Quantitative Trait Loci Conferring Grain Selenium Nutrient in Durum Wheat × Wild Emmer Wheat RIL Population

Jun YAN[1], Wen-tao XUE[2], Rong-zhi YANG[2], Hai-bo QIN[3], Gang ZHAO[1], Fahima TZION[4] and Jian-ping CHENG[2]*

[1]Key Laboratory of Coarse Cereal Processing in Ministry of Agriculture, School of Pharmacy and Bioengineering, Chengdu University, Chengdu, P.R. China; [2]College of Agriculture, Guizhou University, Guiyang, P.R. China; [3]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, P.R. China; [4]Institute of Evolution, University of Haifa, Haifa, Israel
Corresponding author: jpcheng@gzu.cdu.cn

Abstract

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The genetic and phenotypic basis of grain selenium concentration (GSeC) and yield per plant (GSeY) was studied in a tetraploid wheat population consisting of 152 F_6 recombinant inbred lines (RILs) derived from a cross between *Triticum dicoccoides* (accession G18-16) and durum wheat cultivar Langdon (LDN) grown under three different environments over 2 years. Wide genetic variation was found among RILs for GSeC and GSeY. A total of 15 QTL effects on 9 chromosomes associated with GSeC and GSeY were detected, with a logarithm od the odds (LOD) score ranging from ca. 3.2 to 11.7, explaining 1.4% to 18.6% of the phenotypic variation. Higher GSeC and GSeY were conferred by the G18-16 allele at 10 loci and by the LDN allele at 5 loci. Seven QTLs showed interactions with environmental conditions. Five genomic regions harbouring QTLs for grain Se concentrations and yields were selected for further marker-assisted selection programs, facilitating the use of wild alleles for improvement of elite wheat cultivars.

Keywords: grain selenium concentration; grain selenium yield; quantitative trait locus mapping; tetraploid wheat; *Triticum dicoccoides*

Selenium (Se) is essential for formation and activity of the enzyme glutathione peroxidase. This enzyme is necessary to protect the body from inflammatory agents, mutagenic agents, and carcinogens (Combs 2001; Rayman 2008). Se enters the food chain mainly through plants. Researchers have shown that Se is an essential trace element for plants and indeed, Se levels in plant foods are generally low (Rayman 2008). Cereal crops such as wheat, oat, rye and barley are non-accumulators of Se, rarely having more than 0.1 mg Se/kg dry weight (WHO 1987). Nevertheless, on a global level, cereal products contribute a major amount of the dietary Se to humans, especially in developing countries such as China and India (FAO/WHO 2001). There is evidence of Se deficiency in more than 40 countries worldwide. For example, 72% of the areas in China have low or no Se in the soil, and in two-thirds of the population Se intake is deficient (Yan 2010).

Three strategies – dietary diversification, supplementation, and food fortification – are practiced to improve Se nutrient supply, each with its advantages and disadvantages (Ma 2007; Yan 2010). Another strategy was recently explored, biofortification, which aims at increasing nutrition in plant-based foods by breeding. This is a promising and cost-effective approach for diminishing malnutrition (Yan 2010; Velu *et al.* 2014).

Selenium in wheat grain is one of the most bioavailable forms (Hakkarainen 1993). However, wheat has

inherently very low concentrations of micronutrients relative to humans' daily requirements. In cultivated wheat, variation in seed Se concentration is relatively small and does not seem to hold any promise for genetic improvement (Yan 2010). However, wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), the tetraploid progenitor of cultivated durum wheat and bread wheat, is known to harbour wide allelic variation which is relevant for the improvement of various economically important traits in cultivated wheat (Uauy *et al.* 2006). In our previous study, very high concentrations and substantial variations in grain Se content were found among the wild emmer wheat genotypes, and the variation and absolute values among these genotypes for grain Se concentrations (GSeCs) were much higher than those found among hexaploid and tetraploid wheat cultivars (Yan 2010).

