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Genetic variation of some facultative wheat genotypes in terms of accumulation of zinc in the whole grain and endosperm

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Abstract

Malnutrition caused by zinc micronutrient deficiency has become one of the growing problems of developing countries. Using varieties which have high uptake efficiency for this trace element is one of the methods to solve this problem. Two separate trials (with and without zinc foliar application) were conducted in the form of randomized complete block design with three replications and then combined analysis of variance was performed on the results, with the purpose of evaluating the genetic variation in facultative wheat genotypes, in terms of their zinc uptake and translocation efficiency, and to ultimately identify genotypes with high content of zinc in the grain and especially in their endosperm. Zinc was added through foliar application in the amount of 0.68 kg/ha at the end of the vegetative stage and start of reproductive stage. Agronomic traits such as grain yield were measured during the growth period. Also two main traits for trial, the amounts of zinc in the whole grain and in the endosperm, were measured by DTZ (1,5-diphenylthiocarbazone or dithizone) staining and spectrophotometry, respectively. The results of analysis of variance indicated high genetic variation for all the studied traits. Zinc content of the endosperm had the highest genetic and phenotypic coefficient of variation and thousand grains weight had the highest heritability. According to the correlation and regression analysis the amounts of zinc in the whole grain and in the endosperm showed no linear relationship with grain yield and their variations were independent from the variations of grain yield.

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Introduction

The lack of some trace elements in the food products is a growing global challenge. Zinc is one of the important micronutrients in the biological systems, and considering the large number of reports about the zinc deficiency in human populations and plant products, its deficiency has attracted much global attention (Hotz and Brown, 2004; Alloway, 2008; Cakmak, 2008). Zinc deficiency is one of the five major micro-nutrients deficiencies in humans and its deficiency has negative effects on almost a third of the world population (Hotz and Brown, 2004; Stein, 2010). Very small quantities of zinc is vital for humans and plants, and it has an activator or structural catalytic role in many of the plant's enzyme systems and is involved in creation and breakdown of the plant's proteins. Zinc has a vital role in activation of biosynthetic growth hormone, formation of starch, and formation and ripening of seed (Brady and Ray, 2002). Zinc plays the role of catalyst in more than 300 enzymes such as alkaline phosphatase, alcohol dehydrogenase, Cu-Zn superoxide dismutase, carbonic anhydrase, etc. Zinc is necessary for almost all aspects of cell metabolism (Ruz, 2003; Yilmaz, 2007).

Zinc deficiency reduces grain yield and protein percentage and also reduces the nutritional value of plant products. Low concentration of zinc in wheat and products such as bread causes zinc deficiency in humans. Zinc deficiency in humans causes a range of health problems and many diseases and complications such as impaired physical growth, impaired immune system and reduced ability in learning, as well as an increased risk of infection, DNA damage and cancer (Solomons, 2003; Hotz and Brown, 2004; Ho, 2004; Cunningham-Rundles *et al.*, 2005; Gibson, 2006; Prasad, 2007). More than three billion people currently suffer from micronutrient malnutrition (Welch and Graham, 2004). It is estimated that 49% of the world's population are at the risk of not getting enough zinc (Cichy *et al.*, 2005). This deficiency is more concentrated in the semi-arid tropics, particularly in South and Southeast Asia and sub-Saharan Africa (Reddy *et al.*, 2005).

Half of the worldwide areas under wheat cultivation have a soil with low level of available zinc for the plants that consequently result in reducing the amount of zinc in the produced grain (Liu, 1996; Alloway, 2008; Cakmak, 2008). Also it seems that established production of high-yielding varieties (known as Green Revolution products) exacerbates this problem (Cakmak *et al.*, 2010; Stein, 2010; Zhao and McGrath, 2009). High consumption of foods containing zinc deficient grains over a long period may lead to problems caused by zinc deficiency and cause serious health problems. In order to meet the peoples' daily needs for zinc, the amount of zinc must be higher than the optimal level required for the crops themselves, especially in the case of cereals (Welch, 1999; Rengel, 1999; Grusak and Cakmak, 2005). Excessive consumption of food products created from wheat, increases the malnutrition caused by zinc deficiency, because wheat inherently contains very low amounts of zinc but also contains high amounts of substances such as phytate that reduce zinc bioavailability (Cakmak *et al.*, 2010; Welch and Graham, 2004). During the grinding process, the removal of grain's bran -that is rich in zinc and consists of aleurone and embryo- causes a significant part of the grain's zinc to be also removed; therefore, increasing concentration of zinc in wheat grain endosperm has become an important challenge that requires urgent attention (Welch, 1986; Welch and Graham, 1999; Cakmak, 2008; Welch and Graham, 2004; Zhao and McGrath, 2009). Moreover, the processes applied on wheat after its harvest also remove a significant proportion of zinc and other minerals which also leads to further reduction of zinc absorbed by humans (Cakmak, 2008; Kutman *et al.*, 2011; Zhang *et al.*, 2010).

