

NATURAL VARIATION AND IDENTIFICATION OF MICROELEMENTS CONTENT IN SEEDS OF EINKORN WHEAT (*TRITICUM MONOCOCCUM*)

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Abstract: Micronutrient deficiencies in human beings are common problems, especially in developing world. Among the micronutrient deficiencies, zinc (Zn) and iron (Fe) deficiencies are particularly important affecting severely health of humans. Major reason for the widespread occurrence of micronutrient deficiencies in human beings is the high and monotonous consumption of cereal-based foods with very low content of micronutrients. An increase in concentration of Zn and Fe in grain is, therefore, a high-priority research area. Exploitation of large genetic variation for Zn and Fe existing in cereals germplasm is an important approach to minimize the extent of Zn and Fe deficiencies in developing world. In the present study, the variation for seed content of micronutrients (Zn, Fe, Mn and Cu) in 54 accessions of einkorn wheat (*Triticum monococcum*) was tested. The accessions have been first grown under same field conditions in 2 locations in Turkey, and the seeds obtained from the field trials were analyzed for micronutrients. In addition, a mapping population with 168 recombinant inbred lines which were grown in 4 locations in Germany, Turkey and Italy has also been tested for the variation of micronutrients in seeds and analyzed for identification of QTLs associated with micronutrient content in seeds

The results obtained showed existence of large genotypic variation in content of micronutrients. The contents of Zn and Fe among the 54 einkorn wheat accessions varied from 0.21 to 2.16 $\mu\text{g seed}^{-1}$ for Zn with an average of 1.19 $\mu\text{g seed}^{-1}$ and from 0.54 to 3.09 $\mu\text{g seed}^{-1}$ for Fe with an average of 1.15 $\mu\text{g seed}^{-1}$. There was a close positive relationship between seed contents of Fe and Zn. The genetic basis of this variation was elucidated by QTL analysis, using a mapping population comprising 168 recombinant inbred lines that was developed from a cross between 2 cultivated

Einkorn genotypes (e.g., ID-362 bread-making quality poor and ID-331 bread-making quality good). From the parents ID-362 had always more Zn than the other parent in all four locations. The four locations presented different mean values, varying from 1.09 to 2.16 $\mu\text{g seed}^{-1}$ for Zn content, from 0.83 to 1.97 $\mu\text{g seed}^{-1}$ for Fe content, from 1.43 to 1.97 $\mu\text{g/seed}^{-1}$ for Mn content and from 0.14 and to 0.24 $\mu\text{g seed}^{-1}$ for Cu content. Pooling the results of the four trials, a major QTL, common to all four microelements and explaining from 10 to 30% of the variation (depending on the mineral assayed), was observed only on the chromosome 5, and not on the other chromosomes. The Einkorn germplasm tested had a significant variation for micronutrients, especially Zn and this variation could be exploited in breeding programs. Chromosome 5 likely carries the genes affecting micronutrient accumulation in Einkorn seeds

Keywords: *Triticum monococcum*, micronutrients, zinc deficiency, iron deficiency

INTRODUCTION

Currently, half of the world population suffers from micronutrient deficiencies, especially Fe and Zn. Zinc and Fe deficiencies cause very serious health problems such as impairments in the immune system, physical growth, mental and cognitive development and increases in anemia, morbidity and mortality (Welch and Graham 1999, Hotz and Brown 2004). As a main source of calorie intake, cereal-based foods are extensively consumed in the developing world. However, cereals are inherently very poor both in concentration and bioavailability of Zn and Fe in seeds. Welch and Graham (1999) reported that cereal grains are the primary source of Fe and Zn for people in developing countries; however, intakes do not satisfy their mineral requirements. Increasing the total amount and bioavailability of Zn and Fe in food crops is, therefore, a big challenge.

One major approach to minimize micronutrient deficiencies in human beings in developing countries is the selection and development of new plant genotypes with high grain density of micro nutrients in edible parts. Existence of large genetic variation for micronutrients in seeds is essential for a successful breeding program aiming at development of micronutrient-rich new plant genotypes. Several authors have reported a large genotypic variation for Zn and Fe in different cereal species (Peterson et al. 1986, Rengel et al. 1999, Graham et al. 1999, Cakmak et al. 2000). A number of recent studies deal with genetic variation for micro-element content in seeds, such as in bean (Beebe et al. 2000), rice (Gregorio et al. 2000), wheat (Ortiz-Monasterio and Graham 2000, Cakmak et al. 2004) and maize (Banziger and Long 2000). In cultivated wheats, variation in seed Zn and Fe concentration is relatively small and seems to be not promising for a genetic improvement of wheat (Rengel et al. 1999, Cakmak et al. 2004). Compared to cultivated wheat cultivars, wild and primitive wheats, such as *Triticum monococcum*, *T. dicoccon* and *T. dicocoides*, were found to be much more promising genetic donors for micronutrients (Cakmak et al. 2000, 2004, Ortiz-Monasterio and Graham 2000). Among wild

