**Bio-fortification of wheat for improving nutritional security in climate change scenario**

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**Abstract**

In future, wheat production will decrease due to climate change situations with factors that can be related to climate change processes like precipitation-heavy rains, water scarcity, hail storms, drought, land slide; meteorological extremities (temperature anomalies-frost, heat days, high wind, duration of unfavorable periods; air-storms, alterations of radiation and its postulates; and to reduce these risks, the impact of climate change mitigation strategies and management systems for crop adaptation to changing climate conditions should be considered. In developing countries, micronutrient malnutrition is the main health problem, and is the result of deficiencies, excesses or imbalances in diet of a person with respect to nutrients and energy. Approximately, two billion people worldwide suffer from micronutrient deficiencies such as Zinc (Zn) and Iron (Fe) leading to various health impairments. Maintaining grain quality of wheat under climate change is critical for human nutrition, end-use functional properties, as well as commodity value. Bio-fortification strategies viz., genetic and agronomic approaches offer sustainable solution to improve the nutrition status of resource poor people in developing countries. In climate change scenario, developing water use efficient wheat genotypes with high temperatures tolerance will be the main target of plant breeders to improve the production and productivity of wheat crop. In this attempt, high yielding durum wheat varieties with high nutritive traits were developed viz., **HI 8737 (**grain iron of 38.5ppm, grain zinc of 40.0ppm, protein >12.0%); **HI 8759 (**42.1ppm iron, 42.8ppm zinc, >12.0% protein); and **HI 8777 (**48.7ppm iron, 43.6ppm zinc, >14.0% protein); and high yielding climate resilience bread wheat varieties with good chapati, biscuit and bread traits viz., **HI 1605 (**43.0ppm iron, 35.0ppm zinc, >13.0% protein); **HI 1633 (**41.6ppm iron, 41.1ppm zinc, >12.5% protein); and **HI 1634 (**38.0ppm iron, 37.0ppm zinc, >12.0% protein) were developed. The deployment of bio-fortified cultivars during this climate change scenario holds great promise for health and wellbeing of the human population; and will helps to move from food security to nutrition security, where not only calories and proteins but also micronutrients are taken care of. We can make India a healthy and happy nation by targeting bio-fortification in staple food crops during this climate change scenario, that will not only satisfy hunger but also add essential micronutrients to the diet.

**Keywords:** Bio-fortification, climate change, malnutrition, zinc, iron, yield potential,

**Introduction**

Wheat (*Triticum aestivum* L.), a major agronomic crop cultivated worldwide, and has been a chief staple food, supplying approximately 35% of the total food as consumed by the global population (Mohammadi-joo *et al.,* 2015). Its adaptive attributes to varied climatic conditions and environmental stresses make it a remarkable crop contributing to food security in the world (Muslim *et al.,* 2015). Most of people in the world consume wheat and wheat products in the form of chapati, bread, biscuit, pasta or fermented products (Mallick *et al.,* 2013). The world has achieved a global wheat production of 765 million metric tons in the year 2021 (FAOSTAT, 2021). About 30 % of the population of developing countries mainly depends on wheat for nutrition (Ali and Borrill, 2020). In India, the crop is cultivated in 29.55 million hectares to produce the all-time gargantuan output of 107.95 million tonnes with a record average national productivity of 3424 kg/ha (MoA & FW (2020)).

The importance of wheat is undeniable, as humans growing wheat in diverse climates and wide types of soil globally and has three main grain components viz., bran, endosperm and germ. The wheat grain is nutritious due to presence of micronutrients, vitamins, phenolic compounds and protein (Lephuthing *et al.,* 2017). Wheat germ is rich in vitamins B and E, protein, unsaturated fats, minerals and carbohydrates, while the bran consists mostly of insoluble carbohydrates, protein, traces of B vitamins and minerals, and some anti-nutritional factors such as phytic acid. The endosperm is the largest part of the grain and consists mainly of starch and protein (Ram and Govindan, 2020). Though the wheat has several nutritional factors, it is in traces hence the population which is dependent mainly on the wheat diet facing the problem of malnutrition. Malnutrition is a major threat which is allied to agriculture and causing severe penalty to the GDP of developing countries.

In developing countries, micronutrient malnutrition is a serious health problem; and various interventions and solutions are currently used to combat it, but their overall coverage is relatively limited (Qaim *et al.,* 2007). Bio-fortification is the process of breeding nutrients into food crops, provides a sustainable, long-term strategy for delivering micronutrients to rural populations in developing countries. Wheat is being bred for higher levels of micronutrients by both conventional and transgenic breeding methods; and several conventional varieties have been released (Saltzman *et al.,* 2013). The conventional breeding was fruitful as several bio-fortified wheat varieties were released with the collaboration of International and National Research institutes. For example, Zinc Shakti (Chitra) developed by crossing of PBW343 with *Aegilops squarrosa* as a donor for zinc content gene with a 40% increase in Zn content. ‘Zincol 2016’ is zinc fortified variety developed by transferring the genes from *T. spelta* into the Pakistani variety, NARC2011; and ‘WB02’ and ‘HPBW-01’ were developed by transferring the genes from *Ae. squarrosa* and *T. dicoccom* with 25%, 20% and 20% increase in the zinc content respectively (Saini *et al.,* 2020). In Bangladesh, ‘Bari Gom 33’ is released under the collaborative project with International Maize and Wheat Improvement Center (CIMMYT) in 2017, which have 33% higher Zn content (Fig 1). CIMMYT has also released six varieties of bio-fortified wheat viz; Zinc Gahun1, Zinc Gahun2, Bheri-Ganga, Himganga, Khumal-Shalei and Borlaug 2020 in Nepal in the year 2020. In India, the pure line varieties viz., WB 02 (Fig 1), HPBW 01, HI 1605, HI 1633, HI 8759 (d) and HI 8777 (d) developed by ICAR Institutes and SAUs also have higher Fe and Zn content, which showed that conventional breeding has played important role in developing bio-fortified wheat by exploiting available diversity.

