QTLs Identified for Biofortification Traits in Wheat: A Review

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Abstract: Wheat is the essential constituent of cereal-based diets and one of the most significant sources of calories. However, there is an inherently low bioavailability of proteins, mineral, and vitamins in modern wheat grains. Biofortification has earned recognition as an outstanding approach, at the same time as a cure for world hunger. The developments in the identifications of quantitative trait loci (QTL) analysis and understanding of the physiological and molecular basis of QTLs controlling the biofortification traits in wheat has revealed new horizons for the improvement of modern wheat varieties. Within this review, we have compiled the information from the studies carried out in wheat using QTL mapping methodologies that is among the best methods for biofortification traits. We hope this review will serve as an essential reference for the QTLs identified for the several important biofortification traits in wheat.

Keywords: wheat; biofortification; QTLs; 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, biotechnological and genomics approaches [2,3]. Although minerals and vitamins are commonly provided as the dietary supplements, they are out of the reach of most of the people living in the third world [4,5]. Biofortification is a one-time expense that provides a cost-effective, long-term, and sustainable method in combating concealed starvation [6]. People are mostly dependent on the supply of cereals for their dietary requirements; therefore, biofortification of cereals is essential [7]. Wheat is globally traded more than any other crop, and it is the second most produced cereal next to maize [8].

Moreover, wheat annual production has almost tripled since 1940s, and it is anticipated that the yield increment trend of wheat will continue [9]. The biofortified wheat will be useful for the starvation-related malnutrition issue faced primarily by the reduced earnings nations [10].

Large variation for grain iron and zinc concentrations is found in the wild relatives like Aegilops tasuchii of wheat and continues to be exploited for enhancement of modern elite cultivars [11]. Provitamin A continues to be an additional essential nutrient focused for biofortification in wheat via breeding. Accessions of durum wheat are higher in provitamin concentration, and yellow pigment content material due to the presence of carotenoids (xanthophyll and lutein) in durum wheat is an essential trait for improving the content of antioxidant in wheat cultivars [12, 13, 14]. Similarly, enhancement of anthocyanins content in wheat has also an important focus of wheat biofortification programs. Coloured wheat (black, blue, and purple) trait due to the high concentration of phenolics continues to be utilized in several breeding programs and varieties are already 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 indicate high expenses for farmers [18]. Next-generation sequencing is proving useful in the determination of the precise information of cultivated crops. Moreover, developments in next-generation sequencing and statistical methods can aid the identification of the regions within the wheat genome responsible for higher mineral and vitamin content [19, 20, 21]. QTL or linkage mapping methods are employed when the mapping populations are established [22, 23]. Biofortification by breeding continues to be accomplished in crops when genetic variability is readily accessible from the primary, secondary, or tertiary gene pool of crop. When genetic variability is unavailable or difficult to exploit, the genetic transformation is the better approach [24]. Wheat has a large number of wild relatives...
that could contribute to underexploited wild relatives [25, 26]. The different steps involved in the development of biofortification wheat with the aid of marker-assisted breeding are presented in Figure 1.

**Figure 1.** Representation of different strategies for biofortification of the wheat, especially explaining different steps of marker-assisted breeding.

Conventional breeding methods are easy to operate with qualitative traits. These traits depend on a single gene whereas traits like yield are quantitative and therefore are impacted by several genes [27, 28, 29]. There are several techniques for mapping quantitative trait loci (QTL) in an experimental cross [30]. 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 [31]. Experimental design, type of plant population analysed and the level of polymorphisms between parental genomes also affect the predictions of QTLs. Statistical methods to determine quantitative trait loci (QTL) require numerous molecular markers with high-resolution genetic maps [32, 33]. This approach is also related to genomics methods which are geared toward the dissection of complex phenotypes [34].

Genomic resources of wheat have provided important support for functional genomics and conservation biology (by conserving the important landraces) [35, 36]. The wheat genome is complex to interpret simply because of broadly dispersed repetitive sequences, heterozygosity, and polyploidy [37, 38]. Nevertheless, the developments in sequencing methodologies, decreased sequencing price, together with the advancements in the computational resources have permitted the spread of these resources [39, 40]. Besides, the comparative genomics among plant species is demonstrating to be an efficient method for the identification of novel genes regarding the biofortification of modern wheat [41]. Therefore, in this review, we have compiled the QTLs
identified for protein and amino acids, mineral elements and pigments like anthocyanin. This review will be a useful and important resource for the wheat breeders to refer to consider the biofortification of modern wheat.

