Genomic Advances in Biofortification of Iron and Zinc in Wheat (*Triticumaestivum* L.)

Abstract

Majority of population that depend on wheat are found to be deficient in iron and zinc. About 20% of calories worldwide are derived from wheat. Milling of wheat reduces the bioavaibility of Fe and Zn in wheat grains. Micronutrient deficiency is the main cause of malnutrition worldwide and hidden hunger in underdeveloped countries. To overcome this problem it becomes necessary to develop varieties that are rich in iron and zinc content. Biofortification gets around these issues by enhancing the micronutrient content of wheat. The genetic approaches of biofortification particularly the non-conventional methods have proved to be the most efficient ones. These method includes QTL mapping approach, GWAS and transgenics. Although conventional methods for a long time has been used for developing varieties of wheat rich in Fe and Zn but conventional breeding for certain traits may be inconvenient and time-consuming and are prone to errors due to their reliance on traditional phenotyping or visual assessments. Several research have been undertaken to map QTLs of Fe and Zn concentration in wheat grains and have been used in elite genetic backgrounds to improve micronutrient content. Genomic studies revealed through GWAS exhibit high QTL resolution and allele coverage, which can be used for improvement of Fe and Zn content in wheat. Genetic engineering techniques or transgenics approaches allows genetic alteration of crops to improve the nutritional quality of wheat. Genomic approach of biofortification of wheat has proved to be the most efficient methods for increasing iron and zinc content in the crop.

***Keywords**: Wheat, Iron, Zinc, Biofortification, QTL mapping, GWAS, Transgenics*

1. INTRODUCTION

Wheat (*Triticumaestivum* L.) is a worldwide cereal that is farmed, consumed and traded. It ranked third among all the cereals produced. Carbohydrate (78.1%), protein (14.7%), fat (2.1%), minerals (2.1%) all have considerable levels [52-55]. Micronutrient deficiency is the main cause of malnutrition worldwide and has a significant influence on human diets. The hidden hunger in underdeveloped countries is a major cause of health problems. The global significance of mineral micronutrient deficiency in human diets, particularly those of iron and zinc, cannot be overstated. It is found that mostly women and children of reproductive age suffer from anemia which is mainly caused by iron deficiency [2]. In populations where cereal-based foods like wheat contribute a major portion in human diets, iron and zinc deficiency is more severe. This is because grains undergo processing which results in the removal of most of the micronutrient. The increased availability of less nutritious grain has led to "hidden hunger" [1].

Given the state of public health today, it is necessary to develop varieties that are rich in micronutrients. To increase the micronutrient content in human diets, techniques like dietary diversification, mineral supplementation, and post-harvest food fortification can be adopted but unfortunately, these techniques rely on persistent infrastructure and investment. Also, using agronomic strategies such as micronutrient fertilizers application in growing stages of crop for increased micronutrient content have proved be costly [2]. Hence, biofortification solves the problem by enhancing the micronutrient content of the crops by raising mineral levels and bioavailability of micronutrients in the edible portions. The concentrations of Fe and Zn in cereals like wheat can be raised by a variety of biofortification techniques, such as genetic engineering, and traditional breeding [3].

Wheat a widely cultivated and consumed cereal crop, can be a suitable target for Zn and Fe bio-fortification to reduce nutritional deficiency in human diets. Wheat contains abundant minerals in the aleuronelayer, however the bioavailability of zinc and iron is limited due to the existence of phytate in the seeds, which chelates metal ions. Phytate, which includes myo-inositol and phytic acid, has a significant impact on Zn and Fe uptake by the human body. Phylate has a powerful characteristics that allows it to chelate free metal ions and create salt complex that becomes difficult for the body to uptake [3]. The aim is to improve the bioavailability of Zn and Fe in the edible parts of wheat which can be done only if the the anti-nutrient characteristics of phytate is modifed. Hence, there is a need to fix this problem of antinutritional factors of phylate in wheat crop [4].Biofortification of wheat is a cost-effective and sustainable solution to address zinc and iron deficiencies and a better alternative to traditional fortification technologies. This strategy involves developing biofortified crops with easily available Zn and Fe for human consumption at no additional expense [5].

2. NUTRITIONAL IMPORTANCE OF MICRONUTRIENTS (Fe & Zn)

Micronutrients in adequate amount are important for human growth and development. These elements also called trace elements because they are required in very small quantity. These are the cofactors to various enzymes and proteins concerned with various metabolic activities in the body. The human body needs roughly fifty critical micronutrients to carry out all of its vital metabolic processes. The lack of any of these micronutrients adversely affect the development of the human body [6]. Nowadays, there are a number of malnutrition-related health concerns. A primary cause of malnutrition is the absence of essential micronutrients, such as iron (Fe) and zinc (Zn) [7].

One of the most common trace element is zinc whichplays a key role in various biochemical processes that are directly related to the functioning of various proteins associated with metabolic activities like cell growth, cell division, neuron signaling, survival and death. Zinc is necessary for the production of collagen and proteins and is also directly involved in oligomerization process and folding of proteins [8]. Some of the medical symptoms that are the results of zinc deficiency are formation of lesions on the skin, retarded night vision, delayed healing of wounds, retarded mental health, hair loss, diarrhoea, stunted growth, and brainatrophy. However the overuse of zinc can lead to toxicity. Hencea adequate amount of zinc is required in human body to maintain health and prevent diseases [9, 10]. One of the other important micronutrient is iron (Fe) which is responsible for various body functions. Iron is a key component of myoglobin, which stores oxygen in muscular tissues, and the cytochrome system, which is essential for cellular respiration. Fe deficiency is caused due to inefficient absorption of bioavailable iron, fast usage during growth, menstruation, pregnancy and gastrointestinal blood loss [11,12]. Fe deficiency can cause anemia, heart failure, chronic kidney disease, malignancy, and inflammatory bowel issues. The lack of iron affects the synthesis of haemoglomin, myoglobin and cytochromes resulting in lethargy, dyspnea and anemia. Expert panels typically advise treating iron deficiency with 80–200 mg of iron per day [13-14].

