

Identification of QTLs and underlying candidate genes controlling grain Fe and Zn concentration in sorghum [Sorghum bicolor (L.) Moench]

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Abstract

Micronutrient malnutrition or 'hidden hunger' due to consumption of diets poor in iron (Fe) and zinc (Zn) affect over >3 billion people worldwide, mostly women and children in developing countries. Biofortification, increasing grain Fe and Zn by genetic means is one of the sustainable options for combating micronutrient-malnutrition. To identify genomic regions associated with grain Fe and Zn in sorghum, a RIL population (342 individuals) derived from cross-296B × PVK801 was phenotyped for two years at three locations and genotyped with Simple Sequence Repeats (SSRs) and Diversity Arrays Technology (DARTs). Highly significant genotype × environment interactions were observed for both micronutrients; grain Fe showed greater variation than Zn (Table 1). Sorghum genetic map was constructed with 2088 markers covering 1355.52 cM with an average marker interval of 0.6 cM (Figure 1). A large number (167 QTLs) of small main-effect Quantitative Trait Locus (QTLs) controlling Fe and Zn concentration were identified.

A total of 21 QTLs (across seasons and environment) and 118 QTLs (individual environment) were identified (Table 2). A total of 18 QTLs controlling Fe and Zn were found stable across environments. Three QTLs for Fe and fifteen for Zn were identified with phenotypic variance explained (PVE) values ranging from 3.94 to 5.09% and 3.17 to 9.42%, respectively. Of these 18 stable QTLs, 11 were located on chromosome SBI-07. Favorable alleles for 11 QTLs (co-located) for Fe and Zn on chromosome SBI-07 were contributed by parent PVK801-P23. QTLs were analyzed *in-silico* to identify underlying candidate genes, 62 candidate genes involved in Fe/Zn metabolism were identified within QTL interval; 23 were found in QTL with highest phenotypic effect (PVE 9.42%). Identified sorghum genes within QTL interval were used to evaluate gene synteny with *Zea mays* and *Oryza sativa*. Synteny sequence level between *Sorghum bicolor* - *Oryza sativa* ranged from 44% to 97%, while *Sorghum*- *Zea mays* ranged from 49% to 99% (Figure 2). In this present study, identified Fe/Zn QTL positions in sorghum were cross compared with sorghum gene homologue derived from other cereals. The position of 20 QTLs identified in this study (on chromosomes SBI-02, SBI-04, SBI-06, SBI-07, SBI-09 and SBI-10) were same as the Fe/Zn gene homologues positions identified on sorghum genome in our earlier *In silico* homology study (Figure 3).

Methods and materials

- Phenotyping - Sorghum F₆ RIL (309 individuals) population (296B × PVK 801); Multi-location (ICRISAT, IIMR, VNMKV) evaluation; 2 seasons (*Rabi* 2012-13 & 2013-14); 3-replicate; α -lattice design
- Assessment grain Fe/Zn - ICP-OES Method
- Statistical Analysis-Mixed Model, GxE & Pearson correlation coefficient
- Genotyping - SSR - ICRISAT, India; DArT and DArTseq (SNPs) - Diversity Arrays Technology Pvt Ltd (DArT P/L), Australia Analyzed
- Genetic map - Joinmap 4.0 software
- QTLs mapping - Win Cartographer V2.5 software
- In-silico* candidate genes search - Phytozome 10.3 > JBrowse > bicolor v2.1
- Gene synteny - Phytozome 10.3 > JBrowse > bicolor v2.1>Gene info> Homologues.

Results

Table 1. Means (1a) and, variances and heritability (1b) for grain Fe and Zn in Sorghum (296 B × PVK 801)-derived RIL population.

