Theor Appl Genet (2014) 127:1643–1651
DOI 10.1007/s00122-014-2327-6

ORIGINAL PAPER

Zinc and iron concentration QTL mapped in a *Triticum spelta* × *T. aestivum* cross

Jayasudha Srinivasa · Balasubramaniam Arun · Vinod Kumar Mishra · Gyanendra Pratap Singh · Govindan Velu · Raman Babu · Neeraj Kumar Vasistha · Arun Kumar Joshi

Received: 2 December 2013 / Accepted: 5 May 2014 / Published online: 28 May 2014

Abstract

Key message **Ten QTL underlying the accumulation of Zn and Fe in the grain were mapped in a set of RILs bred from the cross *Triticum spelta* × *T. aestivum*. Five of these loci (two for Zn and three for Fe) were consistently detected across seven environments.**

Abstract The genetic basis of accumulation in the grain of Zn and Fe was investigated via QTL mapping in a recombinant inbred line (RIL) population bred from a cross between *Triticum spelta* and *T. aestivum.* The concentration of the two elements was measured from grain produced in three locations over two consecutive cropping seasons and from a greenhouse trial. The range in Zn and Fe concentration across the RILs was, respectively, 18.8–73.5 and 25.3–59.5 ppm, and the concentrations of the two elements were positively correlated with one another (rp =+0.79). Ten QTL (five each for Zn and Fe accumulation) were detected, mapping to seven different chromosomes. The chromosome 2B and 6A grain Zn QTL were consistently expressed across environments. The proportion of the phenotype explained (PVE) by *QZn.bhu-2B* was >16 %, and the locus was closely linked to the SNP marker *1101425|F|0*, while *QZn.bhu-6A* (7.0 % PVE) was closely linked to DArT marker *3026160|F|0*. Of the five Fe QTL detected, three, all mapping to chromosome 1A were detected in all seven environments. The PVE for *QFe.bhu-3B* was 26.0 %.

Communicated by Yunbi Xu.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2327-6) contains supplementary material, which is available to authorized users.

J. Srinivasa · B. Arun · V. K. Mishra · N. K. Vasistha · A. K. Joshi (✉)
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India
e-mail: a.k.joshi@cgiar.org

G. P. Singh
Division of Genetics, Indian Agricultural Research Institute, Pusa, New Delhi, India

G. Velu
International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

R. Babu
CIMMYT, India C/O ICRISAT, Patancheru, Hyderabad, India

A. K. Joshi
CIMMYT, South Asia Regional Office, Kathmandu, Nepal

Introduction

A healthy human diet requires a minimal intake of energy, protein and minerals, and this cannot be fully met by a wholly cereal-based diet (Welch and Graham 2004). It has been estimated that at least 40 % of the world's population, with a heavy representation of women and children in developing countries, suffers from a dietary deficiency in zinc (Zn) and iron (Fe) (Liu et al. 2006; Welch and Graham 2004). Wheat is globally the world's second most important cereal, representing ca. 44 % (estimated 2012/13) of global cereal consumption (FAO 2013), therefore its biofortification with respect to Zn and Fe could have a measurable impact on levels of malnutrition.

Breeding for a higher accumulation of minerals, as for any heritable trait, requires the availability of relevant genetic variation. The genetic basis of quantitative traits of this nature can be most easily obtained by linkage mapping, taking advantage of increasingly informative molecular

marker platforms. The successful identification of quantitative trait loci (QTL) underlying grain Zn and Fe concentration has the potential to accelerate crop improvement via the deployment of marker-assisted selection, and beyond this to the isolation of the gene(s) responsible for the QTL (Salvi and Tuberosa 2005).

Although variation of grain Fe and Zn concentration has been established to have a firm genetic basis (Gregorio 2002). The trait is also highly environmentally dependent, and is particularly sensitive to the concentration of these elements in the soil (Fiel et al. 2005). As a result, efforts to map the genes involved have frequently highlighted the genotype × environment interactions (Trethowan 2007; Trethowan et al. 2005; Joshi et al. 2010). Across the Eastern Gangetic Plains, the major wheat growing area of India, soil analyses have shown that the quantity of available Fe is generally less variable than that of Zn (Joshi et al. 2010). The quantitative inheritance of grain Fe and Zn in wheat, alongside the low heritability of the trait and the large environment and genotype × environment interactions associated with them, have slowed the progress in achieving genetic gain. Here, we report the mapping of several grain Zn and Fe concentration QTL, using a set of recombinant inbred lines (RILs) bred from a cross between a Zn and Fe accumulator (*Triticum spelta*) and a Zn and Fe poor *T. aestivum* cultivar. The material was tested across three locations and over two consecutive cropping seasons, as well as in a controlled greenhouse trial.

