International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-3/320-327
ISSN 2227-670X ©2015 IJACS Journal

IJACS

Zinc Biofortification, Preference or Essential?

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ABSTRACT: Zinc (Zn) is a micronutrient, essential for plants, animals, and humans. Requirement of Zn is very less; but if sufficient amounts of this element be not available, plants will suffer from physiological stresses due to deficiency of different enzymatic functions and other metabolic problems, and finally plants will have fewer grain yields and will produce low quality grain. Because of nutrition with such grains, the signs of Zn deficiency will be unavoidable in humans which increasing of cell abnormalities, decreasing of immune, decreasing of mental abilities, and children's being short are some of them. Soil's natural Zn deficiency in Iran on one hand, and common diet of people (cereals), of this country on the other hand, make it necessary to have wide-spreading strategic researches and macro programming for increasing Zn amount in Iranians' rations. Several solutions have been proposed for overcoming the above situation which reducing phytic acid of wheat flour, Zn-enriched flour, and finally agronomic biofortification and breading biofortification of wheat are some of them.
Keywords: Zinc (Zn), Wheat, Zinc deficiency, and Biofortification.

INTRODUCTION

Zinc (Zn) is a metallic element which for the first time in 1869 was identified as the growth factor of Aspergilus negra by a French scientist named J. Raulin (Raulin, 1869). In 1926, it was recognized as an essential element in plants by A. L Sommer & C. B. Lipman (Nielsen, 2012) and then its necessity for human body was proved in 1991 (Chakmak, 2008).

The human body contains 2 to 2.5 mg Zn which 70 percent of it is used in bone construction. Red blood cells contain 75 to 85 percent of this element (Zn), white blood cells and platelets contain 3 percent, and the rest is in the blood serum (Villagomez A and Ramtekkar, 2014). Several physiological functions have been identified for zinc in the human body, so it is considered as an essential element for maintaining health. Zn is an antioxidant which plays an important role in oxidative stress reactions and can act as a protective factor against free radicals and oxidant (Gupta et al., 2011). Presence of this element is valuable for immune system, detoxifying of toxic metals entered to the body, DNA construction, and maintaining skin health (Slaton et al., 2001). Zn involves in the construction of more than 200 enzymes and its presence is necessary for accelerating vital reactions of the body. Due to its role in the structuring of Carboxy peptidase enzyme, as a protein breakdown enzyme, the function of Zn is determined in the digestion of proteins (Gupta et al., 2011). Other important enzymes which their construction are related to the presence of Zn are: superoxide dismutase enzyme (which is one of the most important antioxidant enzymes), carbohydras enzyme (which involves in the body's electrolyte, and acid and base balance), and alcohol dehydrogenase enzyme (which causes the decomposition of alcohol in the body) (Harris et al., 2007). Also, Zn participate in constructing collagen that causes the combination of cells together and as the part of collagenase enzyme, it is responsible for the analysis of this composition (Karim et al., 2014).

Nowadays, among the 20 causes of people's death in the world, Zn deficiency is ranked as 11th and among the 10 causes of death in the developing countries, this element is ranked as fifth (World Health Organization, 2002). Estimation conducted in recent years indicate that about half of the global population suffer from Zn deficiency (Chakmak, 2008). According to the studies of Hutz and Braun (2004), on an average, Zn deficiency, with amplitude of 4 to 73 percent, affects more than one third of people of the world in various countries. Zn deficiency can cause different kinds of cancers, decreasing of recovery of immune system (Fageria et al. 2006), disorder in tissue and body growth, especially elongation of the body, reducing of pregnancy and reproduce ability, brain disorders, depletion (lack) of learning abilities, skin diseases, hair loss, slowing the healing of wounds, deformed nails, neurological disorders, loss of sense of taste, etc (Guthrie, 1983).

Figure 1. Symptoms of zinc deficiency in humans (Soumitra et al., 2013).

Like in human body, Zn is an important element in plants. Zn is a component of some non-enzymatic proteins, and also is the cofactor of some enzymes. In crops, several enzymes are involved in the metabolism of carbohydrates and protein synthesis, which their activities are depend on Zn. To the mentioned cases, most of dehydrogenases should be added (Fagria et al., 2003). Zn directly or indirectly participates in starch formation in a way that in plants encountered with Zn deficiency, starch concentration often decreases. This element is involved in nitrogen metabolism, and in Indian rice cultivars, it acts as a catalyst in improving the repeated regeneration (Fagria et al., 2003). Zn has an important role on phytochrome activity, membrane integrity, and tryptophan synthesis (pre-substance of Indole Acetic Acid hormone) (Schachtaman and Berker, 1999). In addition, Zn is considered as a crucial factor for oxidation process in plant cells. Zn element is effective on the conversion of carbohydrates, plays a role in regulating the amount of carbohydrates in plants, and finally deficiency of this substance will stop photosynthesis and metabolism of nitrogen (Gupta, 1995).

