Mapping QTLs for Mineral Element Contents in Brown and Milled Rice Using an *Oryza sativa × O. rufipogon* Backcross Inbred Line Population

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Biofortifying food crops with essential minerals would help to alleviate mineral deficiencies in humans. Detection of quantitative trait loci (QTLs) for mineral nutrient contents in rice was conducted using backcross inbred lines derived from an interspecific cross of *Oryza sativa × O. rufipogon*. The population was grown in Hangzhou and Lingshui, with the contents of Mg, Zn, Fe, Mn, Cu and Se in brown rice measured in both trials and that in milled rice tested in Hangzhou only. A total of 24 QTLs for mineral element contents were identified, including two for both the brown and milled rice, 17 for brown rice only, and five for milled rice only. All the seven QTLs detected for the mineral contents in milled rice and 13 of the 19 QTLs for the contents in brown rice had the enhancing alleles derived from *O. rufipogon*. Fifteen QTLs were clustered in seven chromosomal regions, indicating that common genetic-physiological mechanisms were involved for different mineral nutrients and the beneficial alleles could be utilized to improve grain nutritional quality by marker-assisted selection.

Keywords: brown rice, milled rice, mineral element content, quantitative trait locus

Introduction

Mineral nutrients, such as magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu) and selenium (Se), have many metabolic and cellular functions affecting human and animal health. Human mineral deficiencies in Mg, Zn, Fe, Cu and Se are widespread, and the most prevalent are lack of Zn and Fe (Rawat et al. 2013). Mineral deficiencies impair human health and development with symptoms that include mental retardation, physical malformation, immune dysfunction, anemia and susceptibility to infectious diseases (Stein 2010). Biofortification is an approach to improve the content of mineral nutrients in the edible portions of crops through plant breeding. The mineral biofortification of food crops has attracted a great interest because it is an effective and cheaper alternate

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to traditional ways of combating mineral deficiencies, i.e., food fortification and pharmaceutical supplements (Bashir et al. 2013).

Rice (*Oryza sativa* L.), the staple cereal for over 50% of the world's population, is an important subject for biofortification (Bhullar and Gruissem 2013). Significant genetic variations have been reported for grain mineral contents in various rice genotypes, and genetic analysis has indicated that the genetic components played a major role in determining the grain mineral content variation (Gregorio et al. 2000). This provides a potential to identify genes that increase mineral contents in rice and it should be feasible to breed rice cultivars enriched with mineral nutrients.

Traditional plant breeding is largely based on phenotypic selection, which is labor-intensive, time-consuming and costly for improving grain mineral contents. Molecular markers help to track the genetic locus controlling nutrient contents without phenotype evaluation. This suggests that a marker-assisted selection breeding strategy based on quantitative trait locus (QTL) analysis can effectively promote genetic improvement in rice. QTL analysis is a powerful approach to detect chromosomal regions affecting quantitative traits. Numerous QTL mapping have been conducted for grain mineral nutrients in cereal crops, such as wheat, maize and rice (Peleg et al. 2009; Simic et al. 2012; Zhang et al. 2014). In rice, considerable efforts have been made using different types of segregating populations, including introgression lines of Teqing × Yunnan wild rice (Garcia-Oliveira et al. 2009), recombinant inbred lines of Bala × Azucena (Norton et al. 2010, 2012), Madhukar × Swarna (Anuradha et al. 2012), Lemont × Teqing (Zhang et al. 2014) and Zhengshan 97 × Minghui 63 (Lu et al. 2008), double haploid lines of CJ06 × TN1 (Du et al. 2013), and backcross introgression lines of the Lemont × Teqing (Zhang et al. 2014). QTLs for the contents of Mg, Zn, Fe, Mn, Cu and Se in rice grains were detected on all the 12 rice chromosomes.

Modern rice cultivars have lower mineral nutrient contents in their grains (Anandan et al. 2011), because breeders have constantly pursued high yields in rice and have paid little attention to nutritional quality. Wild species of rice are highly diversified and contain genes that confer valuable qualitative and quantitative traits and thus offer an important gene pool of allelic variation for rice improvement (Tanksley and McCouch 1997). Indeed, it has been reported that mineral nutrient contents were higher in wild rice than in cultivated rice (Jiang et al. 2009). Therefore, the exploitation of favorable alleles from wild rice might improve mineral contents in cultivated rice.

