**Biofortification of wheat for improving nutritional security in climate change scenario**

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**Abstract**

Wheat production will decrease in the future due to climate change situations with factors that can be related to 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; 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. In addition, micronutrient malnutrition is also a serious public health problem in many developing countries, which is the result of deficiencies, excesses or imbalances in daily diet of a person with respect to nutrients and energy. Approximately two billion people suffer from micronutrient deficiencies worldwide and deficiencies in essential micronutrients 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. Biofortification strategies viz., genetic and agronomic approaches offers sustainable solution to improve the nutrition status of resource poor people in developing countries. In climate change scenario, development of water use efficient wheat varieties with tolerance to high temperatures is the main goal of plant breeders for improving 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 biofortified 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:** Biofortification, 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). World nutrition mostly depends on wheat and wheat products viz. chapati, bread, biscuit, pasta and fermented products, as people all over the world consume the wheat product(s) in one of these forms (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 (13.43% of global area) to produce the all-time gargantuan output of 107.95 million tonnes (12.96% of world production) with a record average national productivity of 3424 kg/ha as per the third advance estimates from the MoA & FW (2020).

The importance of wheat is undeniable, as humans growing wheat in diverse climates and wide types of soil globally. It has three main grain compartments such as the bran, endosperm and the germ. The wheat grain is nutritious as it houses several micronutrients, vitamins, phenolic compounds and protein at different levels across various grain compartments (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.

Micronutrient malnutrition is a serious public health problem in many developing countries. Different interventions are currently used, but their overall coverage is relatively limited (Qaim *et al.,* 2007). Biofortification is the process of breeding nutrients into food crops, provides a sustainable, long-term strategy for delivering micronutrients to rural populations in developing countries; and wheat is being bred for higher levels of micronutrients using both conventional and transgenic breeding methods; several conventional varieties have been released, while additional conventional and transgenic varieties are in the breeding pipeline (Saltzman *et al.,* 2013). The conventional breeding was fruitful as several biofortified wheat varieties were released with the collaboration of International and National Research institutes. One of such examples is Zinc Shakti (Chitra) developed by the 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). ‘Bari Gom 33’ is released by Bangladesh Agricultural Research Institute (BARI) in association with International Maize and Wheat Improvement Center (CIMMYT) in the year 2017, which have 33% higher Zn content (Fig 1). CIMMYT has also released six varieties of biofortified 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 evidence that conventional breeding has played a very important role in developing biofortified wheat by exploiting available diversity.

**Figure 1 : Field expression of Biofortified wheat varieties WB02 and BARI Gom 33 .**

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|  |  |
| WB-02 high Zn biofortified 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.

Strong influence of environmental conditions on the majority of quality traits in wheat i.e., with growing zones, latitudes and moisture regimes showing the greatest effects, and genotypic effects were mainly observed for carotene content, zinc content, iron content and SDS volume (Eslemi *et al.,* 2005). Grain quality negatively affected by the high percentages of ash accumulated in the grain. Moisture stress increased mainly 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, however, they can be sorted into two major groups: (1) factors that can be related to 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. It can be classified in two broad categories viz., under-nutrition which deals with a lack of healthy nutrients that can result in stopping growth, both internally as well as on the outside, for example stunting, underweight and second group that has to do with excessive food intake and issues such as obesity, diabetes, heart diseases falls under that category of malnutrition (WHO, 2020). Lack of proper food is not only a development issue but is also a crucial economic issue for any country. In 2018, 34.7% of children under five were stunted, 17.3% were wasted and 33.4% were underweight in India (MOHFW, 2019). Micronutrient deficiencies are commonly known as “hidden hunger” and constitute an important form of human malnutrition. Micronutrients are the essential vitamins and minerals required by human beings to stimulate cellular growth and metabolism. In the Global Hunger Index 2020, India falls in the category of ‘serious hunger’, ranking 94th among 107 countries. India has progressed since the last such ranking, when it stood at 102 out of 117 countries (UNICEF, 2020). Deficiencies of iron, Zinc, iodine and vitamin A represent a serious global health problem because these are affecting more than one- third of the global population, especially in developing countries (FAO, 2013).

