

## Development of Protoplast Culture System of Recalcitrant Mangrove Trees

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### Summary

Mangrove trees are very salt tolerant and can grow even in seawater. They have been recalcitrant for cell and tissue culture except for a few species. No report has been published on protoplast cultures in which cell walls are removed under osmotic conditions and plants regenerated. Establishment of protoplast culture system of mangrove cells will be valuable, not only for basic research of the whole process from single cell to plant regeneration, but also for genetic engineering of unique characteristics of mangrove trees through cell fusion and cell selection, and their utilization for reforestation and improvement of salt-rich soil areas.

The main theme of this study is establishing protoplast culture system from recalcitrant mangrove trees. Another important aspect is to develop unique bioassay system of cell cultures to study the effects of variant chemicals quantitatively in comparison to their effects on whole plants, which takes long period for analysis.

We have developed an efficient surveying method for determination of optimum enzyme combinations and osmotic conditions of leaves of *Avicennia marina* and cotyledons of *Sonneratia alba* for protoplast isolation using multi-well plastic plates. We also investigated the effects of several plant hormones and plant growth regulators, and macro elements in the MS medium on the cell proliferation from *A. marina* and *S. alba* with small scale liquid culture using a flat-bottomed tube and observation of free cells and callus formation using an inverted microscope. Not unexpectedly, major differences in optimum conditions were found among the above mangrove materials, suspension cells of *Bruguiera sexangula* and previously established protoplast cultures of tree species, namely *Populus* and *Betula*.