Theor Appl Genet (2000) 100:1295–1303

P. Wu · C.Y. Liao · B. Hu · K.K. Yi · W.Z. Jin
J.J. Ni · C. He

QTLs and epistasis for aluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages

Received: 29 July 1999 / Accepted: 13 October 1999

Abstract To investigate the genetic background for aluminum (Al) tolerance in rice, a recombinant inbred (RI) population, derived from a cross between an Al-sensitive lowland *indica* rice variety IR1552 and an Al-tolerant upland *japonica* rice variety Azucena, was used in culture solution. A molecular linkage map, together with 104 amplified fragment length polymorphism (AFLP) markers and 103 restriction fragment length polymorphism (RFLP) markers, was constructed to map quantitative trait loci (QTLs) and epistatic loci for Al tolerance based on the segregation for relative root length (RRL) in the population. RRL was measured after stress for 2 and 4 weeks at a concentration of 1mM of Al^{3+} and a control with a pH 4.0, respectively. Two QTLs were detected at both the 2nd and the 4th weeks on chromosomes 1 and 12 from unconditional mapping, while the QTL on chromosome 1 was only detected at the 2nd stress week from conditional mapping. The effect of the QTL on chromosome 12 was increased with an increase of the stress period from 2 to 4 weeks. The QTL on chromosome 1 was expressed only at the earlier stress, but its contribution to tolerance was prolonged during growth. At least one different QTL was detected at the different stress periods. Mean comparisons between marker genotypic classes indicated that the positive alleles at the QTLs were from the Al-tolerant upland rice Azucena. An important heterozygous non-allelic interaction on Al tolerance was found. The results indicated that tolerance in the younger seedlings was predominantly controlled by an additive effect, while an epistatic effect was more important to the tolerance in older seedlings; additionally the detected QTLs may be multiple allelic loci for Al tolerance and phosphorus-uptake efficiency, or for Al and Fe^{2+} tolerance.

Communicated by G. Wenzel

P. Wu (✉) · C.Y. Liao · B. Hu · K.K. Yi · W.Z. Jin · J.J. Ni · C. He
Department of Biological Science, College of Life Science,
Zhejiang University, Hua Jia Chi Campus, Hangzhou, 310029, China
e-mail: Pwu@zjau.edu.cn
Fax: 0086-571-6971323

Key words *Oryza sativa* L. · AFLP markers · RFLP markers · Aluminum tolerance · QTLs · Epistasis

Introduction

Aluminum (Al) toxicity is one of the most important yield-limiting factors for rice grown on acid upland and lowland acid sulphate soils (IRRI 1978). Al toxicity results in a reduced and damaged root system, which in turn causes the affected plants to be susceptible to drought stress and mineral nutrient deficiencies (Foy 1988). It is not sufficient to develop a sustainable production system in acid soils to reduce soil acidity and improve soil fertility by the application of lime and fertilizers due to the impossibility of correcting subsoil acidity and the high phosphorus-fixing capacity.

Genetic variability for Al tolerance has been reported among a large number of important crop species, including rice (Howeler and Cadavid 1976; Fageria et al. 1988; Foy 1988; Khatiwada et al. 1996; Sivaguru and Paliwal 1993; Wu et al. 1997). Upland japonica rice lines with a high tolerance for Al have been identified (Khatiwada et al. 1996; Wu et al. 1997), which makes it attractive to transfer Al tolerance genes in upland rice to superior lowland rice and other Al-sensitive upland crops in order to develop cultivars with enhanced Al tolerance. It is prerequisite to determine the genetics of high Al tolerance in upland rice for exploiting the tolerance genes in a breeding program. A diallel analysis, using tolerant upland rice, including Azucena and IRAT104, and the sensitive lowland rice varieties IR1552 and IR45, was conducted to investigate the genetic characterization of Al tolerance in rice (Khatiwada et al. 1996). The results revealed that Al tolerance is governed by high heretability including both additive and dominance effects, with a preponderance of additive effects. Both general combining ability (GCA) and specific combining ability (SCA) are important in the genetics of Al tolerance, though GCA is more relevant than SCA. As high as 48% of significant GCA and SCA narrow-sense heritability and a much higher

variance of SCA were also detected in a solution culture with 30-mg Al^{3+} stress at pH 4.0 (Wu et al. 1997). Although findings suggest that selection for Al tolerance, as a quantitative trait, can be made in early generations of rice the details of the genetics of tolerance are difficult to investigate using conventional genetic analysis.

DNA markers have proved to be efficient tools to detect important genes controlling quantitative traits in various crops. Restriction fragment length polymorphic (RFLP) markers linked to the Al tolerance gene have been reported in wheat (Reide and Anderson 1996). The well-developed rice molecular-marker linkage map (Causse et al. 1994; Kurata et al. 1994) allows one to detect genes for both quantitative and qualitative traits as well as evaluating their effects on these traits in rice. The DNA markers linked to Al tolerance genes in rice could be used to develop marker-aid selection in breeding programs to exploit Al tolerance genes in upland rice, which could reduce, or eliminate, the need for difficult and time-consuming assays of phenotypic evaluation. The objectives of the present research were to detect QTLs and epistatic loci for Al tolerance and to determine the allelic effects on Al tolerance in upland rice.

Materials and methods

Plant materials

A recombinant inbred (RI) population with 150 lines, derived from a cross between an indica Al-sensitive variety IR1552 and a japonica Al tolerant variety Azucena, used in this research, was kindly provided by Dr. D. Senadhira, a former breeder at the International Rice Research Institute. Briefly, the procedure for development of the RI population is as follows: a few F_1 seeds were produced for the cross and planted in a protected field to produce F_2 seeds. Two seeds per plant were harvested from 500 F2 plants for the next generation. This procedure was repeated until the F_7 generation. Twenty five individual plants of each F_7 line were planted. The RI lines was developed by single-seed decent (SSD) unti the F_9. One hundred and fifty uniform lines, based on morphology, were harvested and used for phenotyping and for RFLP and AFLP map construction.