3. Iron and Zinc in Wheat grains

The fruit of wheat is known as a caryopsis that has a single seed. It has a storage tissue called the endosperm, which is primarily made up of protein and starch, and is used as energy source [15]. Trace elements like iron and zinc are concentrated in the embryo and in the aleuronelayer which is the outer layer of endosperm (Figure 1). Also the distribution of zinc and iron differ slightly inside the grain. Zinc is concentrated more in the embryo and iron in the aleurone layer (Figure 2).

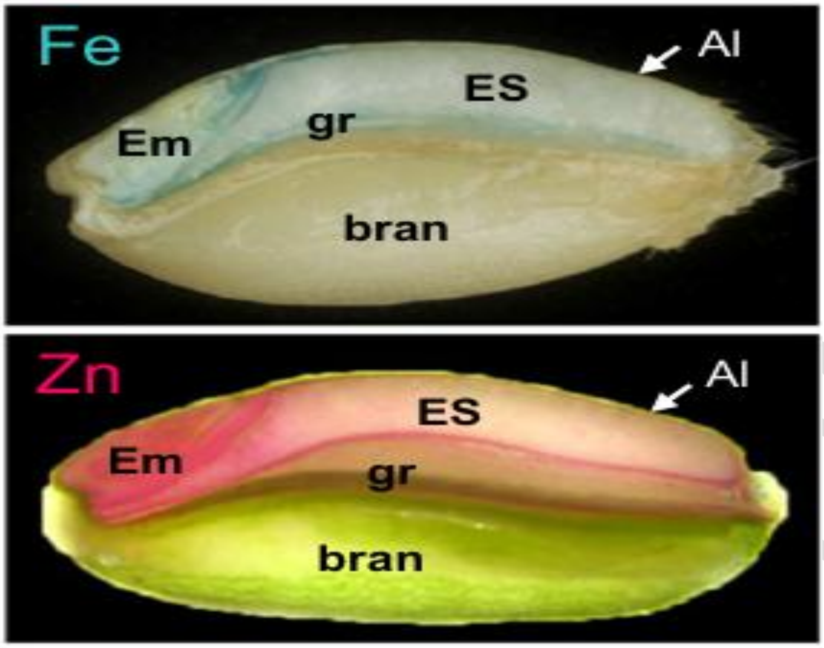

Figure 1: Cross sections through wheat grains displaying the positions of iron and zinc as well as the position of embryo (Em), aluerone (Al), endosperm (ES), bran and groove (gr) **(Source: Balk *et al*. [1]).**

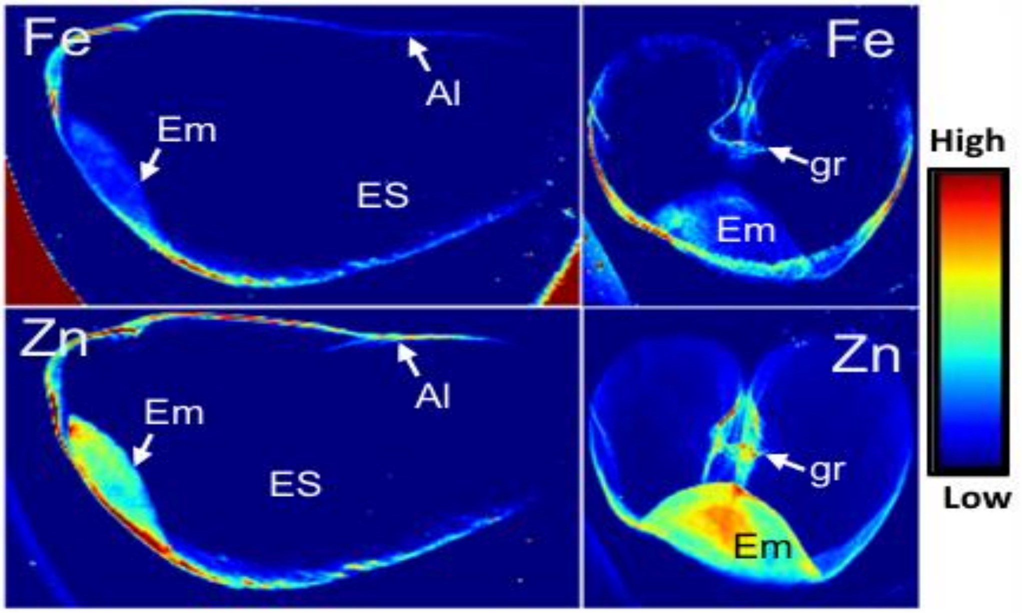

Figure 2: Distribution of iron and zinc in the embryo and aleurone layer **(Source: Balk *et al*. [1]).**

4. Genetic Processes Involved in Absorption and Translocation of Iron and Zinc in Wheat

A good understanding of processes involved in the transport of minerals and nutrients from the rhizosphere to grains is required for genetic biofortification. These processes involves the coordination of various physiological processes like the uptake of iron and zinc by the roots, the long-distance transport of these nutrients from the roots to the shoots and then distribution towards the developing seeds [16].

Plants have developed different mechanism for the uptake of iron from the rhizosphere. Depending on whether the plant belongs to poaceae or non-poaceae family, there are mainly two different strategies for the process. The Strategy I also known as reducing strategy, mainly followed by the non-poaceae members involves ferrous ions (Fe^{+2}). The Strategy II which is known as the chelating strategy involves ferric ions (Fe^{+3}) and are adopted by plants belonging to poaceae [17, 18].