wheat germplasm, the emmer wheat, *T. dicoccoides*, showed the largest variation and the highest concentration of micronutrients, especially for Zn, and is considered a promising genetic source to improve Zn and Fe concentrations of wheat seeds (Cakmak et al. 2004). However, little is known for the diploid wheat *T. monococcum*. Einkorn wheat produces protein equal to durum when grown under adverse condition (Vallega 1979). In addition, the seed amino acid composition in Einkorn is similar to those of other wheats, irrespective of a very large variation in total proteins (Acquistucci et al. 1995). *T. monococcum* was also found to contain high levels of both protein and carotenoids (Borghi et al. 1996). According to Borghi et al. (1996). *T. monococcum* genotypes contain nearly 7 times more carotenoids than cultivated wheat. It is important to study the genetic potential of *T. monococcum* and the mapping populations derived from *T. monococcum* for micronutrients and to characterize the localization of genes and QTLs involved in micronutrient accumulation in seeds.

The main objectives of this study were a) to determine the degree of genetic variability for micro-elements in Einkorn wheat accessions; and b) to identify QTL associated with microelements content (particularly Zn and Fe) in Einkorn wheat, using 168 recombinant inbred lines derived from a cross between ID 362 (poor breadmaking quality) and ID 1331 (good breadmaking quality).

MATERIALS AND METHODS

In the present study, seeds of 54 accession of Einkorn (*T. monococcum*) were used for analysis of Zn, Fe, Mn and Cu. These accessions were kindly obtained from USDA. All lines were grown in 2003–2004 at Adana, Turkey, in two contrasting environments (upland and lowland conditions). The seeds obtained from the field trials were dehulled and analyzed for Zn, Fe, Mn and Cu by using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Seed samples were digested by using a microwave digesting system and then subjected to ICP tests. The measurements were checked using the certified mineral nutrients values in durum wheat flour samples obtained from the National Institute of Standards and Technology. The reference materials used was the durum wheat flour (8436).

The Map

A mapping population has been used to study genetic variation for micronutrients and to identify QTLs which are associated with high micronutrient concentration in seeds. The original consensus map (Fig. 1, Taenzler et al. 2002) was built from two populations of 117 and 168 F₂ plants, respectively, from which F₃ families were derived. Population 1 (117 progenies) was derived from a cross between ID 49, a wild Einkorn line (*T. m. ssp. boeoticum*), and ID 69, a free-threshing, cultivated Einkorn (*T. m. ssp. monococcum* var. *sinskajae*). Population

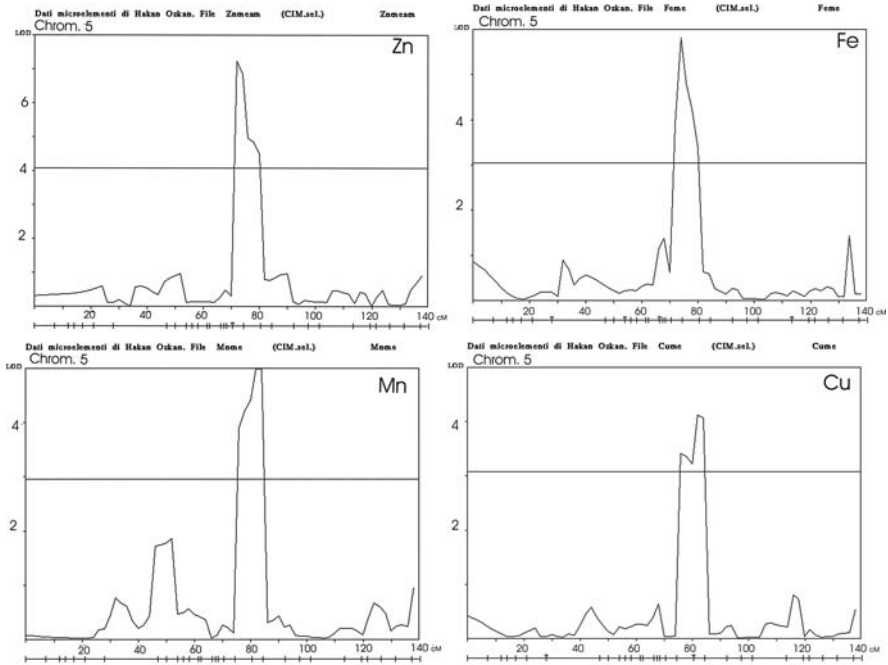


Figure 1. Localisation on einkorn chromosome 5 of QTLs controlling Zn, Fe, Mn and Cu content. The analyses are based on the average contents of four locations. The LOD thresholds corresponding to $P \leq 0.05$ (solid lines) are 4.09, 3.04, 2.96 and 3.08, respectively, while the LOD thresholds corresponding to $P \leq 0.01$ (dotted lines) are 5.22, 4.10, 3.76 and 4.44, respectively

