

Somatic Embryogenesis Tissue Culture for Applying Varietal Forestry to Conifer Species

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ABSTRACT

The use of tree improvement practices to enhance the genetic characteristics of planted seedlings is a forestry practice that consistently shows a high return on investment by increasing yields obtained from planted forests. The use of improved seeds is an effective way of bringing genetic improvement to forest regeneration programs. Seed orchards are currently used to produce seeds in large commercial quantities from trees having

desired genetic traits. However, improved seeds do not provide a method to multiply specific varieties that have desirable traits. Vegetative propagation techniques from full-sib seeds provide the best means for doing varietal forestry by multiplying the improved genetic resource developed from tree improvement programs.

KEYWORDS

vegetative propagation, rooted cuttings, loblolly pine, southern yellow pine

Vegetative Propagation Systems

Two criteria are considered important for the successful implementation of vegetative propagation systems within an operational forestry program. First, the propagation system must have the ability to preserve superior candidate varieties. The ability to propagate elite varieties requires a capacity to maintain varieties in a form capable of regenerating after a minimum period of 5 to 10 years needed in order to test and select varieties in the field. Second, the propagation system has to be able to multiply selected varieties in large enough numbers at a reasonable cost. If these 2 criteria can be reasonably met, the selected vegetative propagation systems can be imple-

mented to incorporate varietal forestry within an operational forestry program.

There have been major advances over the past 50 years in the development of operational vegetative propagation systems for conifer species used in plantation forestry programs. These propagation systems provide a means of bringing new genetic material into forestry programs through the capture of a greater proportion of the genetic gain inherent within a selected tree species. Vegetative propagation systems also provide a method for multiplying superior varieties and/or families identified in tree improvement programs.

Rooted Cuttings

Currently, rooted cuttings are a propagation technique that is available on an operational level to multiply specific varieties that have desirable traits. The primary use of rooted cutting technology is for bulk production of genetically improved materials. This propagation technique is used world-wide to produce tens of millions of rooted cuttings for forest regeneration programs.

Micropropagation through Organogenesis

Tissue Culture

Organogenesis is a tissue culture system that relies on the multiplication of shoots or the *de novo* formation of organs originating from either unorganized callus, preformed shoots, or induced buds. Propagules produced through this system are essentially treated as microcuttings. Thus, shoot propagules are placed in an optimal rooting environment and treated in a similar manner as cuttings. This propagation technique has been used in operational forestry programs in New Zealand on radiata pine (*Pinus radiata*).

Somatic Embryogenesis

Somatic embryogenesis (SE) is a tissue culture approach where proliferative embryo suspensor masses are established from non-meristematic cells and subsequently cultured to produce organized somatic embryos possessing shoot and

root meristems. The term somatic refers to embryos developing asexually from vegetative (or somatic) tissue. This method has been used in horticulture and agriculture on a limited basis, and is now being used to a greater scale in forestry.

Somatic embryogenesis is the only vegetative propagation technology that provides long-term preservation of the selected genetic component of a conifer species that can be used for extended timeframes within an operational forestry program. Thus, somatic embryogenesis is now becoming a viable operational propagation technology for conifer species.

Basic Laboratory Protocols for Somatic Embryogenesis in Conifers

In general, the SE process is divided into several laboratory steps which are performed under sterile conditions to prevent microbial contamination.

Culture Initiation

Mature zygotic embryos are dissected from seeds and placed onto semi-solid medium containing plant growth regulators.

Proliferation

Maintenance of embryonal suspensor mass is characterized by the presence of early-stage somatic embryo structures that are analogous to those occurring during normal seed development. This is followed by a multiplication step when the tissue multiplies and develops as early-stage somatic embryos. Embryogenic cultures can be proliferated in a juvenile form for long periods of time to produce unlimited numbers of propagules from the same variety. At this point, tissue can be allowed to continue to grow or it can be placed into long term storage.

Cryopreservation

Cryopreservation is a means whereby germplasm can be stored. The embryogenic tissue is treated with cryoprotectants, frozen to -35 °C (-31 °F) under a controlled freezing rate, and then



Figure 1. Tubes of embryogenic tissue being removed from cryopreservation storage containers (Courtesy of CellFor Inc).



Figure 2. Nursery production of CellFor Inc bare-root loblolly pine (*Pinus taeda*) somatic seedlings growing at Plum Creek Jesup Nursery, Georgia.

subsequently stored in liquid nitrogen (-196 °C [-321 °F]) (Figure 1). Cryopreserved tissue can be stored indefinitely and then regenerated within a few weeks after a simple thawing process. This long term storage option offers a distinct advantage of somatic embryogenesis tissue culture over rooted cuttings and organogenesis tissue culture.

