

# Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population

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**Abstract** Mineral nutrient malnutrition, and particularly deficiency in zinc and iron, afflicts over 3 billion people worldwide. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, genepool harbors a rich allelic repertoire for mineral nutrients in the grain. The genetic and physiological basis of grain protein, micronutrients (zinc, iron, copper and manganese) and macronutrients (calcium, magnesium, potassium, phosphorus and sulfur) concentration was studied in tetraploid wheat population of 152 recombinant inbred lines (RILs), derived from a cross between durum wheat (*cv.* Langdon) and wild emmer (accession G18-16). Wide genetic variation was found among the RILs for all grain minerals, with considerable transgressive effect. A total of 82 QTLs were mapped for 10 minerals with LOD score range of 3.2–16.7. Most QTLs were in favor of the wild allele (50 QTLs). Fourteen pairs of QTLs for the same trait were mapped to seemingly homoeologous positions, reflecting synteny between the A and B genomes. Significant positive correlation was found

between grain protein concentration (GPC), Zn, Fe and Cu, which was supported by significant overlap between the respective QTLs, suggesting common physiological and/or genetic factors controlling the concentrations of these mineral nutrients. Few genomic regions (chromosomes 2A, 5A, 6B and 7A) were found to harbor clusters of QTLs for GPC and other nutrients. These identified QTLs may facilitate the use of wild alleles for improving grain nutritional quality of elite wheat cultivars, especially in terms of protein, Zn and Fe.

## Introduction

Mineral nutrients play a fundamental role in the biochemical and physiological functions of biological systems. While higher plants obtain their mineral nutrients primarily from the soil, animal and humans depend mostly on higher plants to supply them with mineral nutrients (Grusak and Cakmak 2005). 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). Recently, it has been declared that micronutrient deficiency problems are high priority research area, and their elimination will greatly benefit humanity and contribute to global stability (<http://www.copenhagenconsensus.com>). Enhancement in grain concentrations of mineral nutrients (biofortification), agronomically and/or genetically, is considered the most promising and cost effective approach to alleviate malnutrition and related health problems (Bouis 2003; Welch and Graham 2004; Cakmak 2008; Peleg et al. 2008a). This

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solution, however, requires a comprehensive exploration of potential genetic resources and an in-depth understanding of the physiological and genetic basis of mineral nutrients accumulation in staple food crop.

High seed concentrations of mineral nutrients are a key factor for vigorous germination and successful seedling establishment (Welch 1999; Yilmaz et al. 1998). The amount of minerals in the seed depends on a plethora of processes including absorption from soil, uptake by the roots, translocation and redistribution within the plant tissues and remobilization to the seed (Grusak and Cakmak 2005). Each of these processes is most likely controlled by many genes, which makes the accumulation of minerals in seeds a complex polygenic phenomenon. The advent of molecular markers enables to dissect such complex traits via analysis of quantitative trait loci (QTLs). The identification of QTLs for grain mineral nutrients can accelerate crop improvement through marker-assisted selection and eventually can lead to QTL cloning (Salvi and Tuberosa 2005).

Wheat (*Triticum* spp.) is the major staple food crop in many parts of the world in terms of cultivated area and food source, contributing 28% of the world edible dry matter and up to 60% of the daily calorie intake in several developing countries (FAOstat 2007). Therefore, the composition and nutritional quality of the wheat grain has a significant impact on human health and well-being, especially in the developing world. However, the joint effects of domestication and its associated evolutionary phenomena (i.e., founder effect) following modern breeding processes has eroded the genetic basis of crop species (Ladizinsky 1998; Tanksley and McCouch 1997). Domesticated wheat contains very low levels of minerals and shows a narrow genetic variation as compared with its wild relatives (Cakmak 2008). Using crosses between cultivated and wild species of inbreeding plants, alleles that were “left behind” during the domestication process may be reintroduced into the cultivated genepool (McCouch 2004) for the improvement of grain mineral nutrients.

Wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (körn.) Thell] is the tetraploid ( $2n = 4x = 28$ ; genome BBAA) progenitor of both domesticated tetraploid durum wheat [*T. turgidum* ssp. *durum* (Desf.) MacKey] and hexaploid ( $2n = 6x = 42$ ; BBAAADD) bread wheat (*T. aestivum* L.) (Feldman 2001). Wild emmer germplasm harbors a rich allelic repertoire for improving grain concentrations of micro- and macronutrients in cultivated wheats (Cakmak et al. 2004; Peleg et al. 2008a). A recombinant-inbred line (RIL) population, derived from a cross between durum wheat and wild emmer, was used in the current study to (1) determine the chromosomal location and phenotypic effects of QTLs associated with wheat grain mineral

nutrient concentration; (2) study the phenotypic and genotypic association between the various grain minerals; and (3) identify potential alleles from the wild for future wheat improvement.

## Materials and methods

### Plant material and growth conditions

A population of 152 F<sub>6</sub> RILs was developed by single-seed descent from a cross between durum wheat (cultivar Langdon; LDN hereafter) and wild emmer wheat (accession #G18-16) (Peleg et al. 2008b). The RIL population was tested in the field under three environments over 2 years in the experimental farm of The Hebrew University of Jerusalem in Rehovot, Israel (34°47'N, 31°54'E; 54 m above sea level). The soil at this location is brown-red degrading sandy loam (Rhodoxeralf, American Soil Science Society classification) composed of 76% sand, 8% silt and 16% clay. Seeds were disinfected (3.6% Sodium Hypochloric acid, for 10 min) and placed for vernalization on a moist germination paper for 3 weeks in a dark cold room (4°C), followed by 3 days of acclimation at room temperature. Seedlings were then transplanted into an insect-proof greenhouse protected by a polyethylene top. Water was applied via a drip irrigation system during the winter months (December–April) to mimic the natural pattern of rainfall in the east Mediterranean region. Plants were treated with pesticides to avoid development of pathogens or insect pests and weeded manually once a week. In the winter of 2004–2005, two irrigation regimes were applied: well-watered (750 mm) control (WW05) and water-limited (350 mm) (WL05), using a split-plot factorial (RIL × irrigation regime) block design with irrigation regimes in main plots and genotypes in sub-plots. In the winter of 2006–2007, well-watered (720 mm) treatment (WW07) was applied, using a randomized block design. Each trial was three times replicated with 75 cm long plots, each consisting of five plants.

### Phenotypic measurements

Each plot was harvested as soon as over 50% of the plants reached maturity to minimize seed dispersal. All spikes were harvested, oven-dried (35°C for 48 h) and weighed. A sub-sample of the harvested spikes from each plot (about 20–30 g) was threshed. Grains of each sub-sample were weighed, used to calculate grain yield (GY) and subjected to mineral analyses. Nitrogen in the grain was determined by using a C/N analyzer (TruSpec CN, Leco Co., USA). Grain nitrogen concentration was multiplied by 5.83 to obtain grain protein concentration (GPC) (Merrill and Watt

1973). Grain macronutrients (calcium, Ca; magnesium, Mg; potassium, K; phosphorus, P; and sulfur, S) and micronutrients (zinc, Zn; iron, Fe; copper, Cu; and manganese, Mn) concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Vista-Pro Axial; Varian Pty Ltd, Australia), after digesting samples in a closed microwave system. Measurements of mineral nutrients were checked using the certified values of the related minerals in the reference leaf and grain samples received from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA).

### Statistical analysis of phenotypic data

The JMP® ver. 7.0 statistical package (SAS Institute, Cary, NC, USA) was used for statistical analyses. All phenotypic variables were tested for normal distribution. A factorial model was employed for the analysis of variance, with RILs and blocks as random effects and the trial as a fixed effect. Broad sense heritability estimate ( $h^2$ ) was calculated for each trait across three irrigation regimes using variance components estimated based on ANOVA:

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{g \times e}^2 / e)$$

where  $\sigma_g^2 = [(MS_{RIL} - MS_{RIL \times e}) / e]$ ,  $\sigma_{g \times e}^2 = MS_{RIL \times e}$  and  $e$  is the number of environments and  $MS$  is the mean square. Correlation analyses were used to assess the association among the various grain minerals under each environment. Principal component analysis (PCA) was used to determine the associations among the ten grain mineral concentrations. PCA was based on a correlation matrix and was presented as biplot ordinations of populations (PC scores). Two components were extracted using Eigenvalues  $>1$  to ensure meaningful implementation of the data by each factor.

### QTL analysis

A genetic linkage map of 2,317 cM was previously developed for the 152 RIL mapping population based on 197 SSR and 493 DArT markers (Peleg et al. 2008b). DArT markers that were presented in the above map by clone ID numbers, were renamed with the prefix “wPt”, “rPt” or “tPt” (corresponding to wheat, rye or Triticale, respectively) followed by number. A skeleton map comprised of 307 markers, scattered along the 14 chromosomes (Chr) of tetraploid wheat (one marker per 7.5 cM) was used for QTL mapping. QTL analysis was performed with the MultiQTL package using the general interval mapping for the RIL-selfing population (describe in Peleg et al. 2009). To examine  $G \times E$  interaction, the three-environment QTL model was

compared against a sub-model assuming an equal effect of all environments, using 5,000 permutation tests (such comparison was not applicable in case of a two-QTL solution). The effect of epistatic interaction was examined for each trait by comparison of  $H_0$  ( $\varepsilon = 0$ ), i.e., additive effects of the QTL and  $H_1$  ( $\varepsilon \neq 0$ ), i.e., assuming epistasis (Ronin et al. 1999).

Correspondence between QTLs of different traits was determined using the hypergeometric probability function (Larsen and Marx 1985) according to Paterson et al. (1995):

$$P = \frac{\binom{l}{m} \binom{n-l}{s-m}}{\binom{n}{s}}$$

where  $n$  is the number of comparable intervals;  $m$  is the number of ‘matches’ (QTLs of two traits with  $>50\%$  overlap of their confidence intervals) declared between QTLs;  $l$  is the number of QTLs found in the larger sample and  $s$  is the number of QTLs found in the smaller sample.

