EMBRYOGENETIC STUDIES AS A BASIS FOR STRATEGIES OF REPRODUCTION AND CONSERVATION OF TREE SPECIES

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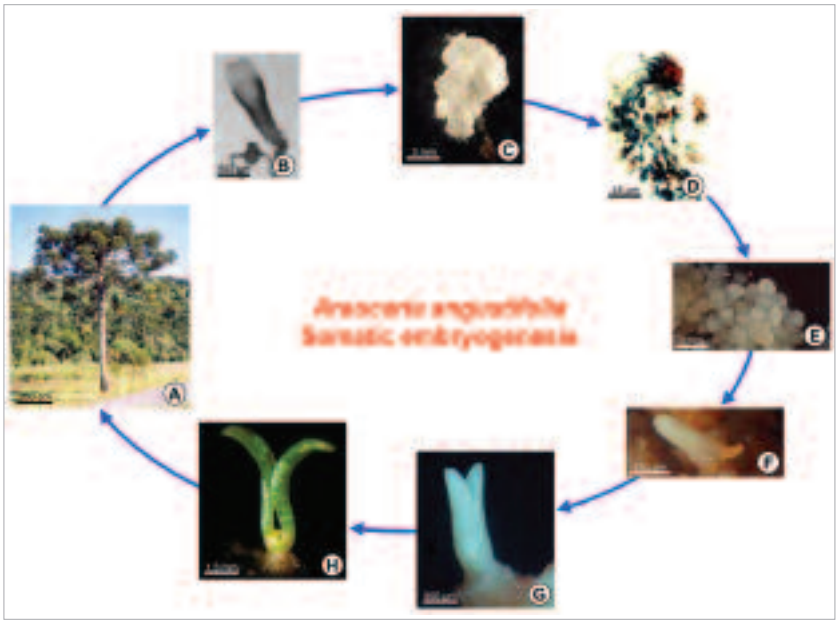
The use of plant cell tissue and organ cultures has emerged as an important tool, when the propagation of tropical woody plants in the reforestation programs is concerned. This technique has been adopted for plants in which the massive propagation has been precluded by low productivity and or low viability of seeds, long-term seed maturation and limited vegetative propagation. In this regard, *in vitro* somatic embryogenesis has been successfully applied in production of somatic cell and viable embryos, in a morphogenetic process closely related to the natural process of zygotic embryogenesis. The major aim of this project is to investigate major physiological, biochemical and molecular changes during the somatic embryogenesis of *Araucaria angustifolia* and *Ocotea catharinensis*, two economically important woody plants in the Atlantic Forest of southern Brazil. The results would pave the way to determine a general biotechnological process required to propagate and to manage important tropical woody plants.



Somatic embryogenesis in A. angustifolia. *(A) Mother tree; (B) Precotyledonary zygotic embryo used as explant; (C) embryogenic culture induced; (D) somatic pro-embryo observed in the maintenance medium; (E - H) somatic embryo on maturation treatment*

Ocotea catharinensis is an endangered native forest tree species of the Southern Brazilian Atlantic Rain Forest. Natural propagation of *O. catharinensis* throughout seeds is hampered by its recalcitrant physiology and, consequently, they cannot be stored for long periods without loss of viability. *Araucaria angustifolia* is the only native conifer of economic importance in Brazil, being the most exploited timber source until the 1970's. Nowadays only relicts of the natural vegetation are found, representing less than 2% of the original area.

As a result of the clear-cutting form of exploitation, *A. angustifolia* and *O. catharinensis* were included in the official list of endangered Brazilian plants, under the "vulnerable" category. Therefore, it is necessary to develop technologies for the conservation and genetic improvement of these Brazilian native species.

Biotechnological tools have a large potential in breeding and biodiversity conservation programs for woody species. Biotechnology is a significant affix to the traditional tree improvement practices, and is the one that utilizes fundamental discoveries in the field of plant tissue culture for clone-forestry, gene transfer techniques, molecular biology, and genomics.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main results of this project concern to the description and analyses of different aspects of embryo development in *A. angustifolia* and *O. catharinensis*.