Maturation

Maturation advances the development of somatic embryos by exposing tissue to phyto hormones and controlled environmental conditions. Within a period of a few months, they are transformed into mature somatic embryos that are analogous to zygotic embryos.

In vitro Germination

The final lab step is *in vitro* germination, in which embryos are placed on a germination medium under controlled environmental conditions. *In vitro* germination occurs within a week and proceeds to the development of true needles. At this point, young somatic seedlings can be transferred to *ex vitro* nursery conditions.

Nursery and Field Performance of Conifer Somatic Seedling Stocktypes

From the early 1990s until the present, germinants from somatic embryogenesis technology have shown continued improvement in their

development into high-quality somatic seedlings in the nursery. In addition, somatic seedling propagation technology has been successfully integrated into both the bare-root (Figure 2) and container seedling production systems. The initial response of germinants to the nursery environment will have a profound influence on subsequent morphological development. Recent nursery performance of somatic seedlings has shown that a proper nursery cultural environment (nutrients, temperature, and moisture) during the initial establishment stage will result in normal morphological development of seedlings. Thus, currently produced somatic seedlings consistently meet morphological standards required for production of operational bare-root or container seedlings. Reforestation site trials have tested the field performance of somatic seedlings and have found that somatic and zygotic seedlings have comparable field performance as a stocktype, indicating that somatic seedlings have all of the traits that are desired in seedlings for use in forest regeneration programs.

Integration into Tree Improvement Programs

There is an opportunity with somatic embryogenesis technology to capture value-added traits at the individual tree or family level. Testing of progeny from selected parents will capture additional gain for improved performance. The value-



Figure 3. Field performance of CellFor Inc loblolly pine trees growing in a forest plantation 21 months (left) after planting and at 6 years of age (right).



added traits that can be captured and propagated through somatic embryogenesis are those that can be identified through any tree-breeding program, and include growth and yield, wood quality, plus stress, pest, and disease resistance. Thousands of varieties can be produced for field trials from selected families having desirable genetic traits. Embryogenic cultures from these varieties can be cryopreserved for long-term storage until field selections are made. Ultimately, a population of varieties, large enough to ensure genetic diversity, can be selected based on field performance criteria. From this type of selection program, CellFor Inc, among others, now commercially offers elite varieties of loblolly pine (*Pinus taeda*) seedlings with yield improvement averaging 42% (Figure 3). At Cellfor Inc, the selected varieties are removed from cryostorage and produced as somatic seedlings in the 10s of millions, and are then deployed operationally to reforestation sites as diverse genetic mixtures.

Operational Somatic Embryogenesis Production System

Commercial acceptance of a novel technology such as somatic embryogenesis requires the ability to develop and implement a successful operational use for the technology. The key components that must be addressed during this stage are as follows:

- 1) development of a cost-effective manufacturing process;
- 2) delivery of high-quality products that provide predictable and reproducible performance;
- 3) technology validation and promotion in the market place.

During the past decade, significant progress has been made towards developing reliable, high-volume, cost-effective somatic embryogenesis production systems. Organizations are working on commercialization programs for spruce (*Picea* spp.), Douglas-fir (*Pseudotsuga menziesii*), loblolly pine, and radiata pine.

Future of this Technology in Operational Forestry Programs

Forestry companies, advance seed production companies, and government organizations around the world are currently working at bringing this tissue culture technology to a point where it can produce conifer somatic seedlings, on a cost effective basis, with desired genetic characteristics because of the potential return on this investment. For example, in the southeastern US, the returns of planting elite varieties of loblolly pine seedlings produced from this tissue culture technology are evident in more tons per acre grown per year, with fewer diseased stems, higher quality, and straighter logs with small knots that will command the highest prices on the sawtimber market. A southeastern US landowner can expect to realize a 10% to 18% return from an investment in seedlings produced from these elite loblolly pine varieties, and harvest revenues that may be 75% greater in terms of 2006 dollars (net present value) than revenues from traditional orchard stock. With these types of potential economic returns, somatic embryogenesis is now a viable vegetative propagation system within operational forestry programs.

In the North American forestry market, CellFor Inc and Arborgen are currently the 2 companies using somatic embryogenesis tissue culture technology to apply varietal forestry in the production of conifer seedlings for the commercial market place. For the 2008 planting season, CellFor Inc will produce 10 million seedlings, while Arborgen will produce 500,000 to 1 million seedlings of southern yellow pines. In the southern US, the current market for southern yellow pine seedlings is 1.2 billion seedlings on an annual basis. It is projected that the market share of elite varieties of yellow pine will be 5% within the next 2 to 3 years. Thus, the application of somatic embryogenesis as a viable operational propagation technology for producing elite varieties of conifer species is just coming of age.

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