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Mapping of genes controlling aluminum tolerance in rice: comparison of different genetic backgrounds

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Abstract Aluminum toxicity is the main factor limiting the productivity of crop plants in acid soils, particularly in the tropics and subtropics. In this study, a doubled-haploid population derived from the rice (*Oryza sativa* L.) breeding lines CT9993 and IR62266 was used to map genes controlling Al tolerance. A genetic linkage map consisting of 280 DNA markers (RFLP, AFLP and SSR) was constructed to determine the position and nature of quantitative trait loci (QTLs) affecting Al tolerance. Three characters – control root length (CRL), Al-stressed root length (SRL) and root length ratio (RR) – were evaluated for the DH lines and the parents at the seedling stage in nutrient solution. A total of 20 QTLs controlling root growth under Al stress and control conditions were detected and distributed over 10 of the 12 rice chromosomes, reflecting multigenic control of these traits. The two QTLs of largest effect, *qALRR-1-1* and *qALRR-8* for root length ratio (a measurement of Al tolerance) were localized on chromosomes 1 and 8, respectively. Three other QTLs in addition to *qALRR-8* were apparently unique in the CT9993 × IR62266 mapping population, which may explain the high level of Al tolerance in CT9993. Comparative mapping identified a conserved genomic region on chromosome 1 associated with Al tolerance across three rice genetic backgrounds. This region provides an important starting point for isolating genes responsible for different mechanisms of aluminum tolerance and understanding the genetic nature of this trait in rice and other cereals.

Keywords QTL mapping · Aluminum toxicity · Rice genetics · *Oryza sativa* L. · Abiotic stress

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Introduction

Soil salinity, acidity and mineral deficiencies will continue to be one of the major problems limiting crop productivity throughout the world. Sanint and Wood (1998) estimated that more than 45.5% of rice produced in Latin America is grown under upland conditions. The upland soils are infertile and mostly acidic in nature. Crops grown in such soils suffer from aluminum toxicity and calcium and phosphate deficiencies (Howeler and Cadavid 1976). Aluminum (Al) toxicity is the most important factor limiting crop productivity in the acid soils which comprise large areas of the world (Kochian 1995), particularly in the tropics and subtropics (Foy et al. 1978; Foy 1984).

The major symptom of aluminum toxicity is rapid inhibition of root growth (Lüttge and Clarkson 1992; Rengel 1992; Delhaize and Ryan 1995). The effect of aluminum toxicity is to arrest or slow down root growth. As a result, stunted or shortened roots are the primary and earliest symptom of aluminum toxicity. Bennett et al. (1987) suggested that the root cap is a site of perception of Al-mediated injury. Roots injured by high Al are usually stubby and thick, and become dark-colored, brittle,

poorly branched and rubberized (Foy 1983). Several techniques have been employed in evaluating Al tolerance in plants, such as measurements of absolute root length and root re-growth, and staining of roots with hematoxylin (Lafever and Campbell 1978, Riede and Anderson 1996; Gallego and Benito 1997). In rice, absolute root length or root length ratio has been widely used as a parameter for evaluating Al tolerance (Coronel et al. 1990; Khatiwada et al. 1996; Wu et al. 1997). It provides major advantages over other techniques: the measurement is simple to perform, and the assessment of root length ratio allows the elimination of genetic differences in root growth under normal conditions (Wu et al. 2000).

By comparing the response of roots and shoots to Al toxicity in wheat, Briggs and Taylor (1993) and Zale (1987) found that Al stress in hydroponic systems affects root characteristics much more than shoot characteristics. Thus, the measurement of root parameters offers the best approach to selecting or screening plant genotypes for Al tolerance.

The physiological and biochemical mechanisms of the toxic effect of aluminum on root elongation have been extensively investigated (Foy and Fleming 1978; Horst et al. 1982; Haug and Shi 1991; Matsumoto 1991; Lüttge and Clarkson 1992; Lazof et al. 1994). However, the genetic mechanisms controlling Al tolerance in crop plants are poorly understood (Aniol and Gustafson 1984; Carver and Ownby 1995). Thus, the inheritance of Al tolerance in barley (*Hordeum vulgare* L.) was reported to be controlled by a single gene (Reid et al. 1969; Minella and Sorrells 1992). In contrast, the genetic system controlling Al tolerance in wheat (*Triticum aestivum* L.) appears to be complex, involving genes of major and minor effect (Aniol and Gustafson 1984; Luo and Dvorak 1996). In corn (*Zea mays* L.), Al tolerance is believed to be governed by a single locus with multiple alleles (Rhue et al. 1978). Major genes for Al tolerance in rye (*Secale cereale* L.), which is the cereal that is most tolerant to most Al (Aniol and Gustafson 1984; Manyova et al. 1988), are located on chromosomes 3R, 4R, and 6RS based on studies using wheat-rye addition lines (Aniol and Gustafson 1984). Information on the genetic mechanisms controlling Al tolerance in rice is limited, but the trait appears to be controlled by many genes (Khatiwada et al. 1996; Wu et al. 1997, 2000; Nguyen et al. 2001).

