Genomic Interventions for Wheat Biofortification

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Wheat is an essential constituent of cereal-based diets, and one of the most significant sources of calories. However, modern wheat varieties are low in proteins and minerals. Biofortification is a method for increasing the availability of essential elements in the edible portions of crops through agronomic or genetic and genomic interventions. Wheat biofortification, as a research topic, has become increasingly prevalent.

wheat biofortification QTLs genomic selection protein minerals

1. Introduction

Malnutrition impacts more than two billion individuals, and Asia and Africa are the most affected regions [1]. Biofortification is an approach for improving the levels of vital ingredients, like vitamins, minerals, and proteins, in the edible portions of crops through conventional breeding, as well as biotechnological and genomics approaches [2][3]. While minerals and vitamins are commonly provided as dietary supplements, they are out of reach for most people living in the developing world [4][5]. Biofortification is a one-time expense that provides a cost-effective, long-term, and sustainable method for combating concealed starvation [6]. Much of the population is dependent on cereals for their dietary requirements; therefore, the biofortification of cereals is essential [7]. Wheat is globally traded more than any other crop, and it is the second most-produced cereal, following maize [8]. Moreover, annual wheat production has almost tripled since the 1940s, and it is anticipated that this trend will continue, as the population continues to grow [9]. Biofortified wheat could help reduce starvation-related malnutrition, with which primarily low-income countries are faced [10].

A large variation in grain iron and zinc concentrations is found in the wild relatives, like *Aegilops tasuchii*, of wheat and continues to be exploited for the enhancement of modern elite cultivars [11]. Provitamin A is an additional essential nutrient, and it is promoted in wheat via breeding through biofortification. Some breeds of durum wheat are higher in their provitamin concentration and yellow pigment content material, due to the presence of carotenoids (xanthophyll and lutein), and this is an essential trait for improving the antioxidant content in wheat cultivars [12][13][14]. Similarly, the enhancement of the anthocyanin content in wheat is an important focus of wheat biofortification programs. Colored wheat (black, blue, and purple), due to the high concentration of phenolics, is utilized in several breeding programs, and certain varieties of it have already been released in several nations [3][15][16].

Several agronomic methods can lead to wheat biofortification [17]. However, augmenting mineral concentrations exclusively via agronomic practices, such as foliar sprays, is associated with high expenses for farmers [18]. This agronomic biofortification is a short-term solution to increasing micronutrient (especially Fe and Zn) availability. As these fertilizers are expensive, they cannot be accessed by resource-poor farmers of low-income countries. In contrast, genetic biofortification is considered a more sustainable solution for the long term. Next-generation sequencing is proving to be useful in the determination of precise information on cultivated crops. Moreover, developments in next-generation sequencing and statistical methods can aid in the identification of the regions within the wheat genome that is responsible for its higher mineral and other biofortification traits [19][20][21][22][23]. Recommended dietary allowances (RDAs) for important minerals, like iron, zinc, and selenium, are given in **Table 1**.

Table 1. Recommended dietary allowances (RDAs) for iron, zinc, and selenium [24].

	Iron (in mg)				Zinc (in mg)				Selenium (in Micrograms)			
Age	**M**	**F**	**P**	**L**	**M**	**F**	**P**	**L**	**M**	**F**	**P**	**L**
Birth to 6 months	0.27	0.27			4	4			15	15		
7 months to 3 years	11	11			5	5			20	20		
4–8 years	10	10			12	12			30	30		
9–13 years	8	8			23	23			40	40		
14–18 years	11	15	27	10	34	34	34	34	55	55	60	70
19 + years	8	18	27	9	40	40	40	40	55	55	60	70

M, F, P and L refer to male, female, pregnancy, and lactation, respectively.

Moreover, this is a cost-effective approach and is known to have practical values. Biofortification by breeding is accomplished in crops where the genetic variability of a trait is readily accessible from its primary, secondary, or tertiary gene pool. Wheat has a large number of underexploited wild relatives that could contribute to the genetic improvement of wheat [25][26].

Qualitative traits are generally governed by a single major gene, whereas, traits, like yield, are quantitative in nature and are, therefore, considered to be governed by several genes [27][28][29]. These qualitative traits are easier to breed than quantitative traits through conventional breeding methods. Conventional breeding, mainly backcross breeding with participatory varietal selection (PVS), has played a significant role in wheat biofortification. Participatory varietal selection (PVS) is particularly used by farmers to identify varieties with better performance. Farmers evaluate multiple traits that are important to them and encourage them through breeding in order to achieve quicker varietal development. The genomic resources of wheat have provided important support for functional genomics and conservation biology (by conserving the important landraces) [30][31]. The wheat genome is difficult to interpret, because it has broadly dispersed repetitive sequences, heterozygosity, and polyploidy [32][33]. Nevertheless, developments in sequencing methodologies, the decreased sequencing price, and advancements in computational resources have permitted the spread of these resources [34][35]. Besides, the comparative genomics

of plant species is proving to be an efficient method in the identification of novel genes with respect to the biofortification of modern wheat [36].

