



Full Length Article

Mapping of Quantitative Trait Loci Associated with Concentrations of Five Trace Metal Elements in Rice (*Oryza sativa*)

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Abstract

Trace metal element concentrations in rice (*Oryza sativa* L.) have great effects on the health of human being. In this study, QTLs for the concentrations of Cd, Cu, Fe, Mn and Zn in brown rice were identified using a recombinant inbred lines (RIL) population. A total of 22 QTLs for the concentrations of Cd, Cu, Fe, Mn and Zn in brown rice were identified with 204 recombinant inbred lines (RILs), including *qCd5* for pot cultivation (PC) with Cd stress and paddy field cultivation (FC) trials in Hangzhou, 9 for PC trial only, and 13 for FC trial only. The phenotypic variance explained by a single QTL ranged as 4.6–9.9% and 2.8–10.4% in PC and FC trials, respectively. Among all QTLs, *qCu4-2* had the largest single effect (10.4% of the phenotypic variance), *qCu7* had the lowest single effect (2.8% of the phenotypic variance). The positive alleles (increasing trace metal element concentrations) at 16 QTLs except *qCd2*, *qCu7*, *qFe7*, *qMn7*, *qZn1-1* and *qZn4* were inherited from the female parent Zhenshan 97 B (ZS97B). These QTLs distributed on chromosomes 1, 2, 4, 5, 7, 8, 10 and 11. Six QTLs were clustered in 1, 4 and 7 chromosomal regions. Especially, *qCd5* was a novel QTL regulated low Cd accumulation in rice. The identification of QTLs provides good candidates for fine mapping and cloning, and could be utilized to improve rice safe and nutritional quality by MAS. © 2018 Friends Science Publishers

Keywords: Trace metal element concentrations; Recombinant inbred lines; QTLs; Mapping; Rice

Introduction

Iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) are essential trace mineral elements to maintain the health of human being (Zheng, 1999). The appropriate intake of trace mineral elements is beneficial to human and animals health, mineral deficiencies harmed human beings health and development with symptoms, such as mental retardation immune dysfunction (Stein, 2010). Trace mineral elements couldn't be biosynthetic *in vivo*, and staple foods are identified as a valid and safe approach to supplement them. Researchers considered that bio-augmentation can improve the mineral nutrients content in crops (Welch and Graham, 2002). Cadmium (Cd) is highly toxic trace metal element to human beings and it could easily enter food chains from polluted crops (Tezuka *et al.*, 2010). With the development of mining and modern industry, Cd pollution is becoming more and more serious, particularly for paddy fields irrigated with Cd-contaminated water, and crops planted in the fields assimilate and accumulate Cd, it may lead destructive consequences for human health (Tezuka *et al.*, 2010). Therefore, crops, especially rice, with low cadmium absorption and accumulation are rush.

Rice, one of the most important food crops, is the staple food for more than half of the world's population and provides a large proportion of mineral nutrients of food in China. Significant genetic variations have been reported for grain trace metal element concentrations in various rice varieties, and genetic analysis has indicated that the genetic components played a major role in determining the grain trace metal element concentration variation, which provides an potential method to breeding new rice varieties with low Cd concentration and essential mineral elements-rich to resolve above problems (Wu *et al.*, 1999).

In recent years, numerous QTLs have been reported for trace metal elements in rice grain. James *et al.* (2007) identified 11 QTLs for mineral nutrients concentrations by using a doubled haploid population derived from the combination of IR64×Azucena. Ishikawa *et al.* (2005) identified QTLs for Cd concentration on chromosomes 3, 6 and 8 by using CSSLs. Huang *et al.* (2015) detected 20 QTLs for Fe, Zn, Se, Cd and Pb concentrations by using 378 accessions of brown rice. Norton *et al.* (2010) detected 77 QTLs, (36 for leaves and 41 for grains) for 17 elements concentrations by using introgression lines of Bala×Azucena. Du *et al.* (2013) detected 32 QTLs for the 7