2. Biofortification for 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 the hexaploid (Triticum aestivum L.) and durum (T. turgidum L. var. durum Desf.) wheat. Based on the extraction methods, grain proteins are grouped into four groups, albumins, globulins, prolamins, and glutelins. The economic value of the wheat grains rely on the GPC; therefore, 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 [42]. Attempts have been made by breeders for improvement in GPC using conventional breeding methods, but the desired outputs are not obtained. The reasons for this include (1) high influence of environment on GPC, (2) negative correlation between GPC and grain yield, and (3) quantitative genetic control of GPC and low heritability [43]. The QTLs for GPC have been identified and mapped on almost all chromosomes of both tetraploid and hexaploid wheat. To our knowledge, for GPC a total of 325 main effect QTL have been reported so far using biparental populations (Table 1). Here, we listed the closely linked markers or marker intervals for the significant QTLs (PVE>10), which may be useful to introgress the target QTLs in elite lines (Table 1). Although major QTLs with a stable effect on GPC across environments are also identified, most of the identified major QTLs were found unstable across the environments.
| Cross | Population type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/chromosome arms | Marker intervals / nearest markers for major QTL (PVE) | References |
|-------|--------------------------|-------------------|----------------------------------|-----------------------------|------------------------------------------------------|------------|
| Durum wheat ('Messapia) × T. turgidum L. var. dicoccoides (MG4343) RILs (65) | 6 | 6.6-27.7 | 4BS, 5AL, 6AS, 6BS, 7BS | - | [44] |
| T. turgidum (L.) var. dicoccoides chromosome 6B RICLs (85) | 1 | 66 | 6BS | Xabg387-6B-Xmgw79-6B (66) | [45] |
| T. aestivum (PH132) × T. aestivum (WL711) RILs (100) | 1 | 18.73 | 2DL | wmc41 (18.73) | [46] |
| T. aestivum (Courtot) × T. aestivum (Chinese Spring) DH lines (187) | 2 | 7.0-17.0 | 1B, 6A | XE38M60200 (17) | [47] |
| T. aestivum (PH132) × T. aestivum (WL711) RILs (106) | 9 | 2.9-7.2 | 2BL, 7AS | - | [48] |
| T. aestivum (PH132) × T. aestivum (WL711) Durum wheat (Messapia) × T. turgidum var. dicoccoides (MG4343) RILs (65) | 7 | 6.5-31.7 | 4BS, 5AL, 6A, 6BS, 7AS, 7BS | Xpsr627 (10.2), Xutv913 (12.6), Pan2 (14.8), Xcdo412 (14.9), Xpsr167 (18.4), Gai-1 (31.7) | [50] |
| T. aestivum (Opata 85) × synthetic hexaploid wheat (W7984) RILs (114) | 2 | 2DS, 7AS | - | - | [51] |
| Breed Combination | Type | Genotypes | RILs | 2017-2018      | 2018-2019      | 2019-2020      | 2020-2021   |
|-------------------|------|-----------|------|----------------|----------------|----------------|-------------|
| *T. aestivum* (Renan) × *T. aestivum* (Récital) | RILs | 1A, 2AS, 3AL, 3BS, 4AS, 4DL, 5BL, 6AL, 7AS, 7DL | (194) | 10 | 4.1-10.4 | [52] |
| *T. aestivum* (WL711) × *T. aestivum* (PH132) | RILs | 2AS, 2BL, 2DL, 3DS, 4AL, 6BS, 7AS, 7DS | (100) | 13 | 2.95-32.44 | [53] |
| *T. turgidum* (L.) var. dicoccoides [LDN(Dic-5B)] × LDN | RICLs | 5B | (133) | 3 | 10.0-33.0 | [54] |
| *T. aestivum* (Renan) × *T. aestivum* (Récital) | RILs | 1AS, 1BL, 1DL, 2AS, 2AL, 2BL, 2DS, 2DL, 3BS, 4AS, 5BL, 5DL, 6DL, 7AL, 7DS | (194) | 3 | 6.2-9.6 | [55] |
| *T. aestivum* (WL711) × *T. aestivum* (PH132) | RILs | 1AS, 2AS, 3AL, 3BS, 4AS, 4DL, 5BL, 6AL, 7AS, 7DL | (100); RILs | 7 + 26 | 8.38-16.58 | [56] |
| Canadian Spring wheat (AC Karma) × *T. aestivum* (87E03-S2B1) | DH lines | 4D, 7B | (185) | 2 | 12.6-32.7 | [57] |
| *T. aestivum* (Opata85) × Synthetic hexaploid wheat (W7984) | 114 RILs | 2AL, 2DS, 5AL, 6DS | | 4 | 15.0-32.0 | [58] |
| *T. aestivum* (Arche) × *T. aestivum* (Recital) | DH lines | 1B, 2AS, 2AL, 2DS, 3B, 3D, 4B, 5A, 5B, 7D | (222) | 13 | 5.5-24.7 | [59] |
| *T. aestivum* (Chuan 35050) × *T. aestivum* (Shannong 483) | RILs | 3BL, 5AL, 6AS | (131) | 3 | 8.64-21.23 | [60] |
| *T. aestivum* (Neixiang188) × *T. aestivum* (Yanzhan) | RILs | 1B, 2A, 2B, 3A, 3B, 4D, 5B, 5D, 7B, 7D | (198) | 16 | 3.2-14.5 | [61] |
| Cross                        | DH/RIL lines   | RIL lines   | Loci                          | p-values (bp) |
|------------------------------|----------------|-------------|-------------------------------|--------------|
| *T. aestivum* (kukri) × *T. aestivum* (Janz) | 13 DH lines (160) |  | 1B, 2A, 3AS, 3B, 4B, 4D, 5A, 5B, 7AL, 7D | [62] |
| Indian durum wheat (PDW 233) × Bhalegaon 4 (a landrace) | 1 RILs (140) | 1 | 9.64 | 7B |
| Durum wheat (Langdon) × Wild emmer accession (G18–16) | 10 RILs (152) | 2.8–9.7 | 2AL, 2BL, 3BL, 4AL, 5AS, 5BL, 6AS, 6BL, 7AL, 7BS | [63] |
| *T. aestivum* (Chara) × an advanced breeding line (WW2449) | 1 DH lines (190) | 1 | 20 | 4A Xstm506tgag–Xgwm165b (20) |
| Durum breeding line (DT695) × Durum wheat cultivar (Strongfield) | 9 DH lines (185) | 9 | 1A, 1B, 2AS, 2BL, 5B, 6B, 7AL, 7B | [64] |
| Chinese hard wheat line (Ning7840) × Soft wheat cultivar (Clark) | 2 RILs (132) | 2 | 11.2–16.8 | 3AS, 4B Xwmc749–Xgwm369 (11.2), Xgwm368–Xwmc617 (16.8) |
| *T. aestivum* (MN98550) × *T. aestivum* (MN99394) | 3 RILs (139) | 3 | 4.5–16.8 | 2BS, 5AL, 6DL Xbarc330–XwPt9094 (11.1), Xwmc245–Xgwm271 (16.8) |
| *T. aestivum* (Huapei 3) × *T. aestivum* (Yumai 57) | 4 DH lines (168) | 4 | 3.09–8.40 | 3A, 3B, 5D, 6D Xwmc749–D_379033 (13.1), BJ236800–Xbarc68 (13.5), D_310555–Xgwm251 (13.9), Xwmc332–Xwm389 (14.8), Xgwm330-D_379033 (13.1), BJ236800–Xbarc68 (13.5), Xgwm330-D_379033 (13.1), BJ236800–Xbarc68 (13.5), D_310555–Xgwm251 (13.9), Xwmc601–Xwmc601 (14.8), TC82001–Xgwm372e (16.2), D_376852–Xgwm601 (17.6), Xwmc633–CA594434a (18.7), D_304657–Xwmc332 (20.4), D_521287–Xgwm389 (20.4), | [65] |
| Durum breeding line (C1113) × Durum cultivar (Kofa) | 15 RILs (93) | 15 | 9.3–21.6 | 1BS, 2AL, 2BS, 3BS, 3BL, 4AL, 5AS, 5BL, 7AS, 7BL | [66] |
| Svevo × Ciccio (both elite durum wheat cultivars) | 11 RILs (120) | 11 | 7.8–40.2 | 1AS, 1AL, 2AS, 2BL, 3BS, 4AL, 4BL, 5AL, 6BS, 7BL | [67] |
| Breed/Population | Type | ID | Yield | Major Alleles | Other Alleles | References |
|------------------|------|----|-------|---------------|--------------|------------|
| Oste-Gata × Massara-1 | F2 derived F3 and F4 lines | [151] | 5.31-9.44 | 1A, 5BL | - | [72] |
| T. aestivum (Weimai 8) × T. aestivum (Jimai 20) | RILs (485) | 9 | 3.06-9.79 | 2B, 3A, 4A, 4D, 5B, 7A | - | [73] |
| T. aestivum (Weimai 8) × T. aestivum (Yannong 19) | RILs (229) | 10 | 6.29-53.04 | 1A, 1B, 2A, 2D, 3A, 4B, 5A, 5D, 6B, 7D | Xgwm66.4–Xgwm234 (10.72), Xcfe254–Xmag972.1 (11.05), Xcfa14.2–ww160.1 (12.04), Xcfa2147–Xcwm109.1 (13.22), Xcfa2163.2–Xcwm216 (53.04) | [73] |
| Synthetic wheat (Am3) × Synthetic wheat (Laizhou953) | BC5F2:F6 families | 9 | 1A, 2D, 3A, 4B, 5D, 6A, 6B, 6D, 7B | - | [74] |
| T. aestivum (BR34) × T. aestivum (Grandin) | RILs (118) | 1 | 16.3 | 5BL | Xbarc234.1–Xfcp273 (16.3) | [75] |
| T. aestivum (Weimai 8) × T. aestivum (Luohan 2) | RILs (302) | 7 | 4.15-9.73 | 2A, 3B, 4A, 5B, 5D, 6B, 7A | - | [76] |
| T. aestivum (Xiaoyan 54) × T. aestivum (Jing 411) | RILs (182) | 5 | 1.14-9.25 | 4B, 4D, 5A, 6A | - | [77] |
| T. aestivum (CO940610) × T. aestivum (Platte) | DH lines (185) | 5 | 5.6-12.3 | 5BS, 6AL, 6BS, 7BS, 7DL | Xgwm540–Xgwm499 (12.3) | [78] |
| T. aestivum (Choteau) × T. aestivum (Yellowstone) | RILs (97) | 2 | 17-19 | 3B, 5B | Barc77 (17), Gwm499 (19) | [79] |
| T. aestivum (Drysdale) × T. aestivum (gladius) | DH lines (68), RILs (182) | 13 | 0.84-10.51 | 1B, 1D, 2A, 2B, 2D, 3B, 4B, 5B, 6D, 7A | Xbarc353–Xbarc296 (10.51) | [80] |
| T. aestivum (Jimai 20) × T. aestivum (gladius) | RILs (155) | 4 | 2B, 2D, 3D, 5A | - | [81] |
| Parentage                                      | Generation | Marker(s)                                      | Ref (ID) |
|-----------------------------------------------|------------|-----------------------------------------------|----------|
| T. aestivum (CD87) × T. aestivum (Katepwa)    | DH lines   | 1D, 2A, 2B, 2D, 4A, 4B, 5A, 5B, 5D, 6A, 6B, 6D, 7A | [82]     |
| T. aestivum (WCB414) × T. aestivum(WCB617)   | RILs       | 1A, 1B, 2B, 2D, 3D, 4B, 5B, 6B, 7B              | [83]     |
| T. aestivum (Ning7840) × T. aestivum(Clark)   | RILs       | 3A, 4BS, 5AL, 5BL                              | [84]     |
| T. aestivum (RAC875) × T. aestivum (Kukri)   | DH lines   | 1B, 2D, 3D, 4B, 5A, 5B, 6D, 7A                 | [85]     |
| T. aestivum (Kitami 81) × T. aestivum (Kachikei 63) | DH lines   | 2BS                                           | [86]     |
| T. aestivum (Berkut) × T. aestivum (Kirchauff) | DH lines   | 1A                                            | [87]     |
| T. aestivum (Chuan 35050) × T. aestivum (Shannon 483) | RILs | 1A, 1D, 2A, 2D, 4A, 4B, 5D, 7D                  | [88]     |
| T. turgidum (Duilio) × T. turgidum (Avonlea)  | RILs       | 1B, 2B, 3B, 4A, 5A, 7A, 7B                     | [89]     |
| T. aestivum CO940610 × T. aestivum (Platte)   | BC3F2;3    | 1B, 6B, 7B                                     | [90]     |

