

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

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

39 chronically hungry meaning that they are undernourished in terms of calories (FAO,2017). When
40 intakes of bioavailable micronutrients are too low to meet the regularity requirement of the body then
41 micronutrient malnutrition develops and it affects the $\frac{1}{3}$ to $\frac{1}{2}$ of the world population (Allen et al.
42 2006). Wheat like many other staple food contain suboptimal quantities of essential micronutrients such
43 as zinc and iron AND more than two billion people worldwide suffer from zinc and iron deficiency
44 because of their PRIMARILY dependence on cereal based diets (Borrill et al. 2014). Due to lack of
45 access to the healthy diets such as fruits and vegetables, about one third of children die under the age of
46 five from malnutrition and one child in four is stunted due to inadequate diet (Hefferon 2015). Many
47 possible strategies like dietary diversification, mineral supplementation and post-harvest food
48 fortifications are used to improve micronutrient intake in the human diet. Biofortification evades
49 these problems by increasing the micronutrient contents in edible part of crops and enhancing
50 their bioavailability and absorption in human body during digestion.(Muhammad et al. 2016).
51 Pregnant women and young children are prone to acute micronutrient deficiency, which reduces
52 physical and mental development in children below 5 years of age, and malnutrition is
53 considered as the largest single contributor to disease in persons of any age (Alanis-guzman,
54 Amaya-guerra, and Saldi 2006). Education, dietary modification, food rationing, supplementation and
55 fortification these are various strategies employed to supplement micronutrient to children and women
56 (best C et al. 2011., Butta et al, 2008). In countries where people depend on cereal based food, high
57 incidence of micronutrient deficiencies has been observed (Muhammad et al. 2016). For the
58 purpose of biofortification it is necessary to increase the bioavailable pool of Zn in cereal grains
59 and not just its total concentration (Khoshgoftarmanesh et al. 2010) .

60 Biofortification is the process of increasing the content and bioavailability of essential vitamins
61 and minerals in staple crops, through plant breeding, transgenic techniques and agronomic
62 practices, to improve nutritional status and alarming rates of malnutrition (Bouis and Saltzman
63 2017). Agronomic biofortification is often considered as a short-term solution to increase
64 micronutrient availability and mainly to complement genetic biofortification (breeding), which is
65 seen as a more sustainable approach(de Valença et al. 2017; Welch and Graham 2004). The main
66 sources of vitamins and minerals (iron, zinc, and vitamin A) for low-income rural and urban
67 populations are staple foods of plant origin that often contain low levels or low bioavailability of
68 these micronutrients. Biofortification aims to develop micronutrient-enhanced crop varieties
69 through conventional plant breeding .Breeding high yielding, high-Fe and -Zn varieties require
70 source materials that shows adequate genetic variation in concentrations of those
71 micronutrients(Meng et al. 2007). Screening studies have shown that modern wheat cultivars are
72 not a good source of genes to enhance the concentration of Zn and Fe(Mondal, Maize, and
73 Rutkoski 2016). The breeding steps include at least (1) Identification of a useful genetic
74 variation and the most promising parents, (2) Long-term crossing and back-crossing activities,
75 (3) stability of the target traits (e.g., high grain Zn concentrations) across the different
76 environments that feature huge variation in soil and climatic conditions, and finally (4)
77 Adaptation of the newly developed biofortified genotypes over a range of crop and soil
78 management practices applied in the target regions or countries(Bouis 2002; Šramková,
79 Gregová, and Šturdík 2009).

80 Biofortification is faced with numerous challenges but also offers new solutions. The initial
81 challenge is to use conventional or molecular breeding to increase the micronutrient content,
82 preferably in the form of bioavailable minerals and biologically active vitamins or vitamin
83 precursors. Supplementary strategies comprise breeding for increased contents of components
84 that promote nutrient uptake and reduce amounts of inhibitors of uptake(Brinch-Pedersen et al.
85 2007). The fundamental drivers of “hidden hunger” quite often fall outside the nutrition sector

86 and are associated with each country's levels of potential resources (i.e. Energy, geography,
87 climate, etc.)(Hoddinott, Rosegrant, and Torero 2014).
88

