# IDENTIFICATION OF QUANTITATIVE TRAIT LOCI CONTROLLING ALUMINUM TOLERANCE IN RICE

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## ABSTRACT

*Quantitative trait loci (QTL) analysis has been carried out to identify genes controlling aluminum tolerance in rice. One hundred and six doubled haploid plants derived from a cross between a japonica variety, Azucena, and an indica variety, IR64, were used for QTL mapping and 175 RFLP and isozyme markers were employed to identify QTLs. QTL analysis revealed the presence of two QTLs, QTL 1 and QTL 2, which located in the middle of chromosome 6 and at the end of chromosome 8, respectively. The two QTLs explained 21.6% of the total phenotypic variation in the population based on a multiple regression model.*

**Key words**: *Oryza sativa* L., rice, QTL, aluminum tolerance

## INTRODUCTION

Rice is the world's most important food crop. It is the staple food for one third of the world's population. Rice is grown in over 110 countries of the world with a total of 146 million ha and 579 million ton of grains in 1990 (Chang 1997). Aluminum toxicity is a major problem limiting crop production in acid sulfate soils, which account for 40% of the world's arable lands ( Ma et al. 1997). When dissolved in acid soils, Al (primarily in the form of $Al^{3+}$) is toxic to many crops including rice (Kochian 1995). The effects of aluminum on plants are numerous, but the primary effect of Al is inhibition of root elongation and consequently damage of root system, resulting in inhibition of nutrient and water uptake (Taylor 1988). Therefore, root tolerance index (RTI), i.e. relative root length calculated as maximum root length in the Al treatment divided by maximum root length in the control nutrient solution, has been suggested to be one of the most important criteria for screening genotypes and cultivars for Al tolerance (Taylor and Foy 1985).

Cereals differently respond to aluminum toxicity, rye (*Secale cereale* L.) being one of the most tolerant and wheat (*Triticum* ssp) being less tolerant (Gallego and Benito 1997). Aluminum tolerance in wheat is considered a monogenic dominant trait (Johnson et al. 1997). However, Lafever and Campbell (1978) indicated that the sensitivity to aluminum was conditioned by a single recessive gene and the inheritance of aluminum tolerance was more complex than a single gene. A study on the inheritance of aluminum tolerance in Atlas 66 wheat revealed that the expression of genes for tolerance to 0.44 mM Al appeared to be more complex than was predicted by the existence of two dominant genes and the genes for tolerance to Al in Atlas wheat were not

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all located on D-genome chromosome (Berzonsky 1992). Moore (1977) showed that wheat exhibited 4 distinct classes of aluminum tolerance, with Brevor and Atlas 66 representing the least and the most tolerant classes, respectively. Riede and Anderson (1996) found that the gene for aluminum tolerance, which accounted for 85% of phenotypic variation for Al tolerance, was located on chromosome 4DL and linked to marker bcd1 230 by a distance of 1.1 cM. In barley (*Hordeum vulgare* L.), one major gene has been reported to control differential aluminum tolerance (Reid 1969). However, the aluminum tolerance in sorghum (*Sorghum bicolor* L.) showed predominantly additive genetic effects with some degree of dominance (Boye-Goni and Marcarian 1985. Aluminum tolerance in corn (*Zea mays*) was not simply inherited (Rhue and Grogan 1977). However, Rhue et al. (1978) showed that aluminum tolerance in corn was a dominant trait and controlled at a single locus with a multiple allelic series and there was no cytoplasmic effect on this trait.

In rice (*Oryza sativa* L.), a partial dominant and one group of gene were detected to control root length under aluminum toxicity (Khatiwada et al. 1996). Wu et al. (1997) reported that the higher additive effect might be the genetic characteristics of tolerance to Al in rice. Due to polygenes controlling aluminum tolerance in rice, progress in the identification of tolerant genotypes using conventional breeding method is slow. So far no molecular work on aluminum tolerance in rice has been reported. Therefore, identification of markers linked to the genes controlling aluminum tolerance in rice would accelerate the breeding program for acid sulfate soil areas.

## MATERIALS AND METHODS

### Plant materials

A population of 106 doubled-haploid (DH) lines used was derived from a single cross between the irrigated indica variety IR64, an aluminum susceptible variety, and the upland japonica variety Azucena, an aluminum tolerant (Khatiwada et al. 1996). These varieties were reconfirmed as susceptible and tolerant, respectively at Plant Molecular Genetics Laboratory of Plant and Soil Science Department, Texas Tech University. An RFLP map of the population was constructed by Huang et al. (1997) from 135 DH lines using 175 markers covering 2005cM with an average distance of 11.5 cM between pairs of markers. This map was used for QTL analysis in our study.

