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Application of Plant Breeding and Genomics for Improved Sorghum and Pearl Millet Grain Nutritional Quality

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1. INTRODUCTION

Micronutrient malnutrition along with overweight and obesity is among the greatest global challenges of current times, leading to double burden of malnutrition on nations and more so on developing countries. The irony is that in a single household, both micronutrient malnutrition and obesity can coexist. To address dietary-induced micronutrient malnutrition, efforts are underway for dietary diversification, food fortification, and supplementation but with only partial success. It is also important to develop low-cost sustainable solutions to address this problem globally, and biofortification of major staples offers an opportunity for this. Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth largest cereal crop grown globally and is the staple for more than 500 million people over 30 countries in Africa and Asia. In India, for example, per capita, sorghum consumption goes as high as 75 kg/year in major sorghum growing areas, though the per capita consumption at national level is decreasing. It is one of the "nutri-cereals" among the cheapest sources of energy, protein, iron, and zinc, contributing more than 50% of the iron and zinc requirement in low-income group populations in India (Parthasarathy Rao et al., 2006; Ashok Kumar et al., 2011, 2013).

Sorghum as a crop in addition to its wide adaptability and resilience to drought and heat shows large variability for grain iron and zinc concentration. Therefore development of cultivars with improved micronutrient quality can contribute greatly towards achieving nutritional goals. This is made possible by understanding the extent of variability, inheritance pattern, trait associationships, and exploring opportunities for application of genomics for improving nutritional quality. In addition, the natural variation for protein, fat, and starch content can also be exploited to improve macronutrient quality through crop improvement programs. Sorghum with its small genome size (730 Mb) and the availability of its complete genome sequence (Paterson et al., 2009) makes it an amenable crop for the application of genomics-based breeding methods. In recent years the rapid reduction in costs of genotyping for genome-wide association studies for identification of single-nucleotide polymorphisms (SNPs), using high-density SNP array technologies (Batley and Edwards, 2007) or genotyping-by-sequencing (GBS) (Chia and Ware, 2011; Davey et al., 2011; Elshire et al., 2011; Morris et al., 2013) or next-generation sequencing (NGS) platforms (Metzker, 2010), has opened the way for genomic selection (GS) (Jannink et al., 2010) or predictive breeding strategies (Riedelsheimer et al., 2012). Such techniques have the potential to considerably accelerate selection gain and improve the effectiveness of breeding with more and more advances in recent years (Grusak and Della Penna, 1999; Bekele et al., 2013). Studies on genetic control of quality traits and mutations have contributed greatly to the increased understanding of nutritional quality traits in sorghum. Mutation in sorghum genes such as *waxy*, producing starch that is very high in amylopectin with little to no amylose, increased protein, and improved starch digestibility

(Karper, 1933; Lichtenwalner et al., 1978; Rooney and Pflugfelder, 1986), *sugary*, which has increased sucrose content (Martin, 1936; Boyer and Liu, 1983), and *high lysine*, which has increased lysine content and protein digestibility (Singh and Axtell, 1973), were some of the findings that have contributed to our knowledge of the genetic controls of grain composition. Furthermore, Quantitative Trait Loci (QTLs) for polyphenol (Rhodes et al., 2014) and mineral traits (Shakoor et al., 2016) have been identified. Linkage and association studies have identified several loci controlling sorghum grain composition (Sukumaran et al., 2012; De Alancar et al., 2010; Rami et al., 1998), and the genes underlying the *waxy* mutation has been fine mapped to 1.8 Mb on chromosome 10 (McIntyre et al., 2008), but more work needs to be done to precisely identify genes responsible for natural variation of grain composition. Surveying the natural variation of grain composition in the sorghum germplasm and finding loci underlying the variation can aid efforts to improve the nutritional value of sorghum. New sources of genetic variation can be used for crop improvement, especially in developing countries where technologies for improving the nutritional value of grain, such as commercial fortification, are not accessible or affordable (Seleka et al., 2011; Nestel et al., 2006; Horton, 2006).

Pearl millet is a nutritious cereal, capable of growing in harsh and marginal environments where no other cereals may give economic returns. More than 70% of its production is used as food, and remainder for feed purposes through informal markets. Nutritionally, it is a richer and a less expensive source of protein, dietary protein, fat, calcium, phosphorus, essential amino acids, iron, and zinc in comparison to other cereal crops. In India, as an example, the contribution of pearl millet to the total nutrient intake (especially iron and zinc) from all foods varies widely. However, in some parts of rural India (Rajasthan, Maharashtra, and Gujarat), the contribution of pearl millet to micronutrient (iron and iron) intake is very high up to 50% (Parthasarathy Rao et al., 2006). Also, the energy value of pearl millet grain is relatively higher compared to the major cereal grains like rice, wheat, or maize. However, in rural areas of India, for example, its per capita consumption has declined from 11.5 kg (during 1972) to 3.06 kg (by 73.4%) in 2010, and from 4 kg (during 1972) to 1.13 kg (by 71.8%) in 2010 in urban areas in India (NRAA, 2012). Nevertheless, pearl millet is fast in gaining popularity as a "nutri-cereal" in Western and Asian countries.

The carbohydrates of the millets comprise largely starch (65%—70%) and a good amount of nonstarchy polysaccharides (NSP; 16%—20%). The NSP, which constitute nearly 95% of the dietary fiber content of the grain, are derived not only from bran portion but also from the endosperm cell walls (Jain et al., 2016). Pearl millet grain generally possesses a high iron concentration and a fairly high zinc concentration. However, not all commercial cultivars have high iron and zinc concentrations, as the primary focus of breeding is always high yield. For instance, the average of Indian commercial cultivars so far released contains 42 ppm Fe and 32 ppm Zn, as expressed as a concentration in the grain (Rai et al., 2013). Notwithstanding this, pearl millet has large variability for iron and zinc concentration, that is, three to four times higher than that of rice and wheat. Furthermore, pearl millet is known to impart several health benefits especially for the management of noncommunicable diseases (NCDs). Consumption of pearl millet is known to help prevent diabetes because of its low glycemic index, high fiber content, and high proportion of slowly digestible starch (SDS) and resistant starch (RS) (Kam et al., 2016).

Keeping in mind all the previously mentioned facts about sorghum and pearl millet, it becomes necessary to explore more about the availability of genetic and genomic resources which will accelerate the crop improvement programs for enhanced nutritional quality. Hence, in this chapter, we briefly review the variability for nutritional quality and genetic control, progress in breeding for quality improvement, progress in genomics and biotechnology for nutritional quality, and recent advances in use of phenomics and genomics tools for quality nutritional improvement and propose an integrated breeding scheme for nutritional quality improvement in sorghum and pearl millet.

