P	P	L	0	8	4	1		B	Dispatch: 15.11.06	Journal: PPL	CE: Suchitra
Journal Name				Manuscript No.					Author Received:	No. of pages: 9	ME: Varma

Physiologia Plantarum 0: 1–9. 2006

Multiple QTL-effects of wheat *Gpc-B1* locus on grain protein and micronutrient concentrations

Assaf Distelfeld[a], Ismail Cakmak[b], Zvi Peleg[a,c], Levent Ozturk[b], Atilla M. Yazici[b], Hikmet Budak[b], Yehoshua Saranga[c] and Tzion Fahima[a,*]

[a]The Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31905, Israel
[b]Sabanci University, Faculty of Engineering & Natural Sciences, Istanbul, Turkey
[c]The R.H. Smith Institute of Plant Science & Genetics in Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Correspondence
*Corresponding author,
e-mail: fahima@research.haifa.ac.il

Received 19 September 2006; revised 2 October 2006

doi: 10.1111/j.1399-3054.2006.00841.x

Micronutrient malnutrition afflicts over three billion people worldwide and the numbers are continuously increasing. Developing genetically micronutrient-enriched cereals, which are the predominant source of human dietary, is essential to alleviate malnutrition worldwide. Wheat chromosome 6B derived from wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell] was previously reported to be a source for high Zn concentration in the grain. In the present study, recombinant chromosome substitution lines (RSLs), previously constructed for genetic and physical maps of *Gpc-B1* (a 250-kb locus affecting grain protein concentration), were used to identify the effects of the *Gpc-B1* locus on grain micronutrient concentrations. RSLs carrying the *Gpc-B1* allele of *T. dicoccoides* accumulated on average 12% higher concentration of Zn, 18% higher concentration of Fe, 29% higher concentration of Mn and 38% higher concentration of protein in the grain as compared with RSLs carrying the allele from cultivated wheat (*Triticum durum*). Furthermore, the high grain Zn, Fe and Mn concentrations were consistently expressed in five different environments with an absence of genotype by environment interaction. The results obtained in the present study also confirmed the previously reported effect of the wild-type allele of *Gpc-B1* on earlier senescence of flag leaves. We suggest that the *Gpc-B1* locus is involved in more efficient remobilization of protein, zinc, iron and manganese from leaves to the grains, in addition to its effect on earlier senescence of the green tissues.

Introduction

Micronutrient malnutrition, and particularly deficiency in Zn and Fe, afflicts over three billion people worldwide, particularly in developing countries (Welch and Graham 2004), resulting in overall poor health, anemia, increased morbidity and mortality rates and lower worker productivity (Black 2003, Cakmak et al. 2002, Holtz and Brown 2004). Many of the countries that suffer from micronutrient deficiencies in human's diet have also large areas of micronutrient-poor soils (White and Zasoski 1999). From an agronomic point of view, large seed reserves of mineral-nutrients can be important for the

Abbreviations – DIC, *Triticum turgidum* ssp. *dicoccoides* accession # FA-15-3; DIC-6B, the substitution of *T. dicoccoides* chromosome 6B into LDN; d.f., degrees of freedom; GFeC, grain iron concentration; GMnC, grain manganese concentration; GPC, grain protein concentration; Gpc-B1, a locus affecting GPC mapped to chromosome 6B; GY, grain yield; GZnC, grain zinc concentration; HD, heading date; LDN, Langdon; n.s., non-significant; PCA, principal component analysis; QTL, quantitative trait locus; RSL, recombinant chromosome substitution line; TKW, 1000 kernel weight.

early establishment of a crop, especially in micronutrient-poor soils (Asher 1987, Welch 1999).

Wheat is an important staple food for human and livestock in many parts of the world, contributing 28% of the world edible dry matter (FAOSTAT data; FAO 2005). Therefore, developing genetically micronutrient-enriched cereals and improving their bioavailability (biofortification) using genetics and genomics tools are considered promising and cost-effective approaches for diminishing malnutrition (Bouis 2003, Cakmak 2002, Fernie et al. 2006, Ghandilyan et al. 2006, Poletti et al. 2004, Tucker 2003, Welch and Graham 2004). Implementing these strategies, however, requires a comprehensive exploration of potential genetic resources and an in-depth understanding on their micronutrient accumulation mechanisms.

Wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell], the tetraploid (genome constitution AABB) progenitor of cultivated durum wheat [*T. turgidum* ssp. *durum* (Desf.) MacKey, AABB] and bread wheat (*Triticum aestivum* L., AABBDD) (McFadden and Sears 1946), is known to harbor a wide allelic variation relevant for the improvement of various economically important traits in cultivated wheats (e.g. Fahima et al. 1998, Nevo et al. 2002, Peleg et al. 2005), including grain mineral concentrations (e.g. Cakmak et al. 2004).

Triticum dicoccoides (accession # FA-15-3; DIC hereafter) from Israel was used by Joppa and Cantrell (1990) as a progenitor for the substitution of individual chromosomes into genetic background of the durum wheat cv. Langdon (CItr 13165, LDN hereafter). The substitution of *T. dicoccoides* chromosome 6B into LDN (DIC-6B hereafter) induced the highest grain protein concentration (GPC) among all other chromosome substitution lines. Cakmak et al. (2004) showed that the same substitution line also accumulated the highest grain Zn concentration (GZnC, hereafter).

