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# Genetic Variation of Bread Wheat Varieties in terms of Zn and Fe Accumulation in grain under Zinc Foliar Application

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**ABSTRACT: The micronutrient malnutrition is being increasingly extended due to rapid growth of human population beside the expansion of agronomy systems. One approach of resolving this problem is employing cultivars with high absorption efficiency for these elements. This Study was designed to evaluate the effect of zinc foliar application and its accumulation in wheat grain on agronomic traits and iron accumulation in the grain of facultative and winter wheat varieties that were conducted in two separate experiments based on RCB design with three replications. The results explained a significant positive effect of zinc foliar application on all traits except grain iron content. The growth type had significant effect on all traits. The effect of variety within the growth type was significant in terms of all traits and indicated high genetic variation among the varieties. Back Cross Roshan, Shahpasand and Mihan were the best varieties in term of grain yield. Bisotoun, Kaveh, Tous and Mahdavi varieties performed the highest content of grain zinc and Back Cross Roshan, Shahpasand and Tous varieties had the highest content of grain iron. Also Rashid, Navid, Alvand and Sayson varieties bilaterally had relative high grain zinc and iron contents. Totally, Sayson and karaj-3 varieties tripartitely performed relative high grain yield and also high grain zinc and iron contents. Grain yield was negatively correlated with grain zinc and positively with grain iron contents.**

**Keywords:** Grain Iron Content, Grain Zinc Content, Wheat.

## INTRODUCTION

As a plant that can be cultivated in all continents and in most soils, wheat requires various nutrients such as zinc and iron to grow. Lack of these elements not only reduces the plant' yield but also lack of such nutrients foods like wheat will decrease its absorption in humans and animals, and consequently, causes various diseases and lowers health status among people (Welch and Graham, 1999). Soil experiments conducted in Iran revealed that 37% of the irrigated wheat fields have problems in terms of availability to iron, 40% of zinc, 25% of manganese, and 24% of absorbable copper. The global statistics have also shown that soils under cereal cultivation across the world suffer the trouble of availability to zinc (50%) and iron (30 %) (Balali *et al*., 2000). More than three million people worldwide suffer from zinc and iron deficiency nowadays. High and uniform application cereal with low concentrations of energy- saving elements is among numerous reasons of the universal expansion of zinc and iron deficiencies in the developing countries. An increased concentration of iron and zinc in plant products is regarded as a major challenge to improve human health worldwide.

Iron is an essential element in plant nutrition, so any disruption in its ability will reduce its growth. Iron deficiency in plants leads to yellowing of leaves and reduction of photosynthesis and chlorophyll concentrations (Mahmoodi *et al.*, 2005), shoot and root dry weight (Tabatabai *et al.*, 2008), change in iron concentration and content (Tabatabai *et al.*, 2008) and other metallic elements in plant tissues (Chen *et al*. 2004). Of course, such traits are closely related to crop yield. Energy-saving and other essential elements play a significant role in plant growth. Stimulating formation of carbohydrates, proteins and DNA, Zinc is involved in RNA metabolism and ribosomal content of plant cells. In fact, it is required for manufacture of tryptophan, the IAA precursor which activates the growth inducing substance (Amberger, 1982). Zinc has three catalytic, co-catalytic, and structural functions (Valle and Auld, 1990; vallee and Falchuck, 1993). Even recently, its contribution with protein molecules involved in DNA transcription has been more considered (Coleman, 1992).

In addition, this element plays an important role in biomass production (Kaya and Higgs, 2002; Kakmak, 2008). Likewise, Zinc has a vital physiological function during seed germination and early seedling growth (Kakmak, 2008).

According to the reports, the average concentration of zinc in wheat grain in different countries is between 20-35 mg kg (Kakmak *et al.,* 2004). The highest content of zinc is located in seed embryo and aleurone, but its concentration in endosperm is very low (Ozturk *et al.*, 2006). According to Welch and Graham's reports (2002), zinc concentrations in the fetus and aleurone layer of wheat grain was about 150 mg/ kg whereas in endosperm, it was just 15 mg/ kg. The reported iron concentration was also 28.8-56.5 mg/ kg (with an average of 37.2 mg/ kg). According to the report, due to be formally sufficient genetic variation in concentrations of iron and zinc in wheat grain can be significantly increased through breeding methods. In the studied wheat varieties, despite significant interaction effect between genotype and environment in terms of concentration of iron and zinc, the genetic part of changes for zinc and iron accumulation was remarkable in seeds. Moreover, a negative correlation was reported between iron and zinc contents in the above- mentioned varieties which can leave negative effect on it in accordance with simultaneous increase of concentration of these two elements in grain seeds.

