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RE-EVALUATION OF ROLE OF VACUOLE DURING SALT ADAPTATION IN HIGHER PLANT CELLS.

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Salt stress by the addition of 150 mM NaCl into the NaCl-unadapted cultured cells of mangrove plant (*Bruguiera sexangula*) induces a rapid influxes of Na^+ into vacuole within a day. Simultaneously, volume of vacuoles *in situ* swelled to 200%. We have proposed that the dynamic changes in the vacuolar volume is a new feature of the salt-adaptation mechanims in higher plant cells.

In the present study, we measured the changes in the activities of acid phosphatase as a protein of vacuolar sap and H^+-ATPase as a tonoplast protein. Increases of both enzyme activities suggest that the change in the vacuolar volume is an active process dependent on the salt-stress. We have further analyzed the importance of this mechanism in other higher plants.

200(3aD10)

CONTROLLED ION UPTAKE MEDIATED BY K^+ TRANSPORTER OF *Arabidopsis thaliana* UNDER NaCl STRESS

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Potassium is an essential macro nutrient required for plant growth, and its uptake into plant root cells is mediated by K^+ transporter. In the plant suffering from salt stress, the Na^+ influx into plant cell is mediated by this transporter.

We introduced the K^+ transporter of *Arabidopsis thaliana* into the yeast that were deleted original K^+ transporters(*trk1*Δ*trk2*Δ) and into the yeast that were deleted both of K^+ transporters and calcineurin (CaN)(*trk1*Δ*trk2*Δ*can*Δ). The internal ion concentration was measured by atomic absorption methods. Na^+ uptake of *trk1*Δ*trk2*Δ*can*Δ was twice more than *trk1*Δ*trk2*Δ. K^+ uptake of *trk1*Δ*trk2*Δ *can*Δ decreased more rapidly than *trk1*Δ*trk2*Δ just after the stress treatment.

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A NOVEL SALT-RESISTANT FACTOR, MANGRIN, FROM A MANGROVE PLANT

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Mangrove is a general term for salt-tolerant woody plants growing along the seashore in tropical and subtropical areas. The reason why mangrove plants can grow in such habitats is not investigated at molecular levels. We postulate that mangrove plants have got specific proteins essential for the salt tolerance in its evolutional process. Based on this hypothesis, functional screening of mangrove cDNAs encoding such proteins was performed using *Escherichia coli* as a host organism. Twenty-nine *E. coli* transformants, which showed remarkable growth under the salt-stress conditions, were obtained from 1 x 10^6 *E. coli* transformants. Analysis on the fragment patterns by restriction endonuclease digestion and on determination of their partial nucleotide sequences showed that twenty-three clones have an identical nucleotide sequence. A full-length cDNA is 1018 bp and the ORF encodes a protein of 141 amino acids including 28 serines (19.86%). It is revealed that there are no similar proteins of all other entries in databases. We designated this protein to "mangrin".

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EXPRESSION OF "MANGRIN" IN YEAST AND TOBACCO

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cDNAs encoding a novel salt-resistant factor, mangrin, were isolated from a mangrove cDNA library using *E. coli* gene expression system. In order to survey the effect of mangrin on salt-tolerance of eucaryotic cells, a full-length mangrin cDNAs ligated with *GAL1* and 35S promoter were introduced into yeast and tobacco suspension-cultured cells, respectively. The growth rates of both cells expressing mangrin were enhanced as compared to the control cells. It is possible that mangrin may function to give salt-tolerance to diverse organisms at cellular level. A mangrin cDNA driven by 35S promoter was introduced into tobacco plants. Growth of control transformants was strongly inhibited by addition of 150 mM NaCl to the medium. In contrast, transformants expressing mangrin showed remarkable well growth as compared to the controls in medium containing 150 mM NaCl. Discovery of mangrin will contribute to elucidate salt-tolerance mechanisms in mangrove plants at molecular biological level. Biosynthesis of mangrin will open a new window to enhance salt-tolerance of higher plants.

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