The existence of large genetic variation for micronutrients in grains is essential for the success of breeding programs aimed at developing new micronutrient-rich plant genotypes. The identification of quantitative trait loci (QTLs) may assist these breeding programs; moreover, the application of genetic modifications is being made possible by the increasing availability of information on the biochemistry of mineral accumulation (Peleg *et al.* 2009). QTL analysis is a powerful tool in agricultural studies, indicating the chromosomal location of genes suitable for breeding programs. For example, a major QTL from wild emmer wheat, pinpointing the chromosomal location of *Gpc-B1*, a gene associated with increased grain protein, zinc (Zn), and iron (Fe) contents, was found and subsequently cloned (Uauy *et al.* 2006). A few studies on QTLs for minerals, e.g. Zn, Fe, copper (Cu), manganese (Mn), phosphorus (P), potassium (K), nitrogen (N), sulphur (S), calcium (Ca) and magnesium (Mg), in wheat grains have been reported in recent decades (Peleg *et al.* 2009; Yan 2010; Pu *et al.* 2014; Tiwari *et al.* 2016). However, studies on QTL mapping of GSeC are scarce. Therefore, our objective was to conduct genetic mapping of QTLs for grain Se density with a view to developing near-isogenic lines (NILs) by backcrossing for introgression of genes associated with high GSeC derived from the wild emmer wheat gene pool into elite wheat cultivars.

MATERIAL AND METHOD

Material. A mapping population consisting of 152 F_6 recombinant inbred lines (RILs) derived from a cross between the durum wheat cultivar Langdon (LDN, as female), and wild emmer wheat accession G18-16 (as male) originating from Gitit in Israel was used in the current study for genetic mapping of grain mineral QTLs. The RILs were tested in the field under three environments over 2 years as described by Peleg *et al.* (2008). The environments were WL05 (water-limited /350 mm/ control in 2005), WW05 (well-watered /750 mm/ treatment in 2005), and WW07 (well-watered /720 mm/ treatment in 2007). The wet treatment was irrigated weekly with total amount of 750 or 720 mm, whereas the dry treatment was irrigated every other week with total amount of 350 mm. Water was applied during the winter months (December–March) to mimic the natural pattern of rainfall in the eastern Mediterranean region (Peleg *et al.* 2008). 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 concentration analyses (Peleg *et al.* 2009).

Se determination. The Se concentration in the wheat grains was determined by hydride generation-atomic fluorescence spectrometry (HG-AFS) following the protocol described in Yan *et al.* (2011). GSeC was expressed as μg/kg dry weight. Grain Se yield (GSeY) was calculated from GSeC and GY, and expressed as μg/plant (grain Se concentration per plant).

Statistical analyses. Statistical analyses of the data were conducted using the JMP® Ver. 6.0 statistical package. Broad-sense heritability estimate (h^2) was calculated for each trait across the three irrigation regimes, as described in Peleg *et al.* (2009).

QTL analysis. A genetic linkage map of 2317 cM was previously developed for the 152 F_6 RIL mapping population based on 197 single-sequence repeats and 493 diversity array technology (DArT) markers (Peleg *et al.* 2008).

MultiQTL Software v. 2.6 (http://www.multiqtl.com) was used to identify promising QTLs for GSeC and GSeY. QTL detection was carried out with a structured multistep scheme embedded in the software as described in Peleg *et al.* (2009) and Yan (2010).

RESULTS

Phenotypic variations in the RIL mapping population for GSeC and GSeY under different environmental conditions. Analysis of variance

Table 1. Mean values, ranges and heritability estimates (h^2) for grain Se concentration and yield (GSeC and GSeY, respectively) in 152 recombinant inbred lines (RILs), as well as the two parental lines of tetraploid wheat, under three environmental conditions (WL05, WW05, WW07)

Trait	WL05				WW05				WW07				h^2
	RILs		parents		RILs		parents		RILs		parents		
	mean	range	LDN	G18-16	mean	range	LDN	G18-16	mean	range	LDN	G18-16	
GSeC (µg/kg)	69.9	20.7–125.8	54.5	78.5	69.0	22.3–123.7	54.9	83.6	51.0	22.8–95.6	29.3	34.8	0.63
GSeY (µg/plant)	1.90	0.32–3.97	1.23	1.86	3.88	1.15–9.51	2.02	4.21	1.54	0.64–3.53	1.73	0.45	0.56