Solution culture experiment

Ten seedlings of each parent and four seedlings of each of the 150 RI lines were used in a solution-culture experiment. The seeds were germinated on quartz sand after sterilization treatment. The roots of 7-day old seedlings were cut off, leaving 1 cm in order to standardise the root length before stress. Seedlings were transplanted to plastic culture containers with standard rice culture solution (Yoshida et al. 1971) and Al stress at a concentration of 1 mM of Al^{3+}. The pH of the solution was ajdusted to 4.0 by using 1 N NaOH or 1 N HCl each day. The solution was replaced every 3 days and nitrogen concentration was increased to 60 mg N/l after the 3rd week of culture. The entire experiment was conducted in a greenhouse from May 15 to June 20, 1997, at Zhejiang University, Hua Jia Chi Campus, Hangzhou, China. The highest and lowest temperatures during the experiment in the greenhouse were 41°C and 21°C, and the humidity ranged from 55% to 75%.

Screening of RILs for Al tolerance

Relative root length (RRL), defined as the ratio of the maximum root length under Al stress divided by the maximum root length in the control (Ganesan et al. 1993; Mckendry et al. 1996; More et al. 1977; Polle et al. 1978), was used to evaluate genotypic variation in Al tolerance. The maximum root length was calculated as an average of the greatest-length and the second greater-length root of each plant. RRL was measured for the parents and the entire population lines after stress for 2 and 4 weeks, respectively.

AFLP and RFLP map construction

Genomic DNA was isolated from fresh leaf tissue and AFLP and RFLP analysis was conducted between the parents following the method of Vos et al. (1995) with minor modifications (Maheswaran et al. 1997). An *Eco*RI/*Mse*I system was used to generate polymorphic AFLP markers, and a total of 16 primer-pair combinations were used to identify 245 polymorphic AFLPs. A set of 135 RFLP markers were detected between the parents and used as anchors to construct a AFLP/RFLP linkage map. Finally, 103 RFLP and 104 AFLP markers were mapped on all 12 chromosomes using the program Mapmaker at LOD>3.0 (Lander 1993).

Mapping QTLs for Al tolerance

To identify the QTLs for Al tolerance at different growth stages, unconditional and conditional QTL mapping was conducted based on the phenotypic means of RRL measured at the 2nd week of stress and the 4th weeks of stress. The conditional means of RRL were obtained by the mixed-model approach (Zhu 1995). QTLs for the variation in RRL at the 2nd and 4th weeks after stress across the population, and the molecular markers linked to the putative QTLs, were analyzed by Qgene (Nelson 1997). The threshold for declaring a putative QTL for Al tolerance was LOD >2.4.

Epistasis analysis

Epistasis analysis between all possible loci on the map was conducted by using the program QTLmapper (Wang et al. 1999).

Results

Phenotypic performance

Parental performance and the segregation for RRLs within the population was shown in Fig. 1. After 2 weeks of stress, the mean RRLs of IR1552 and Azucena were about 0.5 and 1.0, respectively. The unconditional RRLs of IR1552 and Azucena at the 4th week stress were about 0.6 and 1.0, while conditional RRLs at the 4th week after stress, given the RRL at the 2nd week after stress, were about 0.7 and 0.9, respectively. The results indicated that IR1552 is a Al-sensitive and Azucena Al-tolerant, which is consistent with the earlier report of Khatiwada et al. (1996). The difference in conditional RRLs between the parents was smaller than in the unconditional RRLs, which reveals that the earlier seedlings response to Al stress may mainly control the phenotypic performance under Al stress in rice. The RRL of the population was normally distributed at both the 2nd and the 4th weeks after stress. The segregation range for the conditional RRL at the 4th weeks after stress was smaller than the unconditional segregation, but the average RRL was identical with the unconditional value. Tolerant transgressive variation was observed, but IR1552 performed as extremely sensitive (Fig. 1).