Dongxiang wild rice (*O. rufipogon* Griff., hereafter referred to as DWR) is the most northerly-distributed common wild rice in the world (latitude: 28°14' N), but this close relative of cultivated rice has not been used to improve grain mineral nutrients. The present study was conducted to determine QTLs for mineral element contents in brown and milled rice using an interspecific cross between a rice cultivar and an accession of DWR, with the objective to identify potential alleles from *O. rufipogon* for improving mineral nutrients of rice.

Materials and Methods

Plant materials and field experiments

The mapping population consisted of 202 backcross inbred lines (BILs), which was de-rived from a backcross using rice cultivar Xieqingzao B (*Oryza sativa* L., hereafter re-ferred to as XB) as the recurrent parent and an accession of DWR as the donor parent (Chen et al. 2006, 2010).

In the rice-growing seasons from November in 2011 to April in 2012 and from May to October in 2012, the 202 BILs and the recurrent parent XB were grown in experimental fields at the China National Rice Research Institute in Lingshui (LS), Hainan (latitude: 18°30' N, longitude: 110°02' E) and Hangzhou (HZ), Zhejiang (latitude: 30°04' N, longi-tude: 119°55' E), respectively. In each trial, 12 plants per row were transplanted with a spacing of 16.7 cm between plants within a row and 26.7 cm between rows. At maturity, rice grains from the middle 10 plants of each row were bulk-harvested and manually threshed.

Sample preparation

The rice grains were sun-dried and then stored at room temperature for three months be-fore analysis. In the LS experiment, 20 g of rice grains from each line were de-hulled with a testing sheller (THU 35A, Satake Corporation, Hiroshima, Japan). They were then ground with a sample grinder (Cyclotec 1093, FOSS Tecator, Hoganas, Sweden) and passed through a 0.18 mm mesh sieve.

In the HZ experiment, 30 g of grains from each line were de-hulled. Half were ground with a Cyclotec 1093 and passed through a 0.18 mm mesh and the remaining half were milled for 60 s with a laboratory milling machine (Pearlest, Kett electric laboratory, To-kyo, Japan). The milled rice was ground with a Cyclotec 1093 and passed through a 0.18 mm mesh.

Measurement of Mg, Zn, Fe, Mn and Cu contents

About 0.5 g of rice flour was weighed into a 50 mL borosilicate glass tube and added with 8.0 mL 68–70% nitric acid (HNO_3) and 2.0 mL 70% perchloric acid ($HClO_4$). The diges-tion tube was heated at 80 °C for 0.5 h, 150 °C for 2 h, and then at 180 °C until 0.5 mL solution remained. The digestions were performed using a digital block digestion system (model ED54, Labtech Inc., Beijing, China). The digested samples were cooled to room temperature and diluted to a final volume of 25 mL with double de-ionized water. The Mg, Zn, Fe, Mn, and Cu contents were simultaneously measured using an inductively coupled plasma atomic emission spectrometer (IRIS Intrepid II XSP, Thermo Electron Corporation, Milford, MA, USA) according to the manufacturer's instructions.

Measurement of Se content

About 0.25 g of rice flour was weighed into a 50 mL polypropylene tube and digested with 8.0 mL HNO$_3$ using the digital block digestion system. The digestion tube was heated at 80 °C for 0.5 h, and then at 110 °C until 1 mL solution remained. The digested residues were cooled down and added with 2 mL 30% hydrogen peroxide (H$_2$O$_2$) and heated at 110 °C until 0.5 mL solution remained. At the end of the digestion, the digested samples were cooled and diluted to 25 mL with double de-ionized water. The Se content was determined using an inductively coupled plasma mass spectrometer (X Series II, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's instructions.

Data analysis

Descriptive statistics of phenotypic data, including mean trait value, standard deviation, coefficient of variation, minimum and maximum trait values, skewness, and kurtosis, were estimated using the command DSum of the software Windows QTL Cartographer 2.5 (Wang et al. 2012). Pearson's product-moment correlation coefficients between the traits were determined using Microsoft Excel 2007.