Various genomic approaches, such as quantitative trait loci (QTL) mapping, marker-assisted selection (MAS) and genomic selection, have been widely employed for the biofortification of wheat. There are several techniques for mapping QTL in an experimental cross [37]. The molecular basis of QTLs is challenging to dissect, even for model plants, like Arabidopsis and rice, because of the problems in precisely narrowing intervals to single genes [38]. The experimental design, type of plant population analyzed, and the level of polymorphisms between parental genomes also affect predictions of QTLs. Statistical methods for determining quantitative trait loci (QTL) require numerous molecular markers, with high-resolution genetic maps [39][40]. This approach is one of the major genomics methods that is geared toward the dissection of complex phenotypes [41]. Several QTL mapping studies have resulted in the identification of a number of stable and consistent QTLs, which can be helpful in elucidating the genetic basis of biofortification traits. Various mapping populations, such as doubled haploid lines (DH), recombinant inbred lines (RILs) or single seed descent lines (SSDs), F_2-derived F_3 ($F_{2:3}$) or F_4 ($F_{2:4}$), the $BC_3F_{2:3}$ population, and BC_5F_2:F_6 families have been used in mapping QTLs for biofortification traits (**Table 2**, **Table 3** and **Table 4**). All of these populations have their own advantages and limitations [42]. The polyploidy level of wheat has allowed researchers to study some specific homozygous populations, such as recombinant inbred chromosome lines (RICLs) and recombinant substitution lines (RSLs) [43][44][45]. Disomic inter-varietal chromosome (DIC) substitution lines have been used in the development of recombinant inbred chromosome lines (RICLs) for chromosomes 6B and 5B, with the *Triticum turgidum* (L.) var. LDN background. Each of the lines was homozygous for a particular recombined or unrecombined chromosome, and near-isogenic to LDN durum for all of the other remaining chromosomes. These populations served as the best genetic stocks for the precise mapping of the GPC gene/QTLs [43][44] (**Table 2**). An important locus, *Gpc-6B1*, has also been fine mapped (1.5cM proximal) to Xcdo365 and (1.2 cM distal) to Xucw67 using recombinant substitution lines, developed from the cross LDN(DIC-6B) × LDN [45]. Mapped QTLs/genes can be introgressed into the elite lines using MAS. The closer the marker to the QTL/gene, the better will be the prediction.

Table 2. List of the identified quantitative trait loci (QTL) for the grain protein content.

Cross	Population Type and Size	No. of Total QTLs	PVE Range (Additive Effect of QTLs)	Chromosomes/Chromosome Arms	References
Durum wheat ('Messapia) × *T. turgidum* L. *var. dicoccoides* (MG4343)	RILs (65)	6	6.0–23.5	4BS, 5AL, 6AS, 6BS, 7BS	[46]
T. turgidum (L.) var. *dicoccoides* chromosome 6B	RICLs (85)	1	66	6BS	[43]

Cross	Population Type and Size	No. of Total QTLs	PVE Range (Additive Effect of QTLs)	Chromosomes/Chromosome Arms	References
T. aestivum (PH132) × *T. aestivum* (WL711)	RILs (100)	1	18.73	2DL	[47]
T. aestivum (Courtot) × *T. aestivum* (Chinese Spring)	DH lines (187)	2	7.0–17.0	1B, 6A	[48]
T. aestivum (PH132) × *T. aestivum* (WL711)	RILs (106)	9	2.9–7.2	2BL, 7AS	[49]
T. aestivum (PH132) × *T. aestivum* (WL711)	RILs (100) and NILs (10)	1	6.2	5AL	[50]
Durum wheat (Messapia) × *T. turgidum* var. dicoccoides (MG4343)	RILs (65)	7	6.5–31.7	4BS, 6AS, 5AL, 7AS, 7BS, 6BS	[51]
T. aestivum (Opata 85) × synthetic hexaploid wheat (W7984)	RILs (114)	2		2DS, 7AS	[52]
T. aestivum (Renan) × *T. aestivum* (Récital)	RILs (194)	10	4.1–10.4	1A, 2AS, 3AL, 3BS, 4AS, 4DL, 5BL, 6AL, 7AS, 7DL	[53]
T. aestivum (WL711) × *T. aestivum* (PH132)	RILs (100)	13	2.95–32.44	7AS, 2AS, 2DL, 2BL, 3DS, 4AL, 6BS, 7DS	[54]
T. turgidum (L.) var. *dicoccoides* (LDN(Dic-5B) × LDN	RICLs (133)	3	10.0–33.0	5B	[44]
T. aestivum (Renan) × *T. aestivum* (Récital)	RILs (194)	3	6.2–9.6	3A, 4D, 7D	[55]
T. aestivum (WL711) × *T. aestivum* (PH132)	RILs (110)	7	8.38–16.58	2DS, 3AL, 2AS, 1DL, 5AL, 7DL	[56]
Canadian Spring wheat (AC Karma) × *T. aestivum* (87E03-S2B1)	DH lines (185)	2	12.6–32.7	4D, 7B	[57]
T. aestivum (Opata85) × Synthetic hexaploid wheat (W7984)	114 RILs	4	15.0–32.0	2DS,5AL,6DS	[58]
T. aestivum (Arche) × *T. aestivum* (Recital)	DH lines (222)	13	5.5–24.7	2D, 4B, 2A, 1B, 3B, 3D, 5A, 5B, 7D	[59]

Cross	Population Type and Size	No. of Total QTLs	PVE Range (Additive Effect of QTLs)	Chromosomes/Chromosome Arms	References
T. aestivum (Chuan 35050) × *T. aestivum* (Shannong 483)	RILs (131)	3	8.64–21.23	5AL, 3BL, 6AS	[60]
T. aestivum (Neixiang188) × *T. aestivum* (Yanzhan)	RILs (198)	16	3.2–14.5	3B, 2B, 1B, 2A, 2B, 3A, 4D, 5B, 5D, 7B, 7D	[61]
T. aestivum (kukri) × *T. aestivum* (Janz)	DH lines (160)	13		1B, 2A, 3AS, 3B, 4B, 4D, 5A,5B,7AL, 7D	[62]
Indian durum wheat (PDW 233) × Bhalegaon 4 (a landrace)	RILs (140)	1	9.64	7B	[63]
Durum wheat (Langdon) × Wild emmer accession (G18–16)	RILs (152)	10	2.8–9.7	2AL, 2BL, 3BL, 4AL, 5AS, 5BL, 6AS, 6BL, 7AL, 7BS	[64]
T. aestivum (Chara) × an advanced breeding line (WW2449)	DH lines (190)	1	20	4A	[65]
Durum breeding line (DT695) × Durum wheat cultivar (Strongfield)	DH lines (185)	9	16–46	2B, 7A, 1A, 1B, 2A, 5B, 6B, 7A	[66]
Chinese hard wheat line (Ning7840) × Soft wheat cultivar (Clark)	RILs (132)	2	11.2–16.8	3AS, 4B	[67]
T. aestivum (MN98550) × *T. aestivum* (MN99394)	RILs (139)	3	4.5–16.8	5AL, 2BS, 6DL	[68]
T. aestivum (Huapei 3) × *T. aestivum* (Yumai 57)	DH lines (168)	4	3.09–8.40	3A, 3B, 5D, 6D	[69]
Durum breeding line (C1113) × Durum cultivar (Kofa)	RILs (93)	10	9.3–21.6	3BS, 7BL, 5AS, 2BS, 4AL, 5BL, 2AL, 1BS, 7AS, 3BL	[70]
Svevo × Ciccio (both elite durum wheat cultivars)	RILs (120)	10	7.8–40.2	3BS, 2BL, 1AL, 4AL, 2AS, 4BL, 1AS, 6BS, 5AL, 7BL	[71]
Oste-Gata × Massara-1 (durum wheat genotypes)	F_2 derived F_3 and F_4 lines (151)	2	5.31–9.44	1A, 5BL	[72]