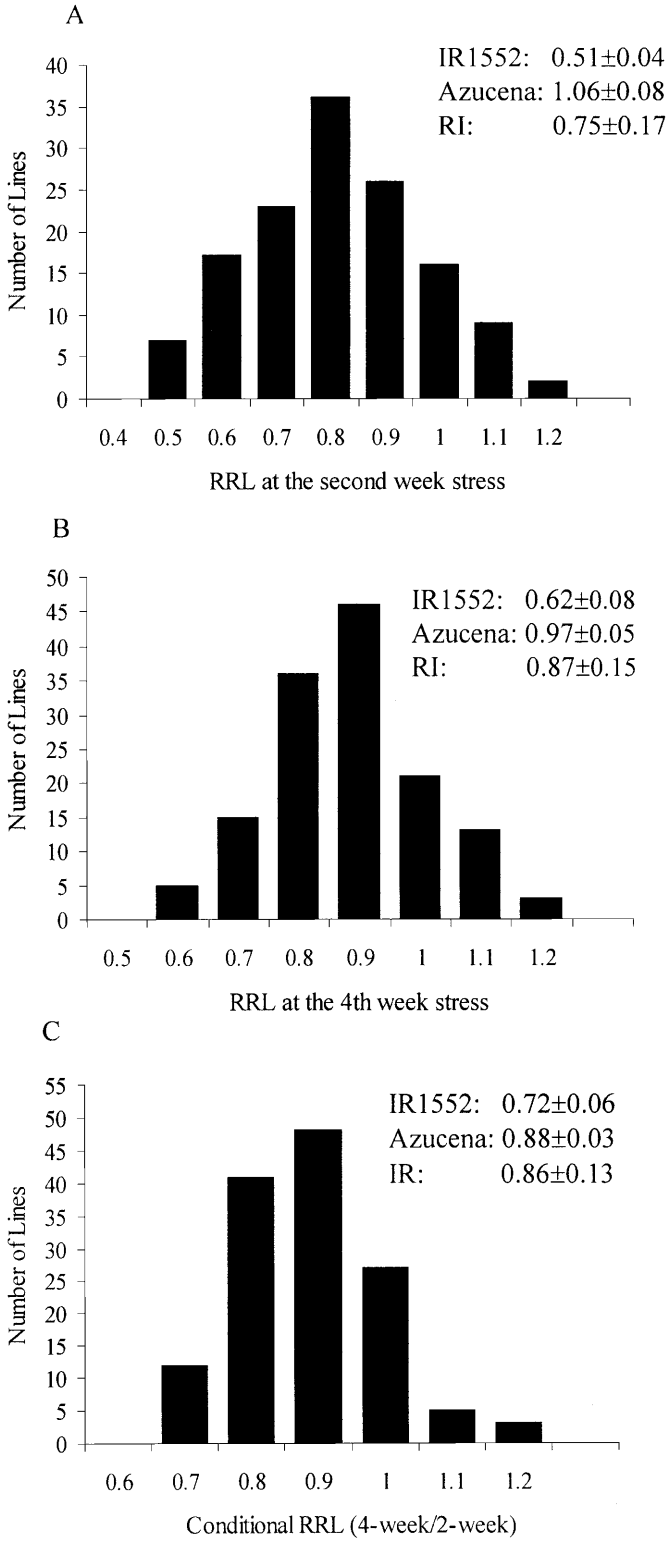

Fig. 1 Means of relative root length (*RRL*) of the parents and the segregation for RRL within the population derived from a cross between IR1552 and Azucena after 2 (**A**)- and 4 (**B**)-weeks stress, and the conditional RRL at the 4th-week of stress given the RRL at the 2nd week after stress (**C**)

Construction and validation of molecular map

A total of 245 polymorphic AFLP bands were detected using 16 primer combinations and 104 were mapped. The number of polymorphic bands was 4–17 per primer pair. The level of polymorphism for individual primer-pair combinations varied from 27.3% to 60.9% . Overall, the polymorphism of AFLP markers in this RI population was about 44.1%, which is 50% higher than that detected using a *Pst*I/*Mse*I system in a double-haploid (DH) population derived from IR64 and Azucena (Maheswaran et al. 1997). Among the mapped 104 AFLP markers, about 56.2% were from IR1552. As many as five chromosomes could be covered by the markers from some primer combinations (Table 1).

The parental survey revealed 135 polymorphic RFLP markers distributed on all 12 chromosomes from 192 rice-genome and cDNA probes (RG and RZ), and the barley cDNA probes (CDO). One hundred and three RFLP markers were mapped to the expected locations as anchors to generate a linkage map, together with the AFLP markers (Fig. 2). The order of anchor markers on each chromosome showed a parallel position and was in agreement with the order in the rice molecular map (Causse et al. 1994). The linkage map has a total length of 2420 cM. The average interval size of the map was 11.7 cM with the smallest interval on chromosome 9 (7.5 cM) and the largest on chromosome 5 (19.0 cM). About 65.7% of the mapped markers deviated from a 1:1 ratio; 62.8% were biased towards IR1552 and 2.9% towards Azucena (Table 2). The segregation ratio of the *indica* allele to the *japonica* allele for the mapped markers is 2:1; therefore, the largest distance of linked markers which can be established is about 29 cM (Manly 1994). The linkage map constructed in this case has only six gaps larger than 29 cM, so that QTL mapping on most of the map regions is reliable.

QTLs for Al tolerance

Single-marker analysis and interval mapping were used to detect putative QTLs for Al tolerance (Table 3). From unconditional mapping, two identical QTLs for Al tolerance were detected on chromosomes 1 and 12, flanked by RZ801 and RG323, and by RG9 and RG457, respectively, at both the 2nd week and the 4th week after stress. The QTL mapped on chromosome 12 explained about 10% and 20% of the total variation in RRL across the population at the 2nd and 4th weeks after stress, respectively, which indicates that the effect of the QTL increased with the increase in stress time from 2 to 4 weeks. In contrast, the effect of the QTL located on chromosome 1 decreased with the increase in stress time. Based on unconditional mapping, the QTL explained about 19% and 15% of the total variation in RRL at the 2nd and the 4th weeks after stress, respectively; but the QTL could not be detected at the 4th week from conditional mapping. These results suggest that the QTL was only expressed at the

