5 and the resulting complex transport in phloem with the help of yellow stripe-like (YSL) transporter family. This YSL transporter gene family is known to aid in unloading iron from xylem to phloem and loading it into developing seeds (Jeong and Guerinot 2009).

10.6.1.2 Fe Deposition in Grains

The distribution of iron in grains has been reported to be heterogeneous in nature. In rice, iron is mainly present in the aleurone layer, scutellum, and integument, whereas in peas the site of accumulation is mainly in the inner and outer epidermal layers of the embryo. Deposition of iron in the developing seed is mainly facilitated by *osYSL2* in rice and *YSL1* and *YSL3* in *Arabidopsis* (Rehman et al. 2021; Tong et al. 2020).

10.6.2 Zinc (Zn)

10.6.2.1 Zn Uptake and Transport

Under high pH conditions, zinc is tightly bound to the soil making it inaccessible for plant uptake. The Zn^{2+} uptake is facilitated by acidification and production of organic chelators like citrate and malate in the plant rhizosphere. The transporter family that contributes to this process belongs to the zinc import protein (ZIP) family (Tong et al. 2020). The zinc ion inside the plant root then makes complexes with nicotinamine and gets radially transported across different root layers which is facilitated by *metal tolerance protein 2 (MTP2)* in *Arabidopsis* (Sinclair et al. 2018). Zinc transport inside the xylem is facilitated by members of the *heavy metal ATPase (HMA)* family of P_{1B}-type ATPases, like *HMA2* and *HMA9* in rice. Once Zn^{2+} gets loaded into the xylem, it then moves to phloem tissues for long-distance Zn transport into the sink. In rice, *OsZIP3/OsHMA2*, *YSL* family transporters, and OsHMA9 are responsible for Zn xylem-to-phloem transport, phloem-to-organs transport, and remobilization, respectively (Tong et al. 2020).

10.6.2.2 Zn Deposition in Grains

Like iron, zinc is concentrated in small vacuoles in aleurone and sub-aleurone layers of the grain. Transcriptional microarray analysis of grain tissues in barley showed expression of heavy metal ATPases (HMAs), metal tolerance proteins (MTPs), and natural resistance-associated macrophage proteins (Nramps), hinting at their role in Zn deposition within the grain (Tauris et al. 2009). Particularly, MTPs, a member of the cation diffusion facilitator (CDF) transporter family, are shown to localize in vacuolar membrane and transport zinc ions to the vacuole (Podar et al. 2012).

Recently, *HvMTP1* has been characterized by overexpression studies in the endosperm of barley grains using endosperm-specific promoters (Menguer et al. 2018). The upregulation of this transporter led to increased zinc concentration in endosperm which opened a new strategy for zinc enrichment in the endosperm of cereal grains.

The molecular mechanism behind Fe and Zn uptake, transport, deposition, and homeostasis has been well characterized in many cereals but not in sorghum. Anuradha et al. (2013) have attempted in silico identification of candidate genes involved in Fe and Zn concentration in grains using reported cereal gene homologs. This study can aid in functional marker development and QTL mapping of grain Fe and Zn concentration in sorghum. Furthermore, the candidate genes can be functionally characterized using overexpression and gene silencing studies to understand their role in case of sorghum.

10.6.3 Provitamin A

The rate-limiting step in isoprenoid biosynthesis is first catalyzed by deoxyxylulose 5-phosphate synthase (DXS). Phytoene synthase (PSY) catalyzes the formation of phytoene from two molecules of geranylgeranyl pyrophosphate. Phytoene is then converted to lycopene by carotene desaturase (CRT-I). Lycopene cyclases ß-LCY and ε-LCY produce ß-carotene (ß,ß-carotene) and α-carotene (ß,ε-carotene). Carotene hydroxylases (CRT-RB) convert a- and ß-carotene to α- and ß-cryptoxanthin and then subsequently to non-provitamin A species like lutein and zeaxanthin. Carotenoids with an unsubstituted ß-ionone ring and all-trans configuration have the potential for conversion to retinol (provitamin A activity) (Lipkie et al. 2013).