89 2.HIDDEN HUNGER CAUSES AND PREVALENCE

90 About 800 million people are chronically hungry worldwide means these are undernourished in terms of
91 calories (FAO et al., 2017). Moreover, two million people are affected by hidden hunger as they suffer
92 from micronutrient deficiency (WHO 2006). Micronutrient deficiencies are more commonly pervasive in
93 the population that mainly depend on the non-diversified plant and cereal based diets which leads to
94 compromised health and economic problems (Gómez-Galera et al. 2010). Over three billion people are
95 currently micronutrient (i.e. Micronutrient elements and vitamins) malnourished, resulting in
96 ATROCIOUS societal costs including learning disabilities among children, increased morbidity and
97 mortality rates, lower worker productivity, and high healthcare costs, all factors diminishing human
98 potential, felicity, and national economic development while Vitamin A deficiency is causing around
99 4500 preventable child deaths daily. Nutritional deficiencies (e.g. Iron, zinc, vitamin A) account for
100 almost two-thirds of the child-hood death worldwide where most of those are dependent on staple crops
101 for their sustenance. Malnutrition also reduces the number of neurons and synapses. When the brain is
102 deprived of an optimal supply of nutrients, there are no discrete lesions. Instead, generalized distortion
103 occurs in those areas that were maturing at the time of nutrient deficit. In other areas of the brain, where
104 the cells have differentiated during prenatal life, malnutrition in infancy reduces the formation of
105 synapses and the branching of dendrites(Alanis-guzman, Amaya-guerra, and Saldı 2006; Dubock 2017;
106 Welch and Graham 2004). Hunger is the outcome of insufficient food intake resulting in decreased health
107 and quality of life while hidden hunger prevails when a person's is getting bellyful food but that food lack
108 nutrients or possess less nutrients that are required for normal functioning of the body(Hoddinott,
109 Rosegrant, and Torero 2014). Recently FAO roughly estimated that about 1/3 of the globally produced
110 food for human consumption is lost or wasted (Gustavsson et al. 2011). Hidden hunger is prevailing in
111 world's populations because of its increase at fast speed, poor economy and poor legislation of
112 governments. Children and pregnant women are more vulnerable to nutrient deficiency because their
113 body require more amount of these nutrients as compared to normal adults (Akhtar et al. 2011). Iron and
114 zinc deficiency are also known as "hidden hunger" results in reduced immunity, rigidity, fatigue,
115 irritability, loss of hairs, compromised psychomotor development, muscle weakening, sterility and in
116 acute case cause death (Wintergerst et al. 2007, Stein 2010). In low income populations, people rely on
117 the inexpensive less caloric monotonous food to meet energy need due to lack of access to the nutrient
118 rich diversify food (Bouis et al. 2011). Eradicating hunger in all its forms, including chronic and hidden
119 hunger, requires good understanding of the problem's magnitude, trends, and determinants. The burden of
120 chronic hunger more than halved since 1990, it remains larger than the burden of hidden hunger. Cost-
121 effective micronutrient interventions that can have an impact in the short to medium term include
122 biofortification, industrial fortification, and food supplementation(Gödecke, Stein, and Qaim 2018).
123 Micronutrient malnutrition ("hidden hunger") now afflicts over 40% of the world's population and is
124 increasing especially in many developing nations. Today, deficiencies of iron and iodine are of most
125 concern to the nutrition community and healthcare officials although other nutrient deficiencies, including
126 zinc, selenium, calcium and magnesium may be prevalent in some global regions(Šramková, Gregová,
127 and Šturdík 2009). In the search for genetic material with high iron and zinc concentration in wheat grain,
128 a significant positive correlation has been found between iron and zinc concentrations, suggesting that
129 these two traits may be combined relatively easily during breeding. The production of semi-dwarf wheat
130 through the introduction of the rht genes has resulted in substantial yield increases. However, this is

131 associated with a reduction in iron and zinc concentrations in some bread wheat genotypes, but not in
132 durum wheat(Meng et al. 2007; Monasterio and Graham 2000).

