

BIO-FORTIFICATION OF WHEAT FOR IMPROVING NUTRITIONAL SECURITY IN CLIMATE CHANGE SCENARIO

Abstract

Wheat production will decrease in the future as a result of climate change issues such as precipitation—heavy rains, insufficient water, hailstorms, droughts, and landslides—meteorological extremes—temperature anomalies—frosts, heat days, high winds, duration of adverse eras; air storms—changes in radiation—and in order to reduce these risks, the effects of climate change extenuation approaches and crop management systems for adaptation to dynamical climate conditions should be taken. The biggest health issue in developing nations is micronutrient deficiency. This is the outcome of a person's diet being deficient, excessive, or out of balance in terms of energy and nutrients. Around two billion individuals worldwide experience micronutrient deficiencies, such as those in zinc and iron, which have a negative impact on their health in many ways. Maintaining the quality of wheat grains in the face of climatic change is essential for human nutrition, end-use functionalities, and artefact prices. Bio-fortification using genetic and agronomic methods offers a practical answer to improving the nutritional status of resource-poor people in developing nations. To increase the output and productivity of wheat crops in the face of climate change, plant breeders will focus on creating wheat genotypes with high temperature tolerance and low water usage. Strong yielding durum wheat varieties with high nutritional features were developed during this endeavour, including 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%), and HI 8777 (48.7ppm iron, 43.6ppm zinc, >14.0%); HI 1605 (43 ppm iron, 35 ppm zinc, >13 % protein), HI 1633 (41.6 ppm iron, 41.1 ppm zinc, >12.5 % protein), and HI 1634 (38.0 ppm iron, 37.0 ppm zinc, >12.0 % protein)

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are three high yielding climate resistance bread wheat types that were developed. In this climate change scenario, the use of bio-fortified variety holds great promise for the safety and health of the human population. It will also assist in the transition from a state of food safety to one of nutrition sanctuary, where not only calories and proteins but also micronutrients are cared for. By focusing on bio-fortification of staple food crops under the current climate change situation, which would not only sate hunger but also add vital micronutrients to the diet, we can make India a healthy and happy country.

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

I. INTRODUCTION

Wheat (*Triticum aestivum* L.), significant agronomic crop growing in several locations worldwide, has long been a staple food, contributing to around 35% of global population food expenditure (Mohammadi-joo et al., 2015). It is a remarkable crop that helps to global food safety because of its capacity to respond to a variety of climatic and ecological stresses (Muslim et al., 2015). The majority of people in the globe eat wheat and wheat-based goods including chapati, bread, cookies, pasta, and fermented foods (Mallick et al., 2013). The globe will produce 765 million metric tonnes of wheat in 2021. (FAOSTAT, 2021). Wheat is the primary source of food for almost 30% of people in developing nations (Ali and Borrill, 2020). In order to produce the unfathomably high production of 107.95 million tonnes, the crop is cultivated on 29.55 million hectares in India with a record average national productivity of 3424 kg/ha (MoA & FW) (2020).

People grow wheat in diverse climates and soil types around the world, and it is composed of three main parts: bran, endosperm, and germ. Micronutrients, vitamins, phenolic compounds, and protein make wheat grain nutritious (Lephuthing et al., 2017). In addition to protein, unsaturated fats, minerals, vitamins A, B, and E, and carbohydrates, wheat bran contains mostly insoluble carbohydrates, protein, traces of B vitamins, minerals, and some anti-nutritional factors. There is a lot of starch and protein in the endosperm of the grain (Ram and Govindan, 2020). The population which is mainly dependent on wheat diets faces the problem of malnutrition because wheat has numerous nutritional factors, but they are present in trace amounts. In developing countries, malnutrition is a major threat allied to agriculture and detrimental to GDP.

To fight micronutrient deficiency, a variety of interventions are now being employed in poor nations, but their total coverage is very limited (Qaim et al., 2007). The process of breeding nutrients into food crops is known as bio-fortification. It offers a long-term, sustainable plan for supplying micronutrients to rural people in developing nations. Both conventional and transgenic breeding techniques are being used to develop wheat for increased levels of micronutrients, and several conventional varieties have already been published (Saltzman et al., 2013). With the cooperation of international and national research institutes, conventional breeding resulted in the introduction of numerous wheat types that were bio-fortified. By using *Aegilops squarrosa* as the donor for the genes encoding the genes for zinc content, PBW343 was crossed with it to develop Zinc Shakti (Chitra), which has a 40% increase in zinc content. The zinc-fortified variety "Zincol 2016" was 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*, respectively, with increases in the zinc content of 25%, 20%, and 20%. (Saini et al., 2020). The Bari Gom 33, which has a 33 percent greater Zn content, was introduced in Bangladesh as a result of the partnership with the International Maize and Wheat Improvement Center (CIMMYT) in 2017. (Fig 1). Additionally, in Nepal in the year 2020, CIMMYT will release six kinds of bio-fortified wheat: Zinc Gahun1, Zinc Gahun2, Bheri-Ganga, Himganga, Khumal-Shalei, and Borlaug 2020. Pure line varieties developed by ICAR Institutes and SAUs in India, such as WB 02 (Fig. 1), HPBW 01, HI 1605, HI 1633, HI 8759 (d), and HI 8777 (d), also have higher Fe and Zn contents, demonstrating the importance of conventional breeding in the development of bio-fortified wheat by utilising readily available diversity.



