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V.T. Nguyen · M.D. Burow · H.T. Nguyen · B.T. Le
T.D. Le · A.H. Paterson

Molecular mapping of genes conferring aluminum tolerance in rice (Oryza sativa L.)

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Abstract Crop productivity on acid soil is restricted by multiple abiotic stress factors. Aluminum (Al) tolerance seems to be a key to productivity on soil with a pH below 5.0, but other factors such as Mn toxicity and the deficiency of P, Ca and Mg also play a role. The development of Al-tolerant genotypes of rice is an urgent necessity for improving crop productivity in developing countries. Inhibition of root growth is a primary and early symptom of Al toxicity. The present study was conducted to identify genetic factors controlling the aluminum tolerance of rice. Several parameters related to Al tolerance, most importantly the relative root growth under Al stress versus non-stress conditions, were scored in 188 F_3 selfed families from a cross between an Al-tolerant Vietnamese local variety, Chiembau, and an Al-susceptible improved variety, Omon269–65. The two varieties are both *Oryza sativa* ssp. indica, but showed a relatively high level of DNA polymorphism, permitting the assembly of an RFLP map consisting of 164 loci spanning 1,715.8 cM, and covering most of the rice genome. A total of nine different genomic regions on eight chromosomes have been implicated in the genetic control of root and shoot growth under aluminum stress. By far the greatest effects on aluminum tolerance were associated with the region near WG110 on chromosome 1. This region does not seem to correspond to most of the genes that have been mapped for aluminum tolerance in other species, nor do they correspond closely to one another. Most results, both from physiological studies and from molecular mapping studies, tend to suggest that aluminum tolerance is a complex multi-genic trait. The identification of DNA markers (such as WG110) that are diagnostic for aluminum tolerance in particular gene pools provides an important starting point for transferring and pyramiding genes that may contribute to the sustainable improvement of crop productivity in aluminum-rich soils. The isolation of genes responsible for aluminum tolerance is likely to be necessary to gain a comprehensive understanding of this complex trait.

Keywords Restriction fragment length polymorphism (RFLP) · Molecular-marker · Rice · Quantitative trait locus (QTL) · Abiotic stress tolerance

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V.T. Nyguyen · B.T. Lee
Institute of Biotechnology, Hanoi, Vietnam

V.T. Nguyen · M.D. Burow · A.H. Paterson
Plant Genome Mapping Laboratory, Texas A & M University, College Station, Texas, 77843-2474, USA

H.T. Nguyen
Plant Molecular Genetics Laboratory, Texas Tech University,

Lubbock, Texas, 79409-0001, USA

T.D. Le
Department of Genetics, National University of Hanoi, Hanoi, Vietnam

V.T. Nguyen · M.D. Burow · A.H. Paterson (✉)
Applied Genetic Technology Center (AGTEC),
Dept. Crop and Soil Science, Dept. Botany, and Dept. Genetics, University of Georgia, 30602 Athens, Georgia, USA
Fax: +1-706-583-0160
e-mail: paterson@uga.edu

Present address:
V.T. Nguyen, Department of Genetics,
National University of Hanoi, Hanoi, Vietnam

Introduction

The tropics contains 58% of the world’s land that is suitable for agricultural production, as well as 73% of the world population (FAO 1991). The adaptation of plants for tropical agriculture is frequently synonymous with adapting plants to soil stress. Soil-fertility stresses or soil-nutrient stresses, including both deficiencies and toxicities, limit agricultural production in the tropics as well as in many temperate regions. Sanchez and Salinas (1981) estimated that approximately 55% of the soil in tropical America, 39% in tropical Africa, and 37% in tropical Asia are acidic, representing 1.6 billion hectares.

Crop productivity on acid soils is restricted by multiple abiotic stress factors. Since the forms of soil alumi-

num (Al) and their solubilities are high, at a pH of 5 or less, aluminum toxicity becomes one of the major growth limiting factors affecting plants on acid soil (Kochian 1995). Symptoms of Al toxicity are not always easily identified; however, the initial and most dramatic symptom of Al toxicity is the inhibition of root elongation as a consequence of toxicity to the root apex (Wallace and Anderson 1984; Taylor 1988, 1991; Ryan et al. 1992, 1993; Delhaize and Ryan 1995; Kochian 1995). Roots injured by high Al are usually stubby and thick, and become dark-colored, brittle, poorly branched and suberized with a reduced root length and volume. Aluminum toxicity may inhibit shoot growth by limiting the supply of nutrients and water due to poorer subsoil penetration or lower root hydraulic conductivity. Shoot growth is also affected by Al toxicity, either due to reduced nutrient and water supply, or to a limited supply of cytokinins from the roots (Pan et al. 1989). Massot et al. (1992) showed that scoring for Al tolerance, using root elongation as a single criterion, may avoid the mis-classification of genotypes which accumulate a large amount of Al in shoots.