mineral elements investigated by using a double-haploid population derived from the cross between Chunjiang 06 and TN1. Zhang *et al.* (2014) detected 134 QTLs associated with the grain concentrations of individual elements by using a recombinant inbred lines and a backcross introgression lines that derived from the cross between Lemont and TeQing. Hu *et al.* (2016) detected 24 QTLs for mineral element concentrations in brown and milled rice by using backcross inbred lines derived from the cross between Xieqingzao B and Dongxiang wild rice. Mahender *et al.* (2016) detected 37 QTLs for rice grain mutational traits through transgenic and advanced genomic approaches. Considering people grow rice for mainly eating grains, while the corresponding molecular regulatory mechanism of trace metal elements of Cd, Cu and zinc, Mn and Fe in brown rice keeps still unclear, therefore, carrying out researches on this aspect of the mechanism are very necessary. According to test design, we planted a set of stable recombinant inbred lines of rice group in different cultural environment, then harvested the grains, and tested the trace metal elements of Cd, Cu and zinc, Mn and Fe content, finally, combined with relevant molecular map, and mapped of the relevant QTLs, which provided the foundation for subsequent further research.

In this study, we identified 22 QTLs of the concentration of Cd, Cu, Zn, Mn and Fe in brown rice using a RIL population with 204 lines derived from the cross between ZS97B, an elite indica maintainer line, and Miyang 46 (MY46), an elite indica three-line restorer, which makes it possible for rice breeders to develop closely linked markers for conducting marker-assisted selection of breeding, and set the stage for the fine mapping and even positional cloning of these QTLs. The identification of QTLs could be utilized to efficient breeding low Cd concentration rice varieties and mineral elements-rich rice varieties by MAS.

Materials and Methods

Plant Materials

An F₁₃ RIL population with 204 lines generated from the cross of ZS97B × MY46 and parental lines were planted in experimental paddy fields and pots with Cd stress at China National Rice Research Institute (CNRRI) in Hangzhou, respectively. In FC trial, 10 seedlings of each line per row were transplanted with a spacing of 20.0 cm between seedlings within a row and 20.0 cm between each two rows. The plants were watered with agricultural irrigated water and managed as traditional pattern of paddy fields from June to October in 2013. In PC trial, 2 seedlings of each line were transplanted with a spacing of 20.0 cm between the two seedlings. The plants were grown in a greenhouse and watered daily with tap water to keep the soil soaked in the water from June to October in 2013. Both trials were designed by random zone and repeated three times. At harvest, rice grains of each line were mix harvested and

manually threshed in both trials. The concentration of trace metal elements in brown rice and experimental soil were determined with inductively coupled plasma optical emission spectrometry (ICP-OES) after digestion, as described below. The trace metal element concentrations of soil in PC and FC trials are listed in Table 1.

Sample Preparation

After sun-dried, the rice grains were stored at room temperature for three months, and then 20 g grains of each line were de-hulled with a testing sheller, after that the brown rice were grinded to powder using a sample grinder and passed through a 0.20 mm mesh sieve.

Measurement of Cd, Cu, Mg, Zn and Fe Concentrations

About 0.5 g of rice powder were weighted into a 50 mL high temperature- and acid-resistant plastic tube and added with 5.0 mL guaranteed reagent nitric acid (HNO₃), then these samples were digested with microwave digestion instrument MARS 5 after shaking gently, and next the digestion tubes were heated from room temperature to 120°C for 5 min, at 120°C for 3 min, and then from 120°C to 180°C for 6 min, at 180°C for 10 min. When the tubes were cooled at 90°C, taken off their lids and then heated at 150°C until about 0.5 mL solution remained. The digested samples were cooled and diluted to imported centrifugal tubes of 50 mL with double de-ionized water. Finally, according to the manufacturer's instructions, the concentrations of Cd, Cu, Mg, Zn and Fe were simultaneously measured by ICP-OES.

