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IDENTIFICATION OF QTL UNDERLYING CADMIUM TOLERANCE AT SEEDLING STAGE USING TWO SETS OF RECIPROCAL INTROGRESSION LINES IN RICE

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Cadmium (Cd) is a toxic heavy metal which has been threatening food security worldwide. In the present study, two sets of reciprocal introgression lines were used to dissect the genetic basis of Cd tolerance at seedling stage. Although the two parents had no significantly difference on Cd tolerance, the introgression progenies showed large variations. A total of 15 main-QTL (M-QTL) and 53 digenic epistatic QTL pairs (E-QTL) were detected for plant height, leaf length, leaf width and root length under Cd stress condition, each explaining 2.48-31.55% of phenotypic variations. E-QTL had larger effects than M-QTL for most traits, suggesting that E-QTL was the main genetic component for Cd tolerance at seedling stage. No common QTL, either M-QTL or E-QTL, was identified independent of background, indicated that both M-QTL and E-QTL were highly sensitive to genetic background. Two pleiotropic QTL in the regions of 13.31-15.56 Mb on chromosome 2 and 7.34-7.59 Mb on chromosome 11 were detected in the two backgrounds respectively, and further validated their effects on three traits by two introgression lines, indicated that they were both genetic true. Our results will give valuable QTL for improving Cd tolerance at seedling stage by molecular breeding.

Keywords: Cd tolerance; introgression line; main-effect QTL; digenic epistatic QTL pair; genetic background; pleiotropic QTL.

INTRODUCTION

In recent two decades, with industry fast development and dramatic increasing commercial phosphorus fertilize application, our environment has been seriously polluted, including a large number of heavy metal accumulation in soil, such as cadmium (Cd) (Deng *et al.*, 2009; Kashiwagi *et al.*, 2009; Canli, 2019). Cd is toxic heavy metal to both plants and animals including rice (Khasanah and Rachmawati. 2020). Superfluous Cd causes rice grow slowly (Chen and Kao, 1995), destroy photosynthesis system (Deng *et al.*, 2009), reduce biomass yield, and finally reduce yield and quality (Grant *et al.*, 2008; Xue *et al.*, 2008a). Moreover, Cd could be concentrated in human liver and kidney through food chain, and seriously threatens human health (Lin *et al.*, 2009). Thus, breeding Cd tolerant rice varieties with low Cd accumulation ability is an effective way to manage Cd threaten.

There is significant difference of Cd tolerance at seedling stage among different varieties (Wu *et al.*, 2006), indicated that Cd tolerance was controlled by genetic component. Cd tolerance at seedling stage is a typical quantitative trait which controlled by multiple genes, but few researches focused on this field. Only a few quantitative trait loci for Cd tolerance have been mapped (Xue *et al.*, 2008a; Lin *et al.*, 2009; Ueno *et al.*, 2009). At present, 12 genes were cloned associated with Cd tolerance, including *OsPDR9* (Moons, 2003), *OsIRT1*, *OsIRT2* (Nakanishi *et al.*, 2006), *OsHMA9* (Lee *et al.*, 2007), *OsHMA3* (Ueno *et al.*, 2010; Miyadate *et al.*, 2011), *OsNramp1* (Takahashi *et al.*, 2011a; Takahashi *et al.*, 2011b), *OsLCT1* (Uraguchi *et al.*, 2011), *OsABCG43* (Oda *et al.*, 2011), *LCD* (Shimo *et al.*, 2011), *OsNramp5* (Ishimaru *et al.*, 2012; Sasaki *et al.*, 2012), *OsHMA2* (Satoh-Nagasawa *et al.*, 2012; Yamaji *et al.*, 2013) and *OsMTP1* (Yuan *et al.*, 2012), and most of them participate in transaction of Cd in rice. However, none of them was natural variation, and difficult to be used in current rice breeding.

