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Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.)

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Abstract This study was conducted to identify and map the quantitative trait locus (QTL) controlling Al tolerance in rice using molecular markers. A population of 171 F_6 recombinant inbred lines (RILs) derived from the cross of *Oryza sativa* (IR64), the Al susceptible parent, and *Oryza rufipogon*, the Al tolerant parent, was evaluated for Al tolerance using a nutrient solution with and without 40 ppm of active Al^{+3}. A genetic map, consisting of 151 molecular markers covering 1,755 cM with an average distance of 11.6 cM between loci, was constructed. Nine QTLs were dentified including one for root length under non-stress conditions (CRL), three for root length under Al stress (SRL) and five for relative root length (RRL). *O. rufipogon* contributed favorable alleles for each of the five QTLs for RRL, which is a primary parameter for Al tolerance, and individually they explained 9.0–24.9% of the phenotypic variation. Epistatic analysis revealed that CRL was conditioned by an epistatic effect, whereas SRL and RRL were controlled by additive effects. Comparative genetic analysis showed that QTLs for RRL, which mapped on chromosomes 1 and 9, appear to be consistent among different rice populations. Interestingly, a major QTL for RRL, which explained 24.9% of the phenotypic variation, was found on chromosome 3 of rice, which is conserved across cereal species. These results indicate the possibilities to use marker-assisted selection and pyramiding QTLs for enhancing Al tolerance in rice. Positional cloning of such QTLs introgressed from *O. rufipogon* will provide a better understanding of the Al tolerance mechanism in rice and the evolutionary genetics of plant adaptation to acid-soil conditions across cereal species.

Keywords Al tolerance · Abiotic stress · QTL mapping · Wild rice · *Oryza sativa*

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Introduction

Crop productivity in acid-soil areas is restricted by multiple abiotic stress factors. Since the forms of soil aluminum (Al) and their solubilities are high at a pH of 5 or less, Al toxicity becomes one of the major growth-limiting factors in acid soils (Kochian 1995). The initial and most dramatic symptom of Al toxicity is inhibition of root elongation as a consequence of toxicity to the root apex (Kochian 1995). Roots injured by high Al concentrations are usually stubby, thick, dark-colored, brittle, poorly branched, with reduced root length and volume. Al toxicity may inhibit shoot growth by limiting the supply of nutrients and water due to poor subsoil penetration or lower root hydraulic conductivity.

There is an urgent need to broaden the gene pool of rice for enhancing tolerance to Al toxicity. Wild species of *Oryza* are an important reservoir of useful genes for tolerance to biotic and abiotic stresses. *Oryza rufipogon* Griff is an ancestor of cultivated rice (*Oryza sativa* L.) and possesses many valuable genes for Al tolerance, resistance to bacterial blight and tungro virus, and cytoplasmic male sterility (Brar and Khush 1997). However, the progress in breeding rice for Al tolerance utilizing *O. rufipogon* genetic resources has been slow because of the lack of understanding on the genetic control of the trait. With the advent of molecular techniques, it is now

Table 1 Descriptive statistics of three traits in rice, measured on 154 RILs and two parental lines, in four replications

Parameter	Minimum	Maximum	Mean	CV[a] (%)	$LSD^{b}_{0.01}$
Control root length (cm)					
IR64	–	–	11.30**	–	–
O. rufipogon	–	–	7.10**	–	–
RILs	4.48	19.28	9.98	11.12	2.02
Stress root length (cm)					
IR64	–	–	2.56**	–	–
O. rufipogon	–	–	5.50**	–	–
RILs	1.76	9.83	3.99	16.18	1.18
Relative root length[c]					
IR64	–	–	0.20**	–	–
O. rufipogon	–	–	0.77**	–	–
RILs	0.18	0.88	0.41	18.93	14.30

** Significant difference at the 1% probability level
[a] Coefficient of variation
[b] Least significant difference at the 1% probability level
[c] Root length ratio: root length under stress over control conditions

possible to identify and map genes conferring Al tolerance and to develop marker-assisted selection for rice improvement.

The exact nature of the molecular mechanisms for Al tolerance in plants is not well understood. Among cereals, rye is considered to be most tolerant to Al toxicity. Aniol and Gustafson (1984) found that Al tolerance in rye is controlled by major genes located on chromosomes 3R and 6R, with other genes on chromosome 4R. Two dominant loci (*Alt1* and *Alt3*) have been mapped on chromosomes 6R and 4R, respectively (Gallego et al. 1998; Miftahudin et al. 2002). In wheat, both major genes (2–3 dominant genes) as well as a polygene controlling Al tolerance have been reported (Aniol and Gustafson 1984). Riede and Anderson (1996) identified a major gene Alt_{BH} responsible for Al tolerance on chromosome 4DL, which accounted for 85% of the total phenotypic variation. Tang et al. (2000) mapped a gene for Al tolerance on the long arm of chromosome 4H of barley, 2.1-cM proximal to the marker *Xbcd*117 and 2.1-cM distal to the markers *Xwg*464 and *Xcdo*1395. The findings from a number of laboratories have indicated that in maize Al tolerance is controlled by multiple genes (Magnavaca et al. 1987). Studies on the genetic control of Al tolerance in rice are limited. Using molecular techniques, Wu et al. (2000) identified several QTLs conferring Al tolerance in a random inbred mapping population derived from Azucena and IR1552. Nguyen et al. (2001) also detected five QTLs for Al tolerance scattered on five chromosomes with a major QTL located on chromosome 1. In another study, Nguyen et al. (2002) found ten QTLs located on nine chromosomes for Al tolerance using a doubled-haploid population derived from the cross of CT9993 × IR62266. These findings suggest that Al tolerance in rice is a complex character. However, so far, no QTL for Al tolerance has been identified from wild species of *Oryza*.

The objectives of our investigation were: (1) to identify QTLs/genes for Al tolerance from *O. rufipogon*, and (2) to compare the location of genes for Al tolerance across different genetic backgrounds of rice and other cereals.