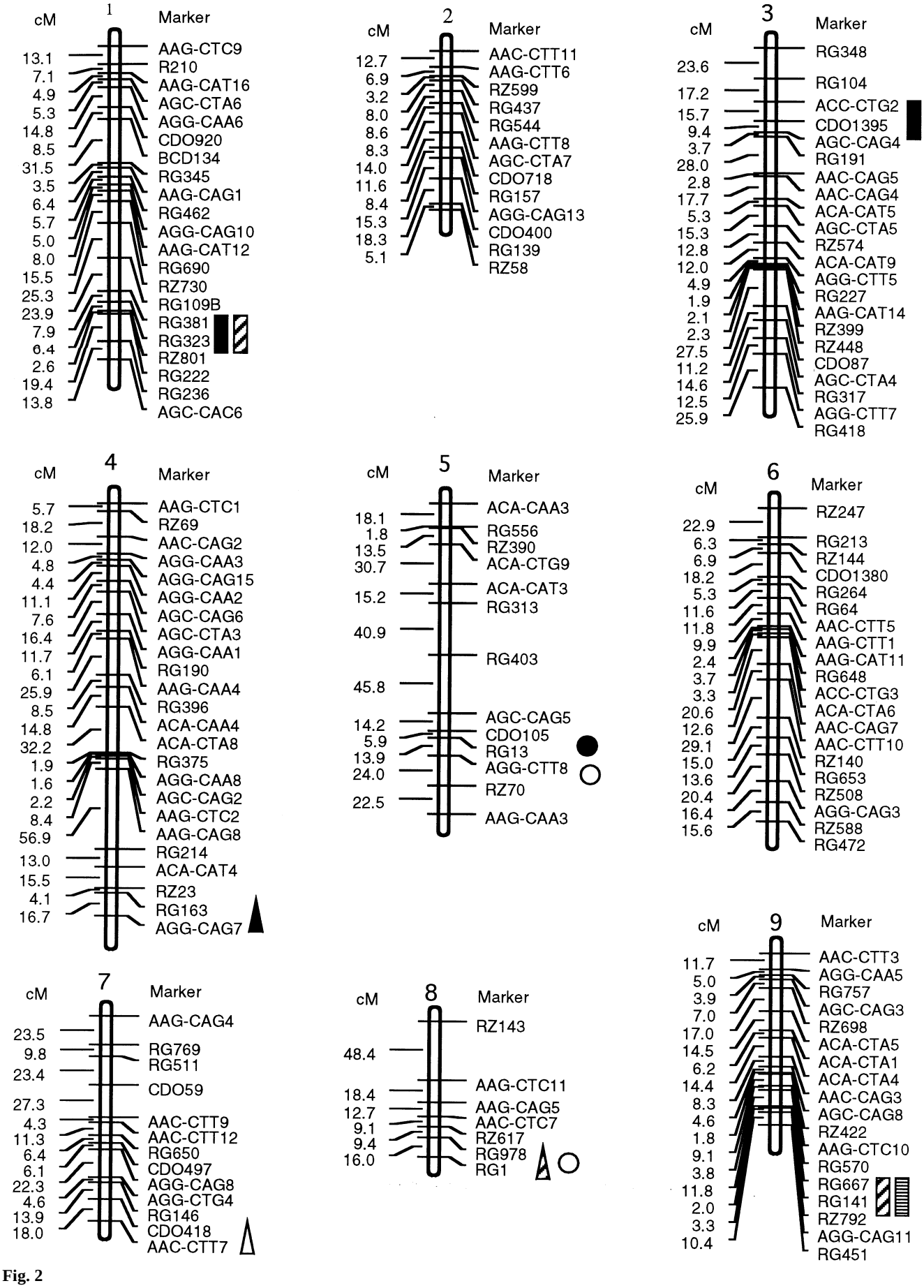
1
cM
Marker
13.1 AAG-CTC9
7.1 R210
4.9 AAG-CAT16
5.3 AGC-CTA6
14.8 AGG-CAA6
8.5 CDO920
31.5 BCD134
3.5 RG345
6.4 AAG-CAG1
5.7 RG462
5.0 AGG-CAG10
8.0 AAG-CAT12
15.5 RG690
25.3 RZ730
23.9 RG109B
7.9 RG381
6.4 RG323
2.6 RZ801
19.4 RG222
13.8 RG236
AGC-CAC6
2
cM
Marker
12.7 AAC-CTT11
6.9 AAG-CTT6
3.2 RZ599
8.0 RG437
8.6 RG544
8.3 AAG-CTT8
14.0 AGC-CTA7
11.6 CDO718
8.4 RG157
15.3 AGG-CAG13
18.3 CDO400
5.1 RG139
RZ58
3
cM
Marker
23.6 RG348
17.2 RG104
15.7 ACC-CTG2
9.4 CDO1395
3.7 AGC-CAG4
28.0 RG191
2.8 AAC-CAG5
17.7 AAC-CAG4
5.3 ACA-CAT5
15.3 AGC-CTA5
12.8 RZ574
12.0 ACA-CAT9
4.9 AGG-CTT5
1.9 RG227
2.1 AAG-CAT14
2.3 RZ399
27.5 RG448
11.2 CDO87
14.6 AGC-CTA4
12.5 RG317
25.9 AGG-CTT7
RG418
4
cM
Marker
5.7 AAG-CTC1
18.2 RZ69
12.0 AAC-CAG2
4.8 AGG-CAA3
4.4 AGG-CAG15
11.1 AGG-CAA2
7.6 AGC-CAG6
16.4 AGC-CTA3
11.7 AGG-CAA1
6.1 RG190
25.9 AAG-CAA4
8.5 RG396
14.8 ACA-CAA4
32.2 ACA-CTA8
1.9 RG375
1.6 AGG-CAA8
2.2 AGC-CAG2
8.4 AAG-CTC2
56.9 AAG-CAG8
13.0 RG214
15.5 ACA-CAT4
4.1 RZ23
16.7 RG163
AGG-CAG7
5
cM
Marker
18.1 ACA-CAA3
1.8 RG556
13.5 RZ390
30.7 ACA-CTG9
15.2 ACA-CAT3
RG313
40.9
RG403
45.8
14.2 AGC-CAG5
5.9 CDO105
13.9 RG13
24.0 AGG-CTT8
RZ70
22.5
AAG-CAA3
6
cM
Marker
RZ247
22.9
6.3 RG213
6.9 RZ144
18.2 CDO1380
5.3 RG264
11.6 RG64
11.8 AAC-CTT5
9.9 AAG-CTT1
2.4 AAG-CAT11
3.7 RG648
3.3 ACC-CTG3
20.6 ACA-CTA6
12.6 AAC-CAG7
29.1 AAC-CTT10
15.0 RZ140
13.6 RG653
20.4 RZ508
16.4 AGG-CAG3
15.6 RZ588
RG472
7
cM
Marker
AAG-CAG4
23.5
9.8 RG769
RG511
23.4
CDO59
27.3
4.3 AAC-CTT9
11.3 AAC-CTT12
6.4 RG650
6.1 CDO497
22.3 AGG-CAG8
4.6 AGG-CTG4
13.9 RG146
18.0 CDO418
AAC-CTT7
8
cM
Marker
RZ143
48.4
AAG-CTC11
18.4
AAG-CAG5
12.7
AAC-CTC7
9.1
RZ617
9.4
RG978
16.0
RG1
9
cM
Marker
11.7 AAC-CTT3
5.0 AGG-CAA5
3.9 RG757
7.0 AGC-CAG3
17.0 RZ698
14.5 ACA-CTA5
6.2 ACA-CTA1
14.4 ACA-CTA4
8.3 AAC-CAG3
4.6 AGC-CAG8
1.8 RZ422
9.1 AAG-CTC10
3.8 RG570
11.8 RG667
2.0 RG141
3.3 RZ792
10.4 AGG-CAG11
RG451

