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QTL underlying iron and zinc toxicity tolerances at seedling stage revealed by two sets of reciprocal introgression populations of rice (*Oryza sativa* L.)

Huan Liu[a,1], *Aijaz Soomro*[b,1], *Yajun Zhu*[c], *Xianjin Qiu*[a], *Kai Chen*[c], *Tianqing Zheng*[b], *Longwei Yang*[a], *Danying Xing*[a,*], *Jianlong Xu*[b,c,d,**]

[a]*Hubei Collaborative Innovation Center for Grain Industry, Yangtze University, Jingzhou 434025, China*

[b]*Institute of Crop Science/National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

[c]*Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China*

[d]*Shenzhen Institute of Breeding for Innovation, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China*

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ABSTRACT

Iron and zinc are two trace elements that are essential for rice. But they are toxic at higher concentrations, leading to severe rice yield losses especially in acid soils and inland valleys. In this study, two reciprocal introgression line (IL) populations sharing the same parents were used with high-density SNP bin markers to identify QTL tolerant to iron and zinc toxicities. The results indicated that the *japonica* variety 02,428 had stronger tolerance to iron and zinc toxicities than the *indica* variety Minghui 63. Nine and ten QTL contributing to iron and zinc toxicity tolerances, respectively, were identified in the two IL populations. The favorable alleles of most QTL came from 02,428. Among them, *qFRRDW2*, *qZRRDW3*, and *qFRSDW11* appeared to be independent of genetic background. The region C11S49–C11S60 on chromosome 11 harbored QTL affecting multiple iron and zinc toxicity tolerance-related traits, indicating partial genetic overlap between the two toxicity tolerances. Our results provide essential information and materials for developing excellent rice cultivars with iron and/or zinc tolerance by marker-assisted selection (MAS).

1. Introduction

Rice is one of the main crops providing nutrition and trace elements to humans. In the face of the rapidly increasing world population, improving production efficiency is an important measure for increasing rice yield. However, many abiotic and biotic stresses limit crop yield capacity. For example, iron and zinc, acting as cofactors for many

* *Corresponding author.*
** *Corresponding author at:* Institute of Crop Science/National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China.
E-mail addresses: xujlcaas@126.com (J. Xu), xingdy_2006@126.com (D. Xing).
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[1] These authors contributed equally to this work.

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enzymes [1], are two trace elements essential for humans, plants and animals, but are toxic to most plants and animals at higher concentrations [2–3]. Ferrous iron (Fe^{2+}) and zinc (Zn^{2+}) toxicity harm rice production, especially in acidic soil in southeast Asia, West Africa, and Brazil [4–6]. In general, yield losses associated with iron toxicity commonly range from 15% to 30%, but complete crop failure can occur in response to severe toxicity at early growth stages [7]. Besides iron toxicity, acidic soil is often associated with zinc toxicity [8]. This problem further damages the health of rice plants, increasing rice yield loss [9]. In fact, iron and zinc toxicities may occur in normal fields at low soil pH when harmful organic acids and hydrogen sulfide accumulate, a problem frequently found in the rice sowing and transplanting periods in China. Breeding rice varieties with iron and zinc toxicity tolerance is the most effective and economic means of minimizing yield loss resulting from iron and zinc toxicity stresses.

Tolerances to iron and zinc toxicities in rice are genetically complex traits, and there is large genotypic variation in the primary rice gene pool. Previous studies screening many iron-tolerant genotypes [4,10–16] have suggested that environmental conditions, timing and level of iron stress, the screening system, and other factors play crucial roles in determining genotype responses to iron toxicity [16]. For this reason, specialized varieties matching individual environments and varieties adapted to a wide range of iron toxicity environments should be bred. For rice zinc tolerance, few screening experiments have been performed. Asominori and Lemont are two relatively tolerant cultivars [9,15], and TY-167 is a zinc-tolerant genotype [17].

Genetic of tolerances to iron and zinc toxicities in rice appears to be quantitatively inherited. Many QTL for iron toxicity tolerance [7,10–11,12,15,16,18,19] and several QTL for zinc toxicity tolerance [9,15] have been identified using biparental populations or germplasm resources. Among them, no QTL has been fine-mapped except for *tLBS5* for iron tolerance on chromosome 1 [20]. Many iron and zinc transporter proteins in rice mediating metal ion uptake, distribution and homeostasis have been cloned, and some play important roles in tolerance to iron or zinc toxicity. For instance, *OZT1* confers rice tolerance to Zn and Cd ion stress [21], and *OsFRO1* enhances tolerance to Fe toxicity of rice in the vegetative stage [22]. However, their value for breeding is uncertain.

Advanced-backcross QTL analysis is a method combining QTL analysis with cultivar development [23]. Following this strategy, our research groups have reported several successful applications of a large-scale backcross (BC) breeding strategy to improve abiotic stress tolerance in rice [24–27], and to identify QTL and mine favorable alleles for complex traits [28–30]. In the present study, QTL tolerances to iron and zinc toxicities were identified using two sets of reciprocal introgression lines (ILs) derived from Minghui 63 × 02,428 and high-density SNP bin markers. The QTL and the elite lines with iron tolerance and/or zinc tolerance will provide essential information and materials for developing new rice cultivars with iron and/or zinc tolerance by marker-assisted selection (MAS).