Among numerous ways to combat iron and zinc deficiencies in human societies is food fortification (Darnton-Hill and Nalobola, 2002). Various researchers have conducted several studies on wheat enrichment. Unfortunately, one of the most significant problems in terms of grain enrichment with zinc and iron is that zinc increase lead to reduction of iron concentration in plants, resulting in reduction of iron uptake (Malakouti and Balali, 2001; Ming and Yin, 1992; Zhang, 1993). Increased zinc gained during iron absorption and transport will provide interference and result in leaves' chlorosis (iron deficiency) (Marschner, 1995). Other research showed that in the plants suffering from zinc deficiency, iron concentration is increased mainly due to increased iron transfer from the roots to the aerial organs (shoots) (Kakmak *et al.,* 1996). Antagonism1 between iron and zinc is quite recognized.

Previous studies have shown the significant role of zinc in absorption and transport of iron. Such interactions are created only when the concentration is high (Alloway, 2008). There are three set ups for this interaction: (1) competition in the uptake and translocation of $Zn^{2+}$ and $Fe^{2+}$ in the root (Kabata-Pendias, 2001), interfere with zinc and iron chelating processes in soil (Kabata-Pendias, 2001), competition in discharge of these two elements from xylem vessels (Alloway, 2008). This combined usage of iron and zinc is among the management methods, which are used to achieve high performances and enhance the quality of wheat grains.

The results offered by Silispour (2005) showed that the combined usage of these elements would both provide significant increase of yield and enhance the grain's qualitative features including protein and zinc contents, which will be ensuring the health of consumer society.

This research was conducted to assess the possibility of combined enhancement of zinc and iron contents in grains along with maintaining or increasing its yield in the Iranian grain cultivars with facultative and winter growing types using modification method and selection of possible superior cultivars.

## MATERIALS AND METHODS

The trial was conducted in the growth season 2011-2012 in agricultural research station of Tabriz University, located in Karkaj region, 12 km east of Tabriz, at 38 05' N and 46 017' E, with 1360 meters elevation above sea level. Climate of the region was mountainous semi-arid cold, and the soil of station was sandy-loam with a slight to moderate alkaline pH (Jafarzadeh *et al.,* 1998). In this study, 16 wheat varieties with facultative growth type (including Bam, Mahdavi, WS-82-9, Navid, Azar, Qods, Alvand, Roshan, Shahriar, Inia 66, Karaj, Azar 2, Zareein, Kaveh, Rashid and Zagros) and 21 winter growth type (including Sayson, Pishgam, Biston, Zare, Karaj 2, MV-17, Omid, Gaspard,, Back Cross Roshan, Mihan, Sardari, Sabalan, Shahpasand, Tous, Alamut, Gaskogen, Shahi, Azadi, Bezostiya, Erom, and Norestar) were studied. The study was conducted in two separate experiments in a completely randomized block design with three replications, in which one of the experiments was done under normal circumstances (without zinc sulfate foliar) and the other was performed with Zinc sulfate foliar.

Before planting, applying fertilizer (according to soil test) was performed so that 64.8 kg/ ha ammonium nitrate ($NH_2NO_3$), 64.8 kg/ ha phosphorus from triple superphosphate with the formula $Ca(H_2PO_4).2H_2O$, 32.4 kg/ ha iron from ferrous sulfate with the formula $FeSO_4.7H_2O$, and 12.96 kg/ ha copper from copper sulfate with the formula $CuSO_4.5H_2O$. In this experiment, 0.68 kg/ ha zinc (Zn) from zinc sulfate with the molecular formula of $ZnSO_4.7H_2O$ was sprayed on the plants during the boots to heading stages. In this study, traits such as grain yield and the amount of zinc and iron in the grain were studied.

PPB staining was used to determine the location and concentration of iron in the grain that produces a blue complex in cases of iron accumulation (Zarcinas *et al.*, 1987). In order to determine the location and concentration of zinc in the grain, the DTZ staining method was used. It also produces a red-purple complex, zinc ditizon.

DTZ or Ditizon's formula is $C_6H_5NHNHCSN = NC_6H_5$ which is used to extract and determine the concentration of Zn. Its other names are Diphenylthiocarbazon-1,5, Ditizonica, Ithizoneda and Ditizon (McNary, 1954).

