Molecular Approaches for Biofortification of Cereal Crops

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Abstract

Cereals are edible grains and the primary staple food crops globally. They are the major source of energy, nutrients, and bioactive substances, which provide potential health benefits in humans and livestock animals. The green revolution enhanced the crop yield potential but did not enhance the nutritional requirements, which can now be overcome by genetic revolution. There is a colossal shortage of micronutrient consumption by a preponderance of the population globally for hidden hunger. Biofortification is a new paradigm, an instrumental intervention for providing nutrients to the people deprived of access to micronutrients while keeping the promise of least cost and sustainability. It can provide enough calories and essential nutrients to meet the requirement for the sound health of the target population.

Meanwhile, there is a continuous increase in population in developing nations, and climate change is a serious challenge to fulfill the food and nutritional requirements. Thus, a more rapid and efficient strategy for biofortification is the need of the hour. Molecular breeding approaches are proven suitable for biofortification by transferring mapped QTLs/genes into nutrient-deficient crops such as QPM maize, high Fe, and Zn-rich rice lines, golden rice enriched with vitamin A, etc. However, molecular breeding advises for limited employment, citing insufficient genetic variation among crops. Furthermore, genetic

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engineering technologies go beyond the agricultural gene pool to increase the concentration of micronutrients. Similarly, recent genome sequencing technologies have paved the way for identifying new regulatory genes and miRNA within the crops, which can be further modified by genome editing approaches using CRISPR-Cas technology. This chapter highlights the strategies of molecular methods for the biofortification of crops substantiated to be precise and effective strategies for potential economic enrichment of nutritional status to overcome the hidden hunger worldwide.

Keywords

Malnutrition · Cereals · Biofortification · Molecular approaches · CRISPR-Cas

2.1 Introduction

Cereals originated from the Latin word "Cerealis" or "Ceres," which is worshiped as "Goddess of Grain." These are the staple foods globally since ancient times and reserve historical significance in human civilization. Cereals with the grains as the economic part belong to the family Gramineae. They pose a massive impact across the globe with the production of 2789.8 metric tons (mt) comprising crops like wheat (734 mt), rice (782 mt), maize (1147.6 mt), millets (31 mt), barley (141.4 mt), sorghum (59.34 mt), oats (23.05 mt), and rye (11.27 mt) (Anonymous 2020). Current estimates of annual cereal consumption are 175–190 and 155 kg/capita, both in developing and developed countries, respectively (OECD/FAO 2019). They are the primary source of various nutrients in both developed and developing countries, viz., energy, carbohydrate (nearly 75%), starch (25–27%), fiber (13%), fat (2%), and protein (6–15%) for humans and livestock (Ram and Mishra 2010). Cereals also contain a range of micronutrients, vitamin E, vitamin B complex, non-starch polysaccharides, and bioactive substances that provide potential health benefits. In contrast, micronutrients are required for proper functioning and essential metabolic activities in humans. Whole grain cereals are considered a healthy food for their nutritional value and worldwide accessibility to a significant proportion of the weak and underprivileged populations.

There is no uniformity in nutritional enrichment globally, which is a worrying concern identified by WHO, especially in children and women concerning essential micronutrients. Micronutrient deficiencies were observed, with an average of 11% in Asia and Africa. The recent reports published as *State of food security and nutrition in the world* (Anonymous 2020) suggested a rise of graph for hungry people since 2014 all across the world. The data shows the trends for 2018 as nearly 700 million or more people that account for 9.2% of the global population facing food insecurity, while ~1.3 billion people, i.e., 17.2% of the whole human population, did not have access to nutritious food at moderately severe levels. Altogether, this scenario prevailed at 26.4% of the population, i.e., 2 billion people in 2018 and 25.9% in 2019. The new report of FAO (Food and Agriculture Organization) suggests that in the last 5 years, 60 million people have joined the line of hunger. Subsequently, this data would cross the 840 million mark by 2030 with the current trends. The report also estimated that before the COVID-19 pandemic, 8.9% of the total population (~690 million) was undernourished. Global economic outlook predicts that post to the pandemic, there may be the addition of around 83–132 million malnourished people in the existing list by the end of the year 2020. This is a horrifying figure adding to the existing burden of malnutrition and thus making the challenge much more complicated. Another report of FAO reveals the facts and figures for the status of various forms of malnutrition which says that in the year 2019, children below 5 years of age were vulnerable to deprived growth as stunted, wasted, and overweight with an estimation of 144 million (21.3%), 47 million (6.9%), and 38.3 million (5.6%), respectively. This figure clears the picture that instead of all the efforts, the world is nowhere close to the SDG target of ending hunger by a stipulated timeline, i.e., 2030. Besides, the rise in obesity among adults goes from developed countries like America to developing countries like India, concisely every part of the world. The rapid rise in the growth of the human population, climatic catastrophe, and unprecedented pandemic like COVID-19 and desert locust attacks are causing setbacks to sustain food security. Consequently, the quality of diet continues to be deteriorating, posing a risk for undernutrition and worsening the food insecurity for the world altogether. In a nutshell, facts about malnutrition are the thumbnails describing the comprehensive efforts taken against malnutrition that are not concrete enough to achieve the target of "zero hunger" and "end malnutrition" to sustain food security by 2030.

We have not lost the battle yet; however, to procure the target, there has to be the assurance of proper accessibility of diets rich with balanced nutrients which are capable of cutting down the expenses of health by 97% and the greenhouse gas emission by 41–74% till 2030 if the healthy diets enriched with balanced nutrition become accessible to the whole world (Anonymous 2020). But the problems lie here, as more than three billion people who dwell in poverty simply cannot afford the expensive healthy food. Low-cost starchy staple food provides only dietary energy needs. However, it is not balanced in nutrients. Simultaneously, most of these people merely possess the expense to have a healthy balanced meal every day, which is roughly five times costlier than any starchy staple food (Anonymous 2020). In a similar context, the FAO annual report mentioned that in Africa and South Asia, nearly 57% of the population cannot make expenses to afford the healthy balanced diets and thus stated the projection of health costs to exceed 1.3 trillion USD per year by 2030 regarding mortality and no-communicable diseases due to deficient diet intake. Cereal crops are the primary food for the people of many developing nations. The recommendation of WHO/FAO for the consumption of vegetables and fruits at the rate of 400 g per person daily is not feasible for countries with a low monetary economy. Therefore, they rely on cereals as the main staple diet, viz., maize, wheat, rice, barley, sorghum, oats, millets, and rye to have wholesome nutrition. Cereals are more accessible and provide most nutrition requirements in every region globally, particularly in Africa and Asia, where cereals are the national staple diet. As more population increases in these regions, cereals can ideally become economically feasible and accessible healthy diets. However, some problems are associated with these crops, such as deficiencies of some micronutrients and nutritional components like inadequate bioavailability of Fe and Zn; lack of vitamin A and essential amino acid, viz., lysine, and tryptophan; and gluten content in wheat cause severe allergic coeliac disease. Enhancing the bioavailability of deficient nutritional content in these cereal crops can efficiently overcome these problems. Biofortification is an efficient way that offers a feasible solution to the nutritional improvement of cereal as a staple food crop and ensures the accessibility of a healthy balanced diet to the target population in the whole world. This is one of the major concerns in the current global scenario to mitigate the prevailing malnutrition status among children and adults belonging to all the world regions. Biofortification of cereal crops can restore food security in the whole world by reducing hunger and malnutrition.

Therefore, a potential economic approach is required to enhance the nutritional components of cereal crops by biofortification. Thus, a molecular approach like molecular marker-assisted breeding, genetic engineering, agrobacterium-mediated transformation, tissue culture techniques, transgenic techniques, and gene editing techniques like CRISPERcas9 presents the efficient opportunity to foster the nutritional component of crops with more certainty and lesser time stipulation than the conventional approaches. Molecular approaches have so far been proven efficient for biofortification in earlier efforts, for example, high vitamin A content-rich rice genotypes, Golden Rice-1 and Golden Rice-2 (IARI-ICAR); high Fe and Zn content-rich rice genotype, BR-29 (IARI-ICAR); high lysine and tryptophan content-rich maize genotypes, HQPM-1 (CCS HAU, against pellagra), HQPM-5, HQPM-7, Protina (IARI), Shaktiman, and Vivek Hybrid-9 (VPKAS Almora), etc. Hence, this chapter imposes the molecular approaches for enhancing the nutrient quotient of cereal crops. Its significant potential is to boost the accessibility of nutrient-enriched diets for the targeted population of all the world regions and to reduce malnutrition to ascertain food security altogether. It is a crucial tool to help us achieve the sustainable goal of zero hunger and end malnutrition by 2030 (Fig. 2.1).

