QTL mapping for Aluminum (Al^{3+}) toxicity tolerance in two sets of Reciprocal Introgression Lines in Rice (*Oryza sativa L.*)

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ABSTRACT— Aluminum toxicity is one of the major environmental constraints limiting rice growth and productivity. To overcome that constraint the genetic study of rice crop-tolerance against Al toxicity is a one of the best approaches to save the time and efficiency. The core objective of the experiment was to identify QTLs for aluminum toxicity tolerance in introgression lines of rice. The experiment was conducted in hydroponic environment with two sets of reciprocal introgression lines derived from the cross of 02428/Minghui63 in japonica 02428 background (02428-ILs) and indica Minghui63 background (MH63-ILs) to evaluate aluminum toxicity tolerance (ATT) at the concentration of 1.5 mmol L-1 at the seedling stage. The relative root elongation (RRE) was recorded as tolerance-criterion. Furthermore, the two sets of RILs were genotyped by 384 evenly distributed SNP markers developed by the two parents. The parent 02428 has greater ATT than that of MH63. In total, fourteen QTLs for all environments on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, and 12 including nine QTLs detected in both populations. Among them, four stable QTLs (QRl1b, QRl2, QRl9 and QRL10) on chromosome 1, 2, 9 and 10 were commonly detected in both backgrounds. The preliminary QTL mapping of ATT will provide useful information for further fine-mapping and marker-assisted selection for rice improvement of Aluminum toxicity tolerance (ATT).

Index Terms /Key words: Rice, Aluminum toxicity, Reciprocal Introgression lines, QTL mapping

Abbreviations

cM=centi Morgan
CR=Crop root (RL2)
IciMapping=Inclusive composite interval mapping
MH63=Minghui 63
P1= MH63 *indica* Parent 1
P2= 02428 *japonica* Parent 2
QR=Quantitative trait loci detected from root
QTLs= Quantitative trait loci/locus
RL1= Root length of control after 10 days of seeding
RL2= Root length of control condition after 25 days of seeding
RL3= Root length under Al^{3+} stress
(for 15 days under Al^{3+} toxicity stress)
RRE=Relative Root Elongation=SR/CR
SR=Stressed root (RL)

1 INTRODUCTION

In acid soil (pH 5.0) aluminum is one of the major constraint which influence plant growth and crop production. There is estimation that over 50% of world's potentially arable lands are acidic [1], [2]. Out of that upto 60% of the acid soils are present in developing countries [1]. These world's arable lands have ~ 40% of aluminum [3], [4], [5]. Therefore it constitutes about 7% of the total amount of the soils in the world [6], particularly in tropics and sub tropics [3], [7].

In acid soils several abiotic stress factors restrict crop pro-

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duction. Aluminum is also abiotic stress factor which is present in different forms in soils, it is highly soluble in acidic pH 5 or less than 5 It is because of this reason, aluminum toxicity has proved one of the major growth limiting factors affecting crop-plants in acid soils [8]. Under severe acid-soils (pH 5.0), Al is soluble into the soil solution as Al^{3+}, which is highly toxic to crop plants, causing a rapid inhibition of root growth that leads to a reduced and stunted root system, thus it has a direct negative effect on the ability of a plant to absorb both water and nutrients [9].

Many researchers have reported the identification of Al-tolerant genotypes in rice and other cereals [10], [11], [12], [13], [14]. The cereal crops have been a primary focus of Al tolerance research. This research has demonstrated that levels of Al tolerance vary widely both within and between species [8], [12]. In wheat, sorghum, and barley, Al tolerance is inherited as a simple trait, controlled by one or a few genes [13]. However, in maize, rice, and Arabidopsis, tolerance is quantitatively inherited [3], [15]. Among the major cereal species that have been extensively studied, such as rice, maize, wheat, barley and sorghum, rice demonstrated superior Al tolerant crop under both field and hydroponic environment [8], [12].

