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Biofortification of Wheat with Iron

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Abstract

In various micronutrient-deficient countries, wheat is used as staple food, comprise more than 50% of the diet. Like many staple foods, wheat contains low concentration of iron (Fe). About two billion people globally have iron deficiency, especially in the regions where staple foods are based on cereal crops such as wheat. Because of high rate of Fe deficiency cases, increased Fe intake through staple food has become the main focused research area globally. As wheat is main source of protein and dietary energy for human beings, its potential to support reducing malnutrition related to Fe can be increased through producing genetically modified wheat varieties with high concentration of Fe. High Fe concentration in cereal crop is a major challenge. Even though during wheat is commonly fortify, promising and more long term solution is biofortification of wheat, which bases on the production of new wheat varieties with characteristically higher concentration of iron. Till now, may researches, which focused on increasing Fe in wheat, aimed at producing natural varieties in progenitor or related species. This review focused on the promising and sustainable approaching to maintain the improve concentration of iron in wheat.

Keywords: Biofortification, iron.

1. Introduction

For all living organism, Iron (Fe) is an essential element as it catalyzes oxidation/reduction reactions. It is attached to protein directly or through a sulfur-iron cluster or a heme group as a prosthetic group [1]. In metalloproteins (e.g. heme-Fe proteins or Fe-S cluster), two redox states of Fe exist i.e. if it loses an electron it becomes ferrous Fe^{2+} (reduced form) and Fe^{3+} (oxidized form) is formed when it gains an electron. These metalloproteins perform essential reactions in the plant and animals body, such as biosynthesis of lipid, DNA and other metabolites, electron transport chain (ETC), photosynthesis and respiration and detoxification of reactive oxygen species. The cellular reactions that take place in plastids, cell wall, cytoplasm and mitochondria need Fe in an adequate amount. Hence, Fe involves in wide range of vital processes in all living organism and itsdeficiency can lead to a number of life threatening diseases [2].

Fe deficiency is one of the most widespread and most severe nutrient deficiencies intimidating human health and affecting approximately two billion people in the world [2]. Fe deficiency causes various physiological diseases, such as anaemia and neurodegenerative disorders [3]. A recent report based on the WHO Database described that nearly 1.6 billion people in the world are effected by anemia in which pregnant women and pre-school children are under a greater risk [4]. Major health concerns regarding Fe deficiency are impairments of immune system, cognitive function and work capacity as well as maternal mortality and increase in infants [5,6]. Countries where people have low meat intake and mostly consume staple crops are more likely to be affected by Fe deficiency diseases. Population including young children, pregnant and postpartum are observed to be more severely affected, as high Fe is needed for infant growth and during pregnancy [2]. By choosing well balanced diet with bioavailable and sufficient Fe and by giving good attention to the composition of food, human health problem related to Fe can be prevented [1]. It is an important and high priority

challenge in research area to improve Fe concentration as well as its bio-availability [7,8].

Several options are there to enrich the food with Fe. These approaches have both advantages and short comings. By consuming Fe salts and chelate in the form of pills, nutrition of Fe in the diet is possible [9]. On the other hand, formulations which are suitable to human health are not only expensive but also difficult to supply across the world especially in underdeveloped areas. Moreover, it needs additional system for purchasing, distribution and transportation which increases its costs.

Fortification of food like flour with Fe salt is also supposed to be an effective way however, it is dependent upon massive industrial processing and distribution [10, 11]. Food diversification with the aim to improve crops such as legume seeds and green leafy vegetables with high Fe contents would be desirable and effective. It is a simplification of the diet with low diversification which is the major cause of deficiency of micronutrients [12]. Therefore, biofortified staple crops seem to be an effective method to reach people needs even in third world countries [13]. Genetic biofortification (plant breeding) and agronomic biofortification (application of fertilizers) are the approaches which are supposed to be cost-effective to the problem [1,14, 15].

Data relative to Zn biofortification provides conclusive evidence in favor of the soil and foliar applications of Zn fertilizers. These fertilizers play an effective role in improvement of gain concentration of Zn [16-19]. On the other hand, Fe fertilizers are not exploited to examine their role for improving Fe concentration in cereal gains. All attempts to understand the soil and foliar application of Fe fertilizers are aimed at restoration of Fe levels, improvement of the yield and reversion of Fe deficiency chlorosis. [20-22].