The increased GPC in DIC-6B line was associated with a quantitative trait locus (QTL) located on the short arm of chromosome 6B (Joppa et al. 1997). Olmos et al. (2003) mapped the source of GPC variation as a single Mendelian locus, designated *Gpc-B1*, within a 2.7-cM chromosome segment. Microcolinearity between rice chromosome 2 and wheat chromosome 6B was used as a stepping-stone for the development of new markers that delimited the *Gpc-B1* locus to a 0.3-cM region (Distelfeld et al. 2004). Using this high-density genetic map and a wheat tetraploid Bacterial Artificial Chromosome (BAC) library (Cenci et al. 2003), a complete physical BAC contig of approximately 250-kb was established for the *Gpc-B1* locus (Distelfeld et al. 2006).

In the current study we used recombinant chromosome substitution lines (RSLs) to examine the effects of *Gpc-B1* locus on micronutrient concentrations in wheat grain under various environments.

Materials and methods

Plant material and experimental design

The plant material used in the present study included the following homozygous RSLs: RSL14, RSL28 and RSL77, developed from the cross DIC-6B × LDN (Joppa et al. 1997), RSL121 developed from the cross RSL65 × LDN (Olmos et al. 2003) and the parental lines LDN and DIC-6B. These RSLs were previously evaluated for GPC in three field experiments in the United States (Olmos et al. 2003) and confer critical recombination events that were used to develop a physical map of the *Gpc-B1* gene region within a 250-kb interval, flanked by markers *Xuhw89* and *Xucw71* (Distelfeld et al. 2006).

In the present study, RSLs were examined under five different environments: (1) the University of Haifa, Israel (Mt. Carmel; 35°01′E, 32°53′N; 480 m above sea level) during the growing season (November–May) of 2003–2004 (Haifa04); (2) and 2004–2005 (Haifa05); (3) the Atlit experimental station, Israel (34°58′E, 32°46′N; 32 m above sea level) in 2004–2005 (Atlit05); (4) The Hebrew University of Jerusalem experimental farm in Rehovot, Israel in 2004–2005 (34°47′N, 31°54′E; 54 m above sea level) under well-watered (approximately 700 mm; Wet05) and (5) water-limited (approximately 250 mm; Dry05) irrigation regimes. A complete randomized block design was employed in each environment, with five and four replications in 2004 and 2005, respectively. Each experimental plot consisted of one row, 80 cm long, with nine plants per plot and 30 cm between rows. All the experiments were fertilized with 100 kg ha^{-1} of N prior to planting and no additional fertilization was applied during the growth period.

Plant sampling and mineral analysis

Two plants from the center of each plot were harvested manually at physiological maturity. Grains were threshed, oven dried (37°C, 72 h), weighed and counted to obtain 1000 kernel weight (TKW) and grain yield per plant (GY). The dried grains were ground and analyzed for their mineral concentration. Nitrogen in the grain was determined by the indophenolblue procedure following Kjeldahl digestion (Jones and Case 1990, Smith 1980). Grain nitrogen concentration was multipled by 5.7 to obtain GPC expressed as percentage of dry grain weight. Grain concentrations of Zn (GZnC) Fe (GFeC), Mn (GMnC), Cu, K and Ca were determined by inductively coupled plasma-optical emission spectroscopy (Varian-Vista-Pro,

Australia). Measurements were calibrated using the certified mineral nutrient values in durum wheat flour samples (8436) obtained from the National Institute of Standards and Technology (Gaithersburg, MD).

The rate of plant senescence was determined in Haifa 2005 field experiment (Haifa05) by counting the number of yellow peduncles in 20 tillers from each plot at 3-day intervals; 24, 27, 30, 33, 36 and 39 days after spike heading date (HD, Zadoks stage 59; Zadoks et al. 1974).

Statistical analysis

The JMP® 6.0 statistical package (SAS Institute 2005) was used for conducting the statistical analyses, unless indicated otherwise. A factorial model was employed for the analysis of variance with genotype and environment considered as fixed effects. Comparison between genotypes was based on Duncan's least significant difference at the 5% probability level. Principal component analysis (PCA) was used to determine the associations among the variables measured using SPSS ver. 14 (SPSS for Windows 2005). PCA was based on the 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. Student's *t*-test was used to compare mean performances of the two genotypic groups under each environment and the mean number of yellow peduncles of the high-and low-GPC groups at each sampling date.

Results

Grain mineral concentrations and associations between traits

Analysis of variance revealed significant effects of environment on all of the measured traits and significant effects of genotype on GPC, GZnC, GFeC and GMnC but not on TKW and GY (Table 1). No significant genotype × environment (G × E) interactions were noted for any of the traits. Other nutrients (potassium, copper and calcium) did not differ significantly between genotypes (not presented) and therefore were excluded from subsequent analyses.