- wPt0266-wPt9299 (10.4), wPt744808-wPt4368 (10.5), wPt1895-wPt6191 (13.7), wPt1924-wPt7872 (16.5), wPt5234-wPt1437 (16.9)
- IWA3069–IWA2023 (11.5), IWA197–IWA6713 (14.7), IWA4662–IWA482 (20.1), IWA482–IWA1846 (20.5), IWA649–IWA7509 (21.5), IWA482–IWA1846 (22)
- RAC875_c35801_905 (11), wsnp_Ex_c2389_4479352 – barc0353b (11), Kukri_c60729_430 – Ra_c9427_300 (17), Ra_c9427_300 – BobWhite_c34551_714 (17)
- Xgpw3215 (28.5), Xwmc245 (29), Xgpm4382 (32.1)*
- Xsrap21–Xwmc44 (11.6), Xswes340a–Xswes342a (11.7), Xgwm495–Xwmc238 (19.5), Xwmc308–Xsrap7c (25.8-27.1), Xgdm67–Xgwm428 (32.7)
- Bx7-MAR (13.2), Xwmc182a (17.7), Xwmc182b (19.2)
2.1. Epistatic Interactions for Grain Protein Content

Development of efficient statistical and genomic tools have allowed the geneticists to identify and map the QTLs involved in epistatic interactions for GPC [56, 63, 69, 70, 77]. Softwares like QTLMapper [91], QTLNetwork [92], and IciMapping [93] were frequently used to locate the epistatic QTLs. Kulwal et al. [56] identified four QTLs which were involved in two digenic QQ epistatic interactions in one RIL population and six E-QTLs involved in three digenic QQ epistatic interaction in other RIL population in bread wheat. However, these interactions accounted for only 2.68% and 6.04% of phenotypic variation in first and second population respectively. Patil et al. [63] identified one pair of epistatic interaction using a RIL population derived from a durum wheat cross between PDW 233 and Bhalegaon 4. Zhao et al., [69] identified two digenic epistatic interaction for GPC, both involving only 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 RIL population obtained from the cross between the UC Davis wheat breeding line UC1113 and the variety Kofa. In another study, Xu et al. [77] identified two significant digenic interaction involving four M-QTL for GPC using a RIL population developed from the Chinese cultivars.

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 [94]. Later on, the same accession was used to produce a complete set of chromosome substitution lines, in the background of modern durum wheat [95]. Using substitution lines, Gpc-B1 gene was later mapped on chromosome arm 6BS which explained 66% of the phenotypic variation for GPC [96]. Gpc-B1 was cloned using map-based cloning approach, and it was found that Gpc-B1 encodes a NAC transcription factor (NAM-B1) that accelerates senescence and also affects grain protein, zinc, and iron content in wheat [97]. Introgression material with functional GPC-B1 allele into the background of elite, varieties is released in different countries [98].

3. Biofortification for Grain Fe and Zn Content

Using agronomical methods, the Zn content of grain can be increased by fertilising the plants with zinc fertilizers. For example, Zhang et al., [99], reported a 58% increase in whole grain Zn, 76% increase in wheat flour Zn using a foliar application of 0.4% ZnSO4·7H2O. In another study, Zou et al., [100] increased grain Zn by 84 % and 90 %, by using Zn as a foliar spray. However, in case of iron, these agronomic approaches have been less effective [101], except if combined with increased nitrogen fertilizers [102] which may not be economically acceptable. At CIMMYT, Mexico (International Maize and Wheat Improvement Center), conventional breeding has been successfully used to increase the zinc content of wheat grains [103]. To date, four Zn biofortified varieties have been released – ‘Zinc Shakti’ (in the background of Indian variety PBW343), ‘Zincol 2016’ (in the background of Pakistani variety NARC2011) and ‘WB02’ and ‘HPBW-01’ having 14, 9, 7 and 7 PPM zinc in their grains, respectively [103]. These four varieties developed in collaboration with CIMMYT, 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 [104]. Although success in Zn biofortification has been achieved, no Fe biofortified variety could be produced so far using conventional breeding.

