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ENRICHMENT OF ZINC IN WHEAT THROUGH BIOFORTIFICATION: A REVIEW

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ABSTRACT
Zinc is an important micronutrient in term of biological metabolism, and is gaining a lot of attention globally because of increasing concerns regarding zinc deficiency in humans. The micronutrient is essential for the normal growth of human. Furthermore, it requires for multiple functioning of human immune system and affect normal processes and development of cell mediated immunity, neutrophil and natural killer cells. Deficiency of zinc is listed among the top five micronutrient deficiencies and greatly affect more than 50% of world population, especially developing countries. Insufficient intake of low zinc based food is main reason for the zinc deficiency in human. Wheat is among the most common cereal crop g, and part of daily diet calories, protein and other micronutrients. In several developing countries, wheat is source of 50% routinely calories intake. An excessive use of low micronutrient based wheat is main reason for zinc deficiency in human as wheat has naturally low zinc quantity and high concentration of phytate which further reduces the availability of zinc. Therefore, it is an immediate challenge to enhance zinc content in cereal crops, especially in wheat.

KEY WORDS: Zinc, zinc deficiency, bioavailability.

INTRODUCTION
Zinc is an important micronutrient that is required by human body. It is important for several proteins in order to maintain their structural stability. It is also important for transcription factors and it acts as a cofactor for more than 300 enzymes (Palmgren *et al*., 2008). Zinc is essential for a healthy and effective immune system and gene regulation (Prasad, 2010). Plants like other organisms need zinc for translation, transcription and regulation of enzymatic activity (White and Broadley, 2011).

Role of zinc in dwarfism and hypogonadism due to zinc malnutrition in Middle Eastern boys was first studied by Prasad *et al*., (1961). Recently, zinc has been recognized as one of the major micronutrient, deficient in human. It is estimated that zinc deficiency is responsible for about 450,000 deaths of children under the age of 5 years annually (Black *et al*., 2008). The biggest reason of zinc malnutrition is the sole dependence on cereals and low intake of fruits, vegetables and meat for nutrition. The processed cereals are a poor source of micronutrients such as zinc, iron, selenium and vitamins (Zhu *et al*., 2007). These are essential to the body, that is they must be taken regularly in the diet as they are not produced by the body itself. Some of the most prominent diseases associated with malnutrition of zinc include scurvy, rickets, anemia and beriberi (Zhu *et al*., 2007). Deficiency of zinc is reported to be a cause of retarded sexual development, stunted growth, impaired brain function, DNA damage, dwarfism and even cancer (Welch and Graham, 1999; Hotz and Brown, 2001; Gibson, 2006; Prasad, 2007). Zn deficient children and adolescents develop an impaired sense of taste along with a poor appetite and mental lethargy (Hambridge, 2000). Deficiency of zinc is a frequent cause of diarrhea and pneumonia in children (Gibson *et al*., 2008; Prasad, 2010). Zinc deficiency is particularly important regarding pregnant mothers as it causes severe consequences in the development of the baby (King, 2000).

Zinc is to be taken cautiously and in a specific amount as zinc if not taken in adequate amount, leads to consequences and if taken in excess becomes toxic. According to the recommended dietary allowances, National Academy Sciences, USA, about 3-5mg/day intake of zinc is required by infants and about 10mg/day is needed by children of age 1 to 10 years. For men, the dietary allowance for zinc is 15mg/day and 12mg/day for females. Slightly more amount about 16 to 19mg/day is required for breast feeding females. Zinc when present in excess binds to sulfur, nitrogen and oxygen containing biological molecules in the body and causes severe damage and inactivation. It may also lead to an uncontrolled displacement of cofactors such as manganese and make the enzyme inactive leading to damage (Palmgren *et al*., 2008).

Various efforts have been made to overcome the problem of zinc malnutrition around the world including fortification of food with zinc and creating awareness among people to take supplements. However, such steps need heavy funding from authorities that makes it impractical in the developing countries where the problem is more severe. Biofortification by producing transgenic plants is the approach that is mostly applied to biofortification of crop such as wheat with iron and zinc. It is seen in the results of a number of surveys and reports that the average concentration of zinc in a whole grain of wheat is about 20-35mg/kg which does not meet the daily requirement of children or adult consuming it (Rengel *et al*., 1999; Cakmak *et al*., 2004). These values fall lower in the areas most prominently, India, Pakistan, China, Iran and Turkey where the soil on which wheat is sown is deficient in zinc. Zinc enrichment of soil is not only important for increasing amount of zinc in the yield but it is also important for wheat sustainable high yields (Cakmak, 2008).