Materials and methods

Plant material

A subset of 171 F_6 recombinant inbred lines (RILs), selected randomly from a population of 312 lines derived from a cross between *O. sativa* cv IR64 and the wild species, *O. rufipogon* (Acc 106424) was used. The RILs were developed by single-seed descent to the F_6 generation at the International Rice Research Institute (IRRI), The Philippines. Panicles were bagged for each generation in order to avoid possible outcrossing. IR64, a high yielding indica rice cultivar with excellent grain quality is widely grown in Asia but is susceptible to Al toxicity (Khatiwada et al. 1996). *O. rufipogon*, a diploid wild species which grows naturally in acidic soils of Vietnam was collected and used in crossing with IR64.

Screening for Al tolerance

The parents along with 171 RILs were screened for Al tolerance in the Plant Molecular Genetics Laboratory, Texas Tech University, in 2000 using a nutrient culture solution following the method reported by Khatiwada et al. (1996) with some modifications. Seeds with uniform size were grown on filter papers soaked with distilled water and kept in the dark at 30 °C for 2 days. Germinated seeds were rolled in a germinating paper and kept at 30 °C for another 36 h. The seedlings with uniform roots were then transferred to a styrofoam sheet with a nylon net bottom with one seedling per hole and ten seedlings in one row per line in each replication. The sheets were floated on 4 l of nutrient solution (Yoshida et al. 1976) in a plastic tray (40 × 25 × 7 cm) for 14 days. Screening was carried out using 0 ppm (non-stress or control condition) and 40 ppm (equivalent to 1.48 mM) of active Al^{+3} from $AlCl_3{\cdot}6H_2O$ at pH 4.0. The nutrient solution was changed daily to avoid fluctuation of the pH. The hydroponic trays and seedlings were maintained in the culture room at 27 ± 2 °C with 12 h of light at 300 PPFD (photo proton flux density).

The experiment was arranged as a randomized complete block design (RCBD) with four replications. At sampling, the longest root of ten seedlings of each RIL in each replication was measured and the mean was determined. The ratio of the average root length under stress over non-stress conditions was used as relative root length (RRL), a measurement of root tolerance to Al toxicity.

Genotypic analysis

Genomic DNA was isolated from lyophilized leaf tissue of 30 day old seedlings of RILs using the potassium acetate method (Tai and Tanksley 1990). Restriction fragment length polymorphism (RFLP) analysis was performed following the procedure of Causse et al. (1994). Five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and

*Xba*I) were used to digest DNA for the RFLP analysis. Microsatellite primer pairs (Research Genetics Inc., Huntsville, Ala. USA) were employed to amplify the simple-sequence length polymorphic DNA according to Chen et al. (1997). DNA bands were visualized via silver staining as described by Panaud et al. (1996).

RNA was isolated from root tips of IR64 and *O. rufipogon* according to Logemann et al. (1987). Root tip samples (0.5–1.0 cm) were collected from IR64 and *O. rufipogon* (Acc 106424) plants treated with 0 and 40 ppm of Al for 4 h. The RNA samples were used for reverse-transcription and differential display analysis (Liang and Pardee 1992, and GeneHunter Corp. Nashville, Tenn., USA). Induced bands of IR64 and *O. rufipogon* under stress conditions were excised, cloned, and amplified according to the GeneHunter's manual. The cDNA fragments with correct size were used to survey for polymorphism between IR64 and O. *rufipogon*.

Map construction and QTL analysis

The program MAPMAKER/EXP v.3.0 (Lander et al. 1987; Lincoln et al. 1992) was employed to establish a genetic linkage map using the Kosambi mapping function (Kosambi 1944). Linkage groups were inferred based on the existing RFLP and microsatellite maps of rice (Causse et al. 1994; Harushima et al. 1998; Temnykh et al. 2001). MAPMAKER/QTL version 1.1 was used to identify loci affecting quantitative traits on the basis of interval analysis (Paterson et al. 1988; Lincoln et al. 1992). A LOD score of 2.4 was selected as the threshold for the presence of a QTL based on the total map distance, and the average distance between markers (Lander and Bostein 1989). With such a threshold, a false positive QTL would be detected anywhere in the genome with a probability of approximately 0.05 (Paterson et al. 1988). The independence test was performed where the initial scan suggested two or more QTLs located on the same chromosome as described by Paterson et al. (1988) and Lander and Bostein (1989). The total phenotypic variation explained by all QTLs was estimated by fitting a multiple regression model in the MAPMAKER/QTL program. The interaction between all possible loci on the map was performed using QTLMapper version 1.0 (Wang et al. 1999).

QTL nomenclature

The QTL was designated as *Q* to indicate that it was detected through QTL mapping, followed by *Al* for Al tolerance and the chromosome number. A final letter was used when more than one QTL affecting the trait was identified on the same chromosome.

Results

Phenotypic evaluation for tolerance to aluminum toxicity

The mean, range and distribution for the three traits, control root length (CRL), stress root length (SRL) and relative root length (RRL), for the RI population and their parents are summarized in Table 1 and Fig. 1. Analysis of variance (ANOVA) showed the presence of significant genetic differences between the two parents and among RI progenies for all three traits (Table 1). IR64 showed a higher CRL than *O. rufipogon* but the latter had a higher SRL and RRL, thus confirming the tolerance of *O. rufipogon* to aluminum toxicity. The frequency distribution of CRL, SRL and RRL of RI progeny did not fit a normal distribution according to the Shapiro-Wilk test. However, the wide range of phenotypic distribution suggested that the traits are quantitative in nature.

