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# HOW SOMATIC PLANT CELL FOLLOWS EMBRYOGENIC PATHWAY DURING CLONING IN MATURE TREES OF CONIFERS?

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Somatic embryogenesis can be considered as an extreme response of somatic plant cells towards specific stress condtions either by cold pretreatment\heat or chemical stress. In tissue culture, differentiated somatic cells acquire embryogenic competence and proliferate as embryogenic cells, and develop into plantlets. Hence, embryogenic cells can be considered totipotent cells based on their aptitude to regenerate or develop into an embryo under certain conditions. Very recently, somatic embryogenesis was successfully induced in many recalcitrant pines using apical meristematic tissue of mature trees of conifers. There are many factors which influence cloning of mature conifers and among them, pH, carbon source, calcium ions, plant growth regulators, smoke saturated water, and salicyclic acid acts are very important signaling molecules. The homeobox transcription factor WUSCHEL (WUS) has been shown to cause dedifferentiation when expressed on somatic cells followed by a production of new cells that can lead to somatic embryogenesis or organogenesis in plants. Future research is very much needed in order to understand mechanism of action of signaling molecules involved in the acquisition of embryogenic competence of somatic cells.

**Keywords**: Competence; Embryogenesis; Gene expression; Somatic cell; Tissue culture.

## Introduction

Somatic cells of many plant species can be cultured under *in vitro* conditions, and induced to form embryos that are able to develop into plants, which are the exact copies of mother plants. Very little is known of the mechanisms that induce the dedifferentiation of a single somatic cell into a totipotent embryogenic cell that can either be regenerated or develop into an embryo and subsequently an entire plant. Therefore, meristem localized cells have supervised freedom and are pluripotent, and that embryogenic cells are unsupervised, autonomous and, hence, freely totipotent. In a classical concept these pluripotent cells gave rise the cells and tissues found in the root and shoot but do not have the ability to form embryos. By contrast, under various stress related conditions, a somatic plant cell can dedifferentiate to give rise to a totipotent embryogenic cell that has the ability to proliferate and\or regenerate an embryo. The regeneration of a complete embryo from a single totipotent somatic cell *via* somatic embryogenesis is a remarkable example of totipotency of plants, which has been known for 40 years. Somatic cells are induced to form embryos by the application of stress and high doses of auxin treatments. Hence, embryogenic cells can be considered totipotent cells based on their aptitude to regenerate or develop into an embryo under certain conditions. Most trees have marked phase changes that result in a decline in their potential for somatic embryogenesis or micropropagation[1-3]. By altering conditions of the source material *ex vitro,* or by changing *in vitro* conditions encountered by the explant, rejuvenation and increased propagation can be accomplished. Stress can have a rejuvenating effect on plant tissues[1]. This review highlights some of the important factors involved in reprogramming the somatic cells into an embryogenic pathway during cloning of mature trees of conifers.

*Background; cloning mature trees of conifers*- Somatic embryogenesis in conifers has been well established by

using various explants such as immature zygotic embryos, mature zygotic embryos, vegetative shoot buds, and secondary needles of mature trees too[4]. There are many disadvantages of using embryo cloning method due to heterozygosity as a result of cross-pollination. The major drawback of embryo cloning are unproven genetically, and very low initiation frequencies of embryogenic tissue which is less than 2 to 3% in most of the conifers. This limits the embryo cloning and deployment of plants for the clonal forestry[5-15]. At present an embryogenic system derived from vegetative shoot apices or secondary needles of mature pines have been well established in at least a few conifers, and an embryogenic system could be used for genetic transformation studies. This is the major breakthrough in forest biotechnology, and certainly solves the current problems of tree breeding. Another important advantage, of using vegetative shoot apices of mature pines as a starting material for somatic embryogenesis, is that cells are actively dividing, hence their higher regeneration capacity serves as the best starting material for genetic transformation studies.

