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

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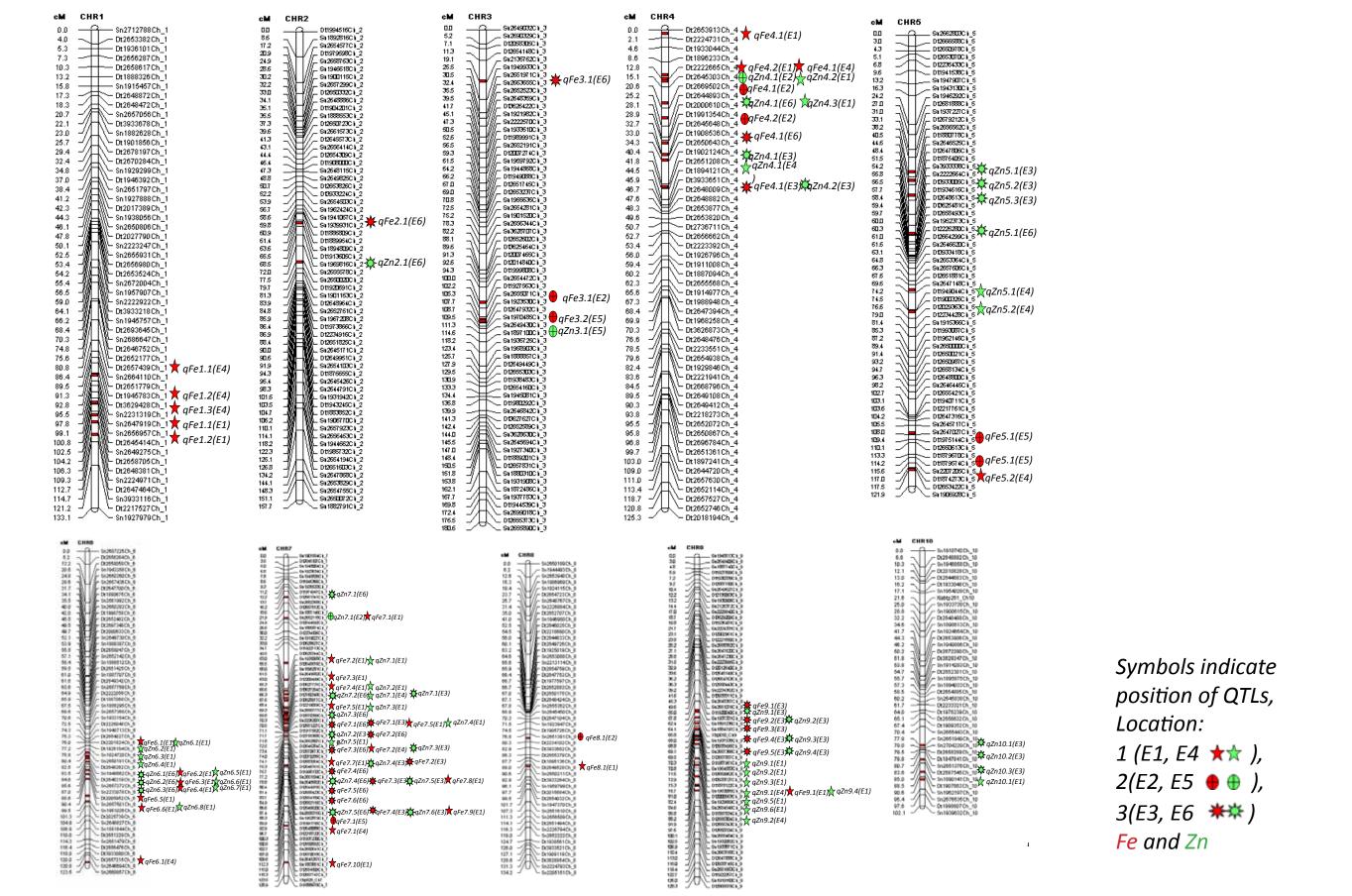
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Abstract

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 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 *Insilco* homology study (Figure 3).