The non- gramineous plants follow the Strategy I where iron in the form of Fe^{+2} are directly absorbed from the rhizosphere. The uptake of iron from roots and its transfer to various parts of plants are mediated by Zinc-regulated trasnsporters (ZRT), Iron-regulated trasnsporters (IRT)-like proteins. Members of the Poaceae family like wheat , rice , and maize , adhere to Strategy II (Fig 3). This method is called as chelating mediated process because the Fe^{+2} ions undergo chelation in the soil by Fe^{+3} based chelation method [19]. Mucineic acid (MA) and phytosiderophores (PSs) released by the roots in strategy-II carries out the chelation of Fe^{2+} in soil. The chelation of Fe^{+2} forms a stable Fe^{+3} chelate in the rhizosphere. The chelated complex of iron when available in rhizosphere is absorbed by the root cells with the help of YSL. Yellow stripe-like (YSL) transpoter proteins are responsible for transferring phytosiderophores –Fe^{+2} complexes into root cells and making them easily absorbed and translocated [3]. (Wani*et al*. 2022). Similarly, there are various protein families that are involved in the transport of iron and zinc [20, 21].

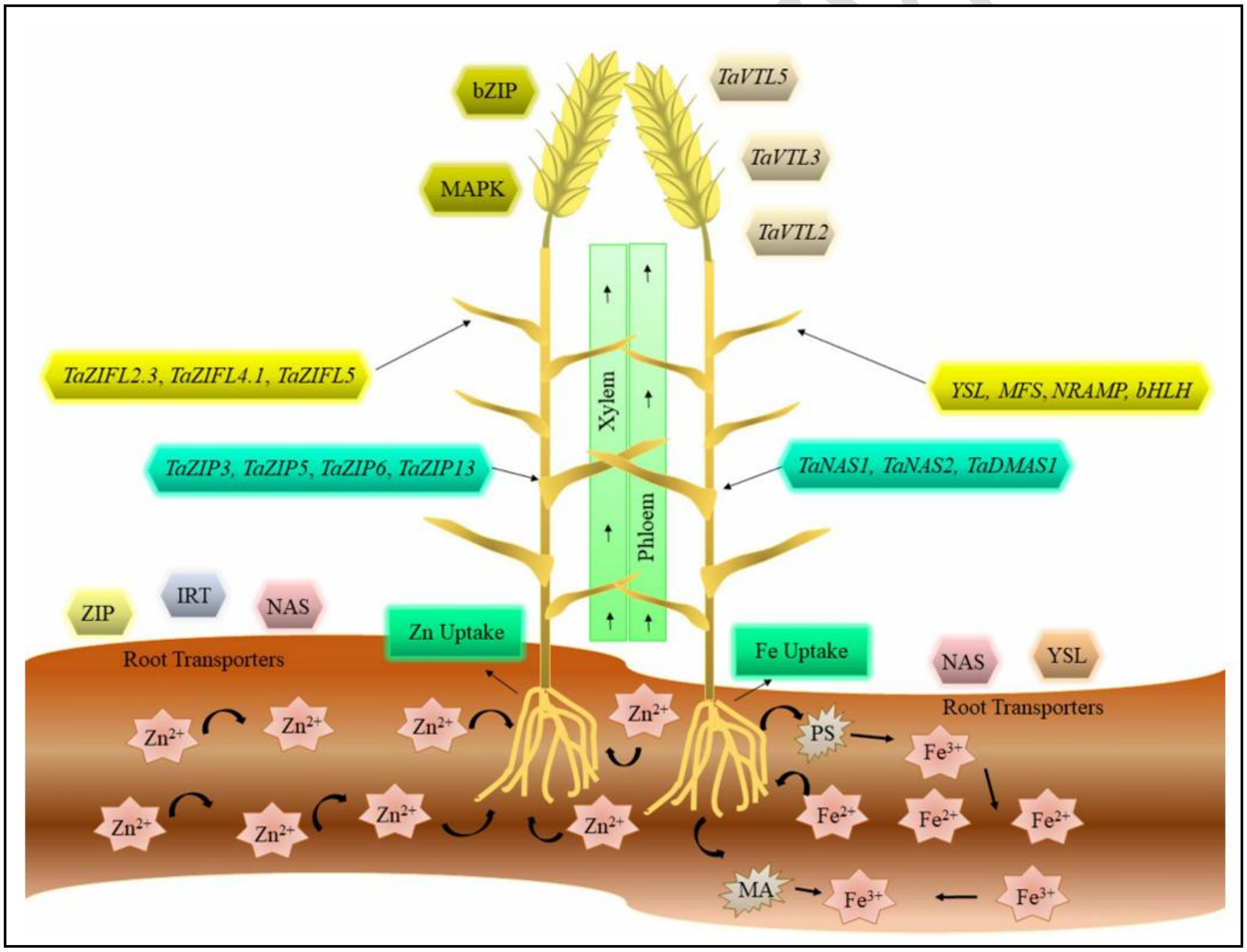

Figure 3: Genetic mechanism involved in iron and zinc regulation governing uptake and translocation of the nutrients to the shoot. The root releases MA and PSs resulting in chelation of iron which makes it available for uptake. The absorption of zinc is governed by ZIP and IRT- like proteins and the translocation of Fe/Zn is done by YSL, NAS and other metal transporters **(Source: Balk *et al*. [1]).**

The uptake and transport of zinc occur either in the form of divalent cation (Zn^{+2}) or as Zn-Ps complex which is formed with an Fe^{+3}chelatorand phytosiderophores which is secreted by the root cells. ZIP like transporters are reported to take up Zn in the plants [22]. Hence, various iron and zinc transporters, including iron-regulated transporter, zinc-regulated transporter-like proteins (ZIP), enable the transfer of Zn^{2+} and Fe^{2+} across the cell membrane [23].Nicotinamide is also an iron and zinc chelator that is responsible for the flow of iron and zinc in the root and further from root to shoot. Various gene families like Zinc-regulated, Iron-regulated trasnsporters (IRT)-like proteins (ZIP), Nicotinamide synthase (NAS), Yellow stripe-like (YSL) transporter protein have shown to control Fe and Zn homeostatis [24]. Various metal transporters have also been reported which are responsible for the translocation of iron and zinc in wheat crop. For example, transporters TaZIPs were involved not only for the translocation of zinc from root to shoot but also to maintain homeostatis of the ions in the crop. Studies shows that TaZIP5, TaZIP6, and TaZIP13 are responsible for zinc uptake [25].