Cross	Population Type and Size	No. of Total QTLs	PVE Range (Additive Effect of QTLs)	Chromosomes/Chromosome Arms	References
T. aestivum (Weimai 8) × *T. aestivum* (Jimai 20)	RILs (485)	9	3.06–9.79	2B, 3A, 4A, 4D, 5B, 7A, 7B	[73]
T. aestivum (Weimai 8) × *T. aestivum* (Yannong 19)	RILs (229)	10	6.29–53.04	5A, 1A, 2D, 1B, 4B, 2A, 3A, 5D, 6B, 7D	[73]
Synthetic wheat (Am3) × Synthetic wheat (Laizhou953)	BC_5F_2:F_6 families (82)	9	2.2–11.5	6A, 1A, 2D, 3A, 4B, 5D, 6B, 6D, 7B	[74]
T. aestivum (BR34) × *T. aestivum* (Grandin)	RILs (118)	1	16.3	5BL	[75]
T. aestivum (Weimai 8) × *T. aestivum* (Luohan 2)	RILs (302)	7	4.15–9.73		[76]
T. aestivum (Xiaoyan 54) × *T. aestivum* (Jing 411)	RILs (182)	5	1.14–9.25		[77]
T. aestivum (CO940610) × *T. aestivum* (Platte)	DH lines (185)	5	5.6–12.3	5BS, 6AL, 6BS, 7BS, 7DL	[78]
T. aestivum (Choteau) × *T. aestivum* (Yellowstone)	RILs (97)	2	17–19	3B, 5B	[79]
T. aestivum (Huapei 3 × Yumai 57; Nuomai 1× Gaocheng 8901; Shannong	DH lines (68), RILs (256), RILs (182)	13	0.84–10.51	2A, 1B, 1D, 2B, 2D, 3B, 4B, 5B, 6D, 7A	[80]

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Cross	Population Type and Size	No. of Total QTLs	PVE Range	Chromosomes	References
T. aestivum Hanxuan10 × *T. aestivum* Lumai 14	DH (119)	Zinc conc.-4 and Zn content-7	5.3–11.9; 4.6–14.6	4D, 5A, 4A, 7A and 7A, 2D, 1A, 3A, 4A, 4D, 5A,	[91]
T. aestivum (RAC875-2) × *T. aestivum* (cascades)	DH (90)	GZn-4; GFe-1		3D, 4B, 6B, 7A, 3D	[92]
T. boeoticum (Tb5088) × *T. monococcum* (Tm14087)	RIL (93)	GZn-2; GFe-3	7.0–12.6; 9.0–18.8	7A, 2A, 7A	[93]
Durum wheat (cv. Langdon) and wild emmer (accession G18-16)	RIL (152)	GZn-6; GFe-11	1.3–23.5; 0.8–17.8	2A, 7A, 5A, 6B, 7B, 5A, 7A, 2A, 2B, 3A, 3B, 4B, 5A, 6A, 6B, 7B	[64]
T. aestivum (Xiaoyan 54) × *T. aestivum* (Jing 411)	RIL (182)	GZn-2; GFe-2	4.23–6.88;	4B, 5A, 5A	[77]

Cross	Population Type and Size	No. of Total QTLs	PVE Range	Chromosomes	References
			3.27–3.43		
T. aestivum (Hanxuan 10) × *T. aestivum (Lumai 14)*	DH (120)	GFe-4	6.1–14.6	5A, 4D, 7A, 7B	[94]
T. aestivum (Tabassi) × *T. aestivum* (Taifun)	RIL (118)	GZn-2; GFe-6	40.22–50.79; 8.94–47	4A, 1A, 7B, 3D, 4D, 2A, 7D	[95]
T. aestivum (PBW343) × *T. aestivum* (Kenya Swara)	RIL (177)	GZn-3	10-15	1BS, 2B, 3AL	[96]
Synthetic hexaploid (SHW-L1) × *T. aestivum* (Chuanmai 32)	RIL (171)	GZn-4; GFe-4	5.5–8.6; 5.4–9.5	2D, 3D, 4D, 5D, 2B, 5B, 5D, 7D	[97]
T. aestivum (Chuanmai 42) × *T. aestivum* (Chuannong 16)	RIL (127)	GZn-3; GFe-4	13.8–15.9; 9.2–19.1	5B, 3D, 4D, 4A, 5A, 4D, 5B	[97]
T. spelta (PI348449) × *T. aestivum* (HUW 234)	RIL (185)	GZn-5; GFe-5	4.25–16.46; 5.6–25.95	2B, 2A, 3D, 6A, 6B, 1A, 3B, 2A	[98]
T. aestivum (Berkut) × *T. aestivum* (Krichauff)	DH (138)	GZn-2; GFe-1	23.1–35.9; 22.2	1B, 2B, 2B	[87]
T. aestivum (SeriM82) × *T. dicoccoides/Ae. Tauschii* (SHW CWI76364)	RIL (140)	GZn-3; GFe-5	8.3–17.3; 7.5–14.5	4BS, 6AL, 6BL, 4BS, 7DS, 2BL, 2DS, 6AL,	[99]
T.aestivum (Adana99) × *T. sphaerococum* (70711)	RIL (127)	GZn-10; GFe-7	9–31; 9–18	1D, 6B, 7B, 7A, 3A, 1B, 2B, 3D, 6A, 6B, 7B, 6B, 2B, 7B, 1B, 2A, 2B, 3A,	[100]
T. spelta (Bubo) × resynthesized hexaploid wheat (Turtur)	RIL (188)	GZn-4; GFe-3	2.86–16.75; 5.49–10.35	1B, 7B, 6A, 3A, 4B, 5B	[101]
Synthetic hexaploid wheat (Louries) × *T. spelta* (Bateleur)	RIL (188)	GZn-12; GFe-7	3.30–32.79; 5.79–21.14	1A, 1B, 3B, 7B, 3D, 4A, 5B, 6A, 7D, 5B, 2A, 4D, 4A, 2B, 3B, 5B	[101]