Large variation between environments was observed for each of the variables (Table 1), demonstrating the

Table 1. Analysis of variance of the effect of genotype and environment on grain protein concentration (GPC), grain zinc concentration (GZnC), grain iron concentration (GFeC), grain manganese concentration (GMnC), 1000 kernel weight (TKW) and grain yield (GY) in four recombinant substitution lines as well as the two parental lines grown under five different environments. Mean values for each genotype and environment are presented above. LDN, Langdon; DIC-6B, the substitution of *T. dicoccoides* chromosome 6B into LDN. **, *** and n.s. indicate significance at $P \leq 0.01$, 0.001 or non-significant effect, respectively. Within each trait, means of genotypes followed by different letters are significantly different by Duncan's least significant difference at 0.05 probability level. †Numbers in parenthesis indicate degrees of freedom (d.f.) in only four environments. ‡Field experiments were conducted under five different environments: Haifa 2004 (Haifa04), Haifa 2005 (Haifa05), Atlit 2005 (Atlit05), Rehovot water-limited treatment 2005 (Dry05) and Rehovot well-watered treatment 2005 (Wet05) field experiments.

		GPC (%)	GZnC (mg kg^{-1})	GFeC (mg kg^{-1})	GMnC† (mg kg^{-1})	TKW (g)	GY† (g plant^{-1})
Genotype							
DIC-6B		14.4 a	60.0 a	44.2 a	53.9 ab	49.5 ab	12.7 a
RSL28		14.1 a	58.6 a	43.6 a	53.4 ab	48.3 ab	10.7 a
RSL121		14.2 a	55.2 a	41.2 a	52.5 a	47.7 ab	11.8 a
LDN		10.8 b	47.5 b	35.9 b	40.9 cd	45.2 b	11.5 a
RSL14		10.4 bc	48.6 b	32.3 b	37.9 d	49.9 a	13.2 a
RSL77		9.9 c	50.5 b	36.1 b	46.3 b	47.8 ab	12.5 a
Environment‡							
Haifa04		10.8 bc	53.1 b	35.1 c	–	46.9 bc	–
Haifa05		11.1 b	63.9 a	37.4 c	40.9 b	43.6 c	12.8 b
Atlit05		7.7 c	35.7 c	26.1 d	32.2 c	43.6 c	6.8 c
Dry05		18.2 a	69.5 a	59.1 a	62.7 a	49.4 b	7.1 c
Wet05		17.8 a	53.1 b	42.4 b	62.8 a	60.9 a	25.5 a
Source of variance	d.f.†	Sum of square					
Genotype (G)	5 (5)	1710***	463**	311**	3871***	72, n.s.	73, n.s.
Environment (E)	4 (3)	11919***	79357***	45067***	15233***	4262***	3712***
G × E	20 (15)	66, n.s.	833, n.s.	611, n.s.	1347, n.s.	411, n.s.	345, n.s.
Experimental error	90 (73)	386	499	1501	2493	1076	768

considerable different conditions imposed at each year, location and irrigation treatment. Nevertheless, despite of the differences between environments, genotypic effects were consistent across all environments as manifested by the non-significant G × E interactions. The mean GPC, GZnC, GFeC and GMnC calculated across the five field environments were significantly higher in DIC-6B than in LDN (Table 1). In addition, RSLs 14 and 77 (LDN allele at the *Gpc-B1* locus) showed micronutrient and protein concentrations similar to LDN or lower (with exception of GMnC in RSL77), while RSLs 28 and 121 (DIC allele at the *Gpc-B1* locus) were similar to DIC-6B. No significant differences in GY were detected among the parental lines and the RSLs. A significant difference in TKW was detected only between LDN and RSL14, both carrying the LDN allele of *Gpc-B1* locus, while all other genotypes showed intermediate TKW values, not differing from either LDN or RSL14.

PCA of the four RSLs and their parental lines (averaged across five environments) extracted two PCs (Eigenvalues >1) that accounted collectively for 91.3% of the variance. PC1 (X-axis, Fig. 1) explained 65.8% of the data set variation, and was loaded positively with GPC, GZnC, GMnC and GFeC and was negatively loaded with GY. PC2 (Y-axis, Fig. 1) explained 25.5% of the data set variation, and was positively loaded with TKW and GY. Significant positive correlation coefficients were obtained between GPC and GZnC ($r = 0.94$, $P < 0.01$), GPC and GFeC ($r = 0.92$, $P < 0.01$) or GPC and GMnC ($r = 0.83$, $P < 0.05$).

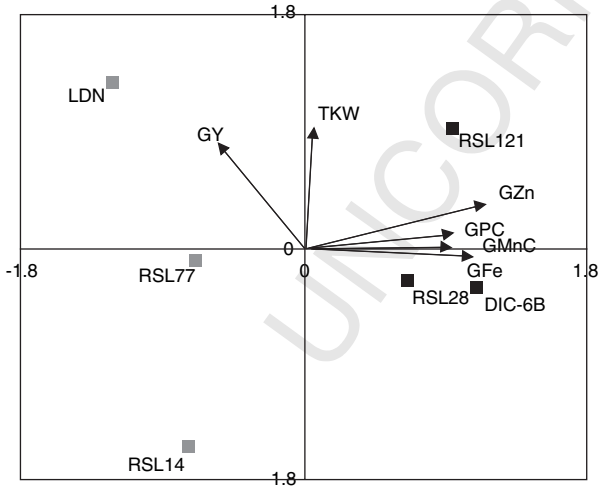

Fig. 1. Principal component analysis (based on correlation matrix) of continuous plant traits recorded on six lines in five field experiments. The three high-*Gpc-B1a* lines are marked with black rectangulars, while the three low-*Gpc-B1b* lines are marked with gray rectangulars. Biplot vectors are trait factor loadings for principal component (PC) 1 and PC2.

