

## Mapping QTLs related to Zn and Fe concentrations in bread wheat (*Triticum aestivum*) grain using microsatellite markers

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### ABSTRACT

Mineral nutrient malnutrition, particularly deficiency in zinc and iron, afflicts over 3 billion people worldwide. Biofortification of food crops is the best approach for conciliating the micronutrient deficiencies. Understanding the genetic basis of their accumulation is the preconditions for enhancing of these micronutrients. In our study, a mapping population of a set of 118 recombinant inbred lines (RILs), derived from a cross between an Iranian local variety "Tabassi", and a European elite variety "Taifun" were analyzed for grain iron and zinc contents. The grains of the RILs population were measured for Fe and Zn concentrations using atomic absorption spectrophotometer. The grain Fe and Zn concentrations in the RILs population ranged from 9.65 to 71.4 and 44.35 to 95.6 mg/kg, respectively. An available linkage map, comprising of 205 simple sequence repeats (SSR) markers and three morphological loci as well, comprising 26 linkage groups, was used for mapping QTLs of interest. For the grain Fe concentration, six significant QTL (LOD $\geq$ 2.5) were detected on chromosomes 2A, 3D, 4D, 7B, and 7D explaining 29.1% of the phenotypic variations of the trait. For the grain Zn concentration, two significant QTL was detected on chromosomes 1A and 4A explaining 51.45% of the phenotypic variations of the trait.

**Keywords:** Bread wheat, Grain Fe, Grain Zn, Microsatellite markers, QTL mapping, Recombinant inbred lines.

### INTRODUCTION

The nutritional health of humans is primarily dependent on plant foods either directly or indirectly (DellaPenna, 1999). Mineral nutrient malnutrition, and particularly deficiency in Zn and Fe, afflicts over 3 billion people worldwide (Welch and Graham, 2004), resulting in overall poor health, anemia, increased morbidity and mortality rates, and low worker productivity (Cakmak, 2002; Hotz and Brown, 2004; Sanchez and Swaminathan, 2005). This nutritional deficiency has been termed as hidden hunger (Welch and Graham, 1999). Biofortification of staple food crops for enhanced micronutrient content through genetic manipulations is the best option available to alleviate hidden hunger with little recurring costs (Welch and Graham, 2004; Monasterio, 2007; Ortiz-Monasterio *et al.*, 2007). Availability of useful variability in germplasm and understanding of its genetic architecture are the prerequisites for a breeding program aimed at biofortification of crop plants (Tiwari *et al.*, 2009). Realizing the importance of biofortification, several studies were undertaken for the evaluation of germplasm and advance breeding lines for grain Fe and Zn content (Cakmak *et al.*, 2000; Monasterio and Graham, 2000; Chhuneja *et al.*, 2006; Morgounov *et al.*, 2007; Rawat *et al.*, 2009). However, only a few studies have focused on studying the genetics of accumulation of micronutrients in the grains of major cereals like wheat and rice (Stangoulis *et al.*, 2007; Shi *et al.*, 2008). Understanding the genetic basis of accumulation of micronutrients in the grains and mapping of the quantitative trait loci (QTLs) will provide the

basis for devising the plant breeding strategies and for improving grain micronutrient content through marker-assisted selection (Tiwari *et al.*, 2009). We analyzed a population of 118 recombinant inbred lines of bread wheat (*T. aestivum*) for grain Fe and Zn concentrations. The present communication describes the mapping of QTL for grain Fe and Zn in this population.

## MATERIALS AND METHODS

### Plant material

The plant materials used for mapping QTLs presumably controlling the grain Fe and Zn concentrations consisted of a set of 118 RILs, derived from a cross between an Iranian local variety Tabassi, and a European elite variety Taifun through the single seed descent method. Detailed information on these RILs and the molecular linkage map generated using this population was described by Kordenaeej (2008). The parents and the RILs were planted in 1.5 m rows, with row–row spacing of 50 cm at University of Natural Resources and Life Sciences, Vienna during 2004–05. Standard agronomic practices were followed for raising the RILs population at this location. Seeds of individual lines were harvested manually and hand threshed to avoid any soil contamination.