Zinc deficiency could be resolved by means of various methods of zinc fertilizer utilization (Cakmak, 2008; Peck *et al.*, 2008), but these methods may not be always considerable as an economical and agronomical solutions. The cost of supplying of fertilizers is the most preventing factor related to apply them, in developing countries (Graham and Ringel, 1993). The alternative and sustainable way to resolve zinc deficiency is selection of tolerant genotypes to zinc deficiency and recognition of genes that are responsible for more efficiency in zinc uptake and translocatin. Many of researchers believe that the breeding methods are more sustainable and economical way for resolving zinc defficiency (Hacisalihoglu *et al.*, 2001; Cakmak *et al.*, 2002; Welch and Graham, 2004; White and Broadley, 2005; Pfeiffer and McClafferty, 2007). The presence of enough genetic variation in terms of zinc uptake and translocation efficiency has an important role in breeding of wheat for this purpose (Graham and Welch, 1996; Palmgren *et al.*, 2008).

In this study, we tried to estimate genetic variation of zinc uptake and translocation efficiency of facultative wheat genotypes by DTZ blotting as a rapid and confident method. To determinate of selection strategy for acquiring both high grain yield and high zinc efficiency, study of relationship among these traits was proposed, too.

Materials and methods

Project location specifications

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°5' N and 46°17' E, with 1360 meters elevation above sea level. Climate of the region was mountainous cold semi-arid, and the soil of station was sandy – loam with a slight to moderate alkaline pH (Jafarzadeh *et al.*, 1998).

Plant materials

The plant material used in this study consisted of 16 varieties of bread wheat with facultative growth habit obtained from the cereal research department of seed and plant improvement institute, Karaj, IR Iran, and 8 recombinant inbred lines (RILs) derived from the cross between Zagros and Norstar varieties. Recombinant inbred lines were provided by the center of excellent in cereal molecular plant breeding, university of Tabriz (Table 1).

Applied fertilizer treatments and zinc foliar application.

The seed bed preparation was based on the soil analysis, so elements required by the plant over the course of trial were used as follows:

- Nitrogen in the form of NH_4NO_3 in the amount of 5.56 kg/ha.
- Phosphorus in the form of triple superphosphate with molecular formula of $Ca(H_2PO_4).2H_2O$ in the amount of 5.56 kg/ha.
- Iron in the form of ferrous sulfate with molecular formula of $FeSO_4.7H_2O$ in the amount of 2.78 kg/ha.
- Copper in the form of copper sulfate with molecular formula of $CuSO_4.5H_2O$ in the amount of 1.12 kg/ha.
- Zinc was added in the form of zinc sulfate with molecular formula of $ZnSO_4.7H_2O$ in the amount of 0.68 kg/ha through a double foliar application at the end of vegetative stage and early reproductive stage.
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Characteristics measured during the growing season

Agronomic traits measured in this study included: Plant height, flag leaf width, flag leaf length, number of fertile tillers, main spike length, 1000-grains weight, straw yield, number of grains per main spike and grain yield.

Determination of the position and the amount of zinc in the whole grain

Numbers of 12 seeds were randomly selected from each experimental unit and were weighted with the purpose of calculating the ratio of number of colored pixels to grain's total weight.

The method of Choi *et al.* (2007) was employed to determine the location and concentration of zinc in the seeds placed on each slide via DTZ reagent. The method was accomplished using Adobe Photoshop software.

Determining the concentration of zinc in the endosperm

Numbers of 20 to 25 seeds of each genotype were placed in distilled water for one day, and then they were ground in a porcelain mortar to remove the seed coat. 200 mg of the prepared flour was poured into micro-tube and then 200 ml of DTZ solution was added. The maximum staining was obtained after 30 minutes. However the solution was stirred several times during this period. After 30 minutes, 2 ml of pure methanol was added to mixture, and then centrifuged in 5000g for 5 minutes. Then the supernatant was analyzed through spectrophotometer in wavelength of 512 nm. Spectrophotometry results were converted to mg/lit through the linear fit of (Y = -62 + 182.9X). In this equation, Y is the zinc concentration, and X is the uptake rate (Ozturk *et al.*, 2006).