Gpc-B1 effects on grain mineral concentration

Olmos et al. (2003) mapped the *Gpc-B1* QTL to approximately 12-cM chromosome segment between markers *Xgwm193* and *Xgwm508* on wheat chromosome 6B (Fig. 2A). The graphical genotype analysis (Young and Tanksley 1989) of the four RSLs with critical recombination events along the *Gpc-B1* gene region is presented in Fig. 2B. The mean phenotypic effects on GPC, GZnC, GFeC and GMnC are presented for each genotype as the percentage difference relative to LDN (Fig. 2C). Olmos et al. (2003) classified RSL28, RSL121 and DIC-6B as high-GPC genotypes, while RSL14, RSL77 and LDN were classified as low-GPC genotypes. The high-GPC group is sharing a 250-kb segment containing the DIC-6B allele of the *Gpc-B1* locus (*Gpc-B1a*, hereafter), whereas the low-GPC group is sharing a 250-kb segment containing the LDN allele of the *Gpc-B1* locus (*Gpc-B1b*, hereafter) (Fig. 2B). This classification was confirmed in the present field experiments (Table 1). The mean GPC value of the *Gpc-B1a* group was higher than the mean GPC value of the *Gpc-B1b* group in each of the five tested environments (Table 2).

A similar classification pattern was also noted for the GZnC, GFeC and GMnC values obtained in the present study (Table 1). The mean GZnC values of the *Gpc-B1a* group were 7–17% higher than the mean values of the *Gpc-B1b* group under the five tested environments (Table 2). The mean grain Fe and Mn concentrations of the two groups followed the same pattern and showed a significant increase of 11–20% in GFeC and 20–40% increase in GMnC for the *Gpc-B1a* group as compared with the *Gpc-B1b* group in all of the tested environments (Table 2). In summary, the results obtained in the present study indicate that the 250-kb *Gpc-B1* chromosome interval is associated with the accumulation of nitrogen, zinc, iron and manganese in the grain.

Early senescence associated with higher GPC, GFeC, and GZnC

A comparison of senescence rate of *Gpc-B1a* and *Gpc-B1b* groups in the Haifa05 environment is presented in Fig. 3. Peduncle senescence was followed by counting the percent of yellow peduncles 24–39 days after spike HD. Yellowing of the peduncles was first observed 27 days after HD. The mean percentage of yellow peduncles was compared between the *Gpc-B1a* and *Gpc-B1b* groups, using separate Student's *t*-tests at each time point. Significant differences were evident between these two groups at 30 and 33 days after HD but not when most of the peduncles were still green (27 days after HD) or when most of the peduncles were yellow (36 days after

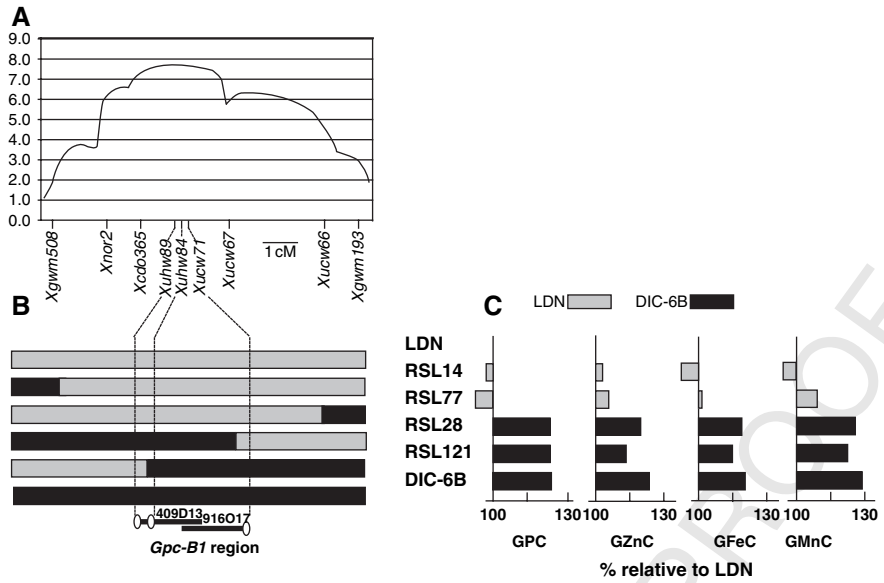

Fig. 2. Map of the effect of *Gpc-B1* locus on grain protein concentration (GPC), grain zinc concentration (GZnC), grain iron concentration (GFeC) and grain manganese concentration (GMnC). Genotypes are shown by a combination of gray bars representing Langdon (LDN) alleles and black bars representing DIC-6B alleles. (A) Quantitative trait locus mapping of *Gpc-B1* on the short arm of chromosome 6B. Adjusted based on results presented in Olmos et al. (2003) and Distelfeld et al. (2006). (B) Graphical genotypes of the recombinant chromosome substitution lines and parental lines used in this study. The distance between *Xgwm193* and *Xgwm508* is about 12 cM. The relative space between markers in this figure is not in proportion to the real distance in the genetic maps published by Olmos et al. (2003) and Distelfeld et al. (2006). The size of the *Gpc-B1* region defined by markers *Xuhw89* and *Xucw71* is 250-kb represented by two overlapping Bacterial Artificial Chromosome clones 409D13 and 916O17 (Distelfeld et al. 2006). (C) Phenotypic effect of each genotype on GPC, GZnC, GFeC and GMnC is shown as the percentage difference relative to LDN.