**Figure 1 : Field expression of Bio-fortified wheat varieties WB02 and BARI Gom 33 .**

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| --- | --- |
|  |  |
| WB-02 high Zn bio-fortified wheat released in India | BARI Gom 33, a zinc-enriched, blast resistant variety released in Bangladesh. |

**Effect of climate change on wheat grain quality**

Global agriculture is facing the probable impact of global warming (Harold, 2015), which is likely to bring local shifts in temperature and in the amount and seasonal distribution of precipitation. It is also likely to result in more extreme weather such as droughts and periods of heavy precipitation. Such changes can affect plant growth, the spread of pests and diseases and water availability in both positive and negative ways (Doll & Baranski, 2011). A recent study estimates the annual costs of adapting to climate change in the agricultural sector to be over USD 7 billion (Nelson *et al.,* 2009). Valizadeh *et al.,* (2014) reported that wheat production will decrease in the future due to climate change; and to reduce these risks, the impact of climate change mitigation strategies and management systems for crop adaptation to climate change conditions should be considered. Temperature and CO2 influence plant growth and development through their effects on stomatal opening and rate of physiological processes. Higher temperatures speed up the biochemical reactions and also increase transpiration losses. Stomatal conductance will decline with increasing CO2 concentration for crop, which fix and reduce inorganic CO2 into organic compounds (C3 plants). Rising atmospheric CO2 concentration provide some counteracting tendencies to the otherwise negative impacts of rising temperature and reduced soil moisture (Lobell & Gourdji, 2012). This seems to benefit more in terms of dry matter production from a higher CO2 level, due to higher leaf expansion, increase in the photosynthetic rate per unit area, increase in water use efficiency and increase in photorespiration rates (Warrick *et al.,*1986). First, higher CO2 has a fertilization effect in C3 species such as wheat, rice and most fruit and vegetable crops, given that photorespiratory costs in the C3 photosynthesis pathway are alleviated by higher CO2 (Lobell & Gourdji, 2012). The lack of expected rainfall has also leaded to water and pasture shortage within the country, which is absolutely one of the biggest problems (Hendrix, 2012). Farmer sensitivity to changing climate and the way they perceive the notable changes in rainfall and temperature condition and its impacts on crop production.

Grain quality is influenced by genetics, management and environment. Maintaining grain quality of wheat under climate change is critical for human nutrition, end-use functional properties, as well as commodity value (Nuttall *et al.,* 2017). In short, low nitrogen levels in soil are known to reduce grain quality in wheat, and this is further exacerbated in high CO2. It is widely recognized that grain quality declines with increase in atmospheric carbon dioxide, so increases in grain yield (weight) do not necessarily indicate increases in global production of grain protein Hatfield *et al.,* (2011). Kimball *et al.,* (2010) reported that grain quality reduced due to low nitrogen and is further reduced by high concentrations of CO2. At low nitrogen levels, protein content was reduced by 39% under elevated CO2 compared to a 33% reduction under ambient CO2. Blumenthal *et al.,* (1991) showed that there was a highly significant positive correlation of grain protein with hours above 35°C during grain filling, and negative correlations with dough strength and loaf volume.

Variation in environmental conditions strongly influence the majority of wheat grain quality traits i.e., with growing zones, latitudes and moisture regimes; and genotypic effects were mainly observed for carotene content, zinc content, iron content and SDS volume (Eslemi *et al.,* 2005). Grain quality showed significant negative affect by the presence of high ash content accumulated in the grain. Moisture stress increasesy protein content and vitreousness; and reduced thousand grain weight and ash content at both latitudes. Climate change is perhaps the most serious environmental threat to the fight against hunger, malnutrition, disease and poverty in Africa, mainly through its impact on agricultural productivity (Enete *et al.,* 2016). Climate change increases child malnutrition and reduces calorie consumption dramatically. Thus, aggressive agricultural productivity investments are needed to raise calorie consumption enough to offset the negative impacts of climate change on the health and well-being of children (Nelson *et al.,*2009). The present problems due to climate change are various, i.e., (1) climate change processes like water scarcity, drought, meteorological extremities (temperature anomalies-frost, heat days, duration of unfavorable periods; precipitation-heavy rains, hail storms, land slide; air-storms, high wind, alterations of radiation and its postulates, (2) economic, social, and policy problems, that may have negative impact on the adaptability to meteorological factors in general and climate change processes in particular regarding food and agricultural production.

**Status of malnutrition in India**

Malnutrition is the result of deficiencies, excesses or imbalances in daily diet of a person with respect to nutrients and energy, which are classified in two broad categories viz., under-nutrition that can result in stopping growth, stunting, underweight and second group i.e., excessive food intake, which results in obesity, diabetes, heart diseases (WHO, 2020). Lack of proper food for the human population in a country is a development issue, as well as crucial economic issue. In 2018, it was observed that 34.7% of children under five were stunted, 17.3% were wasted and 33.4% were underweight in India (MOHFW, 2019). Micronutrients are composed of essential vitamins and minerals required by human beings, which helps in stimulating cellular growth and metabolism. Micronutrient deficiencies (hidden hunger) constitute an important form of human malnutrition. India ranking 94th among 107 countries in Global Hunger Index, which results its falls in the category of ‘serious hunger’, (UNICEF, 2020). More than one-third of the global population, present especially in developing countries are deficient of micronutrients viz., iron, zinc, vitamin A and iodine, which is becoming a serious health problem (FAO, 2013).