*Effect of stress conditions*- Cellular competence is associated with the dedifferentiation of somatic cells that allows them to respond to new developmental signals. It is well accepted that embryogenic competent cells can be morphologically recognized as small, rounded cells with rich cytoplasm and small vacuoles. These types of cells either originate from meristematic tissues under specific stress conditions such as cold or heat or due to the chemical stress or may be due to high doses of auxin and cytokinin. This transient cell state induced by stress conditions can be characterized by extensive cellular reorganization and allows for a developmental switch, if appropriate signals are perceived[2]. Stresses not only promote dedifferentiation, but also can be used to induce somatic embryogenesis using vegetative shoot apices or secondary needles of mature (15 year-old) pine trees e.g *P. kesiya, P. roxburghii, P. wallichiana, P. patula, P. pinea and P. pinaster* [4-24]. In cases where explants from mature trees can not be regenerated under standard conditions, alternative approaches include the application of stress to alter phase change[25]. Stress can have a rejuvenating effect on plant tissues. Rejuvenation of tissues prior to *in vitro* culture has led to regeneration of trees of various ages[24-25]. Cold pretreatment has been used as an environmental stress to promote somatic-embryogenesis in many plant species[6-21]. Therefore, it is clear that stress conditions can be used as a positive inducer of somatic embryogenesis in pines. It was also concluded that cellular functions linked to the stress response can play a role in activation of the embryogenic developmental program. Another important stress factor which plays an important role in the conversion of somatic cells reprogrammed towards embryogenic pathway is the application of high doses of auxin and cytokinin[11-25]. The use of high doses of auxin and cytokinin in the nutrient medium induced the formation of embryogenic tissue in many recalcitrant pines[11-25].

*Factors influencing cloning of mature conifers*- The initiation of embryogenic development in a differentiated cell requires a complete cellular reprogramming. Differentiated functions have to be regulated and, following a transition phase, a new programme leading to embryo development has to be started. Although this reorganization is accompanied by profound morphological and physiological changes, reprogramming of the overall gene expression pattern is of utmost importance. Embryogenesis is associated with artificial conditions, high levels of exogenous growth regulators and many other stress factors. These extreme and stressful conditions may result in a general stress response in cells showing extended chromatin reorganization. There are many factors, which influence the cloning of mature conifers *in vitro,* such as stress conditions, chromatin remodeling, programmed cell death, pH, DNA methylation, calcium ions, plant growth regulators including the use of smoke saturated water, activated charcoal in the nutrient medium, carbon source, salicyclic acid, and finally activation of genes controlling the entire process of somatic embryogenesis. There are few genes known to play a role in the acquisition of embryogenic competence in plant cells. There are many embryogenesis-related genes (*LEA genes, SERK, AGL15, BBM, LEC1, FUS3, AB13*) which expressed in the same manner in both zygotic and somatic embryogenesis. In carrot, SERK expression was shown to be characteristic of embryogenic cell cultures and somatic embryos, but its expression ceased after the globular stage. The homebox transcription factor WUSCHEL (WUS) has been shown to cause dedifferentiation when expressed on somatic cells followed by a production of new stem cells that can lead to somatic embryogenesis or organogenesis in *Arabidopsis* and *Coffea canephora* [26-28]. It was also confirmed that WUS is capable of promoting the vegetative-to-embryonic transition, and eventually somatic embryo formation, suggests that the homeodomain protein also plays a critical role during embryogenesis, in addition to its function in meristem development [28-30].

**Conclusion**

Plant somatic cells under the influence of external environmental stimulus like cold pretreatment or heat or chemical stress leads to the dedifferentiation, and hence

follow embryogenic pathway. Optimum stress conditions promote embryogenic pathway, whereas, higher stress conditions may leads to death of cells[31-32]. However, the signaling pathway or mechanisms involved for acquisition of embryogenic competence are still incompletely known. Recent developments of gene expression studies of WUS\PGA6 also play a key role during embryogenesis, presumably by promoting vegetative-to-embryogenic transition and or\maintaining the identity of the embryonic plant stem cells. Further studies are required to reveal the exact molecular events underlying the developmental switch from the differentiated to the embryogenic cell fate.

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