Cross	Population Type and Size	No. of Total QTLs	PVE Range	Chromosomes	References
T. aestivum (WH542) × synthetic derivative (*Triticum dicoccon* PI94624/*Aegilops sqarrosa* [409]/BCN)	RIL (286)	GZn-5; GFe-4	3.2–14.4; 2.3–6.8	2A, 4A, 5A, 7A, 7B; 2A, 5A, 7A, 7B	[102]
T. aestivum (SHW-L1) × *T. aestivum* (Chuanmai 32),	RILs (171)	4	6.4–28.5	5B, 3D, 7D	[97]

Cross	Population Type and Size	No. of Total QTLs	PVE Range (Additive Effect QTLs)	Chromosomes/Chromosome Arms	References
T. aestivum (Schomburgk) × *T. aestivum* (Yarralinka)	SSD lines (150)	2	13–41	3A, 7A	[106]
T. turgidum L. var. durum (Omrabi5) × *T. dicoccoides* (acc.600545)	RILs (114)	3	6.0–53.0	7AL, 7BL	[107]
T. aestivum (Trident) × *T. aestivum* (Molineux)	DH lines (182)	1	48–77	7B	[108]
T. turgidum L. var durum (W9262-260D3) × *T. turgidum* L. var. duram (Kofa)	DH lines (155)	4	14–23	4B, 2A, 6B, 7B	[109]
T. aestivum (PH82-2) × *T. aestivum* (Neixing 188)	RILs (240)	1	20–28	7AS	[110]
T. turgidum L. var durum (PDW 233) × *T. turgidum* L. var durum (Bhalegaon 4)	RILs (140)	5	5–55.22	7A, 1A, 3B, 5B, 7B	[111]
T. turgidum L. var durum (UC1113) × *T. turgidum* L. var durum (Kofa)	RILs (93)	1		7A	[112]
T. aestivum (PH82-2) × *T. aestivum* (Neixiang 188)	RILs (240)	4	1.5–33.9	1B, 7A, 1A, 4A,	[113]
T. turgidum L. var durum (Latino) × *T. turgidum* L. var durum (Primadur)	F2:F3 families (121)	5	9.4–53.2	2A, 3B, 5A, 7A	[114]
Ajana × WAWHT2074; Carnamah × WAWHT2046;	DH lines (179, 121, 127)	6	4.0–36.0	7B, 2D, 3A, 7A, 4D, 5B, 7B	[115]

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Cross	Population Type and Size	No. of Total QTLs	PVE Range (Additive Effect QTLs)	Chromosomes/Chromosome Arms	References
Ajana × WAWHT2046 (all *T. aestivum*)					
T. turgidum L. var durum (UC1113) × *T. turgidum* L. var durum (Kofa)	RILs (93)	15	6–42.7	1BL, 4AL, 7BL, 6AL, 2AS, 5AS, 5AL, 5BL, 7AS, 7AL, 7BS	[116]
T. aestivum (Chuan 35050) × *T. aestivum* (Shannong 483)	RILs (131)	13	4.1–16.5	5B, 6A, 1A, 1B, 2D, 4A, 4D, 5D, 6D, 7B	[117]
T. turgidum L. var durum (Svevo) × *T. turgidum* L. var durum (Ciccio)	RILs	7	19.3–51.6	1B, 5B, 7A, 2A, 2B, 5A, 7B	[118]
T. aestivum (Gaocheng 8901) × *T. aestivum* (Zhoumai 16)	RILs (176) [123]	16	5.7–30.8	5AL, 2DL, 5BS, 1B.1R, 2AL, 2B-1, 5AS, 5BL, 6BL, 7AS, 7BL	[119]

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genomic selection (GS) or genome-wide selection (GWS), was proposed to overcome the above-mentioned problems, as well as other problems, associated with QTL mapping and/or association mapping [124]. GS predicts the genetic value of selection candidates or individuals based on the genomic estimated breeding values (GEBVs), predicted from highly dense markers, located throughout the genome. Compared to MAS, GEBV captures the largest portion of the genetic variation for the particular trait under selection, owing to the inclusion of all markers, including both minor and major effects, in the determination of the GEBV [124].

2. Biofortification for the Grain Protein Content

Grain protein content (GPC) is among the important traits that contribute to the nutritional value, processing preference, quality of the end products (bread and pasta) and market value of both hexaploidy (*Triticum aestivum* L.) and durum (*T. turgidum* L. var. durum Desf.) wheat. The economic value of wheat grains relies on their GPC; therefore, an improvement in GPC and alteration in the composition of storage proteins in wheat grain have been a significant objective in wheat breeding programs, particularly for those working toward raising the nutritional quality [125]. Several efforts have been made by breeders to improve the GPC in wheat using conventional breeding methods, but the desired outputs could not be obtained. The reasons for this may include: (1) The significant influence of the environment on GPC; (2) negative correlation of GPC with grain yield; and (3) complex quantitative genetic control of GPC and its low heritability [126]. The QTLs for GPC have been identified and mapped on almost all of the chromosomes of both tetraploid and hexaploidy wheat.

To our knowledge, for GPC, a total of 325 main-effect QTLs have been reported so far using biparental populations. Among all the QTLs identified for GPC, the most critical QTL identified so far is *Gpc-B1*. This QTL was first detected in a wild accession (FA-15-3) of tetraploid wheat, *Triticum turgidum* var. dicoccoides [127]. Later on,

the same accession was used to produce a complete set of chromosome substitution lines, with the background of modern durum wheat [128]. Using substitution lines, the *Gpc-B1* gene was then mapped on chromosome arm 6BS, which explained 66% of the phenotypic variation of the GPC [45]. Gpc-B1 was cloned using a map-based cloning approach, and it was found that *Gpc-B1* encodes a NAC transcription factor (NAM-B1), which accelerates the senescence and also affects the grain protein, zinc, and iron content in wheat [129]. The introgression of the functional *GPC-B1* allele in the background of elite lines has resulted in the release of several varieties in different countries [130].

