Review Article

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Current Status and Future Perspectives of Biofortification in Wheat

ABSTRACT

Wheat is the main cereal crop worldwide and about 2 billion people suffer from Zn and Fe deficiency because of their dependence just on cereal crops. Three Billion people are malnourished suffering from mild to severe physical and mental disabilities. Vast genetic diversity of Wheat exist in nature that differs in their mineral compositions. Main deficient micronutrients are Provitamin-A,Zn & Fe which deficiencies cause serious physical and mental abnormalities. Different methods of enhancing mineral contents of plants produce have been used, out of which BIOFORTIFICATION have proved more promising and economical. Bacterial phytoene synthase gene (crtb) and carotene desaturase gene (crti) has been transferred in wheat that has increased carotenoid content but more darker color has less public acceptance.GPC-B1 gene is found to be associated with increase micronutrients but it lowers the overall yield of the plant. Several new methodologies such as oligo-directed mutagenesis, reverse breeding,RNA directed DNA methylation and genome editing have been used for increasing micronutrient composition and their bioavailability .But combination of Plant Breeding methods with Molecular Techniques will be more useful for advancement in this field.

1. INTRODUCTION

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Human and animals require multitude of nutrients for the proper functioning of their body in terms of growth, development and metabolism (Carvalho and Vasconcelos 2013).Wheat is a crop of major importance and together with other staple cereals supply the bulk of calories and nutrients in the diets of a large proportion of the world population(Waters et al. 2009). It is one of the key cereal crops, is grown on 222 million hectares worldwide and is a major source of calories and proteins globally (USDA, 2016).Throughout history, hunger has remained one of the most PREVALENT and SHATTERING problem FACED BY— humankind. Although statistics on food insecurity and malnutrition DIVULGE significant improvement in these areas in the last decade(Hoddinott, Rosegrant, and Torero 2014).At present, 49 nutritional components are known to be essential and indispensable for sustaining human life. These comprise water and carbohydrates, 10 essential amino acids, linoleic and linolenic acids, seven mineral macro elements, 16 mineral microelements and 13 vitamins. Dietary deficiency of essential micronutrients such as zinc (Zn)and iron (Fe) affects more than two billion people worldwide(Šramková, Gregová, and Šturdík 2009; White and Broadley 2005). The problem is most severe in low- and middle income countries, especially in Africa where the estimated risk for micronutrient deficiencies is high for Ca (54% of the continental population),Zn (40%), Se (28%), I (19%) and Fe (5%) (de Valença et al. 2017). Worldwide, about 800 million people are chronically hungry meaning that they are undernourished in terms of calories (FAO,2017). When intakes of bioavailable micronutrients are too low to meet the regularity requirement of the body then micronutrient malnutrition develops and it affects the ⅓— to ½— of the world population (Allen et al. 2006). Wheat like many other staple food contain suboptimal quantities of essential micronutrients such as zinc and iron AND more than two billion people worldwide— suffer from zinc and iron deficiency because of their PRIMARILY dependence on cereal based diets (Borrill et al. 2014). Due to lack of access to the healthy diets such as fruits and vegetables, about one third of children die under the age of five from malnutrition and one child in four is stunted due to inadequate diet (Hefferon 2015). Many possible strategies like dietary diversification, mineral supplementation and post-harvest food fortifications are used to improve micronutrient intake in the human diet. Biofortification evades these problems by increasing the micronutrient contents in edible part of crops and enhancing their bioavailability and absorption in human body during digestion.(Muhammad et al. 2016). Pregnant women and young children are prone to acute micronutrient deficiency, which reduces physical and mental development in children below 5 years of age, and malnutrition is considered as the largest single contributor to disease in persons of any age (Alanis-guzman, Amaya-guerra, and Saldı 2006). Education, dietary modification, food rationing, supplementation and fortification these are various strategies employed to supplement micronutrient to children and women (best C et al. 2011., Butta et al, 2008). In countries where people depend on cereal based food, high incidence of micronutrient deficiencies has been observed (Muhammad et al. 2016). For the purpose of biofortification it is necessary to increase the bioavailable pool of Zn in cereal grains and not just its total concentration (Khoshgoftarmanesh et al. 2010) .