Fe is known to rapidly convert into unavailable forms upon application to calcareous soils and poses poor mobility in phloem, soil or foliar Fe. It is for this reason that Fe is attributed to be less effective than Zn for enrichment of cereal grains [15, 23]. For instance, the increase in grain Fe concentration through foliar spray of $FeSO_4$ or Fe chelates has not been recorded to exceed 36% [18] whilst the foliar application increases grain Zn concentration to a recorded concentration of 2- or 3-fold depending on the plant availability of Zn in soils [15,19]. Some independent studies have also showed that plants exhibit a lack of response to Fe fertilization in terms of grain Fe concentration. In more recent studies, it has been exhibited that the N status of plant plays a significant role in enrichment of ceral grains with Fe. This has been proved through molecular evidence exhibiting that the vegetative tissue remobilization and trans location of Fe/N/Zn into seed are events maintained by similar genetic mechanisms [24,25]. This leads to the creation of a positive correlation between grain Fe and N levels [26,27]. Studies employing the greenhouse and field conditions prove that shoot and grain Fe concentrations can be increased through increment in soil N application [19,28,29]. Moreover, grain Fe may also be enhanced through foliar sprat of urea [29]. It is to be noted that in the conditions cited above, the plants were studied under varying N concentrations, but none of the studies employed soil or foliar applications of Fe fertilizes

To curb Fe deficiency chlorosis in crop plants, various chelated and inorganic forms of Fe fertilizers have been employed and tested. Some examples include $FeSO_4$, FeEDTA, FeDTPA, FeEDDHA, Fe-citrate and FeIDHA (iminodisuccinic acid). Noteworthy in these studies was the pressing fact that effectiveness of Fe compounds in alleviating Fe deficiency chlorosis remains highly variable. The efficiency is dependent on compound stability, penetration ability through leaf cuticle and mobility/translocation following diffusion into leaf tissue. The penetration of Fe within leaf tissue has been stimulated and enhanced through addition of urea within the spray solution of Fe compounds. Whether the coupled use of urea and Fe fertilizers improves the foliar Fe fertilization aimed to increase grain Fe levels is an area yet to be understood. [1].

Another approach of biofortifying wheat and other crop plants is through breeding. This can be done by either breeding industries or government agencies. Second important step is the distribution of these newly bred lines and have they accepted by local farmers. In any case, it seems that in the underworld population, the prevention of deficiency of Fe is strongly dependent on governmental administration, regulation regarding the food quality, quantity and administration.

It should be kept in mind that none of the treatments mentioned above is cheap yet by considering the expenses of preventing diseases like neural dysfunction, fatigue and anemia caused by Fe deficiency is greater, than the expected cost to prevent these diseases [30].

2. Biofortification of Wheat with Iron

Biofortification assigns the natural plant enrichment with nutrients and health supporting factors during their growth. The focus of biofortification is to breed and generate major staple crops that produce edible products which are enriched in micronutrients such as carotenoids, provitamin A and several other known compounds that enhance nutrients efficiency and are useful for human health [31]. The biofortification strategies have been modified for staple food crops like wheat, maize and rice that were, in the past decades, bred for carbohydrates,

characteristics processing and their yield. Better lines of plants that are gives high yield in the field may have poor micronutrient contents in it [14].

By conventional breeding, plants with high micronutrient contents can be produced. Wild varieties with high and beneficial micronutrients are selected and crossed with genetically superior plant lines. This method is labor intensive and can be done by using molecular markers that are closely related to the trait of interest. In most favorable case, the trait's molecular nature is known and can be followed with PCR and sequencing in several breeding steps [32,33]. On the other hand, in addition to conventional breeding, gene technologies can be used to develop biofortified crops with new characteristics. In genetic engineering, interested trait is constructed *in vitro* using molecular marker to mix genes and promoters that together express the trait. These constructs are then transformed in plants, which can be done using *Agrobacterium tumefaciens* or biolistic methods that are based on bombardment of DNA on plant cells. The integration of specific gene in plant genome is confirmed by its expression and hence, these transgenic plants are multiplied [34, 35].

Various challenges have to be overcome for Fe biofortification. These challenges can be acquired if scientists master an understanding in plant physiological mechanism of metal homeostasis [36].

At first, there is a need to increase plant's Fe uptake. Different methods for Fe mobilization in the soil must be used by the plants, depending on the soil properties. In this way, plants can get Fe from the soil in more soluble form. Secondly, Fe should accumulate in plant's edible parts such as fruits and seeds. These plants part should also act as Fe sink. Third, the nutrients in these edible portions should be in the form that human digestive system can digest and use it in a beneficial way [1].