Although majority of QTL/genes could be used in breeding programs, there will be still some failures because of genetic

background effect on QTL expression, such as appearance quality (Qiu *et al.*, 2017a; Qiu *et al.*, 2017b), yield (Zhang *et al.*, 2018; Hu *et al.*, 2018), salt tolerance (Qiu *et al.*, 2015). Besides, digenic epistatic QTL pairs are more sensitive to genetic background (Qiu *et al.*, 2017a).

Cd tolerance at seedling stage is especially important for rice, as it decides rice development and biomass yield and is vital for rice yield (Wu *et al*., 2006). In our previous study, two sets of reciprocal introgression lines derived from *indica* Minghui 63 and *japonica* 02428 were developed, and they had a high-density Bin map (Qiu *et al.*, 2017a; Hu *et al.*, 2018). In the present study, they were measured their Cd tolerance at seedling stage to identify main-QTL (M-QTL), epistatic-QTL pairs (E-QTL), pleiotropic QTL and evaluated genetic background effect on them. Our results will help us better understand genetic basis of Cd tolerance at seedling stage and give us useful gene resources to improve rice Cd tolerance by marker assisted selection.

MATERIALS AND METHODS

***Plant materials*:** In our previous study, two sets of reciprocal introgression lines (ILs) derived from Minghui63 (MH63) and 02428 were developed (Qiu *et al.*, 2017a). The ILs were BC_2F_8 populations and contained 226 and 198 lines the two genetic backgrounds (MH63-ILs and 02428-ILs) respectively. A high-density Bin map containing 4568 Bins was constructed by them.

***Trait evaluation*:** Cd tolerance at seedling stage was measured in the greenhouse in College of Agriculture, Yangtze University in 2017, and followed the methods describes as Xue *et al.* (2008a). After germination, 20 seeds were evenly placed in ten holes in a thin Styrofoam board with a nylon net bottom floated on water in a plastic box with two replications. A week later, one plant in each hole was removed to keep similar growth conditions of all seedlings. They were transferred to standard Yoshida's culture solution. After half a month, 0.2 mol/L $CdCl_2$ was added to the solution. At about 15 days after treatment, eight plants in the middle of each line were harvest, and plant height (PH), leaf length (LL), leaf width (LW) and root length (RL) of each plant were measured. To avoid leaf curling, all harvest seedlings were placed in water before trait evaluation. Another experiment was performed similar with Cd tolerance condition, but all ILs population were growing in Yoshida's culture solution instead of Cd solution, which was represented as control condition. PH, LL, LW and RL of all ILs population were measured at this condition.

***Data analysis*:** Statistical description and correlations among different traits were analyzed in Statistica 5.5 (Morales, 2001). M-QTL and E-QTL were identified by using the inclusive interval mapping (ICIM) function with bi-parental population (BIP) module in IciMapping ver. 4.0 software (Li *et al.*, 2007). Default threshold of LOD was set as 2.5.

***Validation of two pleiotropic QTL*:** The regions of 7.34-7.59 Mb on chromosome 11 and 13.31-15.61 Mb on chromosome 2 were detected to have pleiotropic effects at the two backgrounds, respectively. Two ILs (DQ200 and DQ265 at MH63 and 02428 background respectively) were selected. Twenty seedlings of each of them and their recipient parents were all measured Cd tolerance traits followed the method mentioned above in 2018, and they were test difference using *t*-test with threshold of p=0.05 and 0.01.

RESULTS

***Cd tolerance of two parents and their derived reciprocal introgression lines*:** In either control condition or Cd condition, there were no significant difference between the two parents for PH, LL, LW and RL (Table 1, 2; Fig. 1).

In control condition, all traits of both ILs population had very low variations (CVs of both ILs population were below 3% (Table 2), implying that there were no genetic variations of all traits in this condition. Oppositely, at Cd condition, the variations of all traits in both ILs population were high (above 10%) (Table 1). Besides, all traits in both ILs population showed normal distribution with transgressive segregations, indicated that there was large diversity for Cd tolerance at seedling stage and it was controlled by quantitative trait loci (QTL).