Advances in molecular marker technology have led to the development of detailed molecular linkage maps for many plant species. These maps have allowed the dissection of quantitatively expressed traits into the contributions of Mendelian factors referred to as quantitative trait loci (QTLs), each linked to molecular markers of known map position (Paterson et al. 1988). QTL mapping sets the stage for the acceleration of crop improvement through marker-assisted selection. In addition, QTL mapping also provides insights into comparative genetics and the evolution of genes for Al tolerance among cereals. Molecular markers linked to genes or QTLs conferring Al tolerance have previously been identified in wheat (Riede and Anderson 1996), rye (Aniol and Gustafson 1984; Gallego et al. 1998), maize (Sibov et al. 1999), barley (Tang et al. 2000), and rice (Wu et al. 2000; Nguyen et al. 2001). The main objectives of this study were to map genes controlling Al tolerance in a unique upland rice germplasm and to compare QTLs for Al tolerance across different genetic backgrounds in rice and other cereals.

Materials and methods

Plant material

A total of 146 doubled-haploid (DH) lines from a cross between CT9993-5-10-1-M (abbreviated as CT9993, an upland *japonica* ecotype tolerant to Al toxicity) and IR62266-42-6-2 (abbreviated as IR62266, an *indica* ecotype susceptible to Al toxicity) were used in the present study. The parents were pre-screened for Al toxicity with other rice genotypes known to be Al tolerant, such as Azucena (Khatiwada et al. 1995) and Chiembau (Nguyen et al. 2001), at different Al concentrations. Among several rice lines tested, CT9993 was found to be the genotype most tolerant to Al toxicity (Nguyen et al. 2000). CT9993 was selected under acid soil conditions in the rice breeding program at CIAT. It originated from complex crosses that involved varieties/cultivars that are highly

Table 1. Descriptive statistics of three variables measured on 146 DHLs and the two parental lines in four replications

Trait	Min	Max	Mean[a]	CV (%)[b]	$LSD_{0.05}$[c]	H^2 (%)[d]
Control root length (cm)						
CT9993	–	–	10.66*	–	–	–
IR62266	–	–	7.08*	–	–	–
DHLs	3.91	13.18	9.92	13.55	1.45	86
Stress root length (cm)						
CT9993	–	–	5.56*	–	–	–
IR62266	–	–	1.28*	–	–	–
DHLs	1.29	6.54	3.98	15.32	0.73	92
Root length ratio (%)[e]						
CT9993	–	–	52.97*	–	–	–
IR62266	–	–	18.23*	–	–	–
DHLs	18.86	71.50	45.61	18.52	10.09	88

[a]The *asterisks* indicate that the difference between the parental lines is statistically significant
[b]Coefficient of variation
[c]Least significant difference at the 5% probability level
[d]Broad-sense heritability on a line mean basis
[e]Relative ratio of root length under stress over control condition

tolerant to Al toxicity and low pH, such as Moroberekan, IRAT216, IRAT13, IRAT 120, IRAT121 and the land race 63-83, from Africa and Latin America.

Screening for aluminum tolerance

The parental lines and DH progenies were screened for Al tolerance in the laboratory using a nutrient solution for culture modified after Khatiwada et al. (1996). The experimental design was a randomized complete block with four replications. Seeds of uniform size were sterilized with 15% H_2O_2, rinsed with distilled water, and incubated on filter papers soaked with distilled water in the dark at 30°C for 2 days. Germinated seeds were grown in distilled water for another 2 days in a culture chamber maintained at 27 ± 2°C. Seedlings were then transferred to a styrofoam sheet with a nylon net bottom with one seedling per hole and three seedlings in one row per line in each replication. The styrofoam sheets were floated on a nutrient solution (Yoshida et al. 1976) in a plastic tray containing either 0 (control) or 30 ppm Al (stress treatment). The pH of the solutions was adjusted daily to 4.0 with 1 N NaOH or 1 N HCl. The hydroponic trays and seedlings were maintained in the culture chamber at 27 ± 2°C with 12 h of light at 300 PPFD (photo proton flux density). The longest root of each seedling was measured after 10 days of growth in control or stress solution. The ratio of average root length under stressed versus control conditions for each line in each replication was used as a measure of Al tolerance.

Statistical analysis

Standard analysis of variance (ANOVA) was performed to test the significance of genetic variation among the DH lines for the three traits using SAS (SAS Institute 1988). Broad-sense heritabilities (h^2) were computed from the estimates of genetic ($\sigma^2 G$) and residual ($\sigma^2 e$) variances derived from the expected mean squares of the analysis of variances as , where k is the number of replications.