3.1. QTLs for grain Fe and Zn concentration in Wheat

Marker-assisted breeding can be a potential breeding strategy to develop the Fe and Zn biofortified ears of wheat. The knowledge of the genetic basis for Fe and Zn concentrations are required for successful employment of marker-assisted selection. Various QTL mapping studies have allowed the identification of many QTLs for both Fe and Zn (Table 2). Unfortunately, Most of the QTLs identified for Zn and Fe, were not stable across the studied environments. Shi et al. [105] detected four and seven QTLs for Zn concentration and Zn content, respectively. They suggested a possibility to improve both grain Zn concentration and content simultaneously because all the four QTLs for Zn concentration were co-located with the QTLs for Zn content. QTLs for Zn concentration on chromosomes 4A and 4D and, four QTLs for grain Zn content on chromosome 2D, 3A and 4A were co-located with the QTLs for P contents, indicating a possibility of improving grain Zn and P density simultaneously in wheat.

QTLs for grain zinc and iron have also been mapped in populations derived from crosses between T. boeoticum and T. monococcum [106], durum wheat and wild emmer [64] synthetic hexaploid wheat and T. spelta [107, 108, 109]. Tiwari et al. [106] mapped 2 QTL for grain Fe on chromosomes 2A and 7A explaining...
12.6 and 11.7% of phenotypic variation and 1 QTL for grain Zn on chromosome 7A explaining 18.8% of total phenotypic variation, using a RIL population derived from a cross between T. boeoticum accession ‘pau5088’ and T. monococcum accession ‘pau14087’. Recently in 2017, Crespo-Herrera et al. [108] identified several significant QTLs with a region named as nQGZn.cimmyt-7B_1P2 on chromosome 7B explaining the largest proportion (32.7%) of total phenotypic variance for GZn and one QTL on chromosome 4A (QGFe.cimmyt-4A_P2), explaining the largest (21.14%) proportion of phenotypic variance of the GFe in two RIL populations derived from T. spelta L. and synthetic hexaploid wheat crosses. In other study, Krishnappa et al. [109] mapped four QTLs, explaining 20% of total phenotypic variation and five QTLs, explaining 32% of 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-Xwm407 on chromosome 2A. 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 additive x 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 same additive x additive epistatic interaction with the other QTL (Xgwm617-Xcfa2114) located on the 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 2). Among these studies, some have reported a significant positive correlation between GZn and GFe across different environments indicating co-localization of QTL or pleiotropic effect regulating the concentrations of both GZn and GFe in wheat (Table 2). For instance, Tiwari et al. [106] showed the colocalization of QTLs for GZn and GFe on chromosome 7A between the flanking markers Xcfd31-Xcfa2049, and closest markers viz. wPt-9555 and Xcfa2019 were also mapped on 7A chromosome indicating the association with both GZn and GFe [64, 109]. Colocalization of QTLs for GZn and GFe on other chromosomes such as 2A [109], 2B [87], 4BS [110], 5A [77, 109] and 6B [111]. This colocalization of QTLs provides the opportunity to employ only one MAS programme to elevate the concentrations of both GZn and GFe, simultaneously.
## Table 2. List of quantitative trait loci (QTL) identified for grain zinc (GZn), grain iron (GFe) and grain selenium (Se) content.

| Cross | Population type and size | No. of total QTLs | PVE range | Chromosomes | Marker intervals / nearest markers for major QTL (PVE) | 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 | 1A, 2D, 3A, 4A, 4D, 5A, 7A | Xgwm192—WMC331 (11.9); Xgwm291—Xgwm410 (10.69) [Zn conc.]; P3470.3—P3176.1 (13.4); WMC488—P2071-180 (14.6) [Zn content] | [105] |
| *T. aestivum* (RAC875-2) × *T. aestivum* (cascades) | DH (90) | GZn-4; GFe-1 | 3D, 4B, 6B, 7A, 3D | | Xcfd31-Xcfa2049 (18.8) [GZn]; Xwmc382-Xbarc124 (12.6); Xgwm473-Xbarc29 (11.7) [GFe] | [112] |
| *T. boeoticum* (Tb5088) × *T. monococcum* (Tm14087) | RIL (93) | GZn-2; GFe-3 | 7.0-12.6; 9.0-18.8 | 7A; 2A, 7A | Xcfd31—Xcfa2049 (18.8) [GZn]; Xwmc382—Xbarc124 (12.6); Xgwm473—Xbarc29 (11.7) [GFe] | [106] |
| Durum wheat (cv. Langdon) and wild emmer (accession G18-16) | RIL (152) | GZn-6; GFe-11 | 1.3-23.5; 0.8-17.8 | | wPt8216 (15.4), wPt 9555 (15.7), gwm445 (15.4) [GZn]; gwm154 (14.6), wpt-9555 (10.7), gwm473 (11.7); gwm1054 (11.7) [GFe] | [64] |
| *T. aestivum* (Xiaoyan 54) × *T. aestivum* (Jing 411) | RIL (182) | GZn-2; GFe-2 | 4.23-6.88; 2.37-3.43 | 4B, 5A, 5A | WMC74-Xgwm291 (14.6) | [77] |
| *T. aestivum* (Hanxuan 10) × *T. aestivum* (Lumai 14) | DH (120) | GFe-4 | 6.1-14.6 | 4D, 5A, 7A, 7B | Xgwm4026—Xgwm1081 (40.22), Xgwm3094—Xgwm164 (50.79) [GZn]; Xgwm312—Xgwm817 (12.58), Xgwm3094—Xgwm164 (17.04), Xgwm4670—Xgwm194 (44.60), Xgwm1047—Xgwm383 (44.71), Xgwm767—Xgwm3036 (10.92) | [113] |
| *T. aestivum* (Tabassi) × *T. aestivum* (Taifun) | RIL (118) | GZn-2; GFe-6 | 40.22-50.79; 8.94-47 | 1A, 4A, 2A, 3D, 4D, 7B, 7D | Xgwm4026—Xgwm1081 (40.22), Xgwm3094—Xgwm164 (50.79) [GZn]; Xgwm312—Xgwm817 (12.58), Xgwm3094—Xgwm164 (17.04), Xgwm4670—Xgwm194 (44.60), Xgwm1047—Xgwm383 (44.71), Xgwm767—Xgwm3036 (10.92) | [114] |
| *T. aestivum* (PBW343) × *T. aestivum* (Kenya Swara) | RIL (177) | GZn-3 | 10.00-15.00 | 1B, 2B, 3A | wPt-6174 (10), wPt-3103 (11), wPt-0286 (15) | [115] |
| 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 | Xgwm213—Xbarc216 (13.8), Xbarc6—Xcfe172 (14.5), Xcfa2149—Xbarc48 (15.9) [GZn]; Xwmc468—Xbarc170 (10.3), Xsrap97—Xbarc330 (10.4), Xgwm154—Xbarc108 (19.1) | [107] |
| *T. aestivum* (Chuanmai 42) × *T. aestivum* (Chuannong 16) | RIL (127) | GZn-3; GFe-4 | 13.8-15.9; 9.2-19.1 | 3D, 4D, 5B, 4A, 4D, 5A, 5B | Xgwm213—Xbarc216 (13.8), Xbarc6—Xcfe172 (14.5), Xcfa2149—Xbarc48 (15.9) [GZn]; Xwmc468—Xbarc170 (10.3), Xsrap97—Xbarc330 (10.4), Xgwm154—Xbarc108 (19.1) | [107] |
| *T. spelta* (PI348449) × *T. aestivum* (HUW 234) | RIL (185) | GZn-5; GFe-5 | 4.25-16.46; 5.6-25.95 | 2A, 2B, 3D, 6A, 6B; 1A, 2A, 3B | 989092[F]0-1101425[F]0-16.46 [GZn]; 1708014[F]0-1000008[F]0 (16.55), 3022954[F]0-1102324[F]0 (25.95) [GFe] | [106] |
| Variety Description | DH (138) | RIL (140) | RIL (127) | RIL (188) | RIL (188) | RIL (286) | RILs (171) | RILs (127) | RILs (184) | RILs(171) |
|---------------------|----------|-----------|-----------|-----------|-----------|-----------|-------------|-------------|-----------|-----------|
| T. aestivum (Berkut) × T. aestivum (Kirchauft) | GZn-2; GFe-1 | GZn-3; GFe-5 | GZn-10; GFe-7 | GZn-4; GFe-3 | GZn-12; GFe-7 | GZn-5; GFe-4 | 4 | 1 | 7 | 24 |
| T. aestivum (SerIM82) × T. dicoccoides /Ae. Tauschii (SHW CW176364) | 23.1–35.9; 22.2 | 8.3-17.3; 7.5-14.5 | 9.31–9.18 | 2.86–16.75; 5.49-10.35 | 3.30–32.79; 5.79-21.14 | 3.2–14.4; 2.3-6.8 | 6.4-28.5 | 35.1 | 7.44-15.57 | 4.38-28.38 |
| T. aestivum (Adana99) × T. spelta (70711) | 1B, 2B; 2B | 4BS, 6A, 6L, 6BL, 2BS, 4BS, 6AL, 7DS | 1B, 1D, 2B, 3A, 3D, 6A, 6B, 7A, 7B | 1B, 2A, 2B, 3A, 6B, 7B | 1A, 1B, 3B, 3D, 4A, 5B, 6A, 7B, 7D | 2A, 2B, 3B, 4A, 4D, 5B | wmc036e–cfa2129 (23.1), gwm120–wpt2430 (35.9) | wmc120–wpt2430 (22.2) | rPt-6561 (12), wPt-2698–wPt-0398 (14), wPt-2083–wPt-6083 (15), wPt-733,112 (25), wPt-7161–wPt-9812 (31) | wPt-667,798–wPt-7065 (14), wPt-1394–wPt-7864 (17), wPt-5922 (18) |
| Synthetic hexaploid wheat (Louries) × T. spelta (Bateleur) | 24 | 12; -3; -2 | 32.79; -17.3 | 10; -6 | 14.5 | 35.9 | 11.72 (25.58), wPt-0708 (10.1), wPt-6191-wPt-8658 (23.4), wPt-671684-wPt-667506 (28.5) | Xbarc241-Xsrap15a (35.1) |
| T. aestivum (WH542) × synthetic derivative (Triticum dicoccum PI94624/Aegilops squarrosa [409]/IBC) | Selenium | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D | 3D, 4A, 5B, 7D |
| T. aestivum (SHW-L1) × T. aestivum (Chuanmai 32), T. aestivum (Chuanmai 42) × T. aestivum (Chuanmai 16) | | | | | | | | | | |
| Triticum dicoccoides (Langdon) × Wild emmer wheat (acc. G18-16) | RILs (152) | 15 | 1.4–18.6 | 1A, 1B, 2B, 3A, 4B, 5A, 6A, 7A, 7B |
|---|---|---|---|---|