WL05 – water-limited (350 mm) control in 2005; WW05 – well-watered (750 mm) treatment in 2005; WW07 – well-watered (720 mm) treatment in 2007; LDN – durum wheat cultivar Langdon; G18-16 – wild emmer wheat accession

(ANOVA) indicated significance ($P \leq 0.05$) of genetic variation for the analysed nutrient Se. All variables under each of the environments exhibited normal distribution (Yan 2010). For GSeC and GSeY, under the three environments across 2 years, the wild accession (G18-16) showed higher values than the cultivated durum line LDN, except for GSeY-WW07. Transgressive segregation was common for both GSeC and GSeY (Table 1). Broad-sense heritability estimates (h^2) of 0.63 and 0.56, respectively, indicated that the proportion of phenotypic variation in these two parameters could be attributed to genotypic differences (Table 1).

QTLs for grain Se concentration and yield

GSeC. A total of seven significant QTLs were associated with GSeC, with LOD scores ranging from 3.2 to 9.9, explaining 1.4–18.6% of the variation (Table 2). Higher GSeC was conferred by the G18-16 allele at five loci (1A, 1B, 5A, 7A, 7B) and by the LDN allele at two loci (3A, 7B). Two QTLs showed significant G × E interaction: one QTL (7A) was found only under the WL05 environment (Figure 1).

GSeY. A total of eight significant QTLs were associated with GSeY, with LOD scores ranging from 3.2 to 11.7, explaining 1.4–15.5% of the variation (Table 2). Higher GSeY was conferred by the G18-16 allele at five loci (1A, 1B, 2B, 5A, 7B) and by the LDN allele at three loci (2B, 4B, 6A). Five QTLs showed significant G × E interaction: two QTL (1B, 6A) were found only under WW07 and one (1A) under the WL05 environment (Figure 1).

DISCUSSION

Human health relies on getting sufficient nutrients in the daily diet (Velu *et al.* 2014). Micronutrient malnutrition, also known as "hidden hunger", is caused by a lack of dietary vitamins and minerals, such as vitamin A, Zn and Fe, which are essential for good health (http://www.harvestplus.org). Therefore, our previous and present study adds to our knowledge on QTLs for grain contents of protein and some trace elements (Fe, Zn, Se) (Peleg *et al.* 2009; Yan 2010), as well as their yield, which is of particular importance to human nutrition.

Se is of metabolic importance in some plants, due to its involvement in anti-oxidative processes, although the essentiality of Se to higher plants is still under debate (Germ & Stibilj 2007). Cultivation of plants enriched with Se could be an effective way of producing Se-rich foodstuffs, thereby increasing their health benefits (Lyons *et al.* 2005). This calls for the exploration of desirable genes/QTLs harboured by valuable cereals as a basis for future development of genetically micronutrient-enriched cereals. Nevertheless, reports of QTLs for grain Se density are limited for crops. Several studies have been conducted on rice (*Oryza sativa* L.) and lentil (*Lens culinaris* M.). Norton *et al.* (2010) detected six QTLs for grain Se concentration in rice. Four QTLs were detected for grain Se content in rice by Yan *et al.* (2015). Ates *et al.* (2016) identified four QTLs associated with seed Se concentration in lentil. There is only one study in which a GSeC QTL has been reported for wheat. Using two RIL populations, Pu *et al.* (2014) identified five QTLs for GSeC on five chromosomes: 3D, 4A, 4D, 5B and 7D. In the current study, seven new QTLs (on chromosomes 1A, 1B, 3A, 5A, 7A, 2×7B) for GSeC, and eight QTLs (on chromosomes 1A, 1B, 2×2B, 4B, 5A, 6A, 7B) for GSeY were identified (Table 2, Figure 1). This is the first study reporting QTLs of both GSeC and GSeY using tetraploid wheat RIL populations. These QTLs may be used to conduct fine mapping for grain Se

Table 2. Biometrical parameters of QTLs affecting grain Se concentration and yield (GSeC and GSeY, respectively) in a tetraploid wheat recombinant inbred line (RIL) population (LDN × G18-16)