Linkage map and QTL analysis

A genetic linkage map revised from a previous map (Zhang et al. 2001), consisting of 280 marker loci including 134 RFLPs, 131 AFLPs and 15 SSRs, was constructed based on the 154 DH lines using MAPMAKER/Exp version 3.0. The map covered 1602 cM in length (based on the use of the Kosambi function) with an average distance of 5.7 cM between adjacent markers. QTL analysis was performed according to the method of interval mapping (Paterson et al. 1988; Lander and Bostein 1989) using MAPMAKER/QTL 1.1 (Lincoln et al. 1992). Based on a chromosome number of 12 and the observed map length of 1602 cM, a LOD score of 2.8 was selected as the threshold for declaring presence of a QTL to reduce the experimental false-positive rate to $P < 0.05$ (Lander and Bostein 1989). Independence tests were carried out when there were more than one QTL for the same trait located on the same chromosome (Paterson et al. 1988; Lander and Bostein 1989). QTL designations followed the nomenclature proposed by McCouch et al. (1997). For the best multiple-QTL model, a maximum of seven QTLs is allowed in the MapMaker/QTL program. If more than seven QTLs were detected for one trait, the QTLs which explained the largest portions of phenotypic variation were selected for the regression model.

Results

Phenotypic performance

The mean, range, heritability estimates and distributions for three traits – control root length (CRL), stress root length (SRL), root length ratio (RR) – for the DH population and their parents, are summarized in Table 1 and Fig. 1. The roots of CT9993 and IR62266 plants showed differential responses to aluminum stress: CT9993 has a higher SRL and RR, indicating that it is the more tolerant. The range of progeny means appreciably exceeded that of their parents for the three traits, suggesting transgressive variation among genotypes. The frequency distribution of CRL, SRL, RR of the population was normal according to Shapiro-Wilk test. The broad-sense heritability estimates were 86, 92 and 88%, respectively, for CRL, SRL and RR. High h^2 values suggest the possibility of exploiting the genetic variation in a breeding program.

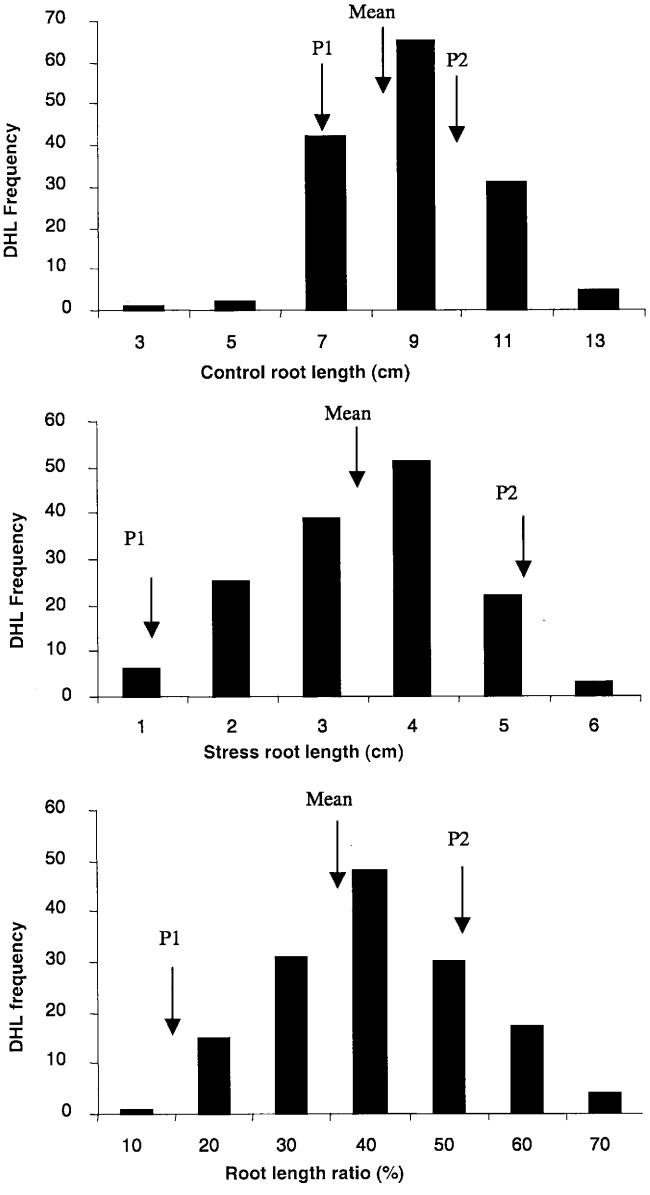


Fig. 1. Frequency distribution for control root length, stress root length, and root length ratio. P1, IR92266; P2, CT9993; Mean is the average of 146 DH lines