Correlation coefficients among difference Cd tolerance traits at seedling stage in both introgression lines was listed in Table 3. All positive correlations among different traits were extremely significant ($p<0.01$), except for correlation between LW and RL (0.20 with $p<0.05$) in 02428 background, and they were consistence in both backgrounds,

Table 1. Statistical descriptions of four Cd tolerance traits at seedling stage at Cd condition in two sets of reciprocal ILs derived from MH63 and 02428.

Trait	parents			MH63-ILs			02428-ILs		
	MH63	02428	MH63-02428	Mean±SD	Range	CV(%)	Mean±SD	Range	CV(%)
PH(cm)	25.64	25.76	-0.12	25.23±3.36	14.77-39.53	13.3	24.88±3.82	15.60-36.16	15.3
LL(cm)	18.17	16.29	1.88	17.49±2.75	11.00-24.83	15.7	15.64±2.88	9.78-24.25	18.4
LW(cm)	0.76	0.74	0.02	0.78±0.16	0.22-1.17	20.6	0.68±0.21	0.12-1.06	30.5
RL(cm)	10.11	10.37	-0.26	10.53±1.98	6.93-15.90	18.8	10.47±1.86	6.10-17.17	17.7

MH63, Minghui63; SD, standard deviation; CV, Coefficient of variation; MH63-ILs, introgression lines at Minghui63 background; 02428-ILs, introgression lines at 02428 background; PH, plant height; LL, leaf length; LW, leaf width; RL, root length.

Table 2. Statistical descriptions of four Cd tolerance traits at seedling stage at control condition in two sets of reciprocal ILs derived from MH63 and 02428.

Trait	parents			MH63-ILs			02428-ILs		
	MH63	02428	MH63-02428	Mean±SD	Range	CV(%)	Mean±SD	Range	CV(%)
PH(cm)	37.12	36.97	0.15	37.18±0.32	34.69-37.92	0.9	37.02±0.54	34.86-38.88	1.5
LL(cm)	24.66	24.75	-0.09	24.69±0.11	22.80-25.33	0.5	24.85±0.14	22.96-26.05	0.6
LW(cm)	01.09	01.08	0.01	01.10±0.03	0.89-1.19	2.7	01.07±0.02	0.91-1.24	1.9
RL(cm)	15.23	15.21	0.02	15.25±0.13	14.09-16.88	0.9	15.30±0.11	12.58-15.59	0.7

MH63, Minghui63; SD, standard deviation; CV, Coefficient of variation; MH63-ILs, introgression lines at Minghui63 background; 02428-ILs, introgression lines at 02428 background; PH, plant height; LL, leaf length; LW, leaf width; RL, root length.

indicated that under Cd stress, seedlings with higher PH would have longer LL, wider LW and deeper RL. Among four traits, the coefficients among PH, LL and LW were higher (all correlation coefficients were above 0.40) than between RL and them, suggesting traits among above-ground had higher correlation than between underground and above-ground.

Table 3. Correlation coefficients of four Cd tolerance traits at seedling stage at Cd condition in two sets of reciprocal ILs derived from MH63 and 02428.

	PH	LL	LW	RL
PH		0.82**	0.44**	0.55**
LL	0.78**		0.45**	0.52**
LW	0.49**	0.42**		0.20*
RL	0.25**	0.22**	0.38**	

Data under and above the diagonal are correlation coefficients in MH63-ILs and 02428-ILs, respectively; * and ** indicate significant differences at the 0.05 and 0.01 levels, respectively; PH, plant height; LL, leaf length; LW, leaf width; RL, root length.