2. Materials and methods

2.1. Development of two sets of ILs population

Minghui 63 (MH63), the male parent of the widely adapted hybrid *indica* variety Shanyou 63, whose distribution covers more than 21° of longitude and 20° of latitude in China [31], was crossed with 02,428, a typical *japonica* with tolerance to low CO_2 concentration stress selected from mutant progenies derived from a cross between two landraces, Pang-Xie-Gu and Ji-Bang-Dao [32]. The F_1 plants were simultaneously backcrossed to the two parents to develop two BC_1F_1 populations, each of around 80 plants. The BC_1F_1 plants were then used as male parents in backcrosses with the corresponding parents to produce two BC_2F_1 populations. The BC progenies were selfed successively for seven generations with no selection from BC_2F_1 to BC_2F_8. Ultimately, two sets of reciprocal ILs, consisting of 198 BC_2F_8 introgression lines (ILs) in the 02,428 background (designated as 02,428-ILs) and 226 BC_2F_8 ILs in the MH63 background (MH63-ILs), were developed after removal of lines with heading dates too late for QTL detection. In addition, $262F_8$ recombinant inbred lines (RILs), derived from the same parents, were developed by single-seed descent from the F_2 population and used to construct a genetic map.

2.2. Phenotyping iron and zinc toxicity tolerances

Evaluations of iron and zinc toxicity tolerances at the seedling stage were performed in turn in the greenhouse in Agricultural College of Yangtze University in year 2014. The method and workflow described by Zhang et al. [15] were applied with slight changes. Plump seeds of each IL and the parents were selected and placed in an oven at 50 °C for 3 days to break dormancy. Seeds were sterilized with 1% sodium hypochlorite solution. After washing twice with distilled water, all seeds were placed into an incubator at 37 °C for 24 h. Germinating seeds were selected and placed in holes in a thin Styrofoam board (10 holes in one row were taken as a replication for one line) with a nylon net bottom, which floated on water in a plastic box. Each line had six replications and each replication had 10 holes with two germinating seeds per hole. After 7 days, one seedling per hole was kept so that all seedlings of a line would experience similar growth conditions. Two replicates selected randomly were then defined as controls and transferred to standard Yoshida's culture solution [33], two replicates were transferred to the same solution supplemented with 300 mg L^{-1} Fe^{2+} (added as $FeSO_4 \cdot 7H_2O$), and the remaining two replicates were transferred to the same solution supplemented with 200 mg L^{-1} Zn^{2+} (added as $ZnSO_4$). The temperature in the greenhouse was set to 30 °C/25 °C (day/night) and the relative humidity was maintained at 50–70%. The solution in plastic boxes was changed every 5 days and the pH was maintained at 4.5. When differences between controls and treatments were clearly observed (about 15 days after treatment), the root dry weight (RDW) and shoot dry weight (SDW) of treatment and control plants were measured. The derived trait, total dry weight (TDW), was calculated as the sum of RDW and SDW. The indexes of toxicity tolerance, relative root dry weight (RRDW), relative shoot dry weight (RSDW) and relative total dry

weight (RTDW) were calculated according to the following formula:

$$\text{Relative value}\ (\%) = (\text{trait value in treatment})/(\text{trait value in control}) \times 100.$$

2.3. Genotyping and map construction

In comparison with reciprocal ILs, which are skewed towards one parent or the other in genome due to successive backcrossing with the recurrent parent, RILs, which are a random mixture of MH63 and 02,428 backgrounds, are more suitable for map construction. Genomic DNA of MH63, 02,428, the two sets of ILs, and the RILs was isolated using a DNeasy mini Kit (Qiagen) and the genotypes of the RILs were determined based on SNPs generated by whole-genome sequencing with the Illumina Genome Analyzer IIx as described previously [34].

MH63 and 02,428 were subjected to whole-genome resequencing and a total of 5,336,108,154 and 5,562,905,674 nucleotides of data were obtained. Alignment was performed against the MSU6.1 assembly of the Nipponbare sequence as the reference genome. In total, 5,062,106,567 and 5,278,080,725 nucleotides of consistent sequence were obtained for MH63 and 02,428, covering respectively 96.57% and 94.03% of the whole genome. Single-nucleotide polymorphisms (SNPs) between these two sequences were identified. Using evidence from more than 3, 4, or 5 reads, respectively 48,498, 42,124, and 36,410 SNPs were found between MH63 and 02,428.

A total of 384 SNPs evenly distributed along the genome were chosen for the design of an Illumina SNP chip [35] for genotyping the two sets of ILs and RILs using their parents and F_1 as control. The framework map was constructed based on genotypic data of the RIL population using QTL IciMapping [36]. Further map filling was performed by restriction association site DNA (RAD) sequencing [37] for the two sets of ILs as well as the two parents. Finally, 58,936 qualified SNPs were identified and integrated into the framework map, with an average distance of 77 kb between adjacent SNPs. A bin was defined as coverage of series of SNPs with same genotype along a chromosome, resulting in a total of 4568 chromosome bins for 12 chromosomes.