Trait	Position (cM)	Nearest marker	LOD	WL05 var (%)	WL05 d	WW05 var (%)	WW05 d	WW07 var (%)	WW07 d	Favourable allele[d]	G × E
GSeC											
1A	169.0 ± 33.3	wPt-5077	5.7**	6.4	8.02 ± 4.00	2.4	2.86 ± 5.05	7.0	6.11 ± 4.23	G	n.s.
1B	152.2 ± 23.2	wPt-5061	5.3**	3.6	2.95 ± 6.03	2.7	−0.03 ± 5.89	8.1	6.73 ± 4.72	G	n.s.
3A	69.8 ± 13.9	wPt-1092	5.4**	6.0	−7.64 ± 4.11	5.4	−7.19 ± 4.33	4.4	−5.18 ± 2.98	L	n.s.
5A	26.7 ± 24.2	gwm293	5.6**	13.2	12.10 ± 4.30	2.7	4.10 ± 4.20	1.4	1.18 ± 3.25	G	*
7A	25.0 ± 39.1	wPt-9926	3.2*	11.4	9.10 ± 7.30	–	–	–	–	G	–
7B-QTL1	85.8 ± 43.1	wPt-11565	9.9*	8.9	5.12 ± 13.95	18.6	11.28 ± 18.42	6.0	1.49 ± 5.46	G	–
7B-QTL2	128.6 ± 23.6	wPt-3730			−5.79 ± 13.03		−6.8 ± 16.64		−0.61 ± 6.76	L	–
GSeY											
1A	149.2 ± 36.1	wPt-4399	3.4*	13.6	0.429 ± 0.262	–	–	–	–	G	
1B	168.3 ± 54.2	wPt-1770	3.6*	–	–	–	–	14.7	0.181 ± 0.312	G	
2B-QTL1	50.4 ± 18.6	wPt-6199	11.7**	13.7	0.311 ± 0.234	15.5	0.601 ± 0.389	3.3	0.008 ± 0.166	G	–
2B-QTL2	113.5 ± 25.1	wPt-1294			−0.242 ± 0.304		−0.484 ± 0.510		0.054 ± 0.135	L	–
4B	74.0 ± 14.6	wPt-7156	6.7**	12.2	−0.472 ± 0.142	10.3	−0.756 ± 0.267	1.4	−0.008 ± 0.129	L	**
5A	88.1 ± 47.6	wmc415a	4.8*	3.2	0.185 ± 0.171	2.6	0.009 ± 0.403	9.6	−0.252 ± 0.232	G	n.s.
6A	56.1 ± 25.5	wPt-4209	3.2*	–	–	–	–	13.9	−0.339 ± 0.083	L	
7B	35.4 ± 45.8	gwm263, gwm537	7.4**	7.4	0.322 ± 0.204	1.9	0.111 ± 0.327	11.4	−0.349 ± 0.116	G	**

LOD – logarithm of the odds scores that were found significant when comparing hypotheses H_1 (there is a QTL in the chromosome) vs. H_0 (no effect of the chromosome on the trait), using 1000 permutations test; var – percentage of explained variation of the trait; d – effect of the QTL; favourable allele – favourable parental allele (from Langdon /L/ or G18-16 /G/) contributing to better grain mineral concentration; G × E – genotype × environment interaction, tested by comparing the model with a new sub-model in which the QTL is assumed to have equal effects in the three environments; this test was not applicable when the QTL was specific for only one environment; *, **, *** and n.s. significant at $P \leq 0.05$, 0.01, 0.001 or non-significant effect, respectively

