Conifer Somatic Embryogenesis and Multi-Varietal Forestry

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Abstract The global forestry sector, managing both natural forests and commercial plantations, is faced with many future challenges, including increased production of wood with desirable attributes, changing to new forest products, adaption to climate change, forest protection, and species conservation and restoration. To meet these challenges, a forest management system should be sufficiently flexible. Such flexibility is offered by the use of emerging tree biotechnologies, such as somatic embryogenesis (SE) and cryopreservation. SE is a tissue culture technique whereby genetically identical trees can be mass produced. Through the implementation of industrial multi-varietal forestry (MVF; the use of tested high-value tree varieties in plantations), it offers a new paradigm in tree breeding and deployment that is more flexible than the current seed orchard system. In addition to gaining economic benefits from MVF, SE enables research to elucidate genetic response to environmental factors, diseases, and insects and provides a tool for species conservation and restoration.

1 Introduction

Tree improvement efforts around the world in the past 50 years have contributed greatly to the productivity and quality of plantation forestry. Increased productivity is delivered through a breeding scheme based on the seed orchard, and this will continue to be the primary means of achieving genetic improvement in the near future. Seed orchard-based tree breeding schemes typically produce about 10 % volume increase per generation (Tosh 2012). Although conventional tree breeding

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provides a substantial increase in productivity, plantation forestry in the future will face new challenges: productivity will be pushed to even higher levels; breeding goals are changing as we search for new products; projected climate change scenarios cast uncertainty about tree adaptation as well as resistance to new pests; and tree breeders are expected to contribute to conservation and restoration of threatened tree species. Thus, tree breeders are required to develop "flexible" breeding and deployment systems to meet these challenges. Recent advances in tree biotechnology for several conifer species have enabled the development of more flexible tree breeding and deployment strategies than conventional seed orchard approaches can offer. In particular, somatic embryogenesis (SE) and cryopreservation offer the implementation of multi-varietal forestry (MVF), which is defined as the deployment of genetically tested tree varieties in plantations. It is also known as clonal forestry, but MVF is considered to be a more descriptive term when applied to industrial plantation forestry (Park 2004). Generally, a clone refers to any genotype with its genetic copies or ramets, whereas *a variety* refers to a clone that is selected or bred for certain attributes (and has field trial data to show to what extent these attributes has been achieved). Despite its many benefits, MVF has not been practiced in conifers due to the inability to produce the same genotypes consistently over time. With the application of SE and cryopreservation, it is now possible to produce the same tested genotypes consistently over time, which is analogous to the production of agronomic and horticultural varieties. The possibility of developing value-added tree varieties for plantation forestry offers a new paradigm in tree breeding and deployment that is more flexible than seed orchards. In addition, the use of other biotechnology tools such as molecular markers can improve the efficiency of such a strategy (Park and El-Kassaby 2006). Development and industrial implementation of a MVF strategy using conifer SE are discussed in this chapter. Application of SE in forest management and research is also discussed.

2 Conifer Somatic Embryogenesis

Simply, SE is a cloning technique based on tissue culture whereby genetically identical copies of a genotype are produced in unlimited numbers. SE in conifers was first reported in 1985 in Norway spruce (*Picea abies*, Hakman et al. 1985; Chalupa 1985), European larch (*Larix decidua*, Nagmani and Bonga 1985), and sugar pine (*Pinus lambertiana*, Gupta and Durzan 1986). Since then, SE has been widely available in many coniferous species, although there are still varying degrees of difficulty in obtaining SE. For several economically important conifers, however, SE is sufficiently refined to the point that it can be implemented in industrial production.

Conifer propagation by SE is accomplished in four stages: initiation and proliferation of embryogenic tissue; maturation of somatic embryos; germination of somatic embryos; and greenhouse/nursery culture (Fig. 1). In general, the initiation of SE is most efficiently obtained by using immature zygotic embryos as the starting material; however, in many spruce species, SE has been obtained from mature or

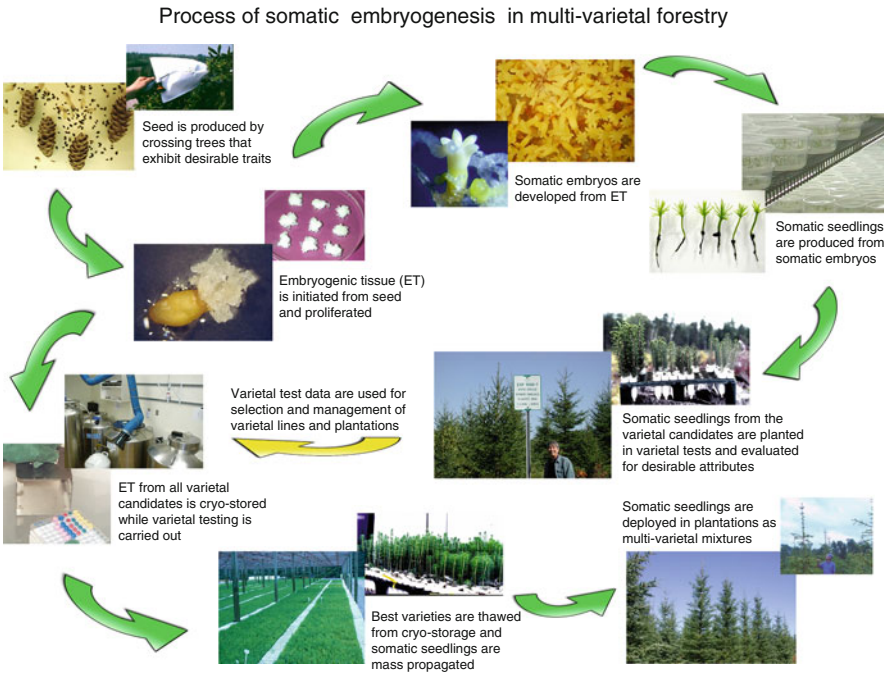

Fig. 1 