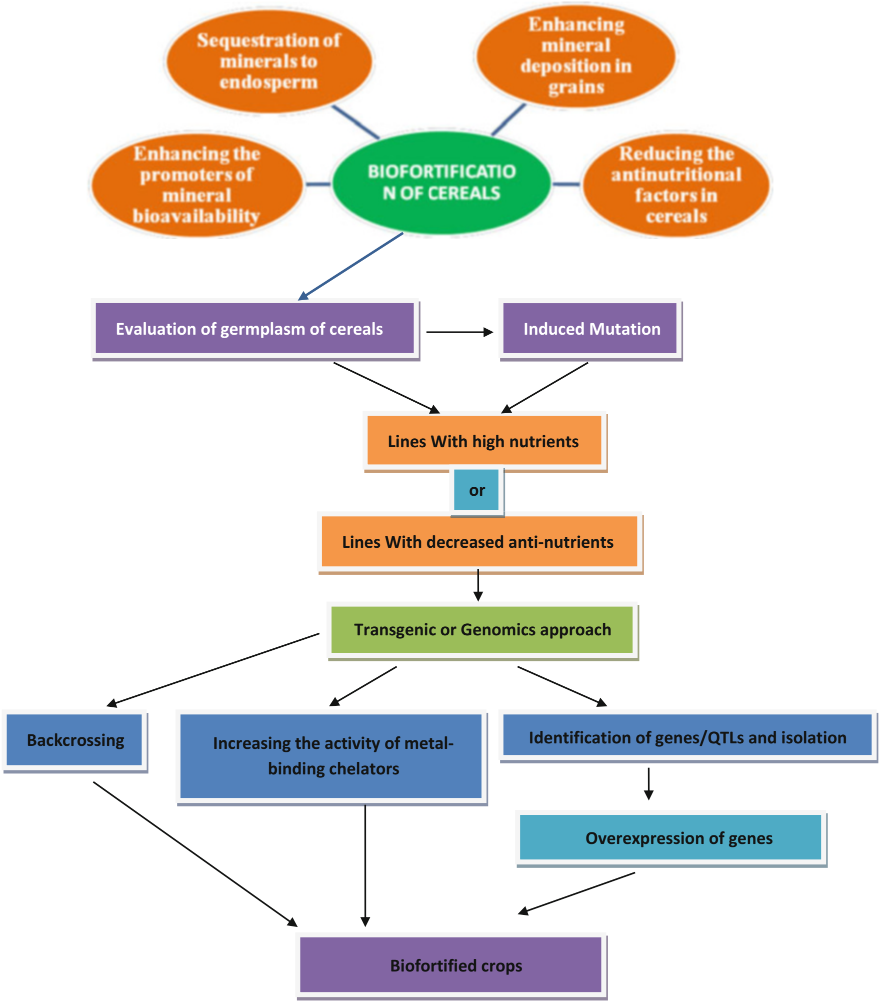

Fig. 2.1 Molecular approaches for quality improvement in cereal crops

2.2 Rice (Table 2.1)

Table 2.1 QTLs identified for different traits for biofortification in cereal crops

Traits/ QTL	No. of QTL	Parentage/cross	Type of population	Marker intervals/closest marker for major QTL	PVE	Chromosome/ chromosome arms/ chromosome position	References
Grain protein content							
qGPC	2	Zhenshan 97 (Indica rice) × Minghui 63 (Indica rice)	RIL—238 lines	C952-Wx-2	6.0–13.0	6, 7	Tan et al. (2001)
qGPC	4	Caiapo (Indica rice) × *Oryza glaberrima* (IRGC103544)	DH—312 lines	RM226–RM297	4.8–15.0	1, 2, 6, 11	Aluko et al. (2004)
qGPC	5	Gui630 (Indica rice) × 02428 (Japonica rice)	DH—81 lines	C22-RG449d, ZG34B-G20, RG435-RG172a	6.9–35.0	1, 4, 5, 6, 7	Hu et al. (2004)
qGPC	1	*Oryza sativa* (V20A) × *Oryza glaberrima* (accession 103544)	BC_3(TC) F_1 families—308 lines		9.0–10.0	8	Li et al. (2004)
qGPC	3	Moritawase (Japonica rice) × Koshihikari (Japonica rice)	RIL—92 lines		2.3–16.3	2, 6, 9	Wada et al. (2006)
qGPC	2	Koshihikari/Kasalath (Indica rice)//Koshihikari (Japonica rice)	BIL—92 lines	R1952 and R2447	14.3–14.8	6, 10	Takeuchi et al. (2007)
qGPC	2	Chuan (Indica rice) × Nanyangzhan (Japonica rice)	RIL—286 lines		2.69–4.50	6, 7	Zhang et al. (2011)

qGPC	5	Xieqingzao (Indica rice) × Milyang 46 (Indica rice)	RIL—209 lines	RM251–RM282, RM190–RZ516	3.9–19.3	3, 4, 5, 6, 10	Lou et al. (2009)
qGPC	9	Zhenshan 97 (Indica rice) × Minghui 63 (Indica rice)	RIL—241 lines		1.60–9.26	2, 3, 5, 6, 7, 10,11, 12	Yu et al. (2009)
qGPC	3	Samgang (Tongil variety) × Nagdong (Japonica variety)	DH—120 lines	RM287-RM26755, 11025-RM287	6.92–22.98	1, 11	Shi et al. (2009)
qGPC	9	Asominori (Japonica rice) × IR24 (Indica rice)	CSSL—66 lines	R1982, XNpb113, C1350, G1149	3.0–53.7	1, 2, 3, 6, 8, 11	Qin et al. (2009)
qGPC	10	Japonica rice (Asominori) × Indica rice (IR24)	RIL—71 lines	R265B-XNpb36, C1003-C688, XNpb212-G1318, C606-XNpb238, R1854-R2373, XNpb24-C562, XNpb338-C796, R758-XNpb15, XNpb268-R411	8.53–23.70	1, 3, 4, 6, 7, 8, 9, 10, 12	Zhang et al. (2011)
qGPC	2	Zhenshan 97B (Indica rice) × Delong 208 (Indica rice)	RIL—188 lines	RM445–RM418	7.2–25.9	1, 7	Zhong et al. (2011)
qGPC	4	Koshihikari/Kasalath (Indica rice)//Koshihikari (Japonica rice)	BIL—182 lines	R250-C746, C16-C809, C847-C596	6.26–12.11	2, 3, 7, 10	Zheng et al. (2011)
qGPC	1	Cheongcheong (Indica rice) × Nagdong (Indica rice)	DH—133 lines	RM12532–RM555	39–41	2	Lee et al. (2014)
qGPC	1	CJ06 (Japonica cultivar) × TN1 (Indica rice cultivar)	DH—116 lines	RM216-RM467	12.3–15.8	10	Leng et al. (2014)
qGPC	3	Cheongcheong (Indica rice) × Nagdong (Indica rice)	DH—133 lines	RM506-RM1235, RM24934-RM25128, RM219-RM23914	39–40	8, 9, 10	Yun et al. (2014)

(continued)

Table 2.1 (continued)

Traits/ QTL	No. of QTL	Parentage/cross	Type of population	Marker intervals/closest marker for major QTL	PVE	Chromosome/ chromosome arms/ chromosome position	References
qGPC	5	M201 (*Oryza sativa*) × JY293 (*Oryza sativa*)	RIL—234 lines	RM423-RM6375, GS3-SLAF13430	6.74–13.50	1, 2, 3, 4	Xu et al. (2015)
qGPC	1	Sasanishiki (Japonica variety) × Habataki (Indica variety)	CSSL—39 lines	RM7124	10.38–15.43	1	Yang et al. (2015)
qGPC	1	Cheongcheong (Indica rice) × Nagdong (Indica rice)	DH—120 lines	RM8261	14	7	Bruno et al. (2017)
qGPC	3	Naveen/*Oryza sativa* (ARC10075)//Naveen (*Oryza sativa*)	BC_3F_5—200 lines	CSCWR_Os01g02590—61041, CSCWR_Os02g10740—65058	6.70–17.35	1, 2, 7	Chattopadhyay et al. (2019)
Phytate concentration							
IP6	2	Azucena (Upland Japonica var) × IR64 (Indica var)	DH—129 lines	RM305–RM178; RM247–RM179	24.3; 15.4	5, 12	Stangoulis et al. (2007)
Iron and zinc							
GZn; GFe	2; 3	IR64 (Indica variety) × Azucena (Japonica variety)	DH—129 lines	**GZn**: RM235–RM17, RM34–RM237; **GFe**: RM270–RM17, RM53–RM300, RM137–RM325A	12–15; 13.8–18	1, 12; 2, 8, 12	Stangoulis et al. (2007)
GZn; GFe	3; 2	Zhengshan97 (Indica cultivar) × Minghui 63 (Indica cultivar)	RIL—241 lines	**GZn:** R3166-RG360, C794-RG118; **GFe**: C472-R2638, RG236-C112	5.3–18.61; 11.11–25.81	5, 7, 11; 1,9	Lu et al. (2008)
GZn; GFe	2; 1	*Oryza sativa* ssp. Indica (Teqing) × *Oryza rufipogon* Griff	IL—85 lines	**Zn**: RM152	5–11; 7	5, 8; 2	Garcia-Oliveira et al. (2009)

GZn; GFe	4; 4	Bala (Indica rice) × Azucena (Japonica rice)	RIL—79 lines	**GZn**: G1082, G20, AB0601, C223; **GFe**: R1440, C949, R1618	11.2–14.8; 9.7–21.4	6, 7, 10; 1, 3, 4, 7	Norton et al. (2010)
GZn	2	ZYQ8 (Indica cultivar) × JX17 (Japonica cultivar)	DH—127 lines	**GZn**: CT206-G177, RZ516-G30	10.83–12.38	4, 6	Zhang et al. (2011)
GZn; GFe	6; 7	Madhukar (Indica rice) × Swarna (Indica rice)	RIL—168 lines	**GZn**: RM501–OsZip2, RM7–RM517, RM260–RM7102, RM234–RM248, RM248–RM8007, RM17–RM260; **GFe**: RM243–RM488, RM488–RM490, RM574–RM122, RM234–RM248 RM248–RM8007, RM17–RM260, RM 260–RM7102	29–35; 69–71	3, 7, 12; 1, 5, 7, 12	Agarwal et al. (2014)
GZn; GFe	3; 8	PAU201 (Indica rice) × Palman 579 (Indica rice)	F_2—247 lines	**Zn**: 8RM474–RM184; RM491–RM519, RM228–RM496, RM53–RM521, RM221–RM208	4.7–19.1; 2.4–26.8	2, 10; 2, 3, 7,10, 12	Kumar et al. (2014)
GZn-4; GFe-1	4; 1	Ce258 (Indica cultivar) × IR75862 (Japonica breeding line)/ZGX1 (Indica cultivar) × IR75862 (Japonica breeding line)	BIL—200 and 201 lines	**GZn**: RM293–RM85, RM407–RM152; **GFe**: RM3–RM340	2–24.4; 10.2–18.3	3, 6, 7, 8; 6, 11	Xu et al. (2012)
GFe	1	Swarna (Indica cultivar) × Moroberekan (Japonica rice)	RIL—60 lines	RM490-RM5	39	1	Indurkar et al. (2015)