Experimental design and statistical analysis

Two separate trials (with and without zinc foliar application) were performed in randomized complete block design with three replications, and then combined analysis of variance was performed on the results. Genetic and phenotypic variances and genetic and phenotypic coefficients of variation for traits were calculated in order to determine the diversity among the studied genotypes. Moreover, the heritability of traits and the standard error of heritability were also estimated. Equations 1 to 6 belong to genetic variance, phenotypic variance, genetic coefficient of variation, phenotypic coefficient of variation, heritability, and standard error of heritability respectively:

$$(1)\ \sigma_g^2 = \frac{MS_g - MS_{Eb}}{rZn}$$

$$(2)\ \sigma_P^2 = \frac{\sigma_{Eb}^2}{rZn} + \sigma_g^2$$

$$(3)\ CV_g = \frac{\sqrt{\sigma_g^2}}{\bar{X}_{00}} \times 100$$

$$(4)\ CV_{Ph} = \frac{\sqrt{\sigma_P^2}}{\bar{X}_{00}} \times 100$$

$$(5)\ h^2 = \frac{\sigma_g^2}{\sigma_P^2}$$

$$(6)\ SE_{h^2} = \sqrt{\frac{2{MS_{Eb}}^2\left(\frac{1}{dfe+2} + \frac{1}{dfg+1}\right)}{MSg^2}}$$

In the above equations, σ_g^2, σ_P^2, MS_g, MS_{Eb}, dfe, dfg, $\bar{X}_{00}$, r and Zn represent genetic variance, phenotypic variance, mean squares of genotypes', mean squared of error, error's degrees of freedom, genotype's degrees of freedom, total mean, number of replications, and level of zinc respectively. Eventually multiple regression analysis was performed to determine the relationship between traits and grain yield. Statistical analysis was performed by IBM SPSS version 21 and Excel version 10 softwares.

Results

Analysis of variance (Table 2) indicated that there was a significant difference between the levels of zinc application (Zinc) in terms of traits number of fertile tillers, plant height, spike length, straw yield, grain yield, zinc content of the whole grain, and zinc content of endosperm.

Table 1. Genotypes studied in the trail.

RILs		Cultivars			
RIL-83	RIL-55	Kaveh	Alvand	Inia66	Bam
RIL-11	RIL-44	Zagros	Shahriar	Azar2	Navid
RIL-51	RIL-49	Rashid	Karaj2	Mahdavi	Qods
RIL-83	RIL-60	WS-82-9	Zarin	Azar	Roshan

There was also a significant difference between genotypes in terms of the all evaluated traits. The Genotype × Zinc interaction only became significant for two traits, zinc content of the endosperm and the zinc content of whole grain. The highest coefficients of variation for error belonged to traits zinc content of endosperm (%42.38), grain yield (%21.88), and straw yield (%18.97) and the lowest to 1000-grains weight (%4.34), plant height (%5.61), flag leaf width (%7.25), and spike length (%7.26).

Table 2. Combined Analysis of variance for studied traits in wheat genotypes.

Sources of variations	Degree of freedom	Mean square: Number of fertile tillers	Plant height	spike length	flag leaf length	Flag leaf width
Zinc	1	10.203*	1710.967*	21.115*	21.623n.s	0.190n.s
Error 1	4	0.853**	105.816**	1.844**	4.315n.s	0.045**
Genotype	23	0.549**	284.281**	1.790**	26.168**	0.053**
Zinc× Genotype	23	0.055n.s	9.491n.s	0.115n.s	0.269n.s	0.003n.s
Error 2	92	0.123	21.540	0.330	2.052	0.006

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

Table 2 (cont.)