[119]
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 the wheat grains with Zn [103, 104]. But, Fe content in wheat grain could not be increased using these methods. For instance, several QTL mapping studies have been conducted and so many QTLs for GFe and GZn, have been mapped on different chromosomes of wheat. So, now, efforts can be made to utilize the QTL information in marker-assisted backcrossing schemes to produce Zn and Fe biofortified wheat. Co-localization of QTLs for GFe and GZn may further provide the opportunity to target them for improving the concentration of both in wheat grains, simultaneously [109]. Moreover, in various QTL studies, some QTLs for these GFe and GZn are also co-localized with those of phosphorus [64, 105], selenium [107], calcium, manganese and magnesium [64] or other agronomically essential traits including grain protein content [77] and thousand grain weight [110].

The genetic variation available for breeders is not limited. For instance, Gorafi et al. [120] assessed 47 synthetic wheat lines derived from crosses between 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. [121], 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. [122] 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 in two clusters, older genotypes and landraces with high GFeC, GZnC were kept in one group and remaining with low GFeC, GZnC were kept in other, these can be utilized as parents in crossing programs.

4. Biofortification for grain selenium content

Selenium (Se), an essential mineral element, incorporates into 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 [123]. In staple crops, Se present at low concentration (<100 mg Se kg⁻¹), so the genetic resources having high amount of Se, as well as the genes/QTLs controlling Se concentration are need to be identified to breed the Se-rich varieties either using conventional or molecular breeding approach such as MAS [118].

There are contrary reports for the amount of genetic variability among wheat cultivars for Se density in grain. Some studies have found no evidence for genetic variability [124, 125], while another found higher Se density in wheat grains [126]. The Se-density in wheat grain was about 16 μg kg⁻¹ which is insufficient to meet the Se requirement for human [125]. In 2005, Lyons et al. [127], surveyed 665 ancestral and wild relatives of wheat, wheat landrace accessions, populations, and commercial cultivars grown in Mexico and Australia for Se concentration in grain. They found grain Se concentrations in the range of 5–720 μg kg⁻¹, but unfortunately, much of this variation was correlated with spatial variation in soil selenium and, no significant genotypic variation in grain Se density was observed. Although, 42% and 35% higher grain Se concentration was found in Aegilops tauschii and rye, respectively. On the other hand, Piergiovanni et al. in 1997 [126], found significant differences between emmer (T. dicoccum Schrank) and spelt (T. spelta L.) accessions, and wheat cultivars for higher contents of Se, Li, Mg, P and Zn. They reported a range of 1.9–5.8 μg/100g with a mean of 3.9 μg/100g and 1.8–3.5 μg/100g with a mean of 2.8 μg/100g in spelt and emmer accessions.

4.1. QTLs for Se Content in Wheat Grain

Knowledge of the underlying genetic mechanism of Se content is the necessary step for Se biofortification of wheat to enhance grain Se content. QTL mapping helps in understanding the genetic basis but, unfortunately, only a small number of QTL mapping studies have been conducted for mapping the QTLs for Se concentration in wheat grain [107, 117, 118, 119]. In 2014, Pu et al. [107] identified a total of 39 QTLs for five micronutrients (Se, Fe, Zn, Cu and Mn) concentrations using two RIL population derived from the crosses between SHW−L1 (synthetic hexaploid wheat) and Chuanmai 32 and, Chuanmai 42 and Chuanmeng 16 respectively. In the first population, they mapped four QTLs on chromosomes 3D, 4A, 5B, 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
genetic variance for Se concentration in wheat grain. Wang et al. [117], mapped 16 QTLs (seven at the seedling stage and nine at the adult stage) for six Se content-related traits on eight chromosomes, 1B, 2B, 4B, 5A, 5B, 5D, 6A, and 7D using a NIL 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 from 7.37 to 20.22% of the total phenotypic variance of Se content. Recently in 2018, for the first time, Pu et al. [118] documented a Se rich synthetic wheat line and mapped a total of 24 QTLs for 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), was explaining the maximum amount (up to 28.38%) of genetic variation.