Due to its ability of remobilization from lower leaves to upper ones, the signs of this element’s deficiency are seen in the older leaves. Normally the signs of mentioned deficiency can be determined by the formation of small and thick leaves which are emerged as rosette. In addition, Zn deficiency obviously intensifies chlorosis phenomenon and this condition is similar to the signs of iron deficiency (Fageria et al., 2006). Zn deficiency will reduce photosynthesis and disorders protein synthesis that the consequence of such process is the gathering of Amino acids and Amides, and disordering of carbohydrates metabolism. Besides, in such a condition, the length of plants and the size of leaves reduce due to the changes in Auxin metabolism, especially Indole Acetic Acid (IAA) (Marschner, 2011).

Figure 2. The effect of Zn deficiency on the plants growth (International Zinc Association, 2014)

Factors which affect the effectiveness of Zn usage are studied by many researchers. Among the factors which influence the absorption of Zn, factors such as total amount of Zn in soil, pH, organic matters, absorption sites, microbial activity, phytosiderophores, soil moisture regimes mainly play important roles. Other factors such as climatic conditions, interaction of Zn and other macro and micro elements are also important (Volvo et al., 2013). Pinta and Aubert (1997) stated that the original materials of soil affect Zn amount. Zn absorbable for plants are mainly accumulated on the surface; and the loss of topsoil (surface) through erosion or excavation can cause Zn deficiency.

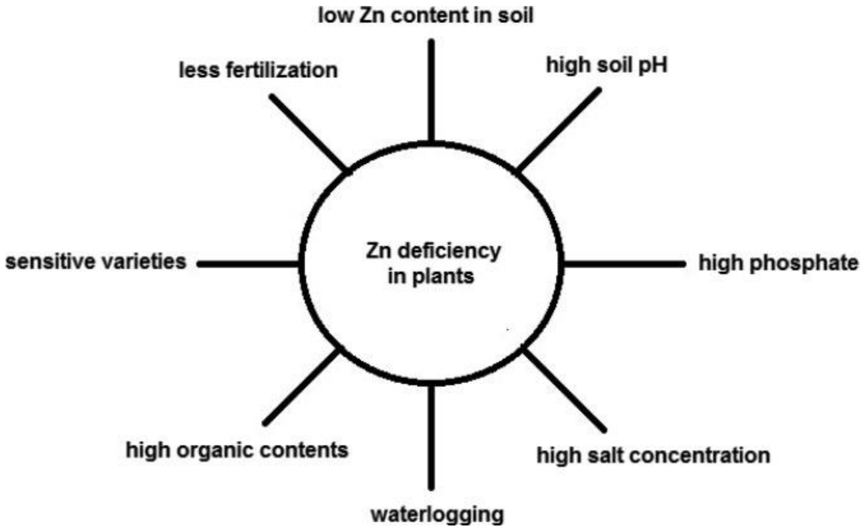

Figure 3. Schematic diagram of the causes of Zn deficiency in crops (Alloway, 2008).

In soil, except in those with high pH that absorption of monovalan of Zn ($ZnOH^+$) in them is possible, the absorption of this element is mainly done as divalent cation ($Zn2^+$). However, high concentration of other divalent cations such as calcium, to some extent prevents its absorption (Marschner, 2011).

For maintaining the balance of Zn, plants use some Zn regulatory networks and the transport networks. These networks contain key sites of ZE which do increasing of the biological supply of Zn element and absorption and transportation of this element from root to stem (Kabir et al,. 2014). Also, there are six different ways for transporting Zn into plant (Fig. 6) that includes ZIP (Zrt and Irt proteins), CDE (cation diffusion facilitator proteins), ATP p kind and CAX (calcium and other divalent cation exchange antiporters).