Fig. 2

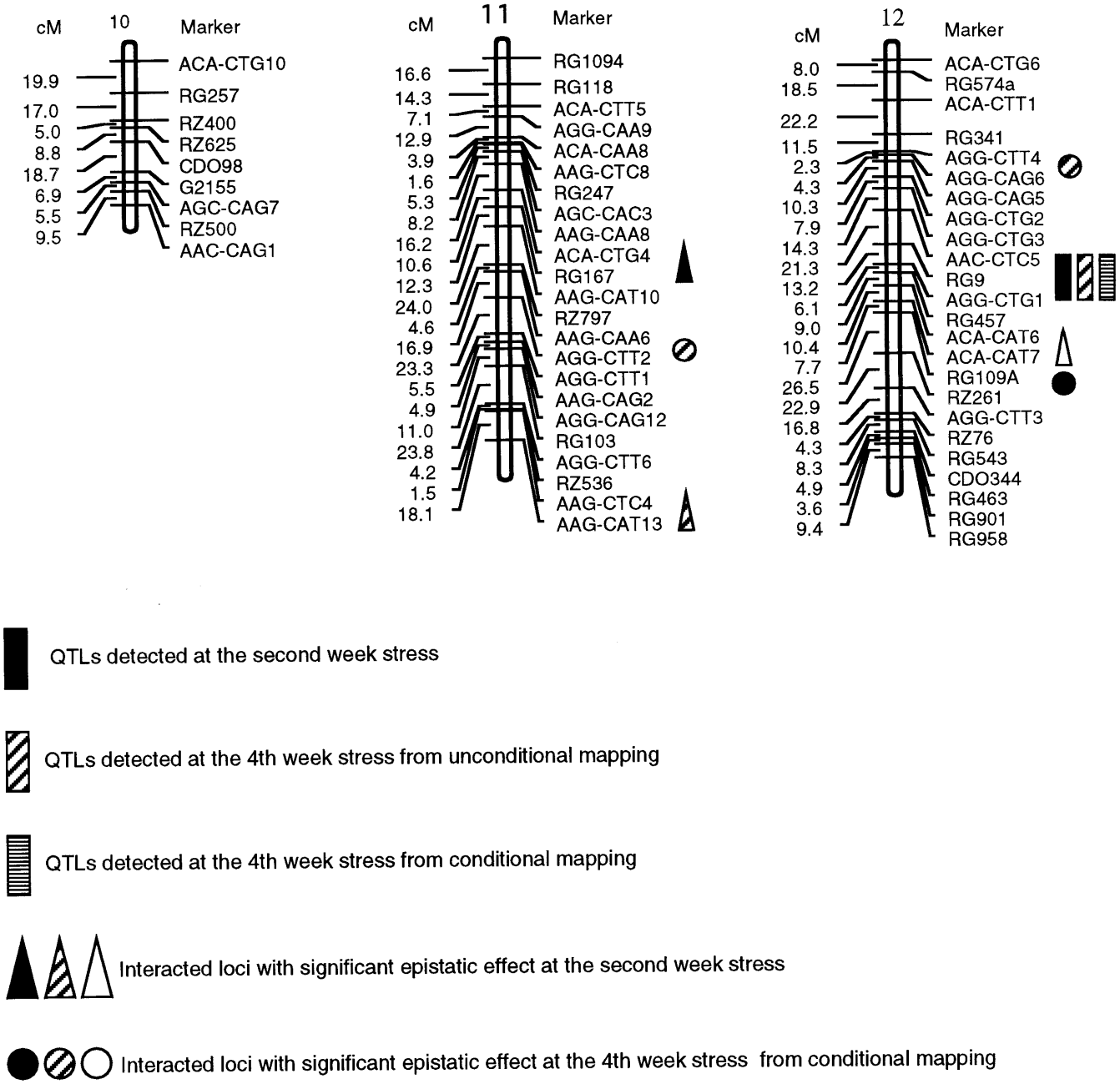

Fig. 2 AFLP/RFLP linkage map of the IR1552/Azucena recombinant inbred population showing the location of QTLs and epistatic effects for Al tolerance. The symbols representing the QTLs and the epistatic loci are as shown above. Designations to the right represent the marker name, and to the left represent the map distance in cM based on the Kosambi function

earlier stage of stress, but that its contribution to tolerance was prolonged to the later growth stage. The third QTL detected at the 2nd week after stress was located on chromosome 3, flanked by CDO1395 and AGC-CAC4, and explained about 9% of the total variation in RRL. At the 4th week after stress, a QTL located on chromosome 9, flanked by RG667 and RG141, was detected by both conditional and unconditional mapping, and explained about 8% of the total variation in RRL. The results indicated that the earlier response of rice seedlings to Al stress may mainly control Al tolerance in rice, but that at least one QTL is important to tolerance over the entire stress period. A mean comparison between marker genotypic classes at the detected QTLs indicated that the positive alleles for Al tolerance were from the Al tolerant upland *japonica* parent, Azucena.

Epistatic effects on Al tolerance

At the 2nd and the 4th weeks after stress, three pairs of non-allelic loci with a significant interaction on Al tolerance were detected, respectively (Table 4). At the 2nd week after stress, the epistastic effect explained about 20% of the total variation in RRL. The conditional epi-

Table 1 Polymorphism, origin and distribution of AFLP markers in the 139 F_9 RI lines of the cross IR1552×Azucena (*Eco*RI/*Mse*I)

Primer pair	Visible bands	Polymorphic bands	Polymorphism (%)	Origin of amplification		Polymorphic bands mapped	Chromosomes covered
				IR1552	Azucena		
AAC-CAG[a]	18	9	50.0	6	3	6	3, 4, 6, 9, 10
AAC-CTC	21	7	33.3	4	3	2	8, 12
AAC-CTT	34	16	47.1	10	6	7	2, 6, 7, 9
AAG-CAA	23	14	60.9	6	8	4	4, 5, 11
AAG-CAG	31	12	38.7	6	6	5	1, 4, 7, 8, 11
AAG-CAT	39	17	43.6	8	9	6	1, 3, 6, 11
AAG-CTC	32	16	50.0	10	6	7	1, 4, 8, 9, 11
AAG-CTT	35	13	37.1	10	3	3	2, 6
ACA-CAA	24	9	37.5	7	2	3	4, 5, 11
ACA-CAT	33	9	27.3	6	3	6	3, 4, 5, 12
ACA-CTA	22	10	45,5	5	5	5	4,6, 9
ACA-CTG	22	10	45,5	7	3	4	5, 10, 11, 12
ACA-CTT	29	9	31,0	3	6	2	11, 12
ACC-CTG	21	8	38,1	6	2	2	3, 6
AGC-CAC	28	9	32,1	3	6	3	1, 4, 11
AGC-CAG	23	9	39,1	5	4	6	3, 4, 5,9, 10
Total	555	245		130	115	104	12
Mean	26.4	11.7	44.1	6.2	5.5	5.0	3.7
Range	17–39	4–17	23.5–81.0	3–12	1–14	2–10	2–8