Biofortification is the process of increasing the content and bioavailability of essential vitamins and minerals in staple crops, through plant breeding, transgenic techniques and agronomic practices, to improve nutritional status and alarming rates of malnutrition (Bouis and Saltzman 2017). Agronomic biofortification is often considered as a short-term solution to increase micronutrient availability and mainly to complement genetic biofortification (breeding), which is seen as a more sustainable approach(de Valença et al. 2017; Welch and Graham 2004). The main sources of vitamins and minerals (iron, zinc, and vitamin A) for low-income rural and urban populations are staple foods of plant origin that often contain low levels or low bioavailability of these micronutrients. Biofortification aims to develop micronutrient-enhanced crop varieties through conventional plant breeding .Breeding high yielding, high-Fe and -Zn varieties require source materials that shows adequate genetic variation in concentrations of those micronutrients(Meng et al. 2007). Screening studies have shown that modern wheat cultivars are not a good source of genes to enhance the concentration of Zn and Fe(Mondal, Maize, and Rutkoski 2016).— The breeding steps include at least (1) Identification of a useful genetic variation and the most promising parents, (2) Long-term crossing and back-crossing activities, (3) stability of the target traits (e.g., high grain Zn concentrations) across the different environments that feature huge variation in soil and climatic conditions, and finally (4) Adaptation of the newly developed biofortified genotypes over a range of crop and soil management practices applied in the target regions or countries(Bouis 2002; Šramková, Gregová, and Šturdík 2009).

Biofortification is faced with numerous challenges but also offers new solutions. The initial challenge is to use conventional or molecular breeding to increase the micronutrient content, preferably in the form of bioavailable minerals and biologically active vitamins or vitamin precursors. Supplementary strategies comprise breeding for increased contents of components that promote nutrient uptake and reduce amounts of inhibitors of uptake(Brinch-Pedersen et al. 2007). The fundamental drivers of “hidden hunger” quite often fall outside the nutrition sector and are associated with each country’s levels of potential resources (i.e. Energy, geography, climate, etc.)(Hoddinott, Rosegrant, and Torero 2014).

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2.HIDDEN HUNGER CAUSES AND PREVILENCE

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About 800 million people are chronically hungry worldwide means these are undernourished in terms of calories (FAO et al., 2017). Moreover, two million people are affected by hidden hunger as they suffer from micronutrient deficiency (WHO 2006). Micronutrient deficiencies are more commonly pervasive in the population that mainly depend on the non-diversified plant and cereal based diets which leads to compromised health and economic problems (Gómez-Galera et al. 2010). Over three billion people are currently micronutrient (i.e. Micronutrient elements and vitamins) malnourished, resulting in ATROCIOUS societal costs including learning disabilities among children, increased morbidity and mortality rates, lower worker productivity, and high healthcare costs, all factors diminishing human potential, felicity, and national economic development while Vitamin A deficiency is causing around 4500 preventable child deaths daily. Nutritional deficiencies (e.g. Iron, zinc, vitamin A) account for almost two-thirds of the child-hood death worldwide where most of those are dependent on staple crops for their sustenance. Malnutrition also reduces the number of neurons and synapses. When the brain is deprived of an optimal supply of nutrients, there are no discrete lesions. Instead, generalized distortion occurs in those areas that were maturing at the time of nutrient deficit. In other areas of the brain, where the cells have differentiated during prenatal life, malnutrition in infancy reduces the formation of synapses and the branching of dendrites(Alanis-guzman, Amaya-guerra, and Saldı 2006; Dubock 2017; Welch and Graham 2004). Hunger is the outcome of insufficient food intake resulting in decreased health and quality of life while hidden hunger prevails when a person's is getting bellyful food but that food lack nutrients or possess less nutrients that are required for normal functioning of the body(Hoddinott, Rosegrant, and Torero 2014). Recently FAO roughly estimated that about ⅓ of the globally produced food for human consumption is lost or wasted (Gustavsson et al. 2011). Hidden hunger is prevailing in world's populations because of its increase at fast speed, poor economy and poor legislation of governments. Children and pregnant women are more vulnerable to nutrient deficiency because their body require more amount of these nutrients as compared to normal adults (Akhtar et al. 2011). Iron and zinc deficiency are also known as ''hidden hunger'' results in reduced immunity, rigidity, fatigue, irritability, loss of hairs, compromised psychomotor development, muscle weakening, sterility and in acute case cause death (Wintergerst et al. 2007, Stein 2010). In low income populations, people rely on the inexpensive less caloric monotonous food to meet energy need due to lack of access to the nutrient rich diversify food (Bouis et al. 2011). Eradicating hunger in all its forms, including chronic and hidden hunger, requires good understanding of the problem's magnitude, trends, and determinants. The burden of chronic hunger more than halved since 1990, it remains larger than the burden of hidden hunger. Cost-effective micronutrient interventions that can have an impact in the short to medium term include biofortification, industrial fortification, and food supplementation(Gödecke, Stein, and Qaim 2018). Micronutrient malnutrition ("hidden hunger") now afflicts over 40% of the world's population and is increasing especially in many developing nations. Today, deficiencies of iron and iodine are of most concern to the nutrition community and healthcare officials although other nutrient deficiencies, including zinc, selenium, calcium and magnesium may be prevalent in some global regions(Šramková, Gregová, and Šturdík 2009). In the search for genetic material with high iron and zinc concentration in wheat grain, a significant positive correlation has been found between iron and zinc concentrations, suggesting that these two traits may be combined relatively easily during breeding. The production of semi-dwarf wheat through the introduction of the rht genes has resulted in substantial yield increases. However, this is associated with a reduction in iron and zinc concentrations in some bread wheat genotypes, but not in durum wheat(Meng et al. 2007; Monasterio and Graham 2000).