(continued)

Table 2.1 (continued)

Traits/ QTL	No. of QTL	Parentage/cross	Type of population	Marker intervals/closest marker for major QTL	PVE	Chromosome/ chromosome arms/ chromosome position	References
GZn; GFe	6; 3	*Oryza sativa* (XB) × *Oryza rufipogon* (DWR accession)	BIL—202 lines	**GZn**: RG172-RM340; RG123-RG172, RG510-RZ251	5.3–11.8; 6.1–28.2	3, 4, 6, 7, 10, 12; 3, 6, 9	Hu et al. (2016)
GZn	4	Nipponbare (*Oryza sativa*)/ W1627 (*Oryza meridionalis*)// Nipponbare	BRIL—151 lines	RM171-RM590, RM573, RM6, RM24085-RM566	15.0–21.9	2, 9, 10	Ishikawa et al. (2017)
GZn; GFe	8; 1	PSBRc82 (Indica cultivar) × Korean rice (Joryeongbyeo & PSBRc82) × IR69428 (Indica breeding line)	DH—130 and 97 lines	**GZn**: 2140834-2147095, 13048465-13057679, 8803052-8832534, 6025827-6047367, 606341-id6006214, 2110566-id2009463, 2783884-2785595, 10858811-id11000778	7.5–22.8; 9.4	2, 3, 6, 8, 11, 12; 4	Swamy et al. (2018)
GZn	8	IR64 (Indica cultivar) × IR69428 (Breeding line) and BR29 (Indica cultivar) × IR75862 (Breeding line)	DH—111 and 146 lines	**GZn**: wd9002310-9831169, 5645339-5648872, 2048774-2054640, 3538410-3548096, 7062019-7089136, 5027770-5077125, 10907196-id11001107	8.6–27.7	2, 3, 5, 7, 8, 9,11	Descalsota-Empleo et al. (2019)
GZn; GFe	1; 5	PAU201 (Indica rice) × Palman (Indica rice)	F_4—579 lines	**GZn**: RM585-RM3; **GFe**: RM2488-RM440, RM440-RM31, RM440-RM31, RM432-RM429, RM566-RM434	25; 34.6–95.2	6; 5, 7, 9	Kumar et al. (2019)

2 QTL clusters	2	Zhenshan 97 (Indica rice) × Nanyangzhan (Indica rice)	RIL—190 lines	**Tyr/Pro/Thr/Gly/Ala/Glu/Asp/Val/Met/Lys/Ser/Asp/Ile/Leu/Phe/His/Arg/Cys:** RM472-RM104	4.05–33.3	1, 7	D'Mello et al. (2003)
10 (His) + 8 (Arg)	10; 8	Zhenshan 97 (Indica rice) × Minghui 63 (Indica rice)	RIL—241 lines	**His:** R321–RM55, RZ398–RM204, RG101–G393, C1003B–RG103, RG118–C794, RM53–RZ599, RM258–RG561, RG424–R2549, RG528–RG128, RM20b–C732; **Arg:** C734b–RZ649, R321–RM55, RG424–R2549, RM258–RG561, R3203–RM20A, RM53–RZ599, RG528–RG128, RM20b–C732	12–35 (His); 16–33 (Arg)	1, 2, 3, 6, 7, 10, 11, 12 (His); 2, 3, 5, 6, 7, 10, 11, 12	Zheng et al. (2011)
12	12	Zhenshan 97 (Indica rice) × Minghui 63 (Indica rice)	RIL—241 lines	**Ser**: R2632–C39, Val: RG173–RM81A, **Met**: RZ536–TEL3	3.4–48.8	1, 11	Lu et al. (2008)
3 QTL clusters	3	Zhenshan 97B (Indica rice) × Delong 208 (Indica rice)	RIL—188 lines	**Asp/Thr/Ser/Gly/Val/Ile/Phe/Lys/Taa:** RM328–RM107, **Asp/Thr/Ser/Glu/Gly/Ala/Cys/Val/Met/Ile/Phe/Arg/Pro/Taa:** MRG186–MRG4499, **Asp/Thr/Glu/Gly/Ala/Val/Leu/Phe/Arg/Pro/Taa:** RM493–RM562	4.2–31.7	1, 7, 9	Zhong et al. (2011)
6	6	Dasanbyeo (*Oryza sativa*) × TR22183 (*Oryza sativa*)	RIL—172 lines	id3015453-id3016090, id3001422 fd10	10.2–12.4		Yoo et al. (2017)

(continued)

Table 2.1 (continued)

Traits/ QTL	No. of QTL	Parentage/cross	Type of population	Marker intervals/closest marker for major QTL	PVE	Chromosome/ chromosome arms/ chromosome position	References
Other micro-minerals							
As: qAs1.1; qAs10.1	1; 1	PSBRc82 × Joryeongbyeo/ PSBRc82 × IR69428	DH (130 and 97 lines)	RM206639-RM214137 (11.2); RM10721590-RM10722207 (7)	27; 25.8	1 (11 cM), 10 (124 cM)	Swamy et al. (2018)
Ca: qCa2.1	1			RM2094246-id2009186 (40.4)	37	2 (5 cM)	
Co: qCo1.1; qCo4.1	1; 1			id1013855-RM827062 (15.8); id4008544-RM4572241 (10.8)	29.2; 29.2	1 (67 cM), 4 (114 cM)	
Cu: qCu4.1	1			RM4285667-RM4314701 (31.8)	30.4	4 (17 cM)	
Mn: qMn2.1	1			2094246-id2009186 (59.5)	43	2 (5 cM)	
Mo: qMo1.1; qMo1.2	2; 1			id1014853-854218 (20.1); id1014853-854218 (29.2); 815155-id1014853(24.3)	51.2; 42.8; 31.5	1 (80 cM); 1 (88 cM)	
Na: qNa1.1; qNa10.1	1; 1			id1006604-267954 (10.7); 10594456-10635878 (8.4)	30.7; 26.7	1 (10 cM); 10 (105 cM)	

PVE phenotypic variance effect

2.3 Wheat

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
Grain protein content (Gpc-b1)					
6	Messapia (Durum wheat) × *Triticum turgidum* L. var. dicoccoides (MG4343)	RIL—65 lines	4BS, 5AL, 6AS, 6BS, and 7BS	6.0–23.5	Blanco et al. (2012)
1	*Triticum turgidum* (L.) var. dicoccoides	RICL—85 lines	6BS	66	Joppa et al. (1997)
1	*Triticum aestivum* (PH132) × *Triticum aestivum* (WL711)	RIL—100 lines	2DL	18.73	Prasad et al. (1999)
2	*Triticum aestivum* (Courtot) × *Triticum aestivum* (Chinese Spring)	DH—187 lines	1B, 6A	7.0–17.0	Perretant et al. (2000)
9	*Triticum aestivum* (PH132) × *Triticum aestivum* (WL711)	RIL—106 lines	2BL, 7AS	2.9–7.2	Dholakia et al. (2001)
1	*Triticum aestivum* (PH132) × *Triticum aestivum* (WL711)	RIL—100 lines; NIL—10 lines	5AL	6.2	Singh et al. (2001)
7	Messapia (Durum wheat) × *Triticum turgidum* L. var. dicoccoides (MG4343)	RIL—65 lines	4BS, 6AS, 5AL, 7AS, 7BS, 6BS	6.5–31.7	Blanco et al. (2002)
2	*Triticum aestivum* (Opata 85) × synthetic hexaploid wheat (W7984)	RIL—114 lines	2DS, 7AS		Börner et al. (2002)
10	*Triticum aestivum* (Renan) × *Triticum aestivum* (Récital)	RIL—194 lines	1A, 2AS, 3AL, 3BS, 4AS, 4DL, 5BL, 6AL, 7AS, 7DL	4.1–10.4	Groos et al. (2004)
13	*Triticum aestivum* (WL711) × *Triticum aestivum* (PH132)	RIL—100 lines	7AS, 2AS, 2DL, 2BL, 3DS, 4AL, 6BS, 7DS	2.95–32.44	Prasad et al. (2003)
3	*Triticum turgidum* (L.) var dicoccoides (LDNDic-5B) × LDN	RICL—133 lines	5B	10.0–33.0	Gonzalez-Hernandez et al. (2004)
3	*Triticum aestivum* (Renan) × *Triticum aestivum* (Récital)	RIL—194 lines	3A, 4D, 7D	6.2–9.6	Groos et al. (2003)

(continued)