Materials and methods

Parental lines, mapping population and grain analysis

A mapping population comprising 185 RILs was developed from the cross *T. spelta* accession H^+ 26 (PI348449) × *T. aestivum* cv. HUW 234. The latter has been a popular cultivar in the North-Eastern Plains Zone of India over the last two decades; its grain accumulates only low levels of both Zn and Fe, unlike those of H^+ 26. The *T. spelta* parent H+26 was identified from the collection of >300 spelt accessions in CIMMYT gene bank. All *T. spelta* lines were not good Zn and Fe accumulators. There was huge genetic diversity within spelt gene pool, and H+26 was one of the best Zn and Fe accumulator. The RILs were developed following methods set out by Singh and Rajaram (1991) and Joshi et al. (2004). The population was field grown in three locations, namely, Banaras Hindu University (BHU), Varanasi (25.25°N, 82.99°E), Rajiv Gandhi South Campus (RGSC), Mirzapur (25.13°N, 82.56°E) (RGSC) and Indian Agriculture Research Institute (IARI), New Delhi (28.64°N, 77.16°E) over two consecutive cropping seasons (2010–2011 and 2011–2012). A further trial was conducted under greenhouse (GH) conditions in 2011. Planting of the RILs, along with the two parents, was carried out in the first week of December in a randomized complete block design with two replications. Each plot comprised three 2-m rows, with an inter-row spacing of 20 cm. The plants were provided with adequate N, P and K fertilizer (120 kg N, 60 kg P_2O_5 and 40 kg K_2O per ha), along with an application of 25 kg per ha Zn. The K_2O and P_2O_5 fertilizer were provided at sowing, but the N application was split between 60 kg at sowing, 30 kg 21 days after sowing and 30 kg 45 days after sowing. The GH experiment soil was fertilized at a rate equivalent to 120 kg N, 80 kg P_2O_5, 60 kg K_2O and 20 kg Zn per ha. At harvest, 20 intact spikes were recovered from each RIL and parent plot, and hand threshed. The grain was subjected to Zn and Fe analysis using X-ray fluorescence (EDXRF spectrometer X-Supreme8000) (Paltridge et al. 2012).

Statistical analysis of phenotypic data

Analyses of variance (ANOVA) for grain Zn and Fe concentration in each of the seven environments were performed using the PROC GLM procedure included within the SAS v9.2 package (SAS Institute Inc.). Estimates of the broad-sense heritability, both within and across the environments, were obtained from the ANOVA using the formulae $h^2 = \sigma_g^2/(\sigma_G^2 + \sigma_e^2/r)$, and $h^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{GE}^2/E + \sigma_e^2 8rE)$, respectively, (Hallauer and Miranda Filho 1981). Here, σ_G^2, σ_{GE}^2 and σ_e^2 represent, respectively, the genotypic variance, the genotype × environment interaction and the error variance, while E and r represent, respectively, the number of environments and the number of replications. Phenotypic and genotypic correlations between traits were calculated following Messmer et al. (2011), and Pearson correlation coefficients between grain Zn and Fe concentration were calculated using the PROC CORR procedure included within the SAS package.

Genotyping, linkage mapping and QTL analysis

Genomic DNA was extracted from 18-day-old seedlings using the Diversity Arrays Technology protocol described online at http://www.diversityarrays.com/sites/default/files/pub/DArT_DNA_isolation.pdf. The resulting DNAs were used for genotyping by 13,460 single nucleotide polymorphism (SNP) and 14,791 DArT loci at DArT Pty. Ltd. (Canberra, Australia). The linkage map was assembled from the genotypic data using QTL IciMapping v3.2 software (http://www.isbreeding.net), applying a LOD threshold of 3.0 between adjacent markers (Li et al. 2007). QTL were identified with the inclusive composite interval mapping (ICIM) algorithm for additive gene effects implemented in QTL IciMapping v.3.2 software. The QTL expressed in each environment separately were defined, as also was the

set of QTL which were stable across all the environments. For both procedures, the walking step was set to 1 cM and a relaxed LOD threshold of 2.5 was applied to call significance (Ribaut et al. 1997; Tuberosa et al. 2002). Stability was inferred when the LOD of the QTL × environment interaction (LOD_{QEI}) was <2.5. QTL nomenclature was standard (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm). QTL × QTL interaction was also investigated.

Results

Performance across environments

The grain of the *T. spelta* parent H^+ 26 accumulated significantly more Zn (62.4 ppm) and Fe (54.9 ppm) than that of the *T. aestivum* parent cv. HUW 234 (respectively, 30.5 and 35.2 ppm) (Table 1). The range in grain Zn concentration among the RILs was 8.8 ppm (BHU2011) to 73.5 ppm (GH), and in Fe concentration from 25.3 ppm (BHU2011) to 59.5 ppm (GH) (Table 1). Zn and Fe accumulation was generally higher in GH than in any of the field experiments. The broad-sense heritability for Zn accumulation ranged, across the environments, from 0.34 to 0.86, and that for Fe from 0.46 to 0.73. Averaged over the full set of environments, the two heritabilities were, respectively, 0.80 and 0.66. The Zn and Fe concentrations were positively correlated with one another, both at the phenotypic (r_p) and the genotypic (r_g) level at six of the seven environments (the exception was BHU2012) (Table 1). The distribution of grain Zn and Fe concentration across the RILs was continuous (Fig. 1). A Shapiro–Wilk test with respect to both Zn ($W = 0.97$, $P = 0.08$) and Fe ($W = 0.99$, $P = 0.76$) revealed that the distributions were normal, and that the range lay between the parental values (Table 1; Fig. 1). The ANOVA showed that both the genotypic and the genotype × environment interaction components of the variance were significant (Table 2).