As the root takes up iron and zinc they are moved through the xylem into the shoots. There zinc moves freely as ion or as a complex formed with organic acids, where as iron has to be chelated with organic acids .Protein families YSL and ZIP help transfer Zn/Fe from root to shoots and from xylem and phloem to targeted parts during grain filling [26]. The amount of Fe and Zn in cereal grain depends on physiological processes such as root acquisition from soil, transport to shoots, and reintegration of preserved minerals from leaves during grain filling [16].

Biofortification of Wheat

There are several methods by which biofortification of wheat can be done. The methods can be classified into agronomic methods and genetic methods (Figure 4).

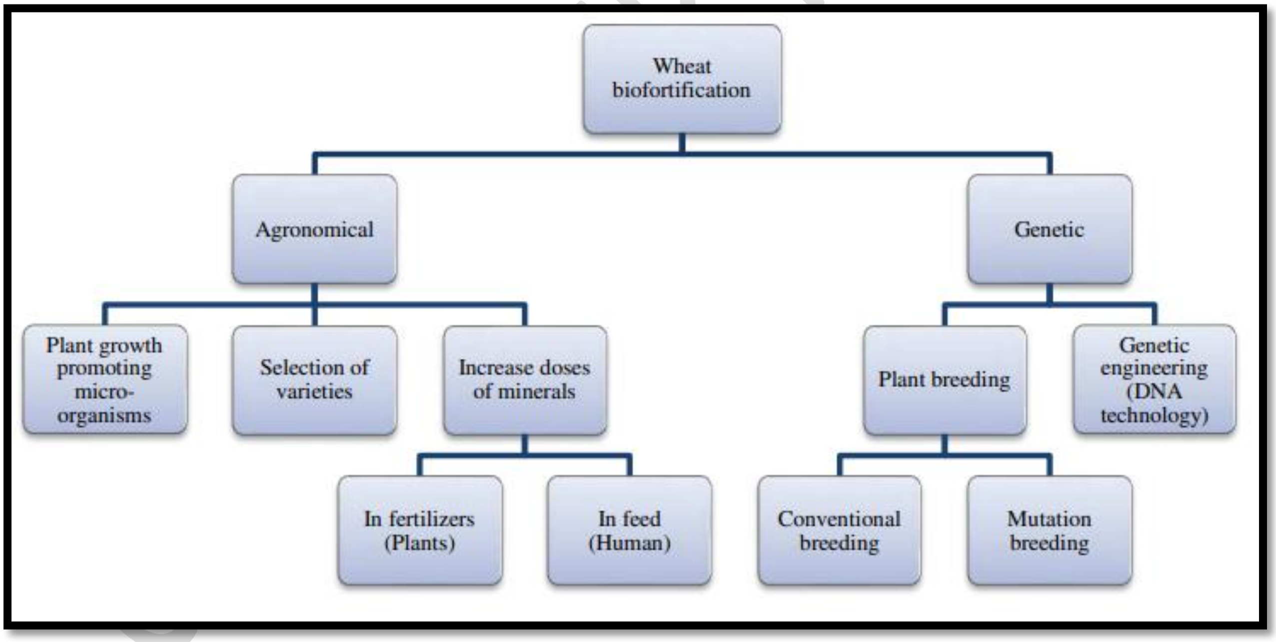

Figure 4: Classification of biofortification method **(Source: Sharma *et al*. [27]).**

5. Genetic biofortification of Wheat

Focusing onto the genetic approaches for biofortification, it can be further divided into conventional methods and non-conventional methods. The schematic display of different breeding strategies that can be adopted for the development of biofortified wheat with increased nutrient concentration is presented in Figure 5.