Among micronutrient deficiencies, iron and zinc are recognized are the most important; and anemia is caused due to iron deficiency and leads to impaired mental development in children, reduced capacity for physical labor in adults. Whereas, zinc is a crucial micronutrient for improving immunity, diabetes control, healing, digestion, reproduction and physical growth. The elements viz., widespread prevalence of stunting, wasting, and nutritional deficiencies among women and children are prevalent in India's profile, when viewed under Global Hunger Index. Malnutrition is the main leading risk factor for death of children under the age of five in India (Vollset *et al*., 2020). Anemia prevalence was also high i.e., 53 % and 54% among all women of reproductive age, and girls with age of 15-19 years, respectively (Anemia *Mukt Bharat* Portal), which indicates that still half of the women in India were affected by anemia. Large variations in degree of malnutrition are observed at sub-national levels i.e., in Haryana, 63% of non-pregnant women, 55% of pregnant women and 72% of children; and are estimated to be anemic (International Institute for Population Sciences, 2017). The serious impact and burden on the India’s food security is the prevalence of under-nutrition and anemia among mainly half of the women, especially pregnant women. Mainly, mothers who are undernourished can trigger cycles of under-nutrition by passing on nutrient and vitamin deficiencies to newly born babies, therefore, under-nutrition in India is normally a gender problem.

Numerous measures were initiated by Indian Government to overcome hunger and malnutrition, viz., National Food Security Mission, National Policy On Farmers, National Nutrition Mission, National Mission on Pulses and Oilseeds, National Horticulture Mission, National Rural Livelihoods Mission, National Rural Health Mission, Mahatma Gandhi National Rural Employment Guarantee Act/Scheme, Integrated Child Development Services (ICDS) for children below 06 years to provide nutrition and pre-school education, Mid-Day Meal (MDM) for children of 06-14 years, Public Distribution System, Janani Suraksha Yojana (Mothers’ Protection Scheme) for pregnant/lactating mothers and Social Assistance to the poor/needy (subsidized food grains, pension, insurance, etc), besides the all-inclusive National Food Security Bill as mentioned earlier. By 2015, India is committed and targeting to achieve the Sustainable Development Goal (SDG) of zero hunger. In 2017, Prime Minister's Overarching Scheme initiated by the Government of India for the Holistic Nutrition (POSHAN) *Abhiyan,* which isa step towards meeting the targets by 2030. The main targets under this programme to be achieved by the year 2022 is to reduce stunting, under-nutrition and low birth weight by two per cent each and anemia by three per cent.

**Bio-fortification of wheat in Indian Scenario**

Under ideal situations, three approaches *i.e.,* food diversity, food supplements and bio-fortification are considered to be effective world-wide to reduce the impact of malnutrition. Whereas, the most sustainable and cost-effective mean for providing the desired levels of nutrients in the diet in natural form is ‘bio-fortification’ (Pfeiffer and Mc Clafferty, 2007). Realizing the paramount importance of nutritional quality in the country, emphasis on bio-fortification in crops was given by Indian Council of Agricultural Research (ICAR) for improvement of nutrition. ICAR aims to develop, scale up production and consumption of nutritious bio-fortified crops in this country of nearly 1.4 billion people. Bio-fortified varieties are also given priority in the ICAR front line demonstrations. ICAR took a crucial step by [establishing minimum levels of iron and zinc](https://www.harvestplus.org/knowledge-market/in-the-news/committed-alleviating-malnutrition-india-declares-minimum-levels-iron) to be bred into all national varieties of pearl millet and extending the same to other important crops (Yadava *et al.,* 2017). Research efforts of ICAR have now led to the development and release of a series of bio-fortified varieties/hybrids through All Indian Coordinated Research Projects (AICRPs) in cereals, millets, pulses, oil seeds, vegetables and fruits. Till 2020, 71 bio-fortified varieties were released in 16 different crops from various institutes of India which is an endorsement of country’s preparedness to alleviate malnutrition through this sustainable approach (Yadav *et al.,* 2020). Indian wheat generally contains 10-11% of protein, 28-32 ppm Iron and 30-32 ppm Zinc (Yadava *et al.,* 2018, 2019). The bio-fortified varieties will provide enough calories in food but also deliver essential nutrient(s) needed for adequate growth and development.

**Breeding approaches for high nutrition wheat :**

Through breeding, the quality levels of staple food crops can be improved to reach the target levels required to meet complete nutritional needs of people without affecting yield and other agricultural characteristics. The process of breeding plants for bio-fortification purposes requires work on 1) determination of genetic diversity of grain iron, zinc and carotene in germplasm and wild relatives, 2) pre-breeding or identification of nutrient-rich parental material, 3) genetic studies to find out genes engineering target traits, 4) development of superior cultivars through breeding methods, 5) testing nutrient-rich elite germplasm in multi-locus traits for G × E interaction and 6) determination of stable genotypes of cereal nutrient traits

1. **Genetic variability for micronutrients in wheat**

Availability of genetic variance for target traits in germplasm is a prerequisite for achieving target levels, and identification of genetic variance serves two main objectives 1) identification of pre-breeding parental lineages for use in crossbreeding programme, genetic studies and development of molecular markers. 2) Identification of elite cultivars or earlier cultivars containing a target concentration of micronutrients with desirable agronomic traits. A recent review on genetic diversity of grain quality traits i.e., micronutrients like iron and zinc in wheat grain showed sufficient genetic variance for these traits. (Table 1).