In order to increase the efficiency of *in vitro* embryo development, a comprehensive understanding of the biochemical and molecular events in somatic and zygotic embryo development is essential. Polyamine (PA) and nitric oxide (NO) metabolisms, as well as amino acids, plant hormones and differential protein expression, seem to be involved in the regulatory mechanisms that play important roles in certain embryo-development processes in *A. angustifolia*. Additionally, biochemical aspects during seed development have been disclosed for *A. angustifolia* and *O. catharinensis*. Some factors, such as indole-3-acetic acid, abscisic acid, polyamines, amino acid and protein contents, were studied during zygotic embryogenesis of these Brazilian species. A better understanding of biochemical alterations during zygotic embryo development, besides providing basic information on seed development, may be useful for further improvement in *A. angustifolia* and *O. catharinensis* somatic embryogenesis.

Somatic embryogenesis in O. catharinensis. *(A) Mother tree; (B) Imature zygotic seed; (C) Isolated immature zygotic embryo used as explant; (D) Direct somatic embryogenesis induced from explant; (E) Globular somatic embryo; (F) Cotyledonary somatic embryo; (G-H) Somatic embryo germination*

MAIN PUBLICATIONS

Santa-Catarina C, Moser JR, Bouzon ZL, Floh EIS, Maraschin M, Viana AM. 2005. Protocol of somatic embryogenesis: *Ocotea catharinensis* Mez. (Lauraceae). In: S. Mohan Jain; Pramod K. Gupta. (Org.). Protocol for Somatic Embryogenesis in Woody Plants. Dordrecht, *The Netherlands: Springer*. **77**:427-443.

Silveira V, Santa-Catarina C, Balbuena TS, et al. 2008. Endogenous abscisic acid and protein contents during seed development of *Araucaria angustifolia*. *Biologia Plantarum*. **52**:101-104.

Steiner N, Santa-Catarina C, Silveira V, Floh EIS, Guerra MP. 2007. Polyamine effects on growth and endogenous hormones levels in *Araucaria angustifolia* embryogenic cultures. *Plant Cell Tissue and Organ Culture*. **89**:55-62.

Santa-Catarina C, Silveira V, Scherer GFE, Floh EIS. 2007. Polyamines and nitric oxide induce morphogenetic evolution in somatic embryogenesis of *Ocotea catharinensis*. *Plant Cell Tissue and Organ Culture*. **90**:93-101.

Silveira V, Santa-Catarina C, Tun NN, Scherer GFE, Handro W, Guerra MP, Floh EIS. 2006. Polyamine effects on the endogenous polyamine contents, nitric oxide release, growth and differentiation of embryogenic suspension cultures of *Araucaria angustifolia* (Bert.) O. Ktze. *Plant Science*. **171**:91-98.

Santa-Catarina C, Silveira V, Balbuena TS, Viana AM, Estelita MEM, Handro W, Floh, EIS. 2006. IAA, ABA, polyamines and free amino acids associated with zygotic embryo development of *Ocotea catharinensis*. *Plant Growth Regulation*. **49**:237-247.

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Floh EIS, Scherer GFE. 2006. Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. *Plant and Cell Physiology*. **47**:346-354.

Silveira V, Floh EIS, Handro W, Guerra MP. 2004. Effect of plant growth regulators on the cellular growth and levels of intracellular protein, starch and polyamines in embryogenic suspension cultures of *Pinus taeda*. *Plant Cell Tissue and Organ Culture*. **76**:53-60.

Silveira V, Balbuena TS, Santa-Catarina C, Floh EIS, Guerra MP, Handro W. 2004. Biochemical changes during seed development in *Pinus taeda L*. *Plant Growth Regulation*. **44**:147-156.

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