WB-02 high Zn bio-fortified wheat released
in India

BARI Gom 33, a zinc-enriched, blast resistant
variety released in Bangladesh.

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

II. CLIMATE CHANGE'S IMPACT ON WHEAT GRAIN QUALITY

According to Harold (2015), the effects of global warming on agriculture are likely to include localised changes in temperature as well as in the quantity and seasonality of precipitation. Additionally, it's likely to lead to more extreme weather, like droughts and periods of excessive precipitation. Such modifications may have both favourable and unfavourable effects on plant growth, the spread of insects and diseases, and water availability (Doll & Baranski, 2011). According to a recent study, the agriculture sector will need to spend more than USD 7 billion year on climate change adaptation (Nelson et al., 2009). According to Valizadeh et al. (2014), climate change will cause a decline in wheat yield in the future; and to reduce these risks, the impact of crop management methods for climate change adaptation to conditions should be taken into account. Plant growth and development are influenced by temperature and CO₂ due to their impact on stomatal opening and the speed of physiological processes. The biochemical processes move more quickly at higher temperatures, which also cause large transpiration losses. For crops that fix and reduce inorganic CO₂ into organic molecules, stomatal conductance will decrease as CO₂ concentration rises (C₃ plants). The adverse effects of increased temperature and decreased soil moisture are partially offset by growing atmospheric CO₂ concentration (Lobell & Gourdj, 2012). Due to faster leaf expansion, an increase in photosynthetic rate per unit area, an increase in water use efficiency, and an increase in photorespiration rates, this appears to gain more from a higher CO₂ level in terms of dry matter production (Warrick et al., 1986). First, as more CO₂ reduces the costs associated with photorespiratory processes in the C₃ photosynthetic pathway, higher CO₂ has a fertilising effect on C₃ species including wheat, rice, and the majority of fruit and vegetable crops (Lobell & Gourdj, 2012). One of the primary issues is that the country is experiencing a water and pasture deficit due to the lack of rain (Hendrix, 2012). Farmers' sensitivity to climate change and how they interpret significant variations in weather conditions, and how such variations affect crop productivity.

Genetics, management, and the environment all affect the quality of grains. For human nutrition, end-use functional qualities, and commodity value, it is crucial to maintain wheat grain quality during climatic change (Nuttall et al., 2017). The quality of wheat grain is known to be decreased by low nitrogen levels in soil, and this is made worse by high CO₂. Increases in grain yield (weight) do not necessarily translate into increases in the world's supply of grain protein because it is well known that grain quality falls with an increase in atmospheric carbon dioxide (Hatfield et al., (2011). According to Kimball et al. (2010), low nitrogen levels cause a reduction in grain quality, which is further impacted by high CO₂ levels. When nitrogen levels were low, protein content reduced under high CO₂ by 39% as opposed to 33% under ambient CO₂. According to Blumenthal et al. (1991), there was an extremely substantial negative association between dough strength and loaf volume and grain protein hours above 35°C during grain filling. The majority of wheat grain quality parameters are substantially influenced by environmental variation, including growing zones, latitudes, and moisture regimes. Genotypic impacts were primarily seen for carotene concentration, zinc content, iron content, and SDS volume (Eslemli et al., 2005). Grain quality was considerably damaged by the high ash level that had accumulated in the grain. At both latitudes, moisture stress decreases thousand grain weight and ash content while increasing protein content and vitreousness. The impact of climate change on agricultural productivity is likely the greatest environmental challenge to the continent of Africa's efforts to combat hunger, malnutrition, illness, and poverty (Enete et al., 2016). Climate change significantly decreases calorie intake and promotes childhood malnutrition. Therefore, in order to increase calorie consumption by a sufficient amount to counteract the detrimental effects of climate change on children's health and wellbeing, significant agricultural productivity expenditures are required (Nelson et al., 2009). The ability to adapt to meteorological factors in general and climate change processes in particular can be negatively impacted by a number of issues at the moment, including (1) water scarcity, drought, meteorological extremes (temperature anomalies-frost, heat days, heavy rains, hailstorms, land-slides, air-storms, high winds, and alterations of radiation and its postulates), (2) economic, social, and policy issues, that can negatively affect the ability to adapt to meteorological factors in general and climate change processes in particular regarding

III. INDIA'S CURRENT MALNUTRITION SITUATION

The minerals and energy in a person's diet can be insufficient, excessive, or unbalanced, which results in malnutrition. It can be divided into two categories: malnutrition, which causes stunting and underweight, and overeating, which causes obesity, diabetes, and heart disease. Population health is important for a country's development and economy. In India in 2018, it was found that 33.7% of children under five were stunted, 17.3% were wasting, and 33.4% were underweight (MOHFW, 2019). The vital vitamins and minerals that humans need make up micronutrients, which aid in promoting cellular growth and metabolism. Unhealthy micronutrient intake (hidden hunger) is a significant form of human malnutrition. Micronutrient deficiencies are known to be most severe when iron and zinc are deficient; anaemia, which is brought on by an iron deficiency, affects children's mental development and adults' ability to perform physical labour. While diabetes regulates healing, digestive, reproductive, and physical growth, zinc is an essential vitamin for boosting immunity. When considered in light of the Global Hunger Index, India's profile reveals a pervasive prevalence of stunting, wasting, and nutritional inadequacies among mothers and

children. Among India, malnutrition is the primary cause of death in children under the age of five (Volls et al., 2020).