Genetic map

The linkage map was constructed using 5,812 informative markers (3,122 DArT and 2,690 SNP). A total of 2,383 loci mapped to A genome chromosomes, 3,019 to B genome chromosomes and 410 to D genome chromosomes. Chromosome 5D was completely unmarked, and chromosome 2D harbored only one marker. The full map covered a genetic length of 20,446 cM with a mean inter-marker distance of 3.5 cM.

Variation in Zn and Fe concentration across environments

An analysis based on the Pearson correlation coefficient (r) revealed that grain Zn concentrations were quite consistent

Table 1 Range, mean, broad-sense heritability and phenotypic and genotypic correlations between grain Zn and Fe concentration measured across seven environments in a RIL population bred from the cross *T. spelta* H^+ 26 × *T. aestivum* cv. HUW 234

Env	Zn (ppm)					Fe (ppm)					Correlations	
	H^+ 26	HUW 234	RILs			H^+ 26	HUW 234	RILs				
	Mean ± SD	Mean ± SD	Range	Mean ± SD	h^2	CV	Mean ± SD	Mean ± SD	Range	Mean ± SD	h^2	CV	r_P	r_g
BHU11	48.6 ± 0.49	21.7 ± 0.71	18.8–48.7	31.5 ± 5.11	0.86	6.0	40.7 ± 0.14	28.9 ± 1.70	25.3–39.7	29.6 ± 2.12	0.46	5.3	0.33**	0.22**
BHU12	51.2 ± 0.78	23.8 ± 0.85	22.8–51.3	34.4 ± 5.36	0.70	8.5	51.0 ± 0.42	32.6 ± 0.57	30.1–48.1	37.0 ± 3.49	0.70	5.1	0.12*	0.01 ns
RGSC11	63.7 ± 7.78	26.8 ± 0.78	24.2–56.8	38.4 ± 6.26	0.64	9.5	53.3 ± 1.20	31.8 ± 1.20	29.8–53.7	38.1 ± 3.32	0.60	5.5	0.71**	0.79**
RGSC12	70.4 ± 1.62	38.9 ± 2.26	30.6–70.0	50.5 ± 5.55	0.56	7.2	58.9 ± 1.83	34.0 ± 1.69	33.1–57.5	44.1 ± 4.25	0.73	5.0	0.25**	0.23*
IARI11	58.0 ± 3.75	22.7 ± 1.56	20.9–60.2	35.6 ± 5.88	0.68	9.3	55.1 ± 0.53	32.1 ± 1.48	30.6–53.9	39.5 ± 3.50	0.57	5.7	0.60**	0.54**
IARI12	70.3 ± 1.48	40.8 ± 0.28	38.9–70.1	51.5 ± 5.17	0.34	8.1	61.5 ± 1.13	45.8 ± 0.35	41.7–58.9	50.2 ± 3.20	0.55	4.3	0.18*	0.22*
GH	74.8 ± 0.42	38.9 ± 1.56	36.9–73.5	53.5 ± 5.80	0.48	7.8	64.2 ± 0.63	41.7 ± 0.50	38.1–59.5	49.2 ± 4.26	0.61	5.4	0.51**	0.49**
Over Env.	62.4 ± 9.70	30.5 ± 8.02	18.8–73.5	42.2 ± 10.26	0.80	8.5	54.9 ± 7.25	35.2 ± 5.70	25.3–59.5	41.1 ± 7.61	0.66	5.3	0.79**	0.92**