Using RFLP (restriction fragment length polymorphism) and SSR (simple sequence repeat) markers, a linkage map has been firstly constructed by Chen et al. (2006) and updated by Huang et al. (2008). The updated map spanned 1306.4 cM using the Kosambi function and consisted of 149 DNA markers, including 41 RFLPs and 108 SSRs. QTLs were determined using Windows QTL Cartographer 2.5 (Wang et al. 2012). Composite interval mapping was conducted using a walking speed of 1 cM and a window size of 10 cM with backward and forward regression. A logarithm of the odds (*LOD*) threshold > 2.0 was used to claim a putative QTL. The QTL was named following the nomenclature recommended by McCouch and CGSNL (2008).

Results

Variation of the mineral element contents in brown and milled rice

Mineral contents of the six elements in brown and milled rice of the BIL population and the recurrent parent XB are summarized in Table 1. Wide variations with continuous distribution were observed in the BIL population for all the traits.

For brown rice which was tested in both trials, the mineral contents were much greater in the HZ trial than in the LS trial for Mn, Cu and Se, while the contents were similar between the two trials for Mg, Zn and Fe. Simple correlation analysis showed that the mineral contents in the brown rice of the BILs were not highly correlated between the two trials. The greatest correlation was found for Zn (*r* = 0.312, *P* < 0.01), followed by Mn (*r* = 0.191, *P* < 0.05). Significant correlation was also observed for Se, but it was negative (*r* = –0.162, *P* < 0.05). No significant correlation was found for the remaining three elements.

Table 1. Phenotypic performance of mineral element contents (mg kg⁻¹) in brown and milled rice in a BIL population of Xieqingzao B (XB)//XB/Dongxiang wild rice

Tissue	Location	Element	Mean	SD	CV	Range	Skewness	Kurtosis	Mean value of XB
Brown rice	Lingshui	Mg	1442	361	0.25	696–2416	0.39	–0.29	1461
		Zn	30.28	4.51	0.15	20.66–43.96	0.42	–0.15	26.70
		Fe	17.42	4.13	0.24	8.00–32.20	0.63	0.94	20.80
		Mn	24.65	5.29	0.21	13.22–41.14	0.48	0.09	27.52
		Cu	1.68	0.62	0.37	0.62–3.43	0.61	–0.39	2.38
		Se	0.034	0.008	0.24	0.010–0.063	0.42	0.73	0.028
Brown rice	Hangzhou	Mg	1339	252	0.19	732–2218	0.67	1.01	1068
		Zn	33.06	4.47	0.14	18.92–45.08	0.19	0.22	25.24
		Fe	14.26	4.51	0.32	3.60–32.40	0.90	1.55	10.20
		Mn	57.45	14.39	0.25	28.46–101.40	1.09	2.64	47.16
		Cu	4.94	0.73	0.15	3.32–8.09	0.82	1.76	3.27
		Se	0.105	0.018	0.17	0.066–0.170	0.62	0.92	0.092
Milled rice	Hangzhou	Mg	465	163	0.35	214–1062	1.45	1.94	261
		Zn	23.66	4.08	0.17	15.08–37.84	0.73	0.72	19.26
		Fe	5.00	2.76	0.55	1.40–17.40	1.52	2.67	5.20
		Mn	23.78	7.04	0.30	12.74–57.38	1.68	4.86	23.80
		Cu	4.04	0.76	0.19	2.70–6.80	1.08	1.40	2.59
		Se	0.101	0.018	0.18	0.063–0.160	0.52	0.35	0.079

For HZ trial where the testing was performed for both the brown and milled rice, the mineral contents were shown to be higher in brown rice than in milled rice, especially for Mg, Fe and Mn (Table 1). As compared with brown rice, the average contents of the BILs in milled rice was reduced by 65.3%, 64.9% and 58.6% for Mg, Fe and Mn, and 28.4%, 18.2% and 3.8% for Zn, Cu and Se, respectively. Similarly, the reduction for XB was 75.6%, 49.0% and 49.5% for Mg, Fe and Mn, and 23.7%, 20.8% and 14.1% for Zn, Cu and Se, respectively. Highly significant ($P < 0.01$) and positive correlations were observed between brown and milled rice for the Zn, Mn, Cu and Se contents, having coefficients of 0.674, 0.546, 0.862 and 0.712, respectively. At the same time, the correlation was shown to be not significant for Mg and Fe.