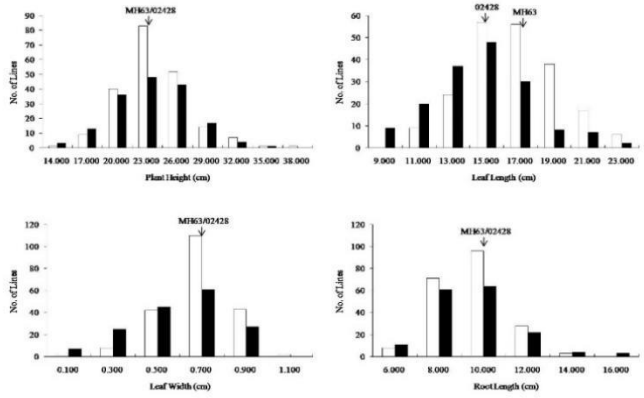

Figure 1. Frequency distributions of four Cd tolerance traits at seedling stage at Cd condition in two sets of reciprocal ILs derived from MH63 and 02428. White and black bars represent introgression line populations with MH63 and 02428 backgrounds, respectively; MH63, Minghui 63.

***M-QTL identification for Cd tolerance at seedling stage*:** No QTL was detected in both IL populations at control condition. At Cd condition, nine QTL for four traits were identified in MH63-ILs population, accounting for 4.48-30.94% of phenotypic variations (Table 4; Fig. 2). A total of 1, 1, 4 and 3 QTL for PH, LL, LW and RL distributed on chromosome 1, 4, 5, 11 and 12 were identified, respectively. Among them, 02428 alleles on *qLW5*, *qRL4* and *qRL12* increased trait values, while 02428 alleles on the rest QTL decreased trait values. The *qRL4* had the largest phenotypic variation rate (30.94%). A total of six QTL were detected in 02428-ILs population, located on chromosome 1, 2, 7 and 8. 1, 2, 1 and 2 QTL were scanned for PH, LL, LW and RL with each explaining 3.74-5.56% of phenotypic variations, respectively. MH63 alleles at all QTL were associated with increased trait values except *qPH2*, *qLL8* and *qRL1*. Among them, phenotypic variation rate of *qLL2* reached the highest (5.56%), and MH63 allele increased LL.

Among identified 15 M-QTL, None QTL was commonly identified in both genetic backgrounds.

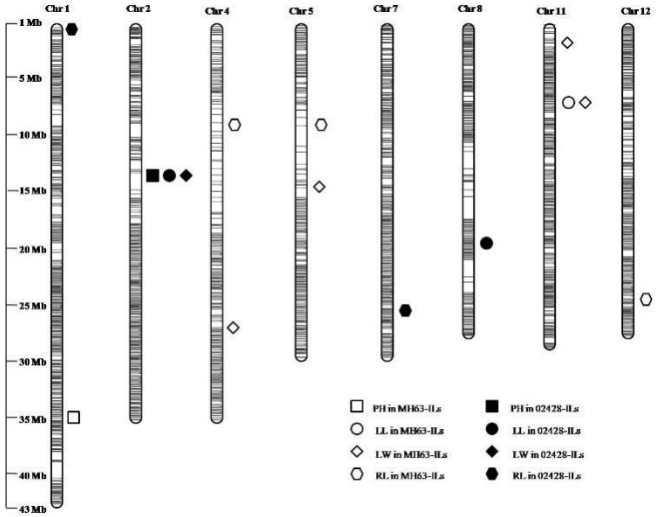

Figure 2. Genome distribution of M-QTL for four Cd tolerance traits at seedling stage in two sets of reciprocal ILs derived from MH63 and 02428. MH63, Minghui63; MH63-ILs, introgression lines at Minghui63 background; 02428-ILs, introgression lines at 02428 background; QTL, quantitative trait locus; PH, plant height; LL, leaf length; LW, leaf width; RL, root length.

Table 4. Main-QTL for four Cd tolerance traits at seedling stage in two sets of reciprocal ILs derived from MH63 and 02428.