2. IMPORTANCE OF SORGHUM AND PEARL MILLET IN NUTRITION

As stated, the double burden of malnutrition is one of the major challenges the world is facing today, which is resulting in increased risk of many chronic diseases and reduced productivity. Stunted growth due to malnutrition and chronic diseases such as type 2 diabetes, cardiovascular disease, and cancer due to overnutrition are becoming more predominant worldwide. Dietary deficiency is among the major reasons for micronutrient malnutrition. Resilient, nutritious staple cereal crops such as sorghum and pearl millet are adapted to a wide range of environments and provide balanced nutrients to the population, significantly improving human health. They are rich in essential vitamins, and minerals like magnesium, potassium, iron and zinc. Both sorghum and millet foods can have low glycemic index and are gluten-free, providing a safe and nutritious diet. The SDS in sorghum is a big boon for diabetics. Also, sorghum and millets are high in polyphenols. Polyphenols are important phytochemicals with strong antioxidant potential, and because of this, they can provide protective action against several NCDs (Aggarwal and Sharma, 2016). In developing regions of the world, a reason behind the re-emergence of sorghum and millets in markets is

because of their good agronomic performance on marginal lands with low fertility without compensating their nutritional value. Today, sorghum is also used as supplement in bakery and confectionary, sports nutrition, infant formula, breakfast cereals, and others.

2.1 Sorghum

Sorghum's grain macronutrient composition is similar to maize and wheat, providing, on average, 70% carbohydrate, 12% protein, and 3% fat. As in other cereals, the sorghum grain is predominantly starch. The endosperm contains essentially all the starch and the majority of the protein, while the germ contains the majority of the fat. Grain sorghum has higher percentage of fat compared with other starch staples such as rice, wheat, and cassava. The complex carbohydrates (starch and dietary fiber) present in sorghum are generally slowly digested and provide fullness and delay the sensation of hunger. Sorghum is an excellent source of nutrition for all age groups. Iron and zinc are two major components required for normal growth of any individual, but these two micronutrients are limiting in developing countries. Sorghum is also a good source of Vitamin B complex vitamins. This vitamin complex plays a crucial role in energy metabolism. Sorghum is particularly rich in thiamin, riboflavin, niacin, pantothenate and vitamin B6. For children aged 1—9 years, sorghum can provide 47%—26% of the World Health Organization recommendations for thiamin, 28%—16% for riboflavin, 49%—24% for niacin, 63%—31% for pantothenate, and 118%—59% for vitamin B6 (Lindsay, 2010). In fact, sorghum's high energy content and ready supply of B-complex vitamins make sorghum an excellent food crop for resource-poor families.

Sorghum also contains a reasonable level of protein, but the amount of protein received by the body depends on the digestibility of the protein. On wet cooking, sorghum protein digestibilities range from 36.4% to 74% (Henley et al., 2010). Arguing that a pepsin digestion model is the preferred in vitro method for assessing sorghum protein digestibility, Mertz et al. (1984) reported digestibility values up to 79% for decorticated/extruded sorghum. Efforts are being made to improve the bioavailability of lysine which is the limiting amino acid in most cereal grains (Jood et al., 1995). Sorghum is also a uniquely good source of phenolic compounds such as flavonoids, 3-deoxyanthocyanins, and condensed tannins that have health protective effects, in comparison to other grains, and fruits, and vegetables (Burdette, 2010; Awika and Rooney, 2004). Tannins are present in sorghum varieties with a pigmented testa layer below the outer pericarp layer of the grains. Red sorghum has high levels of antioxidants with high anti-inflammatory and anticancer activities (Vanamala et al., 2017). Since sorghum is rich in calories, protein, and minerals, it is in the food baskets of millions of people in Africa and Asia (Lindsay, 2010). Both in Africa and Asia, it is grown primarily as food crop, and many food products such as thin and thick porridges, fermented and unfermented breads, lactic acid bacteria fermented and alcoholic beers and nonalcoholic beverages, and malted flours for brewing and products such as malted porridge mixes can be prepared from it. In India, proposals have been made for the use of dehulled (decorticated) sorghum within feeding regimes for infants and children (Pushpama, 1987).

2.2 Pearl Millet

As with most cereals, the first limiting amino acid of pearl millet protein is lysine. A significant relation of lysine content of a protein is its inverse correlation with level of protein in the grain (Deosthale et al., 1971). However, the essential amino acid profile of pearl millet protein shows more lysine, threonine, methionine, and cysteine than in the proteins of sorghum and other millets. Its tryptophan content is also higher. In fact, it has been reported that pearl millet grain contains a 27%—32% higher concentration of indispensible (essential) amino acids than maize, sorghum, and wheat (Ejeta et al., 1987; Davis et al., 2003). Furthermore, it has a less disparate leucine/isoleucine ratio (Hoseney et al., 1987; Rooney and McDonough, 1987) than cereals such as wheat, barley, and rice (Ejeta et al., 1987). The majority of pearl millet lipids are triglycerides (Gupta, 1980). Although the fat content of pearl millet has been reported as factor in flour rancidity, it has been found that the fat content of different genotypes showed variable expression against rancidity, indicating that fat content alone is not responsible for rancidity (Arya et al., 2013). Kim et al. (2003) reported that resistant starch significantly lowers plasma total lipid and cholesterol concentrations in diabetic rats. Hence, the pearl millet's resistant starch may contribute to cholesterol metabolism and optimizing the levels of cholesterol in the human body.

Pearl millet is a good source of niacin, pyridoxine, and B-group of vitamins. The yellowish seed coat pigments generally contribute to the β-carotene content of pearl millet (Jiji et al., 2017). The high polyphenol content of millets has already been mentioned. The other prominent phytochemical in pearl millet is phytate (myoinositol hexaphosphate), which in addition to its effect as an inhibitor of mineral absorption, also can exert some health benefits due to its metal chelating properties, especially "Fe" chelation and thereby hindering free radical formation (Jain et al.,

2016). Minnis-Ndimba et al. (2015) studied the spatial distribution of micronutrients in pearl millet grains and showed that both iron and zinc are predominantly concentrated in the germ, which consists of the scutellum and embryo and in the seed coat, plus the pericarp and aleurone. Since pearl millet can be consumed in the whole grain form, this distribution of these minerals would have no adverse implications on processing.