[a] The first three characters represent the three selective nucleotides at the *Eco*RI end, and the latter three characters represent the three selective nucleotides at the *Mse*I end

Table 2 Distribution of markers on each chromosome, the number of anchors used to construct the RI map, and the number of skewed markers on each chromosome

Chromosome		Number of markers			Av. distance (cM)	Skewed markers	
No.	Length (cM)	Total	AFLP	RFLP		IR1552	Azucena
1	228.7	21	8	13	10.9	10	0
2	120.6	13	5	8	9.3	11	0
3	266.6	22	10	12	12.1	16	0
4	299.8	24	17	7	12.5	12	2
5	246.5	13	6	7	19.0	8	0
6	245.6	20	8	12	12.3	13	0
7	160.9	13	6	7	12.4	7	0
8	114.0	7	3	4	6.3	6	1
9	134.8	18	10	8	7.5	15	0
10	91.3	9	3	6	10.1	8	0
11	246.8	23	16	7	10.7	14	2
12	263.9	24	12	12	11.0	10	1
Total	2419.5	207	104	103	11.7	130	6

Table 3 QTLs and closely linked markers for Al tolerance detected from the segregation of relative root length (RRL) after 2 and 4 weeks stress, respectively, and the conditional segregation after 4-weeks of stress among a recombinant inbred population derived from a cross between IR1552 and Azucena

Marker	Chrom.	Prob.	R^2	LOD	Add.	Genotypic mean		QTL position
						II	JJ	
After 2-weeks stress								
RZ801	1	0.000	0.19	6.93	–0.08	0.71	0.86	RZ801/RG323
CDO1395	3	0.001	0.09	2.62	–0.05	0.74	0.84	CDO1395/AGC–CAC4
RG9	12	0.001	0.10	3.08	–0.06	0.71	0.81	RG9/RG457
After 4-weeks stress								
RZ801	1	0.000	0.15	4.86	–0.05	0.81	0.92	RZ801/RG323
RG141	9	0.001	0.09	3.23	–0.05	0.80	0.91	RG141/RG667
RG9	12	0.000	0.20	6.82	–0.07	0.77	0.89	RG9/RG457
Conditional RRL (4-week/2-week)								
RG141	9	0.001	0.08	2.53	–0.04	0.82	0.88	RG141/RG667
RG9	12	0.000	0.18	4.89	–0.05	0.81	0.91	RG9/RG457

Table 4 Epistasis analysis for gene loci underlying Al tolerance in rice based on the segregation for relative root length (RRL) among a cross between IR1552 and Azucena at the 2nd week after stress and the conditional RRL at the 4th week after stress, given the RRL at the second week after stress

Chrom.	Interval	Site (cM)[a]	Chrom.	Interval	Site (cM)	LOD	R^2
At the 2nd week after stress							
4	RG163/AGG–CAG7	16	11	AAG–CAT10/RG167	2	5.17	0.07
7	CDO418/AAC–CTT7	18	12	CAC–CAT7/ACA-CAT6	4	6.28	0.05
8	RG978/RG1	14	11	AAG–CAT13/AAG–CTC4	18	6.70	0.08
Conditional RRL (4-week/2-week)							
5	CDO105/RG13	4	12	RG109A/ACA–CAT7	0	7.61	0.11
5	AGG–CTT8/RZ70	4	8	RG978/RG1	14	7.67	0.14
11	AAG–CAA6/RZ797	4	12	AGG-CAG6/AGG–CTT4	2	9.25	0.07

[a] Map distance in cM between the first marker locus to the interaction locus on the interval

Table 5 Mean comparison of genotypic combinations between interacting loci with a significant epistasis on Al tolerance. Only the marker loci closely linked to the interacting loci were used for the comparison

Marker loci	Chroms.	Genotypic combination[a]			
		II	IJ	JI	JJ
RRL at the 2nd week after stress					
AGG-CAG7×AAG-CAT10	4×11	0.74B	0.78B	0.88A	0.73B[b]
AAC-CTT×ACA-CAT7	7×12	0.74B	0.81A	0.81A	0.76B
RG1×AAG-CTC4	8×11	0.73B	0.82A	0.83A	0.78B
Conditional RRL (4-week/2-week)					
RG13×RG109A	5×12	0.79B	0.87A	0.88A	0.81B
AGG-CTT8×RG1	5×8	0.88A	0.79B	0.83A	0.85A
RZ797×AGG-CAG6	11×12	0.80B	0.92A	0.83B	0.82B

[a] II=*indica* homozygote, IJ and JI=heterozygote, JJ=*japonica* homozygote
[b] Different characters represent a significant difference the $P<0.01$ level

static effect at the 4th week after stress explained about 32% of the total phenotypic variation. A mean comparison of the combined marker genotypes between the interacting loci which are closely linked to the gene loci indicated that heterozygous genotyopes between the interacting loci had a positive effect on tolerance at the 2nd week after stress, except for one pair of interactions where only the heterozygous genotype of the *japonica* allele at AGG-CAG7 on chromosome 4 and the *indica* allele at AAG-CAT10 on chromosome 11 showed a positive effect. Three types of recombinant genotypes with significant epistasis were detected based on the conditional RRL at the 4th week after stress, given the RRL at the 2nd week after stress. The heterozygous genotype with a combination of *indica* and *japonica* alleles at RG13 on chromosome 5 and at RG109 A on chromosome 12 had a higher RRL, while the heterozygous genotype with an *indica* allele at RZ797 on chromosome 11 and a *japonica* allele at AGG-CAG6 on chromosome 12 also showed a higher RRL. The third pair of interactions with an *indica* allele at AGG-CTT8 on chromosome 5 and a *japonica* allele at RG1 on chromosome 8 gave a negative effect on tolerance (Table 5). The positive effects of three heterozygous genotypes could explain the tolerant transgressive segregation within the population and indicate that the enhanced Al-tolerant cultivar could be selected through a breeding program using Al-tolerant upland rice and superior lowland rice varieties.