## Results

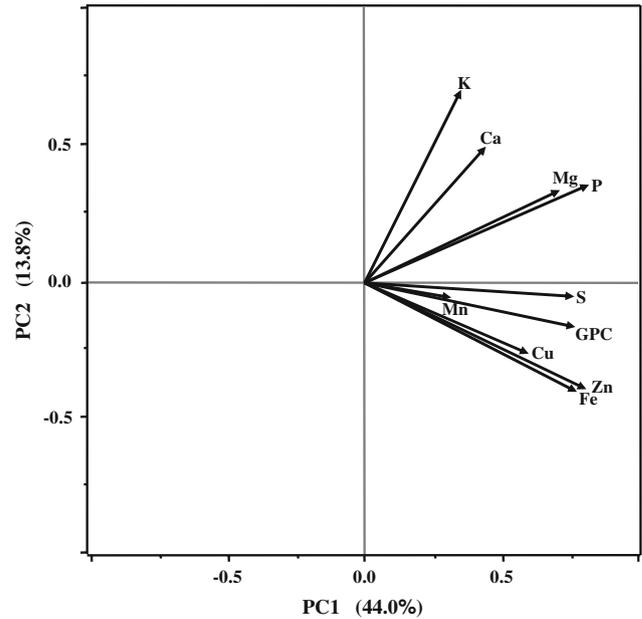
### Phenotypic diversity for grain mineral concentrations

Table 1 presents the mean values, ranges, and heritability estimates of ten grain mineral nutrient concentrations of the two parental lines and the RILs under each of the three environments. Analysis of variance (ANOVA) indicated a high level ( $P < 0.05$ ) of genetic variation for all mineral nutrients analyzed as well as environmental effects (not shown). All variables under each of the environments exhibited normal distribution. Transgressive segregation was common among all traits (Table 1). For example, the highest levels of Zn among the RILs were 46–79% greater than those of their domesticated parental line. Broad-sense heritability estimates ( $h^2$ ) indicates the proportion of phenotypic variance attributable to genotypic difference. Estimates of  $h^2$  for grain mineral concentration ranged from 0.41 (for Mn) to 0.79 (for Ca) (Table 1).

Principal component analysis (PCA) revealed similar patterns when applied for each environment separately (not shown), therefore, a joint PCA was conducted based on genotype means across all environments (Fig. 1). PCA extracted two major principal components (Eigenvalues  $>1$ ) that accounted collectively for 57.8% of the variation. Principal component 1 (PC1, X-axis, Fig. 1) explained 44.0% of the variation among RILs, and was loaded positively with GPC, Zn, Fe, P, Mg, Ca, Cu and S. PC2 (Y-axis, Fig. 1) explained 13.8% of the RILs variation, and

**Table 1** Mean values, ranges and heritability estimates ( $h^2$ ) of grain protein concentration and nine grain mineral nutrient concentrations of 152 recombinant inbred lines (Langdon × G18-16) as well as the two parental lines under each environmental conditions

| Trait                     | Water-limited 2005 |                 |         |         | Well-watered 2005 |                 |        |         | Well-watered 2007 |                 |         |         | $h^2$ |
|---------------------------|--------------------|-----------------|---------|---------|-------------------|-----------------|--------|---------|-------------------|-----------------|---------|---------|-------|
|                           | LDN                |                 | G18-16  |         | LDN               |                 | G18-16 |         | LDN               |                 | G18-16  |         |       |
|                           | RILs               | Mean            | Range   | RILs    | Mean              | Range           | RILs   | Mean    | Range             | RILs            | Mean    | Range   |       |
| GPC (%)                   | 24.1               | 19.8-29.2       | 23.2    | 25.5    | 20.7              | 12.6-27.2       | 18.9   | 25.0    | 16.0              | 12.9-21.1       | 14.1    | 20.4    | 0.63  |
| Zn (mg/kg <sup>-1</sup> ) | 74.9               | 48.5-114.7      | 65.7    | 75.0    | 60.1              | 39.0-105.0      | 58.7   | 89.0    | 55.9              | 39.0-78.0       | 53.5    | 55.5    | 0.62  |
| Fe (mg/kg <sup>-1</sup> ) | 51.4               | 36.0-70.0       | 58.0    | 52.8    | 42.3              | 29.7-80.5       | 45.7   | 59.8    | 25.3              | 17.0-41.0       | 17.7    | 31.8    | 0.69  |
| Cu (mg/kg <sup>-1</sup> ) | 6.7                | 5.0-10.0        | 7.0     | 7.3     | 6.1               | 4.0-8.5         | 5.33   | 8.3     | 7.7               | 5.4-11.7        | 7.2     | 7.7     | 0.76  |
| Mn (mg/kg <sup>-1</sup> ) | 56.5               | 24.5-104.0      | 68.0    | 61.8    | 55.9              | 31.0-91.5       | 57.33  | 69.5    | 27.3              | 16.3-41.0       | 28.5    | 27.5    | 0.41  |
| Ca (mg/kg <sup>-1</sup> ) | 584.9              | 403.0-813.0     | 563.7   | 645.2   | 470.3             | 269.0-697.3     | 466.1  | 610.6   | 400.7             | 269.7-578.0     | 336.8   | 414.4   | 0.79  |
| Mg (mg/kg <sup>-1</sup> ) | 1,476.2            | 1,291.0-1,741.3 | 1,545.0 | 1,561.4 | 1,492.1           | 1,258.0-1,737.5 | 1,436  | 1,629.0 | 1,576.9           | 1,333.1-1,821.9 | 1,469.0 | 1,802.5 | 0.74  |
| K (mg/kg <sup>-1</sup> )  | 5,103.3            | 4,084.0-6,446.0 | 5,378.0 | 4,583.0 | 4,520.9           | 3,660.0-6,568.7 | 4,430  | 4,541.0 | 4,615.9           | 3,926.5-5,754.5 | 4,153.0 | 5,136.0 | 0.58  |
| P (mg/kg <sup>-1</sup> )  | 5,410.2            | 4,390.0-6,415.0 | 5,566.0 | 5,365.0 | 4,893.2           | 3,614.0-5,778.7 | 5,005  | 5,183.7 | 4,776.6           | 4,008.1-5,416.5 | 4,458.0 | 5,175.3 | 0.62  |
| S (mg/kg <sup>-1</sup> )  | 2,278.5            | 1,834.5-2,774.7 | 2,317.0 | 2,631.0 | 2,091.4           | 1,725.3-2,599.0 | 2,030  | 2,399.2 | 1,670.2           | 1,321.6-2,058.6 | 1,564.0 | 2,004.7 | 0.76  |



**Fig. 1** Principal component analysis (based on correlation matrix) of grain protein and nine mineral nutrient concentrations in 152 recombinant inbred lines (Langdon × G18-16) under three environments. Biplot vectors are trait factor loadings for PC1 and PC2

was positively loaded with K and Ca and negatively loaded with Zn and Fe. The PCA showed strong associations between GPC, Zn and Fe (Fig. 1). This association was supported by the high and positive correlations between these variables. Grain Zn concentration correlated with grain Fe concentration ( $r = 0.72$ ,  $P \leq 0.0001$ ,  $r = 0.79$ ,  $P \leq 0.0001$  and  $r = 0.69$ ,  $P \leq 0.0001$  for WL05, WW05 and WW07, respectively), GPC correlated with Zn ( $r = 0.58$ ,  $P \leq 0.0001$ ;  $r = 0.48$ ,  $P \leq 0.0001$ ; and  $r = 0.41$ ,  $P \leq 0.0001$ , respectively), and GPC with Fe ( $r = 0.52$ ,  $P \leq 0.0001$ ;  $r = 0.49$ ,  $P \leq 0.0001$ ; and  $r = 0.33$ ,  $P \leq 0.0001$ , respectively). Strong association was also found between Cu and GPC, Zn and Fe and between Mg and P (Fig. 1).

#### Major characteristics of the detected QTLs

Eighty-two significant QTLs, scattered across all the 14 chromosomes of the tetraploid wheat, were detected for grain protein and nine grain mineral nutrient concentrations characterized under three environments (Table 2). In 50 QTLs (61%) the wild allele (G18-16) contributed to improved grain mineral concentrations and in the remaining 32 QTLs (39%) the domesticated allele (LDN) was favorable. Thirty-eight QTLs exhibited G × E interaction, of which 25 QTLs were detected under all environments (in one case two environments) with different effects and 13 QTLs were detected under one environment, whereas the remaining 44 QTLs (54%) showed no interaction with

**Table 2** Summary of QTLs detected in tetraploid wheat (Langdon × G18-16) RIL population associated with grain protein and nine mineral nutrient concentrations

| Trait, grain concentration | # QTLs | LOD      | Favorable allele |     | Environment          |      |      |      |
|----------------------------|--------|----------|------------------|-----|----------------------|------|------|------|
|                            |        |          | G18-16           | LDN | All env <sup>a</sup> | WL05 | WW05 | WW07 |
| Protein                    | 10     | 3.2–10.4 | 8                | 2   | 7                    | –    | 2    | 1    |
| Zinc                       | 6      | 3.7–16.4 | 5                | 1   | 5                    | 1    | –    | –    |
| Iron                       | 11     | 4.6–16.7 | 5                | 6   | 9                    | –    | –    | 2    |
| Copper                     | 10     | 4.9–10.4 | 6                | 4   | 10                   | –    | –    | –    |
| Manganese                  | 2      | 3.9–4.1  | –                | 2   | –                    | –    | –    | 2    |
| Calcium                    | 9      | 6.0–16.0 | 4                | 5   | 9                    | –    | –    | –    |
| Magnesium                  | 8      | 5.0–9.8  | 6                | 2   | 7                    | 1    | –    | –    |
| Potassium                  | 8      | 3.9–12.2 | 2                | 6   | 5                    | 2    | 1    | –    |
| Phosphorus                 | 8      | 5.3–15.3 | 5                | 3   | 8                    | –    | –    | –    |
| Sulfur                     | 10     | 3.4–9.3  | 9                | 1   | 8                    | –    | –    | 2    |
| Total                      | 82     | 3.2–16.7 | 50               | 32  | 69                   | 4    | 3    | 6    |

<sup>a</sup> In one case QTL conferring Ca (on chromosome 4B) was detected in two environments (WW05 and WW07)

environmental conditions. No significant two-locus epistasis was found between any of the QTLs controlling any of the ten traits.