**Table 1: Genetic variability for grain quality micronutrient traits (iron and zinc) in wheat landraces, germplasms and advance lines**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No** | **Genotypes** | **Fe** | **Zn** | **Phenotypic method** | **Reference** |
| **Mean** | **Min** | **Max** | **Mean** | **Min** | **Max** |
| 1 | 62 translocated lines of “Pavon 76” | 36.6 | 32.0 | 53.0 | 47.1 | 35.6 | 57.6 | ED-XRF | Velu *et al*.,2019 |
| Advance high Zn lines | 40.0 | 30.0 | 52.0 | 53.0 | 35.0 | 72.0 |
| 3 | 245 wheat genotypes derived from landraces | 34.3 | 19.4 | 71.2 | 33.6 | 15.6 | 60.1 | ICP-MS | Khokhar *et al*., 2020 |
| 4 | 150 bread wheat genotypes | 31.9 | 9.2 | 49.7 | 29.0 | 10.7 | 59.4 | ICP-AES | Pandey *et al*., 2016 |
| 5 | 286 RILs of bread wheat | 38.1 | 32.6 | 44.2 | 37.6 | 30.3 | 48.4 | ED- XRF | Krishnappa *et al*., 2017 |
| 6 | 36 Elite wheat genotypes | 37.8 | 6.43 | 85.9 | 29.3 | 3.44 | 60.9 | ICP-MS | Khokhar *et al*., 2018 |
| 7 | 299 winter wheat genotypes | 39.2 | 25.2 | 56.7 | 29.6 | 21.5 | 46.6 |  | Guttieri *et al*., 2015 |
| 8 | 600 core germplasm | 39.65 | 26.3 | 68.8 | 30.4 | 16.8 | 60.7 | ICP-OES | Velu *et al*., 2011 |
| 9 | 37 bread wheats(1st HYPT) | 37.0 | 32.3 | 43.7 | 32.5 | 19.8 | 36.7 | ED-XRF | Velu *et al*., 2012 |
| 10 | 655 Wheat germplasm from China | ICP-MS | Liu *et al*., 2014 |
| Spring | 47.3 | 28.3 | 76.1 | 29.3 | 13.9 | 56.3 |
| Winter | 45.0 | 22.5 | 78.6 | 30.26 | 13.6 | 56.6 |

1. **Relationships of grain micronutrients with yield and other quality traits**

The knowledge of the relationship between traits through correlations is important for simultaneous selection and improvement of traits in genetic improvement. The ultimate goal of the bio-improvement program is to develop micronutrient-rich cultivars in combination with farmers' preferred traits i.e., grain yield, disease and pest resistance along with other grain quality traits. In wheat, a negative weak association was observed between grain yield and its micronutrient concentration (Ficco *et al*., 2009). Significant negative correlations have been observed between glutenin content and Zn and Fe concentration; while strong negative correlation occurred between Fe and plant height and glutenin content indicating that plant with lower glutenin content and shorter height favor higher grain Fe concentration (Gomez-Becerra *et al*., 2010). Moderate negative correlation was observed between zinc and grain yield (Gomez-Becerra *et al*., 2010) and non-significant association between grain yield and micronutrient traits including zinc (Velu *et al*., 2012). A negative correlation was observed between Phosphorus and both Fe and Zn uptake. In wheat grain, approximately 75% of the total Phosphorus is stored as phytic acid, particularly in germ and aleurone layers (Lott and Spitzer, 1980).

Recent studies show that Nitrogen nutrients status of plant also has positive effects on root uptake and shoot transport, retranslocation from vegetative tissues into seed and seed allocation of Fe and Zn (Erenoglu *et al.,* 2011). Increasing soil Nitrogen or foliar application was highly effective in improving root uptake and shoots and grain accumulation of Fe and Zn which was shown from wheat experiment (Kutman *et al.,* 2011). It seems that grain yield and micronutrients have complex relationship which is affected by many external factors like growing conditions, analysis methods and hence strength of relationship is affected by G x E interaction.

1. **Genetic architecture of grain Fe an Zn in wheat**

To overcome the serious malnutrition situation of developing countries, improvement of iron and zinc content in wheat grain along with pro-vitamin A in a major staple crop like wheat through plant breeding is initiated by CGIAR group under Harvest Plus program (www.harvestplus.org). Considering the different parameters like target human population, daily consumption of wheat, average Fe and Zn content in wheat, micronutrient retention after processing, and bioavailability of traits, the target values for iron content is 58 ppm and for zinc content is 38 ppm was decided by Harvest plus. To achieved these targets through breeding program, the knowledge about genetic behaviors of these traits is necessary i.e., information about the heritability of the trait should be known before improvement through conventional breeding. The low heritability values of 0.25 for grain zinc and 0.37 for grain iron were recorded in 20 genotypes of SAMNYT trial in eastern Gangatic plains India (Joshi *et al*., 2010). The high broad sense heritability for first- and second-year experiment of 30 spring wheat genotypes was 0.74 and 0.85 for grain Fe and 0.61 and 0.92 for gain Zn respectively (Khodadi *et al*., 2014). The study of advanced lines from CIMMYT breeding program showed high heritability value for grain Zn (0.79 and 0.83 in 2017 and 2018 respectively) and medium for grain Fe (0.67 and 0.66 in 2017 and 2018 respectively) was recorded for translocated lines of “Pavan 76” by Velu *et al*., (2019).

Initial screening of germplasm, landraces and wild relatives indicates sufficient variability for grain Fe and Zn in wheat, and this available variability as well as the genetic architecture like gene effect, and genotype × environment effect for iron and zinc facilities the breeding approach for improvement for these traits. The generation mean analysis approach in two wheat crosses under normal and stress condition showed that the additive and non-additive gene effects are important for iron and zinc uptake in wheat (Amiri *et al*., 2020). Grain Fe and Zn content are highly quantitative traits and influence by environmental factors. The breeding programme for high micronutrient concentration in the wheat grain is highly influenced by the soil compositions (Trethowan., 2005) and based on the availability of iron and zinc content in the soil, the uptake of micronutrient from soil and mobilization into the grain will be limited (Ortiz-Monaterio *et al*., 2007).