133 2.1.Wheat Grain Composition

134 The mature wheat Grain comprises of three major component such as carbohydrates, protein and cell wall
135 polysaccharides which together account for the 90% of the dry weight and minor component include
136 lipids, phenolics, vitamins and minerals. However distribution of these components vary within the grains
137 (Shewry et al. 2013). Wheat grains are generally oval shaped, although different wheats have grains that
138 range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and
139 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally
140 connected to the wheat flower. The wheat grain contains 2-3% germ, 13-17% bran and 80-85% mealy
141 endosperm. The bran (outer layers of wheat grain) is made up of several layers, which protect the main
142 part of the grain. Bran is rich in B vitamins and minerals; it is separated from the starchy endosperm
143 during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises
144 water-insoluble fiber. More than half the bran consists of fiber components (53%). Chemical composition
145 of wheat bran fiber is complex, but it contains, essentially, cellulose and pentosans, polymers based on
146 xylose and arabinose, which are tightly bound to proteins. These substances are typical polymers present
147 in the cell walls of wheat and layers of cells such as aleurone layer. Proteins and carbohydrates each
148 represent 16% of total dry matter of bran. The mineral content is rather high (7-2%). The two external
149 layers of the grain (pericarp and seed coat) are made up of dead empty cells. The cells of the inner bran
150 layer- aleurone layer are filled with living protoplasts. This explains the rather high levels of protein and
151 carbohydrate in the bran. There are large differences between the levels of certain amino acids in the
152 aleurone layer and those in flour. Glutamine and proline levels are only about one half, while arginine is
153 treble and alanine, asparagine, glycine, histidine and lysine are double those in wheat flour. The
154 endosperm is surrounded by the fused pericarp and seed coat. The outer endosperm, the aleurone layer,
155 has a special structure: it consists of single layer of cubic shaped cells. The aleurone layer is rich in
156 proteins and enzymes, which play a vital role in the germination process. The inner endosperm, i.e. The
157 endosperm without the aleurone layer, is referred to as mealy or starchy endosperm. The endosperm
158 mainly contains food reserves, which are needed for growth of the seedling, it is rich in energy-yielding
159 starch. Apart from carbohydrates, the mealy endosperm contains fats (1,5%) and proteins (13%):
160 albumins, globulins and the major proteins of the gluten complex- glutenins and gliadins.- proteins that
161 will form the gluten at dough making. The contents of minerals (ash) and of dietary fibers are low; 0,5%
162 and 1,5%, respectively. The germ lies at one end of the grain. It is rich in proteins (25%) and lipids (8-
163 13%).The mineral level is also rather high (4-5%). Wheat germ is available as a separate entity because it
164 is an important source of vitamin E. Wheat germ has only one half the glutamine and proline of flour, but
165 the levels of alanine, arginine, asparagine, glycine, lysine and threonine are double(Meng et al. 2007;
166 Šramková, Gregová, and Šturdík 2009)

167 2.2. VITAMIN-A DEFICIENCIES

168

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

175 Carotenoids are synthesized de novo by plants, where they play fundamental physiological roles as
176 photosynthetic pigments and precursors for signaling molecules. They are also essential components of a
177 healthy diet, as dietary antioxidants and vitamin A precursors. Wheat like Rice have less diversity of
178 Vitamin-A. Vitamin A has multiple roles in the body including reproduction, vision, cell differentiation,
179 immune function, bone formation and growth. Vitamin A comes from animal sources in the diet such as
180 retinol or retinyl esters and from provitamin A carotenoid found in plant sources. Vitamin A deficiency
181 has been associated with the severity of infections and in the developing world, it is the primary cause of
182 childhood mortality and morbidity particularly in south Asia and Africa. Vitamin-A Deficiency is the
183 leading cause of the preventable blindness in children. The WHO estimates that about 250-500 million
184 children are blind due to VAD and half of these vision loss will die within a year (Bailey, West, & Black,
185 2015) .

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

200 2.3. IRON AND ZINC DEFICIENCY

201 Iron deficiency ranks among the most widespread nutrient deficiencies, affecting over two billion people
202 worldwide. Iron deficiency anemia (IDA) has been linked to maternal and perinatal mortality, and to
203 impairment of cognitive skills and physical activity. Iron deficiency is also associated with enhanced
204 absorptional environmental metal toxins such as cadmium (Cd) (Silver et al. 2013). Zinc is an essential
205 trace mineral influencing gene expression as well as cell development and replication. Approximately
206 800,000 child deaths worldwide per year are attributable to Zn deficiency. Because it significantly
207 increases the risk of diarrhea, pneumonia, and malaria, Zn deficiency has been linked to the morbidity and
208 mortality of children younger than 5. The global average prevalence of Zn deficiency was estimated at
209 31%, with the most severe burden of diarrhea and pneumonia due to this deficiency found in Africa and
210 South Asia. Africa, however, almost exclusively bears the burden of malarial disease attributable to Zn
211 deficiency. Analyses of multiple grain samples from maize and wheat that have been collected by
212 CIMMYT across environments and genotypes suggest that the levels of Fe and Zn are higher in wheat
213 than in maize. However, we are not aware of any specific studies comparing wheat and maize mineral
214 concentrations in the same experiment and in different grain tissues (Elch et al. 2005; Kabir, Swaraz, and
215 Stangoulis 2014; Maqbool 2019; Meng et al. 2007)

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

220 70% of agricultural soils in Pakistan are Zinc deficient. Deficiency of zinc is more frequent in peat,
221 calcareous, saline sodic and highly weathered and intensively weathered soils (Alloway, 2008). In arid
222 and semi-arid areas due high calcium carbonate and less organic matter contents soils are deficient in zinc
223 (Imran et al. 2014).