Discussion

Three nutrient solution techniques have been used in evaluating rice tolerance for Al toxicity: absolute root length, root re-growth, and hematoxylin staining (Howeler and Cadavid 1976; Martinez 1976; Polle et al. 1978). In the absolute root length technique, relative root length (RRL), termed the root tolerance index (RTI), has been widely used as a parameter for evaluating Al tolerance (IRI 1978; Coronel et al. 1990; Khatiwada et al. 1996; Wu et al. 1997), both for its advantages as a simple measurement technique and the elimination of the genetic difference in root growth under normal culture conditions. Rice root-length reduction due to the primary effect of Al toxicity is interactive with nutrient concentration, solution pH, genotype and seedling age. In full-strength solution, the genotypic response to Al is more distinct in 30 mg Al^{3+} l^{-1} at pH 4.0 (Coronel et al. 1990; Khatiwada et al. 1996; Wu et al. 1997). Good segregation for RRL within the population in this case supports the earlier reports.

The critical Al concentration for Al toxicity in soil solution depends on pH value, nutrient status in the soil, and plant genotypes. In acid soils, especially in subsoils, when the pH value becomes less than 4.5, Al toxicity to the plant usually occurs. A wide range from 2 mg Al^{3+} l^{1-} to 30 mg Al^{3+} l^{-1} has been reported as a critical concentration for Al toxicity to different rice genotypes (Coronel et al. 1990; Khatiwada et al. 1996; Wu et al.

1997). It should be noted, however, that in full-strength solution, the high concentration of phosphate will decrease the active Al concentration because phosphate is a good Al-binding ligand. Therefore, the high critical Al concentration for Al toxicity in culture solution must be overestimated in term sof its initial concentration added in the solution.

Three QTLs were detected in this case after 2 weeks of stress, among which one QTL, flanked by RG323 and RZ801, on chromosome 1 explained about 19% of the total variation in RRL. Conditional mapping at the 4th week after stress and unconditional mapping indicated that the expression of QTLs for Al tolerance was changed, with an increase in the Al stress period. The QTL located on chromosome 1 was only expressed at the earlier stress phase, but its contribution to tolerance could be prolonged to later growth. In contrast, expression of the QTL located on chromosome 12 could be detected at both the 2nd and the 4th weeks after stress, and the effect of this QTL increased with increasing stress time. At least one different QTL with a relatively small effect was identified at a different stress time. It has been reported that gene expression appeared to be influenced by the concentration of Al in the nutrient solution in wheat (Bona et al. 1994). Our results suggest that a different Al stress time could induce a different gene for tolerance, or else that the expression of some tolerant genes are developmentally dependent.

Epistasis analysis indicated that Al tolerance in younger rice seedlings is predominantly controlled by an additive effect. The result supports the hypothesis that rice Al tolerance is a quantitative trait controlled by a greater additive effect, which has also been reported in other crops including wheat (Bona et al. 1994), maize (Lima et al. 1995), common bean (Araujo et al. 1992) and soybean (Spehar 1995). About 76% of the total variation in RRL after 4 weeks of stress was explained by the genetic effect, of which 44% could be attributable to an additive effect and 32% to an epistatic effect. The increase in the genetic effect on Al tolerance with an increase in stress time could explain the higher Al tolerance in older seedlings, which was shown both in this case and in an earlier report (Coronel et al. 1990). However, it should be noted that about a half of the additive effect detected at the 4th week after stress was contributed from the QTLs expressed at the 2nd week after stress based on conditional mapping. Although the genetic effect on Al tolerance is increased with the growth of seedlings, the additive effect expressed at the later stress period was decreased, and the epistatic effect became more important to tolerance. It was found that heterozygous genotypes between interacting loci had a higher RRL, which could explain the transgressive tolerance among the population and indicates that higher Al tolerant materials could be selected from crosses between Al tolerant upland rice and superior lowland rice varieties.

The QTL mapped on chromosome 12 was linked to RG9 which was also reported to be linked with the major QTL for phosphorus (P)-uptake efficiency in rice (Ni et al. 1998). The interval on chromosome 1 flanked by RZ801 and RG323, where the QTL for Al tolerance was detected at the earlier stress stage, is also related to the ability of rice the root to exclude excessive Fe^{2+} into the rhizosphere, which contributes to tolerance for Fe^{2+} toxicity (Wu et al. 1998). The positive alleles for P uptake efficiency and for Fe^{2+} tolerance at the two QTLs, respectively, were from *indica* parents, while the positive alleles for Al tolerance at the QTLs detected were from the *japonica* upland rice Azucena. These results suggest that the two QTLs are multiple allelic loci. P-deficiency stress, and Al and Fe^{2+} toxicities are co-existing stress factors in acid lowland rice soils. The QTLs detected for both Al tolerance and P efficiency, or for both Al and Fe^{2+} tolerance, open the door to developing varieties with multiple tolerance for stresses in acid soils.

Two Al tolerance mechanisms have been suggested: Al exclusion and tissue Al tolerance. Al exclusion has been extensively considered to be attributable to a change of rhizosphere pH, a release of organic acids as Al ligands, and root cell-wall characters which would prevent Al^{3+} from associating with the plasma membrane or entering the symplasm. Our understanding of the mechanism of tissue Al tolerance is as yet fragmentary. Low-molecular-weight-binding peptides induced by heavy metals have been considered as phytochelatins of heavy metals and, as such, to play a role in metAl tolerance in plants. The possible induction of Al-binding compounds that confer Al tolerance has received previous attention. Al induced both protein and cDNA within several hours of Al exposure, and specifically in Al-tolerant genotypes, in both wheat (Basu et al. 1994; Richards et al. 1994; Snowden and Gardner 1993) and rice (Zhang et al. 1997); but so far we have no proof to confirm the relation between the induced protein and the Al-tolerant performance. The biochemical-marker QTL analysis may help us to discover the precise relation, based on the co-segregation for Al-induced cDNA probes and the molecular markers linked to the QTLs detected. Molecular investigations into rice Al tolerance are in their infancy but the present results increase our understanding of the molecular background of Al-tolerant upland rice. The QTLs and the epistatic effect identified indicate that enhanced Al tolerance genotypes could be selected through a breeding program using Al tolerant upland rice and lowland rice with superior yield components.