In another study, Yan et al. [119] mapped a total of 15 QTLs on chromosomes 1A, 1B, 2B, 3A, 4B, 5A, 6A, 7A, 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. dicocoides (accession G18-16) and Langdon (Durum wheat). These above findings provide various main effect QTLs and their linked markers, which can be utilized in the MAS program for Se biofortification of wheat grain. QTLs regulating selenium concentration in wheat grains have been mapped on chromosomes 1A, 1B, 2B, 3A, 3D, 5A, 5B and homeologous groups of 4 and 6 chromosomes (Table 2). Pu et al. [107] identified a QTL on chromosome 4D which explained 35.1% of total phenotypic variance in common wheat. Markers associated with identified QTLs have been given in Table 2.

5. Biofortification for the Grain Yellow Pigment Content (GYPC)

GYPC is responsible for yellowness of wheat flour which is regarded better for human health because of antioxidant properties of carotenoids involved in this pigmentation. The yellow pigment in the wheat grain is predominantly associated with carotenoid compounds (carotenes and xanthophyll) which affect the quality and nutritional value of wheat grain products [128].

5.1. QTLs Identified for GYPC in Wheat

Since GYPC is a complex quantitative trait, several efforts have been made to identify the genetic regions associated with the trait. To our knowledge, in last two decades, several QTL mapping studies (mostly in durum wheat crosses) have been conducted to map more than eighty QTLs associated with GYPC in wheat (Table 3). Interestingly, in each mapping studies, at least one QTL has been mapped on chromosome 7 of both T. turgidum L. var durum and T. aestivum explaining a significant part of phenotypic variation [129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141]. Parker et al. [129] identified two regions on chromosomes 3A and 7A, explaining, 13% and 60% of the genetic variation respectively. They further fine mapped the region on chromosome 7A and identified seven additional AFLP markers within the target region. RIL population of a cross between T. turgidum L. var. durum cultivar and T. dicocoides (acc.600545), Elouafi et al. [130] mapped three QTLs on the chromosomal group 7 (one on 7AL and two on 7BL telomeres) explaining 62% of the total phenotypic variation. All three QTLs were consistent and showed a strong genetic effect and a weak QTL x E effect. Pozniak et al. [131] located phytoene synthase 1 (Psy1) and phytene synthase 1 (Psy2) genes to the group 7 and 5 chromosomes, respectively. They also identified four QTLs underlying phenotypic variation in endosperm colour on chromosomes 2A, 4B, 6B, and 7B. *Psy1-1* allelic locus of *Psy* gene co-segregated with the 7B QTL, demonstrating the role of this gene for endosperm colour. This became the first report of mapping *Psy* genes and supporting the role of *Psy1-1* in increased levels of endosperm colour in durum wheat. In another study, Zhang et al. [134] also mapped *Psy-A1* gene on chromosome 7A with PVE value 33.9% and interestingly also detected a QTL region on 1RS (1B.1R translocation) explaining 31.9% of the phenotypic variance.

In 2008, Patil et al. [132] identified four different stable QTLs on chromosome 1A, 3B, 5B, 7B explaining 5–8.75% of phenotypic variation and one most robust QTL on the distal part of chromosome 7AL explaining 55.2% of the variation in yellow pigment content. Markers (ISSR and AFLP) linked with strongest QTL were also converted into SCAR markers for ease of MAS. Blanco et al. [135] detected clusters of QTLs for carotenoid compounds in durum wheat. In 2012, Roncallo et al. [137] reported overlapping of main QTLs affecting flour yellow colour (Fb*) and GYPC on chromosome arms 4AL, 6AL, 7AS, 7AL, 7BS and 7BL. One QTL on 7BL included *Psy-B1* locus also. A novel minor QTL regulating Fb* (located on 7AS) was showing epistatic effect on YPC. One QTL on chromosome 4AL with a strong effect on Fb* was also involved in two digenic epistatic interactions. Colasuonno et al. [139] mapped a total of seven QTLs on different chromosome regions (1B, 2A, 2B, 5A, 5B, 7A and 7B) using a dense map consisted of 5,670 loci comprising 5,019 SNPs, 467 DArT, 182 SSR markers and eight genes. They also identified two candidate genes involved in carotenoid biosynthesis.
pathway [aldehyde oxidase (AO1), and diphosphomevalonate decarboxylase (DMAPD)] by scanning the genome for QTLs and predicting the SNP homology against annotated proteins in wheat and *Brachypodium* genomes. Recently, in 2016, in a common wheat population, Zhai et al. [141] mapped sixteen QTLs on chromosome 1B.1R, 2A, 2B, 2D, 5A, 5B, 6B, 7A and 7B, explaining 5.7 to 30.8% of phenotypic variance for the trait. QTLs for YPC have been mapped on different chromosomes 1A, 1B, 3A, 3B, 7A, 7B and homeologous groups of 2, 4, 5 and 6 chromosomes (Table 3). QTLs, mapped on homoeologous group 7 chromosomes explained up-to 55.22% of the total phenotypic variation in durum wheat [132] and up to 77% in common wheat [140] in various biparental populations (Table 3), whereas the QTLs on chromosomes 5B contributed up to 16.2% [137] and up to 14.8% [141] in durum and common wheat respectively. Zhai et al. [141] also identified a QTL on 1BL.1RS (H20) explaining 30.8% of total phenotypic variance. Markers listed in Table 3, would facilitate the selection of improved durum and common varieties.
Table 3. List of quantitative trait loci (QTL) identified for yellow pigment content.

