

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

Anuradha Kotla^{1*}, Rahul Phuke^{1,4}, Hariprasanna K², Shivaji P. Mehtre³, Abhishek Rathore¹, Radhika K⁴, Tom Hase C⁵, Belum VS Reddy¹, Patil JV², Farzana Jabeen⁴, Shashikanth D², Pagde LS³, Santosh P Deshpande¹ and Ashok Kumar A¹

1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502 324, Telangana, India; 2. Indian Institute of Millet Research, Rajendranagar, Hyderabad, 500 030, Telangana, India; 3. Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, 431 402, Maharashtra, India; 4. Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, 500 030, Telangana, India; 5. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Niamey, Niger, WCA
Address for correspondence: A.Kotla@cgiar.org

Abstract

Micronutrient malnutrition or 'hidden hunger' due to consumption of diets poor in iron (Fe) and zinc (Zn) affect over >3 billion people worldwide, which 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 (DATs). Highly significant genotype × environment interactions were observed for both micronutrients; grain Fe and Zn 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 involved in parent PVK801-P23. QTLs were analyzed *in-silico* to identify underlying candidate genes, 62 candidate genes controlled by 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 chromosome 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

- Evaluation 2 - Sorghum (*F₂* RIL 2012-13 & 2013-14); Multi-location (ICRISAT, IIMR, VNMKV) evaluation; 2 seasons (SBI 12-13 & 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 gene search - Phytozome 10.3 > JBrowse > bicolor > ICRISAT 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, 296B for Fe/Zn measured for parents and RILs means in individual environment		296B (P1)		PVK 801 (P2)		RILs		SD (\pm)	
Trait	Environment	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fe (mg kg ⁻¹)	ICRISAT 12-13 (E1)	28.0	0.69	33.4	0.69	33.6	0.69	5.6	0.69
	IIMR 12-13 (E2)	28.5	0.69	33.0	0.69	33.0	0.69	6.3	0.69
	VNMKV 12-13 (E3)	46.3	0.69	49.4	0.69	49.2	0.69	6.9	0.69
	ICRISAT 13-14 (E4)	26.0	0.69	28.2	0.69	28.0	0.69	4.9	0.69
	IIMR 13-14 (E5)	30.8	0.69	35.9	0.69	35.8	0.69	5.0	0.69
	VNMKV 13-14 (E6)	27.2	0.69	33.6	0.69	34.0	0.69	7.8	0.69
Zn (mg kg ⁻¹)	ICRISAT 12-13 (E1)	21.3	0.51	24.3	0.51	24.6	0.51	4.7	0.51
	IIMR 12-13 (E2)	21.0	0.51	22.0	0.51	24.7	0.51	5.0	0.51
	VNMKV 12-13 (E3)	26.4	0.51	30.4	0.51	31.4	0.51	6.4	0.51
	ICRISAT 13-14 (E4)	14.6	0.51	16.4	0.51	17.3	0.51	3.5	0.51
	IIMR 13-14 (E5)	21.1	0.51	24.8	0.51	25.6	0.51	4.0	0.51
	VNMKV 13-14 (E6)	19.6	0.51	24.0	0.51	24.7	0.51	5.2	0.51

b) Variances and heritability for (296 B × PVK 801)-derived RIL population evolution									
Pooled (across six environments)									
Trait	σ^2_g	SE (\pm)	σ^2_e	SE (\pm)	σ^2_{gl}	SE (\pm)	σ^2_{ge}	SE (\pm)	h^2
Fe	4.18**	0.69	-0.17	0.66	-0.7	0.80	14.32**	1.58	0.59
Zn	4.17**	0.51	0.71**	0.35	-0.14	0.37	5.22**	1.04	0.69

Individual environments									
	ICRISAT 12-13 (E1)			IIMR 12-13 (E2)			VNMKV 12-13 (E3)		
	σ^2_g	SE (\pm)	h^2	σ^2_g	SE (\pm)	h^2	σ^2_g	SE (\pm)	h^2
Fe	15.51**	1.68	0.78	20.79**	2.17	0.81	23.44**	2.85	0.8
Zn	10.00**	1.12	0.74	12.72**	1.33	0.8	27.41**	2.43	0.8

	ICRISAT 13-14 (E4)			IIMR 13-14 (E5)			VNMKV 13-14 (E6)		
	σ^2_g	SE (\pm)	h^2	σ^2_g	SE (\pm)	h^2	σ^2_g	SE (\pm)	h^2
Fe	8.72**	1.05	0.68	10.84**	1.3	0.68	31.36**	3.23	0.77
Zn	5.48**	0.6	0.73	4.60**	0.68	0.56	12.71**	1.4	0.74