2.4. Data analysis

The phenotypic value of each line in a test environment was taken as the average of two replicates. One-way ANOVA in SAS 9.3 (SAS Institute Inc. Cary, NC) was applied to analyze the variances in target traits in the two populations. Before phenotypic data analysis and QTL mapping, the extreme values for each trait were removed from the dataset.

QTL mapping was conducted using the inclusive interval mapping (ICIM) function in the IciMapping 4.0 software [38]. In this function, marker selection is first conducted via stepwise regression considering all SNP marker information simultaneously. Then the phenotypic values are adjusted by all markers retained in the regression equation, omitting the two markers flanking the current mapping interval. The adjusted phenotypic values are then used for one-dimensional scanning. Based on experience, the LOD threshold was set at 2.5 for claiming a putative QTL.

3. Results

3.1. Linkage map and characteristics of introgression in the reciprocal ILs

The linkage map was constructed using the RIL population derived from MH63 × 02,428. The map spanned 1496.3 cM with a mean distance of 0.33 cM between adjacent markers. The inheritance of parental segments across the genomes of ILs was characterized using high-density informative bin markers. The ILs showed marked variations in introgressed segments from donor parents, and almost all ILs contained more recurrent parent genome than donor parent genome. On average, the introgressed donor–genome proportion in 02,428-ILs was 21.9%, with a range from 1.64% to 88.07%, whereas the introgressed donor–genome proportion in MH63-ILs was 7.98%, ranging from 0.02% to 87.06%. The reciprocal sets of ILs were well separated, without overlap, with respect to the frequency distribution of the MH63 genome (Fig. 1).

3.2. Performance of toxicity tolerance of the parents and their ILs under iron and zinc stress conditions

Iron and zinc stress tests were administered to same populations and parents in the greenhouse. Under normal conditions, the RDW, SDW, and TDW of MH63 were all higher than those of 02,428 in both tests, and differences in RDW and TDW between parents were highly significant ($P \leq 0.01$) and significant ($P \leq 0.05$), respectively (Table 1). These results implied that MH63 showed greater growth than 02,428 at seedling stage under normal cultivation conditions. Except that MH63 showed markedly higher RDW under iron stress and SDW under zinc stress, the two parents showed no significant differences in TDW, RDW, and SDW under the two stress conditions. However, the RRDW and RTDW of 02,428 were significantly higher than those of MH63 under both conditions and the RSDW of 02,428 was significantly higher than that of MH63 under the iron stress condition (Table 1, Fig. 2), suggesting that 02,428 had significantly stronger tolerance to iron and zinc toxicities than MH63.

Transgressive segregations were observed for RRDW, RSDW, and RTDW of iron and zinc stresses to normal

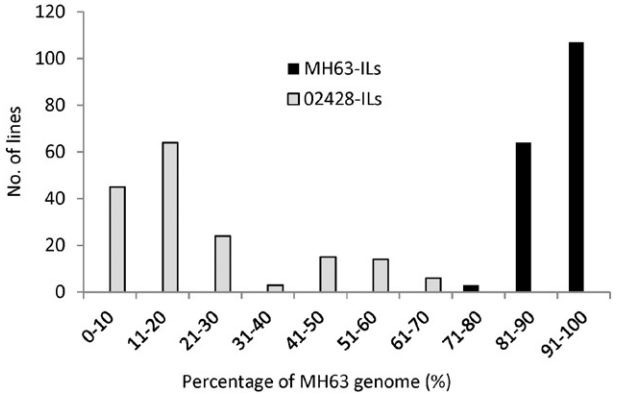


Fig. 1 – The frequency distribution of MH63 genome in two sets of ILs derived from a cross between MH63 and 02,428.

Table 1 – Performance of iron and zinc toxicity tolerance-related traits between two parents under iron and zinc stress conditions.

Condition	Trait [1)]	Parent	Mean ± SD [2)]	Condition	Trait	Parent	Mean ± SD
Control (g)	RDW	02428	0.027 ± 0.005 A	Control (g)	RDW	02428	0.022 ± 0.002 A
		MH63	0.039 ± 0.039 B			MH63	0.032 ± 0.008 B
	SDW	02428	0.152 ± 0.028 a		SDW	02428	0.152 ± 0.036 a
		MH63	0.176 ± 0.031 a			MH63	0.179 ± 0.026 a
	TDW	02428	0.171 ± 0.028 a		TDW	02428	0.182 ± 0.029 a
		MH63	0.212 ± 0.037 b			MH63	0.218 ± 0.031 b
Iron stress (g)	RDW	02428	0.021 ± 0.003 A	Zinc stress (g)	RDW	02428	0.018 ± 0.004 a
		MH63	0.027 ± 0.004 B			MH63	0.016 ± 0.003 a
	SDW	02428	0.111 ± 0.024 a		SDW	02428	0.089 ± 0.019 A
		MH63	0.117 ± 0.028 a			MH63	0.108 ± 0.019 B
	TDW	02428	0.136 ± 0.024 a		TDW	02428	0.113 ± 0.017 a
		MH63	0.148 ± 0.030 a			MH63	0.124 ± 0.019 a
Relative ratio (%)	RRDW	02428	85.26 ± 9.00 A	Relative ratio (%)	RRDW	02428	72.11 ± 4.05 A
		MH63	70.83 ± 3.74 B			MH63	40.42 ± 3.94 B
	RSDW	02428	70.88 ± 5.87 A		RSDW	02428	56.23 ± 4.01 a
		MH63	54.35 ± 7.35 B			MH63	53.92 ± 6.41 a
	RTDW	02428	72.99 ± 5.37 A		RTDW	02428	59.84 ± 4.47 a
		MH63	61.84 ± 5.41 B			MH63	50.60 ± 5.60 b