### Micronutrient assaying

The grain Fe and Zn concentrations of available seeds of the RILs and the parents were individually analyzed based on the Munson and Nelson protocol (Munson *et al.*, 1990). Measurements of Fe and Zn concentrations at first were done for 20 random grain samples of the population in the two replications. As there was no difference between the replications, the measurement continued only by one replication. Briefly, the grains were crushed in a porcelain mortar. Then 2 g of each powdered sample was placed in an electric furnace. Furnace was set in a way that took an hour to reach 700°C and then samples were heated at this temperature for 8 hours. After this time, the samples were removed from the furnace and crucibles were allowed to be cooled at room temperature. Then 10 ml of 1 M hydrochloric acid was added to the crucibles and heated up to 80°C temperature, until the white colored steam was observed. The crucibles of the contents were passed through a Whatman 42 filter paper. Required volume was made after the completion of digestion process, and digests were analyzed by an atomic absorption spectrophotometer (Analytikjena Grade), and the grain Fe and Zn con-

centrations were computed as mg/kg of seeds.

### Construction of linkage map

The Linkage map was provided by Kordenaeej, (2008) based on a total of 205 wheat SSR markers loci and 3 morphological markers loci including spike awn, waxiness of flag leaf, and spike pubescence on 118 F2:8 lines of the Tabassi × Taifun cross combination, applying the JoinMap version 3.0 (Stam, 1993). The recombination fractions were converted to centi-Morgan with Kosambi's mapping function (Kosambi, 1943). Minimum logarithm of odds (LOD) threshold of 3.0 was chosen to group marker loci.

### Statistical analysis

Pearson correlation coefficient was calculated to study correlation, if any, between Fe and Zn accumulation in the grains of the RILs population. Student's t-test was used to test the significant differences between the parents data.

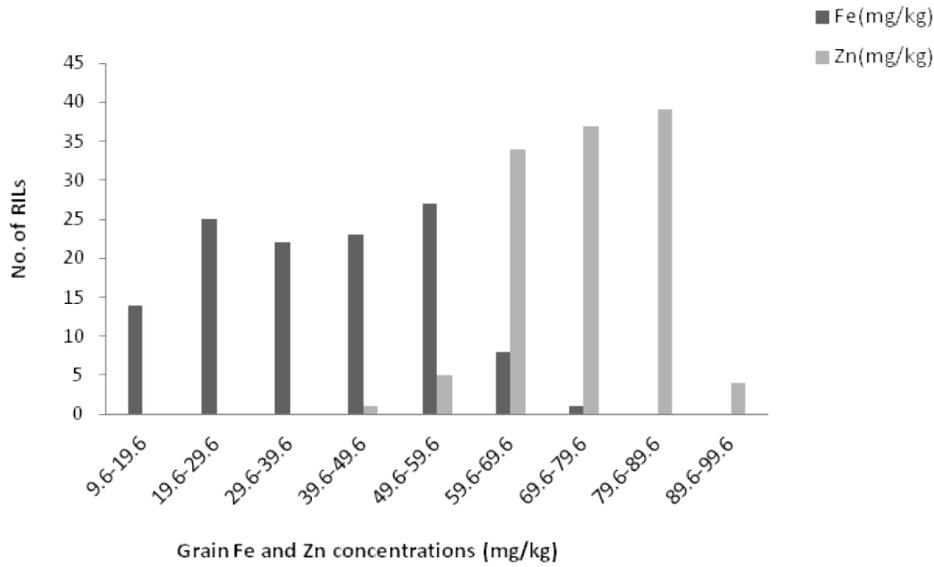
### QTL mapping

The grain Fe and Zn data for all the recombinant inbred lines were individually used for detecting the QTLs governing grain Fe and Zn concentrations in the mapping population. The positions and effects of QTLs were determined following composite interval mapping (CIM) using the software QTL CARTOGRAPHER v. 2.5 (Wang *et al.*, 2012). The significant threshold of the (LOD) scores for detection of the QTLs were calculated based on 1000 permutations at  $P \leq 0.05$  (Churchill and Doerge, 1994). Proportion of observed phenotypic variation explained due to a particular QTL was estimated by the coefficient of determination ( $R^2$ ) using the maximum likelihood method for CIM.