Genotypic variance (σ^2_g), Genotype × Year (σ^2_{gy}), Genotype × Location (σ^2_{gl}), Genotype × Year × Location (σ^2_{gye}) interactions, standard error (SE), heritability (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								
<i>qfe1.1</i>	SBI-01	Dt1937067Ch_1	112.8	Dt2647464Ch_1	112.7	2.84	0.42	3.12
<i>qfe4.1</i>	SBI-04	Dt2646105Ch_4	1.05	Dt224731Ch_4	2.07	3.86	-0.51	4.46
<i>qfe4.2</i>	SBI-04	Dt2650643Ch_4	34.31	Dt1997108Ch_4	33.47	2.65	-0.41	3.05
<i>qfe4.3</i>	SBI-04	Dt3933410Ch_4	40.76	Dt2658021Ch_4	41.15	2.45	-0.39	2.82
<i>qfe6.1</i>	SBI-06	Dt2090541Ch_6	100.23	Dt2657314Ch_6	100.94	3.25	0.45	3.85
<i>qfe6.2</i>	SBI-06	Dt2233502Ch_6	101.39	Sn1918187Ch_6	101.65	2.75	0.42	3.19
<i>qfe7.1</i>	SBI-07	Dt2657812Ch_7	16.22	Dt2653638Ch_7	16.57	2.57	0.41	2.97
<i>qfe7.2</i>	SBI-07	Dt2644692Ch_7	24.90	Sn2646254Ch_7	24.76	3.16	0.44	3.62
<i>qfe7.3</i>	SBI-07	Xtxp525_Ch7	123.47	Sn2653248Ch_7	126.57	5.61	0.60	6.66
Zn								
<i>qzn4.1</i>	SBI-04	Dt2224731Ch_4	2.07	Dt1944553Ch_4	3.64	0.25	-0.43	0.30
<i>qzn4.2</i>	SBI-04	Dt1907645Ch_4	13.59	Dt2645303Ch_4	15.05	4.36	-0.53	5.39
<i>qzn6.1</i>	SBI-06	Dt2005952Ch_6	88.60	Dt2648290Ch_6	88.83	2.49	0.39	3.06
<i>qzn6.2</i>	SBI-06	Sn2657501Ch_6	90.07	Sn2647940Ch_6	91.58	2.64	0.41	3.24
<i>qzn7.1</i>	SBI-07	Dt2657812Ch_7	16.22	Dt2653638Ch_7	16.57	2.57	0.40	3.17
<i>qzn7.2</i>	SBI-07	Dt2645576Ch_7	55.45	Sn2033434Ch_7	56.26	4.49	0.54	5.66
<i>qzn7.3</i>	SBI-07	Sn2650637Ch_7	57.03	Sn1895281Ch_7	57.98	3.76	0.49	4.66
<i>qzn7.4</i>	SBI-07	Sn1895297Ch_7	62.05	Dt2648834Ch_7	62.52	4.16	0.51	5.14
<i>qzn7.5</i>	SBI-07	Dt2649259Ch_7	67.16	Dt3628977Ch_7	67.61	4.43	0.53	5.74
<i>qzn7.6</i>	SBI-07	Dt3627584Ch_7	69.46	Dt2649175Ch_7	69.73	2.84	0.43	3.63
<i>qzn7.7</i>	SBI-07	Sn1937648Ch_7	72.61	Sn1919843Ch_7	73.35	2.91	0.43	3.65
<i>qzn9.1</i>	SBI-09	Sn1950992Ch_9	83.72	Sn1929569Ch_9	82.36	3.58	-0.47	4.38

9 QTLs for Fe - located on chromosomes SBI-01, 4, 6 and 7, 12- QTLs for Zn on chromosomes SBI-04, 6, 7 and 9. q-QTL, PV2- phenotypic variance