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
7	*Triticum aestivum* (WL711) × *Triticum aestivum* (PH132)	RIL—110 lines	2DS, 3AL, 2AS, 1DL, 5AL, 7DL	8.38–16.58	Kulwal et al. (2005)
2	Canadian Spring wheat (AC Karma) × *Triticum aestivum* (87E03-S2B1)	DH—185 lines	4D, 7B	12.6–32.7	Huang et al. (2006)
4	*Triticum aestivum* (Opata85) × Synthetic hexaploid wheat (W7984)	RIL—114 lines	2DS, 5AL, 6DS	15.0–32.0	Nelson et al. (2006)
13	*Triticum aestivum* (Arche) × *Triticum aestivum* (Recital)	DH—222 lines	2D, 4B, 2A, 1B, 3B, 3D, 5A, 5B, 7D	5.5–24.7	Laperche et al. (2007)
3	*Triticum aestivum* (Chuan 35050) × *Triticum aestivum* (Shannong 483)	RIL—131 lines	5AL, 3BL, 6AS	8.64–21.23	Sun et al. (2008)
16	*Triticum aestivum* (Neixiang188) × *Triticum aestivum* (Yanzhan)	RIL—198 lines	3B, 2B, 1B, 2A, 2B, 3A, 4D, 5B, 5D, 7B, 7D	3.2–14.5	Li et al. (2009)
13	*Triticum aestivum* (kukri) × *Triticum aestivum* (Janz)	DH—160 lines	1B, 2A, 3AS, 3B, 4B, 4D, 5A, 5B, 7AL, 7D		Mann et al. (2009)
1	Indian durum wheat (PDW 233) × Bhalegaon 4 (a landrace)	RIL—140 lines	7B	9.64	Patil et al. (2009)
10	Durum wheat (Langdon) × Wild emmer accession (G18–16)	RIL—152 lines	2AL, 2BL, 3BL, 4AL, 5AS, 5BL, 6AS, 6BL, 7AL, 7BS	2.8–9.7	Peleg et al. (2009)
1	*Triticum aestivum* (Chara) × an advanced breeding line (WW2449)	DH—190 lines	4A	20	Raman et al. (2009)
9	Durum breeding line (DT695) × Durum wheat cultivar (Strongfield)	DH—185 lines	2B, 7A, 1A, 1B, 2A, 5B, 6B, 7A	16–46	Suprayogi et al. (2009)
2	Chinese hard wheat line (Ning7840) × Soft wheat cultivar (Clark)	RIL—132 lines	3AS, 4B	11.2–16.8	Sun et al. (2010)
3	*Triticum aestivum* (MN98550) × *Triticum aestivum* (MN99394)	RIL—139 lines	5AL, 2BS, 6DL	4.5–16.8	Tsilo et al. (2010)
4	*Triticum aestivum* (Huapei 3) × *Triticum aestivum* (Yumai 57)	DH—168 lines	3A, 3B, 5D, 6D	3.09–8.40	Zhao et al. (2010)

(continued)

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
10	Durum breeding line (C1113) × Durum cultivar (Kofa)	RIL—93 lines	3BS, 7BL, 5AS, 2BS, 4AL, 5BL, 2AL, 1BS, 7AS, 3BL	9.3–21.6	Conti et al. (2011)
10	Svevo × Ciccio (both elite durum wheat cultivars)	RIL—120 lines	3BS, 2BL, 1AL, 4AL, 2AS, 4BL, 1AS, 6BS, 5AL, 7BL	7.8–40.2	Blanco et al. (2012)
2	Oste-Gata × Massara-1 (durum wheat genotypes)	F_2 derived F_3 and F_4—151 lines	1A, 5BL	5.31–9.44	Golabadi et al. (2012)
9	*Triticum aestivum* (Weimai 8) × *Triticum aestivum* (Jimai 20)	RIL—485 lines	2B, 3A, 4A, 4D, 5B, 7A, 7B	3.06–9.79	Li et al. (2012a)
10	*Triticum aestivum* (Weimai 8) × *Triticum aestivum* (Yannong 19)	RIL—229 lines	5A, 1A, 2D, 1B, 4B, 2A, 3A, 5D, 6B, 7D	6.29–53.04	Li et al. (2012a)
9	Synthetic wheat (Am3) × Synthetic wheat (Laizhou953)	BC_5F_2:F_6 families—82 lines	6A, 1A, 2D, 3A, 4B, 5D, 6B, 6D, 7B	2.2–11.5	Li et al. (2012b)
1	*Triticum aestivum* (BR34) × *Triticum aestivum* (Grandin)	RIL—118 lines	5BL	16.3	Simons et al. (2012)
7	*Triticum aestivum* (Weimai 8) × *Triticum aestivum* (Luohan 2)	RIL—302 lines		4.15–9.73	Wang et al. (2012)
5	*Triticum aestivum* (Xiaoyan 54) × *Triticum aestivum* (Jing 411)	RIL—182 lines		1.14–9.25	Xu et al. (2012)
5	*Triticum aestivum* (CO940610) × *Triticum aestivum* (Platte)	DH—185 lines	5BS, 6AL, 6BS, 7BS, 7DL	5.6–12.3	El-Feki et al. (2013)
2	*Triticum aestivum* (Choteau) × *Triticum aestivum* (Yellowstone)	RIL—97 lines	3B, 5B	17–19	Heo and Sherman (2013)
13	*Triticum aestivum* (Huapei 3 × Yumai 57; Nuomai 1 × Gaocheng 890; 1Shannong 01–35 Gaocheng 9411);	DH—68 lines, RIL—256 lines, RIL—182 lines	2A, 1B, 1D, 2B, 2D, 3B, 4B, 5B, 6D, 7A	0.84–10.51	Deng et al. (2015)

(continued)

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
4	*Triticum aestivum* (Drysdale) × *Triticum aestivum* (gladius)	RILs (155)	2B, 2D, 3D, 5A	–	Maphosa et al. (2015)
12	*Triticum aestivum* (CD87) × *Triticum aestivum* (Katepwa)	DH lines (180)	1D, 2A, 2B, 2D, 4A, 4B, 5A, 5B, 5D, 6A, 6B, 6D, 7A	–	Moore et al. (2015)
11	*Triticum aestivum* (WCB414) × *Triticum aestivum* (WCB617)	RIL—163 lines	6B, 1A, 5B, 2B. 7B, 1B, 2D, 3D, 4B	4.7–16.5	Echeverry-Solarte et al. (2015)
4	*Triticum aestivum* (Ning7840) × *Triticum aestivum* (Clark)	RIL—127 lines	4BS, 5AL, 5BL, 3A	11.5–22	Li et al. (2016)
12	*Triticum aestivum* (RAC875) × *Triticum aestivum* (Kukri)	DH—156 lines	7A, 6A, 3D,1B, 2D, 4B, 5A, 5B, 5D	7.00–17.00	Mahjourimajd et al. (2016)
1	*Triticum aestivum* (Kitami 81) × *Triticum aestivum* (Kachikei 63)	DH—94 lines	2BS	32.1	Terasawa et al. (2016)
1	*Triticum aestivum* (Berkut) × *Triticum aestivum* (Krichauff)	DH—138 lines	1A	17.7	Tiwari et al. (2016)
9	*Triticum aestivum* (Chuan 35050) × *Triticum aestivum* (Shannong 483)	RIL—131 lines	7D, 4A, 4B, 5D, 1A, 1D, 2A, 2D,	4.1–32.7	Sun et al. (2016)
8	*Triticum turgidum* (Duilio) × *Triticum turgidum* (Avonlea)	RIL—134 lines	5A, 3B, 4A, 7B, 1B, 2B, 7A	10–14	Marcotuli et al. (2017)
12	*Triticum durum* var. Svevo × *Triticum turgidum* ssp. *dicoccoides* accession no. Y12-3	RIL—208 lines	4B, 5A, 6B, 1A, 1B, 2A, 3A, 4A, 6A, 7B	2.6–26.6	Fatiukha et al. (2019)
Grain zinc (GZn), grain iron (GFe), and grain selenium (Se) contents					
Zinc conc.-4 and Zn content-7	*Triticum aestivum* Hanxuan10 × *Triticum aestivum* Lumai 14	DH—119 lines	4D, 5A, 4A, 7A and 7A, 2D, 1A, 3A, 4A, 4D, 5A	5.3–11.9; 4.6–14.6	Shi et al. (2008)
GZn-4; GFe-1	*Triticum aestivum* (RAC875-2) × *Triticum aestivum* (cascades)	DH—90 lines	3D, 4B, 6B, 7A, 3D		Genc et al. (2009)
GZn-2; GFe-3	*Triticum boeoticum* (Tb5088) × *Triticum monococcum* (Tm14087)	RIL—93 lines	7A, 2A, 7A	7.0–12.6; 9.0–18.8	Tiwari et al. (2009)

(continued)