HD). Thirty days after HD, the percentage of yellow peduncles was almost six-fold higher (46 vs 8%) in the *Gpc-B1a* group than in the *Gpc-B1b* group. At 33 days after HD, the percentage of yellow peduncles was 2.5-fold higher (85 vs 34%) in the high-*Gpc-B1a* group than in the low-*Gpc-B1b* group. These results indicate that the high-*Gpc-B1a* lines senesced approximately 3 days earlier than the low-*Gpc-B1b* lines. HD and anthesis occurred for all the genotypes at the same time and, therefore, can not account for the differences in senescence rate.

Discussion

Phenotypic effects of the *Gpc-B1* locus

RSLs segregating for the *Gpc-B1* locus have been previously characterized in several independent studies, across different environments, revealing significantly higher GPC in lines carrying the wild emmer chromosome segment (Chee et al. 2001, Joppa et al. 1997, Olmos et al. 2003). In the present study, we have examined additional phenotypic effects of the *Gpc-B1* locus in durum wheat background. GPC values showed consistent increase of more than 20% (Fig. 2; Table 1) in the *Gpc-B1a* lines (RSL28, RSL121 and DIC-6B), carrying the DIC-6B allele, as compared with the *Gpc-B1b* lines (LDN, RSL14 and RSL77), carrying the LDN allele. The chromosomal segment from DIC-6B that is common to the *Gpc-B1a* genotypes is flanked by markers *Xucw71* and *Xuhw89*, 0.2 cM apart (Fig. 2B). This chromosome segment was physically mapped by developing a BAC contig spanning approximately 250-kb (Distelfeld et al. 2006), as part of our efforts toward positional cloning of the *Gpc-B1* gene.

In the present study we have characterize the LDN × DIC-6B RSLs for grain micronutrients (Zn, Fe and Mn) concentrations under five different environments. PCA showed association between GPC, GZnC, GMnC and GFeC (Fig. 1), which was further supported by highly significant correlations. Positive correlation between Zn and Fe concentrations in various *T. dicoccoides* accessions was previously reported by Cakmak et al. (2004). Positive correlation between grain protein and mineral concentration was previously reported for other cereals including bread wheat (Peterson et al. 1986, Raboy et al. 1991), emmer wheat, *Triticum dicoccum* (Gregorio 2002), maize, *Zea mays* L. (Feil and Banziger 1993) and Triticale (Feil and Fossati 1995).

The grain protein, Zn, Fe and Mn concentrations were found significantly higher in the high-*Gpc-B1a* group as

Table 2. Contrasts between the group of genotypes carrying the DIC-6B allele (*Gpc-B1a*) and the group of genotypes carrying the LDN allele (*Gpc-B1b*) in the *Xuhw89-Xucw71* chromosome 6BS interval grown across five environments: University of Haifa 2004 (Haifa04) and (Haifa05), Atlit 2005 (Atlit05), Rehovot water-limited 2005 (Dry05) and Rehovot well-watered 2005 (Wet05) field experiments. DIC-6B, the substitution of *T. dicoccoides* chromosome 6B into LDN; LDN, Langdon; GFeC, grain iron concentration; GMnC, grain manganese concentration; GPC, grain protein concentration; GZnC, grain zinc concentration; TKW, 1000 kernel weight. *, **, *** and n.s. indicate significance at $P \leq 0.05$, 0.01, 0.001 or non-significant effect, respectively. [a]Grain yield (GY) and GMnC were not measured in the Haifa04 experiment.

Traits	Environment	Mean value of *Gpc-B1a* group	Mean value of *Gpc-B1b* group	Significance
GPC (%)	Haifa04	11.4	8.2	**
	Haifa05	13.2	8.6	***
	Atlit05	9.5	6.7	***
	Dry05	21.3	15.4	***
	Wet05	19.2	15.1	***
GZnC (mg kg^{-1})	Haifa04	55.9	50.2	***
	Haifa05	66.7	61.0	*
	Atlit05	36.8	34.5	*
	Dry05	74.1	65.5	**
	Wet05	55.3	47.4	*
GFeC (mg kg^{-1})	Haifa04	38.3	31.8	***
	Haifa05	39.3	35.5	*
	Atlit05	28.3	23.6	***
	Dry05	63.5	54.1	**
	Wet05	44.0	39.2	*
GMnC$^+$	Haifa04	–	–	–
(mg kg^{-1})	Haifa05	37.4	26.9	***
	Atlit05	44.3	36.9	***
	Dry05	73.8	52.8	***
	Wet05	68.8	56.9	*
TKW (g)	Haifa04	46.2	48.0	n.s.
	Haifa05	45.3	42.7	n.s.
	Atlit05	42.9	44.2	n.s.
	Dry05	50.8	47.6	n.s.
	Wet05	60.4	61.3	n.s.
GY[a] (g)	Haifa04	–	–	–
	Haifa05	8.1	7.0	n.s.
	Atlit05	6.3	7.4	n.s.
	Dry05	7.6	6.6	n.s.
	Wet05	26.5	24.8	n.s.

compared with the low-*Gpc-B1b* group (Table 2). These results indicate that the *Gpc-B1* genomic region is responsible for multiple phenotypic effects on the concentrations of grain micronutrients (Fig. 2). In addition to the new phenotypic effects reported here (i.e. GZnC, GFeC and GMnC), the *Gpc-B1* locus was recently shown to affect the senescence rate in both tetraploid and hexaploid wheat backgrounds (Uauy et al. 2006). In that study it was shown that plants differing at the *Gpc-B1* locus, exhibited different senescence rates despite of similar ear emergence and HDs. *Gpc-B1a* genotypes (DIC-6B allele) senesced approximately 4 days earlier than *Gpc-B1b* genotypes (*T. durum* or *T. aestivum* allele) (Uauy et al. 2006). In the present study, we have confirmed these differences in senescence rate (Fig. 3).