While major QTLs, with a large effect on GPCs, have been identified in various studies, most of the identified major QTLs were unfortunately found to be unstable across the environments (**Table 2**). Here, we listed the cross, population and its size, number of total QTLs, including all stable or unstable QTLs over the locations, which were consistent or inconsistent over the years, PVE range of QTLs, and chromosomes/chromosome arms, where QTLs are mapped (**Table 2**).

2.1. Epistatic Interactions for the Grain Protein Content

The development of efficient statistical and genomic tools has allowed geneticists to identify and map the QTLs involved in epistatic interactions for GPC [56][63][69][70][77]. Software, like QTLMapper [131], QTLNetwork [132], and IciMapping [133], have frequently been used to locate epistatic QTLs. In general, there are three different types of epistatic interactions. The first type comprises the interaction between two main-effect QTLs, in which each QTL has a noteworthy effect of its own (M-QTL × M-QTL or QQ epistatic interaction). The second type comprises the interaction between main-effect QTLs and epistatic QTLs (E-QTL), where the E-QTL does not have a noteworthy effect of its own, but it shows a significant effect when it interacts with another QTL (M-QTL × E-QTL). The third type comprises the interaction between two epistatic QTLs (E-QTL × E-QTL). In addition to these epistatic interactions, the interaction of QTLs with the environment is quite common, such as QTL × environment (QE) and QTL × QTL × environment (QQE) interactions [56]. Kulwal et al. [56] identified four QTLs involved in two digenic QQ epistatic interactions in one RIL population and six E-QTLs involved in three digenic QQ epistatic interactions in another RIL population of bread wheat. However, these interactions only accounted for 2.68% and 6.04% of the phenotypic variation in the first and second population, respectively. Patil et al. [63] identified one pair of epistatic interactions using a RIL population derived from a durum wheat cross between PDW 233 and Bhalegaon 4. Zhao et al. [69] identified two digenic epistatic interactions for GPC, both only involving E-QTL, using a population developed from two Chinese wheat cultivars. Conti et al. [70] identified five pairs of epistatic interactions for GPC, in addition to one QQE interaction using a RIL population, obtained from the cross between the UC Davis wheat breeding line, UC1113, and the Kofa variety. In another study, Xu et al. [77] identified two significant digenic interactions, involving four M-QTLs, for GPC using a RIL population developed from Chinese cultivars.

We believe that the QTLs involved in different types of interactions could contribute considerably to the total observed variation of a quantitative trait, and so they should not be ignored. The genetic control of a complex trait can be efficiently understood by dissecting these interactions. The above-mentioned different types of interactions, such as QQ, QE and QQE, observed for the GPC in wheat, make sense in light of the present knowledge on

molecular genetics, where DNA and protein interactions, protein and protein interactions, and epigenetic variations have been revealed to be directed by changes in the environment. It has been evidenced by the characterization of cloned QTLs from higher plants that several QTLs do not directly contribute to the observed variation for the concerned trait, but may instead be either directly involved in interacting with a coding sequence (CDS) or else their gene products, e.g., mRNA or protein may be involved in such interactions [134][135]. It has been suggested that an interaction between a CDS and a regulatory sequence may be significant for the expression of a trait [136].

2.2. Different Prospects of GS for Wheat Biofortification with Protein

Phenotypic selection (PS), MAS, and GS prediction accuracy have been compared in wheat breeding programs. In one study, Heffner et al. [137] compared the above-mentioned three strategies for thirteen agronomic traits, including grain protein content, using a population of 374 winter wheat advanced cycle breeding lines. To evaluate the effects of model selection, the training population size, and the marker density in the presence of a G × E interaction, a cross-validation approach was employed. Heffner et al. observed 28% and 95% higher prediction accuracies with GS, compared to MAS and PS, respectively, providing empirical evidence of the advantage of multifamily GS in wheat breeding. In another study, they compared the phenotypic- and marker-based prediction accuracy of the genetic value of nine different grain quality traits, including flour protein content in two doubled haploid biparental soft winter wheat populations, ‘Cayuga’ × ‘Caledonia’ and ‘Foster’ × ‘KanQueen’ [138]. They reported a significantly greater prediction accuracy with GS, compared to MAS, for all nine traits. With the training population sizes of 96, 48, and 24, they achieved GS accuracy to PS accuracy average ratios of 0.66, 0.54, and 0.42, respectively.

The simultaneous improvement of GPC and grain yield has been a major challenge in wheat breeding, owing to a severe negative trade-off. Simultaneous GS, for grain yield and GPC, using various breeding strategies in the form of selection indices, may be a good approach to mitigating the negative trade-off between these two important traits [139]. Michel et al. compared two breeding strategies based on various “genomic selection indices, viz., (a) to select high-protein genotypes with acceptable yield potential, and (b) to develop high-yielding varieties, while maintaining protein content”. They achieved promising results using the second breeding strategy. In 2016, at CIMMYT, Battenfield et al. [140] developed and validated whole genome prediction models for phenotypes with an end-use quality in the CIMMYT bread wheat breeding program and also tested the accuracy of the developed model using forward prediction on breeding lines, tested in unbalanced yield trials from 2009 to 2015 at three different locations. They phenotyped a total of 5520 breeding lines for nine different traits, including grain and flour protein. They reported a substantial increase in prediction accuracy over time, as the data available to train the model increased. The forward prediction accuracies for the studied parameters ranged from 0.32 to 0.62, and the expected genetic gain was 1.4 to 2.7 times higher than PS for all traits. The predictive abilities of the genomic predictions (GPs) can be improved using genetically correlated traits in multi-trait models [141][142]. Michel et al. [141] phenotyped more than 400 genotyped wheat lines for protein content and baking quality traits, in multi-environment trials from 2009 to 2016, and applied GS to select the best individuals in terms of their good protein content and baking quality traits, as well as grain yield. They achieved an average prediction accuracy r = 0.39 across three independent validation populations, which could be increased to r = 0.47 by modelling the major QTLs, as fixed