2.1.Wheat Grain Composition

The mature wheat Grain comprises of three major component such as carbohydrates, protein and cell wall polysaccharides which together account for the 90% of the dry weight and minor component include lipids, phenolics, vitamins and minerals. However distribution of these components vary within the grains (Shewry et al. 2013). Wheat grains are generally oval shaped, although different wheats have grains that range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally connected to the wheat flower. The wheat grain contains 2-3% germ, 13-17% bran and 80-85% mealy endosperm. The bran (outer layers of wheat grain) is made up of several layers, which protect the main part of the grain. Bran is rich in B vitamins and minerals; it is separated from the starchy endosperm during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises water-insoluble fiber. More than half the bran consists of fiber components (53%). Chemical composition of wheat bran fiber is complex, but it contains, essentially, cellulose and pentosans, polymers based on xylose and arabinose, which are tightly bound to proteins. These substances are typical polymers present in the cell walls of wheat and layers of cells such as aleurone layer. Proteins and carbohydrates each represent 16% of total dry matter of bran. The mineral content is rather high (7-2%). The two external layers of the grain (pericarp and seed coat) are made up of dead empty cells. The cells of the inner bran layer- aleurone layer are filled with living protoplasts. This explains the rather high levels of protein and carbohydrate in the bran. There are large differences between the levels of certain amino acids in the aleurone layer and those in flour. Glutamine and proline levels are only about one half, while arginine is treble and alanine, asparagine, glycine, histidine and lysine are double those in wheat flour. The endosperm is surrounded by the fused pericarp and seed coat. The outer endosperm, the aleurone layer, has a special structure: it consists of single layer of cubic shaped cells. The aleurone layer is rich in proteins and enzymes, which play a vital role in the germination process. The inner endosperm, i.e. The endosperm without the aleurone layer, is referred to as mealy or starchy endosperm. The endosperm mainly contains food reserves, which are needed for growth of the seedling, it is rich in energy-yielding starch. Apart from carbohydrates, the mealy endosperm contains fats (1,5%) and proteins (13%): albumins, globulins and the major proteins of the gluten complex- glutenins and gliadins.- proteins that will form the gluten at dough making. The contents of minerals (ash) and of dietary fibers are low; 0,5% and 1,5%, respectively. The germ lies at one end of the grain. It is rich in proteins (25%) and lipids (8-13%).The mineral level is also rather high (4-5%). Wheat germ is available as a separate entity because it is an important source of vitamin E. Wheat germ has only one half the glutamine and proline of flour, but the levels of alanine, arginine, asparagine, glycine, lysine and threonine are double(Meng et al. 2007; Šramková, Gregová, and Šturdík 2009)

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2.2. VITAMIN-A DEFICIENCIES

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Vitamin A (retinol) is a fat-soluble micronutrient and is mainly FOUND in eggs, liver and butter. Vitamin A precursors such as β -carotene, and other carotenoids, are produced in green and yellow vegetables. B-carotene is the major provitamin A carotenoid, and theoretically enzymatic cleavage of one β-carotene molecule generates two molecules of retinal, compared with only one retinal molecule on cleavage of all other provitamin A carotenoids. The retinal is further converted to retinol and then to retinoic acid as required. (Hess, Thurnham, and Hurrell n.d.; Šramková, Gregová, and Šturdík 2009).

Carotenoids are synthesized de novo by plants, where they play fundamental physiological roles as photosynthetic pigments and precursors for signaling molecules. They are also essential components of a healthy diet, as dietary antioxidants and vitamin A precursors. Wheat like Rice have less diversity of

Votamin-A. Vitamin A has multiple roles in the body including reproduction, vision, cell differentiation, immune function, bone formation and growth. Vitamin A comes from animal sources in the diet such as retinol or retinyl esters and from provitamin A carotenoid found in plant sources.Vitamin A deficiency has been associated with the severity of infections and in the developing world, it is the primary cause of childhood mortality and morbidity particularly in south Asia and Africa. Vitamin-A Deficiency is the leading cause of the preventable blindness in children. The WHO estimates that about 250-500 million children are blind due to VAD and half of these vision loss will die within a year (Bailey, West, & Black, 2015) .

