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## Plant Regeneration and Morphological Characterization during *in vitro* Organogenesis from *Vitex rotundifolia* L. fil.

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### Objectives

*Vitex rotundifolia* L. fil. is an important dune plant to Korea valuable as a perennial for the purpose of repair at destructed areas in dunes. This plant is known to propagate by seeds or rhizomes in natural saline condition. But it was proved that the seeds was too recalcitrant to germinate by conventional methods, we examined a vegetative propagation of *V. rotundifolia* by using tissue culture technique.

We also investigated the callus differentiation at some incubation stages with LM and SEM.

### Materials and Methods

Fresh leaves of *V. rotundifolia* were collected at Anmyeon-Do dune. The leaves were washed thoroughly under running tap water. Following of a 10-sec incubation in 70% ethanol, leaves were surface-sterilized with an aqueous solution of sodium hypochlorite (approx. 2% active chlorine) for 15 min and rinsed three times in sterile distilled water.

We investigated callus and shoot induction with phytohormones treatment in Nitsch medium. Induction of callus formation was observed from leaves culture with auxins, 2,4-D, IAA and NAA. Thereafter, we focused on shoot regeneration at

auxins or cytokinins.

For shooting of the leaf tissue and callus, Nitsch media with various concentrations of growth regulators were tested. The cultures were kept for 4 weeks at 25°C ± 1°C and under a 16 h/8 h photoperiod.

### Results and Discussion

We first examined the effects of auxins on the callus induction using leaf explants. The kinds of plant growth regulators have a different potential for organ development in the culture of *Vitex rotundifolia*.

Leaf explants cultured showed high callus induction rate in auxin (2,4-D, IAA and NAA), with embryogenic callus induction in IAA and NAA except 2,4-D treatment.

We supposed that cytokinins would active on plantlet formation from leaf explants. So, to investigate the effects of cytokinin on organogenesis, leaf explants were cultured on Nitsch medium supplemented with BA, TDZ and 2iP. Among them, BA showed the highest frequency, TDZ showed lower frequency than BA in shoot induction.

Shoot development and tissue patterns during callusing was examined with the SEM, by the method slightly modified from Martinelli et al. (2001), and with LM, respectively.