| Cross | Populatio n type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/ chromosome arms | Marker intervals / nearest markers for major QTL (PVE) | References |
|-------|---------------------------|-------------------|---------------------------------|-----------------------------|-------------------------------------------------------|------------|
| *T. aestivum* (Schomburgk) × *T. aestivum* (Yarralinka) | SSD lines (150) | 2 | 13-41 | 3A, 7A | (Xbcd828D3A) (13), Xwua16D7A.5 (41) | [129] |
| *T. turgidum* L. var. durum (Omrabí5) × *T. dicoccoides* (acc. 600545) | RILs (114) | 3 | 6.0-53.0 | 7AL, 7BL | Xgwm63e (13), Xgwm34 (53) | [130] |
| *T. aestivum* (Trident) × *T. aestivum* (Molineux) | DH lines (182) | 1 | 48-77 | 7B | Xgwm273–Xgwm146 (48-77) | [140] |
| *T. turgidum* L. var durum (W9262-260D3 × *T. turgidum* L. var. durum (Kofa)) | DH lines (155) | 4 | 14-23 | 2A, 4B, 6B, 7B | Xgwm495 (14-20), Xgwm425 (15-21), Xgwm193 (15-21), Psy1-1 (20-23) | [131] |
| *T. aestivum* (PH82-2) × *T. aestivum* (Neixing 188) | RILs (240) | 1 | 20-28 | 7AS | Xwmc809 (20-28) | [142] |
| *T. turgidum* L. var durum (PDW 233) × *T. turgidum* L. var durum (Bhalegaon 4) | RILs (140) | 5 | 5-55.22 | 1A, 3B, 5B, 7A, 7B | Xscar3362 (22.61-55.22) | [132] |
| *T. turgidum* L. var durum (UC1113) × *T. turgidum* L. var durum (Kofa) | RILs (93) | 1 | 7A | Xcfa2293-7A - Xwmc116-7A | [133] |
| *T. aestivum* (PH82-2) × *T. aestivum* (Neixing 188) | RILs (240) | 4 | 1.5-33.9 | 1A, 1B, 4A, 7A | Sec1-HVM23 (31.9), Xwmc809-YP7A (33.9) | [134] |
| *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 | Xgwm372-wPt-9797 (11.1-24.5), Xbarc84-Xgwm299 (11.5-16.2), Xgwm282-wPt-4345 (19.8-30.4), D_304196-PsyA1 (42-53.2) | [135] |
| Ajana × WAWHT2074; Carnamah × WAWHT2046; Ajana × WAWHT2046 (all *T. aestivum*) | DH lines (179, 121, 127) | 6 | 4.0-36.0 | 2D, 3A, 4D, 5B, 7A, 7B | Xbarc2634-wPt9215 (10-21), wPt4038-cfa2257a (21-36), | [136] |
| *T. turgidum* L. var durum (UC1113) × *T. turgidum* L. var durum (Kofa) | RILs (93) | 15 | 6-42.7 | 1BL, 2AS, 4AL, 5AS, 5AL, 5BL, 6AL, 7AS, 7AL, 7BL | BE443797_436–barc302 (10.8), Lpx-A3–wmc617 (12), wmc219–psr573.2 (12), cfa2040–barc1073 (15), wmc311–wmc276 (16.9), barc146–gwm132 (16.8-42.7), | [137] |
| *T. aestivum* (Chuan 35050) × *T. aestivum* (Shannong 483) | RILs (131) | 13 | 4.1-16.5 | 1A, 1B, 2D, 4A, 4D, 5B, 5D, 6A, 6D, 7B | wPt-3931-wPt-665267 (12.1), wPt-3247-wPt-1695 (16), | [138] |
| Parentage                                                                 | RILs | 19.3-51.6* | 1B, 2A, 2B, 5A, 5B, 7A, 7B | IWB73028 (19.3), tPt-1253 (21.8), IWB59875 (51.6) |
|--------------------------------------------------------------------------|------|------------|----------------------------|--------------------------------------------------|
| **T. turgidum** L. var durum (Svevo) × **T. turgidum** L. var durum (Ciccio)** | 7    |            |                            |                                                  |
| **T. aestivum** (Gaocheng 8901) × **T. aestivum** (Zhoumai 16)**           | 16   | 5.7-30.8   | 1B.1R, 2A, 2B, 2D, 5A, 5B, 6B, 7A, 7B | BS00043474_51 (14.1), Ra_c30725_808 (14.8), Tdurum_contig17230_619 (14.8), BS00065690_51 (17.2), 1BL.1RS (H20) (30.8), |
5.2. Allelic Variation and Marker Assisted Selection

Allelic variation for GYPC at Psy1-A1, Psy1-B1/Psy1-S1 and Psy1-D1 loci in wheat have been studied in much detail [136, 143]. In 2009, Wang et al. [144] identified 27 alleles associated with variation in GYPC in wheat. Although several gene/allele-specific markers have been developed with their potential use in improvement of yellow pigment in durum and common wheat [132, 142, 145], studies on marker-assisted development of advanced wheat breeding lines with improved GYPC are lacking [146]. In one study, a short terminal 7EL segment (from *Lophopyrum ponticum*) translocated to 7A, including Lr19 and Y gene, has been transferred to durum wheat by marker-assisted backcrossing [147]. Patil et al. [148] identified eight alleles of Psy-A1 SSR showing significant association with GYPC and seven alleles for Psy-B1SSR having no association with GYPC in 222 wheat accessions. Further, they introgressed Psy-A1 SSR allele from PDW233 to durum wheat cultivars MACS 3125 and HI 8498, and achieved 89 to 98% gain in GYPC over recurrent parents.

6. Biofortification for Grain Phytic Acid Content

Phytic acid (PA) is primarily the storage form of phosphorus in cereal and legume seeds and accounts for 70% to 80% of the total P in grains [149, 150]. Phytases are found in the aleurone layers of cereal grains and are activated due to the moisture and hence are inactive in dry cereals due to lack of moisture for their activation. Because of the strong ability of phytic acid to make insoluble complexes of multi-charged metal ions, the consumption of great quantities of food containing high phytic acid levels could produce a deficit in the absorption of these dietary minerals [151]. Cook et al. [152] reported that because of the high phytate content of cereal porridges, iron absorption of native iron and fortification iron might be deficient. Barbro et al. [153] reported that when the phytate is absent zinc absorption rate from our food will increase by 20 percent and magnesium absorption will increase by 60 percent.

6.1. Genetic Variation and Breeding Strategies

Breeding opportunities for low phytic acid crops depend on the extent of variation for the same present in germplasm. Ram et al. [154] observed 3.4 fold variations in phytase levels among varieties and 5.9 fold among synthetic hexaploids while investigating a total of four hundred wheat genotypes including released varieties in India, advanced lines and synthetic hexaploids. On the other hand, they observed lower variability (1.6 fold in varieties and 2.2 fold in synthetic hexaploids) in phytate levels. Shitre et al. [155] observed a range of phytic acid from 4.97 mg/g to 15.02 mg/g (mean of 9.58 mg/g) in 100 advanced breeding lines. This genetic variation for the trait can be utilized for potential breeding of low phytic acid in wheat. Low phytic acid can also be achieved by manipulating the genes involved either in its biosynthesis or its transport in the vacuoles. Genes involved in the late stages of phytic acid biosynthesis pathway are known in crops like maize, soybeans and barley, but none could have been reported from wheat. For the first time, Bhati et al. [156] identified six insilico wheat genes that might be involved in the biosynthesis of inositol phosphates.

Furthermore, they identified a homolog of Zmlpa-1, an ABC subclass transporter protein (TaMRP3) that is putatively involved in phytic acid transport. Bhati et al. [157] revealed the involvement of ABCC13 transporter in wheat grain development, phytic acid accumulation and lateral root formation. Naidoo et al. [158] successfully introgressed the *lpa1-1* gene into tropical and subtropical adapted germplasm using marker-assisted selection. They significantly increased the efficiency of detection of the homozygous recessive (99.58%) and heterozygous (99.59%) genotypes using SNP as well as improved the recovery of the recurrent parent (92.15%) in the BC2F1 generation using AFLP markers in a maize backcross breeding programme. Same types of effort can be made for generating low phytate wheat.