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
GZn-6; GFe-11	Durum wheat (cv. Langdon) × wild emmer (accession G18-16)	RIL—152 lines	2A, 7A, 5A, 6B, 7B, 5A, 7A, 2A, 2B, 3A, 3B, 4B, 5A, 6A, 6B, 7B	1.3–23.5; 0.8–17.8	Peleg et al. (2009)
GZn-2; GFe-2	*Triticum aestivum* (Xiaoyan 54) × *Triticum aestivum* (Jing 411)	RIL—182 lines	4B, 5A, 5A	4.23–6.88; 3.27–3.43	Xu et al. (2012)
GFe-4	*Triticum aestivum* (Hanxuan 10) × *Triticum aestivum* (Lumai 14)	DH—120 lines	5A, 4D, 7A, 7B	6.1–14.6	Shi et al. (2013)
GZn-2; GFe-6	*Triticum aestivum* (Tabassi) × *Triticum aestivum* (Taifun)	RIL—118 lines	4A, 1A, 7B, 3D, 4D, 2A, 7D	40.22–50.79; 8.94–47	Roshanzamir et al. (2013)
GZn-3	*Triticum aestivum* (PBW343) × *Triticum aestivum* (Kenya Swara)	RIL—177 lines	1BS, 2B, 3AL	10–15	Hao et al. (2014)
GZn-4; GFe-4	Synthetic hexaploid (SHW-L1) × *Triticum aestivum* (Chuanmai 32)	RIL—171 lines	2D, 3D, 4D, 5D, 2B, 5B, 5D, 7D	5.5–8.6; 5.4–9.5	Crespo-Herrera et al. (2016)
GZn-3; GFe-4	*Triticum aestivum* (Chuanmai 42) × *Triticum aestivum* (Chuannong 16)	RIL—127 lines	5B, 3D, 4D, 4A, 5A, 4D, 5B	13.8–15.9; 9.2–19.1	Crespo-Herrera et al. (2017)
GZn-5; GFe-5	*Triticum spelta* (PI348449) × *Triticum aestivum* (HUW 234)	RIL—185 lines	2B, 2A, 3D, 6A, 6B, 1A, 3B, 2A	4.25–16.46; 5.6–25.95	Srinivasa et al. (2014)
GZn-2; GFe-1	*Triticum aestivum* (Berkut) × *Triticum aestivum* (Krichau)	DH—138 lines	1B, 2B, 2B	23.1–35.9; 22.2	Tiwari et al. (2016)
GZn-3; GFe-5	*Triticum aestivum* (SeriM82) × *Triticum dicoccoides/Aegilops Tauschii* (SHW CWI76364)	RIL—140 lines	4BS, 6AL, 6BL, 4BS, 7DS, 2BL, 2DS, 6AL,1D, 6B, 7B, 7A, 3A, 1B, 2B, 3D, 6A, 6B, 7B, 6B, 2B, 7B, 1B, 2A	8.3–17.3; 7.5–14.5	Velu et al. (2017)
GZn-10; GFe-7	*Triticum aestivum* (Adana99) × *Triticum sphaerococum* (70711)	RIL—127 lines	2B, 3A	9–31; 9–18	Pu et al. (2018)

(continued)

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
GZn-4; GFe-3	*Triticum spelta* (Bubo) × resynthesized hexaploid wheat (Turtur)	RIL—188 lines	1B, 7B, 6A, 3A, 4B, 5B	2.86–16.75; 5.49–10.35	Krishnappa et al. (2017)
GZn-12; GFe-7	Synthetic hexaploid wheat (Louries) × *Triticum spelta* (Bateleur)	RIL—188 lines	1A, 1B, 3B, 7B, 3D, 4A, 5B, 6A, 7D, 5B, 2A, 4D, 4A, 2B, 3B, 5B	3.30–32.79; 5.79–21.14	Krishnappa et al. (2017)
GZn-5; GFe-4	*Triticum aestivum* (WH542) × synthetic derivative (*Triticum dicoccon*) PI94624/ *Aegilops sqarrosa* (409/ BCN)	RIL—286 lines	2A, 4A, 5A, 7A, 7B; 2A, 5A, 7A, 7B	3.2–14.4; 2.3–6.8	Crespo-Herrera et al. (2017)
4	*Triticum aestivum* (SHW-L1) × *Triticum aestivum* (Chuanmai 32)	RIL—171 lines	5B, 3D, 7D	6.4–28.5	
1	*Triticum aestivum* (Chuanmai 42) × *Triticum aestivum* (Chuannong 16)	RIL—127 lines	4D	35.1	
7	*Triticum aestivum* (TN18) × *Triticum aestivum* (LM6)	RIL—184 lines	2B, 5B	7.44–15.57	Wang et al. (2017a)
6	Synthetic wheat (SHW-L1) × *Triticum aestivum* (Chuanmai 32)	RIL—171 lines	3D, 5A	8.17–28.38	Pu et al. (2014)
7	*Triticum dicoccoides* (Landon) × Wild emmer wheat (accession no. G18-16)	RIL—152 lines	7A, 5A, 7B, 1A, 1B, 3A	1.4–18.6	Yan et al. (2018)
QFe. pau-2A	*Triticum boeoticum* accession pau 5088 × *Triticum monococcum* accession pau14087	RIL—93 lines	2A , Xwmc382-Xbarc124	23.6	Tiwari et al. (2009)
QFe. pau-7A			7A, Xgwm473-Xbarc29	153.8	
QFe. pau-7A			7A, Xcfd31-Xcfa2049	72.6	
QZn. pau-7A			7A, Xcfd31-Xcfa2049	72.6	
QZn. pau-7A			7A, Xgwm473-Xbarc29	153.8	
Grain yellow pigment content					
2	*Triticum aestivum* (Schomburgk) × *Triticum aestivum* (Yarralinka)	SSD—150 lines	3A, 7A	13–41	Parker et al. (1998)

(continued)

No. of QTLs	Parentage	Population type and their size	Chromosomes position/ chromosome arms	PVE range for the major QTLs	References
3	*Triticum turgidum* L. var. *durum* (Omrabi5) × *Triticum dicoccoides* (acc.600545)	RIL—114 lines	7AL, 7BL	6.0–53.0	Elouafi et al. (2001)
1	*Triticum aestivum* (Trident) × *Triticum aestivum* (Molineux)	DH—182 lines	7B	48–77	Kuchel et al. (2006)
5	*Triticum turgidum* L. var *durum* (PDW 233) × *Triticum turgidum* var *durum* (Bhalegaon 4)L	RIL—140 lines	7A, 1A, 3B, 5B, 7B	5–55.22	Patil et al. (2008)
1	*Triticum turgidum* L. var *durum* (UC1113) × *Triticum turgidum* L.var *durum* (Kofa)	RIL—93 lines	7A		Zhang and Dubcovsky (2008)
4	*Triticum aestivum* (PH82-2) × *Triticum aestivum* (Neixiang 188)	RIL—240 lines	1B, 7A, 1A, 4A,	1.5–33.9	Zhang et al. (2009)
5	*Triticum turgidum* L. var *durum* (Latino) × *Triticum turgidm* L. var *durum* (Primadur)	F_2:F_3 families—121 lines	2A, 3B, 5A, 7A	9.4–53.2	Blanco et al. (2011)
6	Ajana × WAWHT2074; Carmah × WAWHT2046; Ajanax WAWHT204 (*Triticum aestivum*)	DH—179, 121 and 127 lines	7B, 2D, 3A, 7A, 4D, 5B, 7B	4.0–36.0	Crawford et al. (2011)
15	*Triticum turgidum* L. var *durum* (UC1113) × *Triticum turgidum* L. var *durum* (Kofa)	RIL—93 lines	1BL, 4AL, 7BL, 6AL, 2AS, 5AS, 5AL, 5BL, 7AS, 7AL, 7BS	6–42.7	Roncallo et al. (2012)
13	*Triticum aestivum* (Chuan 35050) × *Triticum aestivum* (Shannong 483)	RIL—131 lines	5B, 6A, 1A, 1B, 2D, 4A, 4D, 5D, 6D, 7B	4.1–16.5	Zhang and Dubcovsky (2008)
7	*Triticum turgidum* L. var *durum* (Svevo) × *Triticum turgidum* L. var *durum* (Ciccio)	RIL	1B, 5B, 7A, 2A, 2B, 5A, 7B	19.3–51.6	Colasuonno et al. (2014)
16	*Triticum aestivum* (Gaocheng 8901) × *Triticum aestivum* (Zhoumai 16)	RIL—176 lines	5AL, 2DL, 5BS, 1B. 1R, 2AL, 2B-1,5AS, 5BL, 6BL, 7AS, 7BL	5.7–30.8	Zhai et al. (2016)

PVE phenotypic variance effect

2.4 Maize

S. No	Trait	Parentage	Population type with size	No. of QTL	Chromosome/ chromosome bin	chromosome position (cM)	Closest marker to the QTL	LOD	Reference
Micromineral content									
1	**P**	B84 (BSSL line) × Os6-2 (relative line of C103)	F_4 families (294 lines)	8	3-05; 3-09; 4-08; 6-03; 6-05; 8-05; 9-02; 10-07	10; 48; 48; 20; 34; 26; 16; 32	umc59e; bnlg1257; ZM0819; umc1887; ZM1367; ZM0353; bnlg0244; bnlg1839	5.98; 6.48; 6.69; 4.89; 5.42; 4.50; 8.39; 8.67	Šimić et al. (2012)
2	**Fe**			3	2-05; 6-03; 8-06	40; 24; 32	ZM1368; ZM0960; ZM0825	4.44; 4.93; 4.44	
3	**Fe/ P**			7	1-05; 2-05; 3-05; 4-07; 6-01; 6-03; 10-06	34; 40; 16; 32; 10; 24; 26	ZM0845; ZM1368; bnlg1456; bnlg1784; bnlg0426; ZM0960; ZM1315	4.55; 7.82; 6.54; 4.73; 4.73; 6.75; 5.95	
4	**Zn**			1	4-08	44	ZM1362	5.19	
5	**Zn/ P**			1	3-08	16	bnlg1456	5.21	
6	**Mg**			4	5-03; 6-01; 8-05; 9-07	18; 16; 24; 44	bnlg1046; bnlg426; bnlg1782; bnlg0128	4.44; 4.65; 5.03; 7.96	
7	**Mg/ P**			8	1-01; 1-03; 3-05; 4-08; 5-03; 9-02; 9-07; 10-04	0; 20; 16; 48; 12; 16; 34; 12	bnlg1014; phi109275; bnlg1456; ZM0819; ZM0215; bnlg244; umc1675; ZM0363	6.00; 5.96; 5.65; 5.34; 14.00; 5.28; 5.02; 12.77	