### Aluminum tolerance screening

The procedure used to screen rice seedlings for aluminum tolerance was followed Khatiwada et al. (1996) with some modifications. Seeds were surface sterilized with 0.1% $HgCl_2$ for 2-3 minutes and rinsed thoroughly with distilled water. Sterilized seeds were soaked in distilled water for 24h and then incubated at 30$^{o}$C for 48h. Geminated seeds were rolled in germinating paper and kept at 30$^{o}$C for another 36h. The uniform root seedlings were selected and sown on a tyrofoam sheet with nylon net bottom with one seed per hole and one row per line. The sheets were floated on Yoshida solution (Yoshida 1986) for 14 days. Two concentrations of aluminum (0 and 40ppm) were applied for all entries at

pH 4.0. The culture solutions were renewed weekly and the pH of the solutions was maintained daily at 4.0 with 1N HCl or NaOH. The experiment was designated as randomized complete block design (RCBD) with 8 replications. The temperature and light intensity during the experiment were maintained at 27$^{o}$C and 250 PPFD, respectively. At harvest the longest roots of three plants were measured and averaged. The root tolerance index (RTI), calculated as the maximum root length in Al stress culture divided by maximum root length in control (Wu et al. 1997, Taylor and Foy 1985), was used as an indicator to evaluate Al tolerance.

**Statistical analysis**

Analysis of variance (ANOVA), mean comparisons, and coefficients of variation (CV) of each line were performed using SAS programs (SAS institute Inc. 1989). F tests were used to determine the significance of variance components, and LSD values were computed for comparison of the mean between lines. Broad-sense heritability at the genotype mean level was computed as $H^2_F = \delta^2_G/(\delta^2_G + \delta^2_e/n)$ where $\delta^2_G$ and $\delta^2_e$ were the estimates of genetic and residual variances, respectively, derived from the mean squares of the analysis of variance and n was number of replications. Normal distribution was evaluated using Shapiro-Wilk test.

Two methods of detection of putative QTLs were employed, single-point analysis using the general linear model (GLM) procedure of SAS (SAS institute Inc. 1989) and interval mapping using the MAPMAKER/QTL program (Paterson et al. 1988). The single-point analysis used to test the significance of the association at each locus between marker genotype and trait values over all plants using the F-test (Renoda and Mackill 1996). The probability level of 0.01 was used for the F-test. MAPMAKER/QTL was used to identify loci affecting quantitative traits on the basis of interval analysis. An LOD threshold of ≥ 2.0 was used to detect the presence of putative QTL in a given genomic region. The proportion of total phenotypic variance explained collectively by all defined QTLs for the trait was obtained by fitting the model containing all QTLs for the trait in MAPMAKER/QTL.

**RESULTS AND DISCUSSION**

**Phenotypic variation**

The frequency distribution of phenotypic values of the root tolerance index of the doubled-haploid population and its parents are presented in Fig. 1. The distribution of root tolerance index (RTI) of population was normal using Shapiro-Wilk test. RTI of DH lines ranged from 0.331 to 1.062 with a mean of 0.6596, which was well below the mid-parental value of 0.7117. There were only 2.7% of population belonging to Azucena group and 15.7% of population belonging to IR64 group. Most of the rest were intermediate of the parents. Only 4.54% of the population were extremes. Analysis of variance showed highly significant differences ($P<0.001$) among the lines for root tolerance index after 14 days of treatment with 40ppm $Al^{+3}$. Coefficient variance values of experiment were 12.58% and of all the lines were below 20%.

**Heritability**

The genetic component of variation is important since only this component is transmitted to the next generation. Heritability is the ratio of genetic variation to the total variation. The heritability was very high (0.8318) for root tolerance index on family mean basis, which indicates the possibility of genetic gain in selection for Al tolerance based on root tolerance index under Al toxicity condition. This result was in agreement with Khatiwada et al. (1996) and Wu et al. (1997).

**QTL identification**

The location of two QTLs associated with this trait is shown in table 3 and Fig.2. QTl 1 located in the middle of chromosome 6 explained 12.5% phenotypic variation with additive effect of 7.61 contributed from Azucena allele. QTL 2 located at the end of chromosome 8 explained 10% phenotypic variation with additive effect of 7.29 also contributed from Azucena allele.