[1] RDW, root dry weight; SDW, shoot dry weight; TDW, total dry weight; RRDW, relative root dry weight; RSDW, relative shoot dry weight; RTDW, relative total dry weight.
[2] Significant differences at $P \leq 0.01$ and 0.05 for upper- and lowercase letters, respectively, between 02,428 and MH63 based on *t*-test.

conditions in the two IL populations (Fig. 3). MH63-ILs had average RRDW, RSDW, and RTDW of 71.67%, 67.21%, and 70.24% with ranges of 29.35–94.97%, 15.66–92.16%, and 18.10–91.58%, respectively, under iron stress, and average RRDW, RSDW and RTDW of 54.83%, 59.66%, and 58.88% with ranges of 19.32–81.40%, 24.07–86.19%, and 23.49–85.86%, respectively, under zinc stress (Table 2). Similarly, 02,428-ILs had average RRDW, RSDW, and RTDW of 75.12%, 72.86%, and 74.07% with ranges of 36.05–95.00%, 23.09–94.16%, and 25.56–94.81%, respectively, under iron stress, and average RRDW, RSDW and RTDW of 74.10%, 68.66%, and 69.65% with ranges of 24.74–94.62%, 34.82–94.63%, and 38.24–93.76%, respectively, under zinc stress (Table 2). As shown in Fig. 2, variations of relative RDW, SDW, and TDW in the two sets of ILs all showed normal distribution or skewed but continuous distribution under the two stress conditions, indicating that these two sets of IL populations had abundant diversity in iron and zinc toxicity tolerances and suggesting that some lines carried QTL for iron and/or zinc toxicity tolerances, in view of their tolerant phenotypes.

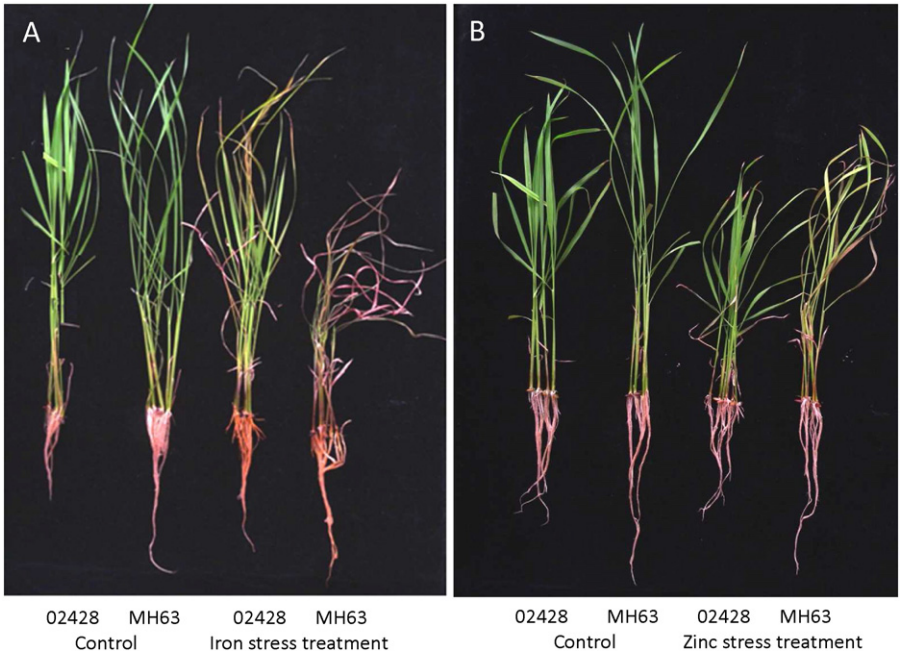


Fig. 2 – Performance of the two parents at 15 days after treatment with iron and zinc stresses.