Iron and zinc have been recognized as the most important among micronutrients. Iron deficiency causes anemia and leads to impaired mental development in children, reduced capacity for physical labor in adults. Whereas, Zinc is crucial for immunity, control of diabetes, healing, digestion, reproduction and physical growth. Malnutrition and the widespread prevalence of stunting, wasting, and nutritional deficiencies among women and children are well-recognized elements of India's profile in the Global Hunger Index. Malnutrition was found to be the leading risk factor for death of children under the age of five in India (Vollset *et al*., 2020). Anemia prevalence was also high, at 53 per cent among all women of reproductive age, and 54 per cent among girls aged 15-19 years (Anemia *Mukt Bharat* Portal), which indicates that still half of the women in India were affected by anemia. At the sub-national level, there are also large variations in degree of malnutrition; in the state of Haryana, for example, 72% of children; 55% of pregnant women; and 63% of non-pregnant women are estimated to be anemic (International Institute for Population Sciences, 2017). The prevalence of under-nutrition and anemia among almost half of the women in India, especially pregnant women, puts a serious burden on the country's food security. This is predominantly because undernourished mothers can trigger cycles of under-nutrition by passing on nutrient and vitamin deficiencies to newly born babies. These statistics indicate that under-nutrition in India is a gender problem.

Indian government had started numerous measures to overcome hunger and malnutrition include National Food Security Mission, National Nutrition Mission, National Policy On Farmers, National Horticulture Mission, National Mission on Pulses and Oilseeds, National Rural Livelihoods Mission, Mahatma Gandhi National Rural Employment Guarantee Act/Scheme, National Rural Health Mission 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. In 2015, India committed to achieve the Sustainable Development Goal (SDG) of zero hunger. As a step towards meeting the targets by 2030, the Government of India launched the Prime Minister's Overarching Scheme for Holistic Nutrition (POSHAN) *Abhiyan* in 2017. Targets were set to reduce stunting, under-nutrition and low birth weight by two per cent each and anemia by three per cent by 2022.

**Biofortification of wheat in Indian Scenario**

Three approaches *i.e.,* food diversity, food supplements and biofortification are considered world wide to reduce the impact of malnutrition. While each of the three approaches is effective under ideal situations, whereas ‘biofortification’ remains the most sustainable and cost-effective mean for providing the desired levels of nutrients in the diet in natural form (Pfeiffer and Mc Clafferty, 2007). Realizing the paramount importance of nutritional quality in the country, Indian Council of Agricultural Research (ICAR) has given emphasis on biofortification of crops for improvement of nutrition. ICAR aims to develop, scale up production and consumption of nutritious biofortified crops in this country of nearly 1.4 billion people. Biofortified 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 biofortified varieties/hybrids through All Indian Coordinated Research Projects (AICRPs) in cereals, millets, pulses, oil seeds, vegetables and fruits. Till 2020, 71 biofortified 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 biofortified varieties not only provide enough calories but also deliver essential nutrient(s) needed for adequate growth and development.

**Breeding approaches for high nutrition wheat :**

Breeding has the potential to increase the nutrient levels of staple food crops to reach the target levels required to meet complete human nutritional needs without affecting yield and other agricultural characteristics. The process of breeding plants for biofortification 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 of the genetic diversity of grain iron and zinc in wheat showed sufficient genetic variance for these traits. (Table 1).