224 There exist large biodiversity for these micronutrients in wheat. Durum wheat is one of the main sources
225 of calories and protein in many developing countries. In a study conducted by (Magallanes-López et al.
226 2017), 46 durum varieties grown under full and reduced irrigation, were analyzed for micronutrients and
227 phytate content to determine the potential bioavailability of the micronutrients. The variation was 25.7–
228 40.5 mg/kg for iron and of 24.8–48.8 mg/kg for zinc.

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

234

235 3. STRATEGIES OF FOOD ENRICHMENT WITH ESSENTIAL 236 NUTRIENTS

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

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

245 3.1. SUPPLEMENTATION

246 Supplementation, however, involves the intake of micronutrients in the form of capsules, tablets or syrup.
247 In fortification and supplementation, manufacturing and/or distribution infrastructure is required, which in
248 the long run may not benefit many, especially those in rural communities. Vitamin A capsules
249 intervention, which started in the 1990s, is an example of supplementation (Elemike et al., 2019.; Dubock
250 2017). The provision of micronutrients to the malnourished population in the form of supplements has
251 proven to be successful (Das et al. 2013; Bhutta et al. 2013) Supplementation is the oral delivery of
252 micronutrients in the form of tablets and syrup, used under chronic deficiencies. Iron absorbed best in the
253 form of ferrous fumarate, ferrous sulphates and ferrous gluconate. Similarly zinc supplied as zinc
254 sulphates, zinc acetate and zinc gluconate (Gómez-Galera et al. 2010).

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

260 3.1.1. MERITS AND DEMERITS

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

271 3.2. FORTIFICATION

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

290

291

292 3.2.1. Agronomic Fortification

293

294 Consumption of diverse food sources, although recommended as a sustainable solution, is unaffordable to
295 the poor populace, who are at risk of malnutrition. The use of industries for the fortification of food
296 nutrients has not been very successful, except for iodized salt(Elemike et al. 2019).There is evidence that
297 agronomic fortification can increase yields and the nutritional quality of staple crops, but there is a lack of
298 direct evidence that this leads to improved human health. Micronutrient fertilization is most effective in
299 combination with NPK, organic fertilizers and improved crop varieties, highlighting the importance of
300 integrated soil fertility management. Agronomic biofortification provides an immediate and effective
301 route to enhance micronutrient concentrations in edible crop products, although genetic biofortification
302 may be more cost effective in the long run(Monasterio and Graham 2000; de Valença et al. 2017). In the
303 soil mineral elements present as dissolved compounds, precipitates, part of lattice structure of clay micelle

304 or present within the soil biota (White & Broadley, 2009). In the soil the major limitation of the
305 biofortification is the low Phyto availability of the mineral micronutrients. Agronomic efforts have been
306 directed towards the mineral nutrient application and their solubilization and mobilization in the soil.
307 Mineral elements with efficient mobility in the soil and plant are considered as successful agronomic
308 biofortification approach.

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

321

322 3.2.2. MERITS AND DEMERITS

323 The nutrients needed by the plants are fortified in the fertilizers, with the belief that they could be
324 absorbed by the plants. The lack of the micronutrients is manifested by abnormal growth of the plant
325 parts; however, sometimes the soil may not be deficient of the micronutrient, rather the roots are unable to
326 absorb and translocate the nutrients due to small root pore size. It is, therefore, essential to explore the
327 strategies of improving crop quality and their essential nutrients to meet the food demands of the growing
328 population. Conventional fertilizers are readily available for plant uptake but also easily lost through
329 leaching, which is a major challenge. NPK and other agrochemicals have been found to have low use
330 efficiency by plants because of fixation, leaching, microbial degradation, photolysis and
331 volatilization(Elemike et al. 2019.; Dimkpa and Bindraban 2016). Limited success of iron fertilization in
332 biofortification, because the applied iron (Fe^{2+}) rapidly oxidized to Fe^{3+} state, which is not absorbed by
333 the plants. (Zhang et al. 2008).