#### QTLs detected for each trait

Detailed biometric parameters of QTLs detected for each of the traits are as follows:

*Grain protein concentration:* A total of ten significant QTLs were associated with GPC with LOD (log of the odds) scores ranging between 3.2–10.4, explaining 1–14% of the variance (Tables 2, 3). Higher GPC was conferred by the G18-16 allele at eight loci (2A, 2B, 4A, 5A, 5B, 6A, 6B, 7A) and by the LDN allele at two loci (3B, 7B). Six QTLs showed significant G × E interaction, one of them (2A) exhibited similar trend across environments, two QTLs (5A, 5B) exhibited a contrasting effect in one of the three environments, two QTLs (2B, 7B) were found only under WW05 and one (6A) under the WW07 environment.

*Grain zinc concentration* A total of six significant QTLs were associated with Zn with LOD scores ranging between 3.7 and 16.4, explaining 1–23% of the variance (Tables 2, 3). Higher Zn was conferred by the G18-16 allele at five loci (2A, 5A, 6B, 7A, 7B) and by the LDN allele at one locus (2A). Three QTLs showed significant G × E interaction, 2 of them (5A, 6B) showed a similar trend across environments and one QTL (7B) was found only under the WL05 environment.

*Grain iron concentration* A total of 11 significant QTLs were associated with Fe with LOD scores ranging between 4.6 and 16.7 explaining 2–18% of the variance (Tables 2, 3). Higher Fe was conferred by the G18-16 allele at five loci (2A, 3B, 5A, 6B, 7A) and by the LDN allele at six loci (2A, 2B, 3A, 4B, 6A, 7B). Five QTLs showed significant

G × E interaction, 2 of them (5A, 6A) exhibited a similar trend across environments, one (3A) had a contrasting effect in one of the three environments, and 2 QTLs (3B, 4B) were found only under the WW07 environment.

*Grain copper concentration* A total of ten significant QTLs were associated with Cu with LOD scores ranging between 4.9 and 10.4, explaining 1–13% of the variance (Tables 2, 3). Higher Cu was conferred by the G18-16 allele at six loci (2A, 4A, 4B, 5A, 6B, 7A) and by the LDN allele at four loci (1A, 3B, 6A, 7B). Four QTLs (5A, 6B, 7A, 7B) showed significant G × E interaction with a similar trend across environments.

*Grain manganese concentration* A total of two significant QTLs were associated with Mn with LOD scores of 3.9–4.1, explaining 11–14% of the variance (Tables 2, 3). In both QTLs higher Mn was conferred by the LDN allele. These 2 QTLs exhibited significant G × E interaction, both found only under WW07.

*Grain calcium concentration* A total of nine significant QTLs were associated with Ca with LOD scores ranging between 5.9 and 16.0, explaining 1–21% of the variance (Tables 2, 3). Higher Ca was conferred by the G18-16 allele at four loci (1A, 4A, 5B, 6B) and by the LDN allele at five loci (2B, 4B, 6A, 6B, 7B). Two QTLs showed significant G × E interaction (2B, 4B), one of them (2B) exhibited a similar trend across environments, and one QTL (4B) was found only under the WW05 and WW07 environments.

*Grain magnesium concentration* A total of eight significant QTLs were associated with Mg with LOD scores ranging between 5.0 and 9.8, explaining 1.1–17% of the variance (Tables 2, 3). Higher Mg was conferred by the G18-16 allele at six loci (1B, 2A, 3A, 5B, 6B, 7B) and by the LDN allele at two loci (6A, 7A). Four QTLs showed significant G × E interaction, three of them (2A, 3A, 5B)

**Table 3** Biometrical parameters of QTLs affecting grain protein and grain mineral nutrient concentrations in tetraploid wheat RIL population (LDN × G18-16)

| Trait                              | Position (cM) | Nearest marker | LOD <sup>a</sup> | Water-limited 2005    |                | Well-watered 2005 |              | Well-watered 2007 |              | Favorable allele <sup>d</sup> | G × E <sup>e</sup> |
|------------------------------------|---------------|----------------|------------------|-----------------------|----------------|-------------------|--------------|-------------------|--------------|-------------------------------|--------------------|
|                                    |               |                |                  | Var. (%) <sup>b</sup> | d <sup>c</sup> | Var. (%)          | d            | Var. (%)          | d            |                               |                    |
|                                    |               |                |                  |                       |                |                   |              |                   |              |                               |                    |
| <b>Grain protein concentration</b> |               |                |                  |                       |                |                   |              |                   |              |                               |                    |
| 2A                                 | 111.3 ± 21.4  | gwm445         | 7.9***           | 0.137                 | 1.20 ± 0.55    | 0.036             | 0.63 ± 0.44  | 0.009             | 0.02 ± 0.28  | G                             | **                 |
| 2B                                 | 95.4 ± 13.8   | gwm1249        | 4.4***           | -                     | -              | 0.090             | 1.10 ± 0.43  | -                 | -            | G                             |                    |
| 3B                                 | 148.1 ± 31.4  | gwm705         | 5.7**            | 0.019                 | -0.36 ± 0.33   | 0.037             | -0.30 ± 0.71 | 0.075             | -0.43 ± 0.67 | L                             | NS                 |
| 4A                                 | 77.2 ± 12.9   | wPt-7558       | 5.9**            | 0.024                 | 0.46 ± 0.32    | 0.097             | 1.21 ± 0.35  | 0.012             | 0.14 ± 0.31  | G                             | NS                 |
| 5A                                 | 11.8 ± 15.1   | gwm154         | 6.4***           | 0.036                 | -0.61 ± 0.31   | 0.051             | 0.86 ± 0.30  | 0.071             | 0.76 ± 0.31  | G                             | **                 |
| 5B                                 | 149.3 ± 21.5  | wPt-11579      | 6.0**            | 0.019                 | 0.39 ± 0.31    | 0.028             | -0.35 ± 0.57 | 0.085             | 0.86 ± 0.27  | G                             | ***                |
| 6A                                 | 57.1 ± 22.1   | tPt-4209       | 3.2*             | -                     | -              | -                 | -            | 0.092             | 0.74 ± 0.33  | G                             |                    |
| 6B                                 | 95.7 ± 11.9   | gwm771         | 10.4***          | 0.120                 | 1.22 ± 0.27    | 0.061             | 0.95 ± 0.30  | 0.038             | 0.56 ± 0.22  | G                             | NS                 |
| 7A                                 | 101.2 ± 10.9  | gwm332         | 6.9***           | 0.056                 | 0.81 ± 0.33    | 0.030             | 0.61 ± 0.35  | 0.096             | 0.93 ± 0.24  | G                             | NS                 |
| 7B                                 | 22.9 ± 11.5   | gwm263         | 3.4*             | -                     | -              | 0.079             | -1.08 ± 0.27 | -                 | -            | L                             |                    |
| <b>Grain zinc concentration</b>    |               |                |                  |                       |                |                   |              |                   |              |                               |                    |
| 2A                                 | 68.3 ± 44.0   | wPt-8216       | 10.5***          | 0.116                 | -5.63 ± 6.42   | 0.154             | -5.19 ± 4.54 | 0.109             | -3.42 ± 4.45 | L                             | -                  |
| 2A                                 | 112.4 ± 35.0  | gwm445         | 10.5***          | 0.116                 | 4.93 ± 7.63    | 0.154             | 2.01 ± 6.19  | 0.109             | 2.82 ± 4.44  | G                             | -                  |
| 5A                                 | 25.8 ± 22.0   | gwm293         | 5.2***           | 0.033                 | 3.54 ± 3.05    | 0.093             | 5.23 ± 1.43  | 0.013             | 0.64 ± 1.42  | G                             | **                 |
| 6B                                 | 133.5 ± 48.6  | gwm1076        | 5.3*             | 0.049                 | 4.55 ± 3.32    | 0.054             | 3.11 ± 2.71  | 0.022             | 0.73 ± 2.11  | G                             | *                  |
| 7A                                 | 65.8 ± 4.6    | wPt-9555       | 16.4***          | 0.090                 | 5.35 ± 2.24    | 0.157             | 6.91 ± 1.34  | 0.235             | 7.54 ± 1.29  | G                             | NS                 |
| 7B                                 | 94.8 ± 18.6   | gwm983         | 3.7*             | 0.110                 | 8.50 ± 1.97    | -                 | -            | -                 | -            | G                             |                    |
| <b>Grain iron concentration</b>    |               |                |                  |                       |                |                   |              |                   |              |                               |                    |
| 2A                                 | 60.1 ± 20.1   | gwm473         | 12.2***          | 0.120                 | -3.34 ± 3.23   | 0.117             | -3.06 ± 2.32 | 0.084             | -1.69 ± 1.62 | L                             | -                  |
| 2A                                 | 95.4 ± 32.2   | gwm1054        | 12.2***          | 0.120                 | 3.55 ± 2.95    | 0.117             | 1.68 ± 2.85  | 0.084             | 0.54 ± 1.30  | G                             | -                  |
| 2B                                 | 122.2 ± 20.1  | wPt-8404       | 6.4**            | 0.060                 | -3.19 ± 1.58   | 0.027             | -1.63 ± 0.95 | 0.031             | -1.13 ± 0.98 | L                             | NS                 |
| 3A                                 | 25.1 ± 28.2   | wPt-2756       | 5.0*             | 0.021                 | -1.61 ± 1.32   | 0.040             | 2.00 ± 1.42  | 0.017             | -0.03 ± 1.11 | L                             | *                  |
| 3B                                 | 196.3 ± 15.1  | gwm1266        | 4.6***           | -                     | -              | -                 | -            | 0.094             | 2.27 ± 0.93  | G                             |                    |
| 4B                                 | 14.3 ± 4.3    | wPt-3255       | 6.8***           | -                     | -              | -                 | -            | 0.123             | -2.75 ± 0.49 | L                             |                    |
| 5A                                 | 7.5 ± 6.2     | gwm154         | 9.0***           | 0.023                 | 1.98 ± 0.99    | 0.146             | 4.28 ± 0.67  | 0.008             | 0.24 ± 0.23  | G                             | *                  |
| 6A                                 | 66.9 ± 22.5   | gwm1150        | 6.7**            | 0.088                 | -4.01 ± 1.42   | 0.029             | -1.39 ± 0.95 | 0.027             | -1.03 ± 0.95 | L                             | *                  |
| 6B                                 | 160.5 ± 25.2  | wPt-5270       | 8.2***           | 0.048                 | 2.97 ± 2.23    | 0.068             | 2.76 ± 1.04  | 0.028             | 1.13 ± 0.86  | G                             | NS                 |
| 7A                                 | 66.5 ± 2.4    | wPt-9555       | 16.7***          | 0.082                 | 3.99 ± 1.01    | 0.107             | 3.63 ± 0.12  | 0.178             | 3.49 ± 0.63  | G                             | NS                 |
| 7B                                 | 46.0 ± 19.3   | gwm400         | 8.2***           | 0.034                 | -2.44 ± 1.14   | 0.058             | -2.64 ± 0.74 | 0.067             | -2.03 ± 0.84 | L                             | NS                 |
| <b>Grain copper concentration</b>  |               |                |                  |                       |                |                   |              |                   |              |                               |                    |
| 1A                                 | 119.6 ± 9.1   | DuPw038        | 10.4***          | 0.116                 | -0.60 ± 0.12   | 0.044             | -0.36 ± 0.12 | 0.047             | -0.42 ± 0.15 | L                             | NS                 |
| 2A                                 | 80.0 ± 14.1   | wPt-8115       | 8.9***           | 0.047                 | 0.35 ± 0.16    | 0.080             | 0.48 ± 0.18  | 0.077             | 0.54 ± 0.19  | G                             | NS                 |
| 3B                                 | 111.4 ± 38.8  | gwm853         | 6.5***           | 0.028                 | -0.13 ± 0.26   | 0.029             | -0.17 ± 0.23 | 0.071             | -0.25 ± 0.38 | L                             | NS                 |