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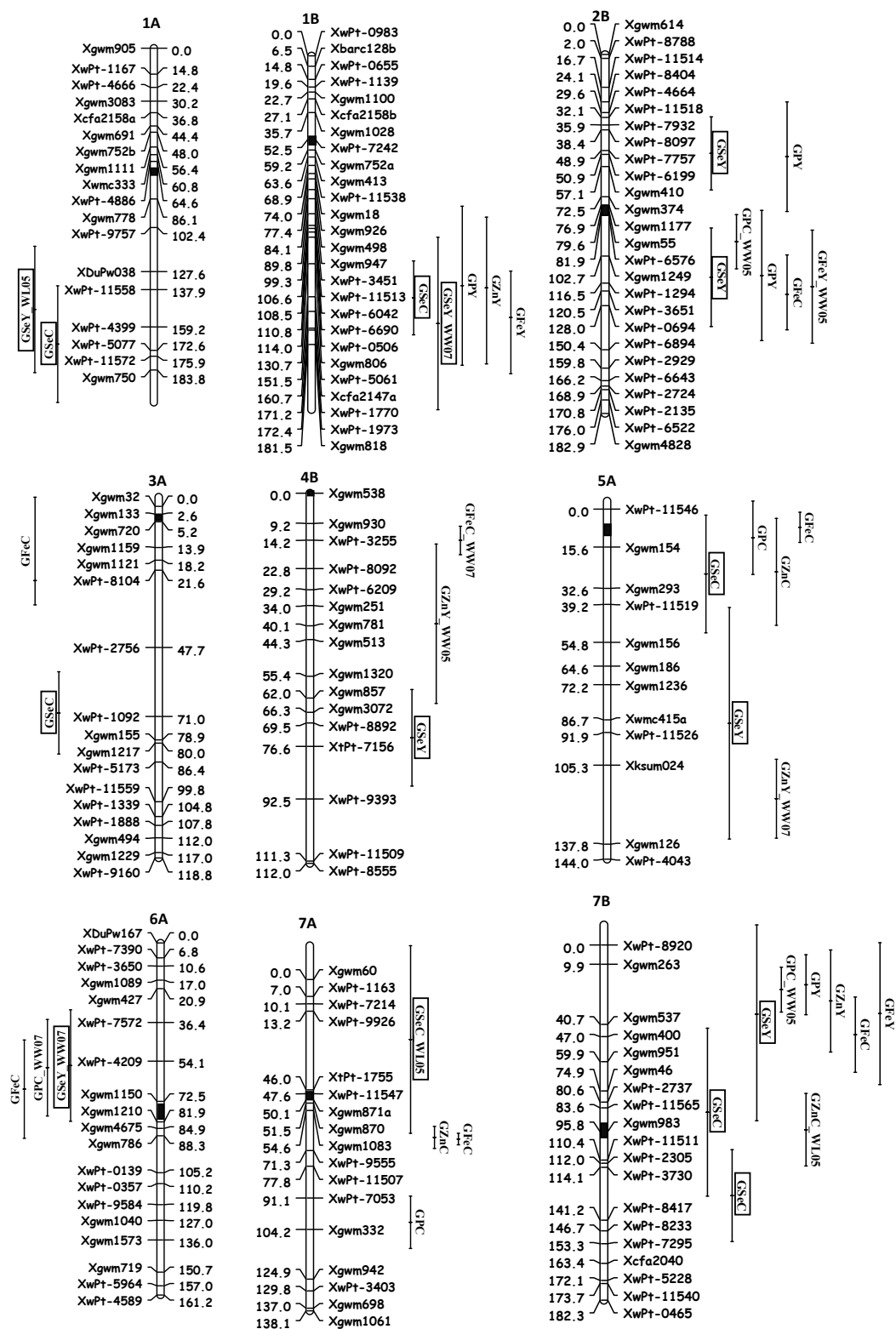
https://doi.org/10.17221/112/2016-CJGPB

Figure 1. Chromosomal intervals for QTLs associated with grain protein concentration (GPC) and yield (GPY), and grain mineral nutrient concentrations and yield, respectively, of zinc (GZnC, GZnY), iron (GFeC, GFeY), and selenium (GSeC, GSeY) in tetraploid wheat recombinant inbred lines under three environments: WL05 – water-limited (350 mm) control in 2005; WW05 – well-watered (750 mm) treatment in 2005; WW07 – well-watered (720 mm) treatment in 2007; the data on GFeC, GZnC and GPC are based on Peleg *et al.* (2009); the data on GFeY, GZnY and GPY are according to Yan (2010)

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density with the aim of transferring chromosome segments carrying high grain Se density alleles into elite wheat cultivars by marker-assisted breeding methodologies.