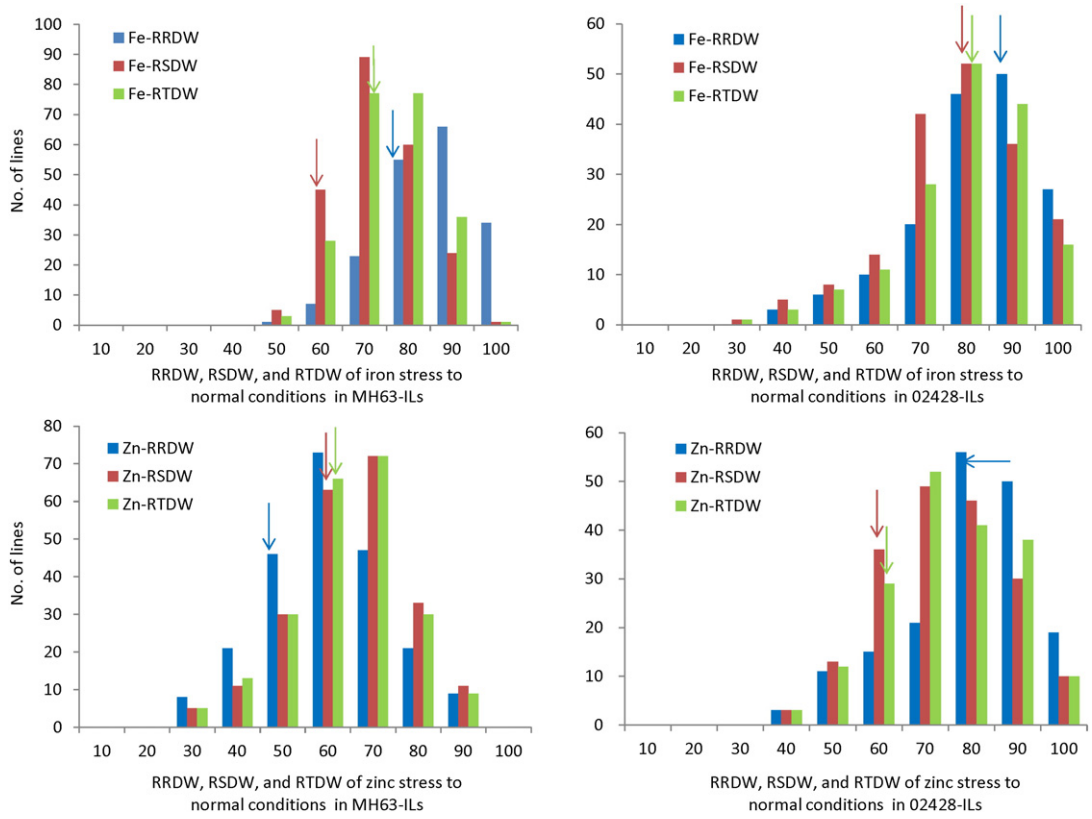


Fig. 3 – Frequency distributions of relative RDW, SDW, and TDW of iron and zinc stresses to normal conditions in two sets of ILs derived from MH63 × 02,428. Arrows point to the averages of the recurrent parents.

3.3. *Detection of QTL for iron toxicity tolerance*

Four and five QTL underlying RRDW and RSDW were identified under iron stress in the MH63-IL and 02,428-IL populations, respectively (Table 3, Fig. 4). Specifically, three QTL (*qFRRDW1–1*, *qFRRDW1–2*, and *qFRRDW2*) for RRDW and one QTL (*qFRSDW11*) for RSDW were identified on chromosomes 1, 2, and 11 in MH63-ILs with favorable alleles all from the donor parent, 02,428. Four QTL (*qFRRDW2*, *qFRRDW3*, *qFRRDW9–1*, and *qFRRDW9–2*) for RRDW and one QTL (*qFRSDW11*) for RSDW were identified on chromosomes 2, 3, 9, and 11 in 02,428-ILs. The favorable alleles at *qFRRDW2* and *qFRSDW11* were from 02,428, whereas those at the other three QTL were from MH63.

Among the above QTL, two QTL, *qFRRDW2* on chromosome 2 and *qFRSDW11* on chromosome 11 were simultaneously detected in both genetic backgrounds, suggesting that expressions of both QTL are independent of genetic background. The average phenotypic variances explained (PVE) of *qFRRDW-2* and *qFRSDW-11* were 12.20% and 11.66%, respectively, indicating that they were major QTL for tolerance to iron toxicity.

Table 2 – Performance for iron and zinc toxicity tolerance-related traits in two sets of ILs under iron and zinc stress conditions.

Condition	Trait	MH63-ILs		02,428-ILs	
		Range (%)	Mean (%)	Range (%)	Mean (%)
Iron stress	RRDW	29.35–94.97	71.67	36.05–95.00	75.12
	RSDW	15.66–92.16	67.21	23.09–94.16	72.86
	RTDW	18.10–91.58	70.24	25.56–94.81	74.07
Zinc stress	RRDW	19.32–81.40	54.83	24.74–94.62	74.10
	RSDW	24.07–86.19	59.66	34.82–94.63	68.66
	RTDW	23.49–85.86	58.88	38.24–93.76	69.65

RDW, root dry weight; SDW, shoot dry weight; TDW, total dry weight; RRDW, relative root dry weight; RSDW, relative shoot dry weight; RTDW, relative total dry weight.