Vitamin-A deficiency affects over 125 million preschool-aged children and 7 million pregnant women in low-income countries. It is the leading cause of preventable pediatric blindness and of over 650 000 early childhood deaths due to diarrhea, measles, malaria, and other infections each year. Wheat only contains trace levels of provitamin A carotenoids. The predominant carotenoid (yellow pigment) in wheat, specifically in durum wheat, is lutein, followed by zeaxanthin. These two carotenoids show no provitamin A activity. Orange-colored wheat seed, which may have some provitamin A carotenoids, have not been identified so far, even after screening thousands of lines in the International Maize and Wheat Improvement Center (CIMMYT) germplasm bank. In the unlikely event that wheat with enhanced provitamin A carotenoid levels is developed, it is important to consider that it will have unusually high yellowish to orange pigmentation, which may have implications for its acceptability to consumers(Giuliano 2017; Meng et al. 2007). Vitamin A deficiency results in the deaths of approximately one million children every year (Rice et al. 2004; Stein et al. 2005; UNICEF and Micronutrient Initiative, 2004b). Provitamin A present only in trace amount in wheat. Lutein followed by zeaxanthin predominantly present in durum wheat (leenhardt et al. 2006).

2.3. IRON AND ZINC DEFICIENCY

Iron deficiency ranks among the most widespread nutrient deficiencies,affecting over two billion people worldwide. Iron deficiency anemia (IDA) has been linked to maternal and perinatal mortality, and to impairment of cognitive skills and physical activity. Iron deficiency is also associated with enhanced absorptional environmental metal toxins such as cadmium (Cd) (Silver et al. 2013). Zinc is an essential trace mineral influencing gene expression as well as cell development and replication. Approximately 800,000 child deaths worldwide per year are attributable to Zn deficiency. Because it significantly increases the risk of diarrhea, pneumonia, and malaria, Zn deficiency has been linked to the morbidity and mortality of children younger than 5. The global average prevalence of Zn deficiency was estimated at 31%, with the most severe burden of diarrhea and pneumonia due to this deficiency found in Africa and South Asia. Africa, however, almost exclusively bears the burden of malarial disease attributable to Zn deficiency. Analyses of multiple grain samples from maize and wheat that have been collected by CIMMYT across environments and genotypes suggest that the levels of Fe and Zn are higher in wheat than in maize. However, we are not aware of any specific studies comparing wheat and maize mineral concentrations in the same experiment and in different grain tissues(Elch et al. 2005; Kabir, Swaraz, and Stangoulis 2014; Maqbool 2019; Meng et al. 2007)

About 37% population in Pakistan is suffering from zinc malnutrition (Nazir et al. 2016). Zinc is responsible for 10% diarrhea, 16% respiratory disorders, 800,000 annual deaths in poor world. Deficiency of zinc also negatively influence the reproductive system, cell growth, immune system, cancer and skin disorders (WHO, 2012). Zinc deficiency mainly occur due to cereal based food which is deficient in Zn.

70% of agricultural soils in Pakistan are Zinc deficient. Deficiency of zinc is more frequent in peat, calcareous, saline sodic and highly weathered and intensively weathered soils (Alloway, 2008). In arid and semi-arid areas due high calcium carbonate and less organic matter contents soils are deficient in zinc (Imran et al. 2014).

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There exist large biodiversity for these micronutrients in wheat. Durum wheat is one of the main sources of calories and protein in many developing countries. In a study conducted by (Magallanes-López et al. 2017), 46 durum varieties grown under full and reduced irrigation, were analyzed for micronutrients and phytate content to determine the potential bioavailability of the micronutrients. The variation was 25.7–40.5 mg/kg for iron and of 24.8–48.8 mg/kg for zinc.

Current strategies towards increasing the iron content of the endosperm are largely based on the expression of legume ferritin genes in an endosperm-specific manner. However, it is apparent that this approach, at least in rice, only allows a two- to three-fold increase in the iron content of the grain due to exhaustion of the iron stores in leaves. Further increases thus have to rely on additional uptake and transport of iron from the root(Brinch-Pedersen et al. 2007).

3.STRATEGIES OF FOOD ENRICHMENT WITH ESSENTIAL NUTRIENTS

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The diets of over two-thirds of the world's population lack one or more essential mineral elements. This can be retrieved through dietary diversification, mineral supplementation, food fortification, or increasing the concentrations and/or bioavailability of mineral elements in plant produce(White and Broadley 2009).

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Supplementation and fortification with micronutrients have met with several difficulties. The initial requirement is to identify the daily need for micronutrients and this is complicated by the fact that the uptake of the micronutrients will be highly dependent on the food matrix as well as on the presence of compounds that may promote or inhibit the uptake. Furthermore, micronutrients are often lost during processing and cooking of the food(Brinch-Pedersen et al. 2007; Gödecke, Stein, and Qaim 2018).