effects, as well as engaging multi-trait prediction models. They observed nearly twice the selection response, compared to the indirect selection, by protein content for the baking quality-related traits, using GS, which was applied 2–3 years earlier than direct phenotypic selection. Recently, in 2019, Kristensen et al. [142] studied multi-trait and trait-assisted genomic prediction models, dealing with two or four traits, including GPC, phenotyped in 1152 advanced winter wheat lines, and compared the GPs of these models with single-trait models. They observed increased predictive abilities for GPC with trait-assisted models. By including phenotypic data for GPC in the trait-associated models, they also reported increased predictive abilities for other traits, such as zeleny sedimentation, which may be advantageous for breeding programs for improving wheat quality. Overall, the above studies suggest that GS is a powerful strategy that can be used to facilitate an early generation selection for end-use quality in wheat, which can provide greater advantages in terms of both quality and yield in wheat breeding programs.

As already mentioned, an effect of the G × E interaction on the grain protein content in wheat is quite common [56][63][69][70]. Different GS models need to be designed to have better accuracy in the presence of different G × E interactions. The prediction accuracy of GS can be enhanced using multi-environment (multi-trait) models, which allow for the borrowing of information across environments (traits). An enhanced prediction accuracy has been reported in a multi-environment (multi-trait) analysis based on both pedigree and marker information, compared to those based on pedigree information only. Burgueno et al. [143] achieved better predictive accuracy than simple linear mixed models by using both marker and pedigree information in multi-environment (multi-trait) models in wheat. These types of the model may be promising for the improvement of the GPC in wheat. Furthermore, in 2015, at CIMMYT, a marker × environment interaction (M.E) genomic selection model was developed. The researchers analyzed three CIMMYT wheat data sets, where more than 1000 lines were genotyped using GBS and evaluated at CIMMYT's research station under controlled environmental conditions using this M·E model, and proposed that the interaction model may provide information on the variants that have stable effects across environments and those which are responsible for G × E interactions [144]. It has also been shown that a sizeable proportion of the prediction accuracy is due to the pedigree or population structure [145]. When prediction equations were trained by predictions from unrelated populations, the prediction accuracy became negligible. However, an increase in the prediction accuracy was achieved when the genomic prediction included modeling GE, in which information from correlated environments was borrowed [145].

As already mentioned, GS is the better option for breeding wheat in terms of producing grain end-use quality traits, as breeding these traits is difficult, owing to the requirement for different assays, which require flour quantities that are only obtainable late in the breeding cycle. Efforts have been made to use near-infrared (NIR) or nuclear magnetic resonance (NMR), requiring a very small amount of flour and only a subset of accessions to obtain GPs of end-use quality in wheat [146]. They derived predictions for nineteen traits with an end-use quality in 398 accessions using a multi-trait approach and obtained increased prediction accuracies, ranging from 0 to 0.69, using NIR/NMR data, compared to the control. This study suggested that the NIR and NMR predictions of quality traits can successfully overcome the chief problem associated with the use of GS in order to hasten the improvement of the traits with a grain end-use quality in wheat.

3. Biofortification for the Grain Fe and Zn Content

The agronomical biofortification method involves fertilizing plants with Zinc fertilizers, which can increase the Zn content of grain. For instance, Zhang et al. [147] reported a 58% increase in whole grain Zn and a 76% increase in wheat flour Zn using a foliar application of 0.4% ZnSO4·7H2O. In another study, Zou et al. [148] increased grain Zn by 84% and 90%, using Zn as a foliar spray. However, in the case of iron, these agronomic approaches have been less effective [149], except if combined with increased nitrogen fertilizers [150], which may not be economically acceptable.

Backcross breeding, with the participatory varietal selection operated by collaboration among CIMMYT, (El Batán, Mexico), the Indian Institute of Wheat and Barley Research (Karnal, India) and Punjab Agricultural University (Ludhiana, India), resulted in a release of zinc biofortified wheat varieties. To date, four Zn biofortified varieties have been released: 'Zinc Shakti' (developed by transferring the genes from *Ae. Squarrosa* into the Indian variety, PBW343), 'Zincol 2016′ (developed by transferring the genes from *T. spelta* into the Pakistani variety, NARC2011) and 'WB02′ and 'HPBW-01′ (developed by transferring the genes from *Ae. squarrosa* and *T. dicoccon*), with 40%, 25%, 20% and 20% increases in the zinc content in their grains, respectively [151]. These four varieties are currently being grown in India and Pakistan. Furthermore, human intervention trials to examine the effectiveness of consuming flour made from Zn biofortified wheat are presently being carried out in Pakistan [152]. While success in Zn biofortification has been achieved, no Fe-biofortified variety has been produced so far using conventional breeding methods.

3.1. QTLs for the Grain Fe and Zn Concentrations in Wheat

Marker-assisted breeding is a potential strategy for the development of Fe- and Zn-biofortified wheat. Knowledge of the genetic basis of Fe and Zn concentrations is required for the successful application of MAS. Genes acting through one or more steps, e.g., root uptake, translocation from root to shoot, storage and remobilization, may be reflected by the QTLs responsible for grain mineral concentrations. Various QTL mapping studies have allowed for the identification of many QTLs for both Fe and Zn (**Table 3**). We list the QTLs identified for both GZn and GFe concentrations in the same place, because many studies reported the QTLs for these two traits simultaneously, and some studies even colocalized the QTLs for these two traits (**Table 3**). Shi et al. [91] detected four and seven QTLs for the Zn concentration and Zn content, respectively. They suggested a possibility for improving both the grain Zn concentration and content simultaneously, because all four QTLs for the Zn concentration were co-located with the QTLs for the Zn content. The QTLs for the Zn concentration on chromosomes 4A and 4D and four QTLs for the grain Zn content on chromosome 2D, 3A and 4A were co-located with the QTLs for the P contents, indicating a possibility for simultaneously improving the grain Zn and P density in wheat.

