Comparative mapping of QTLs for Al tolerance in rice and identification of positional Al-induced genes

MAO Chuan-zao (毛传藻), YANG Ling (杨 玲), ZHENG Bing-song (郑炳松), WU Yun-rong (吴运荣), LIU Fei-yan (刘非燕), YI Ke-ke (易可可), WU Ping (吴 平)

(State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310029, China)

Abstract: Aluminum (AI) toxicity is the major factor limiting crop productivity in acid soils. In this study, a recombinant inbred line (RIL) population derived from a cross between an AI sensitive lowland indica rice variety IR1552 and an AI tolerant upland japonica rice variety Azucena, was used for mapping quantitative trait loci (QTLs) for AI tolerance. Three QTLs for relative root length (RRL) were detected on chromosome 1, 9, 12, respectively, and 1 QTL for root length under Al stress is identical on chromosome 1 after one week and two weeks stress. Comparison of QTLs on chromosome 1 from different studies indicated an identical interval between C86 and RZ801 with gene(s) for AI tolerance. This interval provides an important start point for isolating genes responsible for AI tolerance and understanding the genetic nature of AI tolerance in rice. Four Al induced ESTs located in this interval were screened by reverse Northern analysis and confirmed by Northern analysis. They would be candidate genes for the QTL.

Key words: Aluminum tolerance, Quantitative trait loci (QTL), Expressed sequence tag (EST), Gene, Rice (Oryza sativa L.)

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INTRODUCTION

Aluminum (Al) toxicity is one of the most important yield-limiting factors for crop 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). The physiological and biochemical mechanisms of the toxic effect of Al on root elongation had been extensively investigated (Matsumoto, 2000). The genetic or molecular mechanisms controlling AI tolerance in plants, however, are poorly understood.

Al tolerance has been speculated to be the result of either exclusion of Al from the root apex and/or the tolerance for symplasmic Al. Detoxification of Al by releasing organic acids to chelate Al has been reported in wheat and several other plants (Delhaize et al., 1993; Ma et al., 2002). Furthermore, Al tolerance could be acquired in transgenic tobacco plants by alteration of citrate synthesis (de la Fuente et al., 1997). However, information on Al tolerance mechanisms in rice is limited. Ma et al. (2002) reported that no organic acid except small amount of citrate was induced by Al exposure in rice, and no significant effect on the Al detoxification in both Al-tolerant and Al-sensitive varieties. It means that rice may have different Al tolerance mechanism other than release of organic acids.
AI tolerance is a complex trait controlled by multiple genes in rice (Ma et al., 2002; Nguyen et al., 2001; 2002; 2003; Wu et al., 2000). Five genetic populations have been used for identifying QTLs for AI tolerance on rice. QTLs identified from different background were located on several chromosomes. But one QTL on chromosome 1 was identified in similar position across more than 3 populations.

Various techniques had been used to clone genes for AI tolerance and a number of AI induced genes had been isolated from Wheat (Hamel et al., 1998; Sasaki et al., 2002), Arabidopsis (Richards et al., 1998), Rye (Milla et al., 2002) and Sugarcane (Watt, 2003). It was reported that Al-induced genes could ameliorate AI stress (Ezaki et al., 2000; 2001), which implicated that Al-induced genes could be tolerance genes.

The main objectives of this study were to compare mapping QTLs for AI tolerance across different genetic backgrounds or experimental conditions and to screen AI-induced genes in QTL interval on chromosome 1 for further studying. In this study, QTLs for AI tolerance in rice were mapped, and comparison between different genetic backgrounds was done. Four Al induced EST clones located within the QTL interval on chromosome 1 were identified by reverse Northern analysis.

MATERIALS AND METHODS

Plant material and growth conditions

A recombinant inbreed line (RIL) population composed of 96 lines derived from a cross between an Al-sensitive indica rice variety IR1552 and an Al-tolerant japonica rice variety Azucena developed by single seed decedent were used. Solution culture experiments were performed in a culture chamber at Zhejiang University. The day/night temperature was 30 °C to 24 °C and the relative humidity was 65%-70% and 12 h photoperiod of approximately 300-320 μmol/m²·s provided by 20400 W sodium and metal haloid lamps.

Uniform seeds were rinsed with distilled water, and incubated at 30 °C for 2 days for germination. Germinated seeds were grown in distilled water for another 2 days at 27 °C±2 °C. Seedlings were then transferred to a plastic tray covered by a PVC sheet with nylon screen attached holes. Half strength nutrient solution was used (Yoshida et al., 1976). The pH of the solution was adjusted daily to 4.0 with 1 mol/L NaOH or 1 mol/L HCl. For reverse Northern or Northern analysis, 4-day-old seedlings were used for Al-stress treatment as following: seedlings were exposed to 0.5 mmol/L CaCl₂ solution (pH 4.0) for 2 h, then exposed to 0.5 mmol/L CaCl₂ solution (pH 4.0) containing 0 or 183 μmol/L AlCl₃. Roots and shoots of seedlings sampled at 0 h, 0.5 h, 2 h, 12 h, 24 h, 48 h were cut and quickly frozen in liquid nitrogen, and stored at −70 °C for RNA extraction.

For QTL analysis, the experiment was arranged in a randomized complete block design with 3 replications. Seedlings were transferred to PVC sheet with one seedling per hole and three seedlings in one row per line in each replication. The PVC sheets were laid above a plastic tray with a 1/2 nutrient solution (Yoshida et al., 1976) containing either 0 (control) or 0.556 mmol/L AlCl₃. The pH of the solutions was adjusted daily to 4.0 with 1 mol/L NaOH or 1 mol/L HCl. The longest root of each seedling was measured after 1 and 2 weeks of growth in control (control root length) or stress (stress root length) solution. Relative root length of average root length under stressed versus control conditions for each line in each replication was used as a measure for AI tolerance.

Molecular map construction

A genetic linkage map was constructed based on a previous map (Zhang et al., 2001), consisting of 260 marker loci including 114 restriction fragment length polymorphism (RFLP) markers, 104 amplified fragment length polymorphism (AFLP) markers, 41 microsatellite (SSR) markers and 1 CAPS marker using MAPMAKER/EXP version 3.0. The total map length was 2860 cM with an average distance of 11 cM between adjacent markers. Map units (cM) were derived using the Kosambi function. Forty-one SSR markers and 1 CAPS marker were mapped in this case according to the