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

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

346 3.3. BIOFORTIFICATION

347 Biofortification is the nutrient enrichment of key food crops through genetic enhancement. It differ from
348 fortification (exogenous addition of nutrients) through agricultural interventions such as biotechnology,
349 breeding and agronomy. Agricultural Scientists has lifted the crop production many folds' overs last 100
350 years but nutritive quality of crop products has not been put into research accordingly as a result human in
351 many parts of the world are suffering from malnutrition. The efficient improvement of nutritive quality of
352 important crop species like wheat is dependent on the understanding of the acquisition of micronutrients
353 from soil environment and subsequent translocation and distribution into different tissues(Akhtar et al.
354 2008; Hossain et al. 2018).

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

364 3.3.1. NUTRIENT TRANSLOCATION PATHWAYS

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

368 3.3.2. ACHIEVEMENTS THROUGH BIOFORTIFICATION

369 4: BIOAVAILABILITY

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

376 Bioavailability of micronutrients in the food for the human body is influenced by many factors that can be
377 either food or host related. Dietary intake is an essential factor, as micronutrient bioavailability depends
378 on the chemical form and amount consumed, the nature of the dietary matrix, as well as interaction
379 between nutrients and/or food components that enhance or inhibit absorption in the gastrointestinal tract.
380 Enhancers like ascorbic acid (available in fruits and vegetables) can increase Fe bioavailability, while
381 polyphenols and especially phytate or phytic acid (with high concentrations in staple grains like wheat)
382 are major inhibitors that form complexes with Fe and Zn and limit uptake in the human body. Lutein,
383 zeaxanthin, and b-carotene increase Fe absorption by humans on a maize- or wheat-based diet. Adding
384 1.8 mg of lutein to a maize-based breakfast doubled Fe absorption(Meng et al. 2007; de Valença et al.
385 2017; Welch and Graham 2004). Zinc is bioavailable in Biofortified wheat (Rosado et al., 2009).
386 Foliar application of zinc increases the zinc contents of the cereals as compare to the soil applied zinc.
387 Whole grain Zn concentration increases including endosperm (Cakmak et al., 2010).

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

394 Bioavailability/bioaccessibility of b-carotene from different food matrixes varies greatly, from 2% to
395 70%, being generally higher in fat-rich, cooked matrixes. One crucial aspect that needs further
396 experimentation is whether b-carotene-fortified crops can improve vitamin A status in the main targets of
397 the biofortification efforts, that is, malnourished adults and children. In a recent study, b-carotene-
398 fortified maize was able to improve serum b-carotene levels, but not retinol levels in margin- ally
399 nourished Zambian children(Giuliano 2017; White and Broadley 2005).

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

402 Dietary fibers have a cation-exchange capacity and can reduce the bioavailability of minerals in the small
403 or large intestinal tract producing an increase of final extraction of minerals. In wheat, as in fiber of other
404 cereals, hemicellulose, cellulose, lignin, can influence binding of some minerals. In addition, inulin, a
405 complex carbohydrate, protects Fe and Zn against the sequestering action of phytic acid. Furthermore,
406 organic acids produced by the fermentation of inulin and other non-digestible oligosaccharides in the
407 colon may improve micronutrient solubility and therefore bioavailability. Iron bioavailability is higher
408 from heam Fe sources because of lack of inhibition from the chelating compounds and because its
409 absorption pathways differ from those of non-heam Fe. The bioavailability of dietary iron is affected
410 mainly by the chemical form, by dietary enhancers and inhibitors that affect luminal iron solubility, and to
411 a lesser extent by other cations that may compete for mucosal transport, and by the amount
412 ingested.(Anglani 1998; Harland et al. 1995; Hunt 2005; Meng et al. 2007)

413 4.1. Vitamin-A effect on Zn & Fe Bioavailability

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

424 4.2. MILLING EFFECTS

425 Bioavailability from crop to food is influenced by the crop (variety) which defines whether micronutrients
426 are (re-)localized into edible parts of the crop and by food processing. In rice, Zn and Fe are localized in
427 protein bodies in the outer layer of the grains, which is often removed during processing (dehusking,
428 milling) leaving less Zn and Fe in the consumed rice(de Valença et al. 2017). In an effort to meet
429 consumer demands, the rice industries have intensified the milling process to produce whiter rice, using
430 degrees of milling between 8% and 14%. However, this technique reduces the nutritional value of rice.