3. VARIABILITY FOR NUTRITIONAL QUALITY AND GENETIC CONTROL

3.1 Variability

Sorghum shows extensive variation for several grain quality traits, including the three primary macronutrients (carbohydrate, protein, and fat) across diverse germplasm, and this could be due to adaptation to different environmental factors (Shewayrga, 2012; Sukumaran et al., 2012). The grain Fe concentration in elite parents generally ranges from 20 to 40 ppm, while grain Zn concentration ranges from 20 to 30 ppm (Table 3.1) (Kumar et al., 2013). However, in germplasm lines and diverse breeding lines, higher concentrations have been reported (Table 3.2). Kernel composition studies on global sorghum germplasm showed that the protein ranged from 8.1% to 18.8%, fat content ranged from 1.0% to 4.3%, and starch content ranged from 61.7% to 71.1% (Rhodes et al., 2017). The *durra* and *bicolor-durra* sorghum races had the highest protein (12.6%) and fat (3%) contents and the lowest starch contents (66.1%), while *kafir* sorghum had the lowest protein content (10.9%) and the highest starch content (67.6%). As stated, in general, the nutritive value of protein is determined by the level of lysine, as it is the most limiting indispensable amino acid in cereal proteins. A similar pattern was observed in sorghum where its content of lysine is deficient, but it has high levels of leucine when compared with other cereals (Shewry, 2007; Deosthale et al., 1970). This imbalance in amino acid levels in sorghum may possibly be involved in the etiology of the disease pellagra, which is caused by a deficiency of niacin (vitamin B3) in the diet (Bender, 1983). Thus, identification and selection of sorghum lines with

TABLE 3.1 Variability in Selected Sorghum Parental Lines for Grain Iron and Zinc Concentration

B-Line	Fe (ppm)	B-Line	Zn (ppm)
ICSB 50	50	ICSB 289	29
ICSB 10	48	ICSB 390	29
ICSB 263	47	ICSB 356	28
ICSB 399	47	ICSB 426	28
ICSB 17	45	ICSB 359	27
ICSB 354	45	ICSB 399	27
ICSB 50	45	ICSB 362	27
ICSB 262	43	ICSB 349	27
ICSB 11	43	ICSB 395	27
R-Line	**Fe (ppm)**	**R-Line**	**Zn (ppm)**
ICSR 89035	39	ICSR 89035	26
ICSR 113	38	ICSR 113	28
ICSR 89039	36	ICSR 81	24
ICSR 59	35	ICSR 59	23
ICSR 2	35	ICSR 91001	23
ICSR 91001	35	ICSR 89039	23
ICSR 89040	35	ICSR 94	23
ICSR 142	34	ICSR 100	23
ICSR 90018	34	ICSR 91003	23
ICSR 75	34	ICSR 133	23

TABLE 3.2   Mean and Range for Grain Iron and Zinc Concentration Reported in Sorghum

Entry	Mean	Range	References
FE CONCENTRATION (PPM)			
84	28.00	20.1–37.0	Reddy et al. (2005)
76	59.00	30.0–113.0	Kayode et al. (2006)
20	38.8	29.8–44.2	Ashok Kumar et al. (2010)
1394	42.2	7.7–132.6	Reddy et al. (2010)
14	44.0	28.0–63.00	Nguni et al. (2012)
74	–	21.5–55.5	Ashok Kumar et al. (2013)
222	–	6.3–168.1	Hariprasanna et al. (2014)
ZN CONCENTRATION (PPM)			
84	19.00	13.4–31.0	Reddy et al. (2005)
76	24.00	11.0–44.0	Kayode et al. (2006)
20	27.2	22.2–32.9	Ashok Kumar et al. (2010)
1394	33.5	15.1–91.3	Reddy et al. (2010)
14	33.0	23.0–55.0	Nguni et al. (2012)
74	–	21.5–55.5	Ashok Kumar et al. (2013)
222	–	3.6–87.0	Hariprasanna et al. (2014)

desired amounts of lysine and leucine is a logical approach to improve grain protein quality and help prevent pellagra (Deosthale et al., 1970).

In pearl millet, grain starch content in germplasm generally varies from 62.8% to 70.5%, soluble sugars from 1.2% to 2.6%, and starch amylose content from 21.9% to 28.8% (Jambunathan and Subramanian, 1988). A slightly lower range has been reported for starch (56.3%–63.7%) and starch amylose (18.3%–24.6%) contents among high-yielding Indian pearl millet varieties (Singh and Popli, 1973). In general, crude fiber content is around 2.3% (Ali et al., 2003) and dietary fiber about 7% (Gopalan et al., 1976) to 8.5% (Taylor, 2004). However, an exceptionally high dietary fiber content of 20.4% has also reported in pearl millet, with high levels of 14.2% in sorghum, 17.2% in wheat, and 8.3% in rice (Kamath and Belavady, 1980). The proportion of sucrose in total sugar is lower in pearl millet than in sorghum. Like sorghum, pearl millet generally contains 9%–13% protein, but large variations in protein content, from 6% to 21%, have been observed (Serna-Saldivar et al., 1991). Pearl millet has a relatively high concentration of both iron and zinc, and there is demonstrated potential to increase these levels further by plant breeding. Several reports indicate the existence of large variability for grain iron and zinc concentrations in pearl millet. For example, a study by Pucher et al. (2014) of 72 pearl millet accessions from West and Central Africa assessed in Niger showed moderate ranges in mineral density, 24.2–48.7 ppm for iron, and 19.8–43.4 ppm for zinc. A study focusing on the grain mineral density of 225 Sudanese pearl millet accessions evaluated in Sudan also found wide variation for iron and zinc, ranging from 19.7 to 86.4 ppm for iron and 13.5–82.4 ppm for zinc (Bashir et al., 2014). Rai et al. (2013) found almost twofold variation for iron density (31–61 ppm) and zinc density (32–54 ppm) among 122 commercial and pipeline hybrids of pearl millet developed in India. Other studies on grain iron and zinc densities in pearl millet materials have also reported significant variability (Velu et al., 2007, 2008; Gupta et al., 2009; Govindaraj et al., 2013; Kumar et al., 2016; Anuradha et al., 2017). Understanding natural variation of sorghum and millet grain composition (protein, fat, starch, iron, and zinc) through the analytical and genomic approaches such as X-ray fluorescence (XRF) spectrometry, near-infrared spectroscopy (NIRS), and identifying QTLs can help to improve its nutritional quality through crop improvement programs and marker-assisted selection (MAS).

3.2 Genetics

Biofortification is the process to improve micronutrient concentration such as iron and zinc in plant food staples such as cereal grains like sorghum and pearl millet by genetic means to combat micronutrient malnutrition

(Ashok Kumar et al., 2015). Several biofortification projects have emerged with the aim of reducing mineral deficiency, and the objective of these projects is to increase micronutrient densities, mainly through agronomic intervention and genetic selection (White and Broadley, 2005).