The physiological and biochemical mechanisms of aluminum toxicity are a matter of controversy (Kochian 1995). Because Al can interact with a number of extra-cellular and intra-cellular structures, many different mechanisms of Al toxicity have been hypothesized. Aluminum rhizotoxicity may be related to a disruption of membrane function, probably due to changes in the structure and function of the root-cell plasmalemma (Zhao et al. 1987). Depending on the pH, aluminum can bind to membrane proteins and lipids (Campbell et al. 1994) and participate in the formation of cross-links between proteins and pectins within the cell wall, reducing membrane integrity (Foy 1983). Disturbed mitotic process may also contribute to abnormal root growth (Morimura et al. 1978; Foy 1982a, b). Aluminum is particularly concentrated in the nucleus, and the cell cycle is inhibited, probably at the level of DNA replication (Foy 1974, 1982a, b; Foy et al. 1978). Although aluminum may bind to DNA (Matsumoto 1991) of the root cap cells in particular (Naidoo et al. 1978), the inhibition of cell division is presumably an indirect effect. Rengel (1992) has hypothesized that Al^{3+} blockage of Ca^{2+} channels could prevent the formation of important cytoplasmic Ca^{2+} transients needed for cell division to occur. Al^{3+} is probably an effective cation channel blocker, since it blocks both Ca^{2+} and K^{+} channels in wheat root cells (Gassmann and Schroeder 1994; Huang et al. 1993).

Rice, the world's leading food crop, is profoundly affected by aluminum toxicity. Many researchers have reported the identification of Al-tolerant genotypes in rice, as well as in wheat, maize, sorghum and soybean (Armiger et al. 1968; Howeler and Cadavid 1976; Fageria et al. 1988; Massot et al. 1992; Sivaguru et al. 1992; Sivaguru and Paliwal 1993; Khatiwada et al. 1996; Urrea-Gomez et al. 1996; Bushamuka and Zobel 1998; Massot et al. 1992; Sousa 1998). The identification of DNA markers diagnostic of Al tolerance can accelerate the development of cultivars that can remain productive even under Al stress, and may be the starting point for identifying the specific genes responsible for differences in the response of plant genotypes to toxic aluminum levels.

The main objective of the present study was to use molecular markers to further examine and characterize the genes and QTLs controlling aluminum tolerance in rice, using a cross between an Al-tolerant variety, Chiembau (tropical indica) and an Al-susceptible improved variety, Omon 269–65 (tropical indica).

Materials and methods

Aluminum tolerance screening

The experimental materials were developed by crossing Chiembau, a leading local aluminum-tolerant rice variety (tropical indica) in the north of Vietnam, with Omon 269–65, an improved variety (tropical indica) from the south of Vietnam. From this cross, 188 F_2 plants were randomly selected and selfed to produce 182 F_3 lines. Six F_3 lines did not provide sufficient seed to be used in the progeny test.

The parental lines and 182 self-pollinated F_3 families were screened for Al tolerance using a nutrient-solution culture modified from Khatiwada et al. (1996). Entries were arranged in a randomized complete block design with three 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 room maintained at 27±2°C with 12 h of light at 300 PPFD. Seedlings were then sown on a styrofoam sheet with a nylon net bottom, with one seedling per hole and 18 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 of Al (stress treatment). The nutrient solution was replaced every 5 days. The pH of the solutions was adjusted daily to 4.0 with 1 N NaOH or 1 N HCl. This level of Al stress was optimal for differentiating among rice genotypes based on a preliminary screen of 40 varieties at Al levels ranging from 0 to 200 ppm (data not shown), and is also consistent with the levels used by other workers (Khatiwada et al. 1996) to screen for the Al tolerance of rice.

The longest root of each seedling was measured after 10 days of growth in control or stress solutions. The ratio of average root length under stress versus non-stress conditions for each line in each replication was computed, as follows, as an indicator of the root tolerance index:

$$RR = \frac{SRL}{CRL} \times 100\%$$

where,
RR=root length ratio (%),
SRL=stress root length at 30 ppm Al (cm),
CRL=control root length at 0 ppm Al (cm).

The shoot length ratio (SR), was calculated in the same manner, based on the stress shoot length (cm) at 30 ppm Al (SSL), versus the control shoot length (cm) at 0 ppm Al.