Data Analysis

A total of 256 markers were used to construct a genetic linkage map spanning 1814.7 cM on total 12 chromosomes, with an average interval length of 7.09 cM (Fig. 1). Composite interval mapping analysis of QTL in the RIL population was performed with WinQTLcart 2.5. QTLs were called where their logarithm of the odds (LOD) values exceeded 2.0, and the proportion of phenotypic variation was estimated from the coefficient of determination (R²). Parameter settings for the QTL mapping were as described by Matsubara (Matsubara *et al.*, 2008). The QTL was named following the nomenclature recommended by McCouch and CGSNL (McCouch and CGSNL, 2008). Pearson's product-moment correlation coefficients between the traits were determined using SAS software.

Results

The Traits Performance of ZS97B, MY46 and the RIL Population

ZS97B showed significant higher trait values than MY46 in Cu and Mn, while showed lower trait values in Cd, Fe, no significant difference was identified in Zn concentrations in

Table 1: Trace metal element concentrations (mg/kg) of soil in PC and FC trials

Trials	Cd	Cu	Fe	Mn	Zn
PC	5.88±0.25	38.06±2.89	236.78±14.60	58.71±2.20	23.90±0.86
FC	0.53±0.05	35.53±1.61	114.86±21.70	41.54±5.07	27.15±1.46

PC and FC represent pot cultivation and paddy field cultivation, respectively

Table 2: Trace metal element concentrations (mg/kg) of ZS97B, MY46 and the RIL population

Trace metal elements	Trials	Parents			RIL lines		
		ZS97B	MY46	Mean±SD	Min-Max	Skew	Kurt
Cd	PC	1.82	2.94	2.79±0.97	0.95-6.73	0.935	0.9822
	FC	0.13	0.24	0.15±0.05	0.05-0.31	0.523	-0.0546
Cu	PC	5.82	4.5	2.78±1.89	2.63-6.65	0.4209	0.2423
	FC	4.99	3.95	4.57±0.74	2.79-6.51	0.2492	-0.2246
Fe	PC	7.92	9.33	8.54±2.84	2.56-16.81	-0.2534	-0.2489
	FC	13.84	9.64	10.42±1.39	7.87-15.55	0.9398	1.3033
Mn	PC	41.66	31.42	32.3±6.30	21.19-32.28	0.8108	0.7806
	FC	40.03	28.14	27.09±4.21	16.04-39.93	0.5536	0.0597
Zn	PC	26.81	26.93	27.92±5.23	18.55-49.76	1.1596	2.1708
	FC	25.26	24.74	21.78±2.87	15.90-31.68	0.7752	0.8144

PC and FC represent pot cultivation and paddy field cultivation

PC trial; ZS97B had higher Cu, Fe, Mn, Zn concentration than MY46, but lower Cd concentration in FC trial. Except Fe, the other four trace metal elements concentrations were higher in PC trial than in FC trial (Table 2).