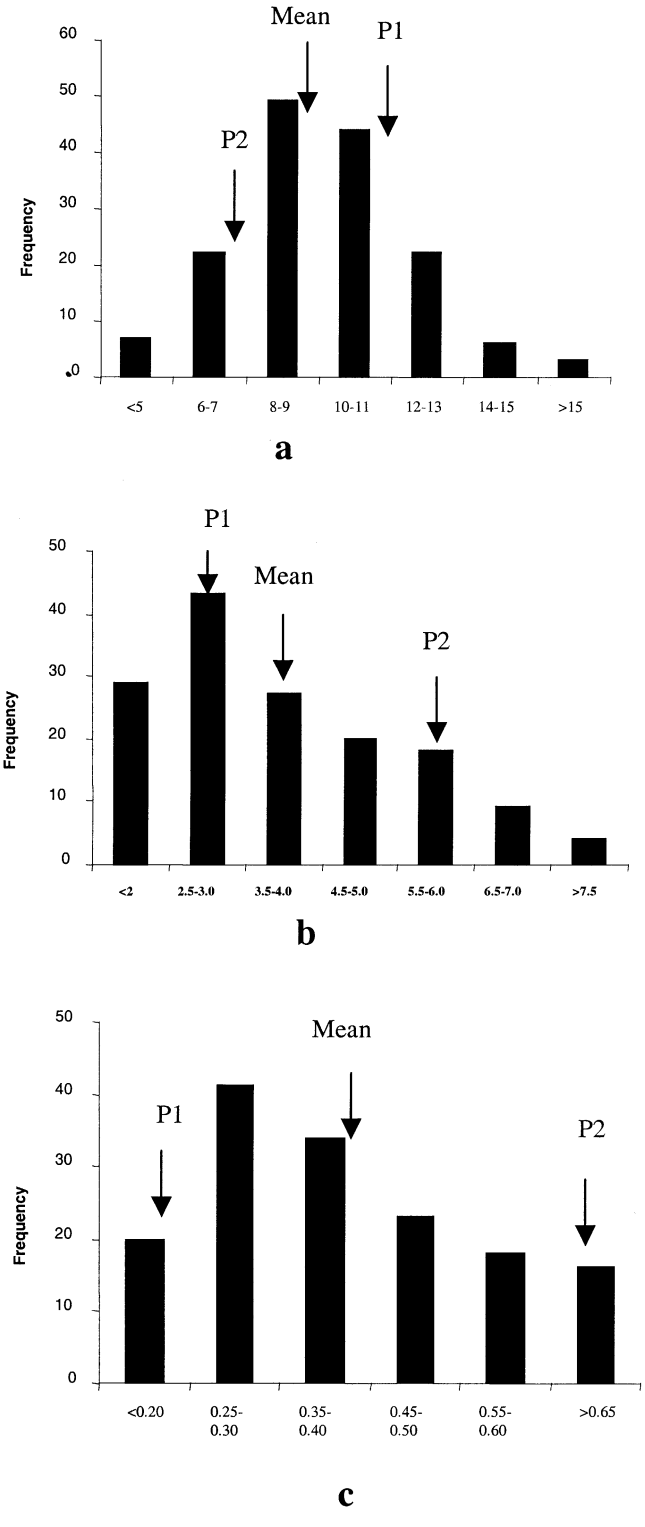


Fig. 1a–c Phenotypic distribution for root traits of 154 RIL-F6 derived from the cross of *O. sativa* cv IR64 × *O. rufipogon* (Acc 106424) under normal and Al stress conditions. **a** Distribution for control root length. **b** Distribution for stress root length. **c** Distribution for relative root length. P1: IR64; P2: *O. rufipogon* (acc 106424); Mean: average value of 154 RILs