Significant at * $P < 0.05$ and ** $P < 0.01$ probability levels

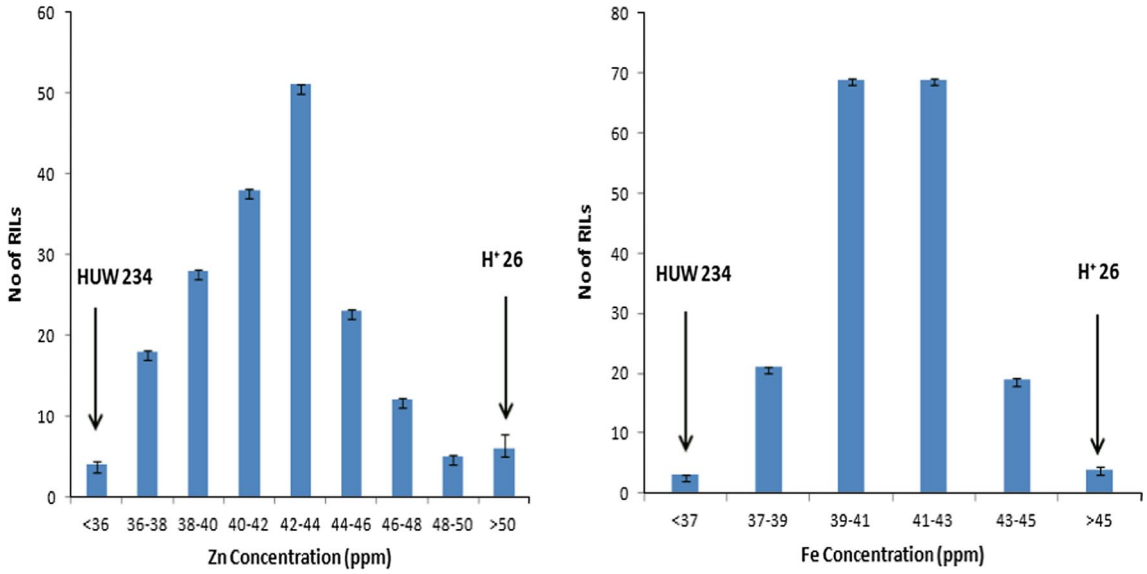

Fig. 1 Distribution of grain Zn and Fe concentration: mean performance over seven environments of the 185 RILs bred from the cross *T. spelta* H^+ 26 × *T. aestivum* cv. HUW 234. *Bars* indicate the standard error ($n = 7$)

Table 2 Analysis of variance for grain Zn and Fe concentration measured across seven environments in a RIL population bred from the cross *T. spelta* H^+ 26 × *T. aestivum* cv. HUW 234

Source	*df*	Zn	Fe
Environment	6	31,912.29**	19,645.44**
Replication	1	219.44**	137.42**
Genotype	184	199.42**	46.33**
Genotype × environment	1,104	25.01**	15.58**
Error	1,294	12.79	4.68

Significant at ** $P < 0.01$

across the seven environments, with *r* ranging from 0.26 to 0.67 (Fig. 2). Similarly, Fe concentrations were consistent across the environments (*r* ranging from 0.09 to 0.35). The grain Zn and Fe concentrations were significantly and positively correlated with one another ($r_p = 0.79$) (Fig. 2), suggesting that the accumulation of these minerals operates via a common genetic mechanism.

QTL analysis of grain Zn concentration

Five QTL for grain Zn content were identified in all, associated with a PVE (Phenotypic variance explained) of between 4.3 and 16.5 % (Table 3). *QZn.bhu-2B* was the most stably expressed QTL (Supplementary fig. 1.1), followed by *QZn.bhu-6A* (Supplementary fig. 1.2). Both of these QTL were detectable in each of the seven environments, and also when performance was averaged across the seven environments. The PVE for *QZn.bhu-2B* was 16.5 % across all environments, while the overall PVE for *QZn.bhu-6A* was 7.0 %. A QTL mapping to chromosome 3D (flanked by the DArT markers *1094214|F|0* and *1057342|F|0*) was detected in six of the seven environments and when performance was averaged across the seven environments; its overall PVE was 4.8 % (Table 3). Another QTL mapping to chromosome 2A (flanked by SNP marker *1126272|F|0* and DArT marker *2255234|F|0*) was detected in four of the environments (PVE = 6.68 % at RGSC12). Finally, a chromosome 6B QTL was detected in only one environment, but was nevertheless significant based on line mean performance over environments (PVE = 9.7 %). *QZn.bhu-2B* was tightly linked (0.68 cM)

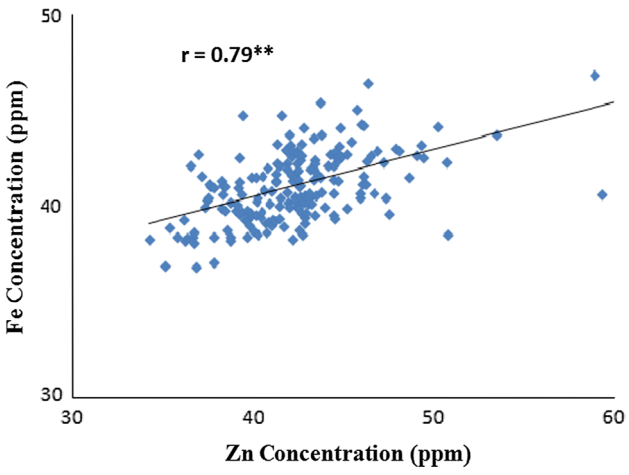

Fig. 2 Phenotypic correlation (*r*) between grain Zn and Fe concentration in the RIL population tested across seven environments

Table 3 QTL for grain Zn and Fe concentration derived from an ICIM-ADD analysis of 185 RILs bred from the cross *T. spelta* H^+ 26 × *T.aestivum* cv. HUW 234