The QTLs for grain zinc and iron have also been mapped in the populations derived from the crosses between *T. boeoticum* and *T. monococcum* [93], durum wheat and wild emmer [64], and synthetic hexaploid wheat and *T. spelta* [97][101][102]. Tiwari et al. [93] mapped 2 QTLs for grain Fe on chromosomes 2A and 7A, explaining 12.6% and 11.7% of the phenotypic variation, and 1 QTL for grain Zn on chromosome 7A, explaining 18.8% of the total

phenotypic variation, using a RIL population derived from a cross between the *T. boeoticum* accession, 'pau5088', and *T. monococcum* accession, 'pau1,4087'. Recently, in 2017, Crespo-Herrera et al. [101] identified several significant QTLs in a region, named *nQGZn.cimmyt-7B_1P2*, on chromosome 7B, explaining the largest proportion (32.7%) of the total phenotypic variance for GZn, and one QTL on chromosome 4A (*QGFe.cimmyt-4A_P2*), explaining the largest (21.14%) proportion of the phenotypic variance of the GFe in two RIL populations derived from *T. spelta* L. and synthetic hexaploid wheat crosses. In another study, Krishnappa et al. [102] mapped four QTLs, explaining 20% of the total phenotypic variation, and five QTLs, explaining 32% of the total phenotypic variation for GFe and GZn, respectively, using a RIL population derived from a cross between an Indian wheat variety, 'WH542', and a synthetic derivative. Further, they identified an association between GFe, GZn and GPC and a region in the interval of Xgwm359-Xwmc407 on chromosome 2A. The QTLs for GZn and GFe co-localized on chromosome 5A (Xgwm126-Xgwm595) and 7A (Xbarc49-Xwmc525). Furthermore, Xu et al. [77] also clearly indicated the role of epistasis in the expression of these traits in wheat grains. One QTL, located on chromosome 2A (Xgwm501-Xgwm156.2), showed an additive × additive epistatic interaction with the other QTL (Xwmc181-Xcfd267.1), located on the same chromosome 2A for GZn concentration, and one QTL on chromosome 2B (Xbarc1138.2-Xcfd238) showed the same additive × additive epistatic interaction with the other QTL (Xgwm617-Xcfa2114), located on chromosome 6A for GFe.

Several studies have identified and mapped QTLs for high GFe and GZn concentrations on different chromosomes, 1A, 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 4B, 4D,5A, 5B, 5D, 6A, 6B, 7A, 7B and 7D, found in different diploid, tetraploid and hexaploid wheat species (**Table 3**). Among these studies, some have reported a significant positive correlation between GZn and GFe across different environments, indicating the co-localization of QTL or pleiotropic effect in regulating the concentrations of both GZn and GFe in wheat grains. For instance, Tiwari et al. [93] showed the colocalization of QTLs for GZn and GFe on chromosome 7A between the flanking markers Xcfd31-Xcfa2049, and the closest markers, viz., wPt-9555 and Xcfa2019, were also mapped on the 7A chromosome, indicating an association with both GZn and GFe [64][102]. The QTLs for GZn and GFe were co-localized on other chromosomes, such as 2A [102], 2B [87], 4BS [99], 5A [77][102] and 6B [100]. The colocalizations or associations between QTLs, affecting different mineral nutrients, may be related to the physiological coupling of certain processes that regulate mineral accumulation in grains. Genomic regions regulating one or a few minerals should not be overlooked, as they might be involved in other mineral-specific mechanisms. Thus, the simultaneous mapping of QTLs for several minerals is advisable, as this will dissect their inter and intra relationships and provide insight into the functional basis of the genomic architecture, physiology and evolution of the system of mineral accumulation in the grain or any other part of wheat.

Furthermore, this colocalization of QTLs provides the opportunity to employ only one MAS program in elevating the concentrations of different minerals, for instance, utilizing both GZn and GFe simultaneously. A shift in the chromosomal locations of the QTLs may also be observed, owing to the contamination that may result in sampling and experimentation, in addition to disturbances caused by variations in the nutrition distribution and the availability of nutrients in the soil [61]. Thus, researchers need to be careful in planning the mapping of QTLs for minerals.

3.2. Breeding Strategies to Develop Zn- and Fe-Biofortified Wheat

Conventional breeding methods have been successfully employed at various research institutes, in collaboration with CIMMYT, to biofortify wheat grains using Zn [151][152]. However, the Fe content in wheat grain could not be increased using these methods. Several QTL mapping studies have been conducted, and many QTLs for GFe and GZn have been mapped on different chromosomes of wheat. Thus, now, efforts can be made to utilize the QTL information in marker-assisted backcrossing schemes to produce Zn- and Fe-biofortified wheat. The colocalization of the QTLs for GFe and GZn may further provide the opportunity to target them to simultaneously improve the concentration of both in wheat grains [102]. Moreover, in various QTL studies, some QTLs for these GFe and GZn are also co-localized with those of phosphorus [64][91], selenium [97], calcium, manganese and magnesium [64], or other agronomically essential traits, including the grain protein content [77] and thousand-grain weight [99]. Using MAS, these specific regions can be transferred to the elite wheat genotypes to simultaneously increase the contents of various minerals.

The genetic variation available for breeders is not limited. For instance, Gorafi et al. [153] assessed 47 synthetic wheat lines derived from the crosses between the tetraploid wheat cultivar, ‘Langdon’, and 47 *Ae. tauschii* lines, collected from different geographical areas. Grain Fe and Zn ranged from 22.2 to 78.5 (mg/kg) and 20.6 to 65.8 (mg/kg), respectively, in these synthetic wheat lines, which can be utilized as potential genetic resources for breeding wheat cultivars with high mineral content. In 2017, Magallanes-López et al. [154], reported a range from 25.7 to 40.5 mg/kg and from 24.8 to 48.8 mg/kg for zinc and iron, respectively, in 46 durum varieties. In a recent study, conducted in Iran, Amiri et al. [155] assessed five elite lines and 75 historical and modern cultivars (released or introduced from 1942 to 2012) and reported a wide range, with a mean of 72.30 ± 0.69 (mg/kg), 39.54 ± 0.51 (mg/kg), 528.92 ± 11.0 (g/ha) and 282.66 ± 5.7 (g/ha) for GFeC, GZnC, GFeY and GZnY, respectively. They grouped all assessed materials into two clusters; older genotypes and landraces with a high GFeC and GZnC were kept in one group, and the remaining genotypes and landraces with a low GFeC and GZnC, which can be utilized as parents in crossing programs, were kept in the other.