In arid and semi-arid soils of the world, grown crops widely suffer from Zinc deficiency, and it causes severe reductions of grain yield and growth in such crops. Normally, zinc deficiency occurs in plants when their soil is poor in organic matter (less than 2 percent) and when the amount of $CaCO_3$ in the soil be higher. The low amount of organic matter is widely known as the main reason of Zn deficiency (Sillanpaa. M and P. L. G. Vlek, 1985). In sum, the regions which their soils suffer from Zn deficiency includes those areas that the sign of this element's deficiency can be seen in humans. For instance, countries like India, Pakistan, China, Iran, and Turkey are considered as such areas (Hutz and Braun, 2004).

In Iran, Zn deficiency and its dangerous effects are seen in most soils, whether in calcareous and alkaline soils of arid and semi-arid areas or in neutral and less acidic soils of North of the country (Salar Dini, 1358). Although the amount of Zn in the soil can reach up to 200 ppm, in alkaline soils, which cover the majority soils of Iran, its amount does not exceed 20 ppm (Salar Dini, 1358). On the other hand, as said before, plants reaction to Zn depends on the soil's pH, in a way that for example in wheat, Zn concentration in the soil solution decreases 30 times for each unit increase in pH with the range of 5 to 7 (Barber. S. A, 1995). Also, Zn amount in topsoil is lesser than its amount in deeper soil. Deficiency of Zn sources, alkaline pH and affluence of calcium carbonate, lack of organic deficiency of soil, high levels of phosphorus and nitrogen of soil are some of the main reasons of Zn deficiency of Iran's soils (Salar Dini, 1358).

Mentioned problems caused that Iran be considered as one of the critical areas as far as Zn deficiency concerns. Iranian anaemia is a dangerous illness that arise due to the lack of Zn and Fe, and indicates critical condition of this element's deficiency in our country. Studies show that stunting disorder along with hypogonadism and chronic anemia, which are often prevalent in the villages of Iran, are some of the consequences of zinc deficiency. Statistics shows that the deficiency of this micronutrient in children under two years is about 20 percent, but in some province of Iran, its deficiency is very severe and in the villages of southern provinces, such as Sistan and Balochestan, Southern Khorasan, and Kerman provinces, its deficiency is more than 70 percent. Unfortunately, the percentage of Iran this deficiency among pregnant women of is also high; so that 40 percent of pregnant women are exposed to the mentioned deficiency. Other important factor which intensifies this critical condition is Iranians' diet. Wheat and its products constitute near to 50 percent of Iran's diet habits and this percentage in the villages is very higher (FAO, 2003). In addition to Zn deficiency in the wheat grown in poor soils, the amount of Zn in wheat grain is inherently low (Chakmak, 2008). However, there are some compounds, such as phytic acid (phytate), fiber and polyphenols, that prevent absorption of the majority amount of grain Zn by human body (Velje et al., 1982). It is noteworthy that when molar ratio of phytic acid to zinc in the diet is over 20, it will have a significant effect on reduction of the absorption of this element (Zn) (Gargari et,. 2007). By linking to Zn, phytic acid reduces solubility and absorption of this element. With considering the distribution of Zn in various parts of the wheat grain, it becomes clear that the amount of this element in the embryo and aleurone layer is higher than other parts of the grain, and because of shell (bran omission) during flour preparation (white flour), flour Zn provided by such wheat confines to the endosperm which has the lowest level of Zn. Of course, it should be noted that in addition to Zn concentration in the bran, the highest amount of phytic acid and protein are also concentrated in this part of the grain which act as obstacles in the way of Zn absorption (Sabravat and Chand, 1999).

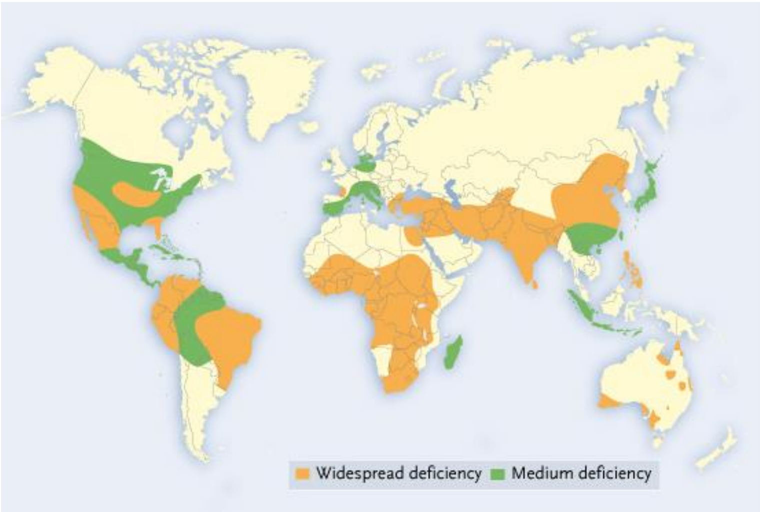

Figure 3. World's soil distribution from the perspective of Zn amounts (Alloway, 2008).