Background	Trait[1]	QTL	Chr.	position (Mb)	LOD	A[2]	R^2(%)[3]
MH63	PH	*qPH1*	1	34.84-35.13	3.28	-1.156	6.55
	LL	*qLL11*	11	7.34-7.59	2.64	-2.246	6.86
	LW	*qLW4*	4	27.01-27.86	2.73	-0.183	12.27
		qLW5	5	14.12-16.12	6.55	0.249	26.97
		qLW11.1	11	2.00-3.24	2.56	-0.073	4.48
		qLW11.2	11	7.34-7.59	4.39	-0.267	16.00
	RL	*qRL4*	4	7.14-11.41	16.10	3.225	30.94
		qRL5	5	8.52-13.02	13.42	-4.225	10.94
		qRL12	12	24.68-25.37	20.17	3.225	5.94
02428	PH	*qPH2*	2	13.31-15.61	2.53	-0.941	3.74
	LL	*qLL2*	2	13.31-15.61	2.86	1.153	5.56
		qLL8	8	19.92-20.28	2.69	-1.068	4.83
	LW	*qLW2*	2	13.31-15.61	3.57	0.058	4.96
	RL	*qRL1*	1	0-0.77	3.72	-0.578	4.67
		qRL7	7	26.59-27.76	2.52	0.662	5.16

[1]PH, plant height; LL, leaf length; LW, leaf width; RL, root length; QTL, quantitative trait locus; Chr., chromosome; MH63, Minghui63; [2]A, additive effect; the additive effects were estimated by the substitution the MH63 allele by the 02428 allele in MH63-ILs and 02428 allele by the MH63 allele in 024283-ILs; [3]R^2, Phenotypic variation explained by the QTL.

Table 5. Digenic epistatic QTL pairs (E-QTL) for four Cd tolerance traits at seedling stage in two sets of reciprocal ILs derived from MH63 and 02428.

Background	Trait[1]	Region1			Region2			LOD	AA[3]	R^2(%)[4]
		Chr.	position (Mb)	M-QTL[2]	Chr.	position (Mb)	M-QTL			
MH63	PH	1	18.72-19.04		3	30.58-31.69		3.04	-1.638	7.29
		1	3.53-3.95		7	27.02-27.71		3.42	3.264	7.13
		2	3.69-3.88		7	4.80-5.18		3.19	-1.589	11.40
		2	8.74-8.86		3	30.58-31.69		3.15	-1.778	9.13
		4	22.38-22.62		12	3.88-5.51		3.33	3.423	7.31
		6	26.23-26.45		9	11.77-14.01		3.38	-1.881	7.23
		7	6.20-7.26		12	3.88-5.51		3.65	3.323	7.37
	LL	1	34.84-35.13		4	7.15-11.41		3.29	-1.618	10.71
		3	4.08-5.42		11	3.71-3.79		3.91	-1.759	11.61
		4	7.15-11.41		8	19.92-20.28	*qLL8*	3.06	1.544	10.53
		7	26.05-26.36		11	1.19-1.53		4.02	-1.483	11.70
		11	7.34-7.59	*qLL11*	12	11.17-12.76		3.57	1.762	10.67
	FW	1	2.363-2.78		11	1.19-1.53		7.83	-0.151	26.90
		2	6.10-6.30		9	11.77-14.01		6.48	0.254	3.15
		2	20.73-22.39		11	0-0.27		3.60	-0.243	2.71
		3	4.08-5.42		11	0-0.27		5.95	-0.218	2.75
		4	7.15-11.41		6	6.22-6.38		5.33	-0.147	2.48
		5	14.12-16.12		11	0-0.27		6.54	0.212	2.74
		6	2.37-3.19		11	1.19-1.53		5.92	0.154	2.67
		8	27.31-27.72		11	1.19-1.53		4.09	-0.152	2.64
	RL	1	22.36-22.84		9	11.77-14.01		7.85	-2.939	3.69
		2	2.33-2.72		9	11.77-14.01		5.33	-3.037	3.79
		2	20.73-22.39		7	27.02-27.71		7.42	-2.257	3.93
		2	20.73-22.39		8	20.94-23.08		6.65	2.949	3.73
		3	17.19-18.15		9	11.77-14.01		3.64	1.527	3.71
		4	7.15-11.41	*qRL4*	6	29.88-30.85		4.59	2.773	3.78
		4	7.15-11.41	*qRL4*	8	20.94-23.08		5.22	-2.762	3.72
		5	8.52-13.02	*qRL5*	9	11.77-14.01		6.04	-2.938	3.76
		6	29.88-30.85		9	11.77-14.01		5.50	2.035	3.81