How plants take up Zinc?

Plants take up zinc via their roots from the soil and pass it on to xylem tissue. It is taken up in the apoplast as Zn ion binds to negatively charged cell wall and phytosiderophores if present. It is usually transported symplastically or apoplastically (White *et al.,* 2002; Broadley *et al.,* 2007). Usually the cell membrane is permeable to zinc and allows easy channel of transport however, the ions influx is majorly mediated through ZRT-IRT-like proteins (ZIPs) and is catalyzed by yellow stripe like proteins (YSLs) specifically in cereal crops (White and Broadley, 2011). The zinc ions can also be precipitated in the form of phytate or phosphate salt if they are taken up in excess (Van Steveninck *et al.*, 1994; Broadley *et al.*, 2007; Straczek *et al.*, 2008; Terzano *et al.*, 2008; Kopittke *et al*., 2011).

The concentration of zinc decreases as it is transported to different parts of the plant and is the richest in the roots as compared to fruits, seeds and tubers (White and Broadley, 2011). The concentration of zinc within the plant varies from plant to plant and often the specific cells within a tissue are sinks for zinc. In cereals such as wheat the zinc has a sink in the seeds embryo, husk or aleurone layer (Lin *et al*., 2005; Ozturk *et al.,* 2006; Liu *et al.*, 2007; Hansen *et al.,* 2009; Persson *et al.*, 2009; Cakmak *et al.*, 2010a,b; Lombi *et al.*, 2011; Stomph *et al*., 2011). In the cytosol, there is negligible amount of zinc in ionic form, it usually forms a complex with organic compounds such as proteins or glutathione. Whereas, the Zn^{2+} form is quiet rich in the leaves and roots (Broadley *et al*., 2007; Roosens *et al.,* 2008; Clemens, 2010; Straczek *et al.*, 2008; Sarret *et al.*, 2009). A mixed concentration of ionic and compound forms of zinc is found in the xylem. Whereas, again little amount of ionic zinc is found in phloem (White and Broadley, 2011).

Plants can take up zinc in both organic and inorganic forms. Usually zinc forms a complex with organic compounds such as proteins, citrate, malate, amino acids or carboxylic acid to be taken up in organic form. It is also found bound to phytate or phosphate salts (White and Broadley, 2011).

Zinc deficient in Wheat

Zinc deficiency occurs in the wheat crop because of the soil on which it is sown, is itself deficient in zinc content. Approximately, 50% of the wheat producing countries lack zinc in their soil (Cakmak, 2002) with the concentration high in arid and semiarid regions (Cakmak *et al.*, 1999; Mirzapour and Khoshgoftar, 2006). The transport of zinc takes place via diffusion (Wilkinson *et al.*, 1968) which is highly dependent upon the pH conditions of the soil. Another important factor is the soil moisture. A soil with poor water content, particularly in secondary stages of plant growth encourages diffusion of zinc (Rattan and Deb 1981; Marschner, 1993; Cakman, 2002). The content of organic matter in the soil also determines the availability of zinc for plant consumption. Soils lacking organic matter are also low in zinc (Marschner, 1993). Soils having high calcium carbonate concentration, high phosphate concentration are also poor in zinc concentrations. Such soils of adverse chemical properties are unsuitable for crop growth.

The wheat grain's own physiology also plays an important role in causing less uptake of zinc. Phytic acid, polyphenols and cellulose causes hindrance in the availability of zinc to human consumption in the digestive tract. Phytic acid present in the seeds is the site of phosphorous storage. It is a strong chelating compound for zinc and forms insoluble compounds that are poorly absorbed by humans. Contradiction occurs on the thought to eliminate phytic acid from the plant to increase zinc absorption through the gut, when it plays key role in fighting against cancers (Somasundar *et al.*, 2005; Vucenik and Shamsuddin 2003). It is also important for the seed germination and seedling vigor (Oltmans *et al*., 2005; Guttieri *et al.*, 2006).

Another reason for low zinc consumption is milling. It is observed in various studies that protein and phytate present in the embryo and aleurone layer are sinks for zinc whereas; the endosperm is generally low in zinc content. These zinc rich parts are removed during milling and therefore, loss of zinc rich content to waste (Cakmak, 2008).