Chr chromosome

2.5 Oats

Character	Position of QTL	Marker interval	Closest marker to the QTL	LOD score	R^2 (%)	Additive effects	Reference
β-glucan	QBg1.jki-A1	17–21	E36M55_1–E36M52_3	7.9	31.27	−0.14	Matthias et al. (2014)
	QBg2.jki-A10	21–83	E33M48_2–E34M49_1	3.51	16.27	0.17	
	QBg1.jki-B1	6–9	E32M55_1–E36M56_a	3.39	24.98	−0.16	
Groat protein	QPr1.jki-A1	20–24	E36M52_3–E35M62_3	14.11	48.84	−0.76	
	QPr1.jki-B1	16–21	E37M47_c–E37M47_b	9.88	45.62	−0.65	

2.6 Pearl Millet

Trait	Position of QTL	Support interval	Marker interval	LOD	R^2 (%)	Additive effects	References
Iron (Fe)	1/54	52–56	pgpb10531-pgpb9130	25.36	31.9	9.7	Kumar et al. (2018)
	1/30	26–36	Xipes017-pgpb12900	6.22	9	4	
	3/20	8–26	Xipes188-pgpb6069	6.59	9.5	0.4	
	4B/8	04–08	pgpb9502-pgpb6039	6.87	10.4	−0.6	
	7/86	84–90	pgpb8427-pgpb13221	8.58	12.2	5.3	
	7/120	118–122	pgpb6825-Xipes195	9.7	14	0.1	
	7/16	0–30	pgpb11956-pgpb9273	7.25	12.5	−1.9	
	7/108	106–110	pgpb11938-pgpb8987	8.83	12.5	4.9	
	E/14	8–14	pgpb10727-Xipes179	9.36	14.3	3.1	
	A/0	0–2	pgpb8445-pgpb11206	7.67	12.4	4	
	D/20	14–24	pgpb10660-pgpb8626	7	11.6	1.2	
	3/110	106–116	*Xpsmp2214-Xipes142*	4.68	19.4	4.5	Kumar et al. (2016)
Fe_OP	1/30	10–38	*Xpsmp322-Xipes181*	4.34	18.1	0.7	
	5/118	94–124	*pgpb11029-pgpb8456*	4.39	18.3	2.6	

(continued)

Trait	Position of QTL	Support interval	Marker interval	LOD	R^2 (%)	Additive effects	References
Zinc (Zn)	1/182	178–186	pgpb10397-pgpb10394	6.5	9.4	1.7	Kumar et al. (2018)
	1/54	52–56	pgpb10531-pgpb9130	23.93	30.4	6.7	
	1/22	14–26	pgpb10483-pgpb11463	6.68	11.6	−2.2	
	4B/8	4–10	pgpb9502-pgpb6039	7.33	11.1	−0.6	
	5/112	108–118	pgpb13229-pgpb12681	8.17	11.6	2.7	
	7/112	110–116	pgpb12329-pgpb9721	7.58	10.9	2.8	
	7/82	74–88	Xipes198-pgpb8427	7.16	10.2	2.7	
	H/16	10–22	pgpb8779-pgpb12691	6.68	11.6	2.1	
	3/110	106–117	*Xpsmp2214-Xipes142*	9.66	35.9	6.8	
Zn_OP	3/110	106–116	*Xpsmp2214-Xipes142*	14.96	50.1	3.7	Kumar et al. (2016)
	7/96	90–98	*Xpsmp2040-pgpb10727*	4.77	197	−1.8	

2.6.1 Molecular Approaches for Biofortification in Cereal Crops

Biofortification strategies for the target nutrient trait improvement among cereal crops and taking time constraints and population growth rates into account may involve one or more molecular approaches. These approaches deal with the genome sequencing of the crop plant and subsequent modification at the genomic level, including alteration or editing of the target sequence and study of expression analysis of the variant. The desirable variants with the transgressive segregation for the particular target trait are selected, and their expression stability is analyzed. Further, the validation at both the molecular and phenotypic levels must be done to ascertain the target trait expression. The molecular approaches for the biofortification of target nutrient traits in cereal crops are as follows:

1. **Genomic Approaches**
    (a) Genome sequencing/resequencing
    (b) RNA sequencing
    (c) QTL mapping

   (d) Micro RNA discovery
   (e) Epigenomics and SNPs
2. **Genome Engineering**
   (a) Molecular breeding
   (b) Mutation breeding
   (c) Cis and transgenics
   (d) Genome editing (TALEN, ZFN, CRISPERcas)

2.6.1.1 Genomic Approaches

(a) *Genome Sequencing/Resequencing*

Sequencing of several crops and non-crop plants has led to the identification of novel alleles for the nutrient trait, which can be incorporated into the crop by various approaches like marker-assisted breeding. Rice is the first milestone achieved in sequencing under the collaborative project on the "International Rice Genome Sequencing Project" in 2005. Since then, over 3000 rice accessions have been resequenced worldwide, resulting in the discovery of the most recent unique genes and QTLs, and thus impeccably moulding the wealthy status of micronutrient quotient in distinct genotypes.

(b) *QTL Mapping*

Liu (1997) had described the QTL as the loci consisting of many genes with a small and cumulative effect that governs the quantitative traits. Hence, the variation so produced is relatively continuous than the discrete, unlike qualitative characteristics. Many other pieces of researches support his description. Gande (2014) and his colleagues emphasized the importance of QTLs regarding the functional expressions like uptake, transport, and packing up of micronutrients into the grain and the coordinated terms of these specific genes/QTLs at the time of deprivation or surplus micronutrient element conditions for employing the layout to improve upon the respective micronutrient concentration. These QTLs can be sequenced and analyzed further using the techniques like *knock out gene* or *overexpression analysis*, which provides valuable information in determining the "gene or sequence of interest." Once the loci get sequenced, the piece of information to design the functional marker can be obtained. In such a way, the QTL can be mapped using fine mapping or used in breeding programs like MAS (marker-assisted selection) to improve the trait.

(c) *Micro RNA Discovery*

MicroRNAs or miRNAs were discovered later, and ever since, various molecular biologists worldwide have conducted research on their functions, expressions, structure, etc., in plant biology. Their role has been clarified after intensive investigations in the various metabolical and gene expression regulation processes. Unlike DNA, it does not carry exons or codes for any functional genes. Instead, its counterpart functions are primarily mediators and helpers, such as meditating in mRNA degradation posttranscriptionally to help regulate gene expression. Recently, some researchers reported their role in the uptake and transport of some nutrients (Kehr 2013; Fischer et al. 2013). This report is supported by some other studies that provide evidence about their potential role in regulating zinc and iron metabolic regulation and signal transduction pathway during the deficiency of any of the essential microminerals in rice crops (Agarwal et al. 2015).

(d) *Epigenetics*

The term epigenetic summarizes the sum of expression caused due to regulation control by the cellular components such as chromatin structure, histone modification, DNA methylation, etc. In a study of stress signaling and pathway, Chinnusamy and Zhu (2009) reported the regulation of gene expression by epigenetic modification in the sequence of DNA upon the arrival of stress signal in the response of stress occurrence as the tolerance or resistance mechanism in crop plants. Duan et al. (2015) reported that Fe plays a determining role in DNA methylation patterns as an act according to epigenetic regulation. The basis of epigenetic regulation for stress resistance/tolerance has been revealed with the advent of tools and techniques of molecular biology, which has opened a new horizon for stress breeding against various abiotic and biotic stresses. The success against the stress through conventional breeding is minimal; however, the concept of epigenetic regulation is being exploited against stress like drought, cold, salinity, pest, etc., which is very useful in reducing crop loss and subsequently increasing yield potential.

2.6.1.2 Genome Engineering

(a) *Mutagenesis or Mutation Breeding*

The term "mutation" refers to the heritable changes in the genetic coding instructions of the DNA. These changes can either be detrimental or beneficial. The beneficial mutants are subjected to mutation breeding. These mutants may be originated spontaneously, naturally, or artificially induced by physical mutagen like ionizing or nonionizing radiation and chemical mutagen like base analogs, alkylating agents, acridine dyes, etc. The mutant so produced causes a new genetic variation that did not exist naturally; thus, it introduces the functional variability that can be exploited in the breeding program to improve any specific traits. Numerous mutants have been reported related to various characteristics, viz., high yield, early maturity, disease resistance, grain quality characters, abiotic stress resistance, etc., among different crops, viz., soybean, cotton, rice, etc. Henceforth, it is capable of shaping nutritional breeding like other traits. In one such study, Wang et al. (2017a, b) reported that "Lilizhi," a mutant rice variety, showed up to a 35% increase in the zinc content than the original rice genotypes by increasing the rate of transport of storage Zn from roots to the grain.

(b) *Molecular Breeding*

This is the most widely used method for biofortification in cereals such as Fe, Zn, provitamin A, grain protein content, in wheat, rice, maize, millets, etc. It requires the gene or allele of interest possessing a specific target trait and set of polymorphic DNA markers such as SSR, SNPs, etc., for the background selection. It reduces the time and effort required in the conventional approach. It selects various stages like foreground selection with the marker at flanking zone, recombinant selection, and background selection to recover the recurrent genome as maximum as possible. On the other hand, genomic selection (GS) with more advantages is a modern approach. It estimates the genotypic breeding value of the individual. GS is more suitable for the transfer of complex QTLs than MAS. The fundamental requisition for this method is the nearest or linked markers to the trait of interest, polymorphic DNA markers, and specific QTL or specific genes.