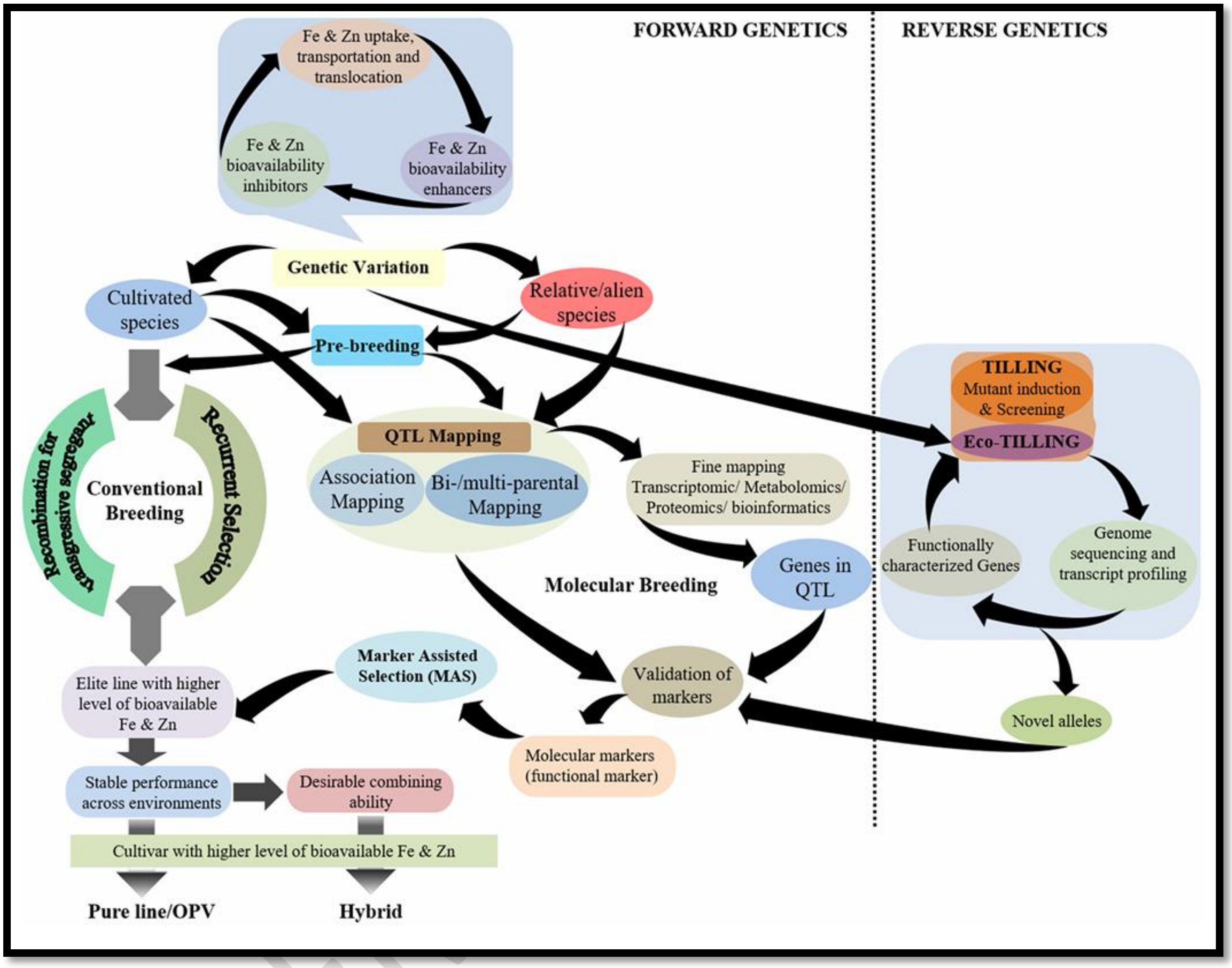

Figure 5: Different genetic approaches for biofortification of iron and zinc in wheat **(Source: Garcia-Oliveira *et al.*[16]).**

Nutrient uptake by plants, nutrient translocation to grain, nutrient storage in the endosperm, reduction of antinutritional compounds, and increased nutrient bioavailability can be improved by effective genetic biofortification [28]. By using marker-assisted breeding, gene discovery, or traditional (conventional) breeding techniques it is possible to exploit genetic variation for increasing nutritional content in grain. Plant breeders or breeding institutions have identified and raised nutrient-dense wheat cultivars that are high in concentrations of iron and zinc as well as other elements that support the bioavailability of these micronutrients. In addition, valuable genes from wild relatives can be transferred using conventional and modern breeding techniques to increase the zinc and iron content of grains [29]. Also, through the use of genetic engineering, genes from any desired source can be directly incorporated into a crop. Recent advancements in genetic biofortification have reduced iron and zinc deficiencies, offering a sustainable diet-based option [30].

5.1 Conventional breeding strategies for biofortification in wheat

Wheat grain yields have steadily increased during the past few decades. Wheat breeders worldwide continue to rely on conventional plant breeding, which has been employed for centuries. The goal is to modify the genotype of a selected crop and to enhance its ability to absorb and accumulate bioavailable nutrients [27]. Using conventional approach a significant contribution has been made in grain quality, yield, agronomic traits governing yield in wheat crop [31].

The International Maize and Wheat Improvement Centre (CIMMYT, Mexico) has employed conventional breeding to create wheat varieties with higher iron and zinc contents [32]. The collaboration of international organization like CIMMYT, Harvest Plus, ICARDA with various public and private sectors have resulted in the development of biofortified varieties of wheat. This collaboration has also resulted in the development of iron and zinc rich varieties of wheat [3]. The production of zinc and iron fortified wheat crop have gained momentum in recent years. In India, the zinc biofortified lines from CIMMYT have a zinc concentration that is 20–40% higher [32]. Five research institutes generated 11 bread wheat and 5 durum wheat varieties with high Fe or Zn content or both , which were released by the central variety release committee, Government of India. The biofortified varieties of wheat developed using conventional approaches along with the nutritional factor improved and institutions responsible are mentioned in table 1 below.

Table 1: Description of improved biofortified wheat using conventional breeding approaches.

VARIETIES	NUTRIENT ENRICHED	INSTITUTIONS	SOURCE
WB 02	Fe and Zn	ICAR-Indian Institute of Wheat and Barley Research, Karnal, India	[33, 34]
HPBW 01	Fe and Zn	Punjab Agricultural university, Ludhiana, India	[33, 34]
HI 1633	Fe and Zn	ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India	[33, 34]
PBW 757	Zn	ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India	[34]
HD 3171	Zn	ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India	[34]
PBW 771	Zn	ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India	[34]
HI 8777	Fe and Zn	Indian Agricultural Research Institute (IARI), Indore	[34]
HI 8759	Fe and Zn	Indian Agricultural Research Institute (IARI), Indore	[34]
HI 8805	Fe and Zn	Indian Agricultural Research Institute (IARI), Indore	[34]
HUW711	Zn	Banaras Hindu University, India.	[3]
MACS 4028	Fe and Zn	Agharkar Research Institute, Pune, India	[3]