Table 3 continued

| Trait                         | Position (cM) | Nearest marker | LOD <sup>a</sup> | Water-limited 2005    |                  | Well-watered 2005 |                  | Well-watered 2007 |                | Favorable allele <sup>d</sup> | G × E <sup>e</sup> |
|-------------------------------|---------------|----------------|------------------|-----------------------|------------------|-------------------|------------------|-------------------|----------------|-------------------------------|--------------------|
|                               |               |                |                  | Var. (%) <sup>b</sup> | d <sup>c</sup>   | Var. (%)          | d                | Var. (%)          | d              |                               |                    |
| 4A                            | 94.1 ± 13.5   | wmc262         | 7.6*             | 0.045                 | 0.35 ± 0.15      | 0.047             | 0.37 ± 0.14      | 0.055             | 0.45 ± 0.15    | G                             | NS                 |
| 4B                            | 66.7 ± 10.4   | gwm3072        | 7.5***           | 0.056                 | 0.40 ± 0.13      | 0.067             | 0.45 ± 0.13      | 0.031             | 0.32 ± 0.17    | G                             | NS                 |
| 5A                            | 12.6 ± 11.2   | gwm154         | 8.8***           | 0.048                 | 0.37 ± 0.13      | 0.131             | 0.65 ± 0.13      | 0.012             | 0.16 ± 0.15    | G                             | *                  |
| 6A                            | 67.5 ± 33.7   | gwm1150        | 4.9*             | 0.046                 | -0.29 ± 0.25     | 0.025             | -0.12 ± 0.22     | 0.027             | -0.07 ± 0.03   | L                             | NS                 |
| 6B                            | 132.2 ± 29.8  | gwm1076        | 5.9*             | 0.026                 | 0.23 ± 0.13      | 0.056             | 0.23 ± 0.18      | 0.056             | 0.37 ± 0.23    | G                             | ***                |
| 7A                            | 86.3 ± 17.3   | wPt-7053       | 5.1*             | 0.032                 | 0.27 ± 0.17      | 0.007             | 0.03 ± 0.11      | 0.062             | 0.47 ± 0.20    | G                             | *                  |
| 7B                            | 72.4 ± 24.2   | gwm46          | 6.7***           | 0.009                 | -0.02 ± 0.17     | 0.013             | -0.14 ± 0.45     | 0.117             | -0.67 ± 0.20   | L                             | ***                |
| Grain manganese concentration |               |                |                  |                       |                  |                   |                  |                   |                |                               |                    |
| 2B                            | 134.7 ± 19.0  | wPt-0694       | 4.1***           | -                     | -                | -                 | -                | 0.112             | -2.90 ± 0.81   | L                             |                    |
| 7B                            | 48.9 ± 30.5   | gwm400         | 3.9**            | -                     | -                | -                 | -                | 0.136             | -3.86 ± 0.21   | L                             |                    |
| Grain calcium concentration   |               |                |                  |                       |                  |                   |                  |                   |                |                               |                    |
| 1A                            | 31.7 ± 26.7   | gwm3083        | 6.0***           | 0.008                 | 2.70 ± 1.50      | 0.019             | 16.70 ± 11.95    | 0.073             | 30.37 ± 9.25   | G                             | NS                 |
| 2B                            | 86.5 ± 6.9    | wPt-6576       | 12.5***          | 0.168                 | -68.97 ± 13.66   | 0.097             | -45.31 ± 9.76    | 0.028             | -17.48 ± 9.29  | L                             | ***                |
| 4A                            | 28.7 ± 2.4    | gwm610         | 13.3***          | 0.065                 | 43.17 ± 11.04    | 0.121             | 51.35 ± 8.14     | 0.048             | 23.32 ± 10.10  | G                             | NS                 |
| 4B                            | 88.1 ± 9.7    | wPt-9393       | 6.0**            | -                     | -                | 0.014             | -19.94 ± 9.89    | 0.103             | -36.45 ± 8.89  | L                             | *                  |
| 5B                            | 54.2 ± 5.9    | gwm371         | 13.3***          | 0.052                 | 38.41 ± 9.84     | 0.081             | 42.10 ± 7.80     | 0.060             | 27.60 ± 7.34   | G                             | NS                 |
| 6A                            | 106.9 ± 20.2  | wPt-0139       | 6.6**            | 0.009                 | -5.91 ± 7.66     | 0.064             | -35.68 ± 12.59   | 0.035             | -19.21 ± 10.48 | L                             | NS                 |
| 6B                            | 22.6 ± 28.1   | wPt-11506      | 13.9***          | 0.190                 | 40.88 ± 32.76    | 0.055             | 21.44 ± 18.48    | 0.016             | 3.75 ± 1.47    | G                             | NS                 |
| 6B                            | 145.4 ± 20.7  | gwm219         | 13.9***          | 0.190                 | -48.32 ± 27.11   | 0.055             | -16.15 ± 16.11   | 0.016             | 3.29 ± 11.76   | L                             | NS                 |
| 7B                            | 23.0 ± 7.8    | gwm263         | 16.0***          | 0.035                 | -29.35 ± 12.67   | 0.172             | -61.87 ± 8.55    | 0.206             | -51.22 ± 10.12 | L                             | NS                 |
| Grain magnesium concentration |               |                |                  |                       |                  |                   |                  |                   |                |                               |                    |
| 1B                            | 132.6 ± 35.8  | gwm806         | 5.3*             | 0.021                 | 18.86 ± 17.15    | 0.069             | 44.89 ± 20.90    | 0.031             | 26.42 ± 15.14  | G                             | NS                 |
| 2A                            | 71.5 ± 9.5    | wPt-8216       | 9.8***           | 0.167                 | 74.11 ± 15.90    | 0.085             | 50.01 ± 22.65    | 0.029             | 25.65 ± 15.45  | G                             | **                 |
| 3A                            | 12.4 ± 7.7    | gwm1159        | 9.3***           | 0.079                 | 50.25 ± 12.13    | 0.011             | 13.91 ± 12.71    | 0.099             | 53.79 ± 12.03  | G                             | *                  |
| 5B                            | 155.6 ± 39.4  | wPt-11579      | 6.8***           | 0.041                 | 31.90 ± 19.58    | 0.014             | 5.98 ± 11.23     | 0.096             | 43.21 ± 32.21  | G                             | **                 |
| 6A                            | 145.8 ± 13.5  | gwm719         | 5.0***           | 0.083                 | -50.79 ± 13.39   | -                 | -                | -                 | -              | L                             |                    |
| 6B                            | 42.9 ± 30.1   | wPt-7748       | 6.5***           | 0.075                 | 46.75 ± 19.36    | 0.023             | 21.71 ± 18.71    | 0.056             | 38.58 ± 15.18  | G                             | NS                 |
| 7A                            | 49.6 ± 18.1   | gwm871a        | 6.0***           | 0.065                 | -40.65 ± 23.57   | 0.024             | -9.21 ± 2.70     | 0.028             | -16.56 ± 13.45 | L                             | NS                 |
| 7B                            | 131.1 ± 29.2  | wPt-8417       | 6.0**            | 0.019                 | 19.66 ± 16.05    | 0.071             | 35.38 ± 24.67    | 0.036             | 25.01 ± 21.03  | G                             | NS                 |
| Grain Potassium concentration |               |                |                  |                       |                  |                   |                  |                   |                |                               |                    |
| 1A                            | 181.5 ± 31.4  | gwm750         | 4.3**            | 0.073                 | 193.39 ± 121.66  | -                 | -                | -                 | -              | G                             |                    |
| 1A                            | 36.1 ± 29.9   | cfa2158a       | 3.9*             | -                     | -                | 0.112             | -296.43 ± 98.20  | -                 | -              | L                             |                    |
| 2A                            | 136.1 ± 16.9  | tPt-3136       | 8.7***           | 0.115                 | 284.99 ± 82.88   | 0.050             | 185.82 ± 87.42   | 0.070             | 130.73 ± 72.13 | G                             | NS                 |
| 2B                            | 88.0 ± 28.9   | wPt-6576       | 5.2*             | 0.065                 | -150.32 ± 166.21 | 0.037             | -127.45 ± 111.87 | 0.026             | -16.30 ± 19.40 | L                             | *                  |