Based on high level of Al tolerance in rice and numerous genetic and genomic resources, rice provides a good model for studying the genetics and physiology of Al tolerance. Recently four mutant genes that lead to Al sensitivity in rice have been cloned, STAR1 (Sensitive to Al rhizotoxicity1), STAR2 (Sensitive to Al rhizotoxicity2), ART1 (Aluminum rhizotoxicity1), and Nrat1 (Nramp aluminum transporter 1) [16], [17]. Seven QTLs for Al tolerance have been reported in rice using 6 different inter- and intra-specific mapping populations [18], [19]. These studies reported a total of 33 QTLs located on all 12 chromosomes, with three intervals on chromosomes [20], [8], [13] being detected in multiple studies. In all of these above referred studies, mapping QTLs for Al toxicity tolerance was estimated based on relative root growth (RRG), and specifically on inhibition of the growth of the longest root (elongation of the longest root in Al treated plants as well as controlled root). The identification of DNA markers diagnostic of Al tolerance can accelerate the development of cultivars that can remain productive even under Al stress, and might be the starting point for identifying the specific genes responsible for differences in the response of plant genotypes to toxic aluminum levels.

Al tolerance trait in rice is mainly controlled by quantitative genes [9]. In rice, root growth under Al stressed condition is controlled by several quantitative trait loci (QTLs). However, two QTLs of largest effect have been identified to explain phenotypic variation for Al toxicity tolerance [21], [22]. A recent study identified two genes STAR1 and STAR2 which function as bacterial-type ATP binding cassette (ABC) transporter to control Al tolerance toxicity in rice [23].

It is noteworthy that our research group (Rice Molecular Breeding) at Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China is first in the world of the agriculture to study these two backgrounds MH63 indica and 02428 japonica together for the detection of QTLs tolerant to different environmental stresses, such as drought and heavy metals. Therefore, the core objectives of this study were to identify QTLs for Aluminum toxicity tolerance at seedling stage in two sets of Reciprocal Introgression lines in rice and to prepare the QTL map showing the position of all detected QTLs on their respective chromosomes.

2 Materials and method

2.1 Development of Plant material

Two sets of introgression lines (ILs) were developed from a cross between MH63 an *indica* variety and 02428 a japonica variety. The F1 hybrids simultaneously back crossed two times with both parents to produce two BC_2F_1 populations. The two BC_2F_1 were allowed to self-produce for $BC_2F_{2:8}$ generation. Finally 2 sets of reciprocal introgression lines (RILs) were developed, consisting of 200 lines in the MH63 indica background and 200 lines in 02428 japonica background. In total these lines of both backgrounds were used in following genotypic and phenotypic study.

The experiment was conducted two times:

1st Preliminary experiment: Preliminary tests with different concentrations of aluminum toxicity were carried out to decide the most suitable concentration of Al toxicity level for the RILs and two parents MH63 indica and 02428 japonica backgrounds.

2nd Regular experiment: On the bases of results obtained from above preliminary experiment, regular experiments were conducted to detect the QTLs for aluminum toxicity tolerance in mapping populations (MH63 indica and 02428 japonica).

2.2 Detailed methodology to conduct the regular study with most suitable concentration of aluminum toxicity

The seed of parents MH63 indica and 02428 japonica and RI lines from MH63 indica and 02428 japonica was surface sterilized with 1% hypochlorite solution for 10 minutes and rinsed well with distilled water. Then the seed was soaked in distilled water in the dark at 30 °C for 48 hours. The most uniform 10 emerged seeds for each RI line per replication were directly sown into perforated Styrofoam sheets covered with nylon net at the bottom. For each experimental condition (control and aluminum treated) most uniform 10 emerged seeds from parents MH63 indica and 02428 japonica were also sown in each container at random. All the material was twice replicated. The Styrofoam sheets were allowed to float on water up to 7 days and then transferred to Yoshida culture solution [24] without applying suitable concentrations of Al^{3+} for first 10 days and then root length for one replication was measured as

base for remaining replications from each condition (control and aluminum treated). On the 11th day of seeding, the Al^{3+} in the form of $AlCl_3$ at the rate of 1.5 mmol L^1 was applied for 15 days. The pH of the solution on alternative day was adjusted to 4.5 with 1 *N* NaOH/HCl. The solution was renewed every fifth day. The experimental materials were laid out in two replications for all experiments (control and treated) in green house of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China at around temperature of 32/25 ^oC in day/night, 70-75% relative humidity and 12 hours photoperiod. The root length was recorded on 15th day of the Al application. The ratio of average root elongation under stress versus non stress conditions for each line in each replication was calculated as follows, as an indicator of the root resistance index:

RRE (%) = SRE/CRE x 100; (19).