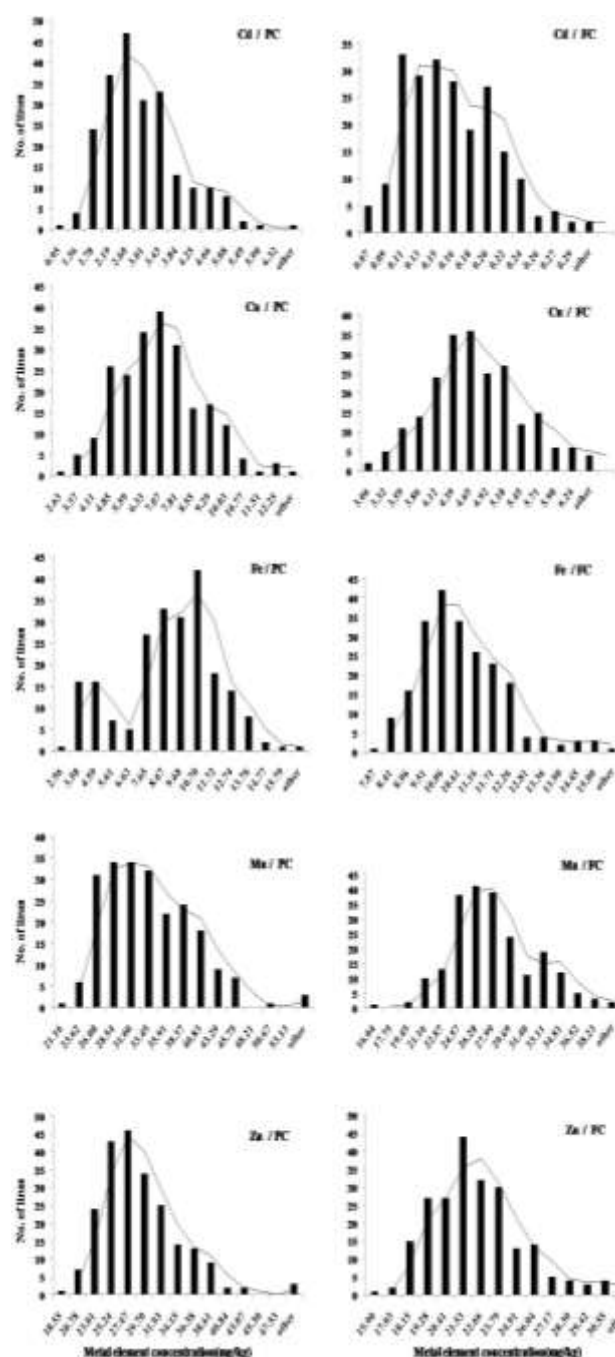
All of these traits showed continuous distribution and transgressive segregation, which suggests that these traits are controlled by QTLs. The absolute value of kurtosis and skewness of these five mineral elements were almost less than one, which indicated the frequency distribution of these traits showed normal variation (Table 2 and Fig. 1).

Correlation Analysis among Different Trace Metal Element Concentrations

Simple correlation analysis showed that Cd and Cu, Cu and Fe/Mn/Zn, Fe and Mn/Zn, Mn and Zn concentrations in brown rice of the RIL population were significant positive correlated in both trials, which implied accumulation and increase of one kind of trace metal element would cause accumulation and increase of other trace metal elements with synergistic effect. In PC trial, except Cd and Mn concentrations, the other trace metal element concentrations had significant positive correlated with each other. In FC trial, except Cd and Fe/Mn/Zn concentrations, the other trace metal element concentrations had significant positive correlated with each other (Table 3).

QTLs Analysis of Trace Metal Element Concentrations in Brown Rice

The interval QTL mapping of five trace metal elements concentrations in brown rice identified 22 QTLs, including 9 QTLs in PC trial and 13 QTLs in FC trial, respectively.

**Fig. 1:** The phenotypic frequency distribution of Cd, Cu, Fe, Mn and Zn concentrations (mg/kg) in brown rice in PC and FC trials, respectively; PC and FC represent pot cultivation and paddy field cultivation

These QTLs distributed on rice chromosome 1, 2, 4, 5, 7, 8 and 11. Except *qCd5*, the other QTLs for trace metal element concentrations in brown rice weren't repeatable detected. The phenotypic variance explained by a single QTL ranged as 4.6–9.9% and 2.8–10.4% in PC and FC trials, respectively (Table 4 and Fig. 2).