Interval mapping can detect QTLs located in intervals up to 50 cM long with only a slight reduction power (Darvasi et al. 1993) and Darvasi and Soller (1994) also indicated that the optimum marker spacing for initial QTL studies can be as wide as 50 cM. In this map, there are only 9 out of 143 interval having more than 30 cM using Kosambi (1944) function with the longest at 43.8 cM. However, Falconer and Mactory (1997) indicated that map distance of 20cM is the limit of resolution, what is detected as a QTL in this region is a segment of this length, which may contain several loci affecting the trait. Fortunately, in this study no QTLs were detected in the regions with interval more than 20cM. The proportion of total phenotypic variation explained collectively by two defined QTLs for the trait was 21.6% using multiple regression model and the interaction between two QTLs was not significant ($P>0.625$) suggesting that there is no epistatic effect and these two QTls have additive effect. At all the detected QTLs for root tolerance index only Azucena parent contributed favorable alleles. These identified QTLs explained a portion of total phenotypic variation despite nearly complete genome coverage by genetic markers along with high heritability estimate suggesting that large number of genes each having small effect are involved in aluminum tolerance which can not be detected because of rather small population size (Tanksley 1993).

Single marker analysis using SAS PROC GLM detected 8 markers associated with root tolerance index. All these marker, which variance- explained ($R^2$) ranged from 6.94% to 10.03%, were located on chromosomes 6 and 8 with the level of probability of 0.01 (table 2). Single-point analysis is as efficient as interval mapping, when the information from flanking makers is considered, only if QTL and marker positions exactly coincide (Lander and Botstein 1989). Hence, single-point analysis was used for confirming the results of interval mapping. Markers found to be associated with root tolerance index using interval mapping (MAPMAKER/QTL) were confirmed by single-point analysis, namely RG162, RG172 and Amp-2.

To locate the genes controlling aluminum tolerance in wheat and rye, Aniol (1984) found that genes for aluminum tolerance

in wheat (Chinese Spring variety) were localized in chromosome arms 6AL, 7AS, 2DL, 3DL, 4DL, and 4BL, and on chromosome 7D. Major genes for aluminum tolerance in rye were found to be located on 3R and 6RS, with other genes on 4R. Molecular marker linked to aluminum tolerance has been mapped in wheat (Riede and Anderson 1996). It has been shown that aluminum tolerance in wheat conditioned by a major gene which is located on chromosome 4DL. Different degrees of tolerance found among wheat varieties reflect the polygenic character of aluminum tolerance, and are confirmed by physiological data suggesting that the mechanism is operating in different subcellular compartments within the wheat root (Aniol 1983). In our study, QTLs controlling aluminum tolerance in rice are located on chromosomes 6 and 8 which are correspondent to chromosomes 7S and 3L in wheat, respectively (Ahn et al., 1993).

It has been hypothesized that correlated traits have a common genetic base (Paterson et al., 1991) and have most significant markers in common (root length in this population) (Courtois et al., 1995). Among the areas common between traits, the chromosomal segment flanked by Amp-2 and CDO999 markers (QTL 2 in this study) on chromosome 8 corresponds to QTLs for tiller numbers (Yan et al., 1998) and brown planthopper resistance (Alam and Cohen, 1998) and relative plant height (Wu et al., 1998) in this population. QTL 2 flanked by RG162 and RG172 marker on chromosome 6 detected in this study is also common with QTLs controlling total root weight and maximum root length (Yadav et al., 1997) in the same population. In reality, the parental lines typically are divergent for many traits, so the tests for linkage to markers are repeated for each trait. This result indicates that these regions are significantly positive associations and play an important role for the rice traits. Therefore, by the introgression of these segments, it is possible to improve several related traits simultaneously.

**Acknowledgements**

Scholarship supported by the Rockefeller Foundation to Nguyen Duy Bay is fully acknowledged. The authors also thank Dr. N. Huang for providing marker data.

Table 1. Descriptive statistics of root tolerance index, measured on 106 recombinant inbred lines and two parental lines in eight replications.

| Parameter | Min | Max | Mean | SD | CV(%) | $LSD_{0.05}$ |
|---|---|---|---|---|---|---|
| DH lines | 0.331 | 1.062 | 0.6596 | 0.0830 | 12.58 | |
| IR64 | 0.368 | 0.586 | 0.4893 | 0.0821 | 16.77 | 0.0815 |
| Azucena | 0.862 | 0.996 | 0.9346 | 0.0503 | 5.38 | |

Table 2. Markers identified as being associated with root tolerance index detected by single-point analysis using SAS PROC GLM

| Marker name | Chromosome # | P | $R^2$ |
|---|---|---|---|
| RG162 | 6 | 0.001 | 10.03 |
| RG172 | 6 | 0.003 | 9.13 |
| RZ144 | 6 | 0.008 | 7.37 |
| RZ667 | 6 | 0.005 | 7.13 |
| AC5 | 8 | 0.006 | 8.21 |
| Amp-2 | 8 | 0.009 | 6.94 |
| Ets-2 | 6 | 0.007 | 7.17 |
| Pgi-2 | 6 | 0.006 | 7.52 |