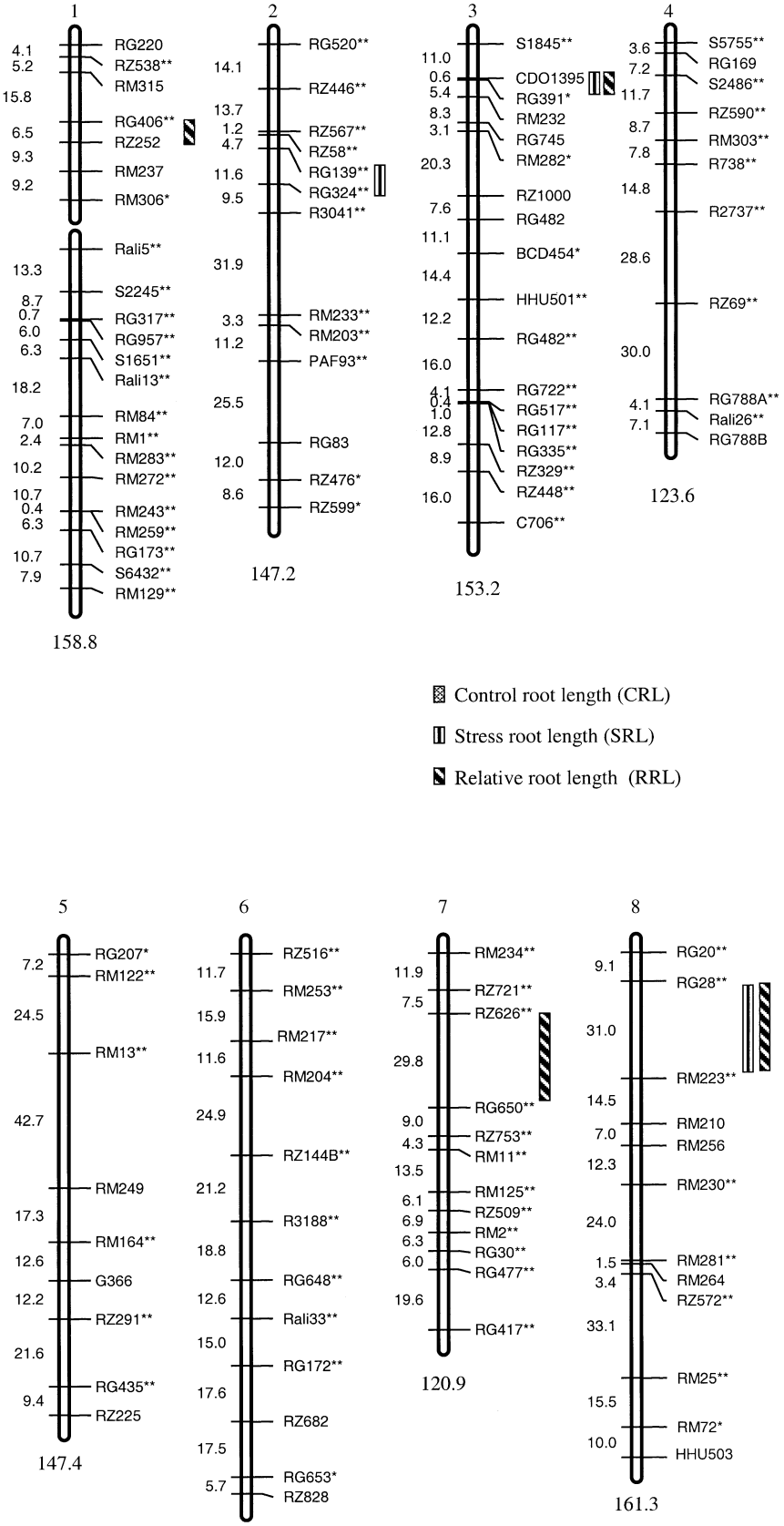
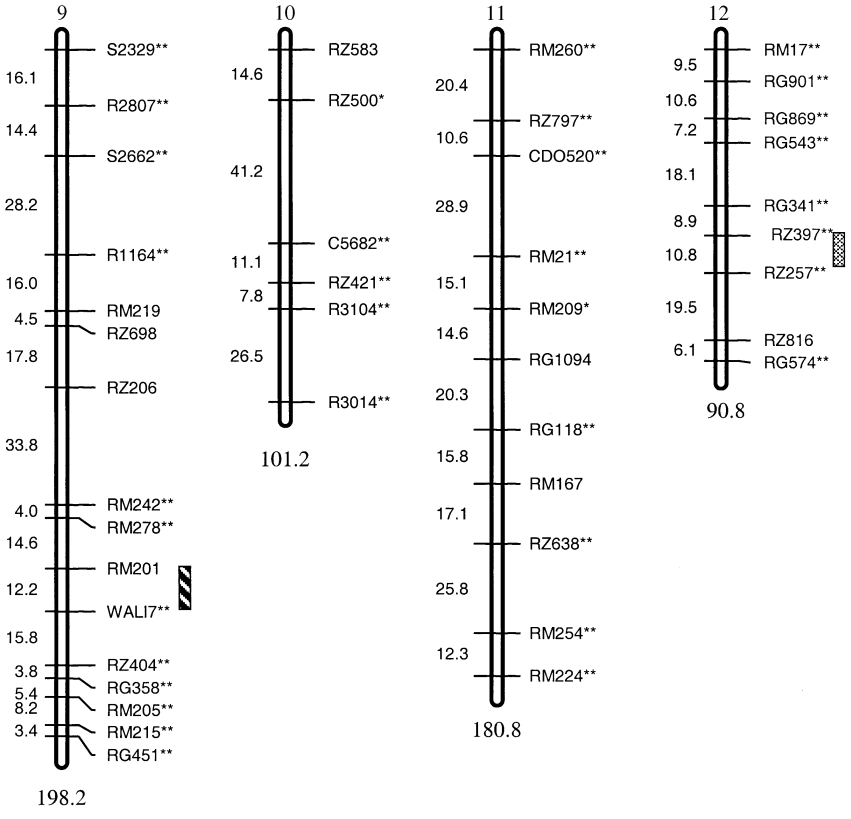


Fig. 2 The molecular linkage map with 151 marker loci constructed from 171 RILs derived from the cross of *O. sativa* cv IR64 × *O. rufipogon* (Acc 106424). Chromosome numbers are indicated at the top. The distance between markers is given in Kosambi centiMorgan. The loci with * and ** show significant segregation skewness at 0.05 and 0.01, respectively. Figures below each chromosome indicate the length of the chromosome in cM. The positions of QTLs are indicated by *vertical bars* drawn equal to the length as detected for the QTL in the MapMaker/QTL

Fig. 2 (continued)



Genetic linkage map construction

Parental polymorphism survey

Five restriction enzymes namely *Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII and *Xba*I, were used to digest genomic DNA of the parents. A total of 400 RFLP rice probes from the Cornell University, New York, and the Rice Genomic Research Program (RGP), Japan, along with 16 differential display fragments in relation to Al stress were used for the parental polymorphism survey. Of these, 274 (65.9%) probes detected polymorphism with at least one of the five restriction enzymes (data not given). The level of polymorphism revealed by each enzyme ranged from 12.5% for *Eco*RV to 27.7% for *Hin*dIII, followed by 19.8% for *Dra*I, 19.5% for *Xba*I and 14.0% for *Eco*RI. Among 168 SSR markers used in the parental survey, 112 (66.7%) were polymorphic between *O. sativa* and *O. rufipogon*. The level of polymorphism detected by microsatellites was similar to that detected by RFLP markers. In addition, among 13 candidate genes for Al tolerance identified from the GenBank database, only four (30.8%) detected polymorphisms between the two parents. This result is not unusual because most of the putative candidate genes are of differential display fragments from wheat and soybean. Overall, 390 markers from a total of 597 (65.3%) were polymorphic between IR64 and *O. rufipogon* (Acc 106424).

Marker segregation and map construction

Two hundred and thirty eight markers showing clear polymorphisms between the two parents were chosen for the progeny survey. Subsequently, 151 marker loci were mapped and used for QTL analysis in the RI lines. Most of the markers showed a single hybridizing band except for RG788, which exhibited two copies. This marker was coded with an A and B suffix. On the average, the non-parental and null alleles (complete lack of signal) were detected among the RI lines at a frequency of 7.3%. These cases were treated as missing data. The segregation of the 151 markers included in the genetic linkage map was tested for goodness of fit using the χ^2 test. The results revealed that most markers (122) deviated from the expected 1:1 Mendelian ratio, which accounted for 80.7% of the total. Most of the markers, 118 out of 151, were skewed toward IR64. Only four were skewed toward *O. rufipogon*, and 29 loci had at least 50% alleles from *O. rufipogon*. The skewness in this RI population (an overall average of 60% IR64 alleles and 40% *O. rufipogon* alleles) affected the ability to map the markers *de novo* based on the segregation data of RI lines alone.