Trait/QTL	Chr	Position (cM)	Confidence interval (cM)	Flanking markers		Direction	QEI		Environment	LOD	R^2	Add
							LOD	R^2				
Zn												
QZn.bhu-2A	2A	146	136.91–150.31	1126272|F|0	2255234|F|0	HUW 234	2.1	0.1	BHU11	2.9	4.98	1.02
									RGSC11	2.91	4.92	1.33
									BHU12	2.68	4.25	1.05
									RGSC12	3.18	6.68	1.31
QZn.bhu-2B	2B	966	948.23–966.68	989092|F|0	1101425|F|0	HUW 234	0.6	0.04	BHU11	3.79	6.85	1.11
									RGSC11	3.6	6.93	1.47
									IARI11	2.73	9.3	1.65
									BHU12	3.57	6.89	1.26
									RGSC12	3.58	10.76	1.6
									IARI12	4.77	15.69	1.74
									GH	4.36	16.02	2.1
									Mean over all Env.	4.81	16.46	2.01
QZn.bhu-3D	3D	57	56.79–61.01	1094214|F|0	1057342|F|0	H^+ 26	1.15	0.02	BHU11	3.22	5.64	1.01
									RGSC11	3.63	6.3	1.41
									BHU12	4.37	7.18	1.29
									RGSC12	2.97	5.9	1.17
									IARI12	2.27	4.83	0.95
									GH	2.18	5.07	1.17
									Mean over all Env.	2.21	4.75	1.06
QZn.bhu-6A	6A	327	317.01–327.98	998265|F|0	3026160|F|0	H^+ 26	1.23	0.01	BHU11	3.84	7.1	1.14
									RGSC11	3.82	6.5	1.43
									IARI11	2.07	5.04	1.2
									BHU12	4.55	7.33	1.3
									RGSC12	4.02	9.47	1.48
									IARI12	2.68	6.77	1.12
									GH	2.47	6.6	1.33
									Mean over all Env.	2.61	6.99	1.29
Zn.bhu-6B	6B	1,488	1,476.65–1,490.85	1001916|F|0	1129916|F|0	H^+ 26	0.89	0.16	IARI11	4.35	13.15	2.11
									GH	2.04	6.3	1.42
									Mean over all Env.	3.41	9.7	1.689
Fe												
QFe.bhu-1A.1	1A	56	49.41–56.09	1046200|F|0	1228280|F|0	HUW 234	0.52	0.04	BHU11	1.83	3.56	0.35
									RGSC11	1.94	3.71	0.57
									IARI11	2	3.64	0.63

Table 3 continued

Trait/QTL	Chr	Position (cM)	Confidence interval (cM)	Flanking markers		Direction	QEI		Environment	LOD	R^2	Add
							LOD	R^2				
									BHU12	2.97	5.67	0.79
									RGSC12	3.4	5.63	0.96
									IARI12	3.41	5.67	0.69
									GH	3.71	6.12	0.97
									Mean over all Env.	3.32	5.56	0.74
QFe.bhu-1A.2	1A	227	218.84–227.11	2289695|F|0	1218555|F|0	HUW 234	0.93	0.03	BHU11	3.47	7.23	0.49
									RGSC11	3.91	8.05	0.84
									IARI11	2.98	5.8	0.74
									BHU12	4.06	7.84	0.9
									RGSC12	4.59	7.71	1.09
									IARI12	4.56	7.69	0.78
									GH	4.37	7.26	1.03
									Mean over all Env.	4.4	7.48	0.84
QFe.bhu-1A.3	1A	346	345.76–355.02	1708014|F|0	1000008|F|0	H^+ 26	2.08	0.1	BHU11	6.68	14.56	0.75
									RGSC11	6.98	15.07	1.24
									IARI11	5.69	11.56	1.13
									BHU12	9.53	19.94	1.55
									RGSC12	9.44	17.03	1.76
									IARI12	9.37	16.99	1.26
									GH	9.72	17.51	1.73
									Mean over all Env.	9.09	16.55	1.35
QFe.bhu-2A	2A	162	161.6–188.49	1081485|F|0	1216621|F|0	HUW 234	0.52	0.03	IARI11	2.66	5.26	0.72
									BHU12	2.33	4.25	0.68
									RGSC12	3.26	5.51	0.95
									IARI12	3.18	5.39	0.67
									GH	3.42	5.75	0.94
									Mean over all Env.	3.27	5.6	0.74
QFe.bhu-3B	3B	1,022	1,015.23–1,022.28	3022954|F|0	1102324|F|0	HUW 234	8.61	0.5	IARI11	3.45	6.97	0.85
									BHU12	2.61	5.06	0.75
									RGSC12	13.96	27.1	2.14
									IARI12	13.82	26.87	1.53
									GH	13.98	26.76	2.07
									Mean over all Env.	13.3	25.95	1.63

LOD likelihood of odds ratio for genetic effects, R^2 total percentage of phenotypic variation explained by each QTL, *Add* the additive effect, *LODQEI* LOD score of the QTL × environment interaction across environments, R^2QEI phenotypic variation explained by main effects of QTL × environment interaction across environments