In order to count the total number of color pixels (TPN) in the grain's red painted zone (to determine zinc content) and in the grain's blue painted area (to determine iron content), Photoshop software was used as follows: (1) in the Menu, click on the Select option and then click on the Colour Range option. 2. Use the marker and place it on the image and click on the red area (to determine zinc content) and the blue area (to determine iron content) in the image, (3) set the red color gamut using the Fuzzy state (the selected degree of fuzziness = 132) and the blue color gamut using the Fuzzy state (the selected degree of fuzziness= 46).

The color contents of the red and blue areas were determined by counting the number of their associated pixels (select the image from the top menu and then, click on the histogram option). The histogram box is opened and the number of pixels in the selected area in the lower left corner of the pixel is shown (Choi *et al.*, 2007). Combined analysis of variance was conducted in a completely randomized block design with three replications with further analysis of sum of squares for variety and cultivar × zinc. To analyze the data, the SPSS, MSTAT-C, and Excel software were used.

## RESULTS AND DISCUSSION

Based on the results of analysis of variance (Table 1), it was revealed that except the iron content of the grain, the effect of zinc foliar on the studied traits was significant. Thus, the average of the traits in zinc foliar condition was different from the mode lacking spraying. Moreover, zinc foliar made no significant change in iron content of the grains. The differences between the cultivars were also significant differences in terms of the studied traits, indicating the existence of high genetic variation among them. Variations in both winter cultivars and cultivars facultative growing types were significant (1%) in terms of the studied traits such as zinc and iron contents in the grains. For all traits, the mutual effect of genotype × zinc was significant representing the coordinated response of all genotypes to zinc foliar.

**Table 1: Analysis of variance for measured traits in wheat genotypes.**

| Variation Sources | Degree of Freedom | Grain yield | Grain's Zinc content | Grain's iron content |
|---|---|---|---|---|
| Zinc | 1 | 12.86** | 1472.981** | 0.967 |
| Error 1 | 4 | 0.246 | 18.622 | 6.949 |
| cultivar | 36 | 7.344** | 808.271** | 42.53** |
| winter | 20 | 5.085** | 691.373** | 28.335** |
| facultative | 15 | 1.193** | 828.035** | 50.975** |
| growth type difference | 1 | 144.789** | 2849.771** | 199.75** |
| cultivar× Zinc | 36 | 0.22 | 70.955 | 3.289 |
| Zinc × winter | 20 | 0.211 | 66.807 | 2.318 |
| Zinc × facultative | 15 | 0.24 | 80.226 | 4.775 |
| Zinc × growth type difference | 1 | 0.1 | 14.85 | 0.419 |
| Error 2 | 144 | 0.23 | 53.096 | 3.293 |
| %CV | | 14.89 | 23.28 | 40.14 |

* and ** represent no-significance, significance at the 5% and the 1% levels, respectively.

**Table 2: The mean of traits of winter and facultative wheat cultivars in average foliar application condition and without zinc foliar application.**

| Traits | Grain yield | Grain's Zinc content | Grain's iron content |
|---|---|---|---|
| winter | 4.09 | 24.9 | **2.29** |
| facultative | 2.09 | 39.7 | **1.59** |
| LSD0.05 | 0.64 | 9.85 | **2.45** |

Due to the significance of the growing- type difference in terms of all traits, the average of the traits for both facultative growing types is mentioned in Table 2. Grain yield and iron content in the winter growing type varieties was higher than facultative growing type ones, yet zinc content of the grains in the facultative growing type cultivars was higher than the winter growing type. Based on the comparison of the means in both cases of with and without zinc foliar regarding the studied traits (Table 3), in all traits (except iron content), a significant difference was observed in conditions with and without foliar application. In fact, traits such as grain yield and zinc content of the grain in foliar condition was more valuable than the cases without foliar application. To confirm this claim, Khoshgoftarmanesh *et al*. (2001) reported that after using zinc sulfate, wheat grains showed a different reaction in terms of yield. Malakoti (2004) also examined the effect of different zinc sources on grain and reported that with the use of zinc, grain yield increases. Malakoti and Balali (2001) stated that due to increased grain yield after foliar, the effect of zinc on chlorophyll content and hormone Indole-3-Acetic Acid was positive. According to Erdal *et al*. (2002), zinc foliar results in increased grain's zinc content in durum wheat. Using different methods of zinc usage in various wheat cultivars, Yilmaz *et al.* (1997) concluded that zinc sulfate usage will not only increase the seed yield significantly but it will also enhance the concentration of this element in wheat grain causing its enrichment.