The strategy for constructing a linkage map from a skewed population suggested by Wang et al. (1994) was followed. First, the markers belonging to the same linkage group were assigned based on the high-density genetic linkage maps of rice (Causse et al. 1994; Harushima et al. 1998; Temnykh et al. 2001). Then, the most-likely order and recombination fraction on the chromosome

Table 2 Putative QTLs detected for control root length (CRL), stress root length (SRL) and relative root length (RRL)

Trait	QTL	Interval	Chromosome	Length[a]	Position[b]	Additive effect[c]	LOD[d]	R^2 (%)[e]
CRL	*QAlCr12.1*	RG341-RZ397	12	8.9	4.0	1.834 (I)	2.8	10.3
SRL	*QAlSr2.1*	RG139-RG324	2	11.6	8.0	3.235 (O)	2.9	26.4
	QAlSr3.1	CDO1395-RG391	3	0.5	0.0	1.478 (O)	6.2	18.7
	QalSr8.1	RG28-RM223	8	31.0	18.0	1.915 (O)	3.1	20.8
Best multiple QTL model							45.6	
RRL	*QAlRr1.1*	RG406-RZ252	1	6.5	6.0	0.100 (O)	2.4	9.0
	QAlRr3.1	CDO1395-RG391	3	0.6	0.0	0.167 (O)	8.3	24.9
	QAlRr7.1	RZ629-RG650	7	29.8	18.0	0.126 (O)	5.4	22.5
	QalRr8.1	RG28-RM223	8	31.0	18.0	0.104 (O)	2.5	20.8
	QAlRr9.1	RM201-WALI7	9	10.0	8.0	0.109 (O)	2.6	9.9
Best multiple QTL model[f]								70.8

[a] Interval between the two markers (cM) where the QTL is located
[b] QTL position from the first marker (cM)
[c] The allelic genetic effect and the O and I meant that the favorable alleles were derived from *O. rufipogon* and IR64, respectively
[d] Maximum-likelihood LOD score for the individual QTL
[e] Phenotypic variation explained by the individual QTL
[f] Phenotypic variance explained by the QTLs collectively

were established by using command 'Three point' and 'First order' at a LOD score of 3.0, based on data from the RI population. For the markers whose locations were unknown, such as markers derived from the differential display analysis or candidate gene, the two-point analysis with a LOD score of 10–12 was applied to find the linkage group. The command 'Try' was used for the unlinked markers. As a result, 151 marker loci were assigned to 13 linkage groups (chromosome 1 had two linkage groups). Chromosomes were oriented with the short arm at the top based on the centromere location (Singh et al. 1996). The order of markers was in good agreement with the maps developed by Cornell University (Causse et al. 1994; Temnykh et al. 2001), the Rice Genome Program (RGP), Japan (Harushima et al. 1998), and the rice maps developed from *O. sativa* and *O. rufipogon* populations (Xiong et al. 1997; Xiao et al. 1998). Two possible inversions were found, one on chromosome 2 among markers RG83, RZ476 and RZ599, another on chromosome 3 between markers RZ1000 and RG482. The total map length was 1,755 cM with the average distance of 11.6 between markers (Fig. 2). Chromosome 10 was the least representative. Although 12 SSR and 20 RFLP markers located on this chromosome were used to survey the parents, only six markers were mapped. The low polymorphism on chromosome 10 suggests that some regions of the cultivated and wild species genomes may be common by descent, or that the *O. rufipogon* may be a derivative of a hybrid between wild *O. rufipogon* and cultivated *O. sativa* resulting from the proximity of wild relatives to farmer's fields throughout Asia.

QTL mapping for Al tolerance

Nine QTLs with a LOD threshold of 2.4 were identified for the three traits. The putative QTLs, their respective chromosomal locations, LOD scores, percentages of variance explained, and additive effects are presented in Table 2. The number of QTLs identified for individual traits ranged from one (for CRL) to five (for RRL) with the phenotypic variation ranging from 9.0 to 26.4%. The locations of these QTLs on the linkage map are shown in Fig. 2.

Control root length (CRL)

Only one QTL, *QAlCr12.1*, located on chromosome 12 flanked by RG341 and RZ397, was identified for controlling root length under the non-stress Al condition. This QTL explained 10.3% of the total phenotypic variation with a favorable allele (longer root length) contributed from IR64. The increase of root length due to this favorable allele is 1.83 cm.

Stress root length (SRL)

Three QTLs affecting root length under stress conditions were detected on chromosomes 2, 3 and 8. The *QAlSr2.1* located on chromosome 2 flanked by RG139 and RG324 had the highest R^2 value (26.4%), followed by *QAlSr8.1* flanked by markers RG28 and RM223 on chromosome 8 (20.8%), and then *QAlSr3.1* on chromosome 3 harbored by CDO1395 and RG391 (18.2%). These QTLs together accounted for 45.5% of the total phenotypic variation. *O. rufipogon* had favorable alleles (longer root length) for all three QTLs. The allelic effects ranged from 1.46 cm for *QAlSr3.1* to 3.24 cm for *QAlSr2.1*.