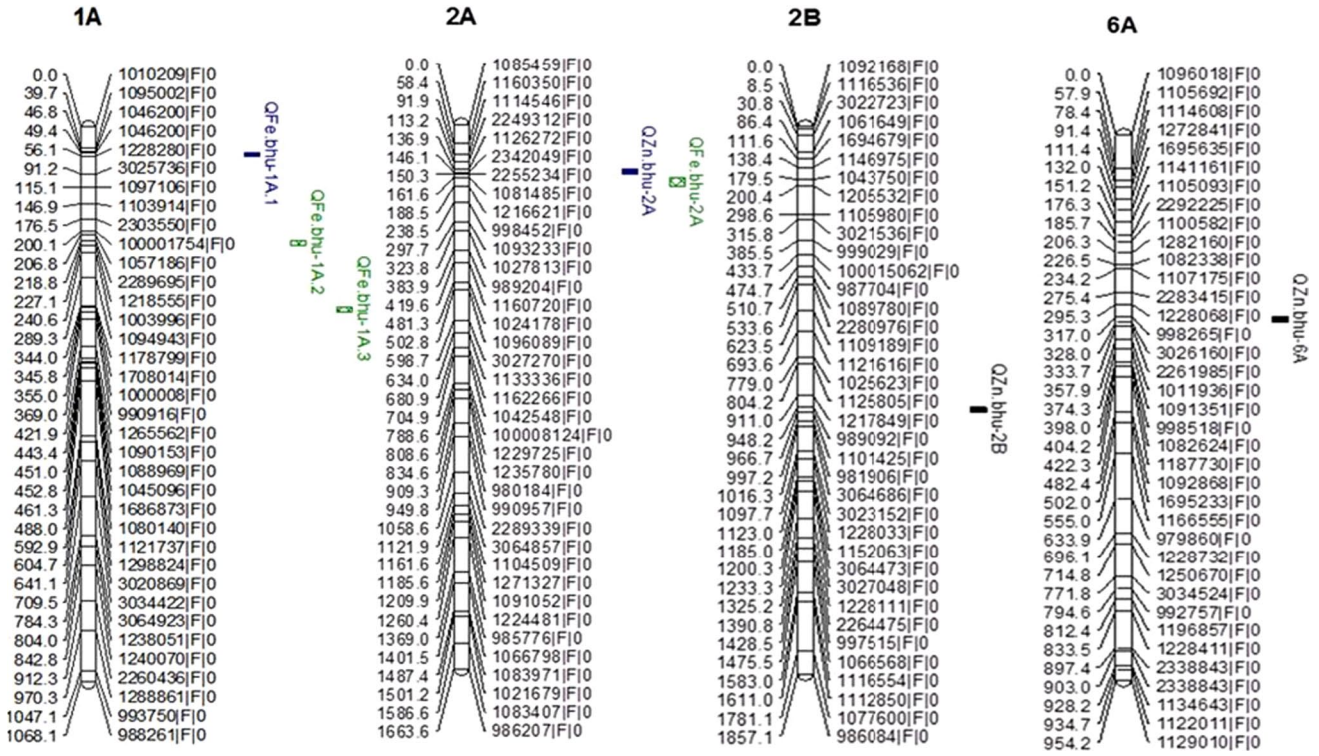

Fig. 3 Partial linkage map derived from the *T. spelta* H^+ 26 × *T. aestivum* cv. HUW 234 RIL population, indicating the location of QTL for grain Zn and Fe concentration

to the SNP marker *1101425|F|0*, *QZn.bhu-6A* was separated by 0.98 cM from DArT marker *3026160|F|0*, and DArT marker *1094214|F|0* mapped within 0.21 cM of *QZn.bhu-3D*. The positive alleles at both *QZn.bhu-2A* and *QZn.bhu-2B* were contributed by the low Zn parent cv. HUW 234, while they were inherited from H^+ 26 at the other three QTL. The significant QTL × environment interactions (QEIs) included the two consistent and stable loci *QZn.bhu-2B* and *QZn.bhu-6A*, although the PVE associated with both was very low (respectively, 0.04 and 0.01 %; Table 3).

QTL analysis of grain Fe concentration

Five QTL were also detected underlying grain Fe content. Their PVEs ranged from 1.8 to 27.1 % (Table 3). *QFe.bhu-1A.2* (overall PVE = 7.5 %) and *QFe.bhu-1A.3* (PVE = 16.6 %) were the most consistent and stable, followed by *QFe.bhu-1A.1* (PVE = 5.6 %). All three of the QTL mapping to chromosome 1A (Supplementary fig. 1.3) were detected in each environment as well as when performance was averaged over the environments. *QFe.bhu-3B* was associated with the highest PVE (27.1 %, at RGSC2012), but also had a major effect at IARI2012 (26.9 %), in the GH trial (26.8 %) and across all environments (26.0 %). *QFe.bhu-2A* was associated with an overall PVE of 5.6 %. The positive allele was inherited from H^+ 26 at *QFe.bhu-1A.3*, but from cv. HUW 234 at each of the other four loci. The nearest markers to the five loci lay, respectively, at a distance of 0.90, 0.24, 0.40, 0.11 and 0.28 (Table 3). For both traits, no QTL × QTL interaction was observed.

Discussion

Grain Zn and Fe concentration are both quantitatively inherited traits, as shown by their continuous distribution across the RIL population. However, the RILs also showed some transgression in both directions suggesting that both parents carried a few different genes with alleles contributing to increased Zn and Fe concentrations (Ozkan et al. 2007; Xu et al. 2012). Grain Zn concentration was rather more variable than grain Fe concentration. Other varietal contrasts have also shown substantial variation for the content of both minerals (Cakmak et al. 2004; Tiwari et al. 2009; Xu et al. 2012). Greenhouse-raised plants accumulated more Zn and Fe in the grain than did the field-grown ones, as similarly observed by Welch et al. (2005). The estimated broad-sense heritability for both grain Zn and Fe concentration varied from medium to high across the seven environments, in accordance with the observations made by

Velu et al. (2012). Nevertheless, both grain Zn and Fe concentrations were quite consistent across the environments.