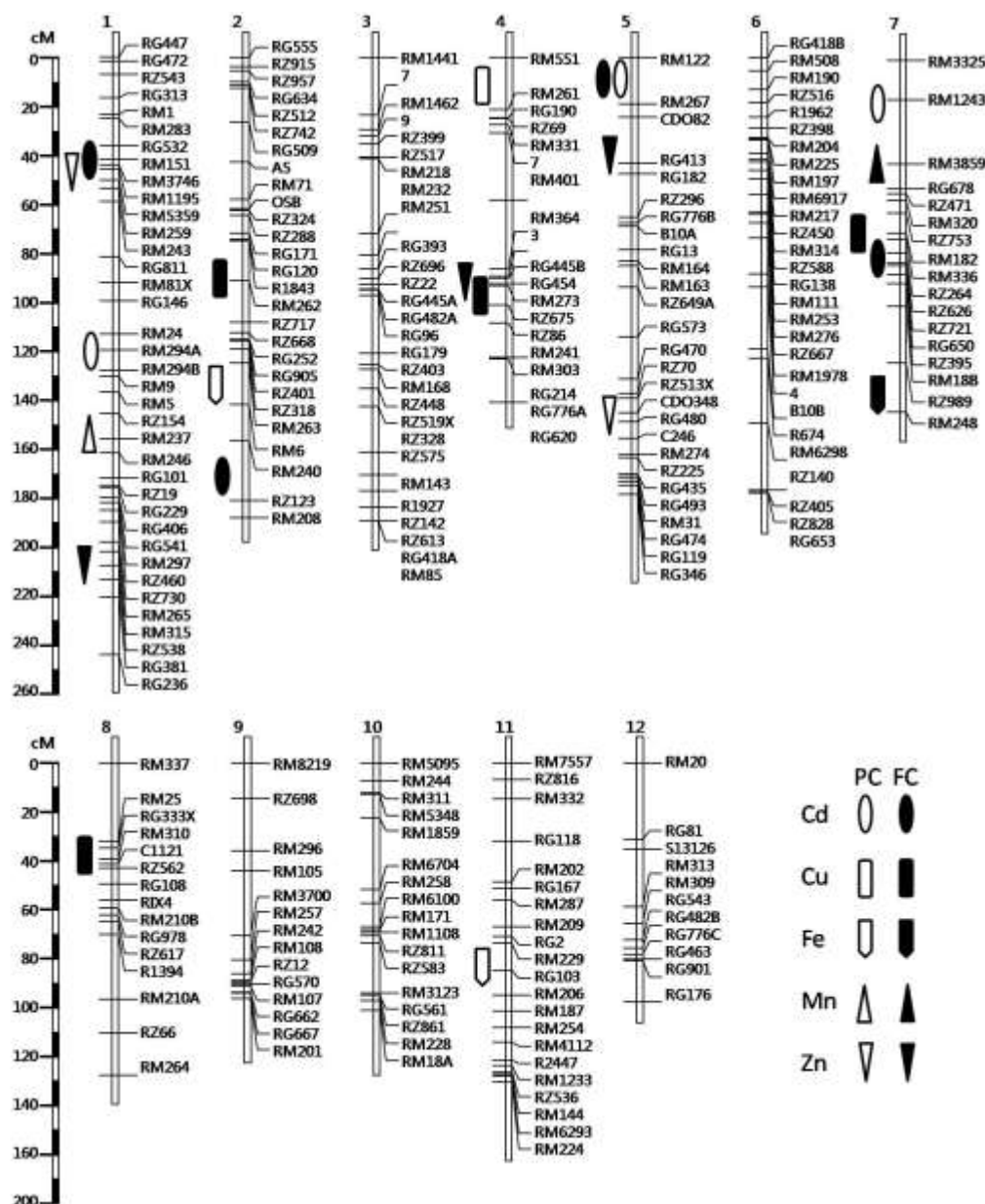


Fig. 2: Chromosomal distribution of QTLs for Cd, Cu, Fe, Mn and Zn concentrations in brown rice in PC and FC trials, respectively; PC and FC represent pot cultivation and paddy field cultivation

QTLs for Cd: Seven QTLs for Cd contents were detected, including one for both trials. In PC trial, *qCd1-1*, *qCd5*, *qCd7-1* were detected. The phenotypic variations caused by each QTL were around 5.9–9.9%, respectively. The positive alleles at these QTLs loci were inherited from ZS97B with low Cd concentration in brown rice. In FC trail, *qCd1-2*, *qCd2*, *qCd5*, *qCd7-2* were identified, explaining 4.8–6.2% of the phenotypic variance, respectively. Except *qCd2*, the positive allele of *qCd1-2*, *qCd5* and *qCd7-2* were inherited from ZS97B. The *qCd5* were detected in both trials.

QTLs for Cu: Five QTLs for Cu concentration were identified. In PC trail, only *qCu4-1* was detected, explaining

about 5.4% of the phenotypic variance. The positive allele of *qCu4-1* derived from ZS97B. The other four QTLs *qCu2*, *qCu4-2*, *qCu7* and *qCu8* were identified in FC trail, explained 2.8–10.6% of the phenotypic variance and had additive value ranging from 2.12 to 5.49. The positive alleles all derived from ZS97B except *qCu7*.