a) The means, standard deviation, ranges for Fe/Zn measured for parents and RILs means in individual environment						
Trait	Environment	296B (P1)	PVK 801 (P2)	RILs	SD (±)	
Fe (mg kg ⁻¹)	ICRISAT 12-13 (E1)	28.0	33.4	33.6	5.6	
	IIMR 12-13 (E2)	28.5	33.0	33.0	6.3	
	VNMKV 12-13 (E3)	46.3	49.4	49.2	6.9	
	ICRISAT 13-14 (E4)	26.0	28.2	28.0	4.9	
	IIMR 13-14 (E5)	30.8	35.9	35.8	5.0	
Zn (mg kg ⁻¹)	VNMKV 13-14 (E6)	27.2	33.6	34.0	7.8	
	ICRISAT 12-13 (E1)	21.3	24.3	24.6	4.7	
	IIMR 12-13 (E2)	21.0	22.0	24.7	5.0	
	VNMKV 12-13 (E3)	26.4	30.4	31.4	6.4	
	ICRISAT 13-14 (E4)	14.6	16.4	17.3	3.5	
ICRISAT 13-14 (E5)	IIMR 13-14 (E5)	21.1	24.8	25.6	4.0	
	VNMKV 13-14 (E6)	19.6	24.0	24.7	5.2	
b) Variances and heritability for (296 B × PVK 801)-derived RIL population evolution						
Pooled (across six environments)						
Trait	$\sigma^2 g$	SE (±)	$\sigma^2 gy$	SE (±)	$\sigma^2 gl$	SE (±)
Fe	4.18**	0.69	-0.17	0.66	-0.7	0.80
Zn	4.17**	0.51	0.71**	0.35	-0.14	0.37
Individual environments						
ICRISAT 12-13 (E1)						
Fe	15.51**	1.68	0.78	20.79**	2.17	0.81
Zn	10.00**	1.12	0.74	12.77**	1.33	0.8
ICRISAT 13-14 (E4)						
Fe	8.72**	1.05	0.68	10.84**	1.3	0.68
Zn	5.48**	0.6	0.73	4.60**	0.68	0.56
Genotypic variance($\sigma^2 g$), Genotype × Year ($\sigma^2 gy$), Genotype × Location ($\sigma^2 gl$), Genotype × Year × Location ($\sigma^2 gyl$) interactions, standard error (SE), heritability's (h^2 , broad-sense), SD= Standard Deviation, all variances ** Significant at 1% level						

Table 2. List of QTLs for grain Fe and Zn concentration in using pooled data (across seasons and environment analysis).

QTL name	Chr.no	Flanking marker (L)	Marker Position (cM)	Flanking marker (R)	Marker Position (cM)	LOD	Additive effect	PV2
Fe								
qfe1.1	SBI-01	Dt1937067Ch_1	112.8	Dt2647464Ch_1	112.7	2.84	0.42	3.12
qfe4.1	SBI-04	Dt2646105Ch_4	1.05	Dt2224731Ch_4	2.07	3.86	-0.51	4.46
qfe4.2	SBI-04	Dt2650643Ch_4	34.31	Dt1997108Ch_4	33.47	2.65	-0.41	3.05
qfe4.3	SBI-04	Dt3933410Ch_4	40.76	Dt2658021Ch_4	41.15	2.45	-0.39	2.82
qfe6.1	SBI-06	Dt2090541Ch_6	100.23	Dt2657314Ch_6	100.94	3.25	0.45	3.85
qfe6.2	SBI-06	Dt223502Ch_6	101.39	Sn1918187Ch_6	101.65	2.75	0.42	3.19
qfe7.1	SBI-07	Dt2657812Ch_7	16.22	Dt2653638Ch_7	16.57	2.57	0.41	2.97
qfe7.2	SBI-07	Dt2644692Ch_7	24.90	Sn2646254Ch_7	24.76	3.16	0.44	3.62
qfe7.3	SBI-07	Xtp525_Ch7	123.47	Sn2653248Ch_7	126.57	5.61	0.60	6.66
Zn								
qzn4.1	SBI-04	Dt2224731Ch_4	2.07	Dt1944553Ch_4	3.64	0.25	-0.43	0.30
qzn4.2	SBI-04	Dt1907645Ch_4	13.59	Dt2645303Ch_4	15.05	4.36	-0.53	5.39
qzn6.1	SBI-06	Dt2005952Ch_6	88.60	Dt2648290Ch_6	88.83	2.49	0.39	3.06
qzn6.2	SBI-06	Sn2657501Ch_6	90.37	Sn2647940Ch_6	91.58	2.64	0.41	3.24
qzn7.1	SBI-07	Dt2657812Ch_7	16.22	Dt2653638Ch_7	16.57	2.57	0.40	3.17
qzn7.2	SBI-07	Dt2645576Ch_7	55.45	Sn2033434Ch_7	56.26	4.49	0.54	5.66
qzn7.3	SBI-07	Sn2650637Ch_7	57.03	Sn1895281Ch_7	57.98	3.76	0.49	4.66
qzn7.4	SBI-07	Sn1895297Ch_7	62.05	Dt2648834Ch_7	62.52	4.16	0.51	5.14
qzn7.5	SBI-07	Dt2649259Ch_7	67.16	Dt3628977Ch_7	67.61	4.43	0.53	5.74
qzn7.6	SBI-07	Dt3627584Ch_7	69.46	Dt2649175Ch_7	69.73	2.84	0.43	3.63
qzn7.7	SBI-07	Sn1937648Ch_7	72.61	Sn1919843Ch_7	73.35	2.91	0.43	3.65
qzn9.1	SBI-09	Sn1950992Ch_9	83.72	Sn1929569Ch_9	82.36	3.58	-0.47	4.38