Where, RRE is the relative root elongation (%), SRE is the stress root elongation at 1.5 mmol L^1 Al and CRE is the control root elongation in control (cm).

1-Relative variation of length=
[(Length of treated plant-Length of control plant/Length of control plant)]X100
2-Relative variation of dry weight=
[(DW of treated plant-DW of control plant/DW of control plant)]X100

2.2. Genotyping and linkage map construction

A total of 265 polymorphic simple sequence repeat (SSR) markers were used to assay the RILs. Marker position and genetic distance among linked markers followed the Cornell map [25], which covered all 12 rice chromosomes with a total genome size of 1132.87 cM and an average distance of 4.27 cM between adjacent markers.

2.3 Data analysis

Phenotypic data of RILs obtained from Control, Al^{3+} stress condition as well as the ratio of Al^{3+} stress to Control condition was used as input data to identify QTLs by using the software Ici-Mapping3.0 [26]. Permutation of 1000 times under the significant level of P=0.05 were used to find the LOD thresholds for different traits in two different backgrounds. Finally, LOD (2.0) values were used as the threshold for claiming the presence of QTLs for related traits in MH63 *indica* background and >1 LOD ranges were used as the threshold for claiming the presence of QTL for related traits in 02428 japonica background. To further examine the extent to which inconsistent QTL detection across the two environments (control and Al^{3+} toxicity stress) actually arose from type II errors, all identified QTLs in one condition were re-examined using the data from the other conditions under the minimum threshold of $p < 0.05$. The test statistics and QTL parameters associated with the QTL are also reported as long as the QTL reached at the minimum threshold [27], [28].

3 RESULTS

3.1 QTLs identified for aluminum toxicity tolerance related trait of root elongation detected in the reciprocal introgression lines of MH63 *indica* background

A total of 13 QTLs were identified on all chromosomes, at threshold LOD scores of 2.00. The identified QTLs are tabulated in Table 1. The QTL (IciM) identified two QTLs *QRl1a* and *QRl1b* on chromosome 1 under all environmental conditions (control and treated) except relative root elongation (RRE) with the LOD 7.07 and additive (Ad) 0.73 under RL1(root length after 10 days of seeding, before application of Al stress), under RL2 (root length of control condition after 25 days of seeding) environmental condition with the LOD 6.45 and Ad 0.76, under RL3 (root length of aluminum treated plants after 25 days of seeding with 15 days aluminum treatment) environmental conditions LOD 4.92 and Additive 0.54. The QTL *QRl1b* was identified on chromosome 1 under all conditions except RRE under RL1 with LOD 2.15 and Ad 0.46, under RL2 conditions with LOD 4.67 and Ad 0.44 and under RL3 with LOD 6.91 and Ad 0.37 (Table 1.). One QTL has been identified on each chromosome 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, namely *QRl2, QRl3, QRl4, QRl5, QRl6, QRl7, QRl8, QRl9, QRl10, QRl11,* and *QRl12*.