2 (168 progenies) was derived from a cross between two cultivated Einkorn lines with different breadmaking quality, ID 362 (poor quality) and ID 1331 (good quality). The integrated map, based on the data sets for both populations and including 477 markers (32 RFLPs, 438 AFLPs, one morphological and six storage-proteins), was assembled using JoinMap version 2.0 (Stam and Van Ooijen 1995). Its total length is of 856 cM, with an average distance of 1.8 cM between markers.

Microelements Content

To assess microelements content, F₃-derived families of Population 2 were grown in Sant'Angelo Lodigiano (Italy) in 1998 (S98) and 2004 (S04), in Cologne (Germany) in 2003 (K04) and in Adana (Turkey) in 2004 (A04), in 10 m² plots, following the agronomic practices described by Castagna et al. (1995). After harvest, the seeds were manually dehulled and microelements content assessed as above described. Data obtained for F₃-derived families were taken as indicators of values for individual F₂ plants.

QTL Analysis

To localize the QTLs responsible for microelements content, the chromosome marker order determined by JoinMap was transferred to the computer program PLABQTL (Utz and Melchinger 1996), together with laboratory data (Fe, Zn, Mn and Cu content) of Population 2. The numbers of markers mapped in Population 2 and considered in our QTL analysis were 36, 28, 33, 18, 37, 26 and 13, respectively, for linkage groups 1 to 7 (data not shown). The allelic average substitution value of the chromosome fragment hosting the microelement-related gene was determined by halving the differences between the genotypic values of the two homozygotic classes.

RESULTS AND DISCUSSION

Genetic Variation for Microelements between Einkorn Accessions

The analysis of microelements concentration and content in seeds of einkorn accessions grown in Adana, in two environments, are presented in Table 1. Clear variation was observed in seed micronutrient concentrations among the accessions. Zinc content of the seeds varied from 0.21 to 2.16 $\mu\text{g seed}^{-1}$, with an average of 1.21 $\mu\text{g seed}^{-1}$ and Fe content varied from 0.54 to 3.09 $\mu\text{g seed}^{-1}$, with an average of 1.27 $\mu\text{g seed}^{-1}$. A similar variation was also found with Mn, but in the case of Cu the genetic variation was lesser.

There was a significant ($p \leq 0.05$) positive correlation between microelements in two locations, most noticeably for Zn, which correlated with Fe, Mn and Cu (data not shown). Similar results were reported by Cakmak et al. (2004) for Zn and Fe. The correlation between Fe and Zn in grain was also reported by Peterson et al. (1986) and Graham et al. (1999). This may point to common genetic mechanisms controlling Zn and Fe uptake and seed deposition. Correlations among microelements indicate that the improvement of one micronutrient (e.g. Zn) may

Table 1. Concentration and content of Zn, Fe, Mn and Cu in seeds of 54 *Triticum monococcum* accessions grown in two different place

Locations	Concentration (mg kg ⁻¹ dry wt.)							
	Zn		Fe		Mn		Cu	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Upland	51	36–76	43	32–61	42	26–60	6.6	4.1–10
Lowland	59	44–84	51	35–85	56	31–92	6.2	3.9–9.1
	Content ($\mu\text{g seed}^{-1}$)							
	Zn		Fe		Mn		Cu	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Upland	1.21	0.37–2.07	1.02	0.54–2.07	1.01	0.53–2.08	0.16	0.10–0.23
Lowland	1.17	0.21–2.16	1.27	0.62–3.09	1.32	0.54–2.23	0.41	0.10–2.56

simultaneously improve the content of other micronutrients (e.g. Fe). The variation for Zn, Fe, Mn and Cu content in seed was much greater when compared to the variation found for the concentration (Table 1). These results suggest that 1) ample genetic variation is detected in the progeny of crosses if the parents have different microelements contents; and 2) it is possible to develop new wheat cultivars with higher Zn and Fe content.

Mapping Population

In the ID 362 x ID 1331 mapping population, the content of four microelements was determined in four different locations: S98, K03, S04 and A04 (see Material and Methods). The parent ID 362 always showed higher content of microelements than the ID 1331 (Table 2); the average lowest and highest values over the locations were 2.06 vs 3.12 for Zn, 1.63 vs 1.80 for Fe, 1.76 vs 2.06 for Mn and 0.12 vs .018 for Cu.