According to the Anemia Mukht Bharat Portal, anaemia prevalence was also high, affecting 53% of women of reproductive age and 54% of girls aged 15 to 19, respectively. This statistic shows that anaemia affects 50% of women in India. At sub-national levels, there are large variations in the severity of malnutrition; for example, in Haryana, it is estimated that 63 percent of non-pregnant women, 55 percent of pregnant women, and 72 percent of children are anaemic (International Institute for Population Sciences, 2017). Anemia and undernutrition affect the majority of women, especially pregnant women, negatively affecting India's food security. The main way that malnourished moms can start cycles of malnutrition is by giving their unborn children nutritional and vitamin deficits. Therefore, gender is typically a factor in under-nutrition in India. The Indian government launched a number of initiatives to combat hunger and malnutrition, including the Mahatma Gandhi National Rural Employment Guarantee Act/Scheme, the National Food Security Mission, the National Mission on Pulses and Oilseeds, the National Horticulture Mission, the National Rural Livelihoods Mission, the National Rural Health Mission, and Integrated Child Development Services (ICDS) for children under the age of six to provide nutrition.

India is committed to and aiming to accomplish the Sustainable Development Goal (SDG) of ending hunger by 2030. Launched in 2017, the Prime Minister's Overarching Scheme for Holistic Nutrition (POSHAN) Abhiyan is a step toward reaching the aim by 2030. By 2022, the key goals of this initiative are to cut the rates of stunting, malnutrition, and low birth weight by 2% each and the rates of anaemia by 3%.

IV. WHEAT BIO-FORTIFICATION IN AN INDIAN CONTEXT

Three strategies, including food diversification, food supplements, and bio-fortification, are thought to be successful globally to lessen the effects of malnutrition in ideal circumstances. While "bio-fortification" is the most economical and environmentally friendly way to add the required amounts of nutrients to the diet in their natural form (Pfeiffer and Mc Clafferty, 2007). The Indian Council of Agricultural Research (ICAR) placed a strong emphasis on bio-fortification in crops to increase nutrition after realising the critical relevance of nutritional quality in the nation. In this nation of around 1.4 billion people, ICAR seeks to develop scale up production of, and consumption of, nutrient-rich bio-fortified crops. The ICAR front line demonstrations prioritise bio-fortified types as well. By defining minimum amounts of iron and zinc to be bred into all national varieties of pearl millet and extending the same to other significant crops, ICAR made a significant advancement (Yadava et al., 2017). Through All Indian Coordinated Research Projects (AICRPs), ICAR's research has now resulted in the creation and release of a number of bio-fortified varieties and hybrids for use in cereals, millets, pulses, oil seeds, vegetables, and fruits. India's institutions have published 71 bio-fortified varieties in 16 distinct crops as of 2020, demonstrating the nation's readiness to address malnutrition using this long-term strategy (Yadav et al., 2020). Protein content in Indian wheat typically ranges from 28 to 32 parts per million Iron and 30-32 ppm Zinc (Yadav *et al.*, 2018, 2019). The bio-fortified variety will offer the necessary nutrient(s) for proper growth and development together with enough calories in diet.

V. BREEDING STRATEGIES FOR WHEAT WITH HIGH NUTRITION

Without impacting yield or other agricultural traits, breeding allows for the improvement of the quality levels of staple food crops to the goal levels necessary to satisfy all of humankind's nutritional demands. Breeding plants for bio-fortification purposes necessitates work on 1) determining the genetic diversity of grain iron, zinc, and carotene in germplasm and wild relatives, 2) identifying nutrient-rich parental material, 3) genetic studies to determine genes engineering target traits, and 4) pre-breeding or identification of nutrient-rich parental material.s, 4) development of superior variety 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. Wheat's genetic variation for micronutrients: A precondition for obtaining target levels is the presence of genetic variance for the desired qualities in germplasm, and the identification of genetic variance serves two basic goals. 1) Identifying pre-breeding parental lineages for a crossbreeding programme, genetic research, and the creation of molecular markers 2) Finding elite variety or earlier variety with a specified level of micronutrients and good agronomic characteristics. In wheat grain, micronutrients like iron and zinc, a recent analysis on the genetic variety of grain quality attributes revealed substantial genetic variance for these variables. (Table 1).

Table 1: Genetic Variability for Grain Quality Micronutrient Traits (Iron and Zinc) in Wheat Landraces, Germplasms and Advance Lines

Sl. 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 <i>et al.</i> , 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 <i>et al.</i> , 2020
4	150 bread wheat genotypes	31.9	9.2	49.7	29.0	10.7	59.4	ICP-AES	Pandey <i>et al.</i> , 2016
5	286 RILs of bread wheat	38.1	32.6	44.2	37.6	30.3	48.4	ED- XRF	Krishnappa <i>et al.</i> , 2017
6	36 Elite wheat genotypes	37.8	6.43	85.9	29.3	3.44	60.9	ICP-MS	Khokhar <i>et al.</i> , 2018
7	299 winter wheat genotypes	39.2	25.2	56.7	29.6	21.5	46.6		Guttieri <i>et al.</i> , 2015
8	600 core germplasm	39.65	26.3	68.8	30.4	16.8	60.7	ICP-OES	Velu <i>et al.</i> , 2011
9	37 bread wheats (1 st HYPT)	37.0	32.3	43.7	32.5	19.8	36.7	ED-XRF	Velu <i>et al.</i> , 2012
10	655 Wheat germplasm from China							ICP-MS	Liu <i>et al.</i> , 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		