As mentioned, there is large genetic variation within sorghum that is suitable for sustainable biofortification (Ashok Kumar et al., 2013). However, to ensure success in research and development, a multidisciplinary approach is necessary for screening and selection of breeding lines with higher concentrations of essential nutrients (Paiva et al., 2017; Vasconcelos et al., 2017). The grain iron and zinc concentrations in sorghum are under polygenic control and show continuous variation. While grain zinc concentration is controlled by additive genes, both additive and nonadditive genes play a major role in controlling grain iron concentration (Ashok Kumar et al., 2013). In sorghum, iron and zinc concentrations are positively correlated ($r = 0.79$; $P < 0.01$), and there is no negative correlation between grain iron and zinc concentration and the grain yield (Ashok Kumar et al., 2013; Phuke et al., 2017; Reddy et al., 2005). Therefore it is feasible to develop micronutrient-dense sorghum cultivars with high yield adapted to various growing conditions. Badigannavar et al. (2016) carried out a survey of mineral concentrations of local sorghum landraces grown in southern and central Indian provinces where they showed that the highest genotypic coefficient of variation and phenotypic coefficient of variation was for calcium content and lowest for magnesium. High heritability and genetic advance for grain yield were found among the sorghum genotypes. The 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 a positive correction of iron content with manganese and calcium. High heritability for grain yield was observed among the sorghum genotypes. Weather condition, soil translocation rate, mineral contents, and genotypes are few factors that could be responsible for the variation among the micronutrients content in sorghum.

In pearl millet, both iron and zinc traits are predominantly under additive genetic control. In fact, highly significant and high positive correlations between performance per se of the hybrids for iron and zinc and their midparental values have provided further support for the concept that these micronutrients are largely under additive genetic control (Velu et al., 2011; Govindaraj et al., 2013). The predominance of additive gene action implies recurrent selection for intrapopulation improvement and open-pollinated variety (OPV) development. Whereas, population genetic analysis (NCD-I) over environments showed that the additive variance was five times higher than that of the dominance for iron and zinc density (Kanatti et al., 2016). The variances due to interaction of additive gene effects with the environment were much less than those arising from dominance × environment interaction for both micronutrients. These greater additive genetic variances along with proportionately less magnitude of genotype - × environment interaction variance translated into higher narrow-sense heritability (always >60%) for iron and zinc density. The association between iron and zinc was highly positively and always significant (often $r = 0.70$), while these two micronutrients were not always associated with grain size in pearl millet (Govindaraj et al., 2013; Kanatti et al., 2016). Therefore, just like sorghum, genetic enhancement of these two micronutrients is highly feasible without compromising the grain size.

4. PROGRESS IN BREEDING FOR NUTRITIONAL QUALITY IMPROVEMENT

Selection, hybridization, mutation breeding, heterosis breeding, and population improvement are some of the breeding methods used in sorghum for developing improved cultivars with high yield and better grain quality. In breeding for micronutrient concentration, establishment of baselines is the first step. For example, when the entire spectrum of sorghum cultivars grown in India was assessed, two important micronutrients, iron and zinc, were found to be low (iron 30 ppm and zinc 20 ppm), which were taken as baselines for improving sorghum grain iron and zinc concentrations. New donors for grain iron and zinc were identified and crossed with elite parents for developing lines with high yield and higher iron and zinc. One of the improved varieties developed, ICSR (ICRISAT Sorghum Restorer Line (R-Line)) 14001, showed its yield superiority in multi-location on-farm testing in Maharashtra State of India and is being released as PVK 1009. Furthermore, this line serves an excellent male parent in hybrid development, and more than 300 hybrids have been developed using it and are being evaluated. Two promising hybrids (ICSH, ICRISAT Sorghum Hybrid 14001 and 14002) are under large-scale on-farm testing in Maharashtra state of India (ICRISAT, 2016; HarvestPlus, 2017). The hybrid, ICSH 14002, was tested in the All India Co-ordinated Sorghum Improvement Project in the 2016 rainy season and showed its superiority for yield and micronutrient density. It is in pipeline for commercial release in Maharashtra State. Grain micronutrient density is mainstreamed in the breeding program. For this, we have identified highly promising yellow pericarp sorghum lines, milo and nonmilo cytoplasm-based improved sorghum hybrids, colored sorghum lines, and white grain

sorghum lines adapted to rainy and postrainy season, possessing higher grain zinc concentration along with higher grain yields (Ashok Kumar et al., 2015). The grain zinc concentration in these lines is much higher than the targeted increment (baseline plus 12 ppm) to be called "first wave" of biofortified products in sorghum (Zn sorghum) compared with the best control PVK 801 (OPV) or CSH 16 (hybrid) and to the baselines for zinc in sorghum (20 ppm). With all these materials in hand, now there is a good number of high yielding sorghum genotypes meeting the targeted increment for zinc in different adaptations (rainy and postrainy season), different cultivar types (OPVs and hybrids), different cytoplasms (milo and nonmilo), in different colored grains (red/brown, yellow, and white grains), and in different basic and intermediate races (*caudatums, durras, durra-caudatums*).

Extensive work has been done to improve protein content, quality, and digestibility in sorghum. As mentioned, lysine is the first limiting amino acid in sorghum. After screening more than 9000 accessions in the world germplasm collection, it was reported that two sorghum lines of Ethiopian origin, IS 11167 and IS 11758, had exceptionally high lysine at relatively high levels of protein (Singh and Axtell, 1973). Both lines were also high in oil (Jambunathan and Subramanian, 1988). The protein efficiency ratio (PER) values obtained for IS 11167 and IS 11758 were 1.78 and 2.06, respectively, compared with the PER of 0.86 for normal sorghum (Fano, 2017). Inheritance studies 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 (Fano, 2017). As the kernels of the high-lysine sorghums are floury in nature, partially dented, and have low seed weight, resulting in low yield potential, attempts were made to transfer the shrunken high-lysine grain of the Ethiopian cultivars to photoperiod-insensitive genotypes with plump, well-filled grains and number of breeding lines developed. A chemically induced high-lysine mutant, P721, developed at Purdue University, has also used in the sorghum breeding programs and improved lines developed.

In pearl millet the first biofortified variety with high iron concentration "Dhanshakti" was released in 2014 and reached 60,000 ha in India (Govindaraj and Rai, 2016). As hybrids are cultivar options in India, many high-Fe test hybrids have been developed with good yield potential. Two of these (ICMH, ICRISAT Millet Hybrid 1202 and ICMH 1301) have been most widely tested and released in 2017. For instance, in terms of performance, over 32 field trials ICMH 1301 had 77 ppm Fe density and 3.3 t/ha grain yield. In addition, truthfully labeled seed production of the promising hybrid ICMH 1201 has been undertaken by the Shakti Vardhak Seed Company since 2014 for commercialization under its brand name Shakti-1201, and it has been adopted by 40,000 farmers, mostly in Maharashtra and Rajasthan. Much greater progress in adopting high-Fe hybrids with high grain yield is expected in the near future, as several biofortified hybrids were advanced to All India Coordinated Pearl Millet Improvement Project Biofortification Initial Hybrid Trial for evaluation in 2017−18 with 66−75 ppm Fe density and 3.0−3.6 t/ha grain yields.