The four locations differed in mean values of micronutrients, varying from 1.09 (A04) to 2.16 (S04) for Zn content, from 0.83 (A04) to 1.97 (K03) for Fe content, from 1.43 (K03) to 1.97 (S98) for Mn content and from 0.14 (S98 and A 04) to 0.24 (S04) for Cu content. Among progenies within locations, the values between samples with low and high microelements content varied 2- to 5-fold; averaged

Table 2. Average values (\pm s.e.) recorded for Population 2 (parents and F3-derived progenies) for microelements content. For F3 progenies, the field of variation covered by single progenies is also reported.2164839550

Micro element	Location						Average
		Genotype	S98	K03	S04	A04	
Zn	ID 362	2.91 \pm 0.04	3.50 \pm 0.00	2.95 \pm 0.18	–*	3.12 \pm 0.19	
	ID 1331	2.40 \pm 0.11	2.04 \pm 0.34	1.74 \pm 0.07	–	2.06 \pm 0.19	
	progenies	1.98 \pm 0.03	1.50 \pm 0.03	2.16 \pm 0.03	1.09 \pm 0.02	1.70 \pm 0.02	
	range	1.29–3.40	0.96–2.85	1.26–3.68	0.49–1.72	1.15–2.33	
Fe	ID 362	1.89 \pm 0.03	2.34 \pm 0.44	1.18 \pm 0.07	–	1.80 \pm 0.34	
	ID 1331	1.78 \pm 0.17	2.03 \pm 0.69	1.08 \pm 0.03	–	1.63 \pm 0.28	
	progenies	1.46 \pm 0.02	1.97 \pm 0.04	1.15 \pm 0.02	0.83 \pm 0.02	1.36 \pm 0.02	
	range	0.92–4.63	0.99–4.59	0.71–1.99	0.33–1.31	0.96–1.96	
Mn	ID 362	2.65 \pm 0.07	1.71 \pm 0.36	1.81 \pm 0.11	–	2.06 \pm 0.30	
	ID 1331	2.30 \pm 0.07	1.60 \pm 0.15	1.39 \pm 0.02	–	1.76 \pm 0.28	
	progenies	1.98 \pm 0.03	1.43 \pm 0.03	1.47 \pm 0.02	1.70 \pm 0.05	1.64 \pm 0.02	
	range	1.28–2.91	0.81–2.50	0.78–2.22	0.62–3.52	1.16–2.40	
Cu	ID 362	0.06 \pm 0.00	0.18 \pm 0.02	0.31 \pm 0.02	–	0.18 \pm 0.07	
	ID 1331	0.05 \pm 0.01	0.12 \pm 0.04	0.20 \pm 0.01	–	0.12 \pm 0.04	
	progenies	0.14 \pm 0.03	0.15 \pm 0.00	0.24 \pm 0.00	0.14 \pm 0.00	0.17 \pm 0.00	
	range	0.04–0.21	0.01–0.27	0.16–0.38	0.06–0.26	0.11–0.24	

* No parents analysed

over location, the progenies with high microelements content showed values double than those with low content. The frequency distribution of Zn, Fe, Mn and Cu level showed considerable transgression in both directions for all microelements (data not shown). This suggests that both accessions carry genes with alleles contributing to an increased content for all microelements tested.

QTL Analysis

For all the traits considered, two LOD score thresholds were computed, the first corresponding to a significance level of $P \leq 0.05$ and varying between 2.96 and 4.08, and the second corresponding to a significance level of $P \leq 0.01$ and varying between 3.76 and 5.22, depending on the microelement considered. The analyses revealed a major QTL on chromosome 5, insisting in the same interval and present in two environments (S98 and K03) for Zn and Mn, and only in K03 for Fe. For Zn and Mn content, second QTL was detected on chromosome 1 in one location (K03). No QTLs were evident for Cu content, even though smaller peaks were present in the same chromosome 5 position of the other microelements. The analyses carried out by pooling the data of the four locations confirmed the existence of a strong QTL ($P \leq 0.05$) between 71 cM and 86cM from the tip of the short arm of chromosome 5 that is associated with Zn, Fe, Mn and Cu content. Three out of four QTLs (for Zn, Fe and Mn) were significant also at LOD scores corresponding to $P \leq 0.01$ (Fig. 1).

The substitution value of the allele originating from ID 362 varied between +0.30 (for Cu) and +0.42 (for Zn), indicating that the allelic segment encompassing the region of the QTL in ID 362 induces an increase of 0.30 to 0.42 units (depending on the microelement) compared with the same allelic fragment of ID 1331. Minor dominance effects (+0.15 and +0.12) of complementary sign to the additive effect were observed in the case of Zn and Fe, respectively.

The results obtained suggest that the *T. monococcum* is a promising genetic resource for working genetic variation and identification of genes for micronutrients, especially Zn. Studies are on-going to collect more information on the localization of genes affecting content of micronutrients (especially Zn) in different genetic stocks derived from *T. monococcum*.

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