Yet another cause of low intake of zinc via diet is that zinc although present in appreciable amounts in the crop plant is unable to make it available for consumption. This is because the zinc is usually localized in the inedible parts of the plant. Thus, for efficient consumption, zinc needs to be readily available in the edible parts. The percentage of population with inadequate intake of zinc is summarized in Figure 1.

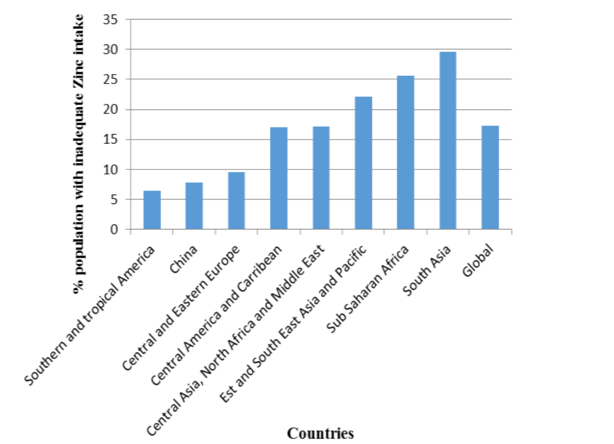

Figure 1: Percentage of population with inadequate Zinc Intake around the globe.

Why Biofortification for Wheat deficient in Zinc?

Fortification of food has been taken as an initiative to overcome the occurrence of diseases and problems associated with malnutrition in public. Also, efforts such as providing supplements to people along with regular diet, or using fertilizers rich in micronutrient such as zinc in the soils those which are deficient in these elements (Cakmak *et al.*, 2010a,b).

Fortifying foods although is an achievement in the process of cutting down deficiency of zinc, it is not very practical. It is not a centralized approach especially in the developing countries where the problem lays the most. Also, in rural areas farmers usually consume their own sown crops and locally milled flour. These fortified foods are not widely available to people residing in the rural and far off places. Nevertheless, the approach is a good substitute for biofortification as biofortification consumes longer time. Efforts to provide supplements to people along with their regular diet requires funding and needs to be widespread in order to reach all people facing the deficiency. This approach thus, cannot be very successful in the developing countries (Cakmak *et al.*, 2010a,b).

Agronomic biofortification that is achieved by using zinc fertilizers is a fast and efficient approach. By this the availability of zinc for consumption is widespread. Genetic biofortification achieved by plant breeding or production of transgenic plants is also a successful approach but is time consuming. Plant breeding is considered as the most sustainable method for enriching wheat (Cakmak *et al.*, 2010a,b). Both the approaches focus on increasing the presence of zinc in the edible portion of plants.

Agronomic Biofortification of Wheat with Zinc

The soil conditions in fields of wheat crops are important factors leading to zinc deficiency in the yield. These include the low or high pH of the soil, prolonged water logging in the fields, high calcium carbonate concentration, high phosphate concentration, salinity and soil type (Alloway, 2008). Various fertilizers are available to overcome this problem in the soil. For a fertilizer to be efficient the water-soluble zinc content should be less than 40% (Slaton *et al.*, 2005). Zinc is usually in the inorganic zinc or chelated zinc form. The most widely used inorganic form of zinc in the fertilizers is zinc sulphate or in the chelated forms ZnO, ZnEDTA and Zn-oxysulfate. The chelated form is however, not very economical to all farmers and is less effective in increasing grain zinc value than inorganic form (Hussain *et al.*, 2010).

It should always be kept in consideration that zinc if taken in excess is toxic. Thus, the amount of macro and micro nutrient should be such as not to imply negative effects to both the crop and its consumer. Addition of zinc to soil is an effective method of increasing the quantity of mineral in the soil. According to Fageria *et al.*, (2009) it is sometimes important to spray the enriched fertilizers directly on the leaves of the crop. Yilmaz *et al.*, 1997 suggested, the use of foliar spray and soil enrichment methods simultaneously to enhance grain yield and zinc content. Yet it is also time and money friendly if the zinc is added to the herbicide sprays as implied by Fageria *et al*., (2009). However, the effects of such an implementation are not clear. Other application methods include the direct soil amendment and pre-sowing seed soaking (Xiwen *et al.*, 2011). The most effective method of enriching zinc content is soil enrichment with fertilizer, foliar application and also pre-sowing seed soaking as this also increases the yield of the wheat (Cakman, 2008). The enriched seeds help plants to acquire better immunity and resistance against stress and disease and their initial growth is more rapid (Nestel *et al.*, 2006).