## RESULTS

### Grain Fe and Zn concentration

The micronutrient analyses of the parental lines showed that Taifun had relatively higher grain Fe and Zn concentrations than Tabassi, because Taifun is an improved and high quality variety and Tabassi is a local unimproved variety (Table 1). For Taifun, the grain Fe and Zn concentrations, averaged over the two replications, were 27.34 and 80.45 mg/kg, respectively. Whereas in Tabassi, the average concentrations were 22.065 and 77.725 mg/kg, respectively. A wide range of variation was observed in the RIL population. The population showed continuous distribution (Figure 1), and trans-



**Figure 1.** Distribution of Taifun / Tabassi RIL population for grain Fe and Zn. Average grain Fe concentrations (milligrams / kilogram) in Taifun and Tabassi were 27.345 and 22.065, respectively, and the average grain Zn concentration (milligrams/kilogram) was 80.45 for Taifun and 77.725 for Tabassi.

**Table 1.** Grain Fe and Zn concentrations (mg/kg) of the parental varieties and the RILs population.

Element	Parents		RILs (range)	Mean	Standard error
	Tabassi	Taifun			
Fe (mg/kg)	22.0265	27.345	9.65-71.4	38.920	1.4
Zn (mg/kg)	77.725	80.45	44.355-95.6	74.529	0.89

gressive segregants were observed for both the Fe and Zn concentrations (Table 1, Figure 1).

**Correlation analysis**

To study the relationship between the grain Fe and Zn concentrations in the RILs, Pearson’s correlation coefficient (r) was determined for all the data. A minus significant correlation was observed between Fe and Zn concentrations in the grains, indicating that the grain Fe and Zn accumulation may affect each other negatively (Table 2).

**QTL analysis for the grain Fe and Zn concentrations**

A framework linkage map already constructed by Kordenaeej (2008), comprising of 205 simple sequence repeats (SSR) markers loci and 3 morphological markers loci including spike awn, waxiness of flag leaf, and spike pubescence on 118 F2:8 lines of the Tabassi ×

Taifun cross combination, was used for mapping QTL of the grain Fe and Zn concentrations. For the grain Fe concentration, 6 significant QTLs (LOD≥2.5) were detected, whereas for the grain Zn concentration, 2 significant QTL were detected (Table 3). The 6 significant QTL for the grain Fe were mapped on the chromosomes 2A, 3D, 4D, 7B, and 7D. The two QTL that were mapped on the chromosome 2A were located in the marker intervals *Xgwm312-Xgwm817* and *Xgwm817-Xgwm630*, respectively. The other QTL detected on the chromosomes 3D, 4D, 7B, and 7D were in the marker intervals *Xgwm1047-Xgwm383*, *Xgwm4670-Xgwm194*, *Xgwm767-Xgwm3036*, *Xbarc184-Xgwm1055*, respectively.

The two QTL on the chromosome 2A, designated as *QFe.shu-2A*, had LOD scores of 3.91 and 3.71 based

**Table 2.** Pearson correlation coefficient between grain Fe and Zn concentrations in the RIL population.

	Fe	Zn
Fe	1	
Zn	-0.365**	1

\*\* Significant at “P < 0.01”.

on the grain Fe content measured in the RIL population, with the respective coefficient of determination (R<sup>2</sup>) values of 12.58 and 17.04. Likewise, the QTL on the chromosomes 3D, 4D, 7B, and 7D designated as *QFe.shu-3D*, *QFe.shu-4D*, *QFe.shu-7B* and *QFe.shu-7D* were detected with LOD scores of 2.76, 2.54, 2.52 and 2.78 with respective R<sup>2</sup> values of 44.71, 44.6, 47.0 and 8.94 respectively (Table 3). The QTLs *QFe.shu-2A*, *QFe.shu-7B*, and *QFe.shu-7D* had positive alleles (allele that increases grain Fe concentration) from Taifun, whereas the QTLs *QFe.shu-3D* and *QFe.shu-4D* had negative alleles from Tabassi (Table 3). For the grain Zn concentration, the two significant QTLs mapped on the chromosomes 1A and 4A in the marker intervals *Xgwm3094-Xgwm164* and *Xgwm4026-Xgwm1081*, respectively (Table 3). The QTL mapped in the marker interval *Xgwm3094-Xgwm164* (Figure 2) was detected at LOD score of 2.97 with respective R<sup>2</sup> value 50.79 (Table 3). This QTL was designated as *QZn.shu-1A*. The other QTL for grain Zn concentration mapped in the marker interval *Xgwm4026-Xgwm1081*. This QTL was designated as *QZn.shu-4A*. The QTL *QZn.shu-1A* had a positive allele from Tabassi, whereas the QTL *QZn.shu-4A* in the marker region *Xgwm4026-Xgwm1081* had a positive allele from Taifun (Table 3).