QTLs for Fe: Three QTLs for Fe concentration were detected. In PC trail, *qFe2* and *qFe11* were detected, which explained about 7.5% and 4.6% of the phenotypic variance, respectively. The positive alleles of *qFe2* and *qFe11* both derived from ZS97B. In FC trail, only one QTL, *qFe7*, was detected, accounting for about 5.3% of the phenotypic

Table 3: Correlation coefficient for trace metal element concentrations in the RIL population

Trace metal elements	Trails	Cu	Fe	Mn	Zn
Cd	PC	0.302**	0.154*	0.118	0.245**
	FC	0.321**	-0.052	0.057	0.01
Cu	PC		0.220**	0.442**	0.661**
	FC		0.157*	0.140*	0.322**
Fe	PC			0.193**	0.351**
	FC			0.300**	0.386**
Mn	PC				0.505**
	FC				0.537**

PC and FC represent pot cultivation and paddy field cultivation; *, ** significant at the level of 0.05, 0.01 respectively

Table 4: QTLs analysis for trace metal element concentrations in brown rice in the RIL population

Trails	Trace metal elements	QTL	Chr.	Interval	LOD	A	R ²
PC	Cd	<i>qCd1-1</i>	1	RM24-RM5	2.91	0.2364	5.9
		<i>qCd5</i>	5	RM122-RM267	3.98	0.2979	9.9
		<i>qCd7-1</i>	7	RM1243-RG678	3.88	0.2641	6.4
	Cu	<i>qCu4-1</i>	4	RM551-RM261	2.03	0.442	5.4
	Fe	<i>qFe2</i>	2	RM263-RZ123	3.2	0.7879	7.5
		<i>qFe11</i>	11	RG103-RM4112	2.15	0.6204	4.6
	Mn	<i>qMn1</i>	1	RZ154-RM264	2.6	1.5899	6.4
	Zn	<i>qZn1-1</i>	1	RG532-RM5359	3.56	-1.356	6.9
		<i>qZn5-1</i>	5	RG470-C246	3.15	1.3474	6.7
	FC	Cd	<i>qCd1-2</i>	1	RM283-RM259	2.67	0.0114
<i>qCd2</i>			2	RM240-RZ123	2.35	-0.0131	6.2
<i>qCd5</i>			5	RM122-RM267	2.54	0.0127	5.3
Cu		<i>qCd7-2</i>	7	RZ753-RZ626	3.55	0.013	5.9
		<i>qCu2</i>	2	R1843-RZ717	2.12	0.163	3.9
		<i>qCu4-2</i>	4	RZ86-RG214	5.49	0.2558	10.6
		<i>qCu7</i>	7	RM320-RM182	2.47	-0.1608	2.8
<i>qCu8</i>		8	RM25-RM310	2.18	0.1442	3.8	
Fe		<i>qFe7</i>	7	RZ989-RM248	2.1	-0.3193	5.3
Mn		<i>qMn7</i>	7	RM1243-RG678	2.78	-1.0979	5.1
Zn		<i>qZn1-2</i>	1	RZ730-RZ538	2.12	0.585	4.4
		<i>qZn4</i>	4	RM3643-RM241	2.48	-0.6165	4.4
		<i>qZn5-2</i>	5	CDO82-RG182	3.09	0.7306	6.4

PC and FC represent pot cultivation and paddy field cultivation, respectively; A represents additive effect. R² represents the proportion of phenotypic variance explained by each given QTL

variance. The positive alleles derived from MY46.

QTLs for Mn: Two QTLs for Mn concentration were identified. In PC trail, one QTL, *qMn1*, was detected, explaining about 6.4% of the phenotypic variance. The positive alleles of *qMn1* derived from ZS97B. In FC trail, one QTL, *qMn7*, was detected, accounting for about 5.1% of the phenotypic variance. The positive alleles of *qMn7* derived from MY46.