QTL detected for mineral element contents in brown and milled rice

At the threshold of LOD > 2.0, a total of 26 QTLs affecting mineral element contents were detected, including nine and ten QTLs for the contents in brown rice tested in the LS and HZ trials, respectively, and seven QTLs for milled rice in the HZ trial. They were distributed on all the rice chromosomes except chromosomes 5, 8 and 11 (Fig. 1). When the locations of the QTLs were compared, it was found that no QTL for mineral contents
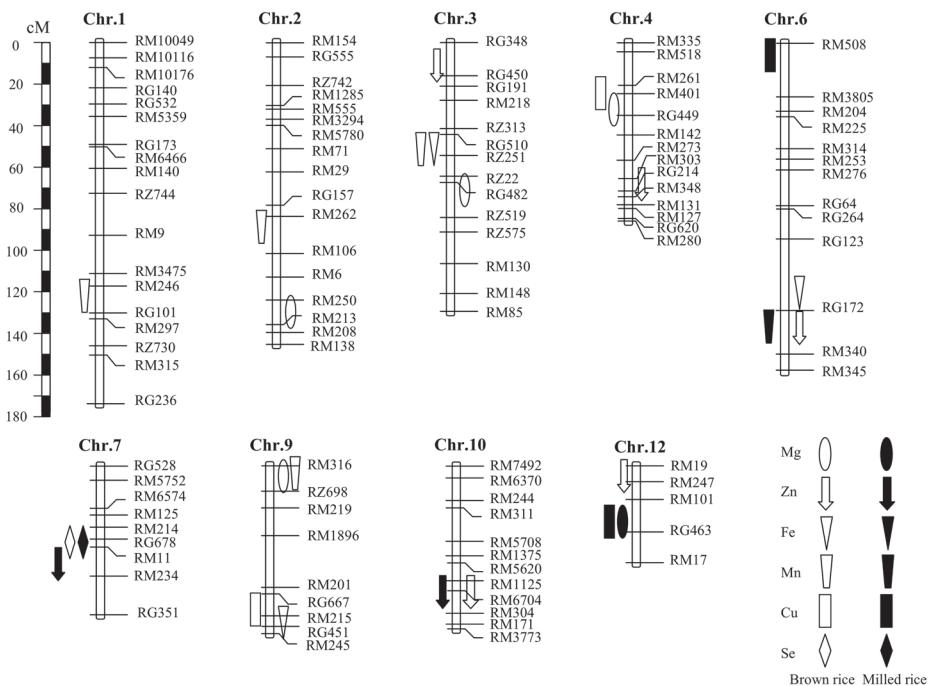

Figure 1. Chromosomal positions of the QTLs conferring Mg, Zn, Fe, Mn, Cu and Se contents in brown and milled rice. QTLs on the right and left hand side of the chromosomes indicate that they were detected in the Lingshui and Hangzhou trials, respectively

in brown rice was simultaneously detected in both trials, whereas two of the QTLs detected for milled rice, *qZn10* and *qSe7*, were also detected for brown rice in the LS and HZ trials, respectively (Table 2, Fig. 1). Thus the total number of QTLs was reduced to 24. While the phenotypic variance explained by a single QTL ranged as 5.3–16.7% and 5.1–28.2% for the brown rice in the LS and HZ trials, respectively, the contribution to the milled rice in the HZ trial was 5.3–56.8%.

Magnesium

Five QTLs were detected for Mg content, including four for brown rice and one for milled rice. In the LS trial, *qMg2*, *qMg3* and *qMg9* were detected, explaining 5.7%, 10.3% and 9.1% of the phenotypic variance, respectively. The enhancing alleles were from DWR at *qMg2* and *qMg3*, and from XB at *qMg9*. In the HZ trial, *qMg4* and *qMg12* were detected for the contents in brown and milled rice, accounting for 6.7% and 56.8% of the phenotypic variance, respectively. The enhancing alleles were from DWR at both loci. Three of the QTLs, *qMg3*, *qMg9* and *qMg12*, were detected with LOD scores greater than 3.0, and their allelic directions were unchanged across all the three estimates for Mg content no matter the effects were significant or not.