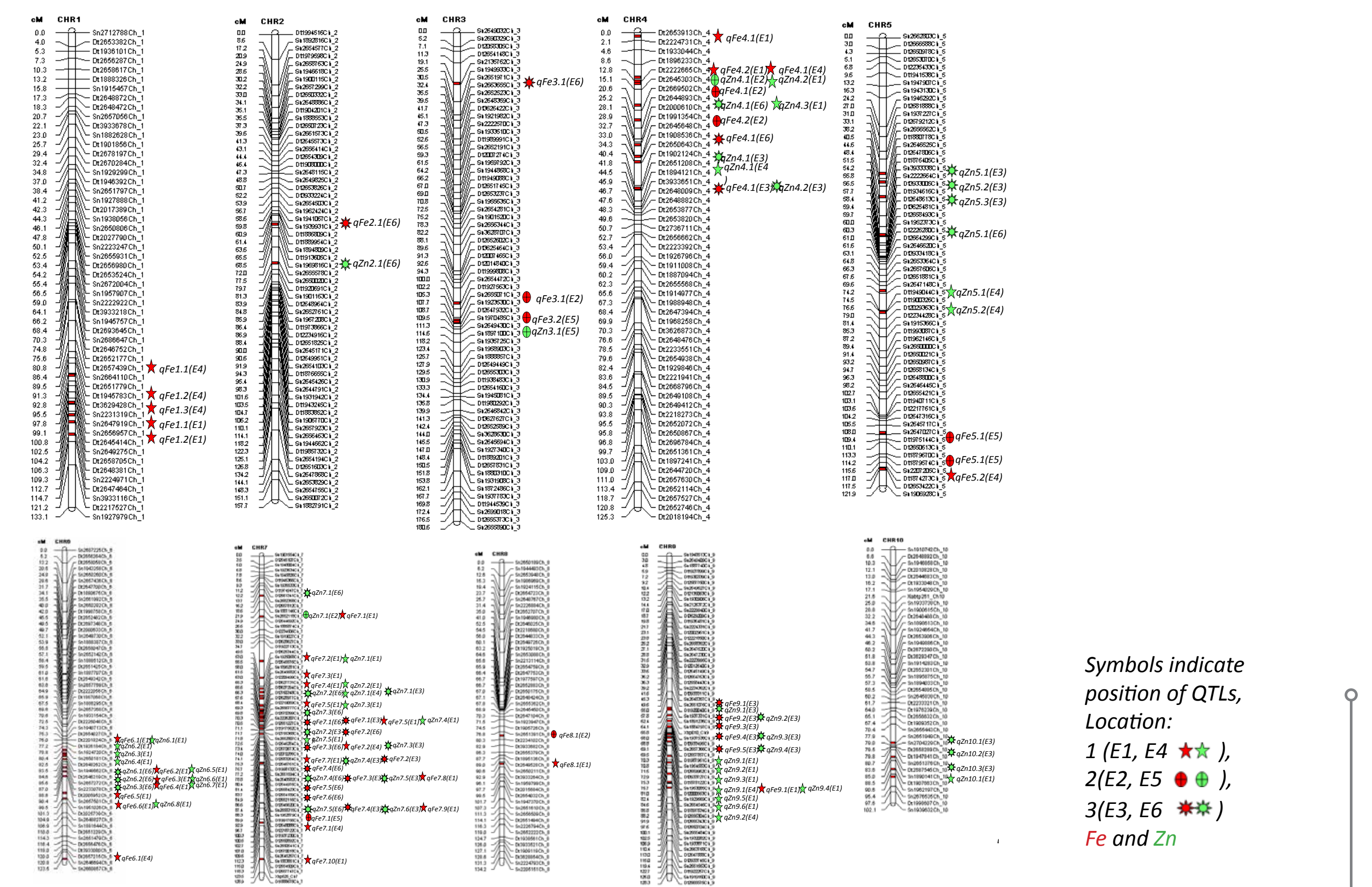


Figure 1. Chromosomal location of putative QTLs for Fe and Zn in sorghum RIL population (309 individuals) derived from (296B × PVK 801). Linkage map 1355.52 cM developed with 2088 polymorphic (1148 DART, 927 DART seq [SNPs] and 13 SSRs) markers.