Acknowledgements This research was funded by The National Natural Science Foundation of China and the Rockefeller Foundation. The authors thank Dr. D. Senadhira, a former breeder at the International Rice Research Institute, for his generous provision of the rice population used in this research.

Reference

Araujo JMD, Santos JBD, Ramalho MAP, Guedes GAA (1992) Genetic control of the tolerance of common bean (*Phaseolus vulgaris* L.) to chemical conditions of the soil under ‘cerrado’ vegetation. Ciencia e Pratica 16:189–196

Basu A, Basu U, Taylor GJ (1994) Induction of microsomal membrane proteins in roots of an aluminum-resistant cultivar of *Triticum aestivum* L. under conditions of aluminum stress. Plant Physiol 104:1007–1013

Bona L, Carver BF, Wright RJ, Baligar VC (1994) Aluminum tolerance of segregating wheat population in acidic soil and nutrient solutions. Commun Soil Sci Plant Anal 25:327–339

Coronel VP, Akita S, Yoshida S (1990) Aluminum toxicity tolerance in rice (*Oryza sativa*) seedlings. In: van Beusichem ML (ed) Plant nutrition-physiology and applications, IRRI, Manila, pp 357–363

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of rice based on an interspecific backcross population. Genetics 138: 1251–1274

Fageria NK, Wright RJ, Baligar VC (1988) Rice cultivar response to aluminum in nutrient solutions. Commun Soil Sci Plant Anal 19:1133–1142

Foy CD (1988) Plant adaptation to acid aluminum-toxic soils. Commun Soil Sci Plant Anal 19:959–987

Ganesan K, Sankaranarayanan C, Balakumar T (1993) Physiological basis of differential aluminum tolerance in rice genotypes. Commun Soil Sci Plant Anal 24:2179–2191

Howeler RH, Cadavid LF (1976) Screening of rice cultivars for tolerance to aluminum toxicity in nutrient solutions as compared with a field screening method. Agron J 68:551–555

Internationa Rice Research Institute (1978) Annual Report for 1977. Los Bavos, The Philippines

Khatiwads SP, Senadhira D, Carpena AL, Zeigler SR, Fernandez PG (1996) Variability and genetics of tolerance for aluminium toxicity in rice (*Oryza sativa* L.). Theor Appl Genet 93: 738–744

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300-kilobase-interval genetic map of rice including 883 expressed sequences. Nature Genet 8: 365–372

Lander ES (1993) Mapmaker/Exp 3.0 and Mapmaker/QTL, 1.1, tutorial and reference manual. Whitehead Institute, 9 Cambridge Center, Cambridge Massachusetts, USA

Lima M, Filho M, Furlani PR (1995) Diallel cross among inbred lines of maize differing in aluminum tolerance. Braz J Genet 18:579–584

Manly KF (1994) Establishing genetic linkage using recombinant inbred lines with an abnormal segregation ratio. Genetics 136: 1433–1434

Mckendry AL, Tague DN, Somers DJ (1996) Aluminum tolerance of 1BL.1RS and 1AL.1RS near-isolines in soft red winter wheat. Crop Sci 36:978–990

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC, Huang N (1997) Polymorphism, distribution, and segregation of AFLP markers in a double-haploid rice population. Theor Appl Genet 94:39–45

Martinez CP (1976) Aluminum toxicity studies in rice (*Oryza sativa* L). PhD thesis, Oregon State University, Corvalis, Oregon, USA

More DP, Kronstad WE, Metzger RJ (1977) Screening wheat for aluminum tolerance. In: Wright MJ, Ferrari SA (eds) Plant adaptation to mineral stress in problem soils. Cornel University Agric Exp Stn, Ithaca, pp 287–295

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed, 3:239–245

Ni JJ, Wu P, Senadhira D, Huang N (1998) Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). Theor Appl Genet 97:1361–1369

Polle E, Konzak CF, Kittrick JA (1978) Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedlings roots. Crop Sci 18:823–827

Richards KD, Snowden KC, Gardner RC (1994) *Wali6* and *wali7*: genes induced by aluminum in wheat (*Triticum aestivum* L.) roots. Plant Physiol 105:1455–1456

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Cop Sci 36:905–909

Sivagure M, Paliwal K (1993) Differential aluminum tolerance in some tropical rice cultivars. II. Mechanism of aluminum tolerance. J Plant Nutr 16:1717–1732

Snowden KC, Gardner RC (1993) Five genes induced by aluminum in wheat (*Triticum aestivum* L.) roots. Plant Physiol 103: 855–861

Spehar CR (1995) Diallel analysis for mineral element absorption in tropical adapted soybeans. Theor Appl Genet 90:707–711

Vos P, Hogers R, Bleeker N, Reijans M, Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang DL, Zhu J, Li ZK, Paterson A (1999) Mapping QTLs with epistatic and QTL×environment interactions by mixed linear model approaches. Theor Appl Gent 99:1255–1264

Wu P, Zhao B, Yan J, Luo A, Wu Y, Senadhira D (1997) Genetic control of seedling tolerance to aluminum toxicity in rice. Euphytica 97:289–293

Wu P, Hu B, Liao CY, Zhu JM, Wu YR, Senadhira D, Paterson A (1998) Characterization of tissue tolerance to iron by molecular markers in different lines of rice. Plant and Soil 203:217–226

Yoshida S, Forno DA, Cock JH (1971) In: Laboratory manual for physiological studies of rice. IRRI, Manila, The Philippines, pp 53–57

Zhang LP, Wu P, Zhu J, Luo A (1997) The expressive difference of inductive genes by aluminum in rice by differential display (English abstract). Sci Agric Sinica 5:71–74

Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. Genetics 141: 1633–1639