5. PROGRESS IN GENOMICS AND BIOTECHNOLOGY FOR NUTRITIONAL QUALITY

The genetic and genomic information in sorghum and pearl millet involving quality traits is limited compared with that concerning the agronomic and stress resistance traits. There is huge variability in the sorghum and pearl millet gene pools for nutritional quality traits which can further be used for various commercial and industrial applications. Genomics can complement the breeding for quality improvement in sorghum and millet. Good progress has been made in improving grain quality traits in sorghum through use of biotechnology for biofortification which can support crop improvement for better nutrition (Ibrahim et al., 1985; Rami et al., 1998; Sukumaran et al., 2012; Rhodes et al., 2017; Kotla et al., 2016). Integrating marker-assisted breeding (MAB) with classical breeding enables the possibility to track the introgression of nutritional quality-associated QTLs and genes into popular cultivars from various germplasm sources.

A number of genetic studies have and are being made for improving grain quality traits across the major cereal crops, including maize (Cook, 2012), rice (He et al., 1999; Aluko et al., 2004; Li et al., 2004), sorghum (Ibrahim et al., 1985; Sukumaran et al., 2012; Rami et al., 1998), and wheat (Huang, 2006; McCartney et al., 2006), and large genetic variation has reported for nutritional quality traits—protein content, amino acid composition, fat and starch contents, and density of micronutrients. Identification of genes influencing sorghum grain composition would help manipulate grain texture and quality to accommodate existing end-use markets and promote new product development (Bean et al., 2016). Key discoveries in the genetic control of grain composition through sorghum mutations include an amylose QTL which is 12 kb away from the *waxy* locus, the primary gene regulating amylose production and improved starch digestibility (Karper, 1933; Lichtenwalner et al., 1978; Rooney, and Pflugfelder, 1986), *sugary* which has increased sucrose content (Boyer and Liu, 1983), and *high-lysine*, which increases lysine content and protein digestibility (Singh and Axtell, 1973). Linkage and association studies have identified several loci for grain composition, but more work needs to be done to identify the genes responsible for variation in nutritional factors

of sorghum. Genome-wide association studies (GWAS) on sorghum grain composition have identified QTLs for polyphenol, mineral traits, and protein, fat, and starch (Rhodes et al., 2014; Shakoor et al., 2016; Rhodes et al., 2017). The majority of protein in sorghum grain is prolamin-type protein. The prolamin of sorghum is called kafirin and is stored in protein bodies in the starchy endosperm (Saito, 2012). 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. The *floury-2* and *opaque-2* (o2) mutants, which were discovered in maize, have major genes regulating kafirin levels and protein digestibility, which can make a valid target to increase sorghum nutrition value for animal feed industry (Singh and Axtell, 1973; Kriegshauser et al., 2006).

Also, significant marker-trait associations (MTAs) have been found, whereby six genes involved in synthesis pathways of starch (Sh2, Bt2, SssI, Ae1, and Wx) or grain storage proteins (o2) showed strong association with different grain quality traits, two yield component traits, and yield (de Alencar Figueiredo et al., 2010). This was the first targeted association mapping study for sorghum grain quality, and these finds can further be used for MAB. Another study identified eight significant MTAs in sorghum lines for traits such as kernel weight, kernel diameter, and acid detergent fiber (Sukumaran et al., 2012). An SNP in the *starch synthase IIa* (SSIIa) gene which is associated with kernel hardness and an SNP in the *starch synthase* (SSIIb) gene which is associated with starch content were of high importance. Yet, another study on 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., 2014). Also, recently, Boyles (2017) found a strong QTL in sorghum 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.

Compared with other cereals, there have been very few gene transfer technology efforts with sorghum. The Africa Biofortified Sorghum project has developed transgenic (genetically modified) sorghum lines with greatly elevated levels of provitamin A (Che et al., 2016), with the aim of combating vitamin A deficiency, which is very prevalent in Africa. Characterization of the kafirin gene family from sorghum resulted in finding its homology with the zein gene family from maize (De Rose et al., 1989). Its expression in the endosperm results in the formation of a complex between starch and protein molecules, making it difficult to completely hydrolyze (Duodu et al., 2003). This results in lower feed digestibility; but at the same time, this is a major boon to diabetics. This gene can be used as marker to accelerate the introduction of high nutrition quality and digestibility traits into different sorghum varieties using genetic engineering. Also, there is a functional gene called dihydropicolinate synthase (*dh-dps-raec1* gene), which is the first enzyme of lysine-specific pathway that has been successfully introduced in the sorghum genome, resulting in improved sorghum with high lysine content and better nutritional quality (Tadesse et al., 2003). Confirmation using ELISA (Enzyme Linked Immmunosorbant Assay) and Western blot analysis showed a 50% increase in lysine in transgenic sorghum plants when compared with the nontransformed ones (Zhao et al., 2000, 2003). In sorghum, two major QTLs on chromosome 1 that are associated with high protein α-kafirin digestibility have been identified, which can further be used for introgression into cultivars of interest with robust agronomic benefits (Winn et al., 2009). Recently, QTLs controlling grain Fe and Zn concentration in sorghum were identified, and they offer new opportunities for early generation selection for high grain Fe and Zn concentration (Kotla et al., 2016).

Up until a few years ago, pearl millet was regarded as a crop with limited genetic and genomic resources. However, since early 2010, substantial progress has been made in generation of genetic stocks and sequencing-based genotyping. It has seen progress from restriction fragment length polymorphism—based markers to the present day SNPs generated from GBS (Genotype By Sequencing), restriction site—associated DNA sequencing, double-digest restriction-associated DNA sequencing (ddRAD-Seq), and large-scale whole genome resequencing efforts. In pearl millet, several $F_{2:3}$ and $F_{2:4}$ mapping populations have been developed from diverse inbred lines of Asian, American, and African origin for study of grain iron and zinc concentrations. Some of these include H77/833-2 × PRLT 2/89-33 (early maturing inbred line, 150 F8 individuals) and ICMB 841 × 863B agronomically elite inbred seed parent, 106 F6 individuals (Kumar et al., 2016). The parent ICMB 841 was bred by pure-line selection for downy mildew resistance (DMR) within an outcrossed seed lot of a maintainer line MS 5141B. ICMB 841 is the seed parent maintainer line (Singh, 1990; Govila et al., 1997). The inbred maintainer line 863B was bred by pure-line selection at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, *Iniadi* landrace material from Togo in West Africa (Rai et al., 2008).