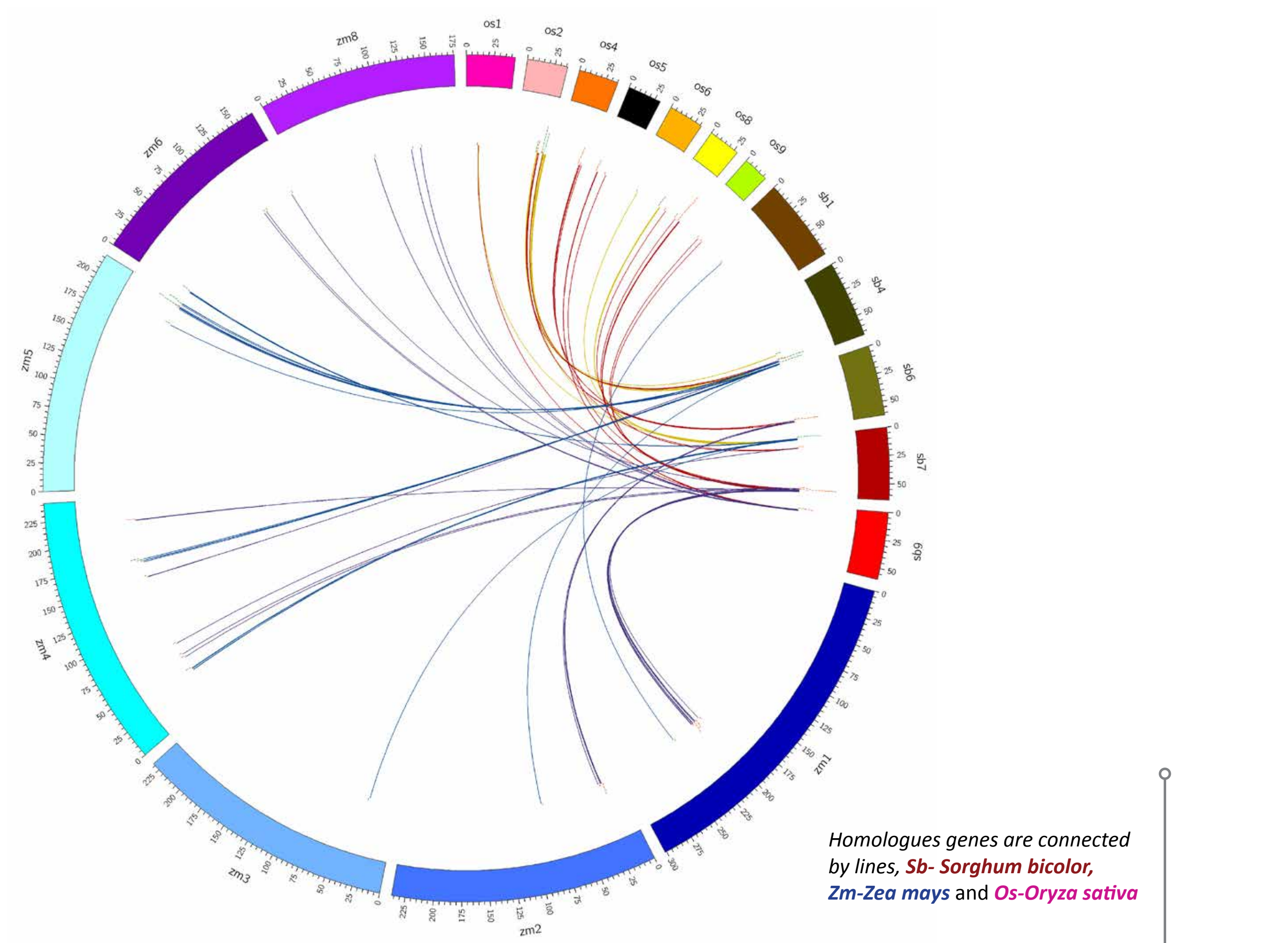


Figure 2. Candidate genes underlying the region associated with grain Fe/Zn QTLs on chromosome SBI-01, SBI-04, SBI-06, SBI-07 and SBI-09 in sorghum showing gene synteny relationship with Zea mays on chromosomes 1, 2, 3, 4, 6, 8 and 10 and Oryza sativa on chromosomes 1, 2, 4, 5, 6, 8, 9, 10 and 11.