1. **Genomics approaches for bio-fortification**

The costly and laborious phenotyping technique and large G × E interaction in conventional breeding leads to slow improvement of micronutrient levels i.e., zinc and iron content in wheat grain; and by identification of linked molecular markers to loci governing variation for the grain micronutrient will leads to selection of genotypes rich in micronutrient without conducting the field phenotyping (Velu *et al*., 2014). The information about the genomic regions, which control zinc and iron content in the grain is very important in order to breed cereals like wheat for biofortifcation using MAS. Several QTL mapping studies were conducted for grain Fe and Zn concentration in wheat. The major gene loci *GPC-B1* mapped on chromosome 6B is closely associated with high Fe, protein and Zn is introgressed from *T. dicoccoides*, which encodes NAC transcription factor (*NAM-B1*) and functions as senescence acceleration and micronutrient mobilization from leaves to grains (Distelfeld *et al*., 2007). Using three different sets of RIL population from CIMMYT-Mexico, a number of QTLs were identified for grain Fe and Zn; and among them a major QTL for grain Zn i.e., *QGZn.cimmyt-7B-1P2* located on chromosome 7B, explaining the phenotypic variation of 32.7% and another major QTL for grain Zn (*QGFe.cimmyt-7A- P2*) located on chromosome 4A, explaining the PVE of 21.4% (Crespo-Herrera *et al*., 2017). Population of 138 lines developed through double haploid (DH) technique from “Berkut and Krichauff” evaluated in eastern Gangetic plains of India, and two QTLs for grain Zn were identified from this population on chromosome 1B and 2B, which explains mean PVE of 23% and 35.9% respectively; and the QTL on chromosome 2B is collocate with grain Fe which explains 22.2% of phenotypic variation for Fe concentration (Tiwari *et al*., 2016). The RIL population developed from Indian old wheat variety WH 542 (286 lines) and synthetic derived genotype (PI94624/Aegilops-squarrosa (409)//BCN) evaluated under six environments for estimation of grain zinc and iron content; and identified four QTLs for grain Fe on chromosome 2A, 5A, 7A and 7B and five QTLs for grain Zn on chromosome 2A, 4A, 5A, 7A and 7B; and these QTLs together explains 20% and 32% phenotypic variation for Fe and Zn in wheat grain, respectively (Krishnappa *et al*., 2017).

The characterization of the full complement of wheat ferritins show that the modern hexaploid wheat genome contains two ferritin genes, tafer1 and tafer2, each represented by three homeoalleles and placed on chromosome 5 and 4, respectively. The two genes are differentially regulated and expressed. The *tafer1* genes are, except in the endosperm, the most abundantly expressed and regulated by iron and abscisic acid status. The promoter of *tafer1*, in contrast to *tafer2*, has iron- and ABA-responsive elements, supporting the expression data. The *tafer1* and *tafer2* genes encode two isoforms, probably functional different and acting in heteropolymer structures of ferritin in cereals. Iron bio-fortification of the wheat grain is possible, Endosperm targeted intragenic over expressing of the *tafer1-A* gene results in a 50-85% higher iron content in the grain (Borg *et al.,* 2012). With carotene genes identified and functional markers developed, there is a growing interest in understanding the molecular basis of QTL underpinning carotenoid content in wheat.

1. **GWAS and genomic prediction for grain Fe and Zn in wheat**

Many biparental approaches were used to dissect the genetic basis of grain Fe and Zn in wheat, but a biparental mapping approach is limited by low resolution of QTLs, limited allelic diversity from two parents, and more time required for development of suitable mapping population. One of the best approaches to dissect the genetic control of complex traits is GWAS, and provides advantage in relation to QTLs resolution, large allelic coverage and use of ancestral diversity through natural germplasm, landraces, elite genotypes and cultivars at same time. GWAS was applied in Harvest plus association mapping panel (HWAM) of 300 bread wheat genotypes for grain Zn phenotyping at range of environments in India and Mexico using Iluminai select 90K Infinitum SNP array, which reveals about 39 marker trait association (MTAs) for grain Zn, and leads to identification of two major QTLs region located on chromosome 2 and 7. Further candidate gene analysis within these major QTLs region reveals zing finger motif alongwith metal ion binding gene associated with the major QTLs (Velu *et al*., 2018). Another study in panel of 246 spring wheat reference set (SWRS) from CIMMYT gene bank Mexico was genotyped with 17,937 SNP markers and phenotype in two locations in India, which revealed eight and six significant MTAs for zinc and iron concentration in grain, respectively (Kumar *et al*., 2018). Alomori *et al*., (2019) perform a GWAS study in 369 European wheat genotypes, 40 MTAs were detected for grain Zn concentration on 12 different chromosomes.

1. **Ploidy Level Effect on Bio-fortification**

Introduction of the high grain protein content (Gpc-B1) locus from the wild tetraploid wheat *Triticum turgidum* ssp. *dicoccoides* into different recombinant chromosome substitution lines resulted in 10–34% higher concentrations of zinc, iron, manganese and protein in the grain compared to lines carrying the allele from cultivated wheat and that the Gpc-B1 locus promoted remobilization of protein, Zn, Fe and Mn from the leaves to the grain (Eide, 2006). In parallel, the ability to access genic sequence through RNA- seq and exome capture (Winfield *et al.,* 2012); and enabling the identification of single nucleotide polymorphisms and the development of publicly available genome-specific markers for genetic mapping in polyploidy wheat (Allen et al.,2013). Recently a comprehensive set of homoeolog-specific gene models for polyploidy wheat has been published (Krasileva *et al.,* 2013). In short, wheat researchers now have access to genome-specific contig assemblies (albeit partial and fragmented), draft reference genomes, gene models and large SNP datasets. Together, these tools should enable more precise mapping and deployment of grain Fe and Zn traits through marker assisted selection.