Restriction fragment length polymorphism (RFLP) genotyping

Genomic DNA of the parents and 188 F_2 progeny was extracted from 2 g of lyophilized leaf tissue, as described by Li et al. (1995). DNA was digested with *Xba*I, *Hind*III, *Eco*RI and *Eco*RV. Electrophoresis, Southern blotting, and autoradiography followed standard procedures (Chittenden et al. 1994).

Data analysis

An RFLP linkage map was constructed using MAPMAKER (Lander et al. 1987). A LOD score of 3.0 was used for two-point analysis and a LOD difference of 2.0 was used for all three-point and multi-point analysis. The assignment of linkage groups or markers to their corresponding chromosomes was based on McCouch and Tanksley (1991). Trait means (for measurements described above), correlations, and heritability were determined using SAS (SAS Institute 1987). The mapping of QTLs 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 1,715.8 cM, a LOD score of 2.5 was selected as the threshold for claiming the presence of a QTL (Lander and Bostein 1989). With such a threshold, the probability that even a single false-positive QTL would be detected anywhere in the rice genome is approximately 0.05. Possible QTLs with a LOD >2.0 were also noted. In all cases where initial scans suggested two or more QTLs on the same chromosome, tests for independence of the QTLs were performed as described (Paterson et al. 1988; Lander and Bostein 1989). The QTLs reported are those that truly represent independent QTLs, rather than correlated effects of the same genetic locus. QTLs were designated with a *Q* to indicate they were detected through QTL mapping, followed by an abbreviation of the trait name and the chromosome number. A final letter was used to accommodate situations where more than one QTL affecting a trait was identified on the same chromosome. Chi-square values were calculated to examine if the observed allelic and genotypic frequencies of the marker loci deviated from the expected ratios. The proportion of total genotypic variance explained collectively by all identified QTLs for each trait was obtained by fitting the model containing all QTLs for the given trait in MAPMAKER/QTL.

Results

Phenotypic performance

The mean value of the CRL, SRL, RR, CSL, SSL and SR from the F_3 population, as well as the SRL of the F_2 population and the two parents, and the broad-sense heritability for each trait are summarized in Fig. 1. The root and shoot length of Chiembau and Omon 269–65 showed a differential response to aluminum stress, as expected. Chiembau has a higher SRL, RR, SSL and SR, indicating its tolerance. Chiembau also had higher root and shoot length of controls (non-stressed plants). Root length was more-sensitive than shoot length to Al stress, as reflected by the smaller values for root length ratio than for shoot length ratio. The frequency distribution of CRL, SRL, RR, CSL, SSL and SR of F_3 and SRL of F_3 progeny was approximately normal. In all cases the range of progeny phenotypes was appreciably greater than the range of parental values, suggesting transgressive variation.

Heritabilities based on replicated tests of F_3 progenies were very high for all traits (0.788–0.942; Fig. 1). By comparison, the heritability of root length under Al stress based on the measurement of single F_2 plants was very low (0.06; Fig. 1), reflecting the need for replicated testing to obtain a reliable assessment of the genetic potential for this trait.

Construction of the linkage map

Two hundred and sixteen loci at intervals of about 8 cM were surveyed in the parents using previously mapped RFLP probes (Causse et al. 1994) generously provided by Steven Tanksley, Susan McCouch (Cornell) and Olin Anderson (USDA-ARS, Albany Calif.), supplemented with heterologous probes available in the Paterson laboratory. Approximately 68% of the probes detected RFLPs. A subset of 137 probes detected 164 RFLP marker loci in the 188 F_2 progeny from the cross Chiembau×Omon 269–65. These loci comprised a map of 19 linkage groups that spanned 1,715.8 cM with an average distance of 10.46 cM (Kosambi 1944) between markers (Fig. 2). There were gaps on chromosomes 1, 2, 3, 4, 8 and 10, but the genome coverage was estimated to be approximately 90% based on alignment to the maps of Causse et al (1994). The order of markers approximately agrees with the map of Causse et al. (1994), Li et al. (1995), and Alam and Cohen (1998). Six possible inversions were found. However, all except one (RG152–RG555) involved very closely linked pairs of loci, or pairs of loci that were flanked by gaps in the map, suggesting probable small errors in one or more maps rather than true differences in chromosome structure. The inversions are as follows: two on chromosome 1 (RZ730b–RG780; RG246a–RG532a), two on chromosome 2 (CDO395–RG139; RG152–RG555), one on chromosome 3 (CDO122–RZ488), one on chromosome 10 (RG421–RG561), and two on chromosome 11 (RG1109–RG353; RG1094–RG2). Among the total of 137 probes, 15 (11%) detected polymorphism at loci that were on different chromosomes from previously mapped locations (RG98, RG118, RG313a, RG323, RG433, RG598, RZ213, RZ244, RZ251, RZ291, RZ455, RZ909, CDO192a, CDO1380a and CDO1395a). This result is approximately consistent with the level of sequence duplication previously reported in rice. We report for the first time (based on a search of the Rice Genes database) the location in rice of CDO1380b, CDO1395b, CSU039, CSU382a, CSU382b, pSB414, pSB108, RG247b, RG313b, RG313c, RG445b, RG996, RZ342 and WG110.