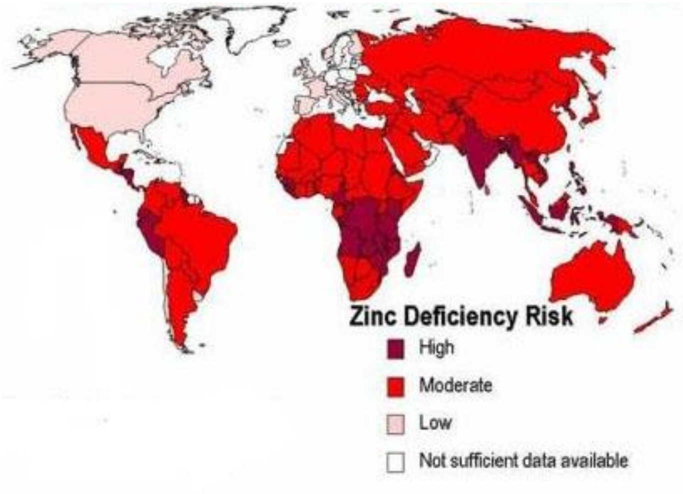

Figure 4. Distribution of Zn deficiency risk in different parts of the world (International Zinc Nutrition Consultative Group)

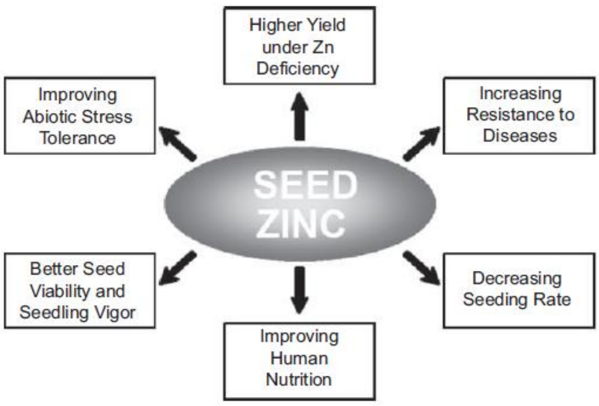

Figure 5. Agronomic and human nutritional benefits resulting from use of Zn-enriched seeds (Cakmak, 2008).

Several solutions have been proposed for overcoming the above situation which reducing phytic acid of wheat flour, Zn-enriched flour, and finally agronomic biofortification, and breading of wheat are some of them (Chakmak, 2008).

Reducing phytic acid of flour

About first solution, it should be mentioned that despite of the advantages of this method, in addition to preventing different kinds of cancer in human (Slaton et al., 2001), phytic acid also induces the increasing of seed germination and seedling vigor in the field condition (Guteri et al., 2006). It should be mentioned that 65 to 75 percent of seed phosphor accumulate as phytic acid (Chakmak, 2008). Actually, measuring grain Zn without regarding phytic acid in it would not be an appropriate indicator to determine body's level of access to Zn. It should be noted that Zn deficiency promotes foundation of phosphor transporter gene in the roots and increases phosphor transferring and remobilization in the phloem (Chakmak, 2008).

Zn-enriched flour

The second solution, which is widely used in some developed countries, is artificially adding Zn element to people's diet. However, using this method due to its high costs is not easily obtainable. It should be noted that according to some estimates, the cost of such process for a population of 50 million people is more than 25 million dollars a year (Bois et al., 2000). On the other hand, compared with this method, using bifortification is more affordable (Chakmak et al, 2008).

Biofortification

The third method (biofortification) is the easiest and safest way to increase seed Zn. Zn-biofortification means increasing grain Zn by using biological strategies which can be done with two methods, i.e. agronomic and breading. Furthermore, by using of Agronomic biofortification, we can reduce seed phytic acid content. In this method, as will be explained, using of Zn in poor soils (poor in respect of Zn), reduces the absorption of phosphorus accumulation (and therefore phytic acid). Such a feature will improve Zn accessibility in the digestive system.