Fertilizers are enriched with zinc by many ways. It is added to a fertilizer by integrating it into the granules during its production or by coating zinc onto granular compound fertilizer. It can also be achieved by blending the two in bulk (Cakmak, 2002).

Genetic Biofortification of Wheat with Zinc

Genetic biofortification can be achieved by either genetic engineering or conventional breeding. Breeding of wheat varieties to attain significant goals is cost effective and successful however, it is very time consuming and the outcome is always uncertain. It is a tedious job to find high yielding and suitable parental varieties that can be bred. Also, once the results are achieved it is a long process to make the genotype stable (White and Broadley, 2005).

Keeping these problems in mind, several modifications in the genetic makeup of wheat can be carried out in order to make plant more efficient in taking up the mineral from soil and making it available in the edible portions for human consumption. Such a technique leads to production of transgenic varieties of wheat. When applying genetic engineering to wheat for biofortification two outcomes are to be achieved i.e. to increase the efficiency of plant's uptake and transport system particularly for the mineral and to increase the amount of bioavailable mineral accumulating in the plant (White and Broadley, 2005).

To apply genetic modification technique genetically variable species are needed that are better at uptake of zinc. It has been studied that wild emmer wheat *Triticum turgidum ssp. dicoccoides,* showed highest concentration of zinc amongst other varieties, typically 14 to 190 mg Zn/kg (Cakmak *et al.,* 2004). Recently it has been reported by Peleg *et al.,* (2008) that new wild emmer varieties have been examined and were found to be rich in zinc and iron in their seeds. Moreover, they are highly tolerant to drought stress and zinc deficiency of soil. Calderini and Ortiz-Monasterio (2003) scrutinized the synthetic wheat derived from *Aegilops tauschii*and observed high concentration of zinc.

It is however, not clear that what is the underlying cause of variation in zinc contents of different varieties amongst wheat. *Triticum dicoccoides* studies have shown that chromosome 6B carries genes that are responsible for high zinc content in the wheat grain and Gpc-B1 locus chromosome 6B effects the concentrations of zinc and protein in the plant (Cakmak, 2008). It has been seen that wild varieties of wheat have a greater nutritional content than the new varieties. It has also been seen that they carry an allele that codes for NAC transcription factor (NAM-B1) whereas, in modern varieties such an allele is nonfunctional. A considerable amount of loss of protein, zinc and iron content was seen when various homologues of NAM were silenced by RNA in transgenic plants produced in experiments. This silencing also brought about a delayed maturation of the grain (Sramkova *et al.,* 2009).

One such approach of producing transgenic plant is over expression of ZIPs particularly *bZIP19* and *bZIP23* transcription factors. This can make the plant accumulate more zinc in its edible portions (Assunção *et al.,* 2010).

Mutations have also been induced in wheat that has led it to make variable amounts of phytic acid (White and Broadley, 2009). Phytic acid naturally present in the seeds chelates zinc and forms insoluble complexes. These insoluble compounds are indigestible in the human digestive system thus, waste of zinc rich portion of the plant.

Advantages of Biofortification of Wheat with Zinc

Biofortified seeds by foliar or other application are active in taking less phosphorous. This makes zinc more accessible to human consumption and digestion. The high zinc content in the seed is not only beneficial to human necessities aspect rather it is very helpful for the plant itself. The high zinc content makes the plant more resistant to environmental stresses such as drought faced at early stages of growth (White and Broadley, 2009). Application of agronomic biofortification in the soil leads to increased resistance of the plant against soil borne pathogens. The seeds with high zinc content have a better seed viability and seedling vigor. Zinc is said to protect and maintain cell membranes structure and stability which are otherwise damaged and porous and leaks organic compounds leading to pathogen attraction to the plant (Cakmak, 2008).

With increased zinc content, many beneficial effects both to plant and animals can be achieved. However, there are many underlying questions to biofortification that needs to be brought to light. With further study and vast implications targets can be successfully achieved.

CONCLUSION

Zinc deficiency in human is a serious concern and has severe consequences. There is a great need to tackle this problem extensively to overcome the hunger problem globally especially in developing countries. Wheat as among the most commonly used crops, need to be given much attention. Novel practical approaches should be introduced to modify wheat to increase the bioavailability of zinc content. It should be given an urgent attention and grants should be given to promote research in the field. The problem of acceptance of genetically modified crops is still in arguments however, actions should be taken to indorse the awareness in common people about benefits these genetically modified crops offer.

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