Segregation of marker loci in the F2 population

The overall genomic composition of the F_2 population showed an average of 50.13% (4.2%) of the genome coming from Chiembau, remarkably close to the expected 50%. A total of 54 (32%) markers, grouped into 30 regions (Fig. 2) on all 12 chromosomes, showed significant deviations (0.05) from expected segregation ratios based on the chi-square test. The most common deviation is an excess of the Omon homozygote (nine cases), followed by an excess of heterozygotes (eight cases), with six cases of excess Chiembau homozygotes, two cases of heterozygote deficiency, and five cases that cannot be evaluated because the diagnostic markers are dominant/recessive.

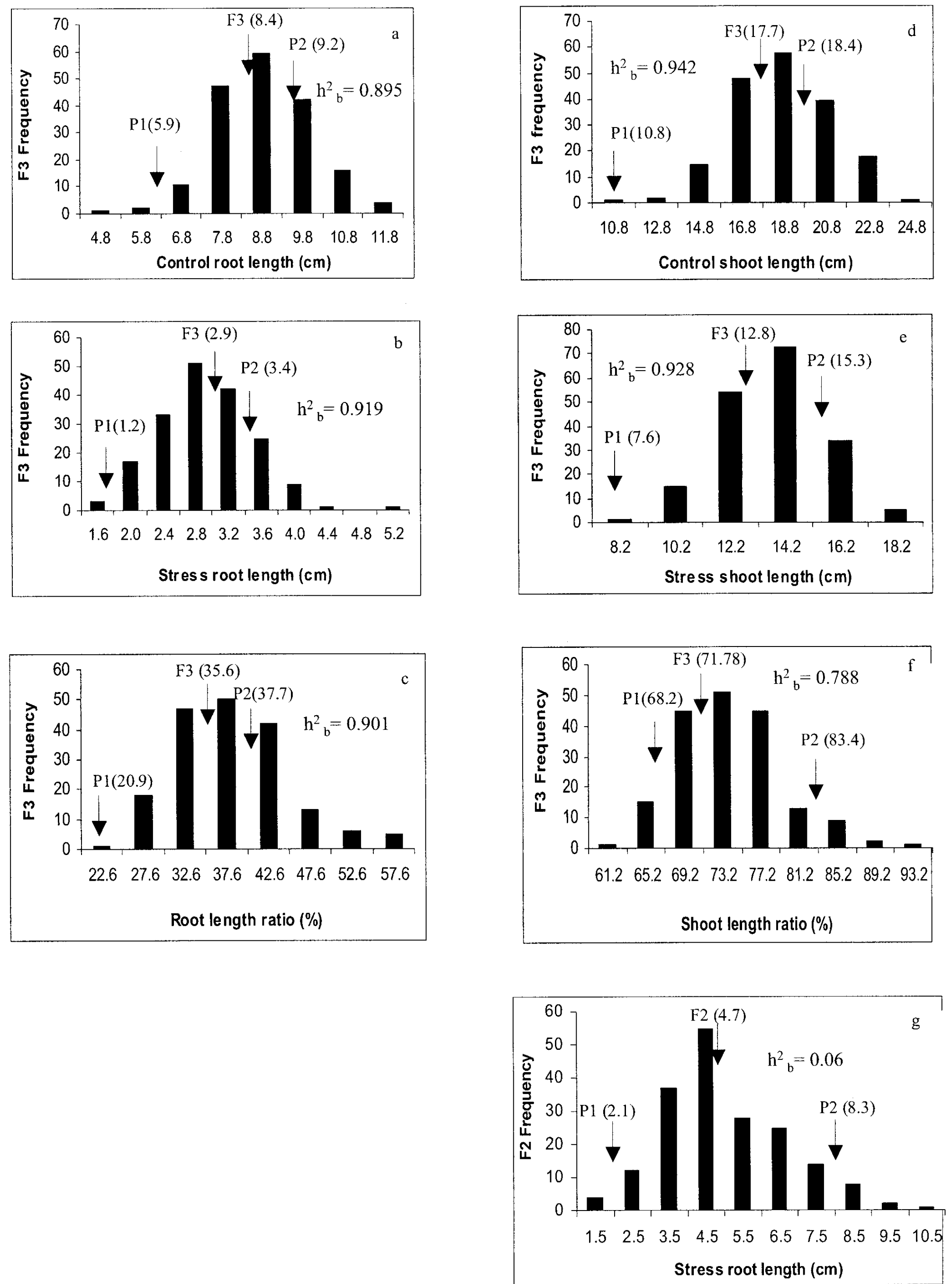

Fig. 1a–g Phenotypic distributions for 182 F_3 lines and 188 F_2 lines from the cross Chiembau×Omon269–65. **a** Distribution for control root length. **b** Distribution for stress root length. **c** Distribution for root length ratio. **d** Distribution for control shoot length. **e** Distribution for stress shoot length. **f** Distribution for shoot length ratio. **g** Distribution for stress root length of 188 F_2 lines. P1: Omon 269–65; P2: Chiembau; h^2_b: broad-sense heritability

Interval mapping of QTLs

Biometrical parameters for each QTL are presented in Table 1. While the primary goal of this experiment is best reflected by the stressed root length, and the ratio of the length of stressed versus non-stressed roots, QTLs that confer different rates of root and/or shoot growth independently of Al stress are also of potential interest to the breeding and genetic communities. Therefore, we have presented QTLs for all measured traits.

Control root length

Two QTLs, *QAlCr2a* and *QAlCr3a*, were identified on chromosomes 2 and 3, and a possible QTL (*QAlCr6a*; based on the 3.0>LOD>2.0 criteria stated in the data analysis) on chromosome 6. Chiembau had the favorable alleles (longer root length) for *QAlCr3a* and *QAlCr6a*, consistent with the difference between the parents. Omon269–65 had the favorable allele for *QAlCr2a*, possibly explaining some of the transgressive variation. A full model containing the three QTLs explained 18.3% of the phenotypic variance.