- 2. Grain micronutrient relationships with yield and other quality factors:** The simultaneous selection and improvement of characteristics in genetic improvement depend heavily on trait correlations. In addition to farmers' favoured qualities, such as grain yield, disease and insect resistance, and other grain quality features, Gram aims to develop micronutrient-rich variety. Grain yield and micronutrient content in wheat showed a marginally negative connection (Ficco et al., 2009). Strong negative correlations between Fe and plant height and glutenin content were found, indicating that plants with lower glutenin content and shorter height favour grains with higher grain Fe concentrations. Significant negative correlations between glutenin content and Zn and Fe concentrations have also been observed (Gomez-Becerra et al., 2010). According to Gomez-Becerra et al. (2010), there is a non-significant link between grain yield and micronutrient characteristics, such as zinc, and a somewhat negative correlation between grain yield and zinc (Velu et al., 2012). The intake of Fe and Zn were shown to be negatively correlated with phosphorus levels. About 75% of the total phosphorus in wheat grain is stored as phytic acid, especially in the germ and aleurone layers (Lott and Spitzer, 1980). Re-translocation of Fe and Zn from vegetative to seed tissues, and seed allocation of Fe and Zn are all positively impacted by nitrogen nutritional status, according to current study (Erenoglu et al., 2011). Increased soil nitrogen or foliar application boosted root absorption and shoot and grain accumulation of Fe and Zn, according to a wheat experiment (Kutman et al., 2011). Grain yield and micronutrients have a complicated connection that is influenced by a variety of external factors, including growth circumstances and analytical techniques. Therefore, the G x E interactions have an impact on the relationship's strength.
- 3. Fe and Zn genetic architecture in wheat:** The CGIAR organisation is attempting to increase the iron and zinc content of wheat grain as well as the vitamin A content of a significant staple crop like wheat through plant breeding as part of the Harvest Plus initiative (www.harvestplus.org). Harvest Plus chose the target values for iron content to be 58 ppm and for zinc content to be 38 ppm after taking into account various factors such as the target human population, daily consumption of wheat, average Fe and Zn content in wheat, micronutrient retention after processing, and bioavailability of traits. Knowledge of these qualities' genetic behaviours, or information on the heritability of the trait, is required in order to attain these goals through conventional breeding programmes. 20 genotypes of the SAMNYT experiment in eastern Gangatic plains of India exhibited low heritability values of 0.25 for grain zinc and 0.37 for grain iron (Joshi et al., 2010). For the first- and second-year experiments using 30 spring wheat genotypes, the high wide sense heritability was 0.74 and 0.85 for grain Fe and 0.61 and 0.92 for gain Zn, respectively (Khodadi et al., 2014). According to Velu et al 2019 .'s research, translocated lines of "Pavan 76" from the CIMMYT breeding programme have high grain Zn heritability (0.79 and 0.83 in 2017 and 2018, respectively) and medium grain Fe heritability (0.67 and 0.66 in 2017 and 2018, respectively).

Initial research on germplasm, landraces, and wild relatives suggests that there is enough variation in wheat grain to accommodate iron and zinc. A breeding strategy for enhancing these qualities is made possible by the available variability and the underlying genetic architecture of genotype x environment impact and gene effect. The generation mean study of two wheat crosses under normal and stressful settings revealed that the major additive and nonadditive gene effects for iron and zinc absorption in wheat (Amiri

et al., 2020). Highly quantitative characteristics like Fe and Zn concentration are controlled by the environment. The availability of iron and zinc in the soil will have a limited impact on the absorption of micronutrients from the soil and mobilisation into the grain in the breeding programme for high micronutrient concentration in wheat grain (Trethowan, 2005). (Ortiz-Monasterio et al., 2007)

- 4. Genomics-based bio-fortification strategies:** However, the discovery of linked molecular markers to the loci governing the micronutrient variation may lead to the selection of genotypes rich in micronutrient without phenotypic testing. In conventional breeding, the time-consuming and expensive phenotyping process combined with the significant G-E interaction leads to slow improvement of micronutrient levels, such as zinc and iron in wheat grain (Velu et al., 2014). To breed cereals like wheat for biofortification using MAS, knowledge of the genomic areas that affect the zinc and iron concentration in grain is crucial. Wheat grain Fe and Zn content was mapped using many QTL analyses. The main gene locus GPC-B1 on chromosome 6B is intimately linked to high levels of iron, protein, and zinc. It was introduced from the fungus *T. dicoccoides* and encodes the NAC transcription factor (NAM-B1), which accelerates senescence and transports micronutrients from leaves to grains (Distelfeld et al., 2007). Several QTLs for grain Fe and Zn were found using three different sets of RIL populations from CIMMYT-Mexico. A major QTL for grain Zn, QGZn.cimmyt-7B-1P2, located on chromosome 7B, explained 32.7 percent of the phenotypic variation, and another major QTL for grain Zn, QGFe.cimmyt-7A-P2, located on chromosome 4A, explained 21.4 percent (Crespo-Herrera et al., 2017). Two QTLs for grain Zn were found from a population of 138 lines developed through the double haploid (DH) technique from "Berkut and Krichauff" evaluated in the eastern Gangetic plains of India. These QTLs are located on chromosomes 1B and 2B, and the QTL on chromosome 2B is associated with grain Fe, which accounts for 22.2 percent of phenotypic variation for Fe concentration (Tiwari et al., 2016). The RIL population was developed from the Indian old wheat variety WH 542 (286 lines) and synthetic derived genotype (PI94624/*Aegilops-squarrosa* (409)/BCN) and evaluated under six environments for estimation of grain zinc and iron content. This work identified four QTLs for grain Fe on chromosomes 2A, 5A, and 7B and five QTLs for grain Zn on chromosomes 2A, 4A, 5, and (Krishnappa et al., 2017). The two ferritin genes, *tafer1* and *tafer2*, are located on chromosomes 5 and 4 respectively, and are each represented by three homeoalleles according to the characterisation of the full complement of wheat ferritins.