**Table 3: Mean for measured traits in with and without zinc foliar application of the studied cultivars wheat.**

| Traits | Grain yield | Grain's Zinc content | Grain's iron content |
|---|---|---|---|
| Condition without zinc (Control) | 2.98 | 28.06 | 2.01 |
| Condition with zinc | 3.46 | 33.88 | 1.96 |
| LSD0.05 | 0.18 | 1.60 | 0.98 |

In general, based on the average of various cultivars in both conditions (with ad without zinc foliar), Shahi cultivar (Fig.1) had the highest grain yield, followed by Back cross Roshan, Shahpasand, and Mihan, respectively. The highest zinc content rates (Fig. 2) belonged to the cultivars of Biston, Kaveh, Alvand, Qods, and Mahdavi, whereas the lowest rates of zinc content of the grain were also related to the varieties of Omid, Back Cross Roshan, Shahpasand, and Shahi.

This indicated that the cultivars with higher yields had lower zinc content. Maralian *et al*. (2007) also reported that there was an inverse relationship between yield and grain quality characteristics, so that any increase of wheat yield will decrease its zinc content. Since these highly produced cultivars are among the most common types in the country, the possible cause of zinc deficiency in the population is somewhat realized.

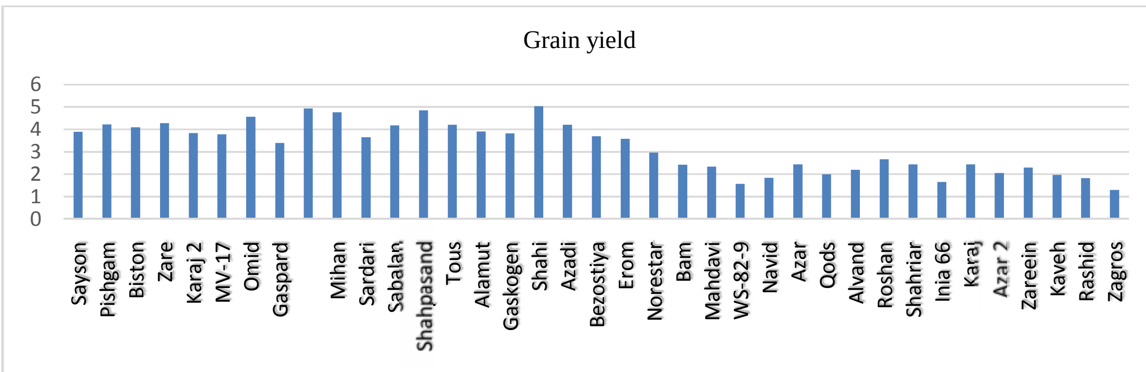


**Fig. 1.** The mean of the studied cultivars wheat of Grain yield in average foliar application condition.

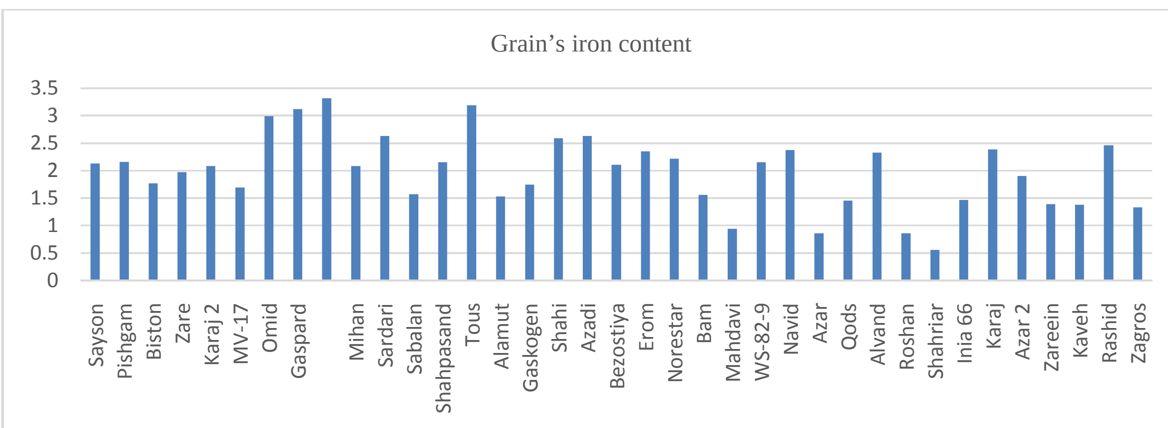


**Fig. 2.** The mean of the studied cultivars wheat of Grain Zinc Content in average foliar application condition.

The highest rates of grain iron (Fig. 3) also belonged to one light cross, Tous, Gaspard, and Omid that are among the varieties with high performance. The lowest iron content belonged to Shahriar and Azar cases. Here, it was found that in general, high-performance varieties had lower zinc content, but their iron content was higher. Malakoti and Ziaeian (2000), Ming and Yin (1992) and Rengel and Graham (1995) reported a negative relationship between iron and zinc. Meantime, Sayson and Karaj 3 allocated a relatively high rate of grain yield and zinc and iron content to themselves trilaterally. Based on the results of regression analysis in conditions with and without zinc foliage (Tables 4 and 5), in both cases, grain yield had a significant positive correlation with its iron content and a significant negative relationship with its zinc content and zinc and iron content showed a significant negative correlation in both conditions.