QTL analysis

A total of 20 QTLs that reached or exceeded the LOD threshold of 2.8 were identified. The putative QTLs, their respective chromosomal locations, LOD scores, percentage of variance explained, and allelic effect are listed in Table 2. The number of QTLs identified for individual traits ranged from 3 (for CRL) to 10 (for RR) with the phenotypic variation varying from 9.2 to 28.7%. The locations of these QTLs are shown in Fig. 2. For CRL, three QTLs (*qCRL-2, qCRL-7 and qCRL-8*) were identified, each explaining between 12.0 and 14.8% of phenotypic variance. These three QTLs together explained 34.5% of the phenotypic variation. Favorable alleles of *qCRL-2* and *qCRL-7* came from CT9993 (longer root length), but for *qCRL-8* the favorable allele was contributed by IR62266. Seven QTLs (*qALSRL-1, qALSRL-6*, *qALSRL-7*, *qALSRL-8*, *qALSRL-9*, *qALSRL-10* and *qALSRL-12*) for SRL were identified on chromosomes 1, 6, 7, 8, 9, 10 and 12, respectively, each explaining from 9.2 to 18.3% of the phenotypic variance. CT9993 contributed the favorable alleles (longer root length) for all seven QTLs. These QTLs explained 39.9% of the total phenotypic variation. A total of 10 QTLs (*qALRR-1-1, qALRR-1-2*, *qALRR-2, qALRR-3*, *qALRR-4*, *qALRR-7*, *qALRR-8*, *qALRR-9*, *qALRR-10,* and *qALRR-12*) were identified for RR. The individual QTLs explained 10.3–28.7% of phenotypic variation. The two QTLs with the largest effect, *qALRR-1-1* and *qALRR-8*, individually explained 24.1% and 28.7% of the phenotypic variation, respectively. CT9993 contributed favorable alleles (less impaired by stress) for nine QTLs (*qALRR-1-1, qALRR-1-2*, *qALRR-3*, *qALRR-4*, *qALRR-7*, *qALRR-8*,

Table 2. Putative QTLs detected for control root length (CRL), stress root length (SRL), root length ratio (RR) by interval mapping with MapMaker/QTL in a doubled-haploid population obtained from the cross CT9993 × IR62266

Trait	Locus[a]	Flanking markers	Chromosome	QTL length[b]	QTL position[c]	Additive effect[d]	LOD score[e]	Fraction of variance explained (%)[f]
CRL	*qCRL-2*	TGMSP2 and ME97	2	6.7	4.0	–1.87	2.95	12.0
	qCRL-7	RG650 and ME71	7	11.4	4.0	–1.39	4.14	14.8
	qCRL-8	RZ997 and EM141	8	14.9	14.0	1.18	3.71	12.0
								34.5
SRL	*qALSRL-1*	ME1014 and RG109	1	4.4	0.0	–0.85	4.58	13.4
	qALSRL-6	R2549 and RG109	6	10.5	0.0	–0.78	3.25	10.9
	qALSRL-7	EM165 and RG404	7	8.9	0.0	–0.76	3.05	9.2
	qALSRL-9	RM201 and RG667	9	6.2	6.0	–1.09	6.28	18.3
	qALSRL-10	RG257 and ME516	10	5.1	4.0	–0.87	4.51	14.1
	qALSRL-12	ME1017 and ME415	12	0.3	0.0	–0.76	3.28	9.8
								39.9
RR	*qALRR-1-1*	CDO345 and ME1014	1	6.1	4.0	–12.39	8.06	24.1
	qALRR-1-2	RG1028 and RZ543	1	1.3	0.0	–10.63	6.47	18.5
	qALRR-2	C1408 and C1419	2	1.8	0.0	10.63	4.54	13.4
	qALRR-3	ME82 and CDO122	3	5.2	4.0	–9.02	4.05	12.8
	qALRR-4	RG190 and EM153	4	5.6	0.0	–11.82	7.08	20.1
	qALRR-7	ME43 and EM1511	7	9.0	0.0	–8.78	3.45	10.3
	qALRR-8	ME53 and C1121	8	15.4	6.0	–13.37	8.23	28.7
	qALRR-9	RG667 and RM215	9	5.4	2.0	–12.20	5.97	19.3
	qALRR-10	EM169 and G333	10	15.9	12.0	–10.33	4.59	17.7
	qALRR-12	RG323 and ME29	12	4.8	2.0	–12.86	6.20	19.7
								60.5

[a]Individual QTLs are designed with "q" indicating QTLs with LOD > 2.8, an abbreviation of the trait name and the chromosome number (followed by another number in cases where more than one QTL affecting a trait were identified on the same chromosome

[b]The map distance between the two markers flanking the QTL

[c]Distance from the first marker in centiMorgan (cM)

[d]Additive effects of homozygotes are calculated as: (IR62266–CT9993)/2. A positive effect indicates better growth of the IR62266 homozygote, and a negative effect indicates better growth of the CT9993 homozygote under conditions of aluminum stress

[e]Maximum LOD score (likelihood odds ratio)