Differentially regulated and expressed are the two genes. Except for in the endosperm, the *tafer1* genes have the highest levels of expression and are controlled by the levels of iron and abscisic acid. Contrary to *tafer2*, *tafer1*'s promoter has iron- and ABA-responsive components, corroborating the expression results. The *tafer1* and *tafer2* genes have two isoforms that likely serve distinct roles in the heteropolymer ferritin complexes seen in wheat. It is feasible to bio-fortify wheat grains with iron. *Tafer1-A* gene overexpression in the endosperm increases the grain's iron content by 50–85 percent (Borg et al., 2012). Understanding the molecular basis of the QTL underlying the carotenoid content of wheat is becoming more and more important as carotene genes have been discovered and functional markers have been developed.

- 5. Genomic prediction and GWAS for wheat grain Fe and Zn:** Several biparental techniques were employed to analyse the genetics of grain Fe and Zn in wheat, however a biparental mapping strategy is constrained by poor QTL resolution, constrained allelic diversity from two parents, and longer development times for acceptable mapping populations. GWAS offers benefits in terms of QTL resolution, high allelic coverage, and simultaneous use of wild germplasm, landraces, elite genotypes, and variety to unravel the genetic basis of complex characteristics. Using an Illumina select 90K Infinium SNP array, GWAS was conducted on the Harvest plus association mapping panel (HWAM) of 300 bread wheat genotypes for the purpose of phenotyping grain Zn in a variety of environments in India and Mexico. This revealed about 39 marker trait associations (MTAs) for grain Zn and led to the identification of two major QTL regions on chromosomes 2 and 7. Further candidate gene analysis inside these key QTL areas identified a metal ion binding gene and zinc finger motif as being connected to the main QTLs (Velu et al., 2018). Two sites in India were used for phenotyping and genotyping 246 samples of spring wheat reference set (SWRS) from the CIMMYT gene bank, and the results showed eight and six significant MTAs for the amounts of zinc and iron in grain, respectively (Kumar et al., 2018). In a GWAS analysis conducted by Alomori et al. (2019) on 369 European wheat genotypes, 40 MTAs for grain Zn content on 12 distinct chromosomes were identified.
- 6. The impact of ploidy level on bio-fortification:** In comparison to lines carrying the allele from cultivated wheat, the high grain protein content (Gpc-B1) locus from the wild tetraploid wheat *Triticum turgidum* ssp. *dicoccoides* resulted in 10-34 percent higher concentrations of zinc, iron, manganese, and protein in the grain. The Gpc-B1 locus also promoted the remobilization of protein, Zn, Fe, and Mn from the leaves to the grain (Eide, 2006). In addition, the discovery of publically accessible genome-specific markers for genetic mapping in polyploid wheat has made it possible to identify single nucleotide polymorphisms and access genic sequence by RNA-seq and exome capture (Winfield et al., 2012). (Allen et al., 2013). A thorough collection of homoeolog-specific gene models for polyploid wheat has just been published (Krasileva et al., 2013). In summary, wheat researchers now have access to draught reference genomes, gene models, massive SNP datasets, and genome-specific contig assemblies (even if they are incomplete and fragmented). Together, these methods should make marker-assisted selection more accurate at mapping and deploying Fe and Zn characteristics in grain.

VI. AGRONOMIC BIO-FORTIFICATION

Although genetic bio-fortification may be more cost-effective in the long run, it offers an immediate and efficient way to increase micronutrient concentrations in edible crop products. This can be done by adding micronutrients to the soil through fertiliser and directly applying them through foliar applications to the crop's leaves (De Valenca et al., 2017). These methods provide quick, complementary fixes for zinc and iron deficiencies, as well as grain quality, in crop production. The poor phyto-availability of the mineral micronutrients in soil is the fundamental barrier to bio-fortification. Approaches to agronomic bio-fortification rely on minerals with effective soil and plant mobility. The most alluring agronomic bio-fortification technique involves addressing soil salinity, raising beneficial soil microbes, and implementing crop rotation strategies, together with the foliar delivery of mineral fertiliser to the plants in photo-available form (Bouis and Saltzman 2017). By simply giving the plants

zinc salts, agronomic techniques may be used to boost the zinc content of grains. For instance, foliar treatment of ZnSO₄ raised total grain zinc by roughly 60%. (Zhang et al., 2008). Such agronomic techniques are less successful for iron, though, unless they are paired with greater nitrogen fertilisation (Aciksoz et al., 2011), which may not be feasible from an economic or environmental standpoint. Workers at CIMMYT, Mexico, employed conventional breeding to release wheat variety with higher iron and zinc concentrations in wheat grain (Velu et al., 2018).