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3.1. SUPPLEMENTATION

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Supplementation, however, involves the intake of micronutrients in the form of capsules, tablets or syrup. In fortification and supplementation, manufacturing and/or distribution infrastructure is required, which in the long run may not benefit many, especially those in rural communities. Vitamin A capsules intervention, which started in the 1990s, is an example of supplementation(Elemike et al., 2019.; Dubock 2017). The provision of micronutrients to the malnourished population in the form of supplements has proven to be successful (Das et al. 2013; Bhutta et al. 2013) Supplementation is the oral delivery of micronutrients in the form of tablets and syrup, used under chronic deficiencies. Iron absorbed best in the form of ferrous fumarate, ferrous sulphates and ferrous gluconate. Similarly zinc supplied as zinc sulphates, zinc acetate and zinc gluconate (Gómez-Galera et al. 2010).

Fortification of wheat flour is simple and major strategy for preventing anemia. A premix of micronutrients added to the flour at a uniform rate through screw feeder located at the end of the milling process. Premix directly can be added into the flour by gravity and air convection using pneumatic system. In Pakistan wheat flour is the only way of iron fortification because 80% of the production of wheat consumed in the form of least expensive products such as chapattis.

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3.1.1. MERITS AND DEMERITS

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Fortified foods may only be accessible to urban consumers, who can easily see and buy them. It is also very essential at crisis period, where food supply is inadequate and unbalanced. Thus, these fortified diets rich in minerals and vitamins are distributed to avoid malnutrition. However, it may be difficult to get to the rural consumers who cannot afford or have access to them. Thus, the need for biofortification of crops is conceived as a strategy for nutrient fortification in crops or staples while in the field the primary priority in fortification should constitute fortification of locally available food sources, while food supplementation should be an interim measure. Biofortification is intended to cater to the poor populace, low-income earners and everyone at large(Bouis et al. 2014; Elemike et al. N.d.). Change in the dietary habits of the people and high cost of the diets with readily bioavailable iron and zinc suffers the dietary diversification and modification(Rawat, Neelam, Tiwari, & Dhaliwal, 2013).

3.2. FORTIFICATION

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Currently due to an issue of availability, affordability and access, food systems fail to supply sufficient micronutrient rich food (Global Panel on Agriculture and Food Systems for Nutrition, 2016). Among the other nutrient specific efforts, fortification is one of the parameter to improve the quality of diet that helps to mitigate the malnutrition of micronutrients among whole population specially including vulnerable groups (Bhutta et al. 2013) Nutrient deficiency in food crops is seriously affecting human health, especially those in the rural areas, and nanotechnology may become the most sustainable approach to mitigate this challenge. There are several ways of fortifying the nutrients in food such as dietary diversification, use of drugs and industrial fortification. However, the affordability and sustainability of these methods have not been completely achieved. Plants absorb nutrients from fertilizers, but most conventional fertilizers have low nutrient use and uptake efficiency(Elemike et al. 2019). Food fortification strategies are categorized into three main approaches by WHO such as mass, targeted and market driven. Mass fortification involves widely consumed food such as wheat, salt, sugar while target approach fortify food consumed by specific age group that has a unique risk of nurtrient deficiency like infant complementary food and market driven approach fortifies food for particular consumer niche (Allen et al. 2006). Regular consumption of the staple food consistently fortified with essential nutrients that are lacking in regular diet derive great benefits to the individual at the risk of severe deficiencies. Iodine fortified salt prevent the irreversible reduction to the IQ of young children and brain disorders (Mannar, Garrett, and Hurrell 2018)

3.2.1. Agronomic Fortification

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Consumption of diverse food sources, although recommended as a sustainable solution, is unaffordable to the poor populace, who are at risk of malnutrition. The use of industries for the fortification of food nutrients has not been very successful, except for iodized salt(Elemike et al. 2019).There is evidence that agronomic fortification can increase yields and the nutritional quality of staple crops, but there is a lack of direct evidence that this leads to improved human health. Micronutrient fertilization is most effective in combination with NPK, organic fertilizers and improved crop varieties, highlighting the importance of integrated soil fertility management. Agronomic biofortification 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(Monasterio and Graham 2000; de Valença et al. 2017). In the soil mineral elements present as dissolved compounds, precipitates, part of lattice structure of clay micelle or present within the soil biota (White & Broadley, 2009). In the soil the major limitation of the biofortification is the low Phyto availability of the mineral micronutrients. Agronomic efforts have been directed towards the mineral nutrient application and their solubiliztion and mobilization in the soil. Mineral elements with efficient mobility in the soil and plant are considered as successful agronomic biofortification approach.