The accumulation of Zn was positively correlated ($r_p = 0.79^{**}$) with that of Fe, suggesting a shared genetic basis for the two traits. Comparing the derived QTL did reveal that the Zn locus on chromosome 2A co-localized with an Fe one (Fig. 3), which offers an opportunity to jointly improve both traits (Welch and Graham 2004). The existence of a positive correlation between grain Zn and Fe accumulation has been reported repeatedly in both bread wheat (Pomeranz and Dikeman 1983; Peterson et al. 1986; Raboy et al. 1991; Graham et al. 1999; Rengel et al. 1999; Balint et al. 2007; Peleg et al. 2009; Genc et al. 2009, Zhao et al. 2009; Xu et al. 2012), wild emmer (Cakmak et al. 2004; Morgonuov et al. 2007; Peleg et al. 2008), domesticated emmer (Gregorio 2002) and triticale (Feil and Fossati 1995). However, to date, the co-localization of grain Zn and Fe QTL has only been observed in tetraploid wheat (Peleg et al. 2009).

Although grain of the *T. spelta* parent H^+ 26 accumulated much more Zn than that of cv. HUW 234, positive alleles at two of the five grain Zn QTL identified were inherited from the *T. aestivum* parent. Similar locations for four of the QTL specifically, *QZn.bhu-2A* (Cakmak et al. 2004; Peleg et al. 2009), *QZn.bhu-3D* (Xu et al. 2012), *QZn.bhu-6A* (Cakmak et al. 2004) and *QZn.bhu-6B*: (Cakmak et al. 2004; Distelfeld et al. 2007; Genc et al. 2009; Peleg et al. 2009) have been previously identified in other populations. The wild emmer allele of the grain protein locus *Gpc-B1* has been shown to also enhance grain Zn and Fe content, and maps to a position consistent with that of *QZn.bhu-6A* (Uauy et al. 2006). With respect to grain Fe concentration, the positive allele at only one of the five QTL was inherited from H^+ 26. The location of two of the *QFe-bhu* loci coincides with previously mapped ones, namely, *QFe-bhu-2A* (Cakmak et al. 2004; Xu et al. 2012; Peleg et al. 2009; Tiwari et al. 2009) and *QFe-bhu-3B* (Peleg et al. 2009); Both H^+ 26 and HUW 234 alleles contributed an additive effect on grain Zn and Fe concentration, showing that the positive alleles were dispersed across the two parents; as a result, transgressive segregation occurred.

Biofortification of wheat can be achieved through plant breeding without affecting the yield or quality (Velu et al. 2012). It is also a more sustainable and cost-effective solution (White and Broadley 2005). Significant knowledge has been gained on the molecular mechanisms affecting the accumulation of Fe (Bauer et al. 2004; Cakmak 2002) and Zn (Hacisalihoglu and Kochian 2003) in plants. In future, these researches could be applied to develop crops with enhanced mineral concentration through functional (DNA sequence) markers in conventional breeding or molecular targets for genetic engineering (Hacisalihoglu and Kochian 2003). In this study, five QTL for grain Zn were identified, each mapping to a different chromosome, while the five Fe QTL mapped to just three chromosomes. Multi-environmental experiments in wheat have led to the recognition of a number of QTL underlying grain micronutrient concentrations, which are effective across a range of environments (Peleg et al. 2009; Tiwari et al. 2009). Such loci would represent the prime target of any marker-aided selection effort aimed at enhancing grain Fe and/or Zn content, avoiding loci associated with large QEIs (Jansen et al. 1995). The difficulty of using conventional breeding to improve grain mineral content means that marker-aided selection would be an attractive proposition, provided that robust QTL can be identified (Gupta et al. 2010).

Acknowledgments The authors are grateful for constructive suggestions made by Wolfgang Pfeifer (HarvestPlus) and Ravi P. Singh and Hans J. Braun (CIMMYT, Mexico) in the course of this study. Support rendered by Uttam Kumar, CIMMYT in molecular analysis is also gratefully acknowledged.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All experiments complied with the current laws of the India, the country in which they were performed.

References

Balint AF, Roder MS, Hell R, Galiba G, Borner A (2007) Mapping of QTLs affecting copper tolerance and the Cu, Fe, Mn and Zn contents in the shoots of wheat seedlings. Biol Plant 51:129–134

Bauer P, Thiel T, Klatte M, Bereczky Z, Brumbarova T, Hell R, Grosse I (2004) Analysis of sequence, map position, and gene expression reveals conserved essential genes for iron uptake in Arabidopsis and tomato. Plant Physiol 136:4169–4183

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

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

Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T (2007) Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. Physiol Plant 129:635–643

FAO (2013) Food outlook. November 2013. Biannual report on global food markets. FAO trade and markets division. FAO, Rome. http://www.fao.org/docrep/019/i3473e/i3473e.pdf

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

Fiel B, Moser S, Jampatong S, Stamp P (2005) Mineral composition of the grains of tropical maize varieties as affected by pre-anthesis drought and rate of nitrogen fertilization. Crop Sci 45:516–523