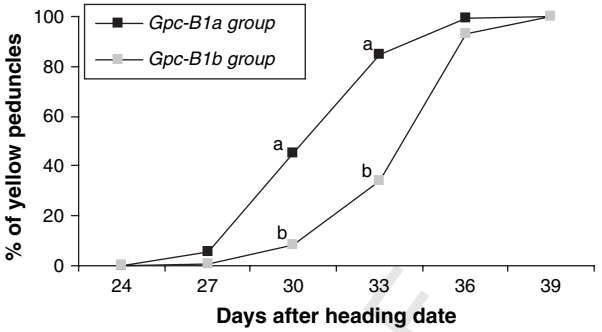

Fig. 3. Comparison of senescence rate of *Gpc-B1a* vs *Gpc-B1b* groups. Peduncles senescence was followed by counting the percent of yellow peduncles 24–39 days after heading date. Student's *t*-test was used to test for differences between the two alleles at each time point, separately. Values marked by different letter represent significant difference at 5% level.

Considering the small genomic region (250-kb) of the *Gpc-B1* locus affecting GPC, GZnC, GFeC, GMnC and senescence, this chromosome segment is likely to carry either a few tightly linked genes or a single gene that affects all of the evaluated traits. The *Gpc-B1* locus is tightly linked (0.1 cM) to a high-throughput co-dominant marker, *Xuhw89*. A 4-bp deletion present in the DIC allele was absent in a collection of 117 cultivated tetraploid and hexaploid wheat germplasm (Distelfeld et al. 2006). Hence, the *Xuhw89* marker could be useful for markers assisted breeding programs aimed to increase GPC, GZnC, and GFeC and GMnC.

Proposed mechanism for the phenotypic effects of *Gpc-B1* locus

Mineral accumulation in grains is determined primarily by the amount absorbed by the roots from soil during vegetative development and later on by the proportion of that amount remobilized to the grain from vegetative tissues (e.g. stem and leaves) during the grain filling period. It should be noted that all minerals transported into the wheat grain must at some stage pass through the phloem because of the xylem discontinuity in the grain stalk (O'Brien et al. 1985).

Kade et al. (2005) reported that a high-GPC RSL68, exhibited at maturity a 12% increase in GPC and a 10% decrease in straw N concentration as compared with LDN. Kade et al. (2005) suggested that the *Gpc-B1a* allele is responsible for more efficient translocation of the available nitrogen from the leaves into the grain, during grain development and maturation. Most of the translocated N originates mainly from degradation of chloroplast proteins occurring during leaf senescence (Hortensteiner and Feller 2002). In light of the results obtained in the present study, we suggest to extend the possible mechanism responsible for more efficient nitrogen remobilization to include also Zn, Fe and Mn.

The transport forms of Fe, Zn and Mn in phloem are still unclear. The possible role of proteins as potential candidates for chelating micronutrients in phloem have been discussed by Grusak et al. (1999) and Van Goor and Wiersma (1976). In addition, von Wiren et al. (1999) suggested that Zn and Fe are most likely chelated by nicotianamine during phloem transport. A more efficient remobilization of minerals through the phloem into the grain could lead to higher mineral concentrations in the *Gpc-B1a* lines, accompanied by an accelerated senescence rate. This model would assume that the *Gpc-B1* locus may harbor gene(s) possibly encoding for (either one or more) transporters, chelators, chelator biosynthesis enzymes, regulatory factors such as protein kinases, membrane receptors or transcription factors.

Another possible mechanism is through the effect of the *Gpc-B1* locus on senescence-related processes in green tissues as presented by Uauy et al. (2006), and confirmed in the current study. These processes could be influenced by repression of housekeeping genes and activation of genes related to chloroplast degradation. These processes may produce higher levels of nutrients that are available for remobilization from the senesced tissues into the grain and as a consequence to the accumulation of higher levels of these minerals in the matured grains. The results of the present study are consistent with this model by showing that accelerated senescence in the *Gpc-B1a* lines was associated with higher levels of grain proteins and minerals (e.g. Zn, Fe and Mn).

Interaction between mineral accumulation, environmental conditions and GY

When selecting for high grain minerals concentration, breeders should take into consideration the impact of selection on yield components. The effect of chromosome 6B introgression on yield was presented in several other studies that showed negative or no correlation of GPC with yield (Cantrell and Joppa 1991, Chee et al. 2001, Steiger et al. 1996). In the present study, GY or TKW were not associated with grain protein or mineral concentrations (Table 1, Fig. 1), thus excluding reduction in grain size or yield as a possible cause for the increased concentrations of grain protein and minerals.