(c) *Transgenics*

Since the achievement of nutritional targets through conventional breeding is marginal and quite complicated, it draws attention to other alternatives that are target-specific and ascertain the success. One such option is "transgenics"; however, it delineates the turmoil while it comes to the ethical perception, yet significant achievements have been garlanded with transgenic technology. Many researchers have reported the enhancement of storage Fe in rice seeds with a range of 1.5- to 3.7-fold due to ferritin through introgressing the gene *OsGluB1 proSoya-ferH1* using transgenic technology in various researches (Goto et al. 1999; Lucca et al. 2002; Vasconcelos et al. 2003; Qu et al. 2005; Paul et al. 2012). Various researchers have reported two- to fourfold enhancement of Fe translocation due to overexpression of NAS genes by introgressing gene *OsActin1 Pro-HvNAS1* and *35S Pro-HvNAS1* (Masuda et al. 2009; Lee et al. 2012; Ishimaru et al. 2010; Johnson et al. 2011). Similarly, enhancement of Fe and Zn has been reported by overexpression of "OsNAS2" and "OsZIP4" genes by various researchers such as Ishimaru et al. (2007). Wirth et al. (2009) reported enhancing transgenes, NAS, and ferritin by 6-fold and 1.3–1.5-fold, respectively. In such a way, transgene portrays the efficient method to improve complex traits in less time frame and with high precision. There is an implicit need for a legitimate framework to address the use of transgene for dire complications regarding nutritional improvement programs.

(d) *Genome Editing*

A genome represents the complete set of DNA, all the genes, and information needed to build and maintain an organism. The function of these genes can be elucidated through genome editing by manipulating the gene sequences. Genome editing requires a "molecular scissor," i.e., an engineered nucleases enzyme that cuts any specific line at specific points. These cleaved sequences were then inserted, replaced, or removed accordingly. The nuclease cleaving follows different specificity such as DSB (double-strand break), homologous recombination, and NHEJ (nonhomologous end-joining). Genome editing, however, engrosses three different forms of nucleases, viz., TALEN (transcription activator-like effector nucleases), and ZFN (zinc finger nuclease), and the recent one are CRISPER (clustered regularly interspaced short palindromic repeats). ZFN consists of two protein domains, viz., DNA-binding domain for a transcription factor and the nuclease domain comprises FokI restriction enzyme for specific cleavage. TALEN contains an NLS, N-terminal translocation signal, and transcriptional activator domain. CRISPER has three components, viz., protospacer adjacent motif (PAM), CRISPER RNA

(Cr RNA), and trans-activating Cr RNA (trscr RNA). It is associated with "cas" protein. Together, it produces precise incisions at a specific sequence. Precision, cleavage, and ligation specificity facilitate the genome editing process to create mutation or substitutions in a cell. It is helpful in gene transfer, transcriptional modifications, resistance against abiotic and biotic stresses, multiple gene editing, gene silencing, etc., and thus, a convenient tool in creating controlled desirable gene expressions like promoter sequence modification or regulatory gene sequence modification. Such a powerful concept is, therefore, being applied broadly for various traits by many researchers.

2.6.2 Molecular Strategies Achieved for Biofortification in the Following Cereals

2.6.2.1 Rice

Rice is the foremost energy package add-on for almost the hemisphere's population, predominantly in Asian countries. Being staple food should essentially supply wholesome nutrition to the consumers; however, it is not so. It lacks a few essential nutritional components such as Fe, Zn, provitamin A, and protein, which stands for the growth and development of young children and infants. Hence, it poses a worrying census regarding malnutrition status at a considerable scale. To surmount this vulnerable problem, various molecular strategies have been employed, which served promising results. The development of "golden rice" using three genes (from "daffodil" and bacteria *Erwinia caratovora*) for the biosynthesis of β-carotenoids in the edible grains with the help of transgenic technology earned praises all over the world. Many of the novel laureates worldwide recommend using golden rice in developing countries to overcome malnutrition.

Similarly, introgression of genes and QTLs for "NAS" and "ferritin" using transgenic technology in several programs produced a noticeable increase in Fe and Zn content by two- to sixfolds. The bioavailability of the nutrients has been an issue that is circumvented by using transgenic assays by many researchers. Mutagenesis has been very useful in producing numerous "mutants" shown to enhance the higher protein and mineral content by increasing the transport of storage minerals from roots to grains. Genome editing added the magical tool in the bag of molecular breeding, with the advent of "CRISPERcas9" editing for the complex genome trait expression, and regulation is pretty handy. Being the first fully sequenced cereal crop, most of the genes and QTLs in rice have been studied, and most molecular markers have been designed. The sequence data is available in various database banks such as NCBI (National Center for Biotechnological Information), DDBJ (DNA Database Bank of Japan), etc.; apart from this, software like SSRit/Gramene is also available to provide complete information about SSR markers. It paves an easy way for marker-assisted breeding to address the improvement of rice breeding programs. Several QTLs/genes are available for nutritional traits. The use of marker-assisted breeding like MAS, MARS, MABB, and genomic selection has reduced the time and enhanced the precision with which even the complex traits can be improved.

2.6.2.2 Wheat

Wheat has an impetus position in the global market. The *hexaploid* wheat and *diploid* wheat are widely consumed wheat varieties. They show significant variation for the Fe and Zn content. Grain protein content in the wheat grain is a concern for wheat breeders. Deficiencies in micro minerals content, viz., Fe, Zn, and Se (selenium), grain protein, and provitamin A are significant nutritional gaps in wheat. For the biofortification of wheat, the most successful and used method is marker-aided selection (MAS). Grain protein content (GPC) is the most concerning nutrient in wheat. Several QTLs have been mapped by various researchers worldwide. *Gpc-B1* is the most accountable QTL identified for the grain protein and mineral content that has been transferred from parent *dicoccoides* species to cultivar *hexaploid* species. However, the nature of QTLs depends upon the uptake, transport, and packing of the nutrient in the grain.

Due to such complexity of these QTLs, conventional breeding could not be part of the winning side. Yet, a variety like "Zinc Shakti" with up to 40% high Zn content has been developed by conventional back cross-breeding; still, no such variety could be developed for Fe content. Mapping study reveals that the Fe and Zn are associated together in the same loci. The QTLs have been mapped for Fe, Zn, and selenium. Marker-assisted selection can unarguably improve these traits by introgressing these QTLs. However, the complex polygenic nature of the QTLs and their interaction with the environment poses problems for MAS. Recently, the announced method of "genomic selection" or genome-wide selection (GWS) can counteract such issues as the genetic breeding value is estimated in this method.

2.6.2.3 Maize

Maize withholds a virtuous place among the cereal crops. Primarily, maize is used as a staple food in corn. Grains of maize critically are deprived of amino acids *lysine* and *tryptophan*. The deficiency of such amino acid causes severe diseases like pellagra upon consumption. Lack of these amino acids subsequently leads to insufficient protein in the endosperm. Another concern is the poor bioavailability of micro minerals like Fe and Zn content and the lack of provitamin A and *phytate* in kernels. Phytate content is responsible for seedling growth and germination. Besides, it also inhibits various types of cancer upon consumption. However, it inhibits the bioavailability of micro minerals such as Fe, Zn, Mg, etc. However, several QTLs have been reported, which govern the high accumulation of micro minerals, viz., Fe and Zn. The endosperm protein content has been improved with the intervention of mutant *opaque-2*. The first QPM maize "Vivek QPM-9" has been developed by the introgression of the opaque-2 mutant using MAS. Similarly, low phytase mutants, viz., *lpa1, lpa2,* and *lpa3,* with a significant reduction in *phytase* have been reported and introgress in cultivars using MAS.

2.6.2.4 Sorghum

Sorghum is an essential hardy cereal crop after rice, wheat, and maize and is the cheap source of energy and nutrients, primarily in semiarid tropical areas. The main obstacle in sorghum is the presence of antinutritional factors like polyphenols and phytate, which inhibit the bioavailability of Fe and Zn. Wide variability is reported for the micro mineral content in sorghum. However, lines with high Fe and Zn content have been developed under the HarvestPlus programs. Much of the molecular strategies have not been exploited in this crop. Yet, the prospect holds the scope for molecular approaches like MAS, GS, genome engineering, etc., for the precise and immediate development of sorghum cultivars.

2.6.2.5 Millets

Millets are the chief source of calories after cereals in Asia and Africa's arid and semiarid parts. They are a staple food, nutrient-rich, and resistant to extreme ecological conditions. Millets play a crucial role in nutritional security as they provide an appreciable amount of proteins, dietary fiber, vitamins, and minerals. To combat micronutrient malnutrition, the biofortification of significant food crops was shown to be an economical method. The HarvestPlus group noticed the need for biofortification in millets. So, to tackle iron deficiency, the HarvestPlus group included pearl millet as one of the staple crops under the Biofortification Challenge Program (BCP) (Fig. 2.2).