Zinc

Six QTLs were detected for Zn content, including one for both the brown and milled rice, four for brown rice only, and one for milled rice only. The common QTL *qZn10* was detected with LOD scores of 3.39 and 2.70 for milled rice in HZ and brown rice in LS, with the DWR allele increasing the Zn content by 2.41 and 1.66 mg kg^{-1}, respectively. It also had a marginal LOD score of 1.98 for brown rice in HZ, with the DWR allele increasing the Zn content by 1.34 mg kg^{-1}. The other QTLs were *qZn4* and *qZn6* for brown rice in LS, *qZn3* and *qZn12* for brown rice in HZ, and *qZn7* for milled rice in HZ. Individually, these QTLs explained 5.3–11.8% of the phenotypic variance and had additive effects ranging from 1.60 to 2.13 mg kg^{-1}. The enhancing alleles were all derived from DWR except *qZn4*. The allelic direction at each locus was unchanged across all the three estimates for Zn content despite that the effects were not always significant.

Iron

Three QTLs were detected for Fe content. They were all responsible for the content in brown rice. Two of the QTLs, including *qFe3* detected in HZ and *qFe6* detected in LS, had LOD scores higher than 3.0 and contributed 28.2% and 16.7% to the phenotypic variance, respectively. At both loci, the enhancing alleles were derived from DWR. The remaining QTL, *qFe9* detected in HZ, had a LOD score of 2.47 and explained 6.1% of the phenotypic variance. The enhancing allele at this locus was derived from XB. Different from QTLs detected for Mg and Zn, none of the QTLs for Fe was shown to have a constant allelic direction across the three estimates.

Table 2. QTLs for mineral element contents in brown and milled rice detected in the BIL population

Trait	QTL	Trial	The trial showing the highest significance				Effect estimated in other trials							
			Interval	LOD	A	R²	Trial	LOD	A	R²	Trial	LOD	A	R²
Mg	qMg2	B-LS	RM250-RM213	2.02	118.2	5.7	B-HZ	0.49	−43.1	1.6	M-HZ	0.02	−7.0	0.1
	qMg3	B-LS	RG482-RZ519	3.45	172.9	10.3	B-HZ	0.59	102.0	3.7	M-HZ	1.09	48.9	5.0
	qMg4	B-HZ	RM401-RG449	2.16	101.8	6.7	B-LS	0.42	57.3	1.0	M-HZ	0.10	−14.0	0.3
	qMg9	B-LS	RM316-RZ698	3.45	−127.3	9.1	B-HZ	0.16	−17.9	0.4	M-HZ	0.18	−13.1	0.5
	qMg12	M-HZ	RM101-RG463	4.48	199.1	56.8	B-LS	0.15	77.3	1.0	B-HZ	0.09	32.1	0.5
Zn	qZn3	B-HZ	RG348-RG450	2.41	1.60	5.5	B-LS	1.10	1.32	3.6	M-HZ	0.76	0.79	1.6
	qZn4	B-LS	RM303-RG214	2.39	−2.00	5.3	B-HZ	0.76	−1.12	1.7	M-HZ	0.23	−0.57	0.6
	qZn6	B-LS	RG172-RM340	3.41	2.02	11.8	B-HZ	0.28	0.73	1.5	M-HZ	0.66	0.80	2.2
	qZn7	M-HZ	RM11-RM234	2.07	1.86	7.1	B-LS	1.35	1.58	4.1	B-HZ	0.07	0.41	0.3
	qZn10	M-HZ	RM1125-RM6704	3.39	2.41	8.1	B-LS	2.70	1.66	7.3	B-HZ	1.98	1.34	5.0
	qZn12	B-HZ	RM19-RM247	2.86	2.13	9.2	B-LS	0.03	0.19	0.1	M-HZ	0.63	0.80	1.6
Fe	qFe3	B-HZ	RG510-RZ251	3.30	5.41	28.2	B-LS	0.08	−0.69	0.6	M-HZ	0.02	0.18	0.1
	qFe6	B-LS	RG123-RG172	3.99	2.01	16.7	B-HZ	0.43	−0.54	1.2	M-HZ	0.09	−0.15	0.2
	qFe9	B-LS	RM215-RG451	2.47	−1.59	6.1	B-HZ	0.28	0.60	0.8	M-HZ	0.10	0.22	0.3
Mn	qMn1	B-HZ	RM246-RG101	3.22	5.22	9.6	B-LS	0.47	0.72	1.3	M-HZ	0.18	0.58	0.4
	qMn2	B-HZ	RM262-RM106	2.34	−4.81	5.1	B-LS	0.01	0.13	0.0	M-HZ	0.68	−1.31	1.6
	qMn3	B-HZ	RG510-RZ251	2.67	20.73	18.6	B-LS	0.03	−0.41	0.1	M-HZ	0.59	3.00	2.8
	qMn6	M-HZ	RG172-RM340	2.15	2.41	6.6	B-LS	0.84	1.34	3.5	B-HZ	1.78	5.47	7.9
	qMn9	B-LS	RM316-RZ698	3.14	−1.83	9.0	B-HZ	0.70	−2.26	1.8	M-HZ	0.43	−0.87	1.2
Cu	qCu4	B-HZ	RM261-RM401	2.90	−0.29	6.5	B-LS	0.09	−0.05	0.3	M-HZ	0.43	−0.11	1.0
	qCu6	M-HZ	RM508-RM3805	3.67	0.27	10.4	B-LS	0.84	0.10	2.3	B-HZ	1.92	0.18	5.4
	qCu9	B-HZ	RG667-RM215	2.76	0.27	7.9	B-LS	0.23	−0.08	0.8	M-HZ	0.73	0.16	2.5
	qCu12	M-HZ	RM101-RG463	2.78	0.81	32.6	B-LS	0.53	0.29	4.9	B-HZ	1.51	0.53	14.1
Se	qSe7	B-HZ	RG678-RM11	2.54	0.008	6.5	B-LS	0.41	−0.002	1.2	M-HZ	2.10	0.007	5.3