The influence of the *Gpc-B1* locus on senescence (Fig. 3) could provide a possible explanation for the negative effect of *Gpc-B1* on yield observed in other studies. Early senescence caused by the *Gpc-B1a* allele may result in a shorter grain filling period. Spano et al. (2003) have shown that just a few days difference in senescence may have significant effects on grain size, yield and GPC. In addition, Hurkman et al. (2003) reported that high temperatures during grain development reduced the duration of starch accumulation. In the Mediterranean-type of environments, starch synthesis period is restricted by water availability and high temperatures at the end of the season (i.e. terminal drought), thus the effect of *Gpc-B1a* on early senescence is not expected to affect grain size. In mild climates, however, the environmental conditions permit longer starch accumulation and grain filling period and therefore the early senescence may cause reduction in grain size and result in a negative correlation between mineral concentrations (e.g. N, Zn, Fe and Mn) and GY.

Conclusions and prospects for wheat improvement

Our results suggest that the *Gpc-B1a* allele of *T. dicoccoides*, already known to increase GPC, is also playing an important role in grain mineral accumulation. In the present study we have shown that the effect of the *Gpc-B1a* on protein and mineral concentrations was consistent across five different environments. The incorporation of the *Gpc-B1a* into commercial wheat cultivars has the potential to improve both protein and micronutrient concentrations in the grain. The recently developed marker, *Xuhw89* (Distelfeld et al. 2006), could accelerate this process, while future positional cloning of the *Gpc-B1* gene may expand this advantage into other cereals, as well.

Acknowledgements – This study was supported by Research Grant No. US-3573-04C, the United States–Israel Binational Agricultural Research and Development Fund, Biofortification Challenge Program-HarvestPlus (http://www.harvestplus.org), the State Planning Organization of Turkish Republic and equipment grants from Israel Science Foundation (No. 048/99 and 1478/04). The authors wish to thank A. Avneri, L. Beloborodov, I. Ben-Israel, Y. Givati-Rap and Y. Shkolnik for excellent technical assistance. A. Distelfeld is indebted to Vaadia-BARD Postdoctoral Fellowship Award No. FI-386-06.

References

Asher CJ (1987) Crop nutrition during establishment phase: role of seed reserves. In: Wood IM, Hazard WH, Rom F (eds) Crop Establishment Problem in Queensland Recognition Research and Resolution. The Australian Institute of Agricultural Science and Technology, Brisbane, pp 88–106

Black MM (2003) Micronutrient deficiency and cognitive function. J Nutr 133: 3927S–3931S

Bouis HE (2003) Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proc Nutr Soc 62: 403–411

Cakmak I (2002) Plant nutrition research: priorities to meet human needs for food in sustainable ways. Plant Soil 247: 3–24

Cakmak I, Graham R, Welch RM (2002) Agricultural and molecular genetic approaches to improving nutrition and preventing micronutrient malnutrition globally. In: Cakmak I, Welch RM (eds) Encyclopedia of Life Support Systems. Eolss Publishers, Oxford, pp 1075–1099

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol AB, Nevo E, Braun HJ, Ozkan H (2004) *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Sci Plant Nutr 50: 1047–1054

Cantrell RG, Joppa LR (1991) Genetic analysis of quantitative traits in wild emmer (*Triticum turgidum* L. var. *dicoccoides*). Crop Sci 31: 645–649

Cenci A, Chantret N, Kong X, Gu Y, Anderson OD, Fahima T, Distelfeld A, Dubcovsky J (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor Appl Genet 107: 931–939

Chee PW, Elias EM, Anderson JA, Kianian SF (2001) Evaluation of a high grain protein QTL from *Triticum turgidum* L. var. *dicoccoides* in an adapted durum wheat background. Crop Sci 41: 295–301

Distelfeld A, Uauy C, Olmos S, Schlatter AR, Dubcovsky J, Fahima T (2004) Microcolinearity between a 2-cM region encompassing the grain protein content locus *Gpc-6B1* on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct Integr Genomics 4: 59–66

Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. New Phytol 169: 753–763

Fahima T, Nevo E, Röder MS, Grama A (1998) Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96: 187–195

FAO (2005) FAOSTAT data. http://apps.fao.org/

Feil B, Banziger M (1993) Nitrogen and cultivar effect on the minerals element concentration in the grain of spring wheat. Eur J Agron 2: 205–212

Feil B, Fossati D (1995) Minerals composition of Triticale grains as related to grain yield and grain protein. Crop Sci 35: 1426–1431

Ghandilyan A, Vreugdenhil D, Aarts MGM (2006) Progress in the genetic understanding of plant iron and zinc nutrition. Physiol Plant 126: 407–417

Gregorio GB (2002) Progress in breeding for trace minerals in staple crops. J Nutr 132: 500S–502S

Grusak MA, Pearson JN, Marentes E (1999) The physiology of micronutrient homeostasis in field crops. Field Crop Res 60: 41–56

Holtz C, Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25: 94–204

Hortensteiner S, Feller U (2002) Nitrogen metabolism and remobilization during senescence. Exp Bot 53: 927–937

Hurkman WJ, McCue KF, Altenbach SB, Korn A, Tanaka CK, Kothari KM, Johnson EL, Anderson OD, DuPont FM, Bechtel DB, Wilson JD (2003) Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. Plant Sci 164: 873–881