## DISCUSSION

The accumulation of micronutrients in cereal grains involves a plethora of processes, including mobilization from the soil, absorption from the rhizosphere, translocation from roots, remobilization from vegetative tissues, and deposition in bioavailable forms in cereal grains, each of which is controlled by numerous genes (Bouis and Welch, 2010). Thus it has the quantitative inheritance pattern and can be mapped by QTL mapping techniques. However, there is limited information about the physiological and the genetic bases of these processes (Welch and Graham, 2004), and further basic research is needed (Xu *et al.*, 2011). So far, only a few

studies have reported the genetics of accumulation of Zn and Fe in the wheat grains (Shi *et al.*, 2008; Chhuneja *et al.*, 2006). The parental lines used by Shi *et al.* (2008) had grain Zn concentration of 34.4 (Hanxyuan 10) and 25.9 (Lumai 14) mg/kg, and the doubled-haploid (DH) population generated from this cross, showed transgressive segregation, with some DHs having grain Zn as high as 50.8 mg/kg. The cultivated hexaploid wheat germplasm has a narrow range for grain Fe and Zn concentrations. Morgounov *et al.* (2007) reported grain Fe concentration in the range of 39-48 mg/kg in a set of 25 spring wheat cultivars and 34-43 mg/kg for 41 winter wheat cultivars with the exception of one spring wheat cultivar, Chelyaba, that had grain Fe concentration of 56 mg/kg. Similarly, the Zn concentrations reported in the winter wheat and the spring wheat sets were 23-33 and 20-39 mg/kg, respectively (Tiwari *et al.*, 2009). Although the grain Fe and Zn concentrations in Taifun and Tabassi were in the same range as reported for the cultivated hexaploid wheat germplasm (Morgounov *et al.*, 2007), the grain Fe and Zn concentrations in Taifun were relatively higher than Tabassi. This can be explained from the fact that Taifun is an improved and high quality variety and Tabassi is a local unimproved variety. A few RILs had grain Fe of 59.6-69.6 mg/kg and Zn concentration of 89.6-99.6 mg/kg. One RIL had grain Fe concentration of 71.4 mg/kg exceptionally. Also 3 RILs had grain Zn concentration more than 90 (90-96) mg/kg. The transgressive segregation in the RIL population is an evidence of presence of different sets of genes in the parental lines for the target traits. Of the six significant QTL detected for the grain Fe, four inherited positive alleles from Taifun on the chromosomes 2A, 7B, and 7D and the other had negative alleles from Tabassi on the chromosomes 3D and 4D (Table 3). Of the two significant QTL detected for Zn, one had a positive allele from “Taifun” on the chromosome 4A and the other had a negative allele from Tabassi on the chromosome 1A (Table 3). The QTL for grain Fe and Zn mapped in the present research, explain 29.10% and 45.51% of the total phenotypic variation, respectively.

Similar to our results, Tiwari *et al.* (2009) using a set of 93 RILs, detected two significant and one suggestive QTL for grain Fe concentration on chromosomes 2A and 7A. Also they found one significant and one suggestive QTL for grain Zn concentration on chromosome 7A. These QTL explained only 25-30% of the total phenotypic variation. In addition Shi *et al.* (2008)