The mineral composition of cereal grain is determined by factors such as type of cereal, soil conditions and fertilizing practices. Fertilization has a greater effect on the mineral content results in grain , with higher levels found in the grain than in the roots, stems and leaves of the plant. P and Ca contents increase with N fertilization, while Zn content decreases. In the grain, fertilizer P additions decrease Zn content but increase P, K, Mg and Mn. In wheat Fe content was significantly influenced by date of planting but not by seeding rates or nitrogen fertilization. Late planting and irrigation significantly increased the Fe and Zn contents but not those of Cu and Mn. The Fe, Zn and Cu contents of wheat were negatively correlated with yield and positively related to protein content. Mn content was independent of yield or protein content(Anglani 1998; Walker and Waters 2011). The most attractive agronomic biofortification strategy is the foliar application of mineral fertilizer to the plants in phytoavailable form, correcting soil salinity, increasing beneficial soil microorganisms and adopting crop rotation practices (White and Broadley, 2011).

3.2.2. MERITS AND DEMERITS

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The nutrients needed by the plants are fortified in the fertilizers, with the belief that they could be absorbed by the plants. The lack of the micronutrients is manifested by abnormal growth of the plant parts; however, sometimes the soil may not be deficient of the micronutrient, rather the roots are unable to absorb and translocate the nutrients due to small root pore size. It is, therefore, essential to explore the strategies of improving crop quality and their essential nutrients to meet the food demands of the growing population. Conventional fertilizers are readily available for plant uptake but also easily lost through leaching, which is a major challenge. NPK and other agrochemicals have been found to have low use efficiency by plants because of fixation, leaching, microbial degradation, photolysis and volatilization(Elemike et al. 2019.; Dimkpa and Bindraban 2016). Limited success of iron fertilization in biofortification, because the applied iron (Fe2+) rapidly oxidized to Fe3+ state, which is not absorbed by the plants. (Zhang et al. 2008).

For the plant biofortification the major drawback of the fertilization strategy is the need of frequent application which makes the approach economically not feasible, potentially negative for the environment and difficult in logistic term (bulky and heavy products) (Hirschi, 2009; White & Broadley, 2009; Winkler, 2011). In addition due to the risk of the reserves exhaustion the availability of certain nutrients are limited.

The only known case that clearly showed a direct effect of agronomic biofortification on human micronutrient status comes from Finland, where nationwide agronomic Se biofortification was practiced since 1985. This program resulted in significantly increased cereal grain Se concentrations, which in turn led to increased human and animal Se intake and significantly decreased Se deficiencies among the population. The average dietary intake doubled from 0.04 mg Se/day/10 MJ in 1985 to 0.08 mg Se/day/10 MJ in 2014, which is above nutrition recommendations leading to an average human plasma Se concentration of 1.4 µmol/L and reflecting an optimal Se status(de Valença et al. 2017).

3.3. BIOFORTIFICATION

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Biofortification is the nutrient enrichment of key food crops through genetic enhancement. It differ from fortification (exogenous addition of nutrients) through agricultural interventions such as biotechnology, breeding and agronomy. Agricultural Scientists has lifted the crop production many folds' overs last 100 years but nutritive quality of crop products has not been put into research accordingly as a result human in many parts of the world are suffering from malnutrition. The efficient improvement of nutritive quality of important crop species like wheat is dependent on the understanding of the acquisition of micronutrients from soil environment and subsequent translocation and distribution into different tissues(Akhtar et al. 2008; Hossain et al. 2018).

The density of minerals and vitamins in food staples eaten widely by the poor may be increased either through conventional plant breeding or through the use of transgenic techniques, a process known as biofortification. In broad terms, three things must happen for biofortification to be successful. First, the breeding must be successful i.e. High nutrient density must be combined with high yields and high profitability. Second, efficacy must be demonstrated i.e. The micronutrient status of human subjects must be shown to improve when they are consuming the biofortified varieties as normally eaten. Thus, sufficient nutrients must be retained in processing and cooking and these nutrients must be sufficiently bioavailable. Third, the biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition in significant numbers(Bouis et al. 2014).

3.3.1. NUTRIENT TRANSLOCATION PATHWAYS

Phytates, and to some extent the lower isomers of inositol phosphate insp5, insp4 and insp3, are strong chelators and bind positively charged proteins, amino acids and minerals in insoluble complexes in the digestive tract(Brinch-Pedersen et al. 2007).