Sources of variations	Degree of freedom	Mean square: Straw yield	number of grains per main spike	Grain yield	1000-Grains weight	Zinc content in the whole grain	Zinc content in the endosperm
Zinc	1	429022.817*	138.137n.s	432741.186*	10.666n.s	1410.935*	1024.528*
Error 1	4	22186.835**	22.262n.s	36175.743**	16.497**	100.295**	118.376n.s
Genotype	23	32729.247**	103.265**	16005.626**	86.836**	265.611**	1214.367**
Zinc× Genotype	23	5222.831n.s	11.805n.s	3349.600n.s	3.166n.s	72.469**	183.332*
Error 2	92	4451.766	9.459	4232.640	2.838	25.172	94.058

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

The difference between the levels of zinc application for the means of traits number of fertile tillers, plant height, spike length, straw yield, grain yield, zinc content of whole grain, and the zinc content of endosperm was significant, but for the other traits was not (Table 3).

Table 3. The mean value of zinc levels in terms of evaluated traits.

	Number of fertile tillers	Plant height (cm)	spike length (cm)	flag leaf length (cm)	Flag leaf width (cm)
Condition with zinc	2.33	86.17	8.30	12.63	1.07
Condition without zinc (Control)	1.80	79.27	7.53	11.86	0.99
$LSD_{0.05}$	0.328	3.655	0.483	0.738	0.075

Table 3 (cont.)

	Straw yield (g)	number of grains per main spike	Grain yield (g)	1000-grain weight (g)	Zinc content in the whole grain (pixels per gram)	Zinc content in the endosperm (milligrams per gram)
Condition with zinc	406.29	25.98	352.22	39.05	45.12	25.55
Condition without zinc (Control)	297.12	24.02	242.58	38.50	38.85	20.22
$LSD_{0.05}$	52.928	1.677	67.584	1.443	3.559	3.866

In the condition of zinc foliar application, RILs 55 and 44 had the highest and Zagros and Roshan varieties had the lowest zinc content of whole grain. Under control condition, RILs 83 and 55 had the highest and Azar and Roshan varieties had the lowest amounts of zinc content of whole grain. There were considerable advance due to zinc application for RIL44, RIL 55, Azar, Alvand, and Navid varieties in terms of zinc content of whole grain (Fig. 1). Under zinc foliar application, RIL 11 and Zagros and Roshan varieties had the highest and RILs 49 and 60 and Iniya66 and Navid varieties had the lowest zinc content of endosperm (fig. 5). Under control condition, Zarin, Roshan, Qods and Alvand varieties had the highest amounts of zinc in the endosperm and Iniya66 and Navid varieties, and RILs 51 and 60 had the lowest value. RILs 11 and 49, Zarin, Zagros, Mahdavi, and Qods varieties showed the most advance due to zinc application in view point of zinc content of endosperm (Fig. 2).

The highest genetic and phenotypic coefficients of variation (Table 4) belonged to the zinc content of endosperm. The lowest value of heritability (0.74) belonged to grain yield and 1000-grains weight, zinc content of endosperm, and zinc content of whole grain showed the highest heritability.

Table 4. Genetic coefficient of variation, phenotypic coefficient of variation, genetic variance, phenotypic variance, and heritability of studied traits in wheat genotypes.

Traits						SE(h^2)
Number of fertile tillers	0.07	0.09	12.90	14.64	0.78	0.07
Plant height	43.79	47.38	8	8.32	0.92	0.02
spike length	0.24	0.30	6.23	6.90	0.82	0.06
flag leaf length	4.02	4.37	16.38	17.06	0.92	0.03
Flag leaf width	0.01	0.01	8.58	9.12	0.89	0.04
Straw yield	4712.91	5454.88	19.52	21	0.86	0.04
Number of grains per spike	15.63	17.21	15.81	16.59	0.91	0.03
Grain yield	1962.16	2667.60	14.90	17.37	0.74	0.08
1000-grains weight	13.97	14.44	9.64	9.80	0.97	0.01
Zinc content of whole grain	40.07	44.27	15.08	15.85	0.91	0.03
Zinc content of endosperm	186.72	202.40	59.72	62.17	0.92	0.03

σ^2_g,σ^2_{ph} ,CV_g , CV_{ph}, h^2 and SE(h^2) represent genetic variance, phenotypic variance, genetic coefficient of variation, phenotypic coefficient of variation, , heritability and the standard error of heritability, respectively.

Correlation analysis (data don't shown) indicated that in both conditions grain yield had a significant positive relation with spike length, straw yield and number of grains per spike. In condition of zinc foliar application, there was a higher correlation between the yield and yield components. In both conditions grain yield did not have any significant correlation with zinc content of whole grain and endosperm.

