

Molecular evolution of mangrin

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Summary

To analyze the mechanisms of salt tolerance in the mangrove plant, *Bruguiera sexangula*, functional screening for cDNAs encoding proteins essential for salt tolerance was performed using *Escherichia coli* as the host organism. A transformant expressing a protein homologous to *Lycopersicon* (tomato) allene oxide cyclase (AOC) displayed enhanced salt tolerance. However, this unusual trait is not conferred by *Lycopersicon* AOC or its *Arabidopsis* homolog. Analysis of the functional region revealed a sequence of only 70 amino acids, which contains an unusual sequence that is essential for the salt-tolerant phenotype. On the basis of its unusual function, the mangrove AOC homolog is designated "mangrin". Furthermore, expression of mangrin driven by the *GALI* promoter and the 35S cauliflower mosaic virus (CaMV) promoter in *Saccharomyces cerevisiae* and tobacco cell lines, respectively, also gave rise to enhanced salt tolerance. Mangrin transcripts increased in cultured *B. sexangula* cells in response to salt stress. Furthermore, molecular evolution of mangrin was done by error prone PCR and DNA shuffling. 11 clones of mutated mangrin showed enhanced activity.

We propose that mangrin plays an important role in the salt-tolerance mechanism of *B. sexangula*, and that the biosynthesis of mangrin might be an effective means of enhancing salt tolerance in higher plants.