Methods and materials

- Phenotyping Sorghum F_c 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

ICRISAT 12-13 (E1)

VNMKV 12-13 (E3)

IIMR 12-13 (E2)

- 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

Zn

(mg kg⁻¹)

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 296B (P1) PVK 801 (P2) RILs SD (±) Environment Trait 5.6 28.0 33.4 33.6 ICRISAT 12-13 (E1) Fe (mg kg⁻¹) IIMR 12-13 (E2) 6.3 28.5 33.0 33.0 46.3 49.2 VNMKV 12-13 (E3) 49.4 6.9 4.9 ICRISAT 13-14 (E4) 28.0 26.0 28.2 IIMR 13-14 (E5) 30.8 35.8 5.0 35.9 VNMKV 13-14 (E6) 7.8 27.2 34.0 33.6

24.6

24.7

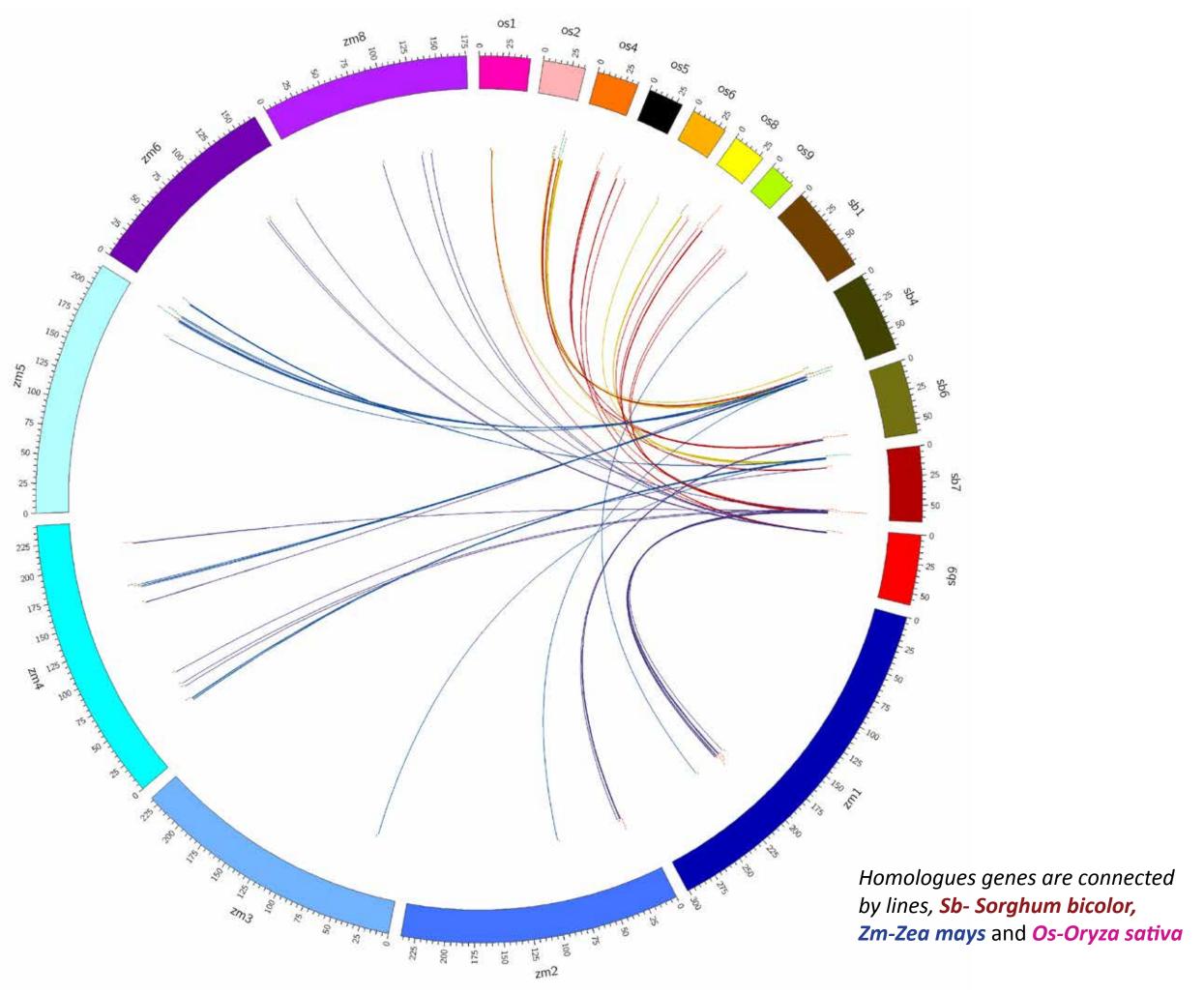
31.4

4.7

5.0

6.4

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.