**Agronomic biofortification:**

It provides an immediate and effective route to enhance micronutrient concentrations in edible crop products, although genetic bio-fortification may be more cost-effective in the long run; and which can be achieved through fertilizer application of micronutrients to the soil and foliar application directly to the leaves of the crop (De Valenca *et al.,* 2017). These approaches offer short-term and complementary solutions in crop production to zinc and iron deficiency along with grain quality. In the soil, the major limitation of the bio-fortification is the low phyto-availability of the mineral micronutrients. Mineral elements with efficient mobility in the soil and plant are considered as successful agronomic bio-fortification approach. The most attractive agronomic bio-fortification strategy is the foliar application of mineral fertilizer to the plants in photo available form, correcting soil salinity, increasing beneficial soil microorganisms and adopting crop rotation practices (Bouis and Saltzman 2017). Using agronomic methods, the zinc content of grain can be increased by simply supplying the plants with zinc salts; for example, foliar application of ZnSO4, which increased total grain zinc by about 60% (Zhang *et al.,* 2008). However, such agronomic practices are less effective for iron, except if combined with increased nitrogen fertilization (Aciksoz *et al.,* 2011), which may not be economically or environmentally acceptable. Conventional breeding has been used by workers at CIMMYT, Mexico to develop varieties of wheat with increased contents of iron and zinc in wheat grain (Velu *et al.,* 2018).

The zinc bio-fortified lines from CIMMYT are currently being grown in Pakistan and India and have 20–40% higher zinc concentration and at least comparable grain yield to the best local cultivars (Velu *et al.,* 2018). Application of iron sulphate (FeSO4), zinc sulphate (ZnSO4) and as alone or in combination either soil and foliar application increased the height of plants, number of tillers, spike length, number of spikelets per spike, number of grains per spike, thousand grain weight, economical yield, biological yield, harvesting index, grain iron, grain zinc and protein contents (Bameri *et al*., 2012). Among different Zn and Fe concentrations applied either soil supplement or foliar spray, combine foliar spray of 0.5% ZnSO4 and 1% FeSO4 significantly improved the maximum growth or quality attributes of wheat (Melash and Mengistu, 2020).

Foliar application method is more appropriate for availability of nutrients to plants for optimum growth as compared to soil application method. Combined application of Zn and Fe (0.5% ZnSO4 and 1% FeSO4) through foliar spray is recommended to enhance the productivity of wheat crop with good quality of grains (Xu *et al.,* 2011). Foliar Zn application at the booting + milking stages significantly increased agronomic traits, grain Zn content and bioavailability in wheat grown on Zn deficient alkaline soils, thereby improving the wheat grain’s nutritional quality for humans (Esfandiari *et al.,* 2016). Though the agronomic fortification helps to increase micronutrient content (Fe and Zn) of the crop, its adaptability and reliability may be wary with different factors such as irrigation, soil and climate. So, the genetic bio-fortification stand as a best option for more efficient fortification of micronutrients.

**Wheat bio-fortified varieties and success stories of India**

Improvement of nutritional quality is one of the thrust areas of research mandate to be achieved by 2025 in ICAR-Indian Institute of Wheat and Barley Research (IIWBR) by manipulating lysine content, starch composition and bio-fortification of micronutrients i.e., zinc & iron; and their enhanced bioavailability and reducing anti-nutritional factors. (Mishra *et al.,* 2007). On World Food Day of 2020, during a ceremony to mark the 75th anniversary of the United Nations Food and Agriculture Organization (FAO), the Prime Minister of India dedicated 17 recently-developed bio-fortified seed varieties of local and traditional crops, including wheat and rice to the nation that are being made available to Indian farmers. In India, twenty-four bio-fortifed wheat varieties were released through AICRIP by the year 2022, which are rich in protein, iron and zinc content. The details of the released bio-fortified varieties are as follows (Gupta *et al.,* 2019).

**Bread wheat :**

**WB 02:** First bio-fortified wheat variety rich in zinc (42.0 ppm) and iron (40.0 ppm) released in India and notified in 2017 for North Western Plains Zone (NWP), which comprises Punjab, Haryana, Delhi, Rajasthan (excluding Kota and Udaipur division), western Uttar Pradesh (except Jhansi division), Jammu and Kathua district of Jammu and Kashmir, Paonta Valley and Una district of Himachal Pradesh and Tarai region of Uttarakhand. Its average grain yield is 51.6 q/ha. It matures in 142 days and is suitable for timely sown irrigated conditions. This bio-fortified variety has been developed by ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana.

**HPBW 01:** A bread wheat variety developed by Punjab Agricultural University, Ludhiana, Punjab, which contains high iron (40.0 ppm) and zinc (40.6 ppm), and released and notified in 2017 for irrigated timely sown conditions of NWP zone. Its average grain yield is 51.7 q/ha and matures in 141 days.

**HI 1605**: Pusa Ujala (HI 1605) is a pure line variety developed by ICAR-IARI, Regional Station, Indore, Madhya Pradesh with high protein (13%), iron (43 ppm) and zinc (35 ppm) along with excellent *chapatti* making quality. It has been released and notified in 2017 for Peninsular Zone of India, which comprises Maharashtra, Karnataka and Tamil Nadu. Its average yield is 35.0 q/ha under timely sown, restricted irrigation conditions.

**HD 3171:** Bread wheat variety developed by ICAR-Indian Agricultural Research Institute, New Delhi was released and notified in 2017, which is suitable for timely sown rainfed conditions of North Eastern Plain Zone, comprising of eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal (excluding Hills), Odisha, Assam and plains of North Eastern States. It’s a medium late variety which matures in 120-125 days with an average grain yield of 28 q/ha; and found to have 47.1 ppm of Zinc content.

**PBW 752:** It is a high yielding bread wheat variety developed by Punjab Agricultural University, Ludhiana, and released for late sown irrigated conditions of North Western Plain Zone. PBW 752 is found to be in rich in protein (12.4 %) in comparison to 8-10 % in popular varieties; and matures in 120 days with an average yield of 49.7 q/ha.