Table 5. Results of the backward regression of studied traits on grain yield, in condition of zinc foliar application.

Traits entered into the model	Standardized regression coefficient
Number of fertile tillers	0.540**
Straw yield	0.255*
The number of grains per spike	0.918**
1000-grains weight	0.513**

* and ** represent significance at the 5% and 1% level, respectively.

Based on backward regression, in control condition (Table 5), the traits number of fertile tillers, straw yield, number of grains per spike, and 1000-grains weight remained in the model which explained %86.8 of variations of grain yield, so they were identified as the variables most effective on the grain yield. In the condition of zinc foliar application (Table 6), traits number of fertile tillers, spike length, number of grains per spike, and 1000-grains weight explained %95.9 of variation of grain yield, and were identified as the variables most effective on the grain yield.

Discussion

Significant differences among the studied genotypes in terms of all traits denoted the existence of high genetic variation among them. Significant Genotype × Zinc interaction about the two traits zinc content of the endosperm and whole grain indicated the different reactions of genotypes to zinc foliar application in terms of these traits. The different response of genotypes to zinc application was previously reported by some researches (Graham and Rengel, 1993; Cichy *et al.*, 2005; Stein *et al.*, 2007; Palmgren *et al.*, 2008; Cakmak *et al.*, 2010).

Table 6. Results of the backward regression of studied traits on grain yield, in control condition.

Traits entered into the model	Standardized regression coefficient
Number of fertile tillers	1.005^{**}
Straw yield	$0.131^{n.s}$
The number of grains per spike	0.840^{**}
1000-grains weight	0.527^{**}

* and ** represent significance at the 5% and 1% level, respectively.

The mean value for most traits including some vegetative characteristics, yield and its components, and grain zinc content under zinc foliar application was higher than the control condition. This difference showed the positive response of genotypes to the zinc application in terms of these traits (Welch, 1986; Rashid and Ryan, 2004; Hossein Abadi *et al.*, 2006; Peck *et al.*, 2008). Increase in grain yield under zinc foliar application, may be due to increasing the amount of growth hormones such as indole acetic acid (IAA) or increasing the amount of chlorophyll (Welch, 1986; Rengel, 1999; Rashid and Ryan, 2004).

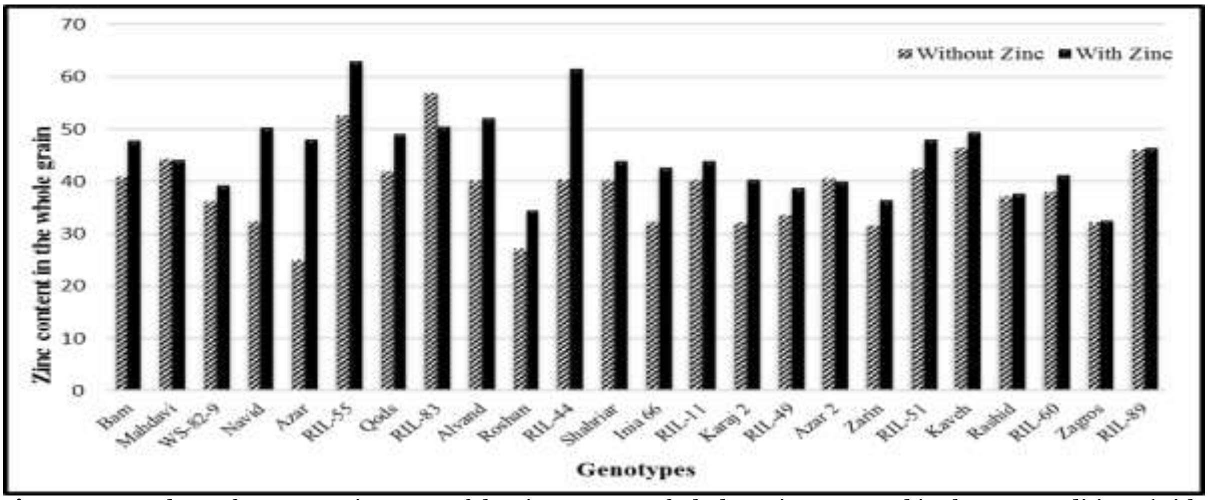

Fig. 1. Mean values of genotypes in terms of the zinc content of whole grain compared in the two conditions (with and without zinc foliar application; $LSD_{0.05}$= 8.136).

