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Title

Isolation of *Arabidopsis thaliana* mutants sensitive to excess boron

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Introduction

Boron (B) is an essential element for plant growth and development; however it can become toxic when it exists in soils in excess. Limitation of crop yield and quality caused by B toxicity is a major agricultural problem in the world (Nable et al., 1997). Toxic effects of excess B in plants were well studied for decades. Although much biochemical and physiological data were accumulated, molecular mechanisms of B toxicity remains unclear. Identification of novel genes which are involved in B toxicity/tolerance is required for elucidation of these mechanisms.

Several researchers had attempted to isolate the molecule that confers B tolerance in plants. Overexpression of *BOR4*, a B efflux transporter, improved tolerance in *A. thaliana* (Miwa et al., 2007). A mapping study of B tolerant and sensitive barley cultivars identified *Bot1*, a B transporter of barley, which provided B tolerance in yeast (Sutton et al., 2007). A similar approach in rice cultivars was also conducted and it found a region in chromosomal 4 that has a major contribution to B tolerance (Ochiai et al., 2008). Actually, transporters can regulate B homeostasis to avoid B toxicity; however these transporters are not likely to give us an answer as to why excess B is toxic.

To identify novel genes involved in B toxicity/tolerance, we conducted new genetic approach. The idea behind it is that mutants with defects in genes important for B tolerance are likely to show growth defects only under high B conditions. Here, we describe the screening and physiological analysis of *A. thaliana* mutants sensitive to excess B.

Materials and Methods

For the first round of screening, EMS mutagenized M2 seeds of *A. thaliana* (ecotype Col-0 *gll-1*) were sown on solid MGRL (Fujiwara et al., 1992) medium containing 1% (w/v) sucrose and 1.5% (w/v) Gellan gum with 3 mM boric acid. After 3 days incubation at 4°C, the plates were placed vertically in the growth chamber (16-h light/8-h, 22°C). After 2 weeks cultivation, plants with very short root compared to wild type were selected and transferred to normal medium (0.03 mM boric acid). Plants recovered root growths were selected. Seeds of the next generation (M3) were subjected to the second round of screening. In the second screening, the seeds were sown both on the normal or high B media and root growths were compared. For the segregation test, reselected plants were crossed with wild type. F₁ progeny of crossed line segregating

3:1 for wild type and short-root plans were identified as excess boron sensitive mutant.

For the growth test, mutants and wild type were grown under various boron conditions (0.03 μ M, 0.03 mM, 3, mM boric acid). After 2 weeks, root length was measured.

Results

After the first round of screening, about 100 lines were obtained from about 20,000 M2 plants. As a result of the second round of screening and segregation test, 7 lines of mutants (M3 generation) were isolated (Fig.1). To test root growth under various B condition, selected mutants and wild type were grown on 0.03 μ M, 0.03 mM and 3 mM boric acid. Except for line 3, mutants root was also shorter than wild type under 0.03 mM boric acid. But, relative root growth of 3mM boric acid compared to that of normal was less than 20 % in all mutants when it was 52 % in wild type. The most severe defect was observed in line 7 (6 %). In boron deficiency (0.03 μ M boric acid), relative root growth of all mutants was similar to that of wild type (about 40 %). These results suggested mutant sensitivity to excess boron.

Further characterization of mutants is expected to provide us with new information for boron toxic action in plants.

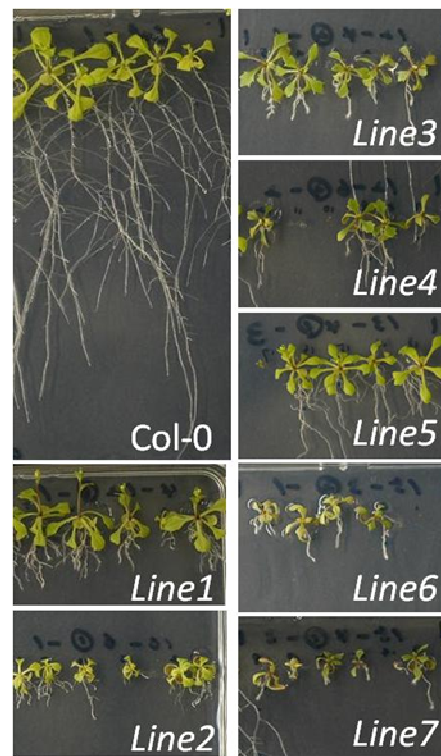


Figure 1: Growth properties of mutants. Plants were grown on MGRL media with 3mM boric acid for 19 days before the pictures were taken.

Acknowledgements

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