Background	Trait[1]	Region1			Region2			LOD	AA[3]	R^2(%)[4]
		Chr.	position (Mb)	M-QTL[2]	Chr.	position (Mb)	M-QTL			
		8	7.49-7.80		9	11.77-14.01		6.89	3.039	3.71
02428	PH	1	2.36-2.65		6	7.49-7.81		2.55	1.420	8.49
		3	0-0.25		7	9.97-10.21		2.75	1.151	7.68
		4	2.50-2.86		4	12.41-14.07		2.72	-1.111	8.96
		6	29.84-31.85		8	9.96-10.12		3.25	-1.409	8.92
		7	7.45-7.64		9	17.49-17.57		2.90	-1.320	9.11
		11	2.50-2.57		12	22.37-22.59		2.83	1.449	8.11
	LL	1	19.58-20.61		2	24.88-25.12		3.65	-1.967	21.82
		1	2.36-2.65		4	19.96-20.28		4.39	-0.807	31.55
		1	7.16-8.64		6	14.94-15.17		9.30	1.858	4.26
		2	24.88-25.08		4	22.42-22.74		3.72	-1.846	14.41
		3	24.95-25.18		4	22.42-22.74		4.33	1.909	16.91
		4	17.14-17.74		5	7.88-13.92		8.18	2.161	4.49
		4	17.14-17.74		7	26.59-27.76		5.69	2.650	4.63
	LW	1	27.50-27.61		11	19.96-20.45		3.34	0.075	8.44
		1	29.96-30.18		7	22.39-22.85		3.35	-0.094	10.48
		2	35.00-35.09		4	27.01-27.86	*qLW4*	3.05	0.079	7.87
		4	17.14-17.74		7	24.92-25.43		3.17	-0.091	9.99
		5	0-0.09		12	22.37-22.59		3.68	-0.084	9.20
		5	14.66-15.68	*qLW5*	7	19.97-20.26		3.64	0.092	9.40
		7	19.97-20.26		11	2.00-3.24	*qLW11.1*	3.06	0.076	8.92
	RL	5	27.49-27.78		12	24.68-25.37	*qRL12*	3.41	-0.765	8.22
		6	14.94-15.17		10	0-0.12		3.25	0.691	8.37
		10	2.33-2.59		11	0-0.27		3.19	0.676	8.13

[1]PH, plant height; LL, leaf length; LW, leaf width; RL, root length; QTL, quantitative trait locus; Chr., chromosome; MH63, Minghui63; [2]M-QTL, main effect QTL listed in Table 4; [3] AA, additive interaction effect; positive value represents E-QTL enhanced trait value, while negative value indicates E-QTL decreased traits value; [4]R^2, Phenotypic variation explained by the QTL.

***E-QTL detection for Cd tolerance at seedling stage*:** In MH63-ILs, 30 digenic epistatic QTL pairs were identified with 7, 5, 8 and 10 for PH, LL, LW and RL respectively, accounting for 2.48-26.90% of phenotypic variations (Table 5). 5 E-QTL occurred between one M-QTL and one locus, and the rest pairs between two loci without main-effects. Among them, 17 E-QTL increased trait values. No E-QTL controlled two or more traits. In 02428 ILs, 6, 7, 7 and 3 digenic epistatic QTL pairs were found for PH, LL, LW and RL respectively, the phenotypic variations explained by E-QTL ranged from 4.26 to 31.55%. 4 pairs were between one M-QTL and one locus, and the rest between two loci without main-effects. 13 E-QTL increased trait values. All E-QTL were associated with only one traits.