At ICRISAT, progress has been made in mapping QTLs for grain iron and zinc concentration using replicated samples of 106 pearl millet Recombinant Inbred Lines (F6) derived from the ICMB 841-P3 × 863-P2 mapping population (Kumar et al., 2016). Two colocalized QTLs for iron and zinc concentration on linkage group (LG) 3 were identified by composite interval mapping. The iron QTL explained 19% of phenotypic variation, whereas the zinc QTL explained 36% of phenotypic variation. Likewise, for open pollinated seeds, the QTL analysis led to the identification of two QTLs for grain iron concentration on LG3 and LG5, and two QTLs for grain zinc concentration on LG3 and LG7. The total phenotypic variance for iron and zinc QTLs in open pollinated seeds was 16% and 42%, respectively (Kumar et al., 2016) (Table 3.3).

Anuradha et al. (2017) used association mapping panel to mine alleles for pearl millet grain increased iron and zinc concentration. A total of 130 diverse lines were evaluated in three pearl millet growing agro-climatic zones of India. MTAs were analyzed with 267 markers (250 Simple Sequence Repeat markers [SSRs] and 17 genetic markers) in both general linear model and mixed linear model. Three SSR markers, *Xpsmp*2261 with an R^2 value of 13.34%, *Xipes* 0180 with an R^2 value of 11.40%, and *Xipes* 0096 with an R^2 value of 11.38%, were found to be associated with grain iron and zinc concentration across three pearl millet growing environments. Upon amplicon sequencing, the *Xpsmp* 2261 amplicon was found to be significantly similar to the intergenic region on pseudomolecule 5, while *Xipes* 0810 was found similar to the aspartic proteinase (*Asp*) gene sequence on pseudomolecule 3 of the pearl millet reference genome.

The validated LG3 high grain iron and zinc density QTLs (Kumar et al., 2016) have been the target for MAB at ICRISAT. Using the linked flanking markers, this QTL together with DMR QTLs have been moved into the genetic background of the pollen parent of HHB 67 Improved. These double QTL introgression lines were crossed with the seed parent of HHB 67 Improved to generate HHB 67 Improved-like hybrids. These QTL introgression lines along with the improved test-cross hybrids are being tested in the national testing system in India with promising results during the 2017 rainy season.

6. RECENT ADVANCES IN USE OF PHENOMIC AND GENOMIC TOOLS FOR QUALITY IMPROVEMENT

Efficient phenotyping is most critical for improving the traits of interest and to make best use of genomic resources in improving crops. Robust, low-cost phenotyping methods are key for accelerating genetic gain, quality improvement, and precise mapping of genetic variation. Recent phenotyping developments have resulted in high-throughput phenotyping platforms that utilize proximal sensing to measure multiple physiological traits. Determining food mineral composition is essential in order to understand its nutritional value. Robinson (1960) showed that atomic absorption spectroscopy was effective in detecting several metals at parts per million level of concentration. However, mineral profiling techniques at tissue level are time-consuming, hence the growing demand for development of modern analytical techniques to help increase plant yields and quality. Nevertheless, understanding the regulation of certain compounds in plants cannot rely solely on one type of analysis and, nowadays, a combined approach is being adopted. For example, combining gene expression analysis with metabolite analysis facilitates the interpretation between key regulators and compound accumulation profile (Wen et al., 2015).

Nuclear magnetic resonance spectroscopy and mass spectrometry (MS) coupled with gas chromatography (GS/MS) or liquid chromatography (LC/MS) are being used to identify plant metabolites such as amino acids, lipids, and carbohydrates (primary metabolites) and polyphenols, alkaloids, terpenes, polyketides, and hormones (secondary metabolites) in sorghum (Guo et al., 2011). In sorghum, high-performance liquid chromatography (HPLC) is frequently utilized in phytonutrient phenotyping of phenolic compounds and flavonoids (Fatima et al., 2015). Additionally, both gas and liquid chromatography can be simultaneously applied, as has, for example, been performed in rice seeds where their antioxidant properties were compared with the content of metabolites (Kim et al., 2014). Furthermore, the technique of ultraperformance liquid chromatography provides better peak separation and higher reproducibility of retention time when compared with HPLC (Guo et al., 2011) and, when coupled with quad time-of-flight (Q-TOF), its sensitivity increase (Farag and Paré, 2013).

Proteomic profiling is an advantageous tool to identify variability between two closely related cultivars. The profiling will thus be based on biochemical composition, protein concentration, and quantification of peptides of interest (Shekhar et al., 2015; Gupta et al., 2015; Morton et al., 2016). Nowadays, lipidomic studies can also be used in the selection of cultivars with optimized composition, through a detailed characterization of lipids and the gathering of quantitative data on lipid class, head group, and acyl group combination (González-Thuillier et al., 2015). Despite

TABLE 3.3 Positions and Descriptions of QTLs Affecting Pearl Millet Grain Fe and Zn Concentration in the (ICMB 841-P3 × 863B-P2)-Derived RIL Population Across the Two Environments at ICRISAT, Patancheru, India

Trait	QTL Positions	Marker Interval	LOD	Partial R2 (%)	Adj. Additive Effects	QEI	R^2 (%)	Additive Effects
Fe	3/110[#]	Xpsmp2214-Xipes142	4.68	20.5	8.3	**	19.4	4.5
	Final simultaneous fit		LOD = 5.04	Adjusted R^2 = 18.9%				
				Adjusted genotypic variation explained = 28.6%				
Zn	3/110[#]	Xpsmp2241-Xipes142	9.66	32.3	8.5	ns	35.9	6.8
	Final simultaneous fit		LOD = 8.54	Adjusted R^2 = 30.9%				
				Adjusted genotypic variation explained = 49.8%				
Fe_OP	Feb-30	Xpsmp322-Xipes181	4.34	0.6	0.6	**	18.1	0.7
	5/118	Pgpb11029-pgpb8456	4.39	14.6	2.9	ns	18.3	2.6
	Final simultaneous fit		LOD = 4.94	Adjusted R^2 = 16.1%				
				Adjusted genotypic variation explained = 26.4%				
Zn_OP	3/110[#]	Xpsmp2214-Xipes142	14.96	34.1	3.7	ns	50.1	3.7
	Jul-96	Xpsmp2040-pgpb10727	4.77	7	−1.2	**	19.7	−1.8
	Final simultaneous fit		LOD = 12.84	Adjusted R^2 = 41.7%				
				Adjusted genotypic variation explained = 94.8%				

$Leading number: Linkage group; Trailing number: QTL position in cM.

