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Research Article

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BIOFORTIFICATION STRATEGIES FOR ENHANCING GRAIN ZINC AND IRON LEVELS IN WHEAT

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Abstract

Wheat is an important source of protein and energy. Modern wheat is deprived of essential micronutrients which lead to mal nutrition in the world. About 2 billion of world population is affected by mal nutrition. The main micro nutrients which are not present in modern hexaploid are iron and zinc. Both of two micronutrients are essential in human physiology, the deficiency of iron and zinc leads to different diseases like anemia, Anorexia, lethargy and diarrhea respectively. Mal nutrition mainly affects children, pregnant women and old ages peoples. So researchers are now mainly focused on biofortification of wheat for improving nutrition value of wheat. Different approaches were used for this purpose like foliar application of fertilizers, but this method is short term method for increasing iron and zinc in wheat. Breeding is another method but it requires a lot of funds, time and resources. Wild emmer wheat was rich in these micronutrients because the genes responsible for enhancing iron and zinc is GPC 1 (NAM B1) was functional but in *Triticum Avestium* it became non functional due to domestication or frame shift etc. so scientists used positional cloning to transfer these genes into modern wheat. In this review we identified that this transgenic method is most reliable and cost less method for improving nutrition content of wheat by conducting feed trail to mice with transgenic wheat containing NAM B1 gene. There is immensely enhancement of these micronutrient or iron and zinc.
Key words: Mal nutrition, GPC (grain protein content), *Triticum Avestium*,transgenic approach.

Introduction

Wheat is the main staple food along with other cereals in the world especially in developing countries. Major Population takes wheat in form of bread for their diet (http://www.cropsreview.com/staple-crops.html). Modern i.e., Hexaploid Wheat contains low concentration of zinc (Zn) and iron (Fe) when people take it as main diet they fall prey of malnutrition. About two billion people suffer from iron and zinc deficiencies (WHO 2015). Malnutrition mainly affects children, women and elder persons. it was calculated by world health organization that malnutrition throughout the world is 17.6% but prevalent in developing countries especially southern Asia and sub-saharan Africa also 29% of population have stunted growth because of malnutrition (WHO 2015). Iron deficiency causes mainly anemia is the most widespread micronutrient deficiency and it results in stunted physical growth as well as mental development, and learning capacity (Bouis 2003). Zinc deficiency is also widespread in world and ranks 5th leading causative agent for diseases in most of developing world (Maret and Sandstead 2006). About 75% of nutrients are lost during milling (Slavin and others 2000; Ozturk and others 2006). Mainly pregnant women and children below the age of five are affected due to malnutrition. In many regions of the world, micronutrient deficiency is a more prevalent problem than poor dietary quality and low energy intake (Stewart et al., 2010). Many national and international organizations like CGIAR consortium is working to alleviate deficiencies of these mineral nutrients by biofortifying staple food crops with essential minerals and vitamins (Welch and Graham, 2004; Bouis, 2007; Cakmak, 2008). One of most common approach to increase micronutrients in staple crops is by applying fertilizers rich in zinc (Zn), Magnesium (Mn) and iron(Fe),but this approach requires some technology and money. One the other hand conventional breeding requires less cost and technology to provide essential nutrients to both developed and developing countries (Graham et al., 2007). Genetic biofortification approach is used to find and exploit genetic variation for mineral content, as well as other approaches involving gene discovery and marker based breeding (Grusak, 2002). There are number of procedures for improving nutrition value in staple crops or main cereals especially wheat. This review is about various methods for improving nutrition value especially zinc and iron of wheat. This review is mainly concerned with genetic biofortification as it is suitable and most effective method for improving nutrition value of wheat.

Various approaches for improving nutrition value:

There are number of strategies for improving nutrition content of wheat like supplementation, biofortication and changing the food habitat of peoples (Zimmerman et. al 2007) but most reliable approaches are Agronomic biofortification and genetic engineering(plant breeding). All the approaches have their own limitations and benefits.