**Table 1: Genetic variation for grain 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. **Association of grain micronutrients with yield and other quality traits**

The degree of genetic and non-genetic association between traits can be calculated through correlation; and knowledge of the relationship between traits 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 such as grain yield, disease resistance and other grain quality traits. The negative albeit weak correlation between grain yield and grain micronutrient concentration in wheat was recorded (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). In case of zinc, the moderate negative correlation with grain yield was recorded (Gomez-Becerra *et al*., 2010) and non-significant association between grain yield and micronutrient traits including zinc was recorded by 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 grain Fe and Zn and provitamin 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 of 58 ppm for grain Fe and 38 ppm for grain Zn were 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 Zn and 0.37 for grain Fe 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 presence of 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 interaction for Fe and Zn facilities the breeding approach for improvement for these traits. The generation mean analysis approach in two wheat crosses under normal and stress condition reported that the additive and non-additive gene effects are important for Fe and Zn uptake in wheat (Amiri *et al*., 2020). Both grain Fe an Zn are highly quantative traits and influence by environmental factors. Breeding for high micronutrient concentration is highly influenced by the soil compositions (Trethowan., 2005) and the uptake of micronutrient from soil and mobilization into the grain is limited by the availability of Fe and Zn in the soil (Ortiz-Monaterio *et al*., 2007).

1. **Genomics approaches for biofortification**

The costly and laborious phenotyping technique and large G × E interaction leads to slow improvement of grain Fe and Zn in wheat; and the identification of linked molecular markers for the 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). 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 associated with high Fe, Zn and protein 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 (*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). The double haploid (DH) population of 138 lines developed from “Berkut and Krichauff” evaluated in eastern Gangetic plains of India identified two QTLs for grain Zn on chromosome 1B and 2B explain 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 of 286 lines developed from Indian old wheat variety WH 542 and synthetic derived genotype (PI94624/Aegilops-squarrosa (409)//BCN) evaluated under six environments for grain Fe and Zn 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 grain Fe and Zn 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 biofortification 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 time required for development of mapping population. To dissect the genetic control of complex traits GWAS is one of the best approaches, which 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 Ilumina i select 90K Infinitum SNP array which reveals 39 marker trait association (MTAs) for grain Zn, which 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 and 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 genotype with 17,937 SNP markers and phenotype in two location India reveled eight significant MTAs for grain zinc concentration and six significant MATs for grain Fe concentration (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) is 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 biofortification may be more cost-effective in the long run; and which can be achieved through micronutrient fertilizer application 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 to the Zn and Fe deficiency in crop production with grain quality. In the soil, the major limitation of the biofortification is the low phyto-availability of the mineral micronutrients. Mineral elements with efficient mobility in the soil and plant are considered as successful agronomic biofortification approach. The most attractive agronomic biofortification 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 International Maize and Wheat Improvement Center (CIMMYT, Mexico) to develop varieties of wheat with increased contents of iron and zinc in wheat grain (Velu *et al.,* 2018).

The zinc biofortified 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 enhance the micronutrient content of the crop, its adaptability and reliability may be wary with different factors such as irrigation, soil and climate. So, the genetic biofortification stand as a best option to do so and makes more efficient fortification of micronutrients.

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

One of the thrust areas of research in the ICAR-Indian Institute of Wheat and Barley Research (IIWBR) mandate is to improve nutritional quality of wheat by manipulating starch composition, lysine content and biofortification of micronutrients (specially Zn, Fe,) and their enhanced bioavailability and reducing anti-nutritional factors by 2025 (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 biofortified seed varieties of local and traditional crops, including wheat and rice to the nation that are being made available to Indian farmers. Till 2020, twenty-two biofortifed wheat varieties were released in India through AICRIP which are rich in protein, iron and Zinc content. The details of the released biofortified varieties are as follows (Gupta *et al.,* 2019).

**Bread wheat :**

**WB 02:** It is the first biofortified wheat variety rich in zinc (42.0 ppm) and iron (40.0 ppm) released in India. It was released and notified in 2017 for 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 which constitutes North Western plain zone of India. Its average grain yield is 51.6 q/ha. It matures in 142 days and is suitable for irrigated timely sown conditions. This biofortified variety has been developed by ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana.

**HPBW 01:** A bread wheat variety containing high iron (40.0 ppm) and zinc (40.6 ppm). It has been released and notified in 2017 suitable for irrigated timely sown conditions of North west Plain Zone. Its average grain yield is 51.7 q/ha and matures in 141 days. This biofortified variety has been developed by Punjab Agricultural University, Ludhiana, Punjab.