3.4. *Detection of QTL for zinc toxicity tolerance*

Four and six QTL for the three traits, RRDW, RSDW, and RTDW, were identified under zinc stress in MH63- and 02,428-IL populations, respectively (Table 4, Fig. 4). Two QTL (*qZRRDW3* and *qZRRDW11*) for RRDW, one (*qZRSDW2*) for RSDW and one (*qZRTDW11*) for RTDW were mapped on chromosomes 2, 3, and 11 in MH63-ILs. The 02,428 alleles at all QTL increased trait values. The *qZRRDW11* for RRDW was located in the region overlapping with that of *qZRTDW11* for RTDW, suggesting the two QTL may be allelic. Two QTL (*qZRRDW3* and *qZRRDW7*) for RRDW, two (*qZRSDW11–1* and *qZRSDW11–2*) for RSDW and two (*qZRTDW3* and *qZRTDW11*) for RTDW were identified on chromosomes 3, 7, and 11 in 02,428-ILs. The 02,428 alleles at all QTL except *qZRTDW3* increased trait values. The *qZRSDW11–2* for RSDW may be allelic to the *qZRTDW11* for

Table 3 – QTL for iron toxicity tolerance identified in MH63-IL and 02,428-IL populations.

Population	Trait [1)]	QTL	Chr.	Marker/physical interval (bp)	LOD	A [2)]	PVE (%)
MH63-ILs	RRDW	*qFRRDW1-1*	1	C1S110–C1S124 10514742–11389991	2.52	0.0482	9.00
		qFRRDW1-2	1	C1S130–C1S142 11788361–12387454	2.51	0.0497	9.24
		qFRRDW2	2	C2S139–C2S143 15875115–16202206	2.57	0.1498	8.68
	RSDW	*qFRSDW11*	11	C11S55–C11S59 4342500–4788363	3.07	0.0814	10.95
02428-ILs	RRDW	*qFRRDW2*	2	C2S139–C2S143 15875115–16202206	3.09	0.0743	15.72
		qFRRDW3	3	C3S256–C3S260 16186851–16524031	2.53	–0.0503	10.41
		qFRRDW9-1	9	C9S119–C9S124 9104463–9312653	2.96	–0.0535	14.75
		qFRRDW9-2	9	C9S185–C9S190 13496238–13788797	3.09	–0.0566	15.02
	RSDW	*qFRSDW11*	11	C11S55–C11S60 4342500–4821401	3.37	0.0864	12.36

[1] RRDW, relative root dry weight; RSDW, relative shoot dry weight.
[2] Additive effect resulting from the substitution of MH63 alleles by 02428 alleles.

RTDW, in view of their location in the adjacent regions sharing the same marker C11S115.

Among the above QTL, *qZRRDW3* for RRDW was simultaneously detected on chromosome 3 in both backgrounds, suggesting that expression of the QTL is independent of genetic background.

3.5. *Genetic relationship between iron and zinc toxicity tolerances*

The QTL mapping results (Tables 3 and 4; Fig. 4) indicated that *qFRSDW11* for RSDW was simultaneously detected under both iron and zinc stress conditions in 02,428-ILs, suggesting a genetic overlap between the two stress tolerances. Additionally, the region of C11S49–C11S60 on chromosome 11 harbored *qFRSDW11*, *qZRSDW11*, *qZRRDW11*, and *qZRTDW11* affecting the iron and zinc toxicity tolerance-related traits RSDW, RRDW, and RTDW and detected in both backgrounds, hinting that the region contains genetically overlapping loci for iron and zinc toxicity tolerances.