Presently being produced in Pakistan and India are CIMMYT's zinc bio-fortified lines, which have 20–40% greater zinc concentrations and at least equivalent grain yields to the best local variety (Velu et al., 2018). Application of iron sulphate (FeSO₄), zinc sulphate (ZnSO₄), and either individually or jointly either foliar and soil application increased plant height, 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). The maximum growth or quality characteristics of wheat were greatly enhanced by foliar sprays of 0.5 percent ZnSO₄ and 1 percent FeSO₄ in combination (Melash and Mengistu, 2020).

Foliar application is better suited for ensuring the availability of nutrients to plants for optimum growth than soil application. Foliar spray applications of Zn and Fe (0.5 percent ZnSO₄ and 1 percent FeSO₄) are advised for wheat crops that produce grains of exceptional quality (Xu et al., 2011). In wheat cultivated on Zn-deficient alkaline soils, foliar Zn spray during the booting + milking phases greatly boosted agronomic features, grain Zn content, and bioavailability, hence enhancing the wheat grain's nutritional quality for people (Esfandiari et al., 2016). Even though agronomic fortification aids in raising the crop's micronutrient content (Fe and Zn), its adaptability and dependability may be hampered by several elements including irrigation, soil, and temperature. Therefore, the best alternative for more effective micronutrient fortification is genetic bio-fortification.

VII. BIO-FORTIFIED WHEAT VARIETIES AND INDIAN SUCCESS STORIES

By adjusting the lysine content, starch composition, and bio-fortification of micronutrients (zinc & iron), ICAR-Indian Institute of Wheat and Barley Research (IIWBR) is dedicated to enhancing nutritional quality by 2025. This is done while lowering anti-nutritional factors and improving the bioavailability of the micronutrients. (2007) Mishra et al. The Indian Prime Minister dedicated 17 recently developed bio-fortified seed varieties of regional and traditional crops, including wheat and rice, to the country during a ceremony to celebrate the 75th anniversary of the United Nations Food and Agriculture Organization (FAO) on World Food Day in 2020. These seeds are being made available to Indian farmers. By the year 2022, 24 bio-fortified wheat variety with high protein, iron, and zinc content will be available in India thanks to AICRIP. The following information pertains to the newly introduced bio-fortified varieties. (Gupta et al., 2019).

1. 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 the North Western Plains Zone (NWP),

which includes 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, WB 02 is the first bio-fortified wheat variety to be released in India. 51.6 q/ha is the average grain yield. It is appropriate for timely irrigation circumstances and matures in 142 days. The ICAR-Indian Institute of Wheat and Barley Research at Karnal, Haryana, produced this bio-fortified variety.

- **HPBW 01:** A bread wheat variety developed by the Punjab Agricultural University, Ludhiana, Punjab that was published and registered in 2017 under irrigated timely seeded circumstances in NWP zone. It has high iron (40.0 ppm) and zinc (40.6 ppm) levels. It matures in 141 days and yields 51.7 q/ha on average.
- **HI 1605:** A pure line variety called Pusa Ujala (HI 1605) was developed by the ICAR-IARI Regional Station in Indore, Madhya Pradesh. It has high protein content (13 percent), iron content (43 ppm), and zinc content (35 ppm), as well as outstanding chapatti-making qualities. It was published and announced in 2017 for India's Peninsular Zone, which includes the states of Maharashtra, Karnataka, and Tamil Nadu. Under conditions of timely sowing and constrained irrigation, its typical yield is 35.0 q/ha.
- **HD 3171:** The ICAR-Indian Agricultural Research Institute, New Delhi, developed a bread wheat variety that was released and notified in 2017. It is suitable for timely sowing in rainfed conditions in the North Eastern Plain Zone, which includes eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal (excluding the hills), Odisha, Assam, and plains of North Eastern States. It is a medium-late variety that takes 120–125 days to reach maturity, yields 28 q/ha of grain on average, and has a zinc content of 47.1 ppm.
- **PBW 752:** It is a high yielding bread wheat variety developed by Punjab Agricultural University in Ludhiana and released for circumstances of the North Western Plain Zone's late seeded, irrigated conditions. PBW 752 matures in 120 days with an average yield of 49.7 q/ha and is shown to be high in protein (12.4%) compared to common varieties' 8–10%.
- **PBW 757:** It is a bread wheat variety that Punjab Agricultural University, Ludhiana developed in 2018 for the North Western Plain Zone's very late-sown irrigation circumstances. It has high zinc content (42.3 ppm), compared to typical kinds' 30.0–32.0 ppm. It is an early maturing variety with a 36.7 q/ha average yield.
- **DBW 187:** : Karan Vandana (DBW 187) is a mega wheat variety developed for the North Eastern and North Western Plains Zones' timely irrigation-sown and fertile circumstances. It may also be grown in these two zones' early sowing, high fertility conditions to produce an average of 75.5 q/ha. Because of its high iron content (43.1 ppm), it is more well-liked by farmers and has the highest breeder seed ID in recent years. It is released by ICAR-Indian Institute of Wheat & Barley Research, Karnal.