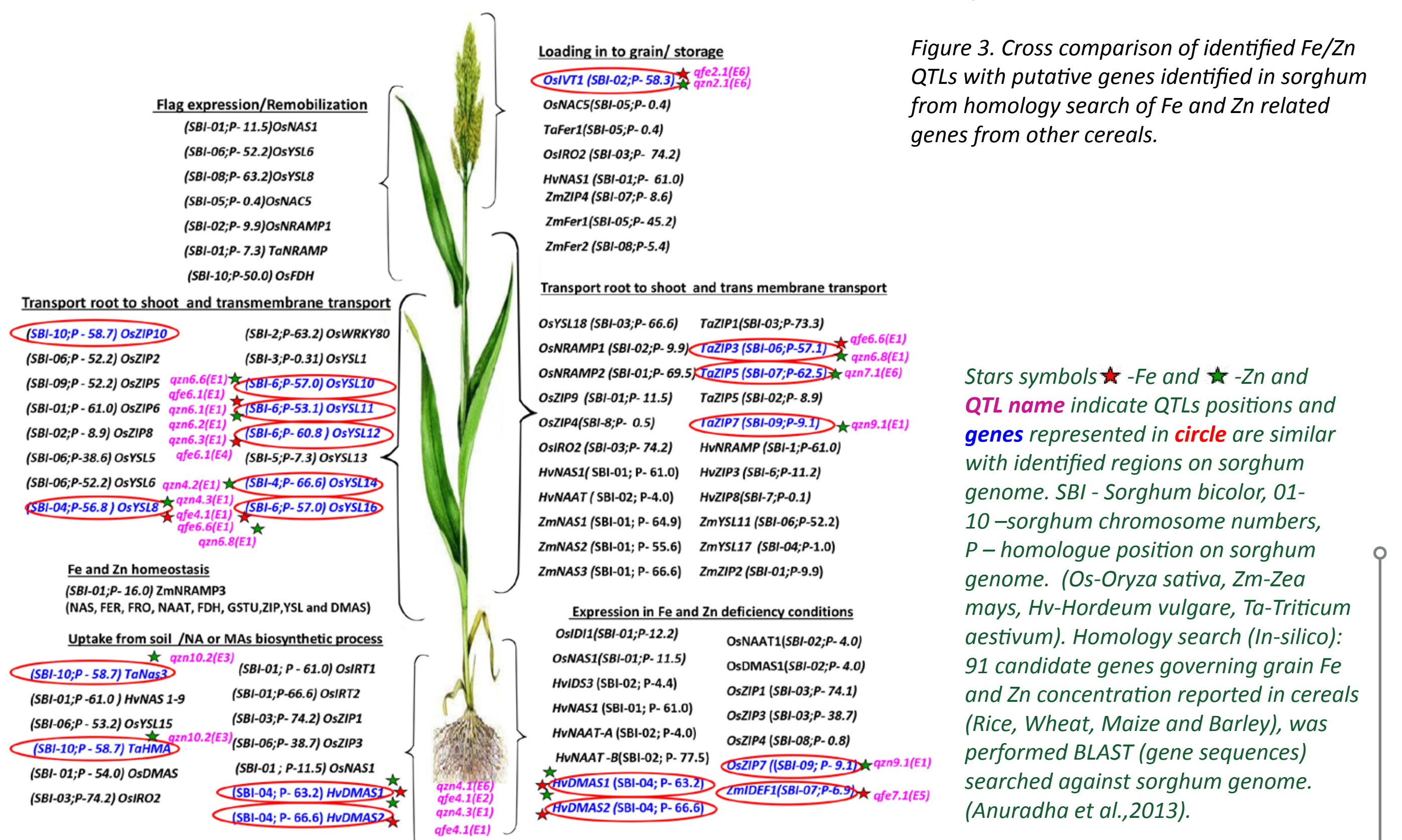


Figure 3. Cross comparison of identified Fe/Zn QTLs with putative genes identified in sorghum from homology search of Fe and Zn related genes from other cereals.

Conclusions

- First report of QTL mapping efforts for grain Fe and Zn concentration in sorghum
- Identified 167 small main-effect QTLs controlling grain Fe and Zn concentration
- Syntenic analysis identified genomic loci harboring grain Fe and Zn concentration and related candidate genes with potential for further functional genomics analysis
- Utilizing integrated approach-conventional breeding and genomic approaches to provide efficient path for development of improved varieties.

Acknowledgement

We thank DBT (Department of Biotechnology, Govt. of India) for providing funding to project on "Bio fortifying sorghum with high grain iron and zinc concentration for combating micronutrient malnutrition, (Project Code: BT/PR 1630/AGR/2/806/2011)" for financial support.

Reference

Kotla Anuradha, Bhanu Prakash, Santosh P Deshpande, Punna Ramu, Trushar Shah and A Ashok Kumar (2013). *In Silico* identification of candidate genes involved for grain Fe and Zn concentration in sorghum using reported cereals gene homologs. Society for Millet Research Global Millet meet 12/2013; DOI: SBI 81-89335-47-2 (www.researchgate.net/publication/259899782_In_Silico_identification_of_candidate_genes_involved_for_grain_Fe_and_Zn_concentration_in_sorghum_using_reported_cereals_gene_homologs)