10.7 Transgenic Efforts for the Development of Biofortified Sorghum

For the development of transgenics in sorghum, various studies have been carried out to detect the type and mode of transformation. Although several explants like immature zygotic embryos, mature embryos, immature inflorescence, and leaf fragments have been suggested, calli derived from immature zygotic embryos have been the explant of choice for the development of sorghum transgenics. Both biolistic and *Agrobacterium*-mediated gene transformation have been employed for the production of transgenic sorghum, but *Agrobacterium*-mediated transformation is preferred to direct transfer methods because of the added advantages in the former (Kennedy et al. 2003). Sorghum is considered as a staple food for sub-Saharan African (SSA) countries because of its drought and heat tolerance nature; however, sorghum lacks important amino acid like lysine, has poor protein digestibility on cooking, and also lacks micronutrients like provitamin A, zinc, and iron. The cases of blindness and anemia are increasing trends in SSA countries. To address these issues and achieve the goal of enriching multiple nutrients in single staple food, the Grand Challenges in Global Health initiative was launched in 2003 funded by the Bill & Melinda Gates Foundation, in association with the National Institutes of Health (NIH). This led to the initiation of the project entitled "nutritionally enhanced sorghum for arid and semi-arid tropical areas of Africa" (Henley et al. 2010; Zhao et al. 2019). The main objectives were (1) to increase iron and zinc bioavailability by 50%, (2) to increase provitamin A levels to up to 20 mg/kg, (3) to increase lysine content by 80–100%, and (4) to improve protein digestibility by 60–80% (Grand Challenges in Global Health 2021). Various efforts were undertaken to achieve these goals, for example, lysine-enriched genetically modified sorghum was reported by Zhao et al. (2003), where they have overexpressed lysine-rich proteins, such as HT12, an analog of barley *hordothionin*, and suppressed a lysine catabolism enzyme, *lysine ketoreductase*, using super binary vectors which enhanced the lysine content by 40–60%. Similarly, for improving protein digestibility of sorghum grain, the seed storage protein, the protease-resistant kafirin, has been targeted. The protein digestibility has been increased by RNAi silencing of the *ϒ-kafirin* (Elkonin et al. 2016) and simultaneous suppression of three genes: *ϒ-kafirin-1*, *ϒ -Kafirin-2*, and *α-Kafiirin -A1* (Grootboom et al. 2014). Furthermore, efforts were taken to increase provitamin A in sorghum for which scientists overexpressed the genes involved in the β-carotene synthesis pathway in the sorghum line (Tx430). The gene constructs encoding the enzymes like 1-deoxyxylulose 5-phosphate synthase (DXS), *Zea mays* phytoene synthase 1 (PSY1), and the *Pantoea ananatis* carotene desaturase (CRTI) were introduced into the sorghum line which resulted in increased ß-carotene level in transgenic plants (up to 9.1 μg/g vs. 0.5 μg/g in non-transgenic control seeds) (Lipkie et al. 2013; Elkonin et al. 2018). However, it was found that the ß-carotene undergoes degradation due to oxidation under storage. To address this challenge, researchers introduced the barley HGGT gene encoding *homogentisate geranylgeranyl transferase*, associated with the synthesis of the vitamin E (antioxidant in nature), along the same gene construct used for ß-carotene enrichment. The co-expression of HGGT and carotenoid biosynthesis genes increased all-trans ß-carotene accumulation (7.3–12.3 μg/g) and alleviate ß-carotene oxidative degradation, resulting in stable provitamin A in transgenic sorghum seeds (Che et al. 2016). Attempts were also made to increase the bioavailability of important micronutrients like Fe and Zn, for which the phytase enzyme was introduced into the sorghum line (Tx430) in order to degrade the phytic acid that acts as a chelating agent for divalent ions. Multidrug resistance-associated protein ATP-binding cassette transporter encoding gene was silenced resulting in lower phytate content (80–86%) compared to their non-transgenic control plants and increased zinc and iron bioavailability (Kruger et al. 2013). In Africa, Biofortified Sorghum lines with enhanced provitamin A, increased Fe and Zn bioavailability, and improved protein digestibility-corrected amino acid score (PDCAAS) have undergone over seven field trials. Additionally, efforts were done to address the key aspects involved in commercialization (Obukosia 2014) (Fig. 10.1).