Agronomic biofortification

Nowadays, the first method is done in various ways which fertilization and foliar of this element are two main ways. It should be considered that fertilizer experiments related to Zn mainly focused on the effects of this element on plants' growth and grain yield, and they study the possible changes in seed's Zn content very rarely. Experiments which have been done in this regard in wheat show that using fertilizers that contain Zn- in addition to increasing the production capacity of the plant- increase the Zn concentration in seed (Chakmak et al., 2008). Also, using fertilizer is more economical in the long term, because fertilizer residues in the soil can provide plants' needs for several years and there would not be necessary to apply fertilizer every year (Yilmaz et al., 1997; Martnz and Vestrman, 1991).

Zn application efficiency in plant depends on type of applied fertilizer, methods of fertilizer application, and the time of its application. Conducted studies reveal that among several types of fertilizers which contain Zn, ZnEDTA is more efficient than other types (Uzturk et al., 2006). But because of its high cost, Zn sulphate fertilizer ($ZnSO_4$) is more common than ZnEDTA. It should be considered that usage percentage of Zn sulphate fertilizer depends on the initial Zn in soil, accessible for plants and also soil's type. For instance, in soils with high calcium carbonate, usage percentage of the fertilizer is higher than sandy soils. In calcareous and poor soils of Turkey, application of 7 kg per hectare of Zn sulphate fertilizer increased grain yield of wheat (Ekiz et al., 1998). Regarding application method of fertilizer, the result of experiments show that the highest grain yield, along with the highest amount of Zn accumulation in grain are obtained in Zn foliar accompanied by fertilization (Chakmak, 2008). However, the fastest and easiest method for increasing Zn content of grain was foliar, especially when Zn was combined with urea (Chakmak, 2008). In an investigation of possibility of applying Zn sulphate as "fertilization-irrigation," Malakoti et al. (2000) show that depending on the clay and limestone percentages of soils, 35 to 60 percent of Zn added to soil's profile by solution fertilizer became non-absorbable and its absorbable part accumulated in 0 to 2.5 cm of soil's surface. In the same way, Zn concentration did not significantly change at the depths more than 5 cm. They suggested that in case of making water of irrigation acidic till pH equal five, Zn sulphate fertilizer can be used as fertilization-irrigation.

Time of the application of fertilizers contain Zn is often related to the foliar. It becomes clear that if the foliar occurs near to the final stages of wheat growth, it will have more effect on the increasing of the Zn concentration in grain. Uzturk et al. (Sabravat and Chand, 1999) showed that the highest content of Zn is obtained when the foliar occurs in the Milky stage. In contrast, there are some reports which show that the best result of foliar of this element is obtained in three stages, i.e. Booting, Milky, and Dough stages (Chakmak, 2008). Interestingly, in the foliar method, applying (using) ZnSO4 is more efficient than other components which contain Zn (Chakmak, 2008).

Several researchers have worked on the wheat biofortification in field. Unfortunately, one of the major problems that we face in grain biofortification with micro-nutrients is the negative interaction between micro-nutrients, especially between Zn with iron and manganese. In a research which have been done in 10 province of the country regarding the role of micro-nutrients in increasing grain yield of wheat, Malakoti et al (2000). observed that increase of micro-nutrients significantly improved wheat's grain yield, but due to negative interaction of Zn with iron and manganese, with an increase in Zn concentration of wheat grain, iron and manganese concentrations decreased (Fig. 2).

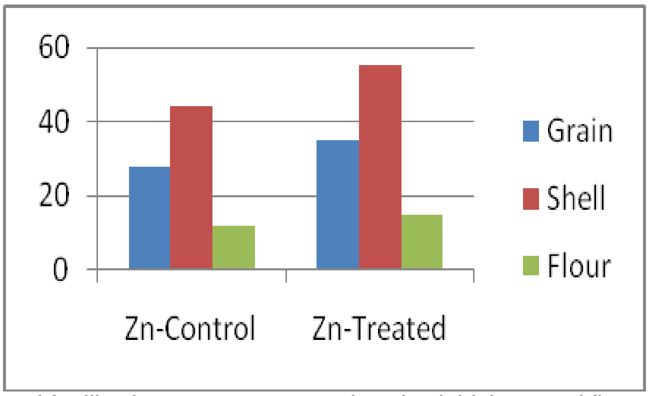

Figure 8. The effect of balanced fertilization on Zn concentrations in yield, bran and flour of wheat in 9 province of the country (Malakoti et al., 2000).