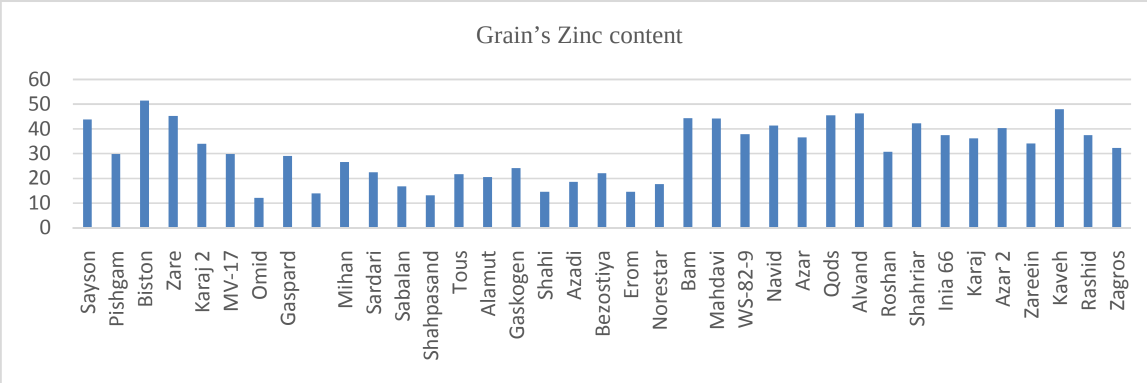


**Fig. 3.** The mean of the studied cultivars wheat of Grain iron Content in average foliar application condition.

**Table 4: Ccorrelation coefficients of evaluated traits in without zinc foliar application.**

| Traits | 1 | 2 | 3 |
|---|---|---|---|
| Grain yield | 1 | | |
| Grain’s Zinc content | -0.538** | 1 | |
| Grain’s iron content | 0.449** | -0.411* | 1 |

* and ** represent no-significance, significance at the 5% and the 1% levels, respectively.

**Table 5: Correlation coefficients of evaluated traits in with zinc foliar application.**

| Traits | 1 | 2 | 3 |
|---|---|---|---|
| Grain yield | 1 | | |
| Grain’s Zinc content | -0.648** | 1 | |
| Grain’s iron content | 0.455** | -0.476** | 1 |

* and ** represent no-significance, significance at the 5% and the 1% levels, respectively.

At the same time, under qualified foliar conditions, the correlation between the grain yield and zinc and iron content was more evident. Therefore, it can be inferred that due to increased grain yield in solution terms, zinc foliar has no direct effect on grain yield, so based on its effect on the rates of chlorophyll and Indole-3-Acetic Acid, zinc can increase grain yield; hence, through increased photosynthesis, this enhanced chlorophyll can increase plant's dry matter (Rashid and Ryan, 2004).

## CONCLUSION

Based on the positive correlation between grain yield and its iron content and their negative correlation with grain's zinc content, it seems that combined selection method is impossible for these three traits in the studied cultivars, so in order to produce high- performance cultivars with considerable zinc and iron grain contents, it is recommended to consider combination between far cases in terms of grain's zinc content followed by selection between the obtained results.

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