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

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

**PBW 752:** It is a high yielding bread wheat variety 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. It matures in 120 days with an average yield of 49.7 q/ha and developed by Punjab Agricultural University, Ludhiana.

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

**UAS 375:** A short duration, drought resistant bread wheat variety developed by University of Agricultural Sciences, Dharwad in 2018. 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) released for late sown irrigated conditions of North Western Plain Zone that matures in 120 days was developed by Punjab Agricultural University, Ludhiana. It is found to be 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 in 2020. It’s high yielding (48.8 q/ha) and iron rich (42.5 ppm) wheat variety maturing in 122 days.

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

**HI 1633:** Pusa Vani (HI 1633) is an early maturing, high yielding bread wheat variety developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore in 2020. It yields at 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). It was released for late sown irrigated conditions of peninsular zone.

**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.

**Durum wheat :**

**HI 8759 (Pusa Tejas):** It is a durum wheat variety with high protein (12%), 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 Madhya Pradesh, Chhattisgarh, Gujarat, Rajasthan and Bundelkhand region of Uttar Pradesh constituting Central Wheat growing zone of India. The average yield of this variety is more than 50.0 q/ha under timely sown irrigated conditions developed by ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Indore.

**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 suitable 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. It yields at an average of 30.4 q/ha and was found to be 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 released in 2020 developed by MACS-Agharkar Research Institute, Pune. 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). It yields at an average of 29.6 q/ha under timely sown restricted irrigated conditions of peninsular zone.

**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).

Apart from Indian Council of Agricultural Research, Harvest Plus project funded by **UK Government; the Bill & Melinda Gates Foundation; the US Government’s Feed the Future initiative and the European Commission supports** the National Agricultural Research System in India to breed, test and release biofortified wheat developed through partnership with the CGIAR institutes viz., International Maize and Wheat Improvement Center (CIMMYT) and (International Crops Research Institute for Semi-Arid Tropics) ICRISAT. This project aims to deploy and promote the biofortified cultivars in India to improve nutrition and public health as these varieties are rich in vitamins and minerals needed for good health. Harvest Plus is part of the CGIAR Program on Agriculture for Nutrition and Health (A4NH), providing global leadership on biofortification evidence and technology (www.harvestplus.org). Under harvest plus project, in November 2019, two commercially available, truthfully labeled (TL) zinc wheat varieties (BHU-31 and BHU-25) were formally launched by seed companies in Bihar, with the intention of reaching more than 1 million farming households with these varieties over five years. Rajendra Prasad Central Agricultural University (RPCAU), Bihar has developed biofortified wheat called Rajendra-Ghehu-3 with 38 ppm of zinc and was released by Bihar State government for use. This is the target level of zinc content for biofortified zinc wheat varieties to have the intended nutrition and health benefit when eaten regularly.

The deployment of biofortified cultivars holds great promise for health and wellbeing of the human population. Several studies have demonstrated the positive effects of these biofortified crops on humans. Special efforts are being made to popularize these biofortified varieties among masses. Quality seeds of biofortified 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 biofortified 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. This efforts by ICAR, NARS, State agriculture Universities, AICRIPs, CGIAR institutes and government to release and promote the naturally biofortified 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 biofortification 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 biofortified crop, it would take about a decade; and when the crops are biofortified 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 biofortified crops. During the manufacture of the biofortified 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 biofortified crops. Also, the more acceptable yield level and persistence to biotic and abiotic stress of these biofortified crop variety. There is no better strategy supporting large-scale prospective studies on the effect of iron biofortified crop and their effective role adopted on decreasing out anemia (Iron deficiency diseases) and also improving better health (Hussain *et al.,* 2010).

**Conclusion :**

Biofortification is a reliable, most economic, and feasible approach of delivering micronutrients to the under-nutrient population of crops. Biofortified 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 biofortification of staple crop, like wheat, is potentially sustainable and cost-effective. Even after the development of biofortified 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. Biofortified wheat varieties can ensure healthy lives during this climate change scenario and promote well being of people of the nation achieving food security, improved nutrition and promote sustainable agriculture. It can be suggested that biofortification 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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