Chapter 6
Use of Biotechnology in Forestry Breeding Programs for Natural Resources and Biodiversity Conservation: Creating Super Trees for the Future

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Abstract Owing to the increasing human population and the increasing global demand for wood, its consumption is exceeding the natural rate of regeneration in many areas worldwide. Despite only 3% of the world's forested land is plantation forest, plantations are highly productive; and with further improvement in genetic composition of planting stock as well as applying biotechnology, additional productivity increases can be obtained. For this reason, it is necessary to enrich traditional breeding programs with biotechnological tools able to increase the quantity and quality of the forestry plants produced. FAO's definition of forest biotechnology encompasses different techniques for cloning forest trees. Forestry companies are currently considering clonal propagation as a good source of forestry plants. Clonal propagation can be achieved by various means: grafting, rooting of cuttings, coppicing, or in vitro propagation. Several methods of clonal propagation are being practiced with conifers. Along this chapter, a summary of some of the different approaches to improve *Pinus* spp. clonal propagation will be described, particularly those made in our laboratory.

Keywords Forestry biotechnology · Breeding programs · Natural resources · Biodiversity conservation

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Introduction

The increase in the human global population leads to an increase on the global demand for wood consumption. At the current rate of consumption, forests in many areas are exceeding their natural regeneration capacities (Fenning and Gershenzon 2002). For this reason, it is necessary to enrich traditional breeding programs with biotechnological tools able to increase the quantity and quality of forestry plants produced (Bonga 2015). Using in vitro technologies, organogenesis is generally restricted to the young seedling as the explant source. For this reason, initially, organogenesis techniques in *Pinus* species in order to produce clonal plants from selected seeds were developed. Then, in order to reproduce exactly the genotype of the donor plant, adult trees were used after applying various rejuvenating pretreatments, e.g., pruning, and spraying with cytokinins, using vegetative buds of different *Pinus* species or fewer needle primordia of 3- and 7-year-old trees. Main problems associated with this technique are low in vitro rooting, small acclimatization percentage, poor growth, etc. For all these reasons, in 2007 we concentrated all our efforts in the development of somatic embryogenesis systems. Somatic embryogenesis is a fascinating developmental pathway through which plants can be regenerated from bipolar structures derived from a single or a few somatic cells and that was first described more than 50 years ago in carrots by Reinert (1958) and Steward (1958). *Pinus* spp. somatic embryogenesis presents different problems, however. During the last few years, we have focused in overcoming some of the problems: the competence window problem, the low initiation frequencies, the low rates of maturation, poor germination rates, low regeneration capacity in conserved cell lines, etc. Moreover, we developed combined systems to increase the efficiency of SE in embryogenic cell lines with recalcitrance to be cryopreserved and procedures in different conifer species including hybrids. In parallel, one of our main research areas of interest was the study of the physiological mechanism controlling the tolerance to drought conditions in *Pinus* species. During the last few years and taking into account all the knowledge generated, as well as the fact that it has been found that using different temperatures during the process of embryo formation produced clonal somatic plants with different phenology, our challenge was being able to modulate the drought tolerance in *Pinus* spp.; Different stressful environmental conditions have been applied along the different stages of somatic embryogenesis (initiation, proliferation, and maturation) in order to obtain clonal plants with tolerance to different degrees of water stress. Preliminary results have showed that somatic plants coming from EMs initiated at lower temperatures showed higher water use efficiency than controls. At the same time, amino acid and sugar analyses and the ultrastructure at cellular level were studied in order to know the structural changes adapt after extreme temperatures (30, 40, 50, and 60 °C) as well as the metabolites involved in the different SE response.

Organogenesis

Seed Organogenesis

Plant propagation through tissue culture may be accomplished by employing callus, organ, cell, and protoplast cultures. Although tissue explants from tree species are generally difficult to grow and differentiate in vitro, the first types of cultures have been experimentally employed with varying degrees of success for micropropagation of a number of tree species (Ahuja 1988). Although initially callus cultures were employed for plantlet regeneration, now mostly organ cultures are employed for clonaI propagation. In this sense, organogenesis is generally restricted to the young seedling as the explant source (Bonga 2017), and these kinds of techniques have been studied for several conifers in the last 30 years (von Arnold and Hawes 1989; Nugent et al. 2001; Tang and Newton 2005).

In 1957, Skoog and Miller (1957) proposed that a balance between auxin and cytokinin determines the morphogenic competence of an explant in in vitro culture. Manipulation of the composition and ratio of these plant growth regulators inside the tissue is often the primary empirical approach to the optimization of in vitro culture. During this process of plant growth regulator optimization, abnormal or unusual organ development is often observed and attributed to plant growth regulator imbalance (Ramage and Williams 2004).

In conifers, seeds usually are the initial explant to induce organogenesis; seed organogenesis involves a four step process: (1) induction and development of adventitious buds on embryonic explants, (2) elongation of shoot buds, (3) multiplication of shoots, and (4) rooting of the shoots and their transfer to ex vitro conditions (Thorpe et al. 1991). In *Pinus radiata*, Aitken-Christie et al. (1988) developed an organogenesis protocol to generate large numbers of meristematic nodules from zygotic embryos. In the aforementioned protocols, shoot induction was achieved in radiata pine with 22 μM benzyladenine (BA) for 3 weeks. Stange et al. (1999) studied the effect of different BA and thidiazuron (T) concentrations to test their effect on the number and quality of the shoots obtained. Currently, BA is the most widely used cytokinin in plant micropropagation due to its effectiveness and affordability, but it has been reported to provoke hyperhydricity in some species (Bairu et al. 2007). Hyperhydricity is a critical factor as hyperhydric explants present several morphological and physiological disorders (Hazarika 2006). These explants are difficult to root, are more susceptible to infections, and present low survival rates when transferred to the greenhouse (von Arnold and Eriksson 1984).

Naturally occurring cytokinins are adenine derivatives found in higher plants (Strnad 1997), and they can be classified by the configuration of their N6-side chain as isoprenoid (zeatin) or aromatic cytokinins (BA and m-T) (Mok and Mok 2001). Meta-topolin (m-T) has been studied as an alternative phytohormone for micropropagation in *Pinus pinaster* (De Diego et al. 2008) and *Pinus pinea* (Moncaleán