**Table 3** continued

| Trait                          | Position (cM) | Nearest marker | LOD <sup>a</sup> | Water-limited 2005    |                  | Well-watered 2005 |                  | Well-watered 2007 |                 | Favorable allele <sup>d</sup> | G × E <sup>e</sup> |
|--------------------------------|---------------|----------------|------------------|-----------------------|------------------|-------------------|------------------|-------------------|-----------------|-------------------------------|--------------------|
|                                |               |                |                  | Var. (%) <sup>b</sup> | d <sup>c</sup>   | Var. (%)          | d                | Var. (%)          | d               |                               |                    |
| 5B                             | 66.7 ± 24.1   | gwm499         | 6.5**            | 0.022                 | -103.60 ± 78.16  | 0.072             | -229.11 ± 85.69  | 0.060             | -119.74 ± 70.62 | L                             | NS                 |
| 6A                             | 85.9 ± 4.1    | gwm4675        | 12.2***          | 0.140                 | -323.92 ± 59.79  | 0.111             | -299.10 ± 66.84  | 0.011             | -34.67 ± 37.90  | L                             | ***                |
| 6B                             | 83.7 ± 26.5   | barc136        | 7.4***           | 0.081                 | -210.30 ± 132.27 | 0.066             | -205.66 ± 115.90 | 0.016             | -44.87 ± 45.11  | L                             | ***                |
| 7B                             | 28.2 ± 16.0   | gwm537         | 4.4***           | 0.114                 | -317.31 ± 86.93  | -                 | -                | -                 | -               | L                             | -                  |
| Grain Phosphorus concentration |               |                |                  |                       |                  |                   |                  |                   |                 |                               |                    |
| 1A                             | 91.3 ± 23.4   | gwm778         | 5.6**            | 0.048                 | -133.51 ± 56.72  | 0.082             | -207.40 ± 17.35  | 0.041             | -90.62 ± 64.66  | L                             | **                 |
| 2A                             | 28.7 ± 23.2   | wPt-6245       | 15.3***          | 0.192                 | -50.64 ± 46.38   | 0.170             | -103.95 ± 110.95 | 0.050             | -37.65 ± 29.89  | L                             | -                  |
| 2A                             | 101.4 ± 31.6  | gwm445         | 15.3***          | 0.192                 | 226.82 ± 64.92   | 0.170             | 154.96 ± 117.89  | 0.050             | 58.27 ± 54.44   | G                             | -                  |
| 4A                             | 48.8 ± 22.5   | DuPw004        | 5.7**            | 0.051                 | 128.98 ± 14.12   | 0.029             | 110.60 ± 72.75   | 0.064             | 127.22 ± 52.63  | G                             | NS                 |
| 4B                             | 41.4 ± 18.7   | gwm781         | 5.4*             | 0.014                 | 49.65 ± 59.47    | 0.076             | 204.74 ± 16.58   | 0.020             | 54.07 ± 55.47   | G                             | **                 |
| 5B                             | 125.2 ± 37.4  | gwm408         | 6.6**            | 0.059                 | -42.34 ± 15.14   | 0.043             | -143.97 ± 66.95  | 0.033             | -54.62 ± 55.10  | L                             | **                 |
| 6B                             | 25.6 ± 23.7   | wPt-3376       | 6.5**            | 0.067                 | 148.50 ± 83.45   | 0.012             | 26.01 ± 8.61     | 0.059             | 121.84 ± 58.19  | G                             | NS                 |
| 7A                             | 97.2 ± 16.9   | wPt-7053       | 8.3***           | 0.054                 | 126.90 ± 87.27   | 0.064             | 185.66 ± 16.43   | 0.079             | 143.02 ± 56.01  | G                             | NS                 |
| Grain sulfur concentration     |               |                |                  |                       |                  |                   |                  |                   |                 |                               |                    |
| 1A                             | 58.9 ± 30.3   | wmc333         | 5.2***           | 0.039                 | 53.49 ± 41.22    | 0.041             | 61.03 ± 32.18    | 0.061             | 55.99 ± 29.03   | G                             | NS                 |
| 2A                             | 105.8 ± 25.8  | gwm445         | 5.9***           | 0.060                 | 76.03 ± 30.17    | 0.058             | 58.94 ± 46.16    | 0.016             | 12.89 ± 13.01   | G                             | NS                 |
| 3A                             | 72.0 ± 14.2   | wPt-1092       | 6.9***           | 0.040                 | 62.57 ± 26.37    | 0.019             | 39.25 ± 22.09    | 0.069             | 65.34 ± 20.33   | G                             | NS                 |
| 4A                             | 75.0 ± 7.5    | wPt-7558       | 9.3***           | 0.060                 | 72.83 ± 24.20    | 0.106             | 100.04 ± 20.73   | 0.032             | 43.94 ± 19.60   | G                             | NS                 |
| 4B                             | 82.5 ± 11.8   | tPt-7156       | 4.7***           | -                     | -                | -                 | -                | 0.142             | -98.20 ± 17.69  | L                             | -                  |
| 5A                             | 95.3 ± 11.5   | wPt-11526      | 6.5***           | 0.034                 | 55.36 ± 24.48    | 0.108             | 108.67 ± 27.14   | 0.012             | 9.51 ± 12.05    | G                             | ***                |
| 5B                             | 121.2 ± 25.2  | wPt-1733       | 3.4*             | -                     | -                | -                 | -                | 0.073             | 70.39 ± 15.29   | G                             | -                  |
| 6B                             | 101.1 ± 25.9  | wPt-11556      | 5.8**            | 0.034                 | 55.12 ± 30.17    | 0.031             | 54.17 ± 25.27    | 0.063             | 59.70 ± 21.14   | G                             | NS                 |
| 7A                             | 54.7 ± 13.8   | gwm1083        | 9.3***           | 0.048                 | 67.91 ± 25.56    | 0.119             | 112.70 ± 23.10   | 0.029             | 37.45 ± 21.20   | G                             | **                 |
| 7B                             | 128.8 ± 27.8  | wPt-3730       | 5.1*             | 0.035                 | 52.18 ± 36.17    | 0.034             | 46.17 ± 43.01    | 0.051             | 44.72 ± 35.21   | G                             | NS                 |

<sup>a</sup> LOD (log- odds) scores that were found significant when comparing hypotheses H<sub>1</sub> (there is QTL in the chromosome) versus H<sub>0</sub> (no effect of the chromosome on the trait), using 1000 permutations test (Churchill and Doerge 1994)

<sup>b</sup> Proportion of explained variance of the trait

<sup>c</sup> The effect of QTL

<sup>d</sup> Favorable parental allele contributing to greater grain protein and mineral nutrient concentrations, Langdon (L) and G18-16 (G)

<sup>e</sup> Genotype × environment interaction, tested by comparing the model with new sub-model in which all environments have equal effect, using 1,000 permutations test. This test is not applicable when QTL is specific for only one environment or in case of the two QTL model

\*, \*\*, \*\*\* and NS indicate significance at  $P \leq 0.05$ , 0.01, 0.001 or non-significant effect, respectively

exhibited a similar trend across environments, and one QTL (6A) was found only under the WW05 environment.

**Grain potassium concentration** A total of eight significant QTLs were associated with K with LOD scores ranging between 3.9 and 12.2, explaining 2–14% of the variance (Tables 2, 3). Higher K was conferred by the G18-16 allele at two loci (1A, 2A) and by the LDN allele at six loci (1A, 2B, 5B, 6A, 6B, 7B). Six QTLs showed significant G × E interaction, three of them (2B, 6A, 6B) exhibited a similar trend across environments, two QTLs (1A, 7B) were found only under WL05 and one (1A) under the WW05 environment.

**Grain phosphorus concentration** A total of eight significant QTLs were associated with P concentration in the grain with LOD scores ranging between 5.4–15.3, explaining 1–19% of the variance (Tables 2, 3). Higher P was conferred by the G18-16 allele at 5 loci (2A, 4A, 4B, 6B, 7A) and by the LDN allele at three loci (1A, 2A, 5B). Three QTLs showed significant G × E interaction (1A, 4B, 5B) with a similar trend across environments.

**Grain sulfur concentration** A total of ten significant QTLs were associated with S with LOD scores ranging between 3.4 and 9.3, explaining 1.2–14.2% of the variance (Tables 2, 3). Higher S was conferred by the G18-16 allele at nine loci (1A, 2A, 3A, 4A, 5A, 5B, 6B, 7A, 7B) and by the LDN allele at one locus (4B). Four QTLs showed significant G × E interaction, two of them (5A, 7A) exhibited a similar trend across environments, and two QTLs (4B, 5B) were found only under the WW07 environment.

## Discussion

Sufficient amount of protein and minerals in the daily diet is essential for human health. While global cereal grain yields have increased dramatically since the Green Revolution, cereal-based diet is short of providing sufficient protein and mineral nutrients, leading to increased percentage of people suffering from nutrient malnutrition (Welch and Graham 2004). Among grain mineral nutrients, Zn and Fe deficiencies are the most important global challenge. According to the World Health Organization, deficiencies in Zn and Fe rank 5th and 6th, respectively, among the risk factors responsible for illnesses in developing countries (WHO 2002).

Little information is available about the genetic control and molecular- physiological mechanisms contributing to high accumulation of macro- and micro-nutrients in the grain. QTL analysis proved a powerful tool in agricultural studies, pointing out the chromosomal location of genes suitable for breeding programs. However, most of the QTL studies conducted so far focused on GPC with very little attention given to other grain nutrients. In wheat, we are

aware of only three publications reporting on QTL mapping of grain minerals: one in *T. monococcum* (Zn, Fe, Cu and Mn; Özkan et al. 2007) and two in bread wheat (Zn and P; Shi et al. 2008; Genc et al. 2008). In the present study, QTL analysis was employed to dissect the genetic basis of grain protein and nine mineral concentrations, using a tetraploid wheat (LDN × G18-16) RIL population. Moreover, the use of a cross between wild and domesticated wheat may facilitate the identification of novel genes that are not present in the domesticated germplasm.