Another technique is the conjugation of Fe and soluble organic ligands which can increase its bioavailability. Some antinutrients such as phytic acid can precipitate Fe if not provided with phytase enzyme [1].

Experiments to target physiological processes of Fe homeostasis are already in process to check the effect of biofortification. Furthermore, assays to check the Fe uptake from plant food items are available [37, 38].

3. Iron Biofortification strategies

3.1 Ferritin content Enrichment

Ferritins are complex of multi proteins formed by peptide chains of ferritin that are arranged in globular manner which contain up to 4000 Fe^{3+} ions inside it. They can be considered as Fe house of a cell. Presents studies suggest that Fe is stored in ferritin for short or long term and used for accumulation of protein which contains Fe. In this way, during development of plant, ferritin supplies Fe and in some species of plant seeds contain high level of ferritin-Fe [39,40]. Briat *et al*[40] suggested that ferritin also help to lessen oxidative stress. On the other hand, Cvitanich *et al*[41] reported that it is not necessary that in every case high ferritin amount is co localized with high level of Fe in seeds. Ferritin localization inside mitochondria or plastids and its coat protein makes it a separate compound from other Fe-binding components. Ferritin is present in all organisms as Fe storage [42-44].

In biofortification strategies, ferritin genes are used, for example leguminous ferritin genes present in bean and soybean, were inserted in other plants and over expression of these gene gave accumulation of ferritin protein in the plants. Ferritin over expression in cereal grains and seeds showed an increased Fe contents in edible parts [45,46] though ferritin over expression did not have same effect in vegetative tissue [47], in other cases Fe deficiency symptoms were observed [48]. Thus over expression of ferritin should be studied in detail and at the same time it may be needed to increase uptake of Fe to have full effectiveness [49].

3.2 Phytic acid content Reduction

A successful approach on which Fe biofortification can be relied on is the reduction of anti-nutrients metabolites (tannins, phenolic polymers and phytic acid) which help in forming Fe complex which are insoluble in the gut [32]. 80 % of the total seed phosphorus content is in the form of phytic acid and its dry mass makes rest of the seed weight [50]. In globoids of many staple crops seeds such as legumes like soybean, aleurone cells and cereal embryo, it accumulates in the form of phosphorous and mineral storage compounds [51]. The frequency of phytic acid in the diet based on plant is supposed to be the major reason of Fe deficiency and anemia in developing countries. However, argumentatively it is also seen negative to reduce phytic acid contents as in balanced diet it enhances immune system and prevent kidney stones [52]. By the disruption of phytic acid biosynthesis chain, phytic acid contents can be reduced which then results in “low phytic acid” (*lpa*) phenotype [53,54]. D glucose- 6-phosphate is a compound which synthesizes phytic acid. It is first transformed into 1d-*myo*-inositol-3-phosphate [Ins(3)P1] [55].

Many biochemical pathways are involved in this transformation depending upon plant species [51,53]. In addition to that, a transporter ABC is required for transport and final step compartmentalization which can be disrupted to disturb biosynthesis pathway of phytic acid [56]. Many mutant lines have been reported in different plant species such as wheat [57], maize [58;59], rice [60,61], soybean [62,63] and Arabidopsis [64,64]. Although, conventional breeding may result in strong reduction and phytic acid and

the *lpa* mutants may also have negative effects such as reduced seedling and slow germination which effect on growth of the plants.

By using gene technology, better mutant can be developed as the function of the genes responsible for phytic acids synthesis can be eliminated. During the life cycle of plant, phytic acid can be reduced by using specific promoter only in specific organs and phases which allow transgenes expression under very controlled conditions [66,67].

On the other hand, the late stages of the synthesis of phytic acid and its transport can be specifically targeted in mutants [64]. For example, two genes for inositol polyphosphate kinases, ATIPK1 and ATIPK2, in Arabidopsis have been disturbed which are needed in the last step of the synthesis of phytic acid. It was observed that mutant produced had 93% less phytic acid contents as compared to controlled groups, while it did not have any effect on seed germination and yield.

As an alternate to this approach depends on plants transformation with phytase enzyme which will break down the phytic acid as it is synthesized. Phytase enzymes are isolated from different micro-organisms. Heat-stability in addition to enzyme activity is important standard to consider food processing procedure [51,68].

Hence, many experiments have to follow to identify a solution to eliminate negative effects of phytic acid as an anti nutrient but also maintain its positive effects on growth of plants.