3.3. Different Prospects of GS in Wheat Biofortification with Fe and Zn

GS may also be a potential strategy for biofortifying wheat with grain zinc and iron, as indicated by Velu et al. [156] in 2016. They recorded genomic and phenotypic data from 330 diverse wheat lines (Harvest Plus Association Mapping panel) to determine the GPs for GZnC and GFeC and two agronomical traits. The estimated genomic predictions ranged from 0.331 to 0.694 for Zn and from 0.324 to 0.734 for Fe. In a study carried out using wheat landraces from Afghanistan, a moderate to high accuracy for major elemental contents (Mg, K, and P) and a low to moderate accuracy for minor elements (Mn, Fe, and Zn) were achieved with the GPs. This level of prediction accuracy is sufficient to consider selecting desirable individuals using marker information alone [157]. Alomari et al. [158] confirmed the usefulness of GPs in exploring the genetic base of GFeC for breeding programs that aim at the biofortification of bread wheat with iron. The GPs for the GFeC trait varied from low to moderate values.

4. Biofortification for the Grain Selenium Content

Selenium (Se), an essential mineral element, is incorporated in proteins to make seleno-proteins, which plays a critical role in human health. These seleno-proteins are necessary antioxidant enzymes that prevent cellular damage from free radicals, resulting in the prevention of chronic diseases, such as cancer and heart disease [159]. In staple crops, Se is present at a low concentration (<100 mg of Se kg^{-1}), so the genetic resources with a high amount of Se, as well as the genes/QTLs controlling the Se concentration, need to be identified to breed the Se-rich varieties, either using the conventional or molecular breeding approach, such as MAS [104].

There are conflicting reports on the amount of genetic variability among the wheat cultivars for the Se density in grain. Some studies have found no evidence of genetic variability [160][161], while another found a higher Se density in wheat grains [162]. A higher density of Se could be due to a more efficient uptake system of the tested species. The Se density in wheat grain was about 16 µg kg^{-1}, which is insufficient to meet the Se requirement for humans [161]. In 2005, Lyons et al. [163] surveyed 665 ancestral and wild relatives of wheat, wheat landrace accessions, populations, and commercial cultivars, grown in Mexico and Australia, for the Se concentration in grain. For the precise assessment of the genotypic variation in the Se density, field trials were also conducted on CIMMYT's field in the Yaqui Valley, near Ciudad Obregon, Sonora, Mexico, under similar conditions. They found the grain Se concentrations to be within a range of 5–720 µg kg^{-1}, but unfortunately, much of this variation was correlated with the spatial variation in soil selenium, and no significant genotypic variation in the grain Se density was observed among commercial bread or durum wheat varieties. However, a Se grain concentrations, that was 42% and 35% higher, was found in *Aegilops tauschii* and rye, respectively. On the other hand, Piergiovanni et al., in 1997 [162], found significant differences between emmer (*T. dicoccon* Schrank) and spelt (*T. spelta* L.) accessions and wheat cultivars with higher contents of Se, Li, Mg, P and Zn. For the Se content, they reported a range from 1.9 to 5.8 µg/100 g, with a mean of 3.9 µg/100 g, and from 1.8 to 3.5 µg/100 g, with a mean of 2.8 µg/100 g, for the spelt and emmer accessions, respectively.

QTLs for the Se Content in Wheat Grain

Knowledge of the underlying genetic mechanism of the Se content is a necessary step for the Se biofortification of wheat. QTL mapping aids in the understanding of the genetic basis, but unfortunately, only a small number of QTL mapping studies, in which the QTLs for the Se concentration in wheat grain are mapped, have been conducted [97][103][104][105]. In 2014, Pu et al. [97] identified a total of 39 QTLs for five micronutrient (Se, Fe, Zn, Cu and Mn) concentrations using two RIL populations derived from the crosses between SHW-L1 (synthetic hexaploid wheat) and Chuanmai 32 and, Chuanmai 42 and Chuannong 16. In the first population, they mapped four QTLs on chromosomes 3D, 4A, 5B, and 7D, explaining 6.4–28.5% of the genetic variance, while in the second population, they mapped only one QTL on chromosome 4D, revealing 35.1% of the genetic variance for the Se concentration in wheat grain. Wang et al. [103] mapped 16 QTLs (seven at the seedling stage and nine at the adult stage) for six Se content-related traits on eight chromosomes, *viz.*, 1B, 2B, 4B, 5A, 5B, 5D, 6A, and 7D, using a RIL population derived from a cross between two Chinese winter wheat varieties (Tainong18 and Linmai6) under both field-grown and hydroponic conditions. Each mapped QTL explained between 7.37% and 20.22% of the total phenotypic variance for the Se content. Recently, in 2018, Pu et al. [104] documented a Se-rich synthetic wheat line for the first time and mapped a total of 24 QTLs for the Se component traits on chromosomes 1B, 3D, 5A, 6A, 6B, 6D and 7D.

Notably, a QTL, located on chromosome 3D (marker interval: 214.00–218.00, Qse.sau-3D), explained the maximum amount (up to 28.38%) of genetic variation.

In another study, Yan et al. [105] mapped a total of 15 QTLs on chromosomes 1A, 1B, 2B, 3A, 4B, 5A, 6A, 7A, and 7B, explaining 1.4% to 18.6% of the phenotypic variation for GSeC (grain Se conc.) and GSeY (grain Se yield) using a RIL population derived from a cross between *T. dicoccoides* (accession G18-16) and Langdon (Durum wheat). These findings provided various main-effect QTLs and their linked markers, which can be utilized in the MAS program for the Se biofortification of wheat grain. QTLs regulating the selenium concentration in wheat grains have been mapped on chromosomes 1A, 1B, 2B, 3A, 3D, 5A, and 5B, as well as homeologous groups of 4 and 6 chromosomes (**Table 3**). Pu et al. [97] identified a QTL on chromosome 4D, which explained 35.1% of the total phenotypic variance for the Se content in common wheat. The details are provided in **Table 3**.

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