Among identified 53 E-QTL, no E-QTL expressed in both backgrounds.

***Validation of two pleiotropic M-QTL for Cd tolerance at seedling stage*:** Among 15 M-QTL detected in either background, two loci controlled two or three traits, which was defined as pleiotropic QTL. The first one was located in the region of 7.34-7.59 Mb on chromosome 11 detected in MH63 background. It controlled both LL and LW with phenotypic variation explained of 6.86 and 4.48% respectively. The second one controlled PH, LL and LW accounting for 3.74-5.56% of phenotypic variations in 02428 background. It was located in 13.31-15.56 Mb on chromosome 2. MH63 allele at both loci increased both trait values except *pPH2*.

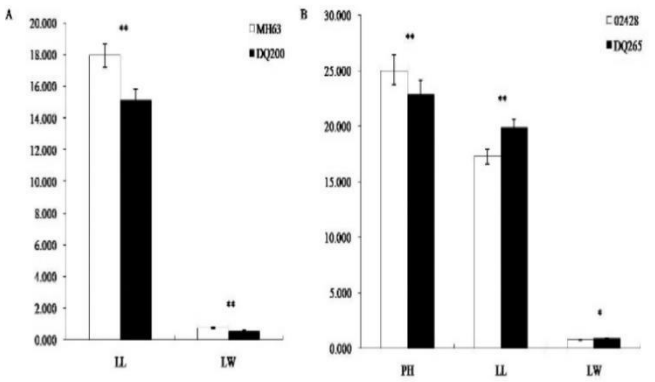

Figure 3. Difference of Cd tolerance traits at seedling stage at Cd condition between an introgression lines harboring two pleiotropic QTL and their parents. A, difference of LL and LW between DQ200 barboring7.34-7.59 Mb on chromosome 11and MH63; B, difference of PH, LL and LW between DQ2265 barboring13.31-15.56 Mb on chromosome 2 and 02428; * and ** indicate significant differences at the 0.05 and 0.01 levels, respectively; PH, plant height; LL, leaf length; LW, leaf width.

To validate these two loci, two ILs, DQ200 from MH63-ILs and DQ265 from 02428-ILs, were selected and measured their Cd tolerance traits. The percentages of current parent genome of them were 92.6 and 93.3% respectively, and there was no other QTL introgressed in backgrounds. The LL and LW of DQ200 were 15.126 cm and 0.553 cm respectively, and they were extremely significantly shorter and slender than MH63 (Fig. 3), suggesting that the region of 7.34-7.59 Mb on chromosome 11 was a true pleiotropic QTL for both LL and LW in MH63 background. The PH, LL and LW of DQ265 were 22.821 cm, 19.853 cm and 1.277 cm, respectively. They were significantly lower, longer and wider than the recurrent parent 02428, indicated that the region of 13.31-15.56 Mb on chromosome 2 was also a real pleiotropic QTL for both PH, LL and LW in 02428 background.

DISCUSSION

In this study, a total of 15 M-QTL were identified for four Cd tolerance traits at seedling stage using two sets of reciprocal introgression lines derived from *indica* three-line restorer MH63 and wide compatible *temperate japonica* variety 02428. Among them, some QTL were near with QTL reported previously. For example, *qPH1* in the region of 34.83-35.13 Mb on chromosome 1, *qRL7* in the region of 26.59-27.76 Mb on chromosome 7 and *qRL8* in the region of 19.92-20.28 on chromosome 8 were located in the adjacent regions of *qRL1.1* for root length, *qSH7* for shoot length and *qCC8* for chlorophyll content (Xue *et al.*, 2008a). *qRL1* in 0-0.77 Mb on chromosome 1 and pleiotropic QTL for LL and LW in 7.34-7.59 Mb on chromosome 11 were near *qSAP-1a* for shoot length, *qLAP-11*/*qSAP-11* for shoot length and leaf length (Lin *et al.*, 2009). We believe with the more studies on this field, there will be more consistent QTL detected in the future. Besides, whether consistent QTL were the same or linkage QTL will need to be further clarified after fine mapping and cloning.