B-LS, B-HZ, and M-HZ represent brown rice in the Lingshui experiment, brown rice in the Hangzhou experiment, and milled rice in the Hangzhou experiment, respectively. A indicates that an additive effect of replacing an Xieqingzao B allele by Dongxiang wild rice allele. R^2 indicates that the proportion of phenotypic variance explained by the QTL effect.

Manganese

Five QTLs were detected for Mn content, including four for brown rice only and one for milled rice only. In the LS trial, *qMn9* were only detected for the content in brown rice, accounting for 9.0% of the phenotypic variance, with the enhancing alleles derived from XB. In the HZ trial, *qMn1*, *qMn2* and *qMn3* for brown rice and *qMn6* for milled rice were detected, explaining 9.6%, 5.1%, 18.6% and 6.6% of the phenotypic variance, respectively. The enhancing alleles were from DWR at *qMn1*, *qMn3* and *qMn6*, and from XB at *qMn2*. Two of the QTLs, *qMn1* and *qMn9*, were detected with LOD scores greater than 3.0, and their allelic directions were unchanged across all the three estimates for Mn content no matter the effects were significant or not.

Copper

Four QTLs were detected for Cu content. They were all detected in the HZ trial. Two of the QTLs, *qCu4* and *qCu9* were responsible for the content in brown rice only, explaining 6.5% and 7.9% of the phenotypic variance, with the enhancing alleles derived from XB and DWR, respectively. Two other QTLs, *qCu6* and *qCu12* were responsible for the content in milled rice only, accounting for 10.4% and 32.6% of the phenotypic variance. The enhancing alleles were from DWR at both loci. One of the QTLs, *qCu6*, was detected with LOD scores greater than 3.0, and the allelic direction was unchanged across all the three estimates for Cu content no matter the effects were significant or not.

Selenium

One QTL, *qSe7*, was detected for Se content in the HZ trial. It showed significant effects for the contents in brown and milled rice with the enhancing allele derived from DWR, explaining 6.5% and 5.3% of the phenotypic variance, respectively.

Discussion

Due to the high consumption of rice, a slight increase in mineral contents in grain would efficiently alleviate ongoing human mineral deficiencies (Gregorio et al. 2000). Knowledge of the genetic variation in grain mineral contents and the genes underlying allelic variation is essential for the improvement of mineral nutrients in rice breeding. In this study, QTL analysis for the contents of six mineral elements in brown and milled rice was conducted using a BIL population derived from an interspecific cross of *O. sativa* × *O. rufipogon*. Totally 24 QTLs were detected, including two for both the brown and milled rice, 17 for the brown rice only, and five for the milled rice only.