- **DBW 173:** ICAR-Indian Institute of Wheat & Barley Research, Karnal developed bio-fortified wheat variety in 2018 that is high in protein (12.5%) and iron (40.7 ppm). It has an average yield of 47.2 q/ha and is a medium late maturing genotype appropriate for late sowing under irrigation in the North Western Plain Zone.
- **UAS 375:** The University of Agricultural Sciences, Dharwad developed and released a short-lived, drought-resistant bread wheat variety in 2018. With an average yield of 21.4 q/ha and a high protein content of 13.8% compared to 8–10% in common variety, it was made available for timely rainfed conditions in the peninsular zone.
- **PBW 771:** For late-sown irrigated circumstances in the North Western Plain Zone, a high yielding bread wheat variety (50.3 q/ha) produced by Punjab Agricultural University, Ludhiana was released. It matures in 120 days and has a high zinc content (41.4 ppm).
- **HD 3249:** In 2020, the ICAR-Indian Agricultural Research Institute will release HD 3249, a bread wheat type that is compatible for the North Eastern Plain Zone's timely irrigated circumstances. It is a 122-day mature wheat variety that has a high yield (48.8 q/ha) and is iron-rich (42.5 ppm).
- **HD 3298:** For extremely late seeded irrigated circumstances in the North Western Plain Zone in 2020, ICAR-Indian Agricultural Research Institute, New Delhi, published HD 3298, a short duration, high yielding bread wheat variety. It yields a yield of 43.7 q/ha on average, 12.1 ppm of protein, and 43.1 ppm of iron.
- **HI 1633 (Pusa Vani):** Early ripening, high yielding bread wheat variety HI 1633 (Pusa Vani) was developed by the ICAR-Indian Agricultural Research Institute, Regional Station, Indore, and released in 2020 for peninsular zone late seeded irrigation circumstances. It produces 41.7 q/ha on average and has high levels of zinc (41.6 ppm), iron (41.6 ppm), and protein (12.4%). (41.1ppm).
- **DBW 303:** DBW 303 is a late maturing, high yielding bread wheat type that works well in the North West Plain zone's high fertility, early sowing circumstances. It had a higher protein content and an average yield of 81.2 q/ha (12.1 %). In 2020, the ICAR-Indian Institute of Wheat and Barley Research, Karnal, developed it.
- **HI 1636 (Pusa Vakula):** Early maturing , high yielding bread wheat variety HI 1636 (Pusa Vakula) was developed by ICAR-Indian Agricultural Research Institute, Regional Station, Indore, and released in 2022 for central zone irrigation-timed sowing circumstances. It produces at a rate of 56.6 q/ha on average and has a high zinc and protein content (12.0% & 44.4 ppm).

2. Durum wheat

- **HI 8759 (Pusa Tejas):** It is a durum wheat variety developed by the ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Indore, with an average yield of about 55.0 q/ha and high protein content (12.0 percent), iron content (42.1

ppm), and zinc content (42.8 ppm); it is used to make pasta, dalia, suji, and chapatti (Indian bread). It was released and notified in 2017 for the central zone's timely sowed irrigation circumstances, which include Madhya Pradesh, Chhattisgarh, and Gujarat. Rajasthan and Bundelkhand region of Uttar Pradesh.

- **MACS 4028:** It is a pure line durum wheat variety with high levels of zinc, iron, and protein (14, 7%, 46, 1 ppm, and respectively) (40.3 ppm). In 2018, it was released and notification for Maharashtra and Karnataka. Under rainfed, timely sown environments, it yields an average of 19.3 q/ha of grain in the Peninsular Zone. It was developed by the MACS-Agharkar Research Institute in Pune, Maharashtra, and matures in 102 days.
- **HI 8777 (Pusa Wheat 8777):** It is an early maturing durum wheat variety that the Indian Agricultural Research Institute (ICAR), Regional station, Indore, released in 2018 for the Peninsular zone's rainfed, promptly sown circumstances. It produced an average of 18.5 q/ha under rainfed circumstances and found to be high in iron (48.7 ppm) and zinc (43.6 ppm), as opposed to common variety' 28.0-32.0 ppm iron and 30.0-32.0 ppm zinc.
- **DDW 47:** ICAR-Indian Institute of Wheat & Barley Research, Karnal developed this durum wheat variety ideal for manufacturing pasta, dalia, and suji in 2020. It is appropriate for timely sowing under constrained irrigation circumstances in the Central zone. It is a medium late maturing genotype with an average yield of 37.3 q/ha and is strong in iron (40.1 ppm), protein (12.7%), and yellow pigment (7.2 ppm).
- **HI 8802 (Pusa Wheat 8802):** A medium tall durum wheat variety was developed by the ICAR-Indian Agricultural Research Institute, Regional Station, Indore, and released in 2020 for the Peninsular zone's timely seeded, constrained irrigation circumstances. It is a variety with early maturation, large yields (29.1 q/ha), and high levels of protein (13.1%) and yellow pigment (6.5 ppm)
- **HI 8805 (Pusa Wheat 8805):** Durum wheat variety released for timely sowing under restricted irrigation circumstances in the Peninsular Zone that is suited for making pasta. It has an average yield of 30.4 q/ha and is high in iron and protein (12.8%) as well as having a high yield (40.4 ppm). It was released in 2020 by the Indore Regional Station of the Indian Agricultural Research Institute (ICAR).
- **MACS 4058:** In 2020, the ICAR-Indian Institute of Wheat & Barley Research, Karnal, released the durum wheat variety, which is high in protein (12.1%) and rich in yellow pigment (7.8 ppm), and is suited for timely sowing in irrigated circumstances in the Peninsular zone. It yielded 47.4 q/ha average yield and reaches maturity quickly (111 days).
- **HI 8823 (Pusa Prabhat):** A durum wheat variety released for Central Zone for timely constrained irrigation circumstances and ideal for pasta production is HI 8823 (Pusa Prabhat). It produces at a rate of 38.5 q/ha on average and is high in protein