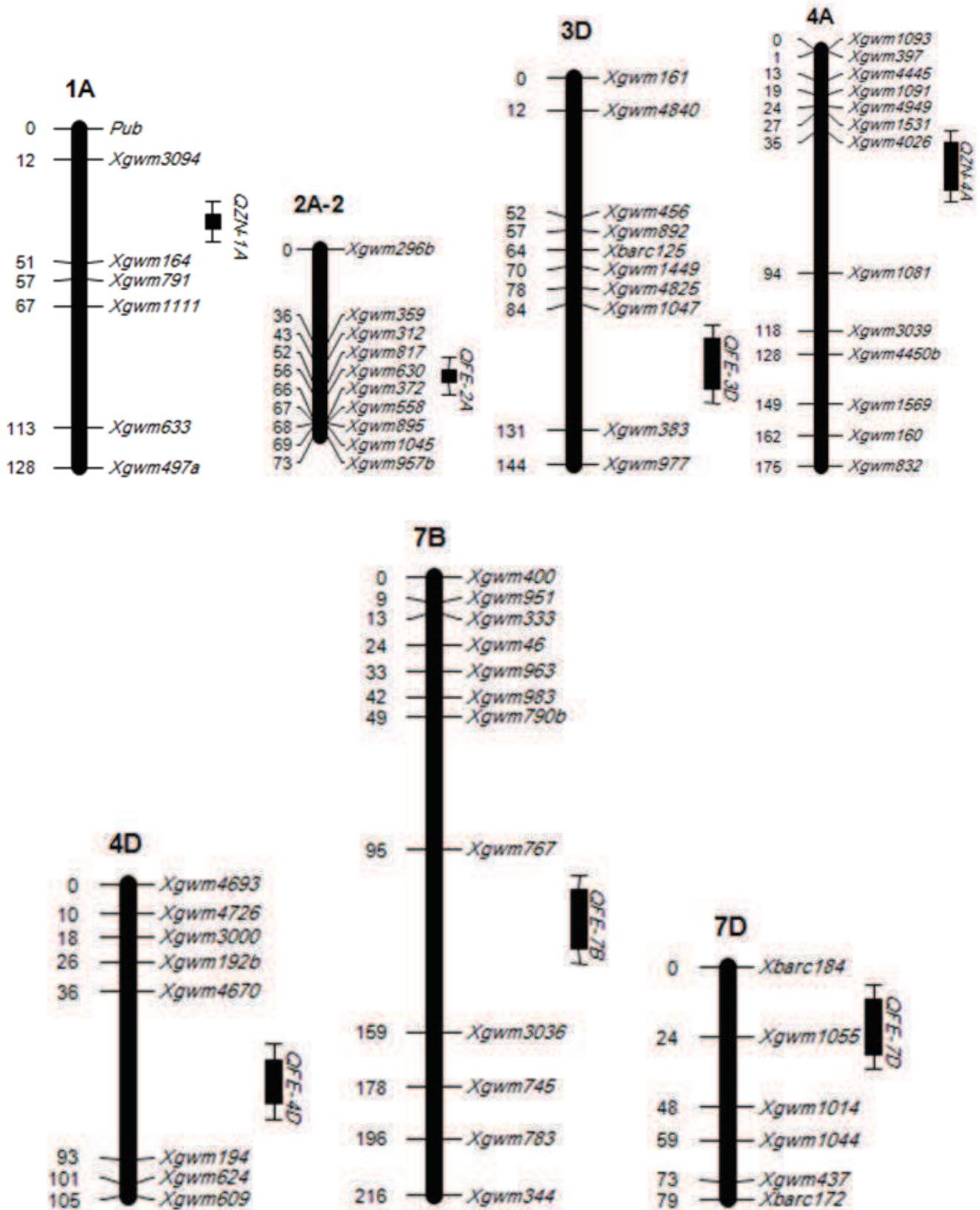


Figure 2. Chromosomal locations of the significant QTLs for grain Fe and Zn concentrations. Values on the left of the map present the centi-Morgan distances of the SSR marker loci.

**Table 3.** Summary of the QTL for grain Fe and Zn concentrations in the RIL population detected using CIM.

Chromosome	Marker interval	Position (cM)	LOD	R <sup>2</sup>	Additive	Favorable allele
QTL for grain Fe						
2A	<i>Xgwm312-Xgwm817</i>	51.5	3.91	12.58	5.84	Tai
2A	<i>Xgwm817-Xgwm630</i>	53.5	3.71	17.04	6.63	Tai
3D	<i>Xgwm1047-Xgwm383</i>	107	2.76	44.71	-10.64	Tab
4D	<i>Xgwm4670-Xgwm194</i>	72.5	2.54	44.60	-10.66	Tab
7B	<i>Xgwm767-Xgwm3036</i>	121	2.52	47.00	10.92	Tai
7D	<i>Xbarc184-Xgwm1055</i>	23.5	2.78	8.94	4.90	Tai
QTL for grain Zn						
1A	<i>Xgwm3094-Xgwm164</i>	39	2.97	50.79	-7.11	Tab
4A	<i>Xgwm4026-Xgwm1081</i>	48.5	2.67	40.22	6.28	Tai

Tab and Tai refer to Tabassi and Taifun alleles, respectively.

