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Plasma membrane Ca^{2+}-ATPase(AtACA8) is involved early cytoplasmic Ca^{2+} homeostasis under aluminum stress

College of Agriculture & Life Science, Chonnam National University, Gwangju 500-757, Korea.
Research Institute for Bioresources, Okayama University, Chuo 2-20-1, Kurashiki 710-0046, Japan.
Sun-Ju Ahn*, Gap-Chae Chung, Hideaki Matsumoto

Objectives

The aluminum (Al) is toxic with soluble form existing in the acid soil. Also it suppresses cell extension and division rapidly (within 1 h) after exposure to Al and diminishes a plant production finally. It has been postulated as a mechanism of Al toxicity by numerous authors that Al may interfere with cellular Ca^{2+}-dependent signal transduction cascades that may be necessary for both cell division and cell elongation. In this study, we report that Ca^{2+}-ATPases of *Arabidopsis* are key regulators of Ca^{2+} ion efflux and PM H^{+}-ATPases are important role after exposure to Al. In the first evidence, in particular, we present data showing that Al-induced changes of cytoplasmic Ca^{2+} concentration are regulated temporally by Ca^{2+}-ATPases by RT-PCR.

Materials and Methods

Seven-day-old *Arabidopsis* seedlings were treated with 200μM $AlCl_3$ and were harvested. To isolate PM vesicles of root and leaf, slightly modified two-phase partitioning method of Palmgren was employed. Ca^{2+}-ATPase and H^{+}-ATPase activity were measured following the method of Ahn et al. For measurement of Al-induced Ca^{2+}-ATPases gene expression using reverse transcriptase-PCR. Immunolocalization of ACA8 proteins was conducted with an antibodies and detected using Confocal Laser scanning microscopy.

Results and Discussion

Interestingly, ACA genes are brodly expressed in any organs such as root, leaf, stem, and flower (Fig.1). Activity of PM Ca^{2+}-ATPase was inhibited about 30% in vesicles prepared from root but did not change that of leaves in the presence of 200μM Al during 24 h (Fig. 2). Also, we found that induced genes(ACA1, 2, 8) and consistently expressed genes(ACA4) by Al treatment in root. In particular, ACA8 was induced very quickly within 30 min (Fig. 3). The ACA8 antibody decorated the PM of whole cells along the root and root hair. No obvious differences of the fluorescence intensity were perceptible in an initial root tip both in control and 2 h Al treatment but decreased significantly after 24 h of Al treatment (Fig. 4). These results suggest that Al-induced changes of cytoplasmic Ca^{2+}concentration are regulated temporally by Ca^{2+}-ATPases.

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Corresponding author : E-mail : asjsuse@chonnam.ac.kr Tel : 062-530-2052

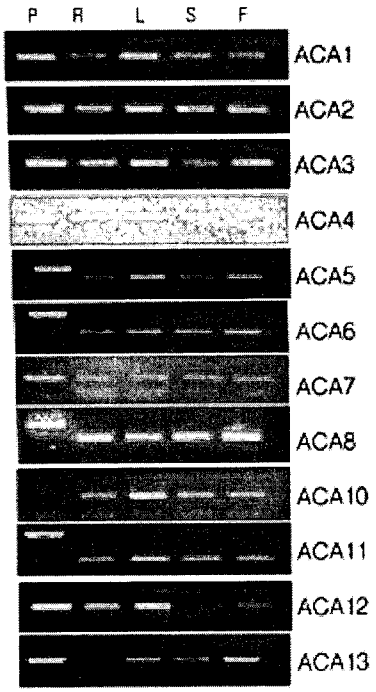

Fig. 1. Expression patterns of AtACA in Arabidopsis root(R), leaf(L), stem(S), flower(F).

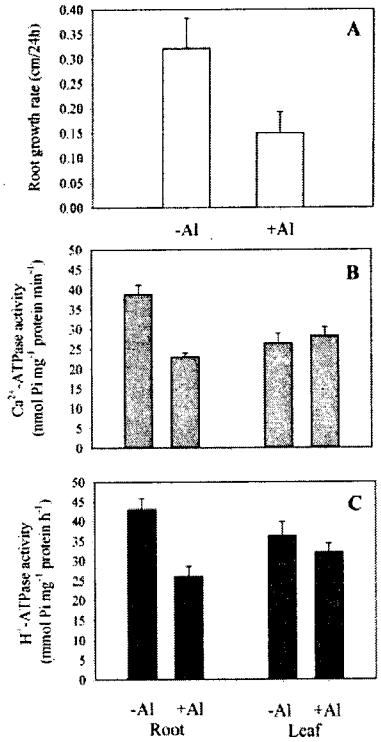

Fig. 2. Effect of Al after 24 h treatment on the RGR and ATPase activity.

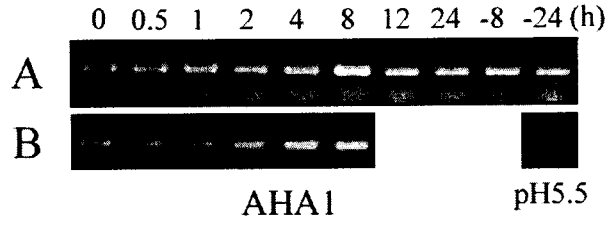

Fig. 3. Expression patterns of AtACA1 in Arabidopsis root after Al treatment during 24 h.

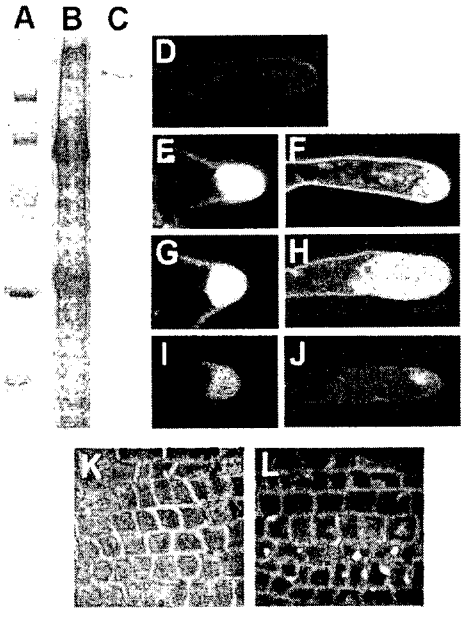

Fig. 4. Analysis of the specificity of ACA8 antibody in root hair and cell.