This study had its wide scope because it has detected some major (common) QTLs in all experimental environments. These are QTL *QRl2* was detected under all experimental environmental conditions, under RL1 condition with LOD 5.77, Ad 0.98, under RL2 with LOD 3.15 and Ad 0.67, under RL3 with LOD 9.97 and Ad 1.12, under RRE with LOD 4.58 and Ad 5.31. The QTL *QRl3* was identified under all environmental conditions, under RL1 with LOD 3.09, Ad 0.64, under RL2 with LOD 2.68 and Ad 0.61, under RL3 with LOD 12.41 and Ad 0.77, under RRE with LOD 2.45 and Ad 3.83. The QTL *QRl4* was also identified in all environmental conditions, under RL1 with LOD 2.2 and Ad 0.29, under RL2 with LOD 3.15 and Ad 0.47, under RL3 with LOD 5.94 and Ad 1.11, under RRE with LOD 2.97 and Ad 4.91. The QTL *QRl5* was detected under all environmental conditions except RRE, under RL1 with LOD 3.16 and Ad 0.75, under RL2 with LOD 3 and Ad 0.74 under RL3 with LOD 5.3, and Ad 1.23. The QTL *QRl6* was identified under all conditions, under RL1 with LOD 2.46 and Ad 1.1, under RL2 with LOD 4.08 and Ad 0.82, under RL3 with LOD 8.66 and Ad 1.21, under RRE with LOD 2.18 and Ad 2.54. The QTL QRl7 was detected under all conditions except RRE, under RL1 with LOD 3.4 and Ad 0.94, under RL2 with LOD 2.58 and Ad 0.46, under RL3 with LOD 8.04 and Ad 1.2. The QTL *QRl8* was detected under all conditions except RRE, under RL1 with LOD 4.4 and Ad 0.96, under RL2 with LOD 4.18 and Ad 0.95, under RL3 15.76 and Ad 1.23. The QTL *QRl9* was identified under all conditions, under RL1 with LOD 2.05

and Ad 0.63, under RL2 with LOD 2.01 and Ad 0.6, under RL3 with LOD 3.51 and Ad 1.17, under RRE with LOD 2.84 and Ad 3.63.(Table 3). The QTL *QRl10* was also detected under all conditions, under RL1 with LOD 10.79 and Ad 0.84, under RL2 with LOD 2.06 and Ad 0.57, under RL3 with LOD 2.93 and Ad 0.53, under RRE with LOD 2.52 and Ad 4.03. The QTL QRl11 was identified under RL1 and RL3, under RL1 with LOD 6.75 and Ad 0.87 and under RL3 with LOD 7.74 and Ad 1.03. The QTL QRl12 was also identified under RL1 and RL3, under RL1 with LOD 4.04 and Ad 0.72, under RL3 with LOD 7.78 and Ad 1.2. The QTLs 2, 3, 4, 6, 9, and 10 were identified under all conditions (Table 1). Therefore, in future these QTLs might be highly useful to develop the Al stress toxicity tolerant lines in MH63 *indica* background.

3.2 QTLs identified for aluminum toxicity tolerance-related trait of root elongation detected in the reciprocal introgression lines of 02428 japonica background

A total of 04 QTLs were detected on four chromosomes 1, 2, 9, and 10 and named as *QRl1, QRl2, QRl9* and *QRl10* respectively from 02428 japonica background, at a threshold LOD score of >1. The identified QTLs are tabulated in Table 2. The QTL *QRl1*was identified under RL1 and RRE conditions, under RL1 with LOD 2.29 and Ad 0.4, under RRE with LOD 1.32 and Ad 1.9. The QTL *QRl2* was detected under RL1 and RL3 conditions, under RL1with LOD 2.27 and Ad -0.21, under RL3 with LOD 1.48 and Ad -0.17. The QTL *QRl9* was identified under all conditions, under RL1 with LOD 1.63 and Ad -0.2, under RL2 with LOD 1.49 and Ad -0.26, under RL3 1.31 and Ad -0.19 and under RRE with LOD 4.58 and Ad 1.63. The QTL *QRl10* was detected under control conditions before aluminum stress application (RL1) with LOD 1.44 and A-0.14.(Table-2). However, these all chromosomes 1, 2, 9, and 10 containing QTLs under 02428 japonica background had been found to be the common chromosomes with those of MH63 *indica* background containing QTLs for root length under aluminum stress toxicity tolerance at seedling stage in reciprocal introgression line populations of rice.

4 Linkage map and QTL distribution on each chromosome as per their position

A total of 265 polymorphic simple sequence repeats (SSR) markers were used to assay the RILs. Marker position and genetic distance among all linked markers followed the cornell map [25], which covered all 12 rice chromosomes with a total genome size of 1132.87 cM and an average distance of 4.27 cM between adjacent markers (Figure1). Chromosome number is indicated at the top. The distance between markers is given in Kosambi cent Morgan [18]. The positions of QTLs are indicated by vertical bars drawn equal to length as detected for the QTL in Map Marker/QTL.