9 QTLs for Fe - located on chromosomes SBI-04, 6 and 7, 12- QTLs for Zn - located on chromosomes SBI-04, 6 and 9.

q-QTL, PV2- phenotypic variance

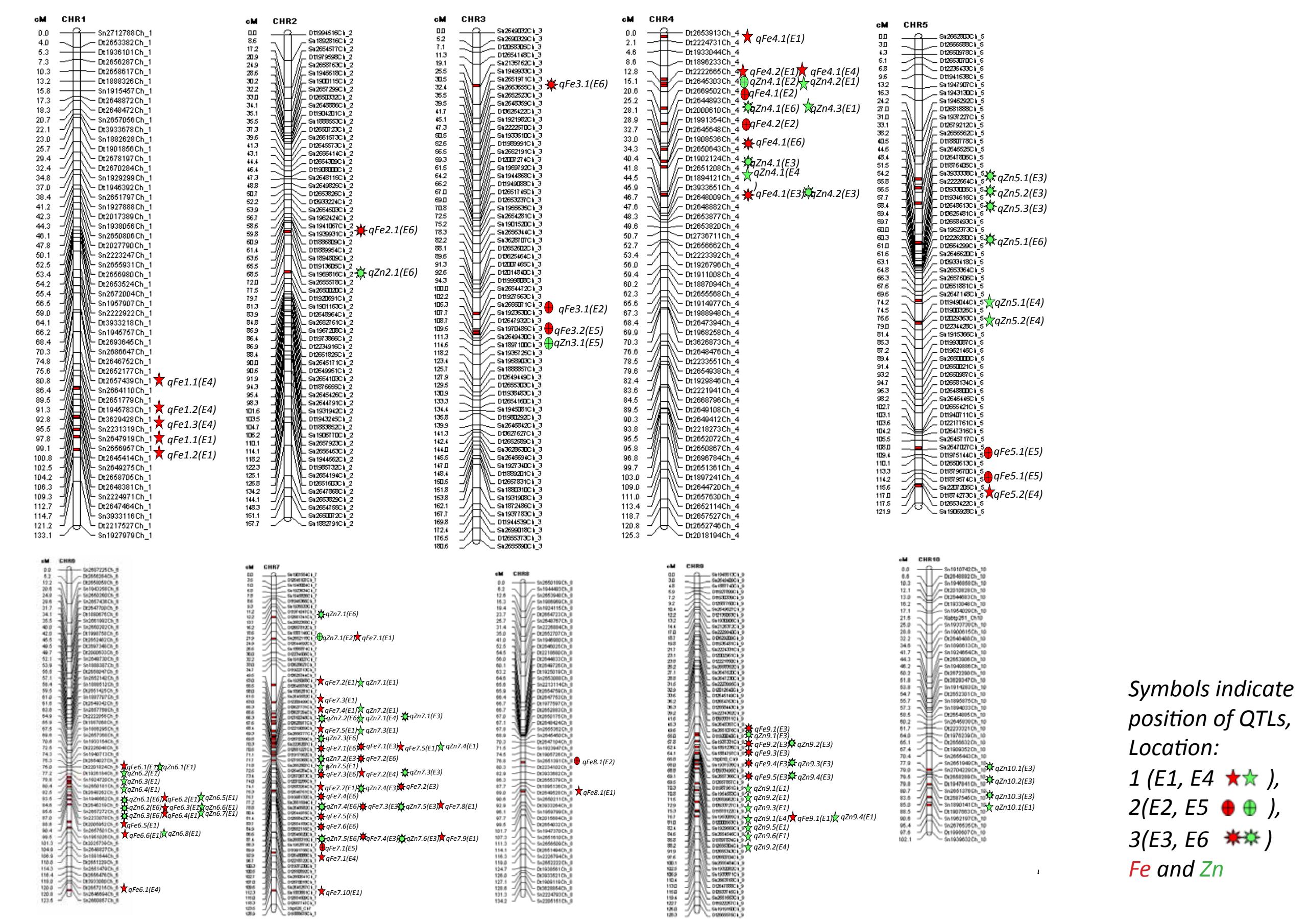


Figure 1. Chromosomal location of putative QTLs for Fe and Zn concentrations in a sorghum RIL population (309 individuals) derived from (296B × PVK 801). Linkage map 1355.52 cM developed with 2088 polymorphic (1148 DArT, 927 DArT seq [SNPs] and 13 SSRs) markers.