[f]Portion of phenotypic variation explained by the QTL. The values shown in *bold* indicate the percentage of the variance explained by the best multiple QTL model

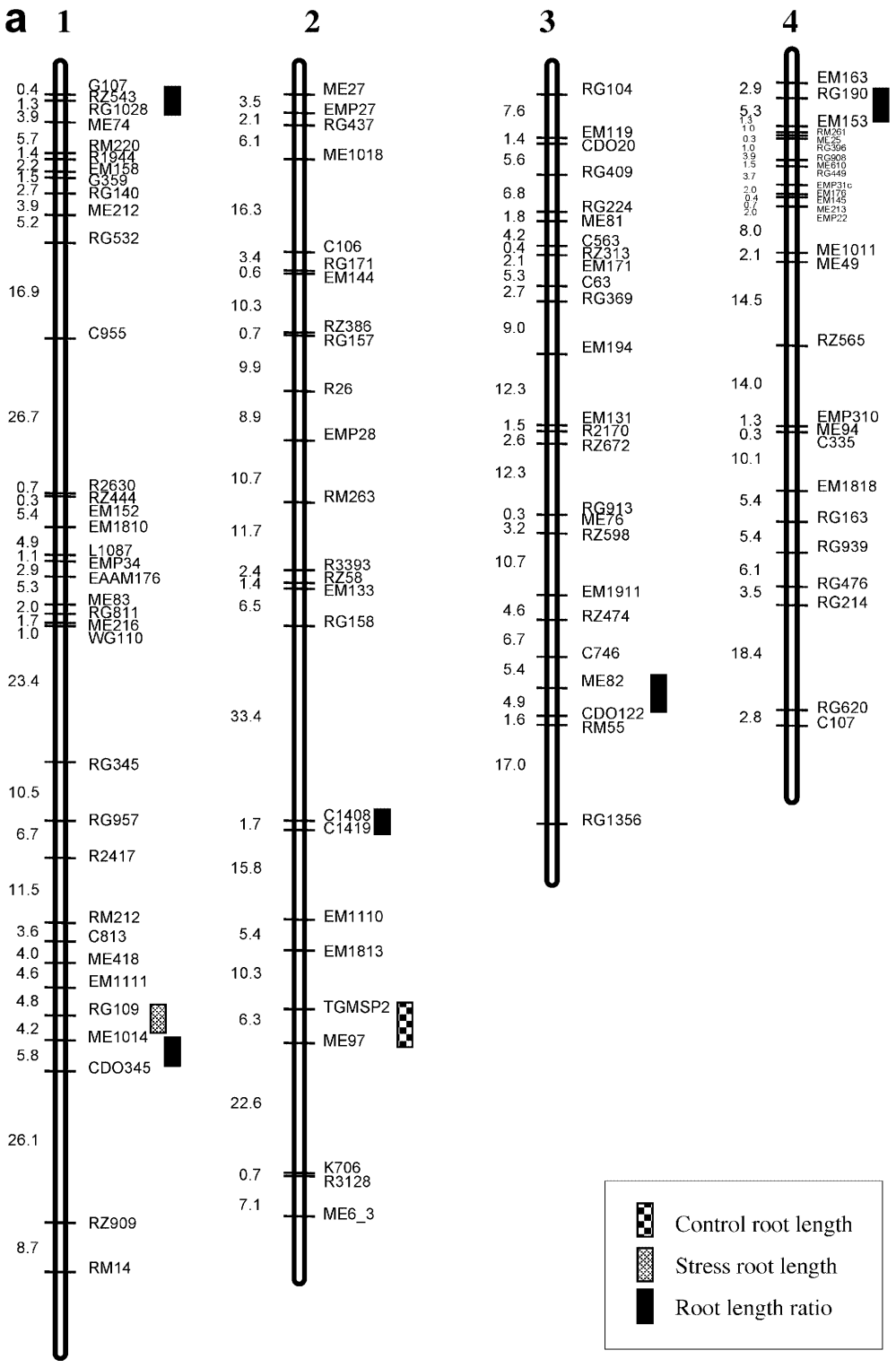


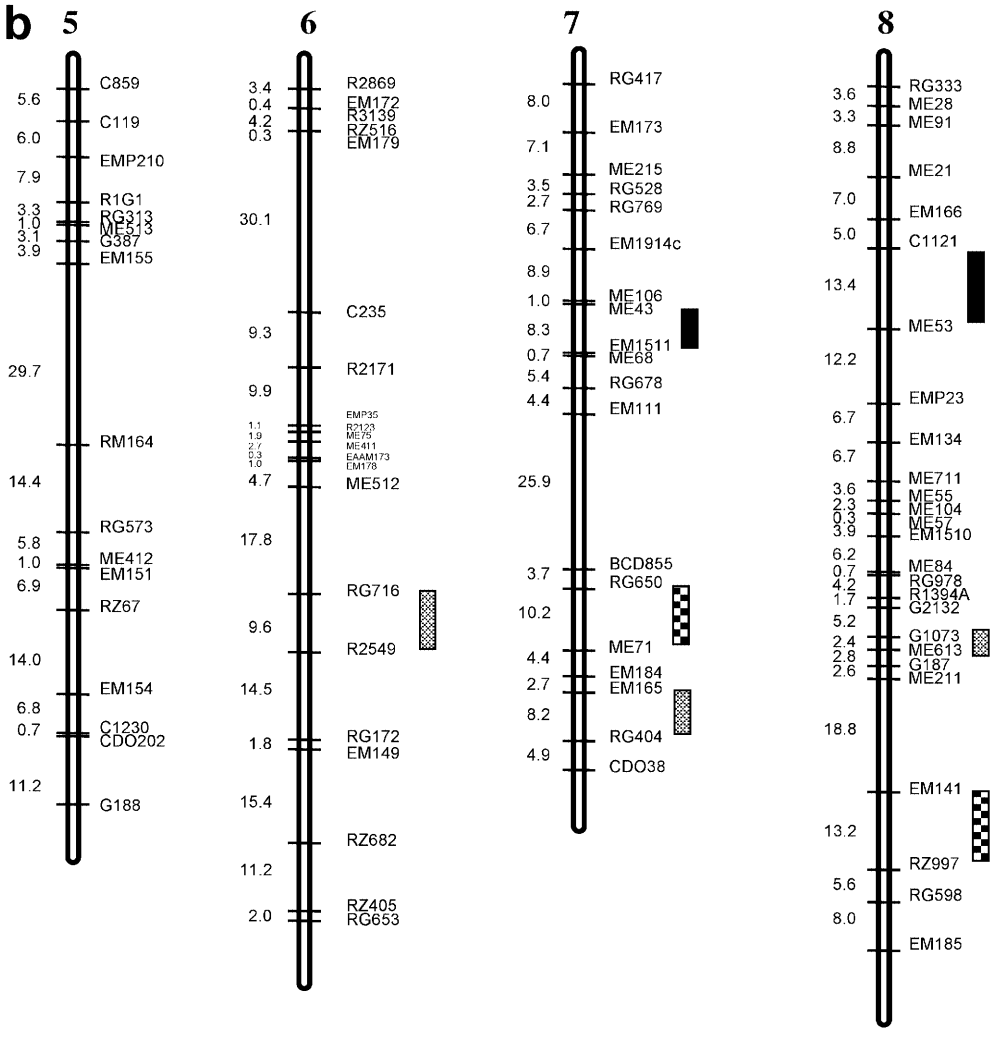
Fig. 2. The molecular linkage map with 280 RFLP, AFLP, and SSR marker loci constructed from 154 DHLs obtained from the cross CT9993 × IR62266. The distance between markers is given in Kosambi centiMorgans. Chromosomal locations of putative QTLs controlling root growth and aluminum tolerance are indicated by *vertical bars* beside the chromosome maps. The *vertical bar length* is equal to the length detected for the QTL by the MapMaker/QTL program

qALRR-9, *qALRR-10*, and *qALRR-12*) and IR62266 contributed the favorable allele for *qALRR-2*, possibly explaining some of the transgressive variation. The best multiple-QTL model containing the seven QTLs with the highest phenotypic variation explained 60.5% of the total phenotypic variance.

Comparison of QTLs for Al tolerance across rice genetic backgrounds

Root length ratio (RR) is the parameter most directly related to Al tolerance in rice and other crops. To determine if there are any common QTLs for RR across

Fig. 2. (Contd.)