Table 2: Improved nutritional content (Fe & Zn) of different varieties of wheat **(Wani*et al*. 2022[3], Yadava*et al*. 2020[34])**

WHEAT VARIETIES	**BIOFORTIFIED NUTRITIONAL CONTENT**
PBW 757	Zinc (42.3 ppm)
PBW 771	Zinc (41.4 ppm)
DBW 187 HI 1605 HD 3249	Iron (> 43 ppm)

HD 3298	
HI 8777	Iron (48.7 ppm) Zinc (43.6 ppm)
HI 8759 HI 8805	Iron(>40 ppm) Zinc(>40 ppm)

In the year 2012, CGIAR's HarvestPlusprogramme collaborated with Banaras Hindu University, India, and CIMMYT to generate high-Zn genotypes with improved agronomic benefits. The developed genotypes were Akshay, BHU 1, BHU 5, and BHU 6. A recent released variety Rajendra-Genhu-4 (BHU-31) is a biofortified zinc variety of wheat and is under commercial cultivation in India. Alongside, the public-private collaboration is commercially being exploited in India. HarvestPlus in collaboration with 'Nirmal Seeds' have commercially exploited two Zn enriched varieties *i.e*, Zinc Shakthi and Akshay. Another seed company named 'Astha Beej' have commercially grown and marketed Zinc Shakthi seeds as "Chitra" [3].

Conventional breeding can exploit genetic variability found in wild types and related species. Nutrient deficiencies can be overcome by crossing with distant relatives and transferring characteristics into commercial cultivars. However, conventional breeding for specific traits may be inconvenient and time-consuming. Although conventional breeding approaches has been used worldwide and has significantly contributed in development of improved varieties but its drawbacks cannot be neglected. These approaches are time-consuming, labor-intensive, imprecise, and prone to errors due to their reliance on traditional phenotyping or visual assessments [35]. Looking into the drawbacks of the above, non-conventional breeding approaches stands as a suitable alternative.

5.2 Non-conventional breeding strategies for biofortification in wheat

Our ability to biofortify crops has improved with a greater understanding and identification of genes responsible for bioavaibility of nutrients and mineral transport. Modern biotechnology has shown that it is feasible to enhance iron and zinc levels by studying genes, their location and molecular markers. Modern breeding techniques requires fewer generations than conventional plant breeding to create new biofortified varieties [28]. The genomic studies done in non-conventional breeding is independent of the stages of crop growth. These techniques are highly precise and reliable as compared to that of non-conventional practices that are based on visual assessment.

Genetic mapping approaches such as QTL mapping and Genome-Wide Association Studies (GWAS) can recognize genes and genomic regions in the DNA that are known to directly affect the traits that govern nutritional factors in wheat. For mapping the locus responsible for iron and zinc content in wheat, genomic resources such as SNP genotyping and reference genome sequence can be used. This will make it easier to develop and deploy molecular markers in elite genetic backgrounds, leading to more efficient bio-fortification breeding programmes [36, 37].

5.3Quantitative Trait Locus (QTL) mapping

Several research have been undertaken to map QTLs of grain Fe and Zn concentration in wheat. In 1997, the first QTL for iron and zinc concentration in wheat was mapped on chromosome 6BS in a RIL (recombinant inbred line) population developed from a hybrid between durum wheat and wild emmer (*Triticumturgidum*) [38]. The QTL, Gpc-B1 showed an increase in iron concentration by 18% and zinc concentration by 12%.Many QTL studies have been undertaken to investigate the genomic areas for iron and zinc contents in wheat.

Several biparental mapping populations have been used to map the iron and zinc concentration in wheat grains. Bi-parental mapping populations were mostly created with bread wheat cultivars as parents, but other hexaploid species such as *Triticumspelta*, stabilised synthetic hexaploid wheat (SHW) lines, and tetraploid parents were also used. Many research use (multi-environment trial) data to identify stable QTL's for grain Fe and Zn content [39]. Several QTLs for grain Fe and Zn content in wheat have been reported and can be used further for biofortification in wheat (Table 3)

Table 3: Various QTLS's for iron and zinc in mapping population of wheat

MAPPING POPULATION	TRAIT	CH. NO.	QTL	FLANKING MARKERS	SOURCE
RILs (PBW343 X Kenya Swara)	Zn	2B 1BS 3AL	QGzncpk.cimmyt-2Bc QGzncpk.cimmyt-1BS QGzncpk.cimmyt-3AL	wPt-6174 wPt-3103 wPt-0286	[40]
RILs (SHW-L1 X Chuanmai 32)	Zn	2D	QZn-2D	wPt-730,057-wPt-671,700	[41]
	Fe	2B	QFe-2B	wPt-7004-wPt-4210	
RILs (Tabassi X Taifun)	Zn	4A 1A	QZn-4 A QZn-1 A	Xgwm4026-Xgwm1081 Xgwm3094-Xgwm164	[42]
	Fe	7B 4D 3D	QFe-7B QFe-4D QFe-3D	Xgwm767-Xgwm3036 Xgwm4670-Xgwm194 Xgwm1047-Xgwm383	
DH (Berkut X Krichauff)	Zn	2B 1B	QZn.bhu-2B QZn.bhu-1B	gwm120–wpt2430 wmc036c–cfa2129	[43]
	Fe	2B	Fe.bhu-2B	gwm120–wpt2430	
RILs (Seri M82 X SHW CWI76364)	Zn	4BS 4BS 4BS	QZn.Y13-14_4BS QZn.Y12-13_4BS QZn.Across_4BS	TP91631-TP81797 TP73864-TP71929 TP91631-TP81797	[44]
	Fe	4BS	QFe.Across_4BS	TP91631-TP81797	