Almost all quantitative traits are controlled by QTL and environments, and QTL could be divided into M-QTL and E-QTL. Large previous studies indicated that they were both important for heterosis (Yu *et al.*, 1997), appearance quality (Qiu *et al.*, 2017a), reproductive isolation (Li *et al.*, 2017) and yield related traits (Hu *et al.*, 2018). In the present study, E-QTL was much more than M-QTL in both IL populations (30 vs 9 in MH63 background and 23 vs 6 in 02428 background respectively), and the majority of them were two loci without main-effects (25 and 19 in MH63 and 02428 backgrounds respectively). Besides, the total phenotypic variations explained by E-QTL were much higher than M-QTL for most Cd tolerance traits at seedling stage (56.86% vs 6.55% for PH and 55.21% vs 6.86% for LL in MH63 background, 51.27% vs 3.74% for PH, 98.08% vs 10.39% for LL, 64.30% vs 4.96% for LW and 24.72% vs 9.83% for RL in 02428 background). Thus, Cd tolerance at seedling stage was mainly controlled by digenic epistatic QTL interations. It was consistent with previous study for yield related traits (Hu *et al.*, 2018).

Using pleiotropic QTL associated with two or more traits to improve traits in molecular breeding would save much time, labor and money, thus they had much higher values than single genes. A large number of pleiotropic QTL were fine mapped and cloned for yield (Xue *et al.*, 2008b; Yan *et al.*, 2011; Yan *et al.*, 2013), grain shape (Song*et al.*, 2007; Shomura *et al.*, 2008; Weng *et al.*, 2008; Mao *et al.*, 2010; Li *et al.*, 2011; Wang *et al.*, 2015a; Wang *et al.*, 2015b; Liu *et al.*, 2017; Qiu *et al.*, 2017a; Zhao *et al.*, 2018), plant architecture (Zhang *et al.*, 2017) and panicle color (Lv *et al.*, 2018). In the present study, two regions of 13.31-15.56 Mb on chromosome 2 and 7.34-7.59 Mb on chromosome 11 were identified and validated as pleiotropic QTL. The former one was for both PH, LL and LW in 02428 background, and the latter one was associated with both LL and LW in MH63 background. The MH63 alleles on both loci increased all trait values except PH in the region of 13.31-15.56 Mb on chromosome 2, suggesting that they could increase Cd tolerance at seedling stage. They would be the most valuable gene resources for improving Cd tolerance at seedling stage.

Rice germplasms carry all kinds of novel genes for almost all traits, and novel genes were intentionally or unintentionally introduced into modern varieties during domestication and modern breeding (Kovach *et al.*, 2007). Although rice varieties don't resistant to Cd toxicity, they may still carry novel genes resistant to Cd toxicity. In this study, there was no significantly difference for Cd tolerance at seedling stage between MH63 and 02428, there were still 15 M-QTL and 53 E-QTL identified. MH63 alleles and 02428 alleles on 10 and 5 M-QTL increased trait values, and all of them could be gene resources for improving Cd tolerance at seedling stage. Among them, two pleiotropic QTL in the regions of 13.31-15.56 Mb on chromosome 2 and 7.34-7.59 Mb on chromosome 11 were further validated, of which MH63 alleles increased almost all trait values, and they would be the most valuable genes in molecular breeding to improve Cd tolerance at seedling stage. Cd tolerance would be largely improved by introducing or pyramiding them into elite varieties. However, it's notable that both of them were background dependent, which means they should be carefully used in other backgrounds, otherwise the Cd tolerance improvement would fail.

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