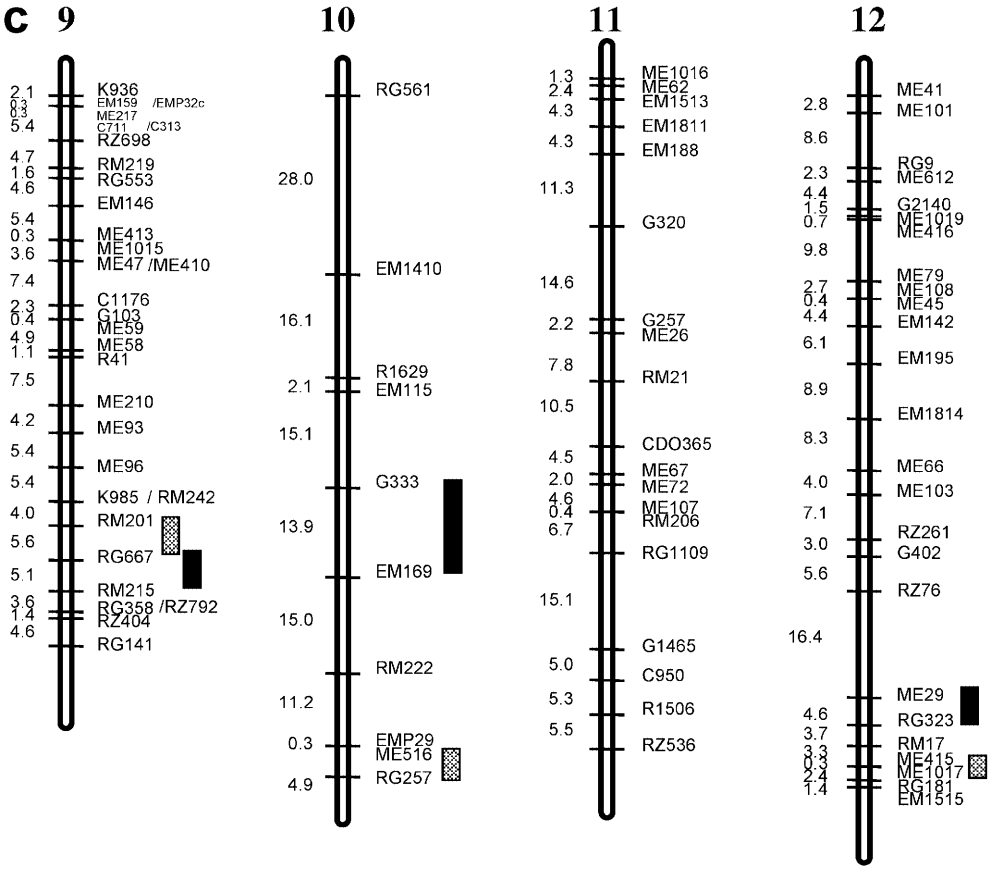


rice genetic backgrounds, results from this study were compared with other reports available in the literature. Of the 10 QTLs for RR, only two appear to be consistent with those identified in other populations. The QTL *qALRR-1-1* ($R^2 = 0.241$) on chromosome 1, one of the QTLs of largest effect for RR, is apparently at the same position as QTLs for Al tolerance found in IR1552 × Azucena (Wu et al. 2000) and OM269 × Chiembau (Nguyen et al. 2001) which also had the largest effect on phenotypic variation (Fig. 3). Another genomic region on chromosome 9 harboring *qALRR-9* in our population was found to lie in the same chromosomal region as a minor QTL ($R^2 = 0.09$) detected in IR1552 × Azucena (Wu et al. 2000). However, the QTL of largest effect on chromosome 8 ($R^2 = 0.287$) does not correspond to any QTL in the IR1552 × Azucena or OM269 × Chiembau population.

Comparison of QTLs for Al tolerance among cereals

To determine whether there are any QTLs for Al tolerance that are common to rice and other cereal species, these results were also compared with those for wheat (Aniol and Gustafson 1984; Riede and Anderson 1996), rye (Aniol and Gustafson 1984; Gallego and Benito 1997; Gallego et al. 1998), maize (Sibov et al. 1999), and barley (Tang et al. 2000) using comparative maps (Ahn and Tanksley 1993; Ahn et al. 1993; Gale and Devos 1998; Wilson et al. 1999) and comparative RFLP probe sets. Two major genes for Al tolerance, *Alm1* and *Alm2*, were found to be located on chromosomes 10 and 6, respectively, in maize (Sibov et al. 1999). The *Alm1* gene was about 20.1 cM from *UMC130* which co-segregated with *RZ141* (Wilson et al. 1999), a marker which maps on rice chromosome 11 (Causse et al. 1994). *Alm2* was located on maize chromosome 6, about 18.5 cM from CSU70, which is closely linked to CDO580 (Wilson et al. 1999), a marker which maps on rice chromosome 5 (Causse et al. 1994). In this study, rice chromosomes 5 and 11 did not contain any QTLs for Al tolerance. Another comparative analysis was performed between rice and the Triticeae. A minor QTL ($R^2 = 0.128$) for Al tolerance in this population, *qALRR-3*, was found on chromosome 3. However, the nearest marker (CDO122) was about 74.8 cM from the

Fig. 2. (Contd.)