Various studies have indicated that more favourable alleles are found in the wild type than in cultivars (Peng *et al.* 2003). Soller and Beckmann (1988) indicated that QTL mapping could uncover "cryptic" genetic variation (beneficial alleles) that is otherwise hidden in a sea of deleterious alleles. Peng *et al.* (2003) identified 75 domesticated QTLs for 11 agronomic traits, and wild QTL alleles of *T. dicoccoides* for 18 (24%) traits with agriculturally beneficial effects. These cryptic alleles, together with genes for resistance or tolerance to biotic and abiotic stresses and high protein content, could advance the utilization of *T. dicoccoides* for wheat improvement. In this and in our previous study, 23 out of the 41 QTLs for wild alleles contributed to the improvement of grain minerals and protein densities. The present results combined with our previous study (Peleg *et al.* 2009; Yan 2010) showed that a total of 14 QTLs conferred by the G18-16 alleles are associated with grain Se, Zn, Fe and protein concentrations and yield on five chromosomes: 1A, 1B, 2B, 5A and 7B (Figure 1). The five genomic regions harbouring QTLs were selected by marker-assisted selection for production of NILs with the most promising QTLs.

There were effects of environment on QTLs of GSeC and GSeY. The genotype × environment interactions (G × E) were noted for QTLs of GSeC and GSeY. There were significant G × E interactions for two QTLs for GSeC on two chromosomes: 1A and 5A, and five QTLs for GSeY on five chromosomes: 1A, 1B, 4B, 6A and 7B. What matters was the fact that there were differences in QTLs for GSeY on three chromosomes: 1B, 6A and 7B between two similar environments (WW05, WW07). We think that soil fertility was lower in WW07 than in WW05, because the RIL wheat in WW07 was planted in the same field as WW05 without application of any fertilizer. Soil fertility could play an important role in Se accumulation in RIL wheat grain. Grain Se content appears to be determined overwhelmingly by soil available Se concentration, which is influenced by pH, redox potential, cation exchange capacity, and levels of organic carbon, S, Fe and Al (Lyons *et al.* 2005). The QTLs for GSeY on two chromosomes 1B, 6A appeared in WW07. It could mean that the lower soil fertility was somehow more beneficial for an increase in GSeY. What the effects of soil fertil-

ity on QTLs of GSeC and GSeY are like is certainly worthy of further study.

The QTL distribution on all chromosomes was highly non-random. A total of 41 QTLs were distributed on 9 of 14 chromosomes and more than one-half of the effects for different grain mineral and protein concentrations and yields were detected on chromosomes 1B, 2B, 5A and 7B. Some of the protein and mineral content QTLs overlapped. Clusters of grain protein and micronutrient QTL effects were distributed on several chromosomes. Colocalisation of the Fe, Zn and Se effects at the same loci (1B, 2B, 5A, 7B) supports the notion that maintaining cation homeostasis requires a network of metal uptake, transport, trafficking and sequestration mechanisms, tightly controlled by several genes, which might not be metal-specific (Clemens 2001). Our results may reflect either linkage or pleiotropy of the corresponding QTLs for protein, Fe, Zn and Se concentrations and yields, similar to the previously mentioned wild wheat allele *Gpc-B1* which is associated with increased grain protein, Zn, and Fe content (Uauy *et al.* 2006). However, coupling tight linkage and pleiotropy could better explain the data, especially due to the high correlation between some traits (Peng *et al.* 2003; Peleg *et al.* 2009). The presence of these clusters, especially those on chromosomes 5A and 7B harbouring colocalised QTL effects for all four target traits, implies that selection and/or breeding for high Zn, Fe and Se levels in seeds may simultaneously result in a high level of protein. The colocalisation of the QTL effects for grain Fe, Zn, Se and protein yields on chromosome 7B is flanked by markers *Xgwm263 - Xgwm537*. The chromosome segment is 10 cM long. Fine mapping of the region harbouring these QTLs can pinpoint the chromosome segment that can be introgressed into elite cultivars, thereby avoiding potential effects of linkage drag of negative traits. Thus, these clustered QTL effects would be highly useful for synchronously improving Zn, Fe, Se and protein densities of wheat grain by introgressing genes from *T. dicoccoides*.

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