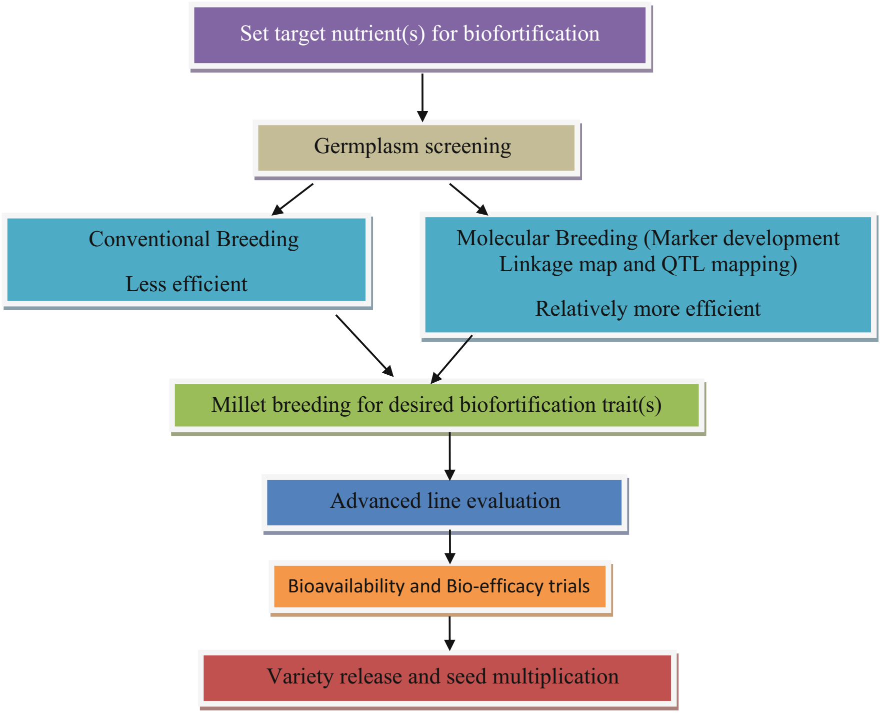

Fig. 2.2 Flow chart for the general breeding procedure of biofortification of millets

(a) **Pearl Millet**
Genetic diversity and QTL mapping studies were conducted for pearl millet genome analysis using RFLP and SSR markers. RFLP and SSR markers were also utilized to construct the first consensus linkage map with four different mapping populations of pearl millet. Accessibility of a high-density consensus molecular map permits us to locate the position of QTLs/genes strictly in the genetic background of several mapping populations. Different marker techniques, viz., single-strand conformation polymorphism–single nucleotide polymorphisms (SSCP-SNPs), conserved intron scanning primers (CISP), and diversity arrays technology (DArT) were also tried in genome studies of pearl millet. However, SNP-based assays and genotyping by sequencing (GBS) technologies were yet to be utilized widely in pearl millet. A severe constrain for genomic studies in pearl millet is the unavailability of high-density genetic maps. The discovery of genes/QTLs responsible for various nutrients (essential amino acids, Fe, and Zn) present in grains of pearl millet can be accelerated by applying high-throughput technologies. The draft genome sequence of the pearl millet is almost ready and expected to be released soon in the public domain. These genomic tools will be handy for analyzing quantitative as well as quality traits.

(b) **Foxtail Millet**
Genomic studies of foxtail millet were mainly done with RFLP and RAPD markers until the generation of SSRs. However, these markers are considered essential genomic tools in foxtail millet to investigate economically important traits at the molecular level. Accelerated creation of structural genomics resources was achieved through the utilization of NGS technologies in foxtail millet. Two research groups have sequenced the foxtail millet genome independently. One was at the Beijing Genomics Institute (BGI), China, and the other at the Joint Genome Institute (JGI) of the Department of Energy, USA. The Beijing Genomics Institute (BGI) research group, China, has done the genome sequencing of foxtail millet cultivar Zhang Gu and variety A-2. Then they identified many SNPs, InDels, and structural variations (for marker development) through comparison of genome sequences of both, i.e., the genome of cultivar Zhang Gu and variety A-2. The other group at the JGI (Joint Genome Institute) of the Department of Energy, USA, has sequenced foxtail millet genotypes Yagu1, A-10, and also a recombinant inbred line (RIL) population derived by crossing Yagu1 and A-10, which leads to the construction of high-density SNP map from resequencing information of the RIL population. Recently, with the help of resequencing information of 916 diverse genotypes of foxtail millet with 0.8 million SNP and SNP haplotype map was constructed. Such a haplotype map may be utilized for the discovery of genes governing grain quality traits employing association mapping-based techniques.

(c) **Other Millets**
Limited research was done in other minor millets at the genomic level. Genetic diversity analysis was done in proso millet with the help of RAPD and AFLP markers. However, SSR markers have been developed in proso millet from

microsatellite enriched genomic DNA libraries and through the transferability of microsatellites from related species of proso millet (wheat, oat, rice, barley, and switchgrass). Some species of millets have only a few shreds of evidence on the utilization of DNA markers, and their genomes are yet to be characterized. Consequently, the immediate focus should be on generating more genomic resources for minor millets, which could be employed to analyze and improve qualitative traits.

2.6.2.6 Oats

The oat (*Avena sativa*) is an annual grass commonly grown in temperate regions requiring lower summer temperatures. Oats are used for human consumption as well as livestock feed. Despite the richness of various essential nutrients, the use of human consumption is limited due to their blend organoleptic taste and spoilage. Though oats are highly nutritious, yet it is not cultivated on a large scale mainly due to reduced market demand and less economic revenue generation compared to other crops. As a result, investments in oat breeding are consistently decreasing. However, the release of biofortified varieties with higher and stable yields can put them in demand.

Mapping studies with RFLP markers revealed that the genomic regions governing β-glucan content in oats are present on chromosomes number 7 and 11. QTLs for traits β-glucan and protein content were mapped with the help of an integrated molecular linkage map constructed by several DNA markers like RFLP, RAPD, SCAR, and AFLP. Though rarely used, CAPS and SCAR were designed for the genomic regions controlling β-glucan and protein content. Like other cereal crops, huge numbers of SSR markers were developed in oat too. High-density linkage maps are needed in oats for high-resolution mapping of quantitative traits due to their large genome size. Genes governing qualitative characteristics could be discovered by oat genome analysis with the constructed SNP consensus map framework.

2.6.3 Limitations in Biofortification Through Molecular Approaches

1. ***Presence of Antinutrient***
   The digestibility of nutrients present in millets is an issue due to the presence of antinutrients. Phytic acid (present in most of the cereals) is one of the essential antinutrients that have a solid ability to inhibit the absorption of crucial minerals, viz., Zn, Ca, and Fe. Antinutritional factors present in crops like phenols, phytase, etc., though they have beneficial functions yet inhibit the expression of other microelements such as Fe, Zn, Mg, Se, etc.
2. ***Yield Reduction with an Improvement of Micronutrient***
   A negative and nonsignificant association was found between Fe and Zn with the grain yield in the breeding lines and cultivars of pearl millet due to their genetic architecture. They were developed through selections for improvement of yield

Table 2.2 Antinutrient profile of millets

Crop	Antinutrient
Pearl millet	• Phytate and goitrogens
Finger millet	• Tannins
	• Oxalates
	• Phytate
	• Non-starch polysaccharides—glucan
	• Protease inhibitors

and not for the micronutrients. But there is a positive correlation between Zn and Fe; therefore, breeding for high-iron pearl millet leads to enhancement of Zn associated with the iron.

3. ***Floral Biology and Mode of Pollination***
   The development of mapping population requires artificial hybridization. Some millets (foxtail millet, finger millet, Kodo millet, etc.) are self-pollinated, which makes artificial hybridization among the selected parental lines tedious. Emasculation and pollination are difficult due to the small flower size.
4. ***Bioavailability of the Micronutrients***
   The bioavailability of micro minerals and nutrients needs to be taken into consideration. The gene/QTLs for deficient traits may enhance the expression; yet, it does not determine the bioaccessibility of the nutrient.
5. ***The Complex Interaction of QTLs with the Environment***
   The complex interaction of QTLs with the climate and additive gene action of multiple loci poses problems in marker-assisted selection (Table 2.2).

2.7 Conclusion

Molecular approaches have reduced the complexity of biofortification of micronutrients in cereal crops, which otherwise was not much virtuous through solemn dependence upon conventional breeding. Mapping studies for the variability among the micro mineral contents like Fe and Zn, grain protein content, vitamin A, and antinutritional factors have revealed several QTLs. Upon introgression, several cultivars yield manifold higher nutrient values. With the advent of molecular markers like SSR, SNPs, etc., marker-assisted breeding has simplified the biofortification of crops, viz., high Fe and Zn, protein, and β-carotenoids. The deficient amino acid content in maize has been improved by developing QPM maize cultivars using MAS. Genomic regions with the coding sequence for target expression have been revealed with the advancement of genome sequencing. Genetic engineering techniques like genome editing via CRISPERcas9, TALEN, and ZFN modify traits' expression of the undesirable genetic sequence. Transgenic technology has been instrumental in resolving complex difficulties to produce genetically novel characteristics such as "Golden Rice-1" and "Golden rice-2" with higher provitamin A concentrations. The expression of Fe and Zn in rice by enhancing expression of NAS genes and introgression of *ferritin* could be possible due to transgenics.

Further, mutagenic breeding added benefits by producing novel mutants for improving traits like high Fe content, low phytase content, etc. Conclusively, the sum of genomic and genetic engineering approaches have enhanced the biofortification in multiple cereal crops, viz., rice, wheat, maize, sorghum, millets, oats, etc. It dealt with all the genetic complexities and turned the roller coaster of biofortification difficulties into a smooth road wide open for easy passage. It also saves time under urgency. Altogether, a slide shifting of paradigm from worrying "hidden hunger" status to sufficient healthy society. In this way, we can achieve the goal of "end malnutrition" and "zero hunger" by 2030 and sustain food security.

2.8 Future Prospects

Genomic selection can be a potential approach in the introgression of complex polygenic traits/QTLs. GWAS (genome-wide association mapping) can map-wide variability and identify many more potential trait-specific QTLs. The various assays to verify the bioavailability of micronutrients using transgenic technologies, *overexpression analysis*, *knockout gene*, etc., can be done in the future to assess the stable expression of introgressing gene/QTL. More strategies for biofortification in crops like sorghum, oats, barley, rye, and millets need to be implemented for an environmentally friendly way of enhancing nutrients.

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