Relative root length (RRL)

Five QTLs, *QAlRr1.1*, *QAlRr3.1*, *QAlRr7.1*, *QAlRr8.1* and *QAlRr9.1*, were identified on chromosomes 1, 3, 7, 8

Table 3 Epistasis analysis for gene loci controlling root length (CRL), stress root length (SRL) and relative root length (RRL) by interval mapping via QTLMapper version 1.0 of the IR64 × *O. rufipogon* (Acc106424) RI population

Trait	Chr.	Interval (*i*)	Chr.	Interval (*j*)	A(*i*)[a]	A(*j*)[b]	AA(*ij*)[c]	LOD
CRL	2	RM233-RM203	5	RM249-RM164	0.03	0.18	1.39**	2.84
	4	S2486-RZ590	12	RM17-RG901	0.43	0.29	1.19**	3.11
	6	Rali33-RG172	12	RM17-RG901	0.18	0.53	1.60**	4.16
	7	RZ509-RM2	9	Wali7-RZ404	0.18	0.32	1.92**	3.82
				R^2 (%)[d]	0.00	0.00	51.21	
SRL	3	S1845-CDO1395	6	RG653-RZ828	1.00**	0.10	0.39	5.20
				R^2 (%)	22.84	0.00	0.00	
RRL	1	RM1-RM283	3	CDO1395-RG391	0.030	0.112**	0.033	7.73
	4	Rali26-RG788B	5	RM249-RM164	0.024	0.065**	0.006	3.34
	9	RM201-Wali7	12	RG543-RG341	0.029	0.036	0.060	3.35
				R^2 (%)	0.00	36.59	0.00	

*, ** Significant levels at the 0.05 and 0.01 probability, respectively

[a] Allelic effect at site (i)

[b] Allelic effect at site (j)

[c] Non-allelic interaction between sites (i) and (j)

[d] Total phenotypic explained by site (i), (j), and the epistatic effect

and 9, respectively, for relative root length. *O. rufipogon* contributed favorable alleles (less impaired by stress) for all five QTLs. The phenotypic variation explained by individual QTLs ranged from 9.0% for *QAlRr1.1* to 24.9% for *QAlRr3.1*. A full model containing the five QTLs explained 70.8% of the phenotypic variation. The allelic effects of the favorable alleles ranged from 10.0% for *QAlRr1.1* to 16.7% for *QAlRr3.1*.

Epistatic analysis

Epistasis is an important genetic factor for complex traits. The interaction between QTLs and background or modifying loci might be the predominant form of epistasis of quantitative traits (Yu et al. 1997). Epistatic analysis between all possible loci in the map for the three traits was performed using QTLMapper version 1.0, and the results are presented in Table 3.

Control root length (CRL)

Four pairs of epistatic loci were identified for the control of root length. The allelic effect either for the testing point *i* or *j* for this trait was found to be non-significant and produced no effect on phenotypic variation. However, the non-allelic interaction effect was found to be highly significant and explained 51.2% of the total phenotypic variation (Table 3). The results suggest that the phenotypic variation of this trait was mainly due to an epistatic effect.

Stress root length (SRL)

One region on chromosome 3 flanked by S1845-CDO1395 was found to have an interaction with the region harbored by RG653-RZ828 on chromosome 6. The allelic effect at site *i* was significant and explained 22.8% of the total phenotypic variation. However, the allelic effect at site *j* and the non-allelic interaction effect between sites *i* and *j* were found to be non-significant and had no effect on phenotypic variation.

Relative root length (RRL)

Six regions on six different chromosomes were found to have an interaction with each other. Only allelic effects at site *j* were found to be significant and explained 36.5% of the total phenotypic variation. Allelic effects at site *i* and epistatic effects between sites *i* and *j* were found to be non-significant and had no effect on phenotypic variation.

Comparison of QTLs for Al tolerance across different genetic backgrounds of rice

Relative root length (RRL) is the parameter most directly related to Al tolerance in rice and other crops. Therefore, QTLs controlling RRL in this study were used to compare with other published reports to identify the common QTLs for Al tolerance across rice genetic backgrounds. Of the five QTLs controlling RRL, two appear to be consistent with the QTLs detected in other populations. The QTL, *QAlRr1.1* (R^2 = 9.0%), on chromosome 1 was apparently located on the same chromosomal region as a major QTL for Al tolerance detected in IR1552 × Azucena (Wu et al. 2000), CT9993 × IR62266 (Nguyen et al. 2002) and OM269 × Chiembau (Nguyen et al. 2001) populations (Fig. 3a). Another genomic region on chromosome 9 harboring *QAlRr9.1* in this population appears to be at the same location with the QTL found in IR1552 × Azucena (Wu et al. 2000) and CT9993 × IR62266 (Nguyen et al. 2002) populations (Fig. 3b).

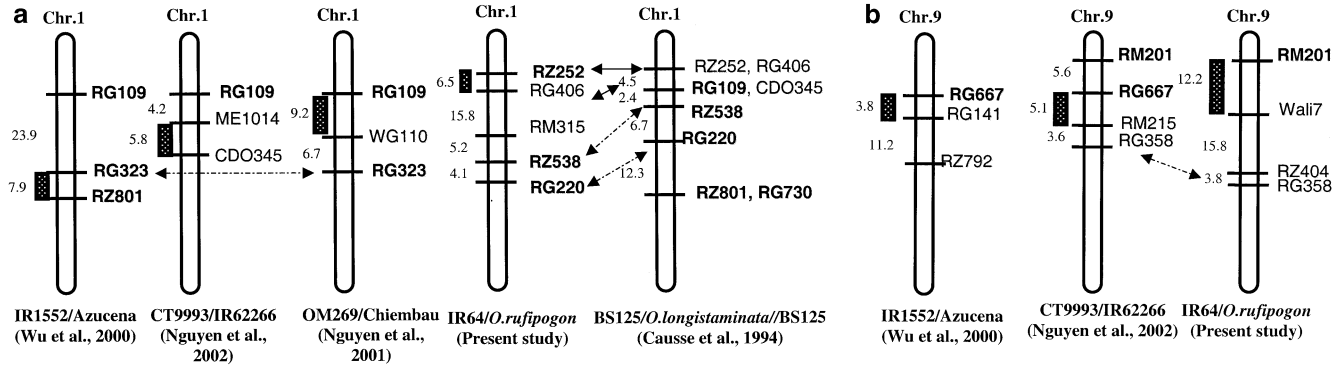


Fig. 3a, b Comparison of QTL controlling Al tolerance across different genetic backgrounds of rice: (**A**); chromosome 1, (**b**) chromosome 9. *Vertical bars* indicate the position of QTLs for Al tolerance