QTLs for Zn: Five QTLs were detected for Zn concentration. In PC trail, *qZn1-1* and *qZn5-1* were detected, explaining 6.9% and 6.7% of the phenotypic variance, respectively. The positive allele of *qZn1-1* derived from MY46, while *qZn5-1* derived from ZS97B. In FC trail, *qZn1-2*, *qZn4* and *qZn5-2* were detected, explaining the phenotypic variances about 4.4–6.4%, respectively. The positive allele of *qZn1-2* and *qZn5-2* derived from ZS97B, while *qZn4* derived from MY46.

Discussion

Some studies revealed that there was interaction among Cd, Cu, Fe, Mn and Zn in the process of absorbing and accumulating in rice (James *et al.*, 2007; Shao *et al.*, 2007; Ishimaru *et al.*, 2012). In our study, the absorption and accumulation of 5 trace metal elements in rice grain presented typical characteristics of quantitative traits, affected by soil environment and there was significant positive correlation between each other. Such as Fe and Zn, Mn and Zn concentrations in brown rice were significant positive correlated in both PC and FC trials, which implied accumulation and increase of one kind of trace metal element would cause accumulation and increase of other trace metal elements with synergistic effect, and it was similar to some other researches' conclusions (Vert *et al.*, 2001; James *et al.*, 2007).

A slight increase of mineral element concentrations and a decrease of Cd concentration in rice grain would efficiently alleviate ongoing human mineral deficiencies and Cd-contaminated rice threatens (Ishimaru *et al.*, 2012). Therefore, making the genetic variation and mechanism of trace metal element concentrations in rice grains clear is essential for improving safe and nutritional quality in rice breeding. The concentrations of trace metal element in rice grain are typical quantitative traits and their heredity are complicated. In this study, we identified 22 QTLs for Cd, Cu, Fe, Mn and Zn concentrations in brown rice, including one for PC and FC trials, 9 for PC trial only, and 13 for FC trial only, using an F₁₃ RIL population derived from the cross of ZS97B×MY46. These QTLs distributed on chromosomes 1, 2, 4, 5, 7, 8, 10 and 11. Among these 22 QTLs, *qCu4-2* had the largest single effect (10.4% of the phenotypic variance), *qCu7* had the lowest single effect (2.8% of the phenotypic variance). The positive alleles (increasing trace metal element concentrations) at 16 QTLs except *qCd2*, *qCu7*, *qFe7*, *qMn7*, *qZn1-1* and *qZn4* were inherited from ZS97B.

In spite of quantitative trait heredity affected by external environment, those QTLs with high genetic ability could be repeatedly detected. In our study, nine of the QTLs detected were located in genomic regions, where QTLs for trace metal element concentrations of the same element had been reported, including two of the five QTLs for Cd concentration, three of the five QTLs for Cu concentration, one of the three QTLs for Fe concentration, two of the five QTLs for Mn concentration, one of the five QTLs for Zn concentration. For Cd, *qCd2* corresponded to a QTL for Cd concentration and *qGcd2* reported by Ueno and Ishikawa (Ueno *et al.*, 2009; Ishikawa *et al.*, 2010), *qCd7-1* overlapped with *qGcd7*, *qGdt7* and *qScd7* identified by Ishikawa (Ueno *et al.*, 2009; Ishikawa *et al.*, 2010; Tezuka *et al.*, 2010), respectively; and *qCd7-2* co-located with *qCDCN7* reported by Shen (Shen *et al.*, 2008). For Cu, *qCu2* co-located with *qCu4-2* reported by Lu (Lu *et al.*, 2008), *qCu4-1* overlapped with *qCu4* identified by Zhang and Hu (Zhang *et al.*, 2014; Hu *et al.*, 2016), and *qCu8* corresponded

to *qSCu8* reported by Ishikawa (Ishikawa *et al.*, 2010). For Fe, *qFe7* corresponded to a QTL for Fe concentration reported by Zhang (Zhang *et al.*, 2014). For Mn, *qMn1* corresponded to *qGMn1* and *qMn1* identified by Ishikawa and Hu (Ishikawa *et al.*, 2010; Hu *et al.*, 2016), and *qMn7* overlapped with *qGMn7* reported by Ishikawa (Ishikawa *et al.*, 2010). For Zn, *qZn5-1* corresponded to a QTL for Zn concentration reported by Zhang (Zhang *et al.*, 2014). These QTLs could be considered to have been consistently detected in different environments and genetic backgrounds.