Genc Y, Verbyla A, Torun A, Cakmak I, Willsmore K, Wallwork H, McDonald G (2009) Quantitative trait loci analysis of zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. Plant Soil 314:49–66

Graham RD, Senadhira SB, Iglesias C, Monasterio I (1999) Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Res 60:57–80

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

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. Mol Breed 26:145–161

Hacisalihoglu G, Kochian LV (2003) How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. New Phytol 159:341–350

Hallauer AR, Miranda Filho JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames

Jansen RC, Van Ooijen JM, Stam P, Lister C, Dean C (1995) Genotype by environment interaction in genetic mapping of multiple quantitative trait loci. Theor Appl Genet 91:33–37

Joshi AK, Kumar S, Ferrara O, Chand R (2004) Inheritance of resistance to spot blotch caused by *Bipolaris sorokiniana* in spring wheat. Plant Breed 123:213–219

Joshi AK, Crossa I, Arun B, Chand R, Trethowan R, Vargas M, Ortiz-Monasterio I (2010) Genotype × environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. Field Crops Res 116:268–277

Li H, Ye G, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Liu ZH, Wang HY, Wang XE, Zhang GP, Chen PD, Liu DJ (2006) Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L.). J Cereal Sci 44:212–219

Messmer R, Fracheboud Y, Banziger M, Stamp P, Ribaut JM (2011) Drought stress and tropical maize: QTL for leaf greenness, plant senescence, and root capacitance. Field Crops Res 124:93–103

Morgonuov A, Gómez-Becerra HF, Abugalieva A, Dzhunusova M, Yessimbekova M, Muminjanov H, Zelenskiy Y, Ozturk L, Cakmak I (2007) Iron and zinc grain density in common wheat grown in Central Asia. Euphytica 155:193–203

Ozkan H, Brandolini A, Torun A, Altintas S, Eker S, Kilian B, Braun HJ, Salamini F, Cakmak I (2007) Natural variation and identification of microelements content in seeds of einkorn wheat (*Triticum monococcum*). In: Buck HT, Nisi JE, Salomon N (eds) Wheat production in stressed environments. Springer, Berlin, pp 455–462

Paltridge NG, Palmer LJ, Milham PJ, Guild GE, Stangoulis JCR (2012) Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grains. Plant Soil 361:251–260

Peleg Z, Saranga Y, Yazici MA, Fahima T, Ozturk L, Cakmak I (2008) Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant Soil 306:57–67

Peleg Z, Cakmak I, Ozturk L, Yazici A, Jun Y, Budak H, Korol AB, Fahima T, Saranga Y (2009) Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. Theor Appl Genet 119:353–369

Peterson CJ, Johnson VA, Mattern PJ (1986) Influence of cultivar and environment on mineral and protein concentrations of wheat flour, bran, and grain. Cereal Chem 63:118–186

Pomeranz Y, Dikeman E (1983) Minerals and protein contents in hard red winter wheat flours. Cereal Chem 60:80–82

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

Rengel Z, Batten GD, Crowley DE (1999) Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res 60:27–40

Ribaut JM, Jiang C, Gonzalez-de-Leon D, Edmeades GO, Hoisington DA (1997) Identification of quantitative trait loci under drought conditions in tropical maize 1 Yield components and marker assisted selection strategies. Theor Appl Genet 94:887–896

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenge Trends. Plant Sci 10:297–304

Singh RP, Rajaram S (1991) Genetics of adult-plant resistance of leaf rust in 'Frontana' and three CIMMYT wheats. Genome 35:24–31

Tiwari VK, Rawat N, Chhuneja P, Neelam K, Aggarwal R, Randhawa GS, Dhaliwal HS, Keller B, Singh K (2009) Mapping of quantitative trait loci for grain iron and zinc concentration in diploid A genome wheat. J Hered 100:771–776

Trethowan RM (2007) Breeding wheat for high iron and zinc at CIMMYT: state of the art, challenges and future prospects In: Proceedings of the seventh international wheat conference Mar del Plata, Argentina

Trethowan RM, Reynolds M, Sayre KD, Ortiz-Monasterio I (2005) Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. Ann Appl Bio 146:405–413

Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002) Identification of QTL for root characteristics in maize grown in hydroponics and analysis of their overlap with QTL for grain yield in the field at two water regimes. Plant Mol Biol 48:697–712

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1301

Velu G, Singh RP, Huerta-Espino J, Peña-Bautista RJ, Arun B, Mahendru-Singh A, Yaqub Mujahid M, Sohu VS, Mavi GS, Crossa J, Alvarado G, Joshi AK, Pfeiffer WH (2012) Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. Field Crops Res 137:261–267

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

Welch RM, House WA, Ortiz-Monasterio I, Cheng Z (2005) Potential for improving bioavailable zinc in wheat grain (*Triticum* species) through plant breeding. J Agric Food Chem 53:2176–2180

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593

Xu YF, An DG, Liu DC, Zhang AM, Xu HX, Li B (2012) Molecular mapping of QTLs for grain zinc, iron and protein concentration of wheat across two environments. Field Crops Res 138:57–62

Zhao FJ, Su YH, Dunham SJ, Rakszegi M, Bedo Z, McGrath SP, Shewry PR (2009) Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. J Cereal Sci 49:290–295