(12.8%) and iron (40.4 ppm) content. It was developed in 2022 by the Indore Regional Station of the Indian Agricultural Research Institute (ICAR).

Apart from Indian Council of Agricultural Research, the Harvest Plus Project, supported by international funding agencies, helps the National Agricultural Research System in India to breed, test, and release bio-fortified wheat developed through a partnership with the CGIAR institutes CIMMYT, Mexico, ICARDA, Morocco, and ICRISAT, Hyderabad, with the aim of promoting bio-fortified wheat varieties in India to improve nutrition and public health. Two commercially available, truthfully labelled (TL) zinc wheat varieties (BHU-31 and BHU-25) were formally launched by seed companies in Bihar during November 2019, with the goal of reaching more than 1 million farming households over the course of five years. Harvest Plus is a component of the CGIAR Program on Agriculture for Nutrition and Health (A4NH), which provides global leadership on bio-fortification evidence and technology. 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 use of bio-fortified variety has a lot of potential to improve human population health and welfare. Studies have shown that these bio-fortified crops have favourable benefits on people, and attempts are being undertaken to increase public awareness of them. For commercial production, high-quality seeds of bio-fortified variety are developed and made available. According to the invoices obtained from the Department of Agricultural Cooperation and Farmers' Welfare, a total of 7466.43 q of breeder seed of bio-fortified types of cereals, millets, oil seeds, etc. were produced over the previous five years (Yadava et al., 2020). By granting licences to several private seed businesses and farmers producers' organisations (FPOs), the seed production of these wheat types is being ramped up so that seeds can be distributed to more farmers. These initiatives by ICAR, NARS, state agriculture universities, AICRIPs, CGIAR institutes, and the government will be essential to the release and promotion of naturally bio-fortified wheat varieties in India in order to achieve "malnutrition free India" and provide a prosperous and healthy future for the nation.

VIII. WAY AHEAD FOR BIO-FORTIFICATION IN WHEAT

The interplay between genotype and environment in relation to grain production and nutrient concentrations has not yet been fully explored. Numerous research projects aimed at improving nutrient usage efficiency have been constrained by pricy and time-consuming phenotyping. Another important aspect of measuring grain quality is the bioavailability of nutrients. Changes in the climate might make the issue worse. A problem with bio-fortification is its high development expenses. The achievable breeding level for various nutrients must be determined in advance. This is a difficult process that takes into account things like the farmers' level of adoption, the amount of food products made from the crop consumed, post-harvest losses, preparation and cooking losses, and the bioavailability of the nutrients. The target breeding level must be certain that there is a positive impact on the recipient's nutritional status (Taylor and Taylor, 2012). It would take roughly ten years for the bio-fortified crop to become publicly available, and there are additional legal and regulatory concerns when the crop is bio-fortified through a genetic transformation process (Birner et al., 2007). Farmers lack incentives and motives to cultivate enhanced crops, and consumers are uninformed that bio-fortified crops may provide high-quality food items.

During the production of the bio-fortified crop, all the research teams should work together to produce an end product with the appropriate nutritional qualities. Improved and palatable cooking quality is required for bio-fortified crops to be well-adapted. Additionally, these bio-fortified crop types have a higher production level and greater resistance to biotic and abiotic stress. Supporting extensive prospective research on the effects of iron bio-fortified crops on reducing anaemia (Iron Deficiency Diseases) and enhancing health is essential (Hussain et al., 2010).

IX. CONCLUSION

A dependable, cost-effective, and practical method of providing crops with the micronutrients they lack is bio-fortification. Mineral concentration in the edible component of bio-fortified crops is higher due to improved mineral absorption from the soil, improved mineral translocation from leaves to grain, and improved mineral sequestration to the endosperm. In addition to beneficial and significant genetic variation in grain Fe and Zn content, wild cousins of wheat exhibit a promising and significant genetic variety. Through both traditional and contemporary breeding techniques, this genetic diversity may be used to boost the concentration and bioavailability of Fe and Zn in contemporary wheat variety. One example of a staple crop that might be genetically improved in an economical and sustainable way is wheat. Even after the creation of bio-fortified crop types, a number of socioeconomic and political issues need to be resolved in order to encourage farmers to grow them and consumers to consume them. Despite these obstacles, scientists and researchers have been attempting to generate new wheat varieties and achieve amazing improvements to the nutritious content of wheat. So, the key to addressing hidden hunger will be multitiered collaboration between researchers, farmers, and consumers (end-user). People in this climate change scenario can live better lives, improve their nutrition, support sustainable agriculture, and attain food security by using bio-fortified wheat variety. We may infer that wheat will predominantly benefit from bio-fortification.

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