Jones JB, Case VW (1990) Sampling, handling, and analyzing plant tissue samples. In: Westerman RL (ed) Soil Testing and Plant Analysis. SSSA, Madison, WI, pp 389–427

Joppa LR, Cantrell RG (1990) Chromosomal location of genes for grain protein content of wild tetraploid wheat. Crop Sci 30: 1059–1064

Joppa LR, Hart GE, Hareland GA (1997) Mapping a QTL for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. Crop Sci 37: 1586–1589

Kade M, Barneix AJ, Olmos S, Dubcovsky J (2005) Nitrogen uptake and remobilization in tetraploid 'Langdon' durum wheat and a recombinant substitution line with the high grain protein gene *Gpc-B1*. Plant Breed 124: 343–349

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37: 81–89

Nevo E, Korol AB, Beiles A, Fahima T (2002) Evolution of Wild Emmer and Wheat Improvement: Population Genetics, Genetic Resources, and Genome Organization of Wheat's Progenitor, Triticum dicoccoides. Springer-Verlag, Berlin, Germany, 364 pp

O'Brien TP, Sammut ME, Lee JW, Smart MG (1985) The vascular system of the wheat spikelet. Aust J Plant Physiol 12: 487–512

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J (2003) Precise mapping of a locus affecting grain protein content in durum wheat. Theor Appl Genet 107: 1243–1251

Peleg Z, Fahima T, Abbo S, Krugman T, Nevo E, Yakir D, Saranga Y (2005) Genetic diversity for drought resistance in wild emmer wheat and its ecogeographical associations. Plant Cell Environ 28: 176–191

Peterson CV, Johnson VA, Mattern PJ (1986) Influence of cultivar and environment on mineral and protein concentration of wheat flour, bran, and grain. Cereal Chem 63: 183–186
Poletti S, Gruissem W, Sautter C (2004) The nutritional fortification of cereals. Curr Opin Biotechnol 15: 162–165
Raboy V, Noaman MH, Taylor GA, Pickett SG (1991) Grain phytic acid and protein are highly correlated in winter wheat. Crop Sci 31: 631–635
SAS Institute (2005) Jmp® Start Statistic (3rd Edn). Thomson Learning, Belmont, CA
SPSS for Windows (2005) Chicago: SPSS Inc
Smith VR (1980) A phenol-hypochlorite manual determination of ammonium-nitrogen in Kjeldahl digests of plant tissue. Soil Sci Plant Anal 11: 709–722
Spano G, Di FN, Perrotta C, Platani C, Ronga G, Lawlor DW, Napier JA, Shewry PR (2003) Physiological characterization of 'stay green' mutants in durum wheat. J Exp Bot 54: 1415–1420
Steiger DK, Elias EM, Cantrell RG (1996) Evaluation of lines derived from wild emmer chromosome substitutions: I. Quality traits. Crop Sci 36: 223–227
Uauy C, Brevis JC, Dubcovsky J (2006) The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. J Exp Bot doi:10.1093/jxb/erl047
Van Goor BJ, Wiersma D (1976) Chemical forms of manganese and zinc in phloem exudates. Physiol Plant 36: 213–216
von Wiren N, Klair S, Bansal S, Briat JF, Khodr H, Shioiri T, Leigh RA, Hider RC (1999) Nicotianamine chelates both Fe^{III} and Fe^{II}. Implications for metal transport in plants. Plant Physiol 119: 1107–1114
Welch RM (1999) Importance of seed mineral nutrient reserves in crop growth and development. In: Rengel Z (ed) Mineral Nutrition of Crops: Fundamental Mechanisms and Implications. Food Products Press, New York, pp 205–226
Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55: 353–364
White JG, Zasoski RJ (1999) Mapping soil micronutrients. Field Crop Res 60: 11–26
Young N, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77: 95–101
Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14: 415–421

Edited by J. K. Schjørring

Author Query Form

Journal: Physiologia Plantarum

Article: ppl_841

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.
Many thanks for your assistance.

Query No.	Query	Remark
1	Please check that references Fernie et al. (2006) and Tucker (2003) are not given in the reference list.	
2	Please check the citation of table footnotes "†" and "‡" in Table 1.	
3	Please check that it is not clear what the superscript + indicates in "GMnC+" in Table 2.	

MARKED PROOF

Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

Instruction to printer	*Textual mark*	*Marginal mark*
Leave unchanged	··· under matter to remain	New matter followed by or or
Insert in text the matter indicated in the margin		
Delete	through single character, rule or underline or through all characters to be deleted	or
Substitute character or substitute part of one or more word(s)	through letter or through characters	new character or new characters
Change to italics	under matter to be changed	
Change to capitals	under matter to be changed	
Change to small capitals	under matter to be changed	
Change to bold type	under matter to be changed	
Change to bold italic	under matter to be changed	
Change to lower case	Encircle matter to be changed	
Change italic to upright type	(As above)	
Change bold to non-bold type	(As above)	
Insert 'superior' character	through character or where required	or under character e.g. or
Insert 'inferior' character	(As above)	over character e.g.
Insert full stop	(As above)	
Insert comma	(As above)	
Insert single quotation marks	(As above)	or and/or or
Insert double quotation marks	(As above)	or and/or or
Insert hyphen	(As above)	
Start new paragraph		
No new paragraph		
Transpose		
Close up	linking characters	
Insert or substitute space between characters or words	through character or where required	
Reduce space between characters or words	between characters or words affected	