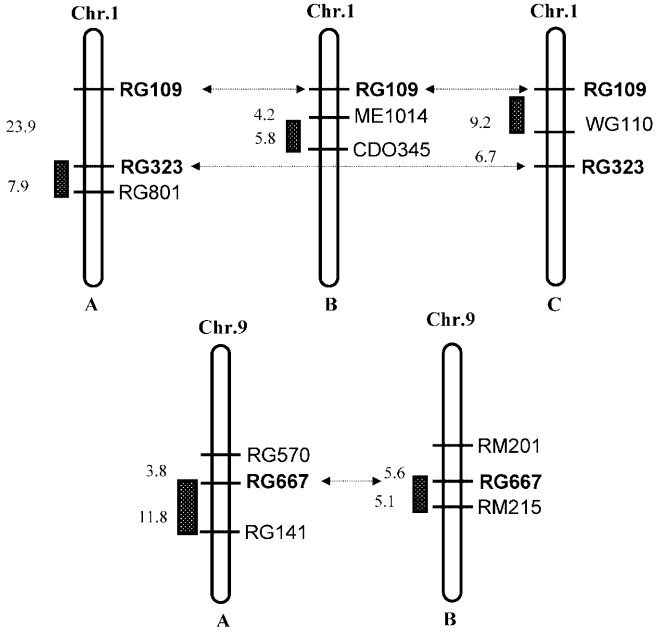


Fig. 3. Genomic regions for aluminum tolerance that are conserved among genetic backgrounds in rice. The *vertical bars* beside the chromosome maps indicate the positions of QTLs for Al tolerance. The partial map A was redrawn from Wu et al. (2000), B is from this study, and C is from Nguyen et al. (2001)

nearest QTL forAl tolerance in Triticeace, indicating that *qALRR-3* does not correspond to the genes controlling Al tolerance in Triticeae. However, a minor QTL ($R^2 = 0.09$) on chromosome 3 in Azucena × IR1552 (Wu et al. 2000) appears to be syntenic with the genomic region carrying a major Al tolerance gene on Group 4 of the Triticeae.

Discussion

An efficient method for evaluating Al toxicity is based on the use of a nutrient solution containing a toxic level of aluminum (Rhue and Grogan 1978; Camargo 1981). Highly significant correlations ($r = 0.64$–0.75) between the Al responses in such a nutrient solution and in the field have been reported in rice (Howeler and Cadavid 1976) and barley (Reid et al. 1971), providing support for the use of the nutrient solution method (Cancado et al. 1999).

The genomic region flanked by CDO345 and ME1014 on chromosome 1 appears to harbor the most important QTL associated with Al tolerance in the rice population we studied. By comparing QTLs for Al tolerance among different genetic backgrounds in rice, we found that the QTL located on chromosome 1, *qALRR-1-1*, appears to correspond to the major QTL detected in rice by Wu et al. (2000) and Nguyen et al. (2001). These results suggest that this genomic region on chromosome 1 contains a

major QTL controlling Al tolerance in several Al-tolerant rice genotypes. Four QTLs with relatively large effects, *qALRR-8* ($R^2 = 0.287$) on chromosome 8, *qALRR-4* ($R^2 = 0.201$) on chromosome 4, *qALRR-12* ($R^2 = 0.197$) on chromosome 12, and *qALRR-1-2* ($R^2 = 0.185$) on chromosome 1, were apparently unique to the CT9993 × IR62266 mapping population. CT9993 was selected in acid soil conditions and its pedigree includes several varieties/cultivars that are highly tolerant to Al toxicity and low pH, such as Moroberekan, IRAT216, IRAT13, IRAT120, IRAT121 and the land race 63-83 (Surapong, personal communication). These QTLs may explain the high level of Al tolerance in CT9993.

The recent development of an integrated genetic and physical map of rice (Chen et al. 2002) will facilitate map-based cloning of important genes in rice. Thus, the genetic marker CDO345 was found to co-segregate with RG109, at a location that is 0.7 cM from the marker R2414 (http://www.shigen.nig.ac.jp/rice/oryzabase). R2414 was found to anchor BAC contig 22 of chromosome 1 on the Clemson rice physical map (http://www.Clemson.edu/projects/rice/fpc/WebFPC). Fine mapping of the QTL on chromosome 1 will help locate the BAC candidate(s) which harbor(s) the gene(s) controlling Al tolerance in rice.

It has been reported (Miftahudin et al. 2002) that there is a conserved genomic region for Al tolerance on the long arm of homoeologous chromosome 4 in wheat (Alt_{BH}), rye (*Alt3*) and barley (*Alp*). The gene controlling Al tolerance in these cereal crops was linked to the markers BCD1230 and CDO1395. It was suggested that the Alt_{BH}, *Alt3*, and *Alp* genes are orthologous loci because of the high level of synteny among chromosome arms 4DL, 4RL, and 4HL, and they may share a common function (Miftahudin et al. 2002). One of the Al tolerance mechanisms in the Triticeae is Al exclusion (Delhaize and Ryan 1995, Kochian 1995, Kochian and Jones 1997). This mechanism is mediated by Al-activated release of organic acids, such as malate or citrate, 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. Homoeologous chromosome 4 of the Triticeae corresponds to chromosome 3 in rice (Gale and Devos 1998). However, the major QTL controlling Al tolerance in different cultivated rice backgrounds was located on chromosomes 1. The mechanism of Al tolerance conditioned by the gene(s) on chromosome 1 in rice may be different from that observed in the Triticeae species. Further investigations into the physiological mechanisms and genes controlling Al tolerance in rice will be beneficial for our understanding of the evolutionary genetics and diversity of Al tolerance mechanisms in rice and other grass species.

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