Table 3. Peak, LOD, percentage of the variation explained of QTLs for root tolerance index using MAPMAKER/QTL

| QTL | Maker interval | Chro. | Position | Distance | LOD | Variance-explained (%) |
|---|---|---|---|---|---|---|
| 1 | RG162 – RG172 | 6 | 2.0 | 5.0 | 2.76 | 12.5 |
| 2 | Amp-2 – CDO99 | 8 | 2.0 | 17.0 | 2.19 | 10.0 |

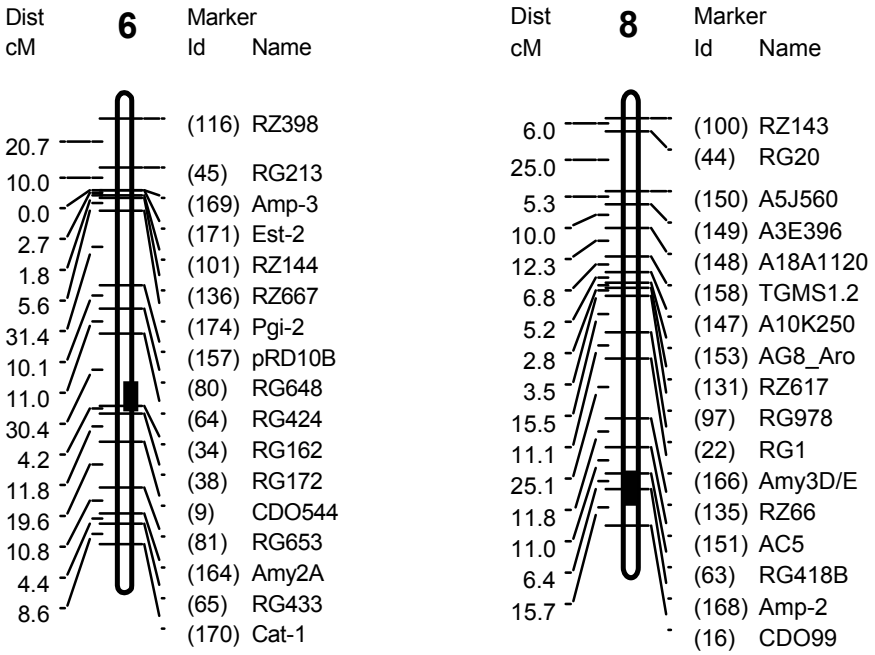


Fig. 2. RFLP map showing locations of QTLs ( ▬ ) for root tolerance index in DH population of IR64 x Azucena.

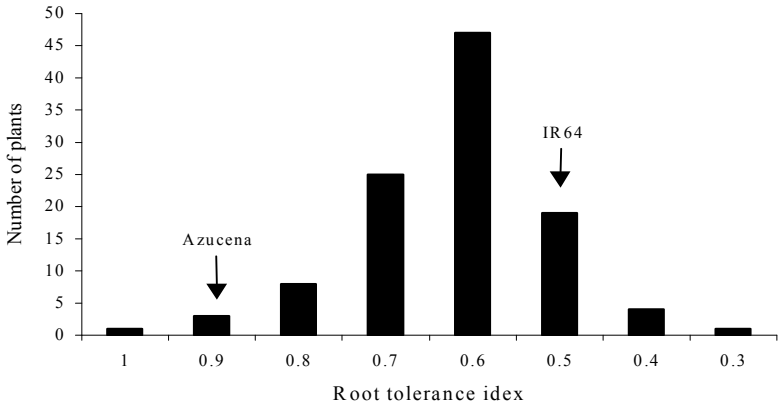


Fig. 1 Frequency distribution of the root toterance index of IR64 x Azucena population after aluminum treatment

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## TÓM TẮT

### Xác định loci di truyền số lượng điều khiển tính kháng nhôm ở lúa

Phân tích loci di truyền số lượng đã được thực hiện nhằm xác định các gen điều khiển tính kháng nhôm ở lúa. Quần thể gồm 106 cây lúa đơn bội kép từ tổ hợp lai của Azucena x IR64 dùng để lập bản đồ gen với 175 đánh dấu RFLP và đánh dấu isozyme. Kết quả cho thấy có 2 loci QTL1 và QTL2 nằm ở khoảng giữa nhiễm sắc thể số 6 và cuối nhiễm sắc thể số 8. Hai loci này đóng góp 21,6% vào biểu hiện kiểu hình.