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 to establish a protoplast culture system from recalcitrant mangrove trees. Another important aspect is to develop a unique bioassay system of cell cultures to study the effects of different additives, such as plant growth regulators and chemicals on mangrove protoplasts. This approach will shorten the time needed to analyze their effects on the environment using a whole plant system.

We have developed an efficient surveying method using multi-well plates for the determination of optimum enzyme combinations and osmotic conditions needed for the isolation of leaf protoplasts from eight mangrove species of three different families. Using multi-well culture plate method, we found stimulatory effects of high concentrations of Mg and Ca salts at various pHs on cell divisions of a mangrove, *Bruguiera sexangula*, protoplasts.

Stimulatory effects of Mg and Ca salts were also first found with leaf culture of *Avicennia marina* by using a flat-bottomed tube method. Free cells and callus formation were subsequently observed under an inverted microscope. Sustainable liquid culture was first obtained from cotyledons of *Sonneratia alba* by using the above method.