detected as many as 4 QTLs for grain Zn concentration (mg/kg) and 7 (including these 4) for grain Zn content (micrograms/grain). The QTL detected by Shi *et al.* (2008) on chromosome 7A, explained the highest level of phenotypic variation. In our study, several individuals with high grain Fe and Zn have been generated from F2:8. In rice, using a DH population, Stangoulis *et al.* (2007) mapped three QTLs for grain Fe accumulation on chromosomes 2S, 8L, and 12L and 2 QTL for grain Zn on chromosomes 1l and 12L. The region where grain Zn QTL was mapped on rice chromosome 1 is orthologous to wheat chromosome 7 (Tiwari *et al.*, 2009). In several studies on the micronutrient analyses in different crops and populations, high positive correlations were reported between Fe and Zn content in grains and other tissues. But the accumulation of the grain Fe and Zn showed a negative correlation in the present study. The notion, that accumulation of Fe and Zn in grains is positively correlated (Shi *et al.*, 2008), may not be true as all the studies were based on estimation of grain Fe and Zn in a set of fixed lines (Morgounov *et al.*, 2007) rather than in the segregating populations (Tiwari *et al.*, 2009). One study in rice by Stangoulis *et al.* (2007), however, using a DH population reported a highly significant positive correlation between grain Fe and Zn concentrations. This may be true for certain loci, as was evident from the Tiwari *et al.* (2009). In their study no correlation was observed between grain Fe and Zn concentrations.

In the current study, two QTLs conferring Zn concentration (1A and 4A) were not mapped in previous studies. Out of the six QTLs for the grain Fe in the current study, three QTLs were mapped also in previous studies corresponding to our results: 2A (Tiwari *et al.*, 2009; Peleg *et al.*, 2009), 3D (McDonald *et al.*, 2008), and 7B (Peleg *et al.*, 2009). A study with diploid (AA), tetraploid (AABB), and hexaploid (AABBDD) wheats showed that Zn efficiency varied with the evolution of wheat. The Zn efficiency for tetraploid wheat was only 36%, while it was 60% for diploid and 64% for hexaploid wheats, respectively (Cakmak *et al.*, 1999b). This variation was attributed to the different ABD genome components. The A and D genomes were shown to contribute to high Zn efficiency (Schlegel *et al.*, 1997; Cakmak *et al.*, 1999a). QTLs conferring high grain mineral concentrations may reflect genes acting in one or more different steps, such as root uptake, root-to-shoot translocation, storage (in leaves or grain) and remobilization, as well as genes that encode regulatory proteins. The identified associations between QTLs affecting different mineral nutrients suggest physiological coupling of certain processes that govern mineral accumulation in wheat grain (Peleg *et al.*, 2009). Nevertheless, genomic regions associated with only one or few minerals should not be overlooked as they may confer other, mineral-specific, mechanisms. The concurrent mapping of QTL for several minerals as well as the dissection of their inter- and intra-relationships provides an insight

into the functional basis of the physiology, genomic architecture and evolution of minerals accumulation in wheat and other cereal (Peleg *et al.*, 2009). Advancements in QTL mapping will facilitate an understanding of the genetic basis controlling micronutrient concentrations in wheat grains. Furthermore, the identification and tagging of major QTLs for the traits in relation to micronutrients with large effects will be helpful in the selection of other QTLs in early generations with the MAS technique, and will greatly accelerate wheat cultivar development for improving mineral concentration in grain (Ortiz-Monasterio *et al.*, 2007). For more precisely identification of the important QTLs regions on the genome, doing a fine mapping project in a subpopulation derived from the present mapping population is also suggested.