**PBW 757:** It is bread wheat variety developed by Punjab Agricultural University, Ludhiana in 2018 for very late sown irrigated conditions of North Western Plain Zone. It contains high zinc (42.3 ppm) in comparison to 30.0-32.0 ppm zinc in popular varieties. It’s an early maturing variety with an average yield of 36.7 q/ha.

**DBW 187:** Karan Vandana (DBW 187) is a mega wheat variety released for timely sown irrigated and fertility conditions of North Eastern Plains Zone and North Western Plains Zones. It is also suitable to grow under early sown high fertility conditions of these two zones to yield an average of 75.5 q/ha. It is known to be rich in iron (43.1 ppm) which makes it more popular among farmers and has the highest breeder seed indent in last few years. It is released by ICAR-Indian Institute of Wheat & Barley Research, Karnal.

**DBW 173:** Bio-fortified wheat variety developed in 2018 by ICAR-Indian Institute of Wheat & Barley Research, Karnal, which is rich in protein (12.5 %) and iron (40.7 ppm). It is a medium late maturing genotype suitable for late sown irrigated conditions of North Western Plain Zone with an average yield of 47.2 q/ha.

**UAS 375:** A short duration, drought resistant bread wheat variety developed and released in 2018 by University of Agricultural Sciences, Dharwad. It was released for timely sown rainfed conditions of peninsular zone with an average yield of 21.4 q/ha and also rich in protein (13.8 %) in comparison to 8-10 % in popular varieties.

**PBW 771:** A high yielding bread wheat variety (50.3 q/ha) developed by Punjab Agricultural University, Ludhiana was released for late sown irrigated conditions of North Western Plain Zone, which matures in 120 days and rich in zinc content (41.4 ppm).

**HD 3249:** Bread wheat variety developed by ICAR-Indian Agricultural Research Institute, New Delhi suitable for timely sown irrigated conditions of North Eastern Plain Zone, and released in 2020. It’s high yielding (48.8 q/ha) and iron rich (42.5 ppm) wheat variety maturing in 122 days.

**HD 3298:** Short duration, high yielding bread wheat variety developed by ICAR-Indian Agricultural Research Institute, New Delhi and released for very late sown irrigated conditions of North Western Plain Zone in 2020. It has an average yield of 43.7 q/ha, protein (12.1 %) and iron (43.1 ppm) content.

**HI 1633 (Pusa Vani):** An early maturing, high yielding bread wheat variety developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore and released in 2020 for late sown irrigated conditions of peninsular zone. It yields an average of 41.7 q/ha and is found to be rich in protein (12.4 %), iron (41.6 ppm) and zinc (41.1ppm).

**DBW 303:** It is a high yielding, late maturing bread wheat variety suitable for irrigated early sown and high fertility conditions of North West Plain zone. It recorded an average yield of 81.2 q/ha and had more protein (12.1%). It was developed by ICAR-Indian Institute of Wheat & Barley Research, Karnal in 2020.

**HI 1636 (Pusa Vakula)**: An early maturing, high yielding bread wheat variety developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore and released in 2022 for timely sown irrigated conditions of central zone. It yields at an average of 56.6 q/ha and is found to be rich in protein (~12.0 %) and zinc (44.4 ppm).

**Durum wheat :**

**HI 8759 (Pusa Tejas):** It is a durum wheat variety developed by ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Indore with an average yield ~55.0 q/ha along with high protein (12.0%), iron (42.1 ppm) and zinc (42.8 ppm); and suitable for making *chapatti* (Indian bread), pasta, *dalia*, and *suji*. It has been released and notified in 2017 for timely sown irrigated conditions of central zone which comprises Madhya Pradesh, Chhattisgarh, Gujarat, Rajasthan and Bundelkhand region of Uttar Pradesh.

**MACS 4028:** It is a pure line durum wheat variety with high protein (14.7%), iron (46.1 ppm) and zinc (40.3 ppm). It has been released and notified in 2018 for Maharashtra and Karnataka. Its average grain yield is 19.3 q/ha under rainfed, timely sown conditions in Peninsular Zone. It matures in 102 days and was developed by MACS-Agharkar Research Institute, Pune, Maharashtra.

**HI 8777 (Pusa Wheat 8777):** It is an early maturing durum wheat variety released by ICAR-Indian Agricultural Research Institute, Regional station, Indore in 2018 for rainfed timely sown conditions of Peninsular zone. Under rainfed conditions, it yielded at an average of 18.5 q/ha and found to be rich in iron (48.7 ppm) and zinc (43.6 ppm) in comparison to 28.0-32.0 ppm iron and 30.0-32.0 ppm zinc in popular varieties.

**DDW 47:** A durum wheat variety suitable for pasta, *dalia* and *suji* making was developed by ICAR-Indian Institute of Wheat & Barley Research, Karnal in 2020 suitable for timely sown restricted irrigated conditions of Central zone. It is a medium late maturing genotype rich in protein (12.7 %), iron (40.1 ppm) and high yellow pigment content (7.2 ppm) along with average yield of 37.3 q/ha.

**HI 8802 (Pusa Wheat 8802):** A medium tall durum wheat variety developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore, and released in 2020 for timely sown restricted irrigation conditions of Peninsular zone. It is early maturing, high yielding (29.1 q/ha) variety and rich in protein (13.0 %) and yellow pigment (6.5 ppm)

**HI 8805 (Pusa Wheat 8805):** A durum wheat variety released for timely sown restricted irrigation conditions of Peninsular zone suitable for pasta making, with an average yield of 30.4 q/ha along with rich in protein (12.8 %) and iron (40.4 ppm). It was developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore in 2020.