3.3.2. ACHIEVEMENTS THROUGH BIOFORTIFICATION

4.~~:~~ BIOAVAILABILITY

Micronutrient bioavailability is the main factor determined the amount of micronutrients absorbed and micronutrient status of individual. Provitamin-A are consumed, absorbed and converted into the vitamin A and retinol. Many factors such as gender, age, health status, genetic factors, food matrix and type and amount of carotenoids in meal affect the efficacy and bioavailability by which provitamin A carotenoids absorbed in the intestine. There is an evidence that Provitamin A, Fe and Zn mutually enhanced their bioavailabilty (Hess et al., 2005)

Bioavailability of micronutrients in the food for the human body is influenced by many factors that can be either food or host related. Dietary intake is an essential factor, as micronutrient bioavailability depends on the chemical form and amount consumed, the nature of the dietary matrix, as well as interaction between nutrients and/or food components that enhance or inhibit absorption in the gastrointestinal tract. Enhancers like ascorbic acid (available in fruits and vegetables) can increase Fe bioavailability, while polyphenols and especially phytate or phytic acid (with high concentrations in staple grains like wheat) are major inhibitors that form complexes with Fe and Zn and limit uptake in the human body. Lutein, zeaxanthin, and b-carotene increase Fe absorption by humans on a maize- or wheat-based diet. Adding 1.8 mg of lutein to a maize-based breakfast doubled Fe absorption(Meng et al. 2007; de Valença et al. 2017; Welch and Graham 2004). Zinc is bioavailable in Biofortified wheat (Rosado et al., 2009). Foliar application of zinc increases the zinc contents of the cereals as compare to the soil applied zinc. Whole grain Zn concentration increases including endosperm (Cakmak et al., 2010).

In the cereal grain, iron and zinc are preferentially stored together with phytate in membrane-enclosed globoids in the protein storage vacuole (PSV) found in the aleurone and the embryo scutellum. The PSV is accordingly central for understanding mineral deposition during grain filling and mobilization of minerals during germination. Recent studies in Arabidopsis have led to the first identification of iron and zinc transporters of the PSV and further illustrate some of the dynamics associated with mineral and phytate transport and deposition into the vacuole(Brinch-Pedersen et al. 2007).

Bioavailability/bioaccessibility of b-carotene from different food matrixes varies greatly, from 2% to 70%, being generally higher in fat-rich, cooked matrixes. One crucial aspect that needs further experimentation is whether b-carotene-fortified crops can improve vitamin A status in the main targets of the biofortification efforts, that is, malnourished adults and children. In a recent study, b-carotene-fortified maize was able to improve serum b-carotene levels, but not retinol levels in margin- ally nourished Zambian children(Giuliano 2017; White and Broadley 2005).

B- carotene makes the Fe more bioavailable by increasing their solubility. Provitamin A activity play important in iron absorption in vitro and in human (Rawat, Neelam, Tiwari, & Dhaliwal, 2013)

Dietary fibers have a cation-exchange capacity and can reduce the bioavailability of minerals in the small or large intestinal tract producing an increase of final extraction of minerals. In wheat, as in fiber of other cereals, hemicellulose, cellulose, lignin, can influence binding of some minerals. In addition, inulin, a complex carbohydrate, protects Fe and Zn against the sequestering action of phytic acid. Furthermore, organic acids produced by the fermentation of inulin and other non-digestible oligosaccharides in the

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colon may improve micronutrient solubility and therefore bioavailability. Iron bioavailability is higher from heam Fe sources because of lack of inhibition from the chelating compounds and because its absorption pathways differ from those of non-heam Fe. The bioavailability of dietary iron is affected mainly by the chemical form, by dietary enhancers and inhibitors that affect luminal iron solubility, and to a lesser extent by other cations that may compete for mucosal transport, and by the amount ingested.(Anglani 1998; Harland et al. 1995; Hunt 2005; Meng et al. 2007)

4.1. Vitamin-A effect on Zn & Fe Bioavailability

Low intake of vitamin A from animal source foods or low intake and/or bioavailability of provitamin A carotenoids from plant foods may negatively impact on iron status through the negative influence of a low vitamin A status on iron metabolism. In such a situation, therefore, increasing the intake of vitamin A or bioavailable provitamin A carotenoids may increase iron utilization, thereby improving iron status. Data on the interactions between zinc and vitamin A deficiencies in humans are more limited and inconclusive than those in animals, and the effect of vitamin A deficiency on zinc metabolism is unknown. A major limitation is that plasma zinc concentration, the only routine zinc status indicator available, is not a useful indicator of zinc status because it is influenced by stress, infection, food intake, and hormonal status and only represents 0.1% of total body zinc.(Christian et al. 2001; Hess, Thurnham, and Hurrell n.d.; White and Broadley 2005)

4.2. MILLING EFFECTS

Bioavailability from crop to food is influenced by the crop (variety) which defines whether micronutrients are (re-)localized into edible parts of the crop and by food processing. In rice, Zn and Fe are localized in protein bodies in the outer layer of the grains, which is often removed during processing (dehusking, milling) leaving less Zn and Fe in the consumed rice(de Valença et al. 2017). In an effort to meet consumer demands, the rice industries have intensified the milling process to produce whiter rice, using degrees of milling between 8% and 14%. However, this technique reduces the nutritional value of rice. This work is the first study to evaluate effects of milling on the folic acid content and fatty acid composition of rice(Monks et al. 2013)

Milling process of cereal grain results in significant quantity of loss of grain zinc. Bran is removed during milling process that contain highest Zn contents. Zinc present in less quantity in remaining portion of grain (Dewettinck et al., 2008).– Endosperm of seed contain 80-85% of carbohydrates and minerals and low zinc concentration.