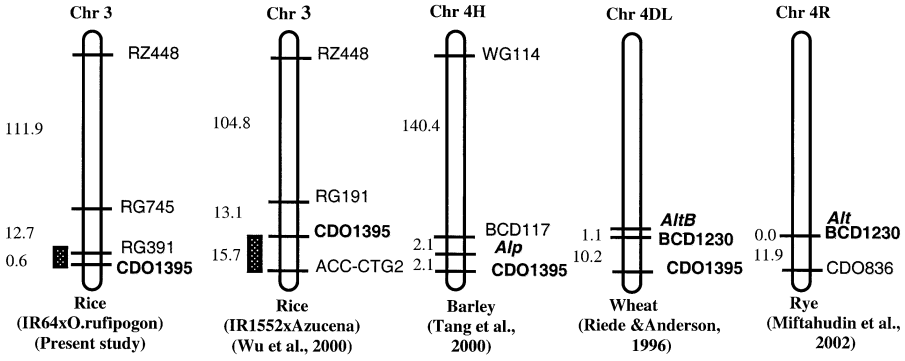


Fig. 4 Comparison of QTLs controlling Al tolerance across cereal crops. *Vertical bars* indicate the position of QTLs for Al tolerance

Comparison of QTLs for Al tolerance among cereal crops

To determine common QTLs between rice and other cereal species for Al tolerance, the results of this study were compared with those of wheat (Riede and Anderson 1996), rye (Miftahudin et al. 2002), maize (Sibov et al. 1998) and barley (Tang et al. 2000) using comparative maps (Ahn and Tanksley 1993; Gale and Devos 1998) and a comparative RFLP probe set from Cornell University. The analysis indicated that the QTL *QAlRr3.1* with the largest phenotypic effect (R^2 = 24.9%) in this population and the QTL with a small phenotypic effect (R^2 = 9.0%) in the IR1552 × Azucena population (Wu et al. 2000) located on rice chromosome 3 appear to be orthologous to the genomic region carrying the major Al tolerance gene on group 4 of the Triticeae (Fig. 4).

Discussion

Identification of QTLs for Al tolerance in *O. rufipogon*

A total of nine QTLs, mapped on seven different chromosomes, were identified for the three root traits (CRL, SRL and RRL) in this population. For CRL, one QTL on chromosome 12 with a favorable allele (longer root length) contributed from IR64 was identified by interval mapping. However, four pairs of epistatic loci were identified for CRL. The main effects of these loci did not produce any phenotypic variance, but when they interacted with each other the interactions produced a pronounced effect on the phenotypic variation (51.2%) for this trait (Table 3). The results suggest that epistasis accounts for a considerable portion of the total phenotypic variance of root length under non-stress conditions. The results are in good agreement with Zhang et al. (2001) where under a solution culture, one QTL explaining 12.2% for seminal root length in rice was found, but five pairs of epistatic loci for root length were identified, which together accounted for 60% of the total variation in this trait. In contrast, three QTLs together explaining 45.6% of the total phenotypic variation were identified controlling stress root length (SRL), with favorable alleles (longer root length) contributed from *O. rufipogon*. Epistatic analysis revealed that this trait is largely controlled by the main effect of the QTLs. Only one pair of epistatic loci was identified; however, it did not have any significant effect on the phenotypic variance.

The analysis revealed three epistatic loci for RRL. The major QTL, *QAlRr3.1*, on chromosome 3 had a non-allelic interaction with region RM1-RM283 on chromosome 1, although this interaction was not significant. It was similar to *QAlRr9.1* on chromosome 9 where the interaction between this QTL and another background locus on chromosome 12 was not significant. The total

phenotypic variation in relative root length was primarily controlled by the main effects of the QTLs. In a different rice population, Wu et al. (2000) detected three pairs of epistatic loci whose effects explained only about 20% of the total variation in relative root length. The epistatic analysis indicated that Al tolerance in rice seedling is predominantly controlled by additive effects. The results support the hypothesis that Al tolerance in rice is a quantitative trait controlled by additive genetic effects, similar to wheat (Bona et al. 1994) and maize (Lima et al. 1995). Results from the QTL and epistatic analyses in this study suggest that high yielding genotypes with enhanced Al tolerance could be obtained through a single cross between *O. rufipogon* as a donor of Al tolerance and an elite line with superior yield components. Furthermore, marker-assisted selection could be used to pyramid QTLs identified in this study for enhancing Al tolerance in rice. It is possible to selectively transfer the major QTL linked with the CDO1395 marker on chromosome 3 identified from *O. rufipogon* for aluminum tolerance.

Comparison of QTLs for Al tolerance across different genetic backgrounds of rice and cereal species

Molecular markers tightly linked to QTLs across different genetic backgrounds and environments would be highly useful in marker-assisted selection and early generation selection of desirable recombinants. So far, four populations have been used to map Al tolerance genes in rice. The QTLs controlling RRL, the most important parameter for Al tolerance, from the present study were compared to those identified in different rice populations. Of the five QTLs detected for Al tolerance, three appear to correspond to the QTLs identified in the previous studies. A QTL located on chromosome 1 in this study, *QAlRr1.1*, apparently mapped to the same chromosomal region with the QTL detected in IR1552 × Azucena (Wu et al. 2000), CT9993 × IR62266 (Nguyen et al. 2002) and OM269 × Chiembau (Nguyen et al. 2001) populations. Chromosome 1 has been partially sequenced by Japanese scientists. Of 418 BAC clones on chromosome 1, 353 clones were sequenced as of April 23rd 2002 (http://www.genome.clemson.edu/projects/rice/ccw/status/seqSum1.html). Using integrated genetic maps (http://shigen.lab.nig.ac.jp/rice/oryzabase/), it was found that RG406, one of the flanking markers of the QTL on chromosome 1, is about 0.3 cM from the R1416 marker. This marker anchored a BAC clone OSJNBa0014K08f on contig 20 in the Clemson physical map of rice. The sequence of this BAC was sent to the rice genome automated annotation system (http://ricegaas.dna.affrc.go.jp/) for gene analysis. No gene sequence related to the organic-acid exclusion mechanism was found in this BAC.