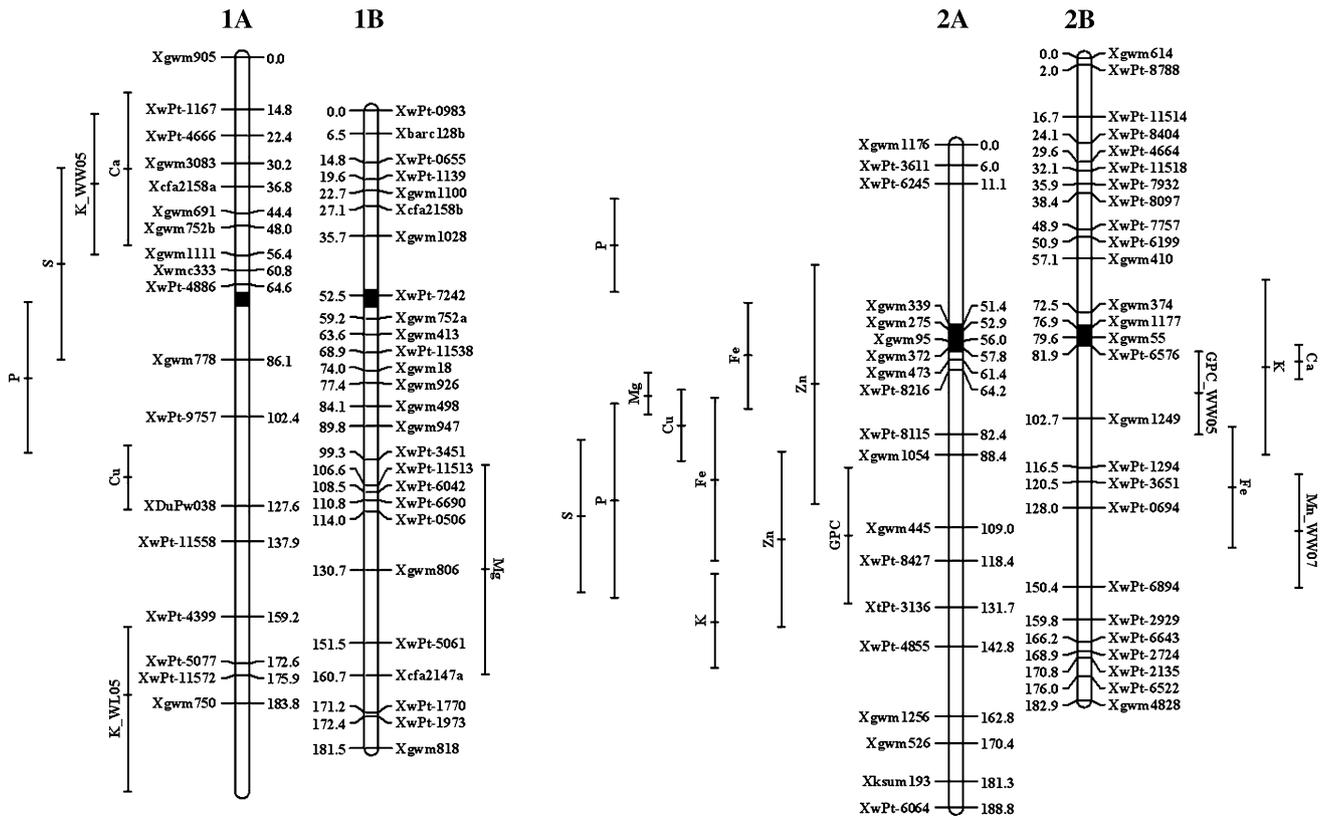
### QTLs conferring grain protein concentration

Application of N fertilizer, the most common approach to enhance GPC in wheat, is biologically limited. Abundant experimental evidence shows that GPC increases with N application up to a certain point, after which it remains stable, while the straw N concentration keeps increasing (Barneix 2007 and references therein). Genetic improvement is the most promising strategy to increase GPC under either optimal or sub-optimal N availability. In the present study, ten QTLs associated with GPC were identified (Fig. 2, Table 3). QTLs affecting variation in GPC were reported in tetraploid and hexaploid wheat on all 14 chromosomes of genome A and B (Joppa et al. 1997; Snape et al. 1997; Prasad et al. 1999; Perretant et al. 2000; Harjit et al. 2001; Zanetti et al. 2001; Börner et al. 2002; Blanco et al. 2002; Groos et al. 2003; Gonzalez-Hernandez et al. 2004; Blanco et al. 2006; Zhang et al. 2008). Because of the multigenic nature of GPC and G × E interactions, most QTLs for GPC, detected in the current and previous studies, accounted for relatively small proportion of the total phenotypic variance. Nevertheless, two major QTLs were associated with higher GPC (2A, 6B), explaining 12–13.7% of the phenotypic variance (Table 3). A major QTL for GPC from wild emmer wheat was localized on chromosome arm 6BS (Joppa et al. 1997) and successfully transferred into bread wheat cultivars (Mesfin et al. 1999; Khan et al. 2000). Recently, this QTL, designated *Gpc-B1*, was cloned (Uauy et al. 2006; Distelfeld and Fahima 2007).

The wild emmer wheat genepool harbors a wide genetic variation for GPC (Avivi 1979; Cakmak et al. 2004; Peleg et al. 2008a), which was considered relevant for improving domesticated wheat (Blanco et al. 2002; Gonzalez-Hernandez et al. 2004; Joppa et al. 1997). In the present study, the wild emmer alleles (G18-16) were favorable in most (80%) QTLs for GPC, thus confirming the potential of wild emmer germplasm for wheat breeding programmes.

### QTLs conferring grain micronutrient concentrations

Micronutrients (Zn, Fe, Cu and Mn) are required by plants at very low concentrations, while at high concentrations



**Fig. 2** Likelihood intervals for QTLs associated with grain protein (GPC) and grain mineral nutrient concentrations of zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P) and sulfur (S) in recombinant inbred

lines of the cross between Langdon and G18-16. QTLs expressed only for the following specific environment are marked: water-limited in 2005 (WL05), well-watered in 2005 (WW05), and well-watered in 2007 (WW07)

they may become toxic. Thus, plants have evolved a complex regulation networks to control minerals homeostasis (Grusak and Cakmak 2005; Grotz and Guerinot 2006).

*Zinc* plays multiple roles in various physiological and metabolic processes in plants including membrane function, protein synthesis and detoxification of reactive oxygen species (Cakmak 2000). In the current study, Zn was conferred by six QTLs (2A, 2A, 5A, 6B, 7A, 7B), with the wild allele being favorable in five cases (Table 2). Five QTLs conferring Zn concentration were mapped in previous studies, with three of them corresponding to our results: 5A (Özkan et al. 2007; Shi et al. 2008), 6B (Distelfeld et al. 2007; Genc et al. 2008), and 7A (Shi et al. 2008; Genc et al. 2008). *Iron* is involved in many enzymatic functions in plants affecting photosynthesis and chlorophyll biosynthesis (Marschner 1995). Fe was conferred in the current study by 11 QTLs, with the wild allele being favorable in five cases. Two QTLs were mapped also in previous studies: 5A (Özkan et al. 2007) and 6B (Distelfeld et al. 2007). *Copper* plays important roles in photosynthesis and pollen (Marschner 1995). Cu was conferred in the current study by ten QTLs, with the wild allele being favorable in six cases. Only one of the mapped

QTLs was previously reported (5A; Özkan et al. 2007). *Manganese* is involved in activities of several enzymes related to photosynthesis, respiration, and nitrogen metabolism (Marschner 1995). Mn was conferred in the current study by two QTLs, both with the domesticated allele being favorable. These two QTLs were found only in the WW07 environment, indicating a pronounced  $G \times E$  interaction. In agreement with this, heritability estimates for Mn were low (0.41) relative to other minerals. We are not aware of a previous report on these two QTLs (2B and 7B), however, two other QTLs conferring Mn were previously reported: 5A (Özkan et al. 2007) and 6B (Distelfeld et al. 2007).

#### QTLs conferring grain macro-nutrient concentrations

Macronutrients (Ca, Mg, K, P and S) are essential elements used by plants in relatively large amounts. Unlike micronutrients, which have only functional roles, macronutrients have both structural and functional roles. We are not aware of previous reports of QTLs for macronutrient concentrations in wheat grain, apart from a single publication on P (Shi et al. 2008).