VNMKV 13-14 (E6)	19.6	24.0	24.7	5.2
IIMR 13-14 (E5)	21.1	24.8	25.6	4.0
ICRISAI 13-14 (E4)	14.6	16.4	17.3	3.5

24.3

22.0

30.4

b) Variances and heritability for (296 B × PVK 801)-derived RIL population evolution

21.3

21.0

26.4

•	•	•	•						
		F	Pooled (across	six environm	ents)				
Trait	σ²g	SE (±)	σ²gy	SE (±)	σ²gl	SE (±)	σ²gyl	SE (±)	h²
Fe	4.18**	0.69	-0.17	0.66	-0.7	0.80	14.32**	1.18	0.58
Zn	4.17**	0.51	0.71**	0.35	-0.14	0.37	5.22**	0.54	0.69
			Individual e	environment	S				
	ICRISAT 12-13 (E1)			IIMR 12-	13 (E2)		VNMKV 1	2-13 (E3)	
	σ²g	SE (±)	h²	$\sigma^2 g$	SE (±)	h²	σ²g	SE (±)	h²
Fe	15.51**	1.68	0.78	20.79**	2.17	0.81	27.44**	2.85	0.8
Zn	10.00**	1.12	0.74	12.72**	1.33	0.8	23.41**	2.43	0.8
	ICRISAT 13-14 (E4)			IIMR 13-	14 (E5)		VNMKV 1	3-14 (E6)	
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
Conotyn	$rac{1}{2}$	\times Voor $(\sigma^2 \sigma u)$	Conotypo v Loca	$(\sigma^2 \sigma l) = G$	anotyno y	Voarvlo	$cation(\sigma^2 \alpha u)$	interaction	c

Genotypic variance(σ^2 g), Genotype × Year (σ^2 gy), Genotype x Location (σ^2 gl), Genotype x Year x Location(σ^2 gyl) interactions, standard error (SE), heritability's (h², 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		Flanking	Marker	Flanking	Marker		Additive	
name	Chr.no	marker (L)	Position (cM)	marker (R) Fe	Position (cM)	LOD	effect	PV2
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	Dt2233502Ch_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	Xtxp525_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

Figure2. 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 syntenic 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.*