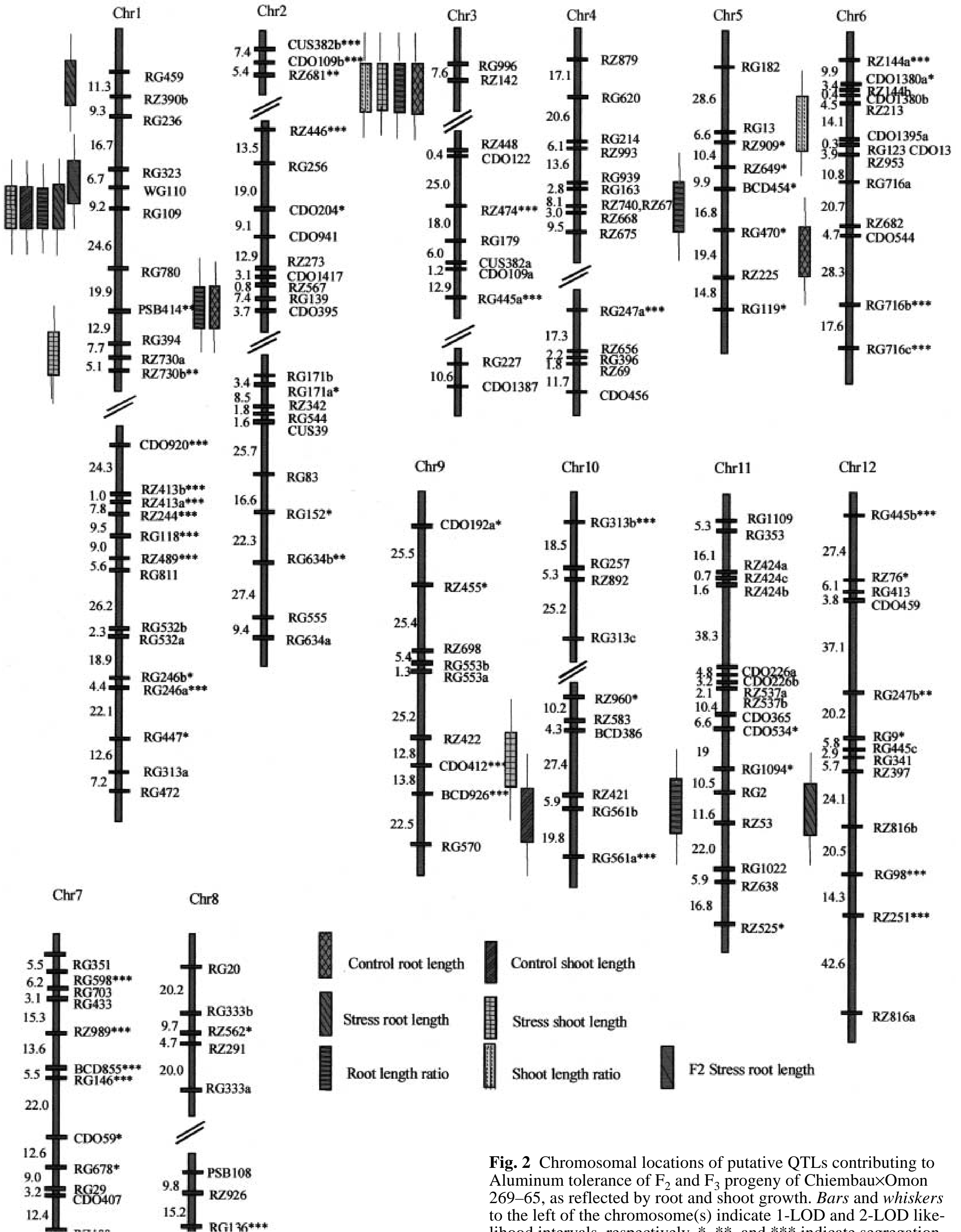

Fig. 2 Chromosomal locations of putative QTLs contributing to Aluminum tolerance of F_2 and F_3 progeny of Chiembau×Omon 269–65, as reflected by root and shoot growth. *Bars* and *whiskers* to the left of the chromosome(s) indicate 1-LOD and 2-LOD likelihood intervals, respectively. *, **, and *** indicate segregation distortion significant at 0.05, 0.01, and 0.005

Stress root length

Three QTLs, *QAlSr1a, QAlSr1b* and *QAlSr12a*, were identified on chromosomes 1 and 12. Chiembau had the favorable alleles (longer root length) for all three, consistent with the difference between the parents. *QAlSr12a* showed evidence of overdominance, with a dominance deviation more than triple the additive effect, possibly explaining some of the transgressive variation. A full model containing the three QTLs explained 38.9% of the phenotypic variance. An additional QTL, *QA1R1a*, was found on chromosome 1 near *QAlSr1b* based on measurement of the F2 plants, barely meeting the significance level. No other QTLs could be detected in the F2 plants.

Root length ratio

Three QTLs, *QAlRr1a, QAlRr2a* and *QAlRr3a*, were identified on chromosomes 1, 2 and 3, and two possible QTLs, *QAlRr5 and QAlRr11a*, on chromosomes 5 and 11. Chiembau had the favorable alleles (less impaired by stress) for *QAlRr1a, QAlRr5* and *QAlRr2a*, consistent with the difference between the parents. Omon269–65 had the favorable allele for *QAlRr11a*, possibly explaining some of the transgressive variation. *QAlRr3a* showed a marked heterozygote disadvantage. A full model containing the five QTLs explained 39.8% of the phenotypic variance.

Control shoot length

Two QTLs, *QAlCs1a* and *QAlCs10a*, were identified on chromosomes 1 and 10, respectively. Chiembau had the favorable alleles (longer shoot length) for both, consistent with the difference between the parents. However, the additive effect of the Chiembau allele for *QAlCs10a* was very small, and the dominance deviation was about 9-times larger, possibly explaining some of the transgressive variation. A full model containing the two QTLs explained 44.9% of the phenotypic variance.

Stress shoot length

Three QTLs, *QAlSs1a, QAlSs1b* and *QAlSs10a*, were identified on chromosomes 1 (two) and 10, respectively. A possible QTL, *QAlSs3a,* was found on chromosome 3. Chiembau had the favorable alleles (longer shoot length) for *QAlSs1a, QAlSs1b* and *QAlSs3a,* consistent with the difference between the parents. However, the additive effect of the Chiembau allele for *QAlSs3a* was very small,

Table 1 QTL for Aluminum tolerance in F_2 and F_3 from the cross Chiembau×Omon269–65