5 Discussion

The plant root growth and its development is usually taken as a valid criteria for the measurement of aluminum (Al^{3+}) toxicity tolerance of rice crop [22], [29], [30]. Therefore, besides genotypic data, the phenotypic data for root length of control (untreated) and treated plants as well as relative root elongation (RRE) were kept as a base for the identification of QTLs for Al^{3+} toxicity tolerance in RILs of both MH63 indica and 02482 japonica backgrounds. In comparison of QTL analysis and their distribution on chromosomes for Al^{3+} stress toxicity tolerance related traits in rice through this study with earlier similar work done by other scientists, for Al^{3+} stress toxicity tolerance in rice, we detected 17 QTLs for the root length trait in both MH63 *indica* and 02428 japonica backgrounds on each chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. However, Nguyen *et al.* (2002) [31] had detected a total of 20 QTLs controlling root growth under aluminum stress control and relative root length conditions in rice on 12 chromosomes except chromosome 5 and 11 had also gave a great strength to our identified QTLs under these all experimental conditions control, Al toxicity stress and relative root elongation (RRE), which would be further unutilized in future research for the development of aluminum toxicity tolerant varieties. Our these results are similar with that of Nguyen *et al* (2002) [31], they had also identified 09 aluminum toxicity tolerant QTLs from root in rice under all experimental conditions control, aluminum toxicity stress and relative root length on chromosomes 1, 2, 3, 7, 8, 9, and 12.

In above referred both studies, the identified QTLs were commonly detected on common chromosomes 1, 2, 3, 7, 8, 9, 10 and 12. But in our study, we could detect some more QTLs on chromosomes 4, 5, 6, and 11, which proved that MH63 *indica* had some more Al toxicity tolerant QTLs than the populations used by afore mentioned scientists. Jian *et al.* (2002) [17] had detected 03 aluminum toxicity tolerant QTLs for relative root elongation in rice; each on chromosome 1, 2, and 6. In terms of relative root elongation (RRE), we had identified a total of nine QTLs one on each chromosome 1, 3, 4, 6, and 10 in MH63 *indica* background and two common QTLs on each chromosome 2 and 9 in MH63 indica and 0248 japonica backgrounds. The QTLs identified in our study for RRE indicated that MH63 indica and 02428 japonica backgrounds had greater number of QTLs than the Kasalath an *indica* and Koshikari japonica populations for same trait of evaluation (root). Mao *et al.* (2004) [32] had identified one QTL in rice for root elongation on chromosome 1and three QTLs for relative root elongation (RRE) one on each chromosome 1, 9 and 12. Our study had also matched with that study of Mao et al., (2004) [32] we had also identified two QTLs on common chromosome 1, and one QTL on chromosome 1 in MH63 *indica* and two QTLs on chromosome 9 in MH63 indica as well as in 02428 japonica backgrounds, showed that these common QTLs could be very much useful in further development of Al toxicity tolerant varieties in future. Adam *et al.* (2011) [20] had detected three QTLs by using total root growth each on chromosome 1, 2, and 12 indicated that these QTLs were strong QTLs which were commonly found by many scientists including our this study.

Nevertheless, our results for detection of Al toxicity tolerant QTLs in rice after 15 days of Al toxicity stress are quietly differing from the QTLs detected by Wu *et al*. (1999) [33], in which they could identify only one QTL on chromosome 1 after two weeks of Al stress and one QTL on chromosome 12 after four weeks of Al stress.

