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# Embryogenic tissue initiation, somatic embryos maturation & plant regeneration in Japanese red pine (*Pinus densiflora* Sieb. ET Zucc.)

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## Objectives

**We have attempted to establish somatic embryogenesis system by initiating of embryogenic tissue from megagametophytes at different seed collection dates and producting somatic embryos from the embryogenic tissue in *Pinus densiflora*.**

## Materials and Methods

1. Material

Plant - *Pinus densiflora*, embryogenic tissue, megagametophytes

2. Methods:

Embryogenic tissue was obtained from immature seeds and was proliferated. Maturation was performed with various combinations and concentrations of ABA & gellan gum (Phytagel, Sigma) for production somatic embryos from 3 lines of embryogenic tissue.

## Results and Discussion

From over 5,700 seeds cultured, initially 10 embryogenic tissue lines (0.17 %) were obtained. However, only three (0.052 %) survived on maintenance medium. The highest tissue initiation (0.36 %) was observed with the seeds collected on July 5. The three viable lines (PD04-6, PD04-8 & PD04-9) were used for further somatic embryo production. As for somatic embryo maturation, the highest number (1,438/g FW) of matured cotyledonary somatic embryos (from line PD04-8) was obtained on a medium containing 80$\mu$M abscisic acid (ABA), 0.2M maltose, and 1.0 % gellan gum. The cotyledonary embryos formed on maturation medium were transferred and germinated on half-strength Litvay medium (LM) plus 0.2% gellan gum. The frequency of whole plantlet (including shoots & roots both) regeneration was varied (30.4~0%) depending on the concentrations of either ABA in the maturation medium or germination medium containing activated charcoal. At present, somatic plants were recovered from the germination medium and transplanted into soil mixtures of perlite, vermiculite & peatmoss.

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