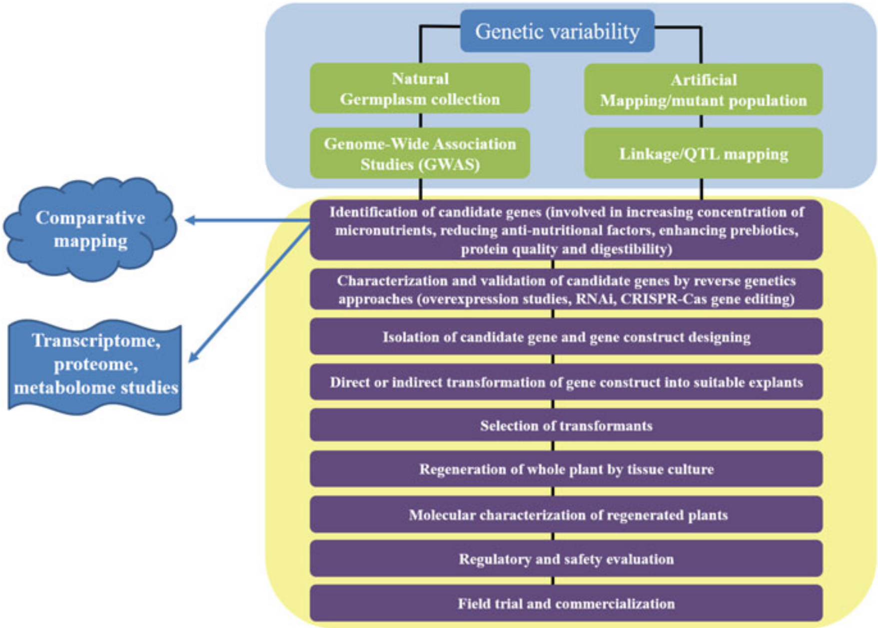

Fig. 10.1 Strategy used for the development of transgenic sorghum

10.8 Challenges for the Public Release of Transgenic Sorghum

The commercialization of transgenics has always been a controversial topic in many countries to date. Some believe that GM has the potential to solve various global challenges, while others pursue GM crops as a risk to the environment and humankind. The ABS sorghum lines are mainly targeted to release in Kenya (Eastern Africa) and Nigeria and Burkina Faso (Western Africa) because of the worst effects of micronutrient deficiencies. A study in Burkina Faso revealed that farmers are quite open to the addition of micronutrients to sorghum since sorghum is a subsistence crop in that region and, at the same time, they want to get rid of the severe micronutrient deficiencies prevailing in that area (Cardona et al. 2018; Chinedu et al. 2018). A market survey showed that farmers are ready to pay more for biofortified sorghum provided it performs better than the local varieties. Moreover, the study pointed out that the farmers who have experienced the benefits of first-generation GM crop (Bt cotton) are more likely to adopt second-generation GM crop, biofortified sorghum (Cardona et al. 2018). A study regarding the adoption of iron-fortified sorghum in Nigeria cited that environmentalists are strongly against the release because of the possible harmful effect on human health and the environment. Secondly, Nigerian consumers are very particular about their food choice like taste, aroma, and color. The vitamin A-enriched sorghum transgenics may change the color of the plant's parts making it less preferable by consumers. Moreover, seeds need to be bought every year and cannot be reused again and again breaking the seed saving culture and tradition of Nigerian farmers. Thirdly, the lack of funding can lead to the withdrawal of the transgenic sorghum project for which the government should support the scientist in this regard to continue their research program. Finally, the biggest challenge for the transgenic biofortification sorghum project in Nigeria is the lack of knowledge and negative perception regarding biotechnology by the stakeholders (Obi et al. 2017).

For successful and early adoption of ABS by SSA farmers, active involvement of farmers is recommended throughout the process of product development. Further, the government should create awareness about the nutritional benefits of the new product, subsidize the product, and participate in seed distribution of the transgenics. The scientists of ABS project are quite confident about the safety of introduced ABS genes hoping to see the light of the day.

10.9 Economical and Social Constraints for the Biofortified Sorghum

According to the UN Sustainable Development Goal 2 (SDG2), by 2030 all forms of hunger end, which is quite a daunting task. Malnutrition or hidden hunger due to micronutrient deficiencies has affected about one-third of the world population and severely endangered economic development. One of the workable strategies to diminish micronutrient malnutrition (MNM) is the biofortification of sorghum and increasing the intake of sorghum. Biofortification is the most promising option to improve the nutrition security of the poor. Biofortified foods can increase the levels of vitamins and minerals in our daily needs, and the nutrition status of vulnerable groups can be raised both at a national and worldwide scale, thus improving human nutrition (Lividini and Fiedler 2015; Meenakshi et al. 2010; Trijatmiko et al. 2016; Zhao and Shewry 2011; Saltzman et al. 2013; Waters and Sankaran 2011).