In this way, our results for QTL analysis had showed a new ray of hope for rice scientists to take a new step into the field of Al toxicity tolerant rice varieties. Our study strongly supported the study of Yong *et al*. (2006) [34] in which they have also identified seven QTLs, including three QTLs for control root elongation on chromosome 6, 8, and 9, one QTL for aluminum toxicity stress root elongation on chromosome 4, and three QTLs for relative root elongation (RRE) on chromosome 5, 9, and 10. However, we had identified twelve QTLs, three of them are common QTLs with their study for control root elongation after application of aluminum toxicity stress on chromosome 6, 8, and 9, for root elongation under stress, we had detected 15 QTLs, one of them is also a common QTL detected by them and us on chromosome 4, for RRE, we had identified 9 QTLs, out of these, two QTLs had been commonly detected in these two studies on chromosome 6 and 10. It is very much interesting to note that chromosomes 1, 2, 9, and 10 containing QTLs under 02428 japonica backgrounds had been found to be the common chromosomes with those of MH63 *indica* background containing QTLs for root length under aluminum stress toxicity tolerance at seedling stage in reciprocal introgression line populations of rice.

6 Conclusion

In spite of toxic effects of aluminum toxicity stress on two sets of reciprocal introgression lines (RILs) and their respective parents in MH63 *indica* and 02428 japonica backgrounds, these all RILs had some important Al^{3+} stress toxicity tolerance QTLs, which might lead us to further enhancement to evolve and release Al^{3+} toxicity tolerant varieties in future. It is also great success of our this study that we could prepare the "QTL map" ever first time for 12 chromosomes showing marker distance between two makers and different legends for different QTLs in each MH63 indica and 02428 japonica backgrounds. This QTL map would also be highly useful to future scientists to study the QTL mapping for other environmental stresses in both MH63 indica and 02428 japonica background populations.

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Table 1. QTLs for related traits of root elongation detected in the reciprocal introgression under MH63 *indica* background

					RL1		RL2		RL3		RRE	
Trait	QTL	Chr	Marker intervals	Position	LOD	Ad	LOD	Ad	LOD	Ad	LOD	Ad
RL	QRl1a	1	M6-M7	35	7.07	0.73	6.45	0.76	4.92	0.54		
	QRl1b	1	M31-M32	122	2.15	0.46	4.67	0.44	6.91	0.37		
	QRl2	2	M39-M40	49	5.77	0.98	3.15	0.67	9.97	1.12	4.58	5.31
	QRl3	3	M70-M71	61	3.09	0.64	2.68	0.61	12.41	0.77	2.45	3.83
	QRl4	4	M103-M104	53.03	2.2	0.29	3.15	0.47	5.94	1.11	2.97	4.91
	QRl5	5	M128-M129	58	3.16	0.75	3	0.74	5.3	1.23		
	QRl6	6	M136-M137	2	2.46	1.1	4.08	0.82	8.66	1.21	2.18	2.54
	QRl7	7	M177-M178	74	3.4	0.94	2.58	0.46	8.04	1.2		
	QRl8	8	M184-M185	42	4.4	0.96	4.18	0.95	15.76	1.23		
	QRl9	9	M198-M199	17	2.05	0.63	2.01	0.6	3.51	1.17	2.84	3.63
	QRl10	10	M221-M223	38	10.79	0.84	2.06	0.57	2.93	0.53	2.52	4.03
	QRl11	11	M243-M244	47	6.75	0.87			7.74	1.03		
	QRl12	12	M258-M259	38	4.04	0.72			7.78	1.2		

Trait= Trait root length (RL) for which QTLs were detected.
QTL= Name given to detected QTLs as per related trait and chromosome number.
Chr= Chromosome number on which QTL was detected.
Marker interval= It is the interval in which QTL is located.
Position= Most probable position of the QTL after analysis.
Control= control environmental condition for which no any stress toxicity was applied.
LOD= Logarithm of odds (LOD score).
Ad= (additive effect) is associated with substitution effect of the recipient allele by the donor allele.

Table 2. QTLs for related traits of root elongation detected in the reciprocal introgression lines under 02428japonica background

					RL1		RL2		RL3		RRE	
Trait	QTL	Chr	Marker intervals	Position	LOD	Ad	LOD	Ad	LOD	Ad	LOD	Ad
RL	QRl1	1	M31-M32	152	2.29	0.4					1.32	1.9
	QRl2	2	M39-M40	48	2.27	-0.2			1.48	-0.17	4.58	1.63
	QRl9	9	M199-M200	36	1.63	-0.2	1.49	-0.26	1.31	-0.19		
	QRl10	10	M209-M210	30	1.44							

Abbreviations and other related information for this Table is as same as for Table1.