	Loading in to grain/ storage OsIVT1 (SBI-02;P- 58.3) afe2.1(E6
Flag expression/Remobilization	OsNAC5(SBI-05;P- 0.4)
(SBI-01;P- 11.5)OsNAS1	TaFer1(SBI-05;P- 0.4)
(SBI-06;P- 52.2)OsYSL6	OsIRO2 (SBI-03;P- 74.2)
(SBI-08;P- 63.2)OsYSL8	HvNAS1 (SBI-01;P- 61.0)
(SBI-05;P- 0.4)OsNAC5	ZmZIP4 (SBI-07;P- 8.6)
(SBI-02;P- 9.9)OsNRAMP1	ZmFer1(SBI-05;P-45.2)
(SBI-01;P- 7.3) TaNRAMP	ZmFer2 (SBI-08;P-5.4)
(SBI-10;P-50.0) OsFDH	Transport root to shoot and tran
(SBI-10;P - 58.7) OsZIP10 (SBI-2;P-63.2) OsWRKY80	OsYSL18 (SBI-03;P- 66.6) TaZIP1(
	OsNRAMP1 (SBI-02;P- 9.9)
(SBI-06;P - 52.2) OsZIP2 (SBI-3;P-0.31) OsYSL1 (SBI-09;P - 52.2) OsZIP5 qzn6.6(E1) (SBI-6;P-57.0) OsYSL10	OsNRAMP2 (SBI-01;P- 69.5) ToZIP5 (
qfe6.1(E1)	OsZIP9 (SBI-01;P- 11.5) TaZIP5 (
(SBI-01;P - 61.0) OSZIP6 qzn6.1(E1) (SBI-6;P-53.1) OSYSL11 qzn6.2(E1) (SBI-6;P-53.1) OSYSL11	OsZIP4(SBI-8;P- 0.5)
(SBI-02;P-8.9) OS2IP8 qzn6.3(E1)	OsIRO2 (SBI-03;P- 74.2) HvNRAM
(SBI-06;P-38.6) OsYSL5 qfe6.1(E4) (SBI-5;P-7.3) OsYSL13	HvNAS1(SBI-01; P- 61.0) HvZIP3
(SBI-06;P-52.2) OsYSL6 qzn4.2(E1) (SBI-4;P-66.6) OsYSL14	HvNAAT (SBI-02; P-4.0) HvZIP8(
SBI-04;P-56.8) OSYSL8 qfe4.1(E1) fe6 6 (E1)	ZmNAS1 (SBI-01; P- 64.9) ZmYSL1
qfe6.6(E1) → ★ qzn6.8(E1)	ZmNAS2 (SBI-01; P- 55.6) ZmYSL1
Fe and Zn homeostasis	ZmNAS3 (SBI-01; P- 66.6) ZmZIP2
(<i>SBI-01;P- 16.0</i>) ZmNRAMP3	
(NAS, FER, FRO, NAAT, FDH, GSTU,ZIP,YSL and DMAS)	Expression in Fe and Zn defi
Uptake from soil /NA or MAs biosynthetic process	OsiDi1(SBI-01;P-12.2) Osi
★ qzn10.2(E3)	OsNAS1(SBI-01;P-11.5) Osl
	HviDS3 (SBI-02; P-4.4) Osi
(SBI-01;P-61.0) HvNAS 1-9 (SBI-01;P-66.6) OsIRT2	HvNAS1 (SBI-01; P- 61.0)
(SBI-06;P - 53.2) OsYSL15 (SBI-03;P- 74.2) OsZIP1 ★ gzn10.2(E3) (SBI-06:P- 38.7) OsZIP3	HvNAAT-A (SBI-02; P-4.0)
(SBI-10;P - 58.7) TaHMA (SBI-00;F - 50.7) OSEIF 5	HUNAAT_R(SRL02, D. 77 5)
(SBI- 01; P - 54.0) OsDMAS (SBI-01; P-11.5) OsNAS1	* UDMAS1 (SPL 04: P. 63.7)
(SBI-03;P-74.2) OsIRO2	qfe4.1(E2) qre4.3(E1)
(SBI-04; P- 66.6) HvDMA52	afe4.1(E1)

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.

trans membrane transport

ZIP1(SBI-03;P-73.3) ZIP3 (SBI-06;P-57.1) ZIP5 (SBI-07;P-62.5) 🙀 qzn7.1(E6) ZIP5 (SBI-02;P- 8.9) ZIP7 (SBI-09;P-9.1) + qzn9.1(E1) NRAMP (SBI-1;P-61.0) ZIP3 (SBI-6;P-11.2) ZIP8(SBI-7;P-0.1) nYSL11 (SBI-06;P-52.2) nYSL17 (SBI-04;P-1.0) nZIP2 (SBI-01;P-9.9) deficiency conditions

OsNAAT1(SBI-02;P- 4.0) OsDMAS1(SBI-02;P- 4.0) OsZIP1 (SBI-03;P- 74.1) OsZIP3 (SBI-03;P- 38.7) OsZIP4 (SBI-08;P- 0.8) OsZIP7 ((SBI-09; P- 9.1) qzn9.1 ZmIDEF1(SBI-07;P-6.9) + afe7.

Stars symbols 🛨 -Fe and 🛣 -Zn and **QTL name** indicate QTLs positions and genes represented in circle are similar with identified regions on sorghum genome. SBI - Sorghum bicolor, 01-10 –sorghum chromosome numbers, *P* – homologue position on sorghum genome. (Os-Oryza sativa, Zm-Zea mays, Hv-Hordeum vulgare, Ta-Triticum aestivum). Homology search (In-silico): *91 candidate genes governing grain Fe* and Zn concentration reported in cereals (Rice, Wheat, Maize and Barley), was performed BLAST (gene sequences) searched against sorghum genome. (Anuradha et al.,2013).

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
- Synteny analysis identified genomic loci harboring grain Fe and Zn concentration and revealed 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

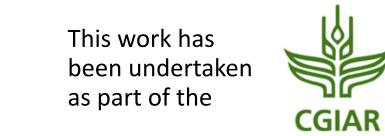
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Reference

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