Trait[a]	Locus[b]	Flanking marker	Chr.	Additive effect[c]	Dominance[d]	Peak LOD score	% Variance explained
$SRLF_2$	*QAlR1a*	RG323-WG110	1	–0.60	–0.31	2.54	7.2
$CRLF_3$	*QAlCr3a*	RG996-RZ142	3	–0.48	0.28	3.47	9.3
	QAlCr6a	RZ682-CDO544	6	–0.39	–0.13	2.16	5.8
	QAlCr2a	RG139-CDO395	2	0.44	0.16	2.93	7.2
$SRLF_3$	*QAlSr1a*	RG459-RZ390b	1	–0.12	–0.26	2.86	7.0
	QAlSr1b	WG110-RG109	1	–0.44	0.14	11.55	27.4
	QAlSrl2a	RZ397-RZ816b	12	–0.10	0.32	3.43	8.3
RRF_3	*QAlRr1a*	WG110-RG109	1	–4.84	1.22	10.71	25.0
	QAlRr3a	RG996-RZ142	3	0.76	–4.12	3.30	10.0
	QAlRr5a	BCD454-RG470	5	–2.03	1.57	2.48	6.1
	QAlRr11a	RG2-RZ53	11	2.33	–1.40	2.19	6.2
	QAlRr2a	RG139-CDO395	2	–2.67	0.38	3.26	7.9
$SSLF_3$	*QAlSs1a*	WG110-RG109	1	–1.89	0.42	25.31	51.2
	QAlSs1b	RG394-RZ730a	1	–1.49	0.75	16.03	34.9
	QALSs3a	RG996-RZ142	3	–0.10	–0.95	2.49	6.9
	QAlSs10a	BCD386-RZ421	10	0.02	1.07	2.54	8.7
$CSLF_3$	*QAlCs1a*	WG110-RG109	1	–2.24	0.66	20.16	41.3
	QAlCs10a	RZ421-RG516b	10	–0.14	1.28	2.82	7.2
SRF_3	*QAlS3a*	RG996-RZ142	3	–2.16	–1.55	3.38	9.3
	QAlS6a	RZ213-CDO1395a	6	–0.53	2.85	2.31	7.3

[a] SRL: Al-stressed root length (stressed); CRL: control root length; RR: root ratio (Stressed/Control); SSL: stressed shoot length; CSL: control shoot length; SRF: shoot ratio (Stressed/Control). F_2 and F_3 indicate the generation in which the phenotype was measured

[b] Individual QTLs are designated with ”Q” indicating QTLs with a LOD>2.5; the abbreviation of the trait name and the chromosome number, is followed by letter to accommodate situations when more than one QTL affecting a trait is identified on the same chromosome. Possible QTLs with a LOD >2.0 are also reported

[c] Additive effects of homozygotes are calculated as: (Omon-Chiembau)/2. A positive effect reflects greater growth of the Omon homozygote, and a negative additive effect reflects greater growth of the Chiembau homozygote

[d] Dominance deviations are calculated as: Heterozygote – [(Omon+Chiembau)/2]. A positive effect reflects growth of the heterozygote that exceeds the midparent, and a negative effects reflects growth that is less than the midparent

and the dominance deviation was about 9-times larger. *QAlSs3a* showed a marked heterozygote disadvantage, while *QAlSs10a* showed virtually no difference between the homozygotes but a large heterozygote advantage, possibly explaining some of the transgressive variation. A full model containing the four QTLs explained 59.7% of the phenotypic variance.

Shoot length ratio

QTL *QAlS3a* was identified on chromosome 3. A possible QTL, *QAlS6a,* was found on chromosome 6. Chiembau had the favorable alleles (longer shoot length) for both, consistent with the difference between the parents. However, the additive effect of the Chiembau allele for *QAlS6a* was small, while the dominance deviation was about 5-times larger, possibly explaining some of the transgressive variation. A full model containing the two QTLs explained 15.5% of the phenotypic variance.

Discussion

A total of nine different genomic regions on eight chromosomes have been implicated in the genetic control of root and shoot growth under aluminum stress. By far the greatest additive effects on aluminum tolerance were associated with the region near WG110 on chromosome 1, in which the Chiembau allele was associated with higher root length under stress, as reflected by the higher root length ratio. The relatively large effect of this genomic region on root length under Al stress was the only one that could be discerned in the (stressed) un-replicated F_2 plants. The region was also associated with differences in shoot-length both in stressed and non-stressed conditions, so shoot length parameters alone could not be considered as indicative of stress tolerance.

A second genomic region near RG996 (chromosome 3) also showed consistent effects on aluminum tolerance; however, the effects of this genomic region were complex. With regard to the root length ratio, the allele from Omon (the less-tolerant parent) showed a slight advantage; however, the heterozygote showed much less tolerance than either homozygote. It is possible that this could reflect a form of 'hybrid breakdown' rather than a truly higher susceptibility of the heterozygote to aluminum. Although both Chiembau and Omon are of the same rice subspecies (*indica*), the relatively high level of DNA polymorphism (68% of DNA probes screened) between them suggests that they are quite divergent. By contrast, with regard to shoot length ratio, the Chiembau allele was favorable and there was some indication of dominance but not overdominance. The difference in gene action between the root length ratio and the shoot length ratio may suggest that there are actually two different (closely linked) genes exerting these effects.