431 This work is the first study to evaluate effects of milling on the folic acid content and fatty acid
432 composition of rice(Monks et al. 2013)

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

437

438 5: FUTURE PATHWAYS OF RESEARCH

439 5.1. Genetics of Biofortification

440 5.1.1. Transgenic Biofortified Organisms

441 (Wang et al. 2014) transferred bacterial phytoene synthase gene (*crtb*) and carotene desaturase gene (*crti*)
442 into the common wheat cultivar Bobwhite. Expression of *crtb* or *crti* alone slightly increased the
443 carotenoid content in the grains of transgenic wheat, while co-expression of both genes resulted in a
444 darker red/yellow grain phenotype, accompanied by a total carotenoid content increase of approximately
445 8-fold achieving 4.76 $\mu\text{g g}^{-1}$ of seed dry weight, a β -carotene increase of 65-fold to 3.21 $\mu\text{g g}^{-1}$ of seed
446 dry weight, and a provitamin A content (sum of α -carotene, β -carotene, and β -cryptoxanthin) increase of
447 76-fold to 3.82 $\mu\text{g g}^{-1}$ of seed dry weight. The high provitamin A content in the transgenic wheat was
448 stably inherited over four generations.

449 5.1.2. Qtls for Biofortification of Wheat

450 The characterization of the full complement of wheat ferritins show that the modern hexaploid wheat
451 genome contains two ferritin genes, *tafer1* and *tafer2*, each represented by three homeoalleles and placed
452 on chromosome 5 and 4, respectively. The two genes are differentially regulated and expressed. The
453 *tafer1* genes are, except in the endosperm, the most abundantly expressed and regulated by iron and
454 abscisic acid status. The promoter of *tafer1*, in contrast to *tafer2*, has iron- and ABA-responsive elements,
455 supporting the expression data. The *tafer1* and *tafer2* genes encode two isoforms, probably functional
456 different and acting in heteropolymer structures of ferritin in cereals. Iron biofortification of the wheat
457 grain is possible. Endosperm targeted intragenic overexpressing of the *tafer1-A* gene results in a 50-85%
458 higher iron content in the grain(Borg et al. 2012).

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

461 5.2. PLOIDY LEVEL EFFECT ON BIO-FORTIFICATION

462 Introduction of the high grain protein content (*Gpc-B1*) locus from the wild tetraploid wheat *Triticum*
463 *turgidum* ssp. *Dicoccoides* into different recombinant chromosome substitution lines resulted in 10–34%
464 higher concentrations of zinc, iron, manganese and protein in the grain compared to lines carrying the
465 allele from cultivated wheat and the authors proposed that the *Gpc-B1* locus promoted remobilization of
466 protein, Zn, Fe and Mn from the leaves to the grains(Brinch-Pedersen et al. 2007).

467

468 5.3. Fe AND Zn Transporters & Related Proteins

469 To increase mineral concentrations in edible tissues, without loss of yield, there must be increased uptake
470 by roots (of minerals present in the soil solution) or leaves (for foliar applied minerals), effective

471 redistribution within the plant to the edible portion, and accumulation in edible tissues in a nontoxic
472 form(White and Broadley 2009).

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

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

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

486 5.4.

487 5.4.1.

488 6.CONCLUSION

489
490 Biofortification is the most reliable and economic approach of overcoming hidden hunger. Increasing the
491 concentration of minerals in the edible parts of the cereals results in the better uptake from the soil and
492 enhanced translocation to grain from the seed and improved endosperm sequestration. Genetic diversity
493 can be utilized to enhance micronutrient composition through conventional and modern breeding
494 approaches. Biofortification of edible produce through genetic strategies is potentially cost effective and
495 will deliver most benefits to the 40% of the world's population who rely primarily on their own food for
496 sustenance. Most recent technologies that have been used in this field for advancement are oligo-directed
497 mutagenesis, reverse breeding,RNA directed DNA methylation and genome editing. But gmos still have
498 little acceptance in the society so first awareness about their usefulness is necessary to make more
499 advancement in this field. Climate change has imposed serious threats for genetic diversity ,so
500 maintaining the genetic diversity of the plants is prerequisite for breeding highly biofortified crops.
501 Maintenance of the post-harvest mineral and elements composition is another area of research that needs
502 attention so that efforts done for breeding biofortified crops don't go wasted. In this short review ,we have
503 tried to discuss some strategies involved in developing biofortified crops, compare different methods of
504 enhancing food nutrition, role of minerals uptake pathways and used of different transgenic techniques to
505 make biofortified crops.

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