3.3 Increase of nicotianamine content

In plants, nicotianamine is an essential compound of metal homeostasis. It is derived by a compound S-adenosyl methionine when an enzyme, nicotianamine synthase, acts on it. Depending on the pH of the environment, nicotianamine can bind to variety of metals including ferric and ferrous. The function of nicotianamine regarding Fe, is to confirm Fe solubility in the cell so Fe can be used by different compartments of cell. In addition to that, nicotianamine is a part of many other essential sub processes of plant metal homeostasis such as detoxification of metals, intercellular and intracellular transport, uptake and mobilization, storage and sequestration. Different studies explained the positive effects of nicotianamine on uptake of Fe and its accumulation in seeds [69-72]. Thus we can consider nicotianamine as an important and potential compound for biofortification of Fe in grains and seeds of agriculture plants.

Lee *et al*[38] showed, over expression of the *OsNAS3*, which is responsible for the synthesis of nicotianamine synthase resulting in increased amount of Fe in seeds and leaves of plants and higher nicotianamine-Fe content in seeds. Furthermore, it is reported that these transgenic seeds provided enhanced source of Fe as compare to wild type seeds. Zheng *et al*[73] demonstrated that nicotianamine synthase expression gene OsNAS1 has seed specific response as higher amount of nicotianamine was found in rice grains. It has been reported that by using human cells, transgenic grain performed better Fe utilization.

Other studies also showed that increased expression of nicotianamine synthase genes may give increased amount of nicotianamine but not necessarily used by plants [74]. In an argument, excess amount of nicotianamine may limit the Fe availability in apoplast [74]. Furthermore, it is observed that over expression of nicotianamine synthase resulted in increased amount of Fe in leaves but not accordingly in seeds.

4. Other factors affecting bioavailability of Fe

The techniques mentioned above explained that more than one gene can be used to increase the bioavailable Fe level. Other multiple factors which are effective for Fe bioavailability can be used in combination for further advantages. Some approaches have been experimented for example Lucca *et al* [45] stated that a metallotionein and bean ferritin were produced containing higher amount of Fe, which might be bioavailaible, when rice expressed *aspergillus* phytase. In another study, maize plants were created which expressed soybean ferritin and aspergillus phytase in the endosperm of kernels at the same time [75]. 20-70% increased Fe content was found in such plant with almost no phyate contents. Very fascinatingly, such kernels are proved to be advantageous to human cells in bioavailability studies [75]. In another study, rice plants were generated which expressed three gene namely; Arabidopsis nicotianamine synthase gene *AtNAS1*, a bean ferritin gene and a phytase, simultaneously [76]. Combination of nicotiamine and ferritin production in excess showed a strong increase of Fe in the grain's endosperm which was attained in transgenic technique with one gene.

5. Non-Transgenic iron Biofortification method

Non-transgenic approach is an alternative to the transgenic approaches. The genetic traits that have ability to accumulate high Fe content can be identified and back crossed to local varieties. The benefit of this method is that there is no supposition trait physiology to be made beforehand. With the help of modern DNA sequencing technologies, the new genes and alleles of interest can be identified in case if transcription factors genes have any effect on their expressions [25]. In these cases, the power of

natural genetic variation is utilized which is based on the natural selection of the best available traits that evolved in the germplasm collection, frequently based on the interplay of multiple genes and specific alleles (quantitative traits). For example, scientist have collected different crops such as bean [77], rice [78] and wild wheat [79] to check the mineral contents variations in them. Moreover, recombinant hybrid line formed from crossing of two distantly related lines may help in mapping and identifying of quantitative and single trait loci like in Medicago [80] and wheat [81]. Different crops such as maize and rice line with novel traits have been screened on the basis of Fe uptake and its biobioavailability analysis [82].

6. Conclusion

Our aptitude to perform basic research and experiment on wheat will be extremely significant to progress studies and researches in model species. The science has been extremely progressive by recent developments in mutant catalog, genomic resources and transgenic strategies. With the identification of gene or genes responsible for the improved production of Fe in wheat, the next will be the transfer of the gene in other wheat varieties to improve the Fe content in wheat. However, there are many challenges to successfully carry out these experiments. Despite these challenges it is believed that scientist working on wheat now have the resources and tools needed to make major improvements of Fe concentration in wheat and produce new wheat varieties. These new varieties can be significant investment in improving the health of billions of people tools to avoid Fe malnutrition.

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