Generally, in the mentioned research, the reasons of negative interaction of Zn with iron and manganese in wheat grain can be summed as follows

Dilution effect: With an increase in grain yield by using one element, concentration of other elements reduces.

Competition effect

Nutrient elements may compete together for occupying similar site and positions on the similar carriers. Nutrient elements, such as Zn, iron, and manganese may compete together in the process of absorption and transportation from roots to shoots (aboveground organs). Wheat needs iron and manganese more than Zn.

One of the other methods of agronomic Zn biofortification is the using of seed priming technique (Haris et al., 2007). It should be noted that priming is related to the application of those treatments that has been done for improving the germination condition and for overcoming environmental difficulties and can finally result the proper placement and grain yield of the plants (Akres et al., 1987). It becomes clear that seed priming with solutions which contain Zn, improves germination process (Haris et al., 2007). For instance, priming of corn seed with the solution of $ZnSO_4$, apart from increasing the grain's Zn content, produced more biomasses and increased grain yield (Haris et al., 2007). Soaking seeds of *Echinacea purpurea* (L.) in the solution of 0.1 percent MnSO4 or 0.5 percent $ZnSO_4$ also increased germination of the plants 36 to 38 percent in the field (condition) and 89 to 91 percent in laboratory (condition) (Babia et al., 1999). In rice also, Saltun et al. showed that priming rice seeds with solution which contain Zn, had several beneficial effects. Most likely, in case of satisfying the desired goals, priming is more economical than previous methods.

Breading biofortification

Breading biofortification is another method for increasing Zn content of wheat grain. Although this method is the cheapest and easiest way for overcoming Zn deficiency, available agronomic wheat seeds have minimum content of Zn and related genetic changes in them is very less (Chakmak, 2008). Mentioned reasons redirected our attention to the wild varieties of Cramineae which have more Zn content. For instance, Zn content of wild Emmer (Triticum turgidum ssp, dicoccoides) varies between 14 to 190 mg per kg (Chakmak et al., 2004). Synthetic wheat which is derived from a type of Aegilops tauschii, also has genetic potential for increasing Zn content of grain. Despite of our little knowledge about the genetic control and molecular mechanisms involved in Zn accumulation ability in the grain, due to the higher correlation of grain's protein and Zn, it seems that gens which are responsible for increasing of protein and Zn, are related together and thus, breading for higher protein and yield simultaneously may result increase of Zn content (Distlfeld et al., 2007). Transgenic approaches can also be used in this regard. In the recent years, many programs are designed for increasing Zn content of grain (Chakmak, 2008). Available evidences (documents) suggest that Zn and iron transporter proteins from ZIP family have potential ability for improving the concentration of micronutrient elements in grains (Sumasender et al., 2005). These proteins play a role in the absorption and transportation of the cationic micronutrients. However, the role of mentioned proteins in genotypic changes for tolerating Zn deficiency or accumulating Zn in seeds is not clear. Thus, doing more breading researches regarding this issue seems necessary.

Also, new methods of biotechnology try to increase the content of elements in the harvestable products. By efforts in the field of molecular biology, genomics tries to increase absorption and transportation of the elements, especially Zn. Several researchers reveal their significant findings regarding the role of molecular biology in increasing the content of elements (Kabir et al., 2014). Now, significant progresses are obtained in the production of transgenic plants with higher ability of Zn absorption. These plants are produced by changing (manipulating) of proteins of ZIP family (White and Brwadly, 2005; Lucka et al., 2006; Gandilian et al., 2006; Boril et al., 2014). In sum, the molecular methods which are common and used for increasing the Zn concentration in the grain, are as follows: 1) Higher Zn concentration in the edible parts of the plants occurs with the higher expressions of ZIP 19 and ZIP 23 gens. 2) Changes of the transporter proteins in the plasma membrane of the roots of wheat. 3) Higher expression of the coding gene of Zn transporter. 4) Higher expression of Nicotine Amide Synthetase gene (NAS). 5) Higher expression of the gens related to siderophores. 6) Higher expression of genes that increase glutathione, phytochelatins, and thiol groups. 7) Change of Aloeron and embryo ligands. 8) Increasing the expression of HMA gene (Broeil et al., 2014; Kabir et al., 2014).

According to the above discussions, it can be concluded that natural Zn deficiency in Iran's soils on one hand, and common diet of the people of this country on the other hand, make it necessary to have wide-spreading strategic researches and macro programming for increasing Zn content in Iranians' rations.

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