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# Direct Somatic Embryogenesis from Epidermal Single Cells of Zygotic Embryos of *Eleutherococcus senticosus* by Plasmolyzing Pretreatment

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## Objectives

Plasmolysis results in the separation of plant cell cytoplasm from the cell wall as a result of water loss and induced by immersing a plant cell in strong saline or sugary solution. Plasmolysis treatment renders the various stresses to plant cells and tissues, however, physiological and chemical changes in cells after plasmolysis treatment are not unclear.

In general, auxin treatment such as 2,4-D is necessary to induced somatic embryogenesis from plant tissues. Here, we report the direct somatic embryogenesis from the pre-plasmolyzed zygotic embryo on MS medium without any growth regulator. We observed the relationship between morphological and cellular changes in the cells of zygotic embryos of *Eleutherococcus senticosus* after plasmolyzing pretreatment and somatic embryogenesis from epidermal single cells.

## Materials and Methods

1. Plant materials : Zygotic embryos of *Eletherococcus senticosus*
2. Methods: Plasmolysis, SEM observation, TEM observation, Confocal microscope observation of callose deposition, RT-PCR.

## Results and Discussion

When zygotic embryos of *Eletherococcus senticosus* were preplasmolyzed through 1 M mannitol or 1 M sucrose, somatic embryogenesis occured on the surfaces of explants on MS medium without any growth regulator. While the control (zygotic embryos untreated) germinated and grew into seedlings. In preplasmolyzed zygotic embryos, somatic embryos developed on the whole surfaces of explants at high frequency, and embryo development was independent each other and comparably dispersed on the whole surfaces of explants. SEM observation showed that the plasmoylsis pretreatment resulted in the random cell arrangement of epidermal cells, probably due to random cell division, and numerous somatic embryos developed from these epidermal cells. Confocal and TEM analysis revealed that plasmolyzing pretreatment enhanced the cell wall thickening and heavy deposition of callose (β-1,3 glucan) between cell wall and plasma membrane. Expression of two callose (β-1,3 glucan) synthase gene enhanced markedly after plasmolyzing pretreatment. The above results indicate that plasmolyzing pretreatment induced the blocking the intercellular spaces by cell wall thickening and callose deposition, which confers direct somatic embryogenesis from epidermal single cells of zygotic embryos.

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