Fe, self-pollinated grain Fe concentration (ppm); Zn, self-pollinated grain Zn concentration (ppm); Fe_OP, open-pollinated grain Fe concentration (ppm); Zn_OP, open-pollinated grain Zn concentration (ppm).

** Significant at 1% level

Adapted from Kumar S., Hash C.T., Thirunavukkarasu N., Singh G., Rajaram V., Rathore A., Senapathy S., Mahendrakar M.D., Yadav R.S., Srivastava R.K., 2016. Mapping quantitative trait loci controlling high iron and zinc content in self and open pollinated grains of pearl millet [Pennisetum glaucum (L.) R. Br.]. Front. Plant Sci. https://doi.org/10.3389/fpls.2016.01636.

FIGURE 3.1 Low-cost phenotyping tools, XRF and ICP-OES, for measuring grain Fe and Zn. *ICP-OES*, inductively coupled plasma—optical emission spectrometry (L); *XRF*, X-ray fluorescence (R).

the individual benefits of these techniques, research studies are currently underway to combine them in order to overcome their individual constraints.

The application of NIRS in sorghum to assess grain quality has been reported, in particular, to measure starch, amylose, protein, lipid, total phenolic and condensed tannin content, and grain hardness and endosperm texture (Guindo et al., 2016). Inductively coupled plasma—optical emission spectrometry (ICP-OES) is probably the most efficient method for analysis of minerals such as P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn (Paiva et al., 2017). The XRF spectrometry analysis is a nondestructive technique, while the ICP-MS is highly sensitive to the different elements and compounds. XRF has standardized for assessing grain iron and zinc, which produces highly reproducible results also highly significantly correlated with ICP results for sorghum ($r = 0.79$ for Fe and $r = 0.90$ for Zn; Ashok Kumar et al., 2013) and pearl millet ($r = 0.90$; Govindaraj et al., 2016a,b; Fig. 3.1).

Another recent advance in phenotyping is the use of a low-altitude unmanned aerial vehicle (UAV) that has a high throughput phenotyping efficiency and also cost-effective. It uses different sensors for fast and nondestructive high throughput phenotyping. It is easy to use and provides on-demand access to data and high spatial resolution. UAV-remote-sensing platforms can be used for studying phenomics and genomics (Watanabe et al., 2017).

Molecular approaches such as MAS and identification of Quantitative Trait Loci (QTLs) in a mapping population has been an informative tool over many years in crop improvement programs and functional genetics. Using this approach, a large number of QTLs for various traits have been identified in sorghum in different genetic backgrounds (Zou et al., 2012; Reddy et al., 2014; Wang et al., 2014). In the era of genomics, new approaches have emerged that include GBS marker systems such as diversity array technique sequencing (DArTseq), when coupled with GWAS is the NGS equivalent of QTL analysis for traits of interest (Mudge et al., 2016). Apart from these, other biotechnological approaches such as RNA interference (RNAi), microRNA, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated system (CAS), transcription activator—like effector nucleases (TALEN), zinc-finger nuclease are being used to introduce the nutritionally important potential genes through genetic engineering (introducing or editing the genome). RNA interference is a natural process which silences specific genes before being translated by degrading messenger RNA. Developments in the technology of RNAi have enabled us to understand the functions of genes and their regulation that controls important traits (Katoch et al., 2017). Until recently, when technologies such as TALEN and Zinc Finger have emerged, the targets were the proteins, but with the discovery of new methods like CRISPR/CAS a gene can be specifically targetted (Cong et al., 2013; Hwang et al., 2013). Using these technologies, the nutritional quality of cereal crops like maize, rice, and wheat are being improved (Chai et al., 2005; Kusaba et al., 2003; Regina et al., 2006; Segal et al., 2003).

Today, the role of domestication and human selection upon sorghum grain starch synthesis and its implications for current breeding programs are being explored by a comprehensive analysis linking sorghum grain genomics and transcriptomics to improve sorghum grain quality and yield (Campbell et al., 2016). This study showed that many genes within the primary starch synthesis pathway had a clear reduction in nucleotide diversity between the landraces and wild lines, demonstrating that the ancestral effects of domestication are still clearly recognizable. Furthermore, identification of nonsynonymous SNPs present within a number of starch synthesis loci can provide

researchers with an opportunity to conduct further starch structural studies and enhance our understanding of starch synthesis (Mudge et al., 2016). This has been made conceivable with the availability of a large number of molecular markers and the advances in high-throughput and cost-effective genotyping technologies, as well as progress in the area of computational analysis. The advances in these approaches in different crops have been unprecedented in the entire history of plant breeding. All these methodologies are being used in many cereals, including sorghum, wheat, maize, rice, barley, and many other crops and have offered new insights into the genetics of different traits of economic importance (Sallam et al., 2015; Xu et al., 2014).

7. INTEGRATED BREEDING SCHEME FOR NUTRITIONAL QUALITY

Crop breeding largely deals with the creation, selection, and fixation of superior phenotypes for the development of improved lines or cultivars to fulfill the needs of farmers and consumers both locally and globally. However, for a long time, grain quality breeding was not a major focus of crop breeders, rather increasing yield per unit area has been the major concern. Recently, considering food and nutritional insecurity, grain nutritional improvement has become essential in major staple crops. The Harvest Plus challenge program coordinated by the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI) initiated a global platform integrated breeding program called biofortification, under the slogan of "better crops for better nutrition." Biofortification, which can be defined as genetic enhancement of bioavailable essential micronutrients, minerals, and vitamins, in edible parts of crops for measurable impact on human health (Bouis, 2003), is an integrated multidisciplinary and sustainable breeding approach. As (explained) in Section 3, considerable variability in the concentration of grain micronutrients in cultivated sorghum and pearl millet varieties has been reported (Ashok Kumar et al., 2012, 2015; Govindaraj et al., 2009, 2016a,b). However, wider variation is found in untapped germplasm and can be used for successful genetic improvement, but difficulties in the use of such new sources in traditional breeding include linkage drag of undesirable alleles. So, like any other quantitative traits, variability for micronutrient content should be balanced by adaptation or performance. Random mating of source populations for recombination could overcome this problem, but it is a lengthy cultivar development process. In general, micronutrient traits are under multi-genic control; hence enabling genomics technologies in biofortification breeding will increase efficiency by avoiding linkage drag and speed up cultivar development.