It is notable that all the seven QTLs detected for the mineral contents in milled rice and 13 of the 19 QTLs for the contents in brown rice had the enhancing alleles derived from the Dongxiang wild rice. The introgression of favorable *O. rufipogon* QTL alleles into the genetic background of the *indica* rice cultivar XB would facilitate genetic improvement of grain mineral nutrition in rice.

Thirteen of the QTLs detected in this study were located in genomic regions where QTLs for mineral contents of the same element had been reported, including three of the five QTLs for Mg content, four of the six QTLs for Zn content, two of the five QTLs for Mn content, and all of the four QTLs for Cu content. For Mg, *qMg2* corresponded to a QTL for Mg content reported by Zhang et al. (2014), and q*Mg3* and *qMg12* overlapped with *qMg3-1* and *qMg12-1* reported by Garcia-Oliveira et al. (2009), respectively. For Zn, *qZn3* corresponded to *qZn3.1* reported by Anuradha et al. (2012), *qZn4* and *qZn10* overlapped with QTLs for Zn content identified by Zhang et al. (2014), and *qZn7* co-located with one of the QTLs for Zn content reported by Lu et al. (2008), Norton et al. (2010) and Anuradha et al. (2012), respectively. For Mn, *qMn6* corresponded to a QTL for Mn identified by Zhang et al. (2014), and *qMn9* overlapped with *qMn9* reported by (Norton et al. 2010, 2012). For Cu, *qCu4*, *qCu9* and *qCu12* corresponded to QTLs for Cu reported by Zhang et al. (2014), and *qCu6* co-located with *qCu6* identified by Garcia-Oliveira et al. (2009) and Norton et al. (2012). These QTLs could be considered to have been consistently detected in different environments and genetic backgrounds.

Similar to many other studies, clustering of QTLs for related traits was evident in this study. Fifteen of the QTLs identified in present study were located in seven clusters distributed on six chromosomes. QTLs for three elements, Zn, Fe and Mn, were located in the vicinity of RG172 on chromosome 6. QTLs for two elements were located in each of the other clusters, including two clusters on chromosome 9 and one clusters each on chromosomes 3, 4, 7, and 12 (Fig. 1). In three regions, QTLs *qZn6*/*qFe6*, *qMg9* and *qFe9* were clustered with *qMn6*, *qMn9* and *qCu9* which have been reported. In addition, *qSe7* located in the cluster on chromosome 7 and *qZn10* which did not co-locate with QTLs for other elements were responsible for the contents in both the brown and milled rice which have been reported. These QTLs provided good candidates for gene fine-mapping and cloning.

Recent studies also showed that many functional genes involved in grain minerals in rice have been cloned and characterized. The *OsZIP3* gene was located in the *qZn4* region, which was expressed in roots and responsible for Zn uptake from soil (Yang et al. 2009). In the *qZn6* and *qMn6* interval, the *OsNRAMP3* gene was constitutively expressed in the node and transported Mn to young leaves and panicles (Yang et al. 2013). The *OsFER2* gene located in the *qZn12* interval was specifically over-expressed in endosperm and contributed to 2.09- and 1.37-fold Fe and Zn contents in milled rice (Paul et al. 2012). It is worth to develop near isogenic lines differing at these gene loci at the genetic background of XB for analyzing their relationship with QTLs detected in the present study.

The XB//XB/DWR BIL population was previously used in QTL mapping for yield traits (Huang et al. 2008). Among the 23 QTLs for grain yield traits detected by Huang et al. (2008), *qTNSP2*, *qNFGP6*/*qTNSP6*/*qSF6* and *qSF9* were located in the regions for *qMn2*, *qCu6* and *qCu9*, but the allelic direction for mineral contents was opposite to the yield traits. Unfavorable relationships between yield traits and grain mineral contents have also been reported in other studies (Anandan et al. 2011). These suggest that *O. rufipogon* alleles beneficial to some traits may sometimes have deleterious effects on other traits. This should be taken into account in the introgression of the *O. rufipogo*n into modern rice varieties.

Acknowledgements

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