At present, many of the functional genes involved in absorption and transportation of trace metal elements in rice grains have been cloned and characterized. The OsNRAMP5, OsHMA3, OsHMA2, OsLCT1 and LCD regulated the uptake, transport and accumulation of Cd in rice (Ueno *et al.*, 2010; Shimo *et al.*, 2011; Uraguchi *et al.*, 2011; Ishimaru *et al.*, 2012; Sasaki *et al.*, 2012; Satoh-Nagasawa *et al.*, 2012; Takahashi *et al.*, 2012). The OsNRAMP1, OsFER2, OsYSL2, OsYSL15, OsYSL18, OsIRT1 and OsIRT2 contributed to transporting of Fe in rice (Curie *et al.*, 2000; Bughio *et al.*, 2002; Koike *et al.*, 2004; Ishimaru *et al.*, 2006; Nakanishi *et al.*, 2006; Inoue *et al.*, 2009; Aoyama *et al.*, 2009; Takahashi *et al.*, 2011; Paul *et al.*, 2012). The OsYSL2, OsYSL6 and OsNRAMP5 regulated to transporting of Mn in rice (Ishimaru *et al.*, 2010; Sasaki *et al.*, 2011; Ishimaru *et al.*, 2012). The OsZIP3 and OsZIP4 contributed to Zn transport (Ishimaru *et al.*, 2005; Yang *et al.*, 2009). Our study revealed the OsNRAMP5, OsNRAMP1 and OsHMA3 genes were located in the *qCd7-1* region, the OsYSL2 and OsYSL15 genes were located in the *qFe2* region, the OsYSL18 gene was located in the *qZn1-2* region and the OsZIP3 gene was located in the *qCu4-2* region, which implied there were some genetic locus regulated trace metal elements uptake and accumulation in ZS97B. On the basis of the QTLs detected repeatedly in different years, environments and genetic backgrounds, developing corresponding molecular markers, using MAS to select some special traits in rice would promote the process of rice breeding. Here, we identified a number of QTLs in different environments, and there were some cloned genes related to trace metal element accumulation in the interval of some detected QTLs. Therefore, we could conducted fine-mapping and cloning of above QTLs, then develop relevant functional molecular markers, and use these makers to efficiently select genes derived from ZS97B regulated trace metal element concentrations in rice breeding, at last efficiently develop safe and nutritional rice new varieties.

Clustering of QTLs for related traits also were found in our study, which was similar to some other studies (Shen *et al.*, 2008; Zhang *et al.*, 2014; Hu *et al.*, 2016). Six of the QTLs identified in this study were located in three clusters distributed on three chromosomes. Two QTLs, *qCd1-2* and *qZn1-1*, were located in the cluster on chromosomes 1. Another two QTLs, *qCu4-2* and *qZn4*, were located in the cluster on chromosomes 4 (Fig. 1). In one region, *qCd7-2*

was clustered with *qCu7* on chromosomes 7, which have been reported (Shen *et al.*, 2008). To molecular breeding, pleiotropy (one gene affecting multiple phenotypes) was meaningful to fine-mapping and cloning of clustered QTLs. On one hand, we need to know the number of related characters between themselves. On the other hand, we need to understand correlation between quantitative traits. Furthermore, we may use molecular makers close linkage to these QTLs to breed new rice varieties by MAS, and simultaneously improve multiple traits in rice.

Conclusion

Trace mental element concentrations in rice are complex quantitative traits. Further studies are needed to fine mapping and clone these novel QTLs for Fe, Mn, Cu, Zn and Cd concentrations in brown rice in the future, and which also could be utilized to improve rice safe and nutritional quality by MAS.

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