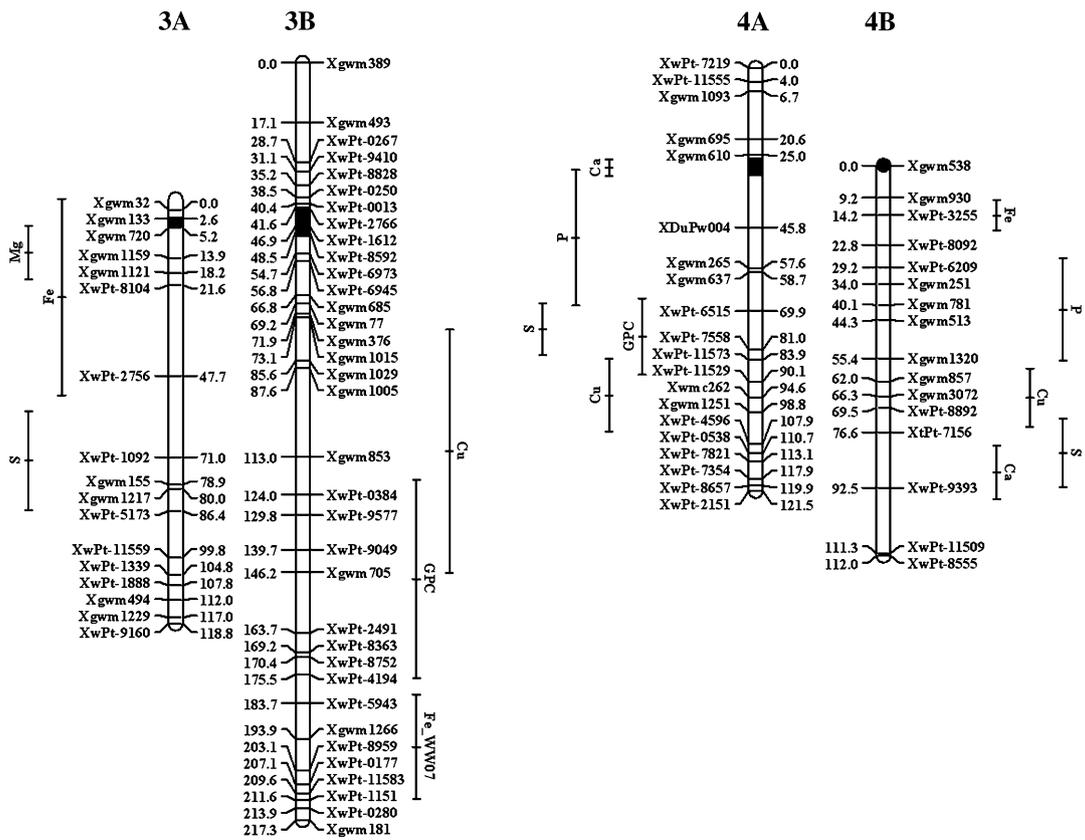


Fig. 2 continued

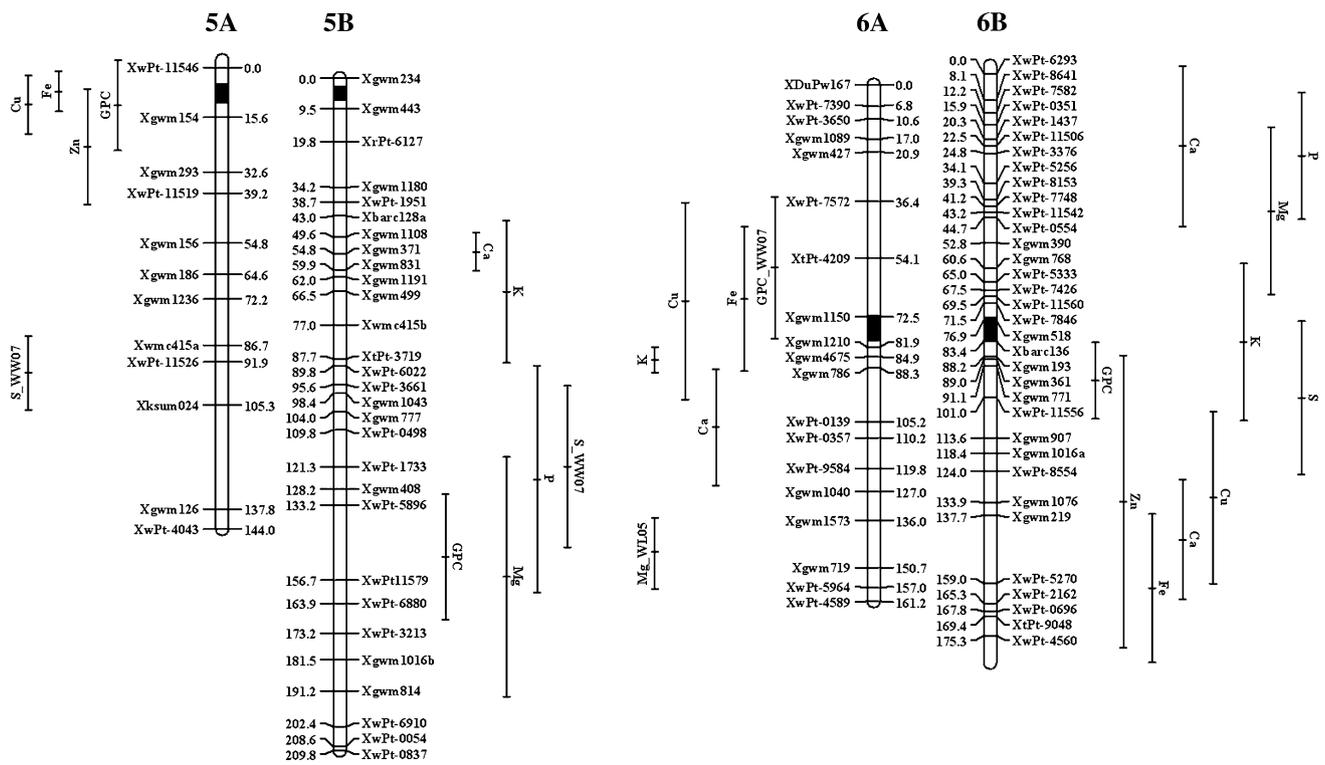


Fig. 2 continued

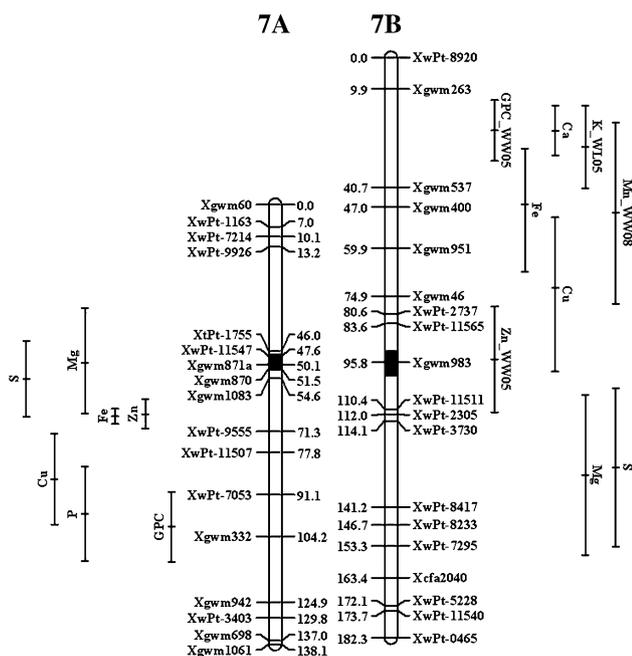


Fig. 2 continued

*Calcium* is a structural element in cell wall and biological membranes (Marschner 1995). Ca was conferred in the current study by nine QTLs, with the wild allele being favorable in four cases. *Magnesium* is a critical structural component of the chlorophyll molecule in plants. Mg was conferred in the current study by eight QTLs, with the wild allele being favorable in six cases. *Potassium* is absorbed by plants in larger amounts than any other mineral element (excluding nitrogen) and is necessary for photosynthetic carbohydrates metabolism and protein synthesis (Marschner 1995). K was conferred in the current study by eight QTLs, with the wild allele being favorable in only two cases. *Phosphorus* is among the key substrates in energy metabolism and biosynthesis of nucleic acids and membranes. P was conferred in the current study by eight QTLs, with the wild allele being favorable in five cases. Three QTLs conferring P concentration were mapped in a previous study, one of which corresponds to our results (4A; Shi et al. 2008). *Sulfur* promotes activity of several co-enzymes, vitamins and proteins, it is involved in chlorophyll formation and improves root growth and seed production (Marschner 1995). S was conferred in the current study by ten QTLs, with the wild allele being favorable in nine cases.

#### Homoeologous QTL loci

Owing to the allopolyploid nature of the wheat genome, a number of important traits such as daylength sensitivity (*Ppd*; group 2), plant height (*Rht*; group 4) and

vernalization requirement (*Vrn*; group 5) are controlled by series of genes on homoeologous linkage groups (Law et al. 1976; McVittie et al. 1978; Scarth and Law 1983). In the current study, as many as 28 QTLs (14 pairs) for the same trait were mapped to seemingly homoeologous positions on five chromosome groups (2, 4, 5, 6, 7) of the tetraploid wheat (Fig. 2). Homoeology was detected for nine traits (excluding Mn) including GPC (groups 2, 6), Zn (group 7), Fe (group 2), K (groups 2, 6), P (group 4), Mg (group 7), S (groups 4, 5, 7), Ca (group 6) and Cu (groups 4, 6). Similarly, in a previous study, with the same RIL population (Peleg et al. 2009), 30 QTLs (15 pairs) for plant productivity and drought related traits were mapped to seemingly homoeologous positions. Both parental lines are allotetraploids comprised of two genomes (A and B) that are presumed to have diverged from a common ancestor 2.5–4.5 million years ago (Huang et al. 2002) and gave rise to the tetraploid genome about 0.5 million years ago (Dvořák and Akhunov 2005). Therefore, although not confirmed by tightly linked markers, it is very likely that the numerous homoeologous-QTLs reflect synteny between A and B genomes.

#### Association among grain protein and mineral concentrations

To test the extent to which different traits were genetically associated, we evaluated the correspondence of QTL confidence intervals. The 82 QTLs discovered in the current study were located in 32 non-overlapping genomic regions (Fig. 2). Relationships between QTLs conferring grain protein and mineral concentrations may shed light on possible common mechanisms influencing mineral concentrations in the grain of wheat and other cereal species.

Quantitative trait loci conferring high GPC were co-localized with QTLs conferring high Zn in three genomic regions (2A, 5A, 6B) and with QTLs conferring high Fe in five genomic regions (2A, 2B, 5A, 6A, 7B) (Fig. 2, Table 4). The likelihood that such associations would occur by chance are  $P = 0.05$  and  $P = 0.008$ , respectively (Larsen and Marx 1985; Paterson et al. 1995). These results were further supported by principal component analysis (Fig. 1) and positive phenotypic correlations between GPC and both Zn or Fe (Table 4). Positive correlation between GPC, Zn and Fe has been reported for several cereals including: wild emmer wheat (Cakmak et al. 2004; Peleg et al. 2008a), emmer wheat, *Triticum dicoccum* (Gregorio 2002), bread wheat, *T. aestivum* L (Peterson et al. 1986; Raboy et al. 1991), and Triticale (Feil and Fossati 1995). Recently, the *Gpc-B1* allele from wild emmer was found to encode a NAC transcription factor (*NAM-B1*) inducing accelerated senescence and increased grain protein, Zn and Fe concentrations. It has been suggested that *NAM-B1*

**Table 4** Genotypic and phenotypic association among wheat grain protein and nine mineral nutrient concentrations and grain yield. The upper values indicate the number of corresponding QTLs (>50% overlap between their confidence intervals) out of the total number of