Figure1.

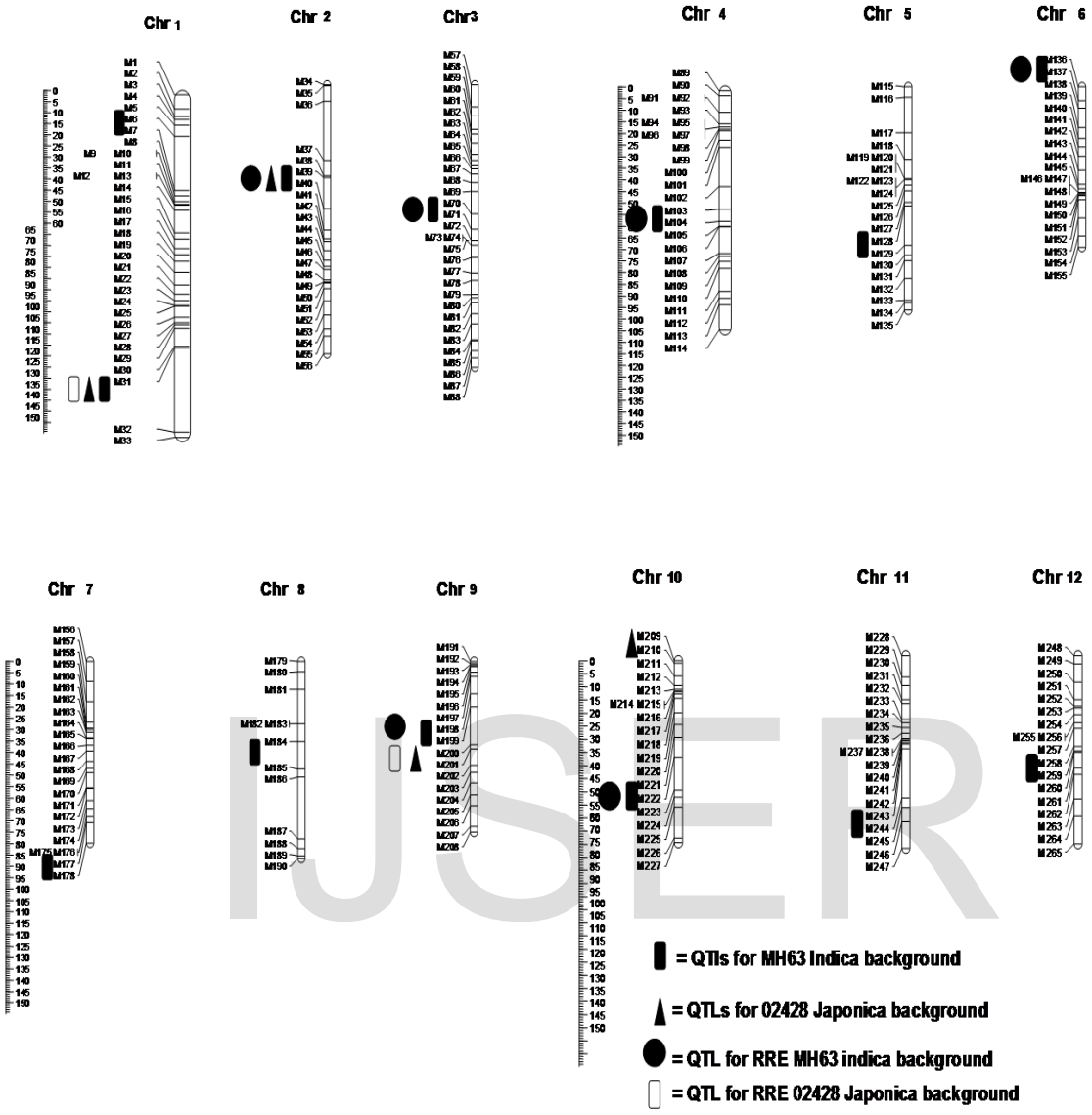

Figure1. Linkage map and distribution of detected QTLs on each chromosome (Chr) for aluminum toxicity tolerance