**MACS 4058:** It is a durum wheat variety developed by MACS-Agharkar Research Institute, Pune and released in 2020 for timely sown restricted irrigated conditions of Peninsular zone. It is a short duration, high yielding (29.6 q/ha) variety which is rich in protein (14.7 %), iron (39.5 ppm) and zinc (37.8ppm).

**DDW 48:** High Protein (12.1%) and yellow pigment rich (7.8 ppm) durum wheat variety released by ICAR-Indian Institute of Wheat & Barley Research, Karnal in 2020 suitable for timely sown irrigated conditions of Peninsular zone. It has recorded an average yield of 47.4 q/ha and matures in a short period (111 days).

**HI 8823 (Pusa Prabhat):** A durum wheat variety released for timely sown restricted irrigation conditions of Central zone suitable for pasta making. It yields at an average of 38.5 q/ha and rich in protein (12.8 %) and iron (40.4 ppm) ontent. It was developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore in 2022.

Apart from Indian Council of Agricultural Research, Harvest Plus project funded by International funding organizations **supports** the National Agricultural Research System in India to breed, test and release bio-fortified wheat developed through partnership with the CGIAR institutes viz., CIMMYT, Mexico and ICRISAT, Hyderabad, which aims to deploy and promote the bio-fortified cultivars rich in vitamins and minerals in India to improve nutrition and public health. Harvest Plus is part of the CGIAR Program on Agriculture for Nutrition and Health (A4NH), which provides global leadership on bio-fortification evidence and technology; and two commercially available, truthfully labeled (TL) zinc wheat varieties (BHU-31 and BHU-25) were formally launched by seed companies in Bihar during November 2019, with the intention of reaching more than 1 million farming households over five years. Rajendra Prasad Central Agricultural University (RPCAU), Bihar has developed bio-fortified wheat called Rajendra-Ghehu-3 with 38 ppm of zinc and was released by Bihar State government for cultivation.

The deployment of bio-fortified cultivars holds great promise for health and wellbeing of the human population. Several studies have demonstrated the positive effects of these bio-fortified crops on humans; and efforts are being made to popularize them among masses. Quality seeds of bio-fortified varieties are being produced and made available for commercial cultivation. In the last five years, a total of 7466.43 q of breeder seed of bio-fortified varieties among cereals, millets, oil seeds etc. have been produced as per the indents received from Department of Agricultural Cooperation and Farmers’ Welfare (Yadava et al., 2020). Seed production and distribution of these wheat varieties are being scaled up by giving license to various private seed companies and farmers producers’ organizations (FPOs), so that seed reaches more farmers. These efforts by ICAR, NARS, State agriculture Universities, AICRIPs, CGIAR institutes and government to release and promote the naturally bio-fortified wheat varieties in India would be a key component for achieving “malnutrition free India” and provide prosperous healthy future to the country.

**Way ahead for bio-fortification in wheat**

Until now, the genotype and environmental interaction with respect to the yield of grain and nutrient concentrations have not been precisely understood. Many research programs for the enhancement of nutrient use efficiency have been restrained by expensive and laborious phenotyping. Moreover, the bioavailability of nutrients is another important factor in determining the grain quality. Changing climate situations may further amplify the problem. Biofortification faces challenges with high a cost of development. In advance, the achievable breeding level of different nutrients is essential to be determined, which is a complex process and involves the determination of the adoption level by farmers, quantity of food products made from the crop consumed, post-harvest and preparation and cooking losses, the bioavailability of the nutrients and nutrients requirements. Thus, the target breeding level should be sure that there is a useful impact on the nutritional status of the recipient (Taylor and Taylor, 2012).

For widely available of the released bio-fortified crop, it would take about a decade; and when the crops are bio-fortified through the genetic transformation process, there occur additional political and regulatory issues that have to be addressed (Birner *et al.,* 2007). There is a lack of incentives and motivations to the farmers for growing improved crops, and consumers, themselves, are unaware to find quality food products from bio-fortified crops. During the manufacture of the bio-fortified crop, all the research teams should work together to produce an effective end product with the desired nutritional property. There should be a better acceptable and good cooking quality for good adaption of bio-fortified crops. Also, the more acceptable yield level and persistence to biotic and abiotic stress of these bio-fortified crop variety. There is no better strategy supporting large-scale prospective studies on the effect of iron bio-fortified crop and their effective role adopted on decreasing out anemia (Iron deficiency diseases) and also improving better health (Hussain *et al.,* 2010).

**Conclusion :**

Bio-fortification is a reliable, most economic, and feasible approach of delivering micronutrients to the under-nutrient population of crops. Bio-fortified crop exhibits increased mineral concentration in their edible portion with better uptake of mineral from the soil, improved translocation of minerals to grain from leaves, and enhanced mineral sequestration to endosperm. There is promising and substantial genetic diversity in wild relatives of wheat, and having useful and wide genetic variation in grain Fe and Zn content. This genetic variability can be utilized to increase both the concentration and bioavailability of Fe and Zn in modern wheat cultivars through conventional and modern breeding approaches. Genetic bio-fortification of staple crop, like wheat, is potentially sustainable and cost-effective. Even after the development of bio-fortified crop varieties, various socioeconomic and socio-political challenges are to be addressed to popularize their cultivation by farmers and their consumption by the end-user. Despite these challenges, scientists and researchers have been working now to make remarkable improvements of nutrient concentration in wheat and produce new wheat varieties. Thus, multitier coordination between researchers, farmers, and consumers (end-user) will play a key role in overcoming hidden hunger. Bio-fortified wheat varieties can ensure healthy lives during this climate change scenario and promote well-being of people of the nation by achieving food security, improved nutrition and promote sustainable agriculture. It can be suggested that bio-fortification of wheat can predominantly help in reducing malnutrition problems of the world and help in harvesting of grain yield with higher quality and reduce the negative impacts during climate change situations.

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