QTLs detected for each trait (indicated in parenthesis). The lower values indicate the coefficients of correlation ( $r$ ) between each pair of traits in the 152 RILs (LDN  $\times$  G18-16) averaged across the three environments

|         | GPC (10) | Zn (6)   | Fe (11) | Cu (10) | Mn (2) | Ca (9)  | Mg (8)  | K (8)   | P (8)    | S (10)  |
|---------|----------|----------|---------|---------|--------|---------|---------|---------|----------|---------|
| Zn (6)  | 3*       |          |         |         |        |         |         |         |          |         |
|         | 0.55***  |          |         |         |        |         |         |         |          |         |
| Fe (11) | 5**      | 5***     |         |         |        |         |         |         |          |         |
|         | 0.59***  | 0.79***  |         |         |        |         |         |         |          |         |
| Cu (10) | 6***     | 5***     | 5*      |         |        |         |         |         |          |         |
|         | 0.38***  | 0.56***  | 0.54*** |         |        |         |         |         |          |         |
| Mn (2)  | 1        | 0        | 2*      | 1       |        |         |         |         |          |         |
|         | 0.19*    | 0.29***  | 0.19*   | 0.03    |        |         |         |         |          |         |
| Ca (9)  | 2        | 1        | 1       | 1       | 0      |         |         |         |          |         |
|         | 0.14     | 0.27***  | 0.19*   | 0.08    | 0.24** |         |         |         |          |         |
| Mg (8)  | 1        | 2        | 3       | 1       | 0      | 1       |         |         |          |         |
|         | 0.46***  | 0.37***  | 0.33*** | 0.37*** | 0.23** | 0.4***  |         |         |          |         |
| K (8)   | 4*       | 0        | 2       | 1       | 1      | 4*      | 0       |         |          |         |
|         | 0.21*    | 0.05     | 0.11    | 0.18*   | -0.03  | 0.28*** | 0.27*** |         |          |         |
| P (8)   | 4*       | 2        | 2       | 2       | 0      | 2       | 3       | 1       |          |         |
|         | 0.61***  | 0.5***   | 0.49*** | 0.38*** | 0.14   | 0.37*** | 0.69*** | 0.53*** |          |         |
| S (10)  | 4*       | 3        | 2       | 3       | 0      | 2       | 3       | 2       | 3        |         |
|         | 0.66***  | 0.58***  | 0.5***  | 0.27*** | 0.2*   | 0.33*** | 0.52*** | 0.11    | 0.54***  |         |
| GY (6)  | 3*       | 1        | 1       | 2       | 1      | 2       | 0       | 1       | 1        | 1       |
|         | -0.29*** | -0.26*** | -0.22** | -0.18*  | 0.04   | 0.03    | -0.23** | -0.11   | -0.33*** | -0.24** |

\*, \*\*, and \*\*\* indicate significant correspondence (Larsen and Marx 1985; Paterson et al. 1995) or correlation coefficient at  $P \leq 0.05$ , 0.01 and 0.001, respectively

controls nutrient remobilization from leaves to grains (Uauy et al. 2006). In the current study, QTLs conferring GPC and Zn were clearly mapped to the same genomic region (6BS) and QTL for Fe was mapped to the same chromosome arm. The common genetic control of GPC, Zn and Fe was further demonstrated in two additional genomic regions (2A, 5A) in which QTLs for these three grain constituents exhibited significant overlap (Fig. 2).

Exceptionally strong association was found between QTLs conferring Zn and QTLs for Fe, with co-localization in five genomic regions ( $P = 0.0009$ ), two of which corresponded also to GPC. These findings are further supported by a significant positive correlation ( $r = 0.79$ ,  $P = 0.0001$ ) (Fig. 1; Table 4), indicating a strong genetic association between mechanisms affecting grain Zn and Fe concentrations. Numerous previous studies reported on positive correlation between grain Zn and Fe concentrations in cereals (e.g., Cakmak et al. 2004; Morgounov et al. 2007; Peleg et al. 2008a), however, only one study reported on co-localization of QTLs for Zn and Fe contents in rice (Stangoulis et al. 2007). In *Arabidopsis thaliana*, QTLs for Zn and Fe were found to be either co-localized (Waters and Grusak 2008) or not associated (Vreugdenhil et al. 2004).

The ten QTLs conferring Cu, found in the current study, were significantly co-localized with QTLs for GPC in six cases ( $P = 0.0006$ ), with QTLs for Zn in five cases ( $P = 0.0007$ ) and with QTLs for Fe in five cases ( $P = 0.01$ ) (Fig. 2, Table 4). These relationships were further supported by the PC analysis (Fig. 1) showing strong positive correlation between Cu concentration and GPC, Zn and Fe (Table 4). Grain Cu concentration was hardly investigated, presumably due to its low nutritional priority, and we are not aware of prior reports on phenotypic or genotypic (QTL overlap) association between Cu and other minerals in wheat grain. In wheat, Fe, Zn and Cu are highly mobile, while Mn is almost immobile in the phloem (Pearson and Rengel 1994; Garnett and Graham 2005). All nutrient transports into the grain must at some stage pass through the phloem due to xylem discontinuity in the grain stalk (O'Brien et al. 1985). This could explain, on the one hand, the close association between Zn, Fe and Cu, and on the other hand the low number of QTLs (2) detected for Mn. Notably, both QTLs for Mn were significantly co-localized with QTLs for Fe (Table 4).

Approximately 75% of the total P in the wheat grain is stored as phytic acid (*myo*-inositol 1,2,3,4,5,6-

hexakisphosphate), mostly in the germ and aleurone layers (Raboy 2000). This relatively small molecule with a high charge density is a strong chelator of positively charged mineral cations such as Fe, Zn, Ca, K and Mg (Raboy 2000; Lott and West 2001). In winter wheat, GPC was strongly correlated with both phytic acid and total P (Raboy et al. 1991). Thus, selection for increased GPC is expected to be associated with increased grain phytic acid. In the current study, four out of eight QTLs for grain P concentration were co-localized with QTLs for GPC ( $P = 0.027$ ), which was also supported by phenotypic association (Fig. 1, Table 4). However, while in three loci (2A, 4A, 7A) the wild allele was associated with higher values of P and GPC (Table 3), in one locus (5B) the wild allele conferred high GPC and low P, thus offering the prospect of improving GPC without increasing phytic acid. Four macronutrients, P, Ca, K and Mg, exhibited positive phenotypic association (Fig. 1, Table 4). QTLs conferring high Ca were co-localized with QTLs for high K in four genomic region ( $P = 0.01$ ), which may reflect the common affinity of Ca and K to phytate. However, QTLs for P and Mg showed no significant genetic association with either QTLs for K or Ca (Table 4). In agreement with these results, correspondence between QTLs for Ca and K co-localized with a known phytate locus was reported in *Arabidopsis thaliana* (Vreugdenhil et al. 2004).

An association between QTLs conferring high S and QTLs for GPC occurred in four genomic regions ( $P = 0.03$ ) (Fig. 2, Table 4). These results are further supported by significant positive phenotypic correlations between S and GPC (Fig. 1, Table 4). In all of these genomic region (2A, 4A, 5B, 6B) the wild allele contributed to increased N or S (Table 2). Plants tend to maintain a relatively constant ratio of organic N to organic S, particularly in their vegetative tissues, even though the ratio of total N to total S can vary widely in response to N and S supply (Dijkshoorn and van Wijk 1967). Therefore, the positive genotypic and phenotypic association between N and S is not surprising.

#### Association between grain mineral nutrient concentrations and grain yield

High yield capacity is a major requirement for any crop cultivar. Therefore, when breeding for other traits, special attention should be given to avoid negative effects on yield. In previous studies in wheat, grain mineral nutrient concentrations exhibited negative associations with GY (Löffler et al. 1983; Cox et al. 1985; Gauer et al. 1992; Groos et al. 2003; Calderini and Ortiz-Monasterio 2003; Oury et al. 2006). However, neither negative nor positive associations between grain mineral concentrations and productivity were found in wild emmer wheat (Peleg et al. 2008a). Recently, the same mapping population

(LDN × G18-16) tested under two contrasting water availabilities (WW05 and WL05), revealed 6 QTLs conferring GY (2B, 2B, 4A, 4B, 5A, 7B; Peleg et al. 2009). GY data under the three environments (WW05, WL05 and WW07) studied here for grain minerals were re-analyzed and showed similar results (not shown). Three out of ten QTLs conferring GPC were significantly associated with QTLs conferring GY (2B, 4A, 7B). High GPC and low yield were conferred by wild alleles in two genomic regions (2B, 4A) and by the domesticated allele in one region (7B). This was further supported by a negative correlation between GPC and GY ( $r = -0.29$ ,  $P = 0.0003$ ) (Table 4).

Abbo et al. (2009) hypothesized that under ancient non-fertilized practices, low nutrient requirement (and grain mineral concentrations) might have conferred GY advantage and was hence unintentionally selected for in farmers' fields. Indeed, modern wheat cultivars have typically lower grain protein and mineral concentrations relative to their wild ancestors (e.g., Peleg et al. 2008a). Yet seven QTLs for GPC (2A, 3B, 5A, 5B, 6A, 6B, 7A) explaining up to 13.7 (per single QTL) of the variation in GPC were not associated with GY. Furthermore, the six genomic region conferring GY (Peleg et al. 2009) were not significantly associated with QTLs for Zn ( $P = 0.23$ ), Fe ( $P = 0.28$ ) or other minerals (Table 4). Thus the introduction of QTLs for improved grain protein and mineral concentrations is not necessarily expected to reduce productivity.

#### Conclusions and implications for wheat improvement

Breeding staple food crops with higher nutrient concentration in the grain is a low-cost, sustainable strategy to alleviate micronutrient malnutrition. Increasing grain concentrations of mineral nutrients is also likely to improve seed germination and seedling development under various stress conditions. Wild emmer wheat was shown to offer abundant genetic diversity for multiple biotic and abiotic stress adaptive traits (Feldman and Sears 1981; Nevo et al. 2002), including grain protein and mineral nutrient concentrations (Avivi 1979; Cakmak et al. 2004; Peleg et al. 2008a). Indeed, in most QTLs detected in the current study the wild parent allele was favorable. Recently, a gene affecting GPC, Zn and Fe (*TiNAM-B1*) originating from wild emmer wheat was cloned (Distelfeld and Fahima 2007 and references therein). Likewise, QTL analysis of segregating population derived from cross between domesticated × wild bean revealed an advantage of the wild parental alleles in seed Zn and Fe content (158 and 180%, respectively) compared with cultivated bean (Guzmán-Maldonado et al. 2003).

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. Few genomic regions (Chr. 2A, 5A, 6B, 7A) were found to harbor clusters of QTLs for GPC and other minerals. These regions offer unique opportunities for synchronous improvement of GPC, Zn, Fe and other mineral nutrients in wheat grain. Nevertheless, genomic regions associated with only one or few minerals should not be overlooked as they may confer other, mineral-specific, mechanisms.

Our results exemplify unique opportunities to exploit favorable alleles that were excluded from the domesticated genepool as a result of the genetic bottleneck involved in the domestication processes. 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 crops.

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