Biofortification is cost-effective as it ensures a nourishing future for all humans including the rural population (Grootboom et al. 2014; Saltzman et al. 2013; Zhao and Shewry 2011). With a one-time investment in biotechnology, farmers can sustain it for many years (Saltzman et al. 2013; Meenakshi et al. 2010). Finally, transgenic biofortification is a viable method of reaching micronutrient-deficient populations in the rural area who often have limited access to diverse, fortified, or supplemented meals. The success of using biofortified sorghum varieties in lowering down the problem of micronutrient malnutrition (MNM) has attracted the attention of communities, but most importantly it depends upon the attention from poor rural population. This can only be possible if the discouraging factors are eliminated. The government and development agencies are following many possible ways to mitigate MNM by increasing dietary diversification, supplementation of minerals, fortification, and enhancing the concentration and bioavailability of plant-based foods (White and Broadley 2009). It is a really very hazardous task to change people's diet. However genetic modification of food (biofortification), in general, has attracted high-rated controversies among scientists and policymakers.

There are four key issues that are expected to play a role in farmers' perception and attitude toward the biofortified sorghum; these include (1) source of seed, (2) market consideration, (3) experience with Bt cotton, and (4) external influence. Chinedu et al. (2018) found that many farmers still practice seed saving, but about 60% indicated that the source of their seed did not matter to them. To be able to strive in the presence of these challenges, the new biofortified sorghum seeds need to possess desirable agronomic attributes that could make it competitive with the local cultivars to encourage farmer's adoption; the biofortified alternative should possess a complete package of attributes including the addition of extra nutrient; it should be early maturing, low-cost, and high-yielding; and it should be provided through government institution to be more accessible to farmers.

Henceforth, there is a need for an extra effort by the government and NGOs to reach the rural people. By providing biofortified seeds, many benefits can be achieved. First, by growing the transgenic seed, it becomes a strategic means of developing micronutrient-dense trait on the major staple food (sorghum) which is widely consumed, from the rural area (Meenakshi et al. 2010). This is unlike food supplementation and fortification which often start from the urban areas. Secondly, during the laboratory production of biofortified crops, higher-yielding and disease resistance attributes can equally be added to biofortified crops, thereby improving their production. Finally, the production surplus generated from the biofortified crops can be marketed in the urban areas, providing more income for the farmers (Miller and Welch 2013; Saltzman et al. 2013).

10.10 Genome Editing Approaches for Biofortification of Sorghum

Although sorghum is recalcitrant to gene transformation as compared to other species (Raghuwanshi and Birch 2010), some genotypes have been successfully transformed with both particle bombardment (Casas et al. 1993) and *Agrobacterium* (Zhao et al. 2000) methods. CRISPR/Cas9 system-mediated targeted gene modification was reported for the first time in sorghum in 2013 (Jiang et al. 2013). Subsequently, various protocols were reported for *Agrobacterium* (Sander 2019; Char et al. 2020) and particle bombardment-mediated CRISPR/Cas-based gene editing (Liu et al. 2019). The practical application of CRISPR-based gene editing in sorghum was reported for improving protein quality and digestibility. Li et al. (2018) have successfully demonstrated editing of an alpha-kafirin gene family which increased the protein quality and digestibility in sorghum. Recently, Meng et al. (2020) have demonstrated an efficient protoplast assay in sorghum that can be used for transient gene expression and editing studies by CRISPR/Cas system. CRISPR-based gene editing holds a huge potential to expedite the goal of multi-nutrition enrichment in staple food like sorghum, but the product commercialization is highly dependent on the public perception and definition of natural products (Fig. 10.2).

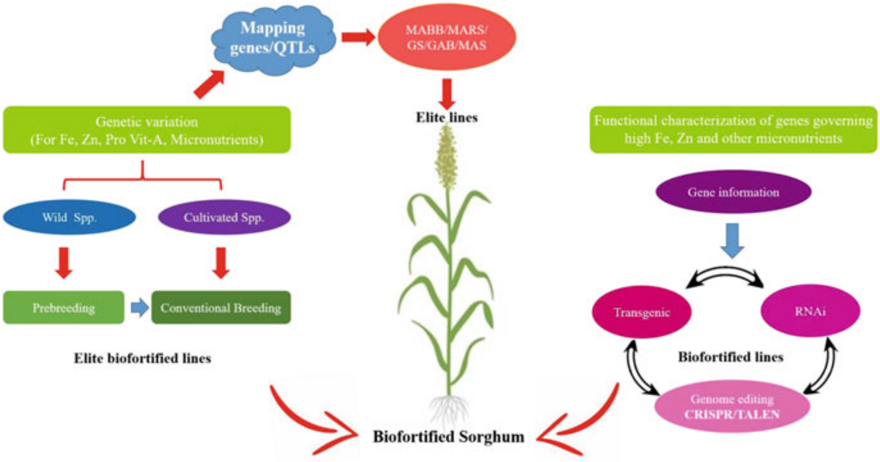

Fig. 10.2 Different approaches for improvement of nutraceutical properties in sorghum

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