Another QTL, *QAlRr9.1*, with $R^2 = 9.9\%$ also appears to correspond to the QTL identified in the IR1552 × Azucena (Wu et al. 2000) and CT9993 × IR62266 (Nguyen et al. 2002) populations, except for the OM269 × Chiembau (Nguyen et al. 2001) population. CT9993 was found to be most tolerant to Al toxicity among several *O. sativa* genotypes tested by Nguyen et al. (2000). A major QTL ($R^2 = 28.7\%$) conferring Al tolerance in the CT9993 × IR62266 population was located on chromosome 8 flanked by C1121 and M53. One of the major QTLs identified in this study ($R^2 = 20.8\%$) was also located on chromosome 8 flanked by RG28 and RZ650. Due to the lack of common markers between two genetic maps, a comparison was performed using an integrated map from the Japanese Oryzabase (http://shigen.lab.nig.ac.jp/rice/oryzabase/). It was found that the marker C1121 was 27.7 cM from RG333, and the marker RG28 was 21.8 cM from RG333. It is likely that these QTLs are located on the same chromosomal region. These QTLs were unique in these two populations, which may explain the high capacity for Al tolerance in CT9993 and *O. rufipogon* Acc. 106424. Further fine-mapping of these QTL regions is suggested to verify that these QTLs are common in different rice genetic backgrounds.

It was interesting to note that the QTL with a largest effect, *QAlRr3.1* ($R^2 = 24.9\%$), in this study and the small QTL ($R^2 = 9.0\%$) in the IR1552 × Azucena population were located on chromosome 3, which corresponds to homoeologous chromosome group 4 of the Triticeae (Gale and Devos 1998). Previous reports showed that there is a conserved genomic region on the long arm of homoeologous chromosome 4 for Al tolerance among wheat (Alt_{BH}), rye (*Alt3*) and barley (*Alp*) (Miftahudin et al. 2002). The gene controlling Al tolerance in wheat and rye was tightly linked to marker BCD1230 and loosely linked to CDO1395; however, the gene controlling Al tolerance in barley was tightly linked to CDO1395. It was suggested that the Alt_{BH}, *Alt3* and *Alp* genes are orthologous loci because of the high level of synteny among chromosomes 4DL, 4RL and 4HL, and they may share a common function. One of the mechanisms for Al tolerance in the Triticeae is Al exclusion (Kochian 1995). This mechanism is mediated by Al-activated release of organic acids (malic acid), which chelate Al^{+3} in the rhizosphere and prevent its entry into the root apex. This physiological evidence is strongly supported by the orthologous loci controlling Al tolerance in the Triticeae.

The major QTL controlling Al tolerance mapped on rice chromosome 3 in this study appears to be orthologous with Al tolerance genes on group 4 of the Triticeae. When genetic mapping in collinear genomes pinpoints similar traits to the same chromosomal regions, there is a good reason to suspect that these loci are encoded by different alleles of a single gene (Bennetzen and Freeling 1997). However, the physiological evidence of Al tolerance in rice is still lacking. The parental screening showed that *O. rufipogon* has the highest Al tolerance capacity as compared to *O. sativa*. These results lead to a hypothesis that one of the mechanisms for Al tolerance in *O. rufipogon* may be common to that in the Triticeae, which is associated with Al exclusion. An attempt on

finding candidate genes conferring Al tolerance in the rice chromosome 3 harboring the Al tolerance QTL of interest was made by searching the rice sequence database. Out of 160 BAC clones on rice chromosome 3, only 53 clones have been sequenced as of April 23rd 2002 (http://www.genome.clemson.edu/projects/rice/ccw/status/seqSum3.html). Unfortunately, most sequenced clones are located on the long arm of the chromosome while the gene for Al tolerance mapped on the short arm of this chromosome. We realize that the whole rice genome sequences have been published (Goff et al. 2002; Yu et al. 2002). However, in order to search for the candidate genes on chromosome 3, a good connection between genetic and physical maps is needed.

Towards positional cloning of Al tolerance QTLs from *O. rufipogon*

The QTLs identified from *O. rufipogon* are potential candidates for the development of near-isogenic lines (NILs) leading to the positional cloning of genes for Al tolerance in rice. Of primary interest is the fine mapping and cloning of the major QTL located on chromosome 3. This QTL explained about 25% of the phenotypic variation and appears to be orthologous with a major Al tolerance gene located on group 4 of the Triticeace chromosome. *QAlRr1.1* on chromosome 1 had a small effect (9.0%) on the phenotypic variation. However, its location appears to be consistent with a major QTL previously detected in three other rice populations. *QAlRr7.1* on chromosome 7 had the second largest effect (22.5%) on the phenotypic variation. It apparently was a unique QTL in this population. *QAlRr8.1* on chromosome 8 also had a large effect (20.8%) on the phenotypic variation. It appears to map in the same chromosomal region with a 'unique' QTL in the CT9993 × IR62266 population, in which CT9993 was found to have the highest Al tolerance capacity among several *O. sativa* genotypes (Nguyen et al. 2000). However, since the genomic regions harboring these QTLs (*QAlRr7.1* and *QAlRr8.1*) are still wide (29.8 and 31.0 cM, respectively), saturation mapping of these regions with additional markers is necessary to be useful for QTL introgression and positional cloning. *QAlRr9.1* on chromosome 9 had a small effect (9.9%) on the phenotypic variation. It, however, appears to be consistent among three different rice populations. It is also tightly linked to WALI7 (2 cM), an Al-induced cDNA from wheat.

The development of NILs would allow breeders to evaluate the effect of *O. rufipogon* alleles introgressed into cultivated rice backgrounds. Moreover, large backcross populations derived from the specific NILs can be developed for fine mapping and eventual cloning of the target QTLs. Further investigation into the physiological mechanisms and genes controlling Al tolerance in rice will provide a better understanding of the evolution and diversity of Al tolerance in rice and other cereal species.

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