7. Biofortification for Anthocyanin Content

Worldwide, grains of common wheat cultivars are amber in colour, and the anthocyanin biofortified wheat is quite uncommon. But now, researchers have started to focus on anthocyanins content in wheat because of their vital importance in human health. These act as antioxidants and prevent from cardiovascular diseases [159], diabetes [160], cancer [161] and obesity [162] etc. Garg et al. [163] developed anthocyanin-rich blue, purple and black wheat lines with alien chromosome or its arm and localized purple and blue colour to the
pericarp and aleurone, respectively. Combination of genes for both purple and blue colours produce black wheat.

7.1. Genetic basis of Purple Coloured Wheat Grains

The genetic base of pericarp in wheat has been explored in some detail, some researchers found monogenic [164] and while others reported digenic inheritance for pericarp colour [164, 165]. Dobrovolskaya et al. [164] attempted crosses between purple-grained hexaploid wheat ‘Purple Feed’ - Pp1Pp1/Pp2Pp2, ‘Purple’ - Pp1Pp1/Pp3Pp3 with non-purple-grained cultivars ‘Novosibirskaya 67’ (‘N67’) and ‘Saratovskaya 29’ (‘S29’) to map the genes Pp1, Pp2, and Pp3. Both Pp2 and Pp3 showing mono-factorial (dominant) inheritance, were mapped in the centromeric region of the chromosome 2A. Therefore, these two genes were suggested different alleles at the same locus and then designated as Pp3a and Pp3b. They further reported mutual interaction (9:7) of two dominant genes, Pp1 and Pp3 in the crosses between purple-grained wheat and ‘S29’. Khlestkina et al. [165] mapped Pp1 gene on the short arm of chromosome 7B in an F2 population from the durum wheat (Triticum durum) cross TRI 15744/TRI 2719, whereas in common wheat it was reportedly located on 7BL. Mapping suggested that Pp3 gene of T. durum is allelic to the Pp3 gene. Tereshchenko et al. [166] suggested that the Pp genes on T. durum chromosome 7B and T. aestivum chromosome 7D are orthologous and designated them Pp-B1 and Pp-D1, respectively. Overall, it can be concluded that the purple pericarp trait in T. aestivum is controlled by genes placed on chromosomes 7D and 2A; still, underlying molecular mechanisms by which they regulate the pericarp colour were remaining unknown.

Marker-assisted backcross strategy was followed to produce a set of bread wheat near-isogenic lines (NILs) carrying various combinations of Pp alleles such as Pp3 (chr. 2A), Pp-A1 (7A) and Pp-D1 (7D) [167]. Based on a qRT-PCR-based study, the authors further suggested that Pp genes up-regulate the transcription anthocyanin synthesis structural genes [Chi (chalcone-flavanone isomerase) and F3h (flavanone 3-hydroxylase)] in contrasting ways.

In 2016, Liu et al. [168] compared the transcriptomes of purple and white pericarps in common wheat and reported significant differential expression of a total of 23,642 unigenes (9945 up-regulated and 13,697 down-regulated). The analysis predicted three unigenes of MYB gene on the long arm of the chromosome 7B and three unigenes of MYC on the long arm of the chromosome 2A as candidate genes for the purple grain trait. Further, they also observed a higher expression level of TaMYC1 in purple wheat grains compared to white (amber) wheat grains. Shoeva et al. [169] isolated TaMYC1 gene encoding MYC-like transcription factor from the bread wheat genome which was co-located with the Pp3 gene regulating purple pericarp colour. Later on, in 2017, Zong et al. [170] gave additional strong evidence of TaMYC1 to be a synonym of Pp3 and found differences between dominant and recessive Pp3 alleles (TaMYC1p and TaMYC1w). In another study, Li et al. [171] isolated an MYB transcription factor gene, TaMYB3, localised on chromosome 4BL which induces anthocyanin synthesis in the pericarp cells. Recently, Jiang et al. [172] characterised two transcription factors, TaPpm1 (purple pericarp-MYB 1) and TaPpb1 (purple pericarp-bHLH 1) in wheat, and it was demonstrated that the interaction of TaPpm1 and TaPpb1 co-regulates the synthesis of anthocyanin in pericarps.

7.2. Development of Anthocyanin Biofortified Purple Wheat

Burešová et al. [173] confirmed different chromatin introgressions, carrying genomic regions associated with the production of blue-aleurone, from Thinopyrum ponticum and Triticum monococcum into the background of blue-aleurone wheat elite lines. They analysed a total of 26 blue aleurone wheat lines and reported introgression (ranging from a ditelosomic addition to a disomic substitution, substitution of entire chromosome arms and various translocations) from T. ponticum in 17 lines and presumably from T. monococcum in nine lines. This study supports a hypothesis that the introgressions activated the blue aleurone trait present in blue aleurone wheat lines, but inactivated, in common wheat germplasm.

Anthocyanin biofortified wheat has attracted the attention of many researchers across the world, but the developed lines exhibit low yield due to linkage drag [174]. In India, Garg et al. [163] developed coloured wheat lines viz. blue, purple and black, with reasonable yield potential and regional adaptation. They transferred genes for blue, purple and wheat pericarps from blue aleurone wheat “TA3972”, purple wheat “TA3851” and black wheat to high yielding, disease resistant and locally adapted Indian wheat cultivars PBW550, PBW621 and HD2967. These developed coloured wheat lines exhibited higher anthocyanin content and antioxidant activity (black>purple>blue>white) than donor wheat lines and, high Fe and Zn content compared to amber wheat cultivars [175].
8. Conclusions

Within this review, we've compiled the information from the studies carried out in wheat using QTL mapping methodology that is among the best methods for biofortification. Micronutrient deficiency or micronutrient malnutrition or hidden hunger is an increasingly severe global challenge to humankind. As wheat is the major staple food crop in temperate countries and frequently consumed in developing countries, it becomes necessary to biofortify the wheat with micronutrients especially, iron and zinc, for fulfilling the requirement of the human for better health.

In general, there are two major biofortification approaches, first, agronomical biofortification and second genetical biofortification (including conventional breeding, molecular breeding and genetic modification). There is a complex genetic mechanism governing the quantitative traits responsible for the biofortification traits in wheat, and improving biofortification traits of wheat via traditional breeding approaches is also complicated. In this post-genomic and computational systems biology era, specifically, QTL mapping research has helped to dissect the molecular basis of biofortification in wheat. Although, molecular mapping techniques also indicate the co-localization of biofortification related QTLs with some undesirable traits. As a result, the utilisation of genetic variation for biofortification traits with molecular breeding strategy appears to become valuable in future.

Additionally, the existence of substantial genetic variability for biofortification traits also presents superior possibilities to raise the bioavailability of vitamins, minerals and proteins in wheat. The summary of significant QTLs with PVE value >20% for biofortification traits are represented in Figure 2. Moreover, biofortification of all relevant QTLs together will require detailed knowledge of the traits and their coexistence; having said that, this also appears to become a promising strategy for the close to future. Therefore, biofortification of wheat may perhaps be enhanced by way of breeding. Additionally, the combination of functional and genetic proof in conjunction with genome sequencing will hopefully deliver additional insights with regards to the emerging biofortification genomics.
Figure 2. Illustration of the locations of various biofortification traits in wheat grain and closest markers/marker intervals on chromosome associated with the QTLs.

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