It was demonstrated that in view point of human nutrition, the practical and profitable part of wheat grain zinc is the portion which saved in endosperm and the other parts will lose during the flour making process (Cakmak, 2008; Palmgren *et al.*, 2008). Therefore, genotypes with higher content of zinc in their endosperms will be much important. In this study Roshan and Zagros varieties showed high zinc content of endosperm under both zinc application and control conditions, but the zinc content of whole grain about these varieties was low. In general, it can be stated that probably, those genotypes with low zinc content of whole grain and contrariwise, high zinc content of endosperm, somehow managed to use particular strategies and mechanisms to transfer zinc from their embryo and aleurone layers into the endosperm. Also, it is possible that the genetic potential of such varieties is in ways that from the very beginning stages of grain creation accumulate more zinc in their endosperm whereas some others accumulate this zinc in their embryo and aleurone layers (Rengel, 1999; Hcisalihoglu *et al.*, 2001; Cakmak *et al.*, 2002; Ozturk *et al.*, 2006; Yilmaz, 2007). The answer to these hypotheses requires more future studies.

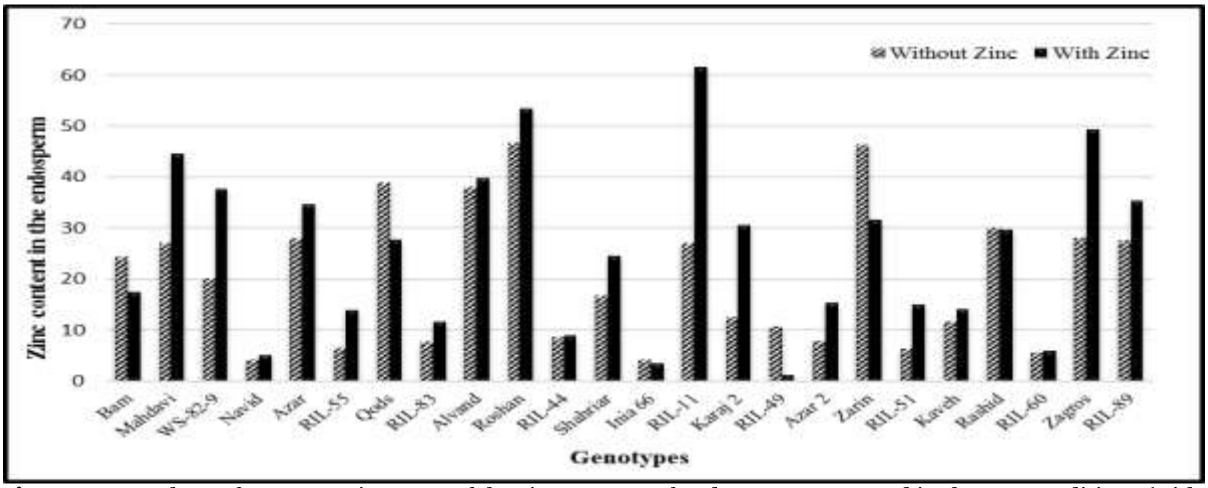

Fig. 2. Mean values of genotypes in terms of the zinc content of endosperm compared in the two conditions (with and without zinc foliar application; $LSD_{0.05}$= 15.730).

Present study showed a high variation and heritability about zinc content of endosperm indicating the potential of improving this trait among genotypes (chahal and Gosal, 2002; stein *et al.*, 2007). Also, correlation and regression analysis expressed the independence of zinc content of endosperm from grain yield. Therefore, it can be stated that considering this condition, selection for this traits can be done independently from grain yield or through index selection method. This point can be considered as an advance for improving grain yield and zinc simultaneously (Richards, 1997; Chahal and Gosal, 2002). Because, the negative correlation of grain yield and micronutrient content of kernel was reported in some studies (Palmgren *et al.*, 2008) that render difficult the selection programs.

Finally, results of this study showed that there was an acceptable genetic variation in the few number of studied genotypes for the two traits zinc content of whole grain and zinc content of endosperm. This result approved the possibility of improving these traits through wheat conventional breeding procedures. Another positive and important point is that the traits zinc content of whole grain and endosperm were not significantly correlated with yield. This indicates the independence of these traits from yield and the possibility of selecting these traits apart from the yield. Of course, the proposed strategy is selection of those varieties which not only are zinc efficient but also have a high yield.

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