On the other hand, the complexity of nutrient estimation in grains and additive nature of inheritance of these quality traits has led to the development of a range of analytical tools. These challenges are being gradually addressed in many crops including sorghum and millet using rapid screening techniques such as XRF and ICP. Being additively controlled traits, intrapopulation and pedigree breeding methods are more effective. The detailed breeding approaches used for quality improvement in sorghum and pearl millet are illustrated in Fig. 3.2. The program follows the usual pedigree breeding and line development with monitoring and initial selection pressure on grain quality traits using advanced tools and techniques. By applying these protocols in the early breeding generations, the population mean or family mean is favorably shifted and increased frequency of homozygous lines with the desired quality characteristics can be expected at the end of the breeding process.

8. WAY FORWARD

Even though an enormous amount of research on molecular markers has been accomplished in the recent past in sorghum and pearl millet, there is still an immense need to develop breeder-friendly high-throughput markers for the important biotic, abiotic traits coupled with nutrient traits like grain iron and zinc concentration. In the current era of rapid genome sequencing, there are great opportunities for marker development since complete genomes can be sequenced in a very short time. Apart from the identified markers for grain iron and zinc density in pearl millet and sorghum, new QTLs/candidate genes need to be identified from different genotypes with higher grain iron and zinc concentration. After necessary validation, these QTLs/genes can be moved in forward breeding programs, marker-assisted backcrossing, and in genomic sequencing using high-throughput genotyping platforms. This will lead to faster and more efficient development of hybrid parent lines and populations adapted to different agro-ecologies with resistance to biotic, abiotic, and quality traits.

Increasing the nutritional quality of sorghum and pearl millet is of paramount importance for providing nutrient-rich food to low-income group families. With the ever-expanding technologies in genomics and bioinformatics, we
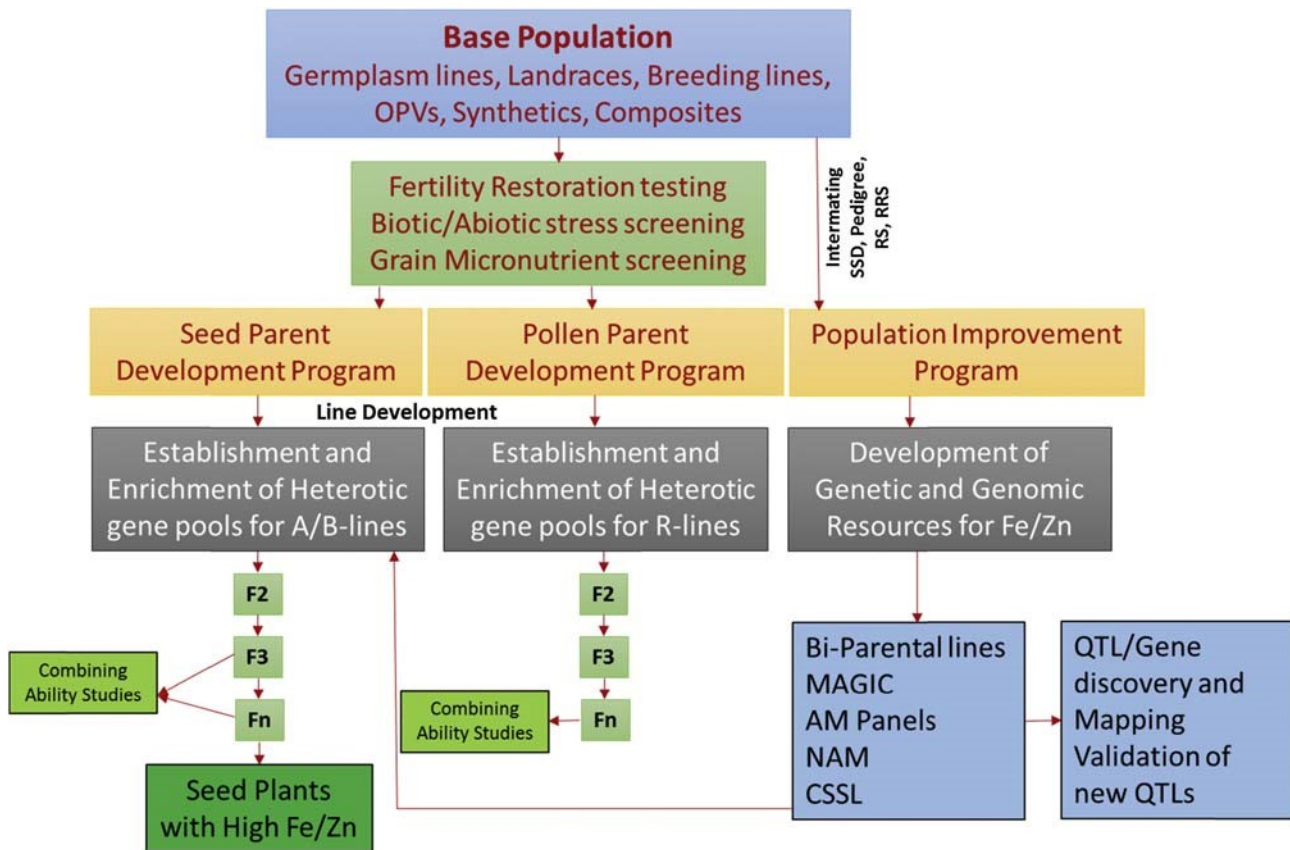
FIGURE 3.2 Integrated breeding program proposed for grain quality traits in sorghum and pearl millet.

can search different genes for enhancing the **nutritional** quality within and across species and study the expression and interaction of genes. The use of **molecular markers** offers increased selection efficiency for the trait of interest aiding the conventional breeding (Vasconcelos et al., 2017). Studies involving molecular markers can reduce the time and resources in developing new varieties with desired traits. Use of molecular markers will effectively complement the ongoing crop improvement programs for enhanced nutritional quality. Gene identification, cloning, and genetic engineering can be used to integrate desirable traits into the sorghum and pearl millet genomes. The main focus is to improve the density of micro- and macro-nutrients in sorghum and millets that are limiting in majority of the diets of people in the semi-arid tropics. Also, **more** precise investigation of genotype-by-environment (GxE) interaction on grain composition and identifying grain quality trait loci may be needed to guide the plant breeders in developing elite sorghum and millet cultivars suited for various end-products without compromising productivity. Thus, biofortifying sorghum and millets using a combination of classical plant breeding and modern biotechnology is the best option to develop improved products to provide better health to consumers. In particular, sorghum and pearl millet have considerable scope to be utilized as complementary (weaning) foods in at-risk populations because they have better nutritional quality and are more available compared with some other cereals. Furthermore, genomic research on shelf-life and processing traits will also enhance the utilization of sorghum and pearl millet in the future.

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