		5BS 7DS	QFe.Y13-14_5BS QFe.Across_7DS	TP91631-TP81797 TP43715-TP37547	
RILs (WH542 X SHW line)	Zn	2A 2A	QGZn.iari-2A QGZn.iari-2A	Xgwm359-Xgwm249 Xgwm359-Xwmc407	[45]
	Fe	7B 2A	QGFe.iari-7B QGFe.iari-2 A	Xgwm577-Xbarc264 gwm359-Xgwm249	
RILs (Adana99 X 70,711) Hexaploid population	Zn	7B 6B 7A 1D 1B 3A	QGZn.ada-7B QGZn.ada-6B QGZn.ada-7A QGZn.ada-1D QGZn.ada-1B QGZn.ada-3A	wPt-733,112 wPt-667,798–wPt-7065 wPt-2083–wPt-6083 wPt-6979–wPt-730,718 rPt-6561 wPt-2698–wPt-0398	[46]
	Fe	2B 6B 2B 7B	QGFe.ada-2B QGFe.ada-6B QGFe.ada-2B QGFe.ada-7B	wPt-1394–wPt-7864 wPt-667,798–wPt-7065 wPt-9812 wPt-5922	
RILs (Saricanak98 X MM5/4) Tetraploid population	Zn	1B 6B	QGZn.sar-1B QGZn.sar-6B	wPt-6434- wPt-1403 wPt-743,099- wPt-5037	[46]
	Fe	5B 3A/3B 5B	QGFe.sar-5BTKM QGFe.sar-3 A/3B QGFe.sar-5BMCO	wPt-81.25 wPt-9504 wPt-0784- wPt-8875 wPt-7400 wPt-8449	
RILs (Bubo X Turtur)	Zn	7B 1B	QGZn.cimmyt-7B_1P1 QGZn.cimmyt 1B_P1	3,945,822; 1132640F0-5CG 3,934,172; 3,934,936	[47]
	Fe	3A	QGFe.cimmyt-3A_1P1	1,234,521; 3034169F0-11AG	
RILs (Louries X Batelur)	Zn	1B 7B	QGZn.cimmyt-1B_1P2 QGZn.cimmyt-7B_1P2	4,991,478; 3,937,490 1,079,651; 1,262,636	[47]
	Fe	2A 4D	QGFe.cimmyt-2A_P2 QGFe.cimmyt-	4,262,668; 1,226,245 2,363,822; 3,961,236	

		4A	4D_P2 QGFe.cimmyt-4A_P2	338,535; 1,211,533	
RILs (Roelfs F2007 X Chinese Parental Line)	Zn	7A 5B	QGZn.co-7 A QGZn.co-5 A	5,356,706 5,325,178|F|0 1,244,217 1,272,027|F|0	[48]
	Fe	5A 3B	QGFe.co-5 A. 2 e QGFe.co-3B.1	1,102,433 988,523 1,089,107 1,127,875|F|0	
RILs (Kachu X Zinc shakti)	Zn	1B 6A	QGZn_Y17_1b QGZn_Y17_6a	13,142,877; 3,954,275 1,092,057; 1,082,014	[49]
	Fe	4A 6B	QGFe_Y17_4a QGFe_Y17_6b	1,099,697; 5,324,893 1,864,870; 2,278,502	

In the study above, each QTLs were frequently distinct from one another because of the variations in populations, environments, and marker sets, all of which had a significant impact on the precise position and identification accuracy of the QTLs. By projecting the markers onto the genome sequence, it should now be easier to compare the actual location of QTLs found in various investigations, using the new reference sequence as a benchmark. This could make it possible to identify the QTLs that consistently produce higher levels of iron and zinc concentration. However, due to practical limitations on the number of markers that may be utilized for multiple QTL's simultaneously using marker-assisted selection might be challenging in large breeding populations. Alternatively, marker-assisted selection could be used to stack small effect QTL within the same genomic area to create a haplotype with many advantageous QTL. Halotype basically means a set of DNA present in a single chromosome that have a tendency to inherit together. By using marker-assisted selection, this haplotype might then be chosen as a single unit, lowering the number of markers needed for further selection stages. Using this method could also add value to marker usage by combining QTL for higher iron and zinc content with QTL for other advantageous traits, such yield or disease resistance, which are more likely to already be under marker-assisted selection [50].

Several QTL studies have been undertaken to investigate the genomic areas for iron and zinc concentration in wheat. A double haploid population crossing Berkut and Krichauff was developed. Zn and Fe concentrations in grains were measured using X-ray fluorescence. Three QTLs were found using composite interval mapping: one for protein (1A), two for Zn with a QTL being co-located for Fe [43]. The QTLs that were found throughout several seasons seemed to be reliable and beneficial for MAS (marker assisted selection).In current investigation at CIMMYT in Mexico, a high grain zinc content line of Chinese provenance was crossed with a Mexican bread wheat cultivar, Roelfs F2007 to create a RIL mapping population for QTL identification. Using the annotated wheat genome sequence, they have also discovered 55 potential genes responsible for the Zn and Fe content of the grain [48]. Also, Four QTLs were discovered for grain Zn and Fe concentration in a RIL population generated from the cross of Indian variety 'WH 542' and the SHW (Synthetic Hexaploid Wheat) line [45].

Large-scale utilization in breeding programmes to generate nutrient-rich elite lines should be prioritized. Studies show that developing countries are the most affected nations of micronutrient deficiencies. Bio-fortifying wheat varieties with QTLs affecting grain Fe and Zn content is a cost-effective and sustainable technique. To increase the Fe and Zn levels, screening unknown landraces, related species, wild relatives, promising SHW lines, and alleles from high-nutrient genetic resources is necessary. Mapping populations across habitats is crucial for understanding genotype-environment interactions and identifying stable QTLs for these traits. Using reported QTLs in elite genetic backgrounds can create nutrient-rich breeding lines, providing a long-term solution to malnutrition.