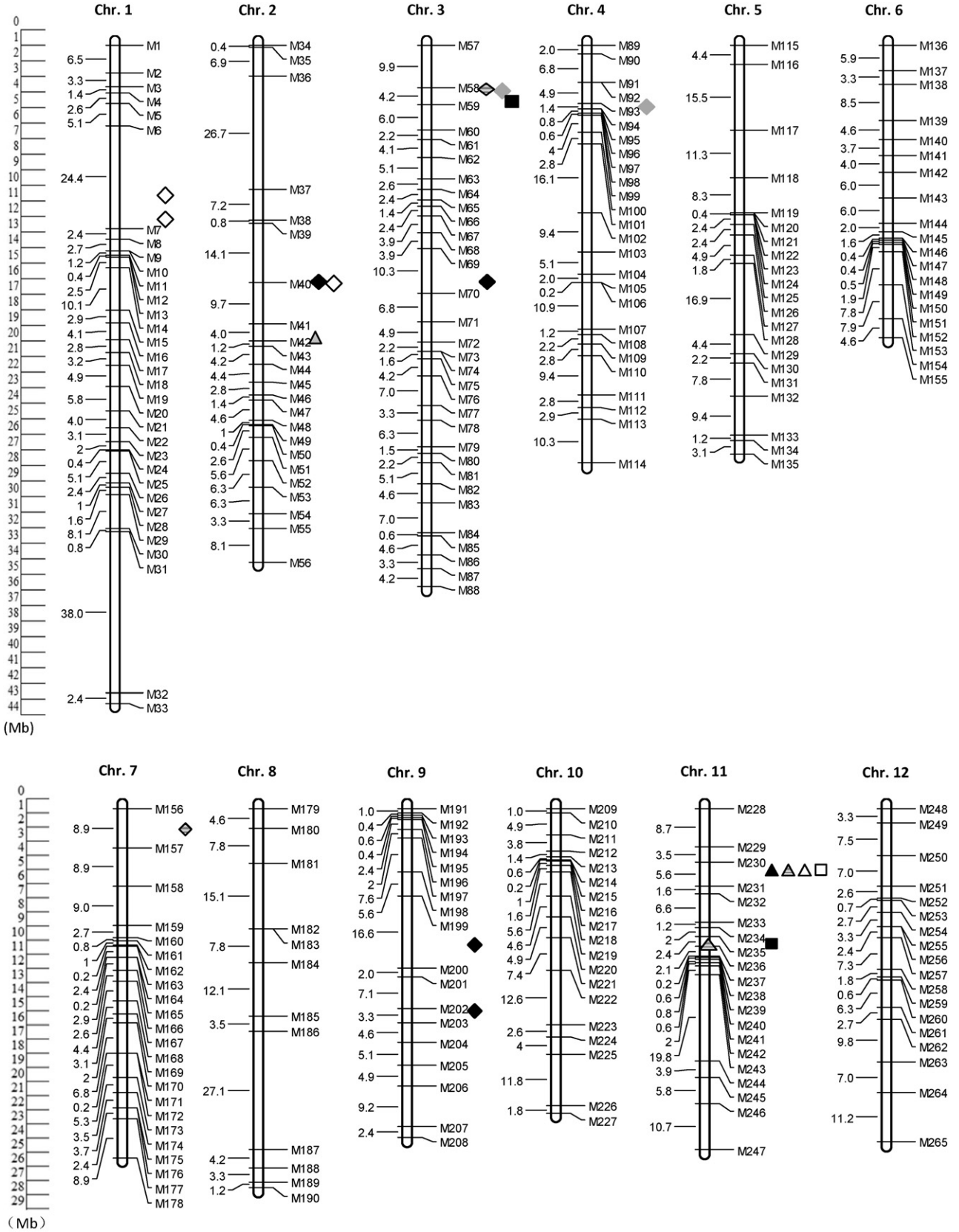
4. Discussion

4.1. *Detection of QTL for iron and zinc toxicity tolerances*

Using RRDW, RSDW, and their derived trait RTDW as indexes of iron and zinc toxicity tolerance as recommended by Wu et al. [16], nine and ten QTL contributing to iron and zinc toxicity tolerances, respectively, were identified in the two sets of reciprocal IL populations in this study (Tables 2 and 3). Comparison of QTL identified in this study with previously reported iron toxicity tolerance QTL on the *japonica* Kato GRAMENE annotation sequence map 2009 [39], revealed that some were located in the same regions as QTL previously reported, or in adjacent regions. For instance, *qFRRDW1–1* with flanking markers C1S110 and C1S124 on chromosome 1, which affected RRDW in MH63-ILs, was mapped in the same region as a QTL for RRDW under iron stress [11], and partially overlapped with *QRdw1* affecting root dry weight under iron and zinc stresses [15]. *qFRRDW2*, located in the region C2S139–C2S143 on chromosome 2, which affected RRDW under iron stress in both MH63- and 02,428-ILs, was mapped in a region adjacent to *qRRL2–2* for relative root length under iron stress [40]. QTL regions for the iron and zinc toxicity tolerances mentioned above that were identified in different mapping populations and diverse environments could be beneficial for MAS breeding of iron and zinc toxicity-tolerant cultivars. It is noteworthy that QTL in the region C11S55–C11S59 on chromosome 11 that affected multiple iron and zinc toxicity tolerance-related traits in both MH63- and 02,428-ILs, was an important QTL with a large additive effect and genetic background independence. It merits confirmation in other populations and fine-mapping for map-based cloning.

4.2. *Effect of genetic background on detection of QTL for iron and zinc toxicity tolerances*

In this study, the reciprocal ILs were skewed towards one parent or the other in genome due to successive backcrossing with the recurrent parent and showed relatively uniform genetic background, thus ensuring that QTL mapping of stress tolerance was not strongly affected by genetic "noise" from cosegregating, non-target traits such as heading date and plant size. Accordingly, background effect on QTL detection can be revealed by comparison of mapping results from the two reciprocal IL populations. Of the 19 QTL affecting iron or zinc toxicity tolerance identified in the reciprocal backgrounds, only two (10.5%) were simultaneously identified in both backgrounds, clearly suggesting that most QTL detected in one background were not identified in another, so that there were genetic background effects on QTL detection for iron and zinc toxicity tolerance. This finding suggests that special care should be taken when QTL mapping information


Chr. 1
Chr. 2
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Chr. 9
Chr. 10
Chr. 11
Chr. 12
(Mb)
QTL underlying RRDW for MH63 and 02428 populations under Fe2+ stress and for MH63 and 02428 populations under Zn2+ stress, respectively.
QTL underlying RSDW for MH63 and 02428 populations under Fe2+ stress and for MH63 and 02428 populations under Zn2+ stress, respective.
Represent QTL underling RTDW for MH63 and 02428 populations under Zn2+ stress, respectively.