Three additional regions on chromosomes 2, 5 and 11 showed an association with the root length ratio, considered to be the most reliable measure of aluminum tolerance. In the case of chromosome 2, the same genomic region also was associated with a difference in the control root length.

Measurement of the shoot length and the shoot length ratio alone are considered misleading with regard to aluminum tolerance (see above). A possible QTL was found on chromosome 6 that might affect the shoot length ratio but not the root length ratio; however, it fell slightly below our (LOD 2.5) significance threshold. We report this finding for the future reference of other researchers, in case future studies should show stronger evidence implicating this genomic region in aluminum tolerance, or should be of interest for the study of shoot growth for other reasons.

Finally, two additional genomic regions on chromosomes 6 and 10 have been associated with differences between Chiembau and Omon that do not appear to be related to aluminum tolerance. The chromosome-6 region affected only the root length of the control treatment, and the chromosome-10 region affected the shoot length of both the control and stressed treatments to similar degrees. Again, we report these QTLs for the benefit of others who may need this information for studies that are not directly related to aluminum tolerance.

We find aluminum tolerance in this population to be predominantly determined by one gene of relatively large effect (by the standards of QTL mapping), but to be modified by several genes of smaller effect. This is reasonably consistent with available data regarding the inheritance of aluminum tolerance in rice; analysis of variance of a 7×7 diallel for relative root length by Khatiwada et al. (1996) showed that high relative root length is governed by both additive and dominance effects with a preponderance of additive effects. Another diallel of 56 F1 progenies derived from 8 male×7 female rice parents with differential Al tolerance suggested inconsistent dominance effects (Wu et al. 1997).

However, as is true for the mechanisms of aluminum tolerance, its inheritance in other crops remains controversial. In maize, Al tolerance has been shown by some authors to be inherited as a complex trait (Magnavaca et al. 1987; Lima et al. 1992), while others have asserted that it is a single major gene (Moon et al. 1997; Rhue et al. 1978). In wheat, rye and triticale, Aniol and Gustafson (1984) associated chromosome arms 6AL, 7AS, 2DL, 3DL, 4DL 4BL and 7D with Al tolerance in 'Chinese Spring', and major genes for tolerance in rye seem to be located on 3R and 6RS, with other genes on 4R. Aniol (1990) showed that Al resistance was linked to at least three different chromosome arms: the short arm of chromosome 5A and the long arms of chromosomes 2D and 4D, the latter two being consistent with earlier data (Takagi et al. 1983). Gallego and Benito (1997) and Gallego et al. (1998) show that Al tolerance in rye is controlled by at least two independent and dominant loci (*Alt1* and *Alt3*) located on chromosomes 6RS and 4R. Others have found Al tolerance in the Triticeae to be monogenic (Delhaize et al. 1993), with the predominant

locus on the long arm of chromosome 4D (Luo and Dvorak 1996), and linked to diagnostic RFLP markers (Riede and Anderson 1996). Johnson et al. (1997) indicated a single dominant gene was transferred from Atlas 66 to Hard Winter Wheat. In dicots, three to five genes may control Al tolerance in an F_4-derived population from soybean PI 416937, (Bianchi-Hall et al. 1998), and several single recessive mutations conferring Al sensitivity have been identified in *Arabidopsis thaliana* (Larsen et al. 1996).

Further confusing the picture, there seems to be little correspondence in the location of aluminum tolerance genes within or between taxa. In addition to the diversity of genes cited from other sources (above), the major gene we report near WG110 does not correspond to most of the genes that have been mapped for aluminum tolerance in other species. WG110, a wheat genomic probe, maps to wheat homoeologous group 3L (http://genome.cornell.edu/cgi-bin/WebAce/webace?db=graingenes), a location that is not associated with aluminum tolerance in any of the wheat studies (above).

The great diversity of results both from physiological studies and from molecular mapping studies, support the notion that aluminum tolerance is a complex multigenic trait, and that there may exist many different tolerance or resistance mechanisms. The identification of DNA markers (such as WG110) that are diagnostic of aluminum tolerance in particular gene pools provide an important starting point for transferring and pyramiding genes that may help to improve productivity in aluminum-rich acid soils. To gain a good understanding of the molecular basis of aluminum tolerance, it appears necessary to isolate genes responsible for several different mechanisms of aluminum tolerance; diagnostic DNA markers represent a first step toward this goal.

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