Genome-wide association studies (GWAS)

QTL mapping has substantially improved understanding of polygenic characteristics, such as Fe and Zn accumulation in wheat grains. Using bi-parental mapping populations from cultivated tetraploid, hexaploid, and SHW lines, many QTLs were discovered for these two micro-nutrients. But the QTLs found in bi-parental populations have low resolution and are limited to genetic variation in the two parental lines utilized to produce the mapping population. Genomic areas revealed through GWAS exhibit high QTL resolution and allele coverage and can be used in elite genetic background to improve nutritional quality of wheat [51].

The genome wide association studies used various association panels, including Harvest Plus Association Mapping Panel, CIMMYTs Spring Wheat Reference Set, European elite wheat varieties, SHW lines, and *Aegilopstauschii* accessions. HPAM panel comprising 330 diverse lines were examined in India and Mexico in 6 trails for Zn content. GWAS identified 39 significant MTAs and two large-effect QTLs on chromosomes 2 and 7 [4]. The same HPAM panel was genotyped for Fe and Zn levels at different growth phases. GWA studies found that 72 and 65 MTAs were substantially linkedwith Zn and Fe concentration [56]. Other study reveals that GWAS was conducted on two panels *i.e*. on 369 European elite wheat varieties (panel) and 183 genotypes (subpanel) using polymorphic SNP markers to determine grain Zn content. There were 40 and 161 MTAs detected in the elite varieties panel and subpanel, with extremely significant MTAs reported on chromosomes 5A and 3B. Previous studies have reported QTLs on these genomic regions [57]. The genetic architecture of grain Fe content was also established using the same two panels. GWAS identified significant MTAs on chromosomes 2 A, 3B, and 5 A for Fe concentration, as well as 137 and 41 significant SNPs in the subpanel and panel respectively [58].

All things considered, these investigations have advanced our knowledge of the genetic makeup of wheat grain Fe and Zn contents. Even though these two characteristics are influenced by soil characteristics and are under polygenetic control, their level can be raised by pyramiding numerous big effect QTLs and also through MAGIC population. These genomics research will not only deepen our understanding of the genetics behind these features, but they will also boost the efficiency of biofortification.

6. Transgenic approach for biofortification

Modern biotechnological techniques have made use of this understanding to show that iron and zinc levels can be raised in both the whole grain and the starchy endosperm in particular [1]. Although the development of transgenic in wheat is still not as high as that of other crops but it is continually rising. In wheat by employing the wheat ferritin gene (TaFer1-A), an attempt was made to increase the Fe content of wheat grain. Due to overexpression of the Ferritin gene, grain Fe content improved by 50–85% (44.5 µg/g in endosperm) [59].

A variety of promoters has been reported which targets transgene expression in certain tissues. Furthermore, a patented method (PureIntro) from Japan has made it possible to transform wheat using Agrobacterium mediated gene transfer technology. But there are still difficulties. This method is costly and is unable to develop genotype-independent transformations as mostly two spring wheat varieties namely, Bobwhite or Fielder has been used for the process as they are susceptible to the bacterium but these varieties are unsuitable for commercial cultivation [60].

Although very less work has been done in wheat using transgenic approach. Transgenic studies have been hampered due to lack of wheat genomic information, but presently that high-quality gene sequences are readily accessible and transformation efficiencies are improving and transgenic studies are likely to play a vital part in developing iron and zinc rich varieties in wheat.

7. Conclusion

Cereal grains feed the majority of the world's population and enhancing their nutritional value can have a direct impact on human health. Wheat (*Triticumaestivum* L.) is a widely cultivated cereal crop. Wheat biofortification can be done in a variety of ways and the most practical of which are QTL mapping and GWAS approaches. Increasing the nutritional content of micronutrients such as iron and zinc *via* traditional methods is difficult due to the intricate and time-consuming process. However, a lot of work has been done using conventional/traditional approaches some of which are WB-02, HI 1633, MACS 4028, HPBW 01 (Fe and Zn enriched varieties of wheat), PBW757, PBW 771, HD 3171 (Zn enriched variety of wheat). In the era of high-throughput genomics and sophisticated statistical analysis, dealing with QTL mapping, GWAS, and other molecular approaches has become more useful. Genetic mapping techniques such as QTL mapping and Genome-Wide Association Studies (GWAS) can identify genes and genomic areas in DNA that are known to have a direct impact on the features that govern nutritional aspects in wheat. Many important QTL's governing iron and zinc content in wheat have been reported in various biparental mapping populations. Techniques such as composite interval mapping were used to find QTL's for the purpose and X-ray fluorescence for determining the content of Fe and Zn in grains. These findings can be utilized to produce iron and zinc biofortified varieties of wheat. With the new era come new techniques, such as transgenic approaches. Although very less work has been done till date in the development of transgenic wheat but currently efforts are made for developing one. Methods like PureIntro from Japan had successfully been able to develop transgenic in two wheat varieties. It has become evident that transgenic research is going to play a vital role in biofortification of wheat crops.Overall, using non-traditional strategies discussed here shows to be an acceptable answer to the aforementioned challenge. Many investigations and researches have been conducted in many institutes related to the subject, and the use of QTL mapping methodologies has consistently produced satisfactory results. Working with such novel and unconventional ways might not only provide the desired outcomes of improving nutrient content in wheat, but it can also aid in understanding gene function and their expression. Many studies have also found that utilizing these strategies can not only alter the intended feature, but also increase the expression of other desirable traits. Biofortification to increase micronutrient levels in crops is becoming increasingly important in order to address the hidden hunger and nutritional deficiencies in the human population.

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