5: FUTURE PATHWAYS OF RESEARCH

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5.1. Genetics of Biofortification

5.1.1. Transgenic Biofortified Organisms

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(Wang et al. 2014) transferred bacterial phytoene synthase gene (crtb) and carotene desaturase gene (crti) into the common wheat cultivar Bobwhite. Expression of crtb or crti alone slightly increased the carotenoid content in the grains of transgenic wheat, while co-expression of both genes resulted in a darker red/yellow grain phenotype, accompanied by a total carotenoid content increase of approximately 8-fold achieving 4.76 μg g–1 of seed dry weight, a β-carotene increase of 65-fold to 3.21 μg g–1 of seed dry weight, and a provitamin A content (sum of α-carotene, β-carotene, and β-cryptoxanthin) increase of 76-fold to 3.82 μg g–1 of seed dry weight. The high provitamin A content in the transgenic wheat was stably inherited over four generations.

5.1.2. Qtls for Biofortification of Wheat

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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 overexpressing of the tafer1-A gene results in a 50-85% higher iron content in the grain(Borg et al. 2012).

With carotenogenes identified and functional markers developed, there is a growing interest in understanding the molecular basis of QTL underpinning carotenoid content in wheat.

5.2. PLOIDY LEVEL EFFECT ON BIO-FORTIFICATION

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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 the authors proposed that the Gpc-B1 locus promoted remobilization of protein, Zn, Fe and Mn from the leaves to the grains(Brinch-Pedersen et al. 2007).

5.3. Fe AND Zn Transporters & Related Proteins

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To increase mineral concentrations in edible tissues, without loss of yield, there must be increased uptake by roots (of minerals present in the soil solution) or leaves (for foliar applied minerals), effective redistribution within the plant to the edible portion, and accumulation in edible tissues in a nontoxic form(White and Broadley 2009).

The cereal grain consists of four major tissues: the embryo, the aleurone, the starchy endosperm and the outer layers (testa and pericarp). Elemental microanalyses of wheat grain sections reveal that phosphate, potassium, calcium, manganese, iron and zinc appear to be distributed in a similar way with the highest concentrations being in the aleurone and the embryo (in particular the scutellum) and a low concentration in the starchy endosperm. In contrast Sulphur, copper and chloride are quite evenly distributed between the different tissues(Brinch-Pedersen et al. 2007).

Knowledge of the molecular mechanisms of iron absorption is growing rapidly, with identification of mucosal iron transport and regulatory proteins. Both body iron status and dietary characteristics substantially influence iron absorption, with minimal interaction between these two factors(Hunt 2005).

Several studies have attempted to increase the iron content of the endosperm by expressing iron-binding proteins such as lactoferrin and, in particular, ferritin. Lactoferrin (LF) is an 80 kda iron-binding glycoprotein related to transferrin which is present in high concentrations (1–2 g/l) in human milk(Brinch-Pedersen et al. 2007).

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6. CONCLUSION

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Biofortification is the most reliable and economic approach of overcoming hidden hunger. Increasing the concentration of minerals in the edible parts of the cereals results in the better uptake from the soil and enhanced translocation to grain from the seed and improved endosperm sequestration. Genetic diversity can be utilized to enhance micronutrient composition through conventional and modern breeding approaches. Biofortification of edible produce through genetic strategies is potentially cost effective and will deliver most benefits to the 40% of the world's population who rely primarily on their own food for sustenance. Most recent technologies that have been used in this field for advancement are oligo-directed mutagenesis, reverse breeding,RNA directed DNA methylation and genome editing. But gmos still have little acceptance in the society so first awareness about their usefulness is necessary to make more advancement in this field. Climate change has imposed serious threats for genetic diversity ,so maintaining the genetic diversity of the plants is prerequisite for breeding highly biofortified crops. Maintenance of the post-harvest mineral and elements composition is another area of research that needs attention so that efforts done for breeding biofortified crops don’t go wasted. In this short review ,we have tried to discuss some strategies involved in developing biofortified crops, compare different methods of enhancing food nutrition, role of minerals uptake pathways and used of different transgenic techniques to make biofortified crops.

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