Table 4 – QTL for zinc toxicity tolerance identified in MH63-IL and 02,428-IL populations.

Population	Trait [1)]	QTL	Chr.	Marker interval (bp)	LOD	A [2)]	PVE (%)
MH63-ILs	RRDW	*qZRRDW3*	3	C3S66–C3S69 3873340–4079319	3.0853	0.0451	5.31
		qZRRDW11	11	C11S55–C11S59 4342500–4788363	3.3696	0.0864	12.36
	RSDW	*qZRSDW2*	2	C2S169–C2S177 19138636–19486553	3.1584	0.0585	8.06
	RTDW	*qZRTDW11*	11	C11S49–C11S60 4043766–4821401	2.6000	0.0756	6.97
02428-ILs	RRDW	*qZRRDW3*	3	C3S66–C3S69 3873340–4079319	2.8634	0.0451	6.05
		qZRRDW7	7	C7S28–C7S30 2677013–2856141	2.5697	0.0509	7.26
	RSDW	*qZRSDW11-1*	11	C11S49–C11S60 4043766–4821401	2.8648	0.0523	8.01
		qZRSDW11-2	11	C11S115–C11S125 8925976–9573028	2.787	0.0518	7.86
	RTDW	*qZRTDW3*	3	C3S70–C3S73 4139255–4251555	2.900	–0.0531	7.43
		qZRTDW11	11	C11S115–C11S127 8925976–9573028	4.600	0.0634	11.79

[1] RRDW, relative root dry weight; RSDW, relative shoot dry weight; RTDW, relative total dry weight.
[2] Additive effect resulting from the substitution of MH63 alleles by 02,428 alleles.

is applied to breeding for iron and zinc toxicity tolerance using MAS, as genetic backgrounds may differ greatly between mapping and breeding populations. It is essential that QTL mapping be combined with MAS-based breeding in the same population, a practice that has been strongly recommended for complex quantitative traits [23,26].

4.3. Genetic overlap between iron and zinc toxicity

Genetic overlap has been previously found in various fields, such as human disease [41] and plant stress tolerance [29,42–43]. Some chromosome regions harbor QTL for tolerance to more than one metal ion stress [9,18,44–45]. In a previous study in our laboratory, Zhang et al. [15] detected two zinc toxicity tolerance-related QTL, *QSdw2a* and *QSdw5*, which were mapped together with a iron toxicity tolerance QTL. In the present study, a 777 kb region flanked by C11S49–C11S60 on chromosome 11 contained one iron toxicity tolerance QTL (*qFRSDW11*) and three zinc toxicity tolerance QTL (*qZRRDW11*, *qZRTDW11*, and *qZRSDW11–1*. *qZRRDW3*) associated with RRDW was mapped together with two QTL for tolerance to iron toxicity in a previous study [10,46]. These results show that there is at least partial genetic overlap between iron and zinc stress tolerances. Recently, functional and comparative genomic studies have revealed many multifunctional metal transporters. For instance, *OZT1* confers plant tolerance to Zn and Cd ions [21], and *OsHMA3* not only reduces the toxicity of Ca^{2+} to rice seedling but also maintains Zn^{2+} balance in the rice stem [47–48]. These reports provide additional evidence of genetic overlap among different metal toxicity stresses. The molecular mechanisms underlying different stress tolerances await deeper investigation.

4.4. Potential application in breeding of iron and zinc toxicity tolerances

During long domestication and artificial selection, some favorable alleles have been intentionally or inadvertently introgressed into modern varieties from wild rice or landrace [49]. 02,428, which was selected from a cross between two landraces, showed tolerance not only to low CO_2 concentration stress [32] but also to iron and zinc toxicities, as demonstrated in this study. Two QTL (*qFRRDW2* and *qFRSDW11*) for tolerance to iron toxicity and two QTL (*qZRRDW3* and *qZRSDW11*) contributing to zinc toxicity tolerance were simultaneously identified in the two genetic backgrounds, and their favorable alleles all came from 02,428. This finding shows that favorable genes for iron and zinc toxicity tolerance "hidden" in 02,428 could be used by introgression and further pyramiding in elite modern cultivar backgrounds using molecular marker technologies.

Acidic soils with iron toxicity are always associated with zinc toxicity [8]. Thus, genetically overlapping loci provide a strategy for development of a cultivar tolerant to both stress toxicities that can not only simplify the process of MAS and reduce its cost but also improve the efficiency of development of a stress-tolerant variety. In this respect, the unique QTL, *qFRSDW11*, which affected iron and zinc toxicity tolerances in MH63- and 02,428-ILs, could be applied in breeding excellent rice varieties with tolerances to iron and zinc toxicities.

Fig. 4 – Distribution of QTL affecting iron and zinc toxicity tolerances identified in MH63- and 02,428-IL populations on a linkage map constructed using 265 framework SNP markers based on RILs derived from 02,428 × MH63.

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