## REFERENCES

- Bouis H. E., and Welch R. M. (2010). Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*, 50: 20-32.
- Cakmak I. (2002). Plant nutrition research: Priorities to meet human needs for food in sustainable ways. *Plant and Soil*, 247: 3-24.
- Cakmak I., Cakmak O., Eker S., Ozdemir A., Watanabe N., and Braun H. J. (1999 a). Expression of high zinc efficiency of *Aegilops tauschii* and *Triticum monococcum* in synthetic hexaploid wheats. *Plant and Soil*, 215: 203-209.
- Cakmak I., Tolay I., Ozdemir A., Ozkan H., Ozturk L., and Kling C. I. (1999b). Differences in zinc efficiency among and within diploid, tetraploid and hexaploid wheats. *Annals of Botany*, 84: 163-171.
- Cakmak I., Ozkan H., Braun H. J., Welch R. M., and Romheld V. (2000). Zinc and iron concentrations in seeds of wild, primitive, and modern wheats. *Food & Nutrition Bulletin*, 21: 401-403.
- Chhuneja P., Dhaliwal H., Bains N. S., and Singh K. (2006). *Aegilops kotschyi* and *Aegilops tauschii* as sources for higher levels of grain iron and zinc. *Plant Breeding*, 125: 529-531.
- Churchill G. A., and Doerge R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, 138: 963-971.
- DellaPenna D. (1999). Nutritional genomics: manipulating plant micronutrients to improve human health. *Science*, 285: 375-379.
- Hotz C., and Brown K. H. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. International Nutrition Foundation: for UNU.
- Kordenaeej A., and Lelley T. (2008). Mapping QTLs related to yield and yield components under drought in bread wheat. Sydney University Press.
- Kosambi D. D. (1943). The estimation of map distances from recombination values. *Annals of Eugenics*, 12: 172-175.
- McDonald G. K., Genc Y., and Graham R. D. (2008). A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yield. *Plant and Soil*, 306: 49-55.
- Monasterio I., and Graham R. D. (2000). Breeding for trace minerals in wheat. *Food & Nutrition Bulletin*, 21: 392-396.
- Monasterio I., Palacios-Rojas N., Meng E., Pixley K., Trethowan R., and Pena R. J. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science*, 46: 293-307.
- Morgounov A., Gómez-Becerra H. F., Abugalieva A., Dzhunusova M., Yessimbekova M., Muminjanov H., and Cakmak I. (2007). Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica*, 155: 193-203.
- Munson R. D., and Nelson W. L. (1990). Principle and practice in plant analysis. In R.L. Westerman (eds), *Soil Testing and Plant Analysis* (pp. 359-387). SSSA. Madison, WI.
- Ortiz-Monasterio J., Palacios-Rojas N., Meng E., Pixley K., Trethowan R., and Pena R. J. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science*, 46: 293-307.
- Peleg Z., Cakmak I., Ozturk L., Yazici A., Jun Y., Budak H., and Saranga Y. (2009). Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. *Theoretical and Applied Genetics*, 119: 353-369.
- Rawat N., Tiwari V. K., Singh N., Randhawa G. S., Singh K., Chhuneja P., and Dhaliwal H. S. (2009). Evaluation and utilization of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content in wheat. *Genetic Resources and Crop Evolution*, 56: 53-64.
- Sanchez P. A., and Swaminathan M. S. (2005). Cutting world hunger in half. *Science*, 307: 357-359.
- Schlegel R., Cakmak I., Torun B., Eker S., Tolay I., Ekiz H., and Braun H. J. (1997). Screening for zinc efficiency among wheat relatives and their utilisation for alien gene transfer. Wheat: Prospects for Global Improvement, Springer: 347-352.
- Shi R., Li H., Tong Y., Jing R., Zhang F., and Zou C. (2008). Identification of quantitative trait locus of zinc and phosphorus density in wheat (*Triticum aestivum* L.) grain. *Plant and Soil*, 306: 95-104.
- Stam P. (1993). Construction of integrated genetic linkage maps by means of a new computer package: Join Map. *The Plant Journal*, 3: 739-744.
- Stangoulis J. C. R., Huynh B. L., Welch R. M., Choi E. Y., and Graham R. D. (2007). Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica*, 154: 289-294.
- Tiwari V. K., Rawat N., Chhuneja P., Neelam K., Aggarwal R., Randhawa G. S., and Singh K. (2009). Mapping of quantitative trait loci for grain iron and zinc concentration in diploid A genome wheat. *Journal of Heredity*, 100: 771-776.
- Wang S., Basten C. J., and Zeng Z. B. (2012). Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC.

- Welch R. M., and Graham R. D. (1999). A new paradigm for world agriculture: meeting human needs: productive, sustainable, nutritious. *Field Crops Research*, 60: 1-10.
- Welch R. M., and Graham R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, 55: 353-364.
- Xu Y., An D., Li H., and Xu H. (2011). Breeding wheat for enhanced micronutrients. *Canadian Journal of Plant Science*, 91: 231-237.