Agronomic biofortification

Wheat cultivation areas are prone to zinc and iron deficiencies which results into zinc and iron deficiencies in grain which is the main cause for malnutrition in humans (Alloway,2009) There are number of elements present on our earth approximately 113 but out that only fourteen are essential micronutrients which are necessary for life processes of both plants and animals, because animals are heterotrops. They dependent on plants. Plants required these minerals for their survival. Out of fourteen essential minerals, eight elements are required in sufficient quantities, others in small quantities; the former referred to as macronutrients and the latter as micronutrients or true elements minerals elements are present as free ions, adsorbed ions and dissolved form making a web like structure in the soil (White & Broadley, 2009). One of the common hindrances in biofortification is mainly meager phytoavailability of mineral micronutrients in the soil. Therefore, the scientists trying to apply fertilizers containing these minerals and also efforts for solubilization and mobilization of mineral elements in the soil. Application of fertilizers give good results in biofortifying wheat or other cereals but this method is not good of organic nutrients like vitamins. Which are synthesized by plant itself. As this approach is of biofortification depends on several factors like soil composition, mineral availability in soil and in plant (Hirschi, 2009; Zhu et al., 2007). Agronomic biofortification is also shot term biofortification by applying zinc and iron enriched with organics like Vermicompost and also recommended dose of nitrogen, by this compost treatment zinc and iron concentration increased from (24.3 to 48.5 %) and (5.9 to 18.4 %) respectively (Yadav et. al., 2011).

Application of fertilizers and foliar spray for increasing zinc and iron concentration

Application of zinc sulfate (ZNSO4) and iron Ferrous sulfate (FeSO4) as a fertilizer to the wheat can increase the concentration of zn and fe in the developing grain but this method is short term solution to the problem(cakmak,2008). As compared to fertilization foliar application of Zn and Fe is most reliable remedy to this problem of biofortification (Cakmak et al., 2010). The concentration of Zn and Fe in endosperm can be further increased by adjusting timing and solute concentration in foliar spray (zhang et al 2010). There are various forms of fertilizers of zinc and iron are used to increase the concentration but znso4 and and feso4 are two important forms (www.harvestzinc.org) also timing of foliar spray determines the concentration of both Zn and Fe in the developing grain. The application of both the above methods i.e., fertilizers and foliar spray can increase the quantity of both Zn and Fe by 70% (cakmak et al 2010). Application of Zn and Fe in the early developing stages of wheat grain enhance the Zn and Fe concentration (zhang et al 2010). High iron and zinc concentration also helps wheat in in its earlier physiology like in developing good growth of roots and helps in defense against soil borne of pathogens (cakmak 2012).

In general, agronomic biofortification of cereal crops can be used as an important agricultural tool to increase human nutrition of people in the developing world. The well known example of biofortification of cereal crops using Se fertilizers comes from Finland; this was a case study whole of country. use of NPK and selenate fertilizers or the crops and pastures starts in 1984. It was the collective program to increase or improve zinc and iron concentration. It is well studied that use of fertilizers in developing countries provides short-term method of biofortification for example FE fertilizers are not quickly dissolved in the soil so, Fe fertilizers are used in large scale or in chelated to organic form. Also use of foliar spray in Fe deficient soil plants to increase Fe concentration which eventually increase yield and indirectly improve human health but these fertilizers causes severe damage to environment. (Murgia et al. 2012, Sperotto et al. 2012).

Breeding approach for increase iron and zinc of wheat

From last 1 decade researchers are mainly concerned about the yield not on nutritional perspective. Many organizations like harvest plus project CGIAR (the consultative group on international Agricultural Research) have developed plants which are resistant to the disease and other essential features (Ortiz et al., 2007) and no one bothered about nutritional look of wheat. Now CIMMYT developed wheat plants with high yielding varieties with high concentration of iron and zinc. Various studies shown that iron and zinc in grain are quantitatively inherited traits in wheat (Trethowan et al., 2005). Now recent focus of CIMMYT is to transferring of genes which encodes for iron and zinc from wild emmer wheat to modern wheat (*Triticum aestivum)*. About 40 elite varieties were tested.

Genetic Engineering

The genetic engineering method of biofortication is most reliable method because till the date the molecular breeding researchers of wheat come under many difficulties due to huge size of wheat genome (16 Gb) which is five folds more than humans also it is hexaploid. Genetic biofortification uses plant breeding techniques to increase zinc and iron concentration and also lowers the level of anti-micronutrient with enhancing level of mineral absorption (Pfeiffer et al 2007**).** The genetic biofortification uses following methods or steps to improve nutrition of the world population.

Gene screening responsible for enhancing zinc and iron concentration in wheat

About 3000 germplasm accessions were screened by the international wheat genome sequencing consortium gene bank to see variation of iron and zinc (Monasterio and Grham 2000). The modern haploid wheat and it ancestors shows different concentration of iron and zinc (cakmak et al 2000). The IWGSC has also shown purified individual chromosome arms by using flow sorting technique (Safar et al 2004). Wild emmer wheat is that to be parental wheat variety with higher concentration of iron and zinc (cakmak et al., 2000). So scientists are trying to find the reason of decline in iron and zinc in modern wheat by observing landraces (Monasterio and Grham 2000). *Triticum dicoccoides,Aegilops taushchii, Tritium monococcum* and *Triticum boeticum* were the varieties of wheat with high concentration of iron and zinc (cakmak et al., 2000). The CIMMYT organization used breeding of wheat species like Triticum turgidum and Titicum dicoccum to develop modern hexploid wheat with higher concentration of iron and zinc (Otiz-Monasterio 2007). The use of modern well defined data sequence, as it reduces cost of sequencing ,offers a new ways and means for investigating genes which are responsible for iron and zinc concentration in the grain. Now a day's researchers used RNA-seq was applied to identify differentially expressed genes in lines with reduced expression of *NAM* genes (Cantu etal.,2011). Data sequencing helps in finding many classes of genes including transporters, hormone regulated genes and transcription factors were identified .the physiology of senescence and nutrient mobilization are related to proportionally to micronutrient content in endosperm of wheat. The reverse genetic shows that NAM regulated genes are present in wheat. The Insilco analysis or homology of various transcriptome revealed a high resolution structure of particular gene are precisely defined (Krasileva et al.,2013) along with RNA sequencing data (Duan et al., 2012).

Transgenic strategy

Transgenic approach is the best approach for increasing iron and zinc in the modern wheat varieties. This strategy requires less cost and resources as compared to other approaches like fertilizers and breeding approaches. Both the approaches requires more fund and also application of fertilizers are harmful to environmental point of view. The breeding program needs a lot of resources and time. Whereas the transgenic approach is most reliable and less cost program for increasing nutrition value specially iron and zinc of modern wheat. In this approach the first and foremost step is to find the gene responsible for increasing concentration of iron and zinc of wheat. To find the gene responsible or gene activity researcher used different markers linked to loci which determines the variation of micronutrients. There were different studies which showed that quantitative trait loci (QTL) linked to iron and zinc. It was identified that ZIP family has a role in increasing iron and zinc or micronutrients density (Schachtman and Barker 1999; Eide 2006). The genes encoding the Fe and Zn transporter proteins are expressed in response to iron and zinc deficiencies, respectively. But it was observed that role of these transporters in variation of genome is not clear. In Arabidopsis thaliana Expression of the genes encoding a Zn transporter protein from roots of a barley genotype resulted in an increase in grain Zn concentration (Ramesh et al. 2004). Wild emmer wheat is ancestor of cultivated is promising source of allele responsible for iron and zinc concentration (Xie and Nevo, 2008). The GPC (NAM B1) was mapped on chromosome 6B and its loci about 250 Kb in size is responsible for increasing iron and zinc from *Triticum dicoccoides.* NAM B1 is a transcription factor that codes for increases senescence and nutrition mobilization from vegetative part to develop grain (Uauy et al., 2006; Distelfeld et al., 2007). NAM B1 is a member if NAC gene family plays important role in developmental processes, auxin signaling, defense and abiotic stress responses, and leaf senescence (Olsen et al., 2005). On the bioinformatic analysis of phylogeny of NAC transcription genes reveals close relatives of ONAC proteins also similarities with No apical meristem which was later assigned as NAM B1(Uauy et al., 2006). Using positional cloning of Gpc-B1, it was clear that quantitative trait locus linked with increased grain protein, zinc, and iron content (Uauy et al., 2006). So when we cloned this NAM B1 g ng gateway cloning and using agrobacterium transformatio he plants we saw increased iron and zinc in transgenic wheat. We conducted a feed trials to the mice with the transgenic wheat (HD3086). There is clearly increase in iron and zinc in these mice (see in table 1).

FEED TRAILS: Inductively coupled plasma mass spectrometry (ICP-MS)

Table 1. C1, C2, C3 and C4 are control plants without NAM B1 gene and T1,T2,T3 and T4 are transgenic plants

S. NO	SAMPLE	IRON	ZINC
1	C1	367	7.7
2	C2	307	5.3
3	C3	275	4.5
4	C4	319	5.5
5	T1	524	11.0
6	T2	500	9.3
7	T3	487	16.5
8	T4	506	10.5

Conclusion

It is clear that genetic, fertilizers and breeding biofortification offer suitable solutions to the enhancing micronutrient of modern hexaploid wheat. Genetic and agronomic biofortification approaches are actually not separate solutions, they are complementary to each other. There is promising, substantial genetic diversity in wild and primitive wheat, which have wide and useful genetic variation in grain iron and zinc. This genetic variation is used for wheat breeding programs to improve both the concentration and bioavailability of iron and Zinc in modern wheat varieties. A number of international and national organization like CIMMYT are working for enhancing iron and zinc concentration. In this review we identify that genetic transformation of GPC 1 (NAM B1) gene from wild emmer wheat to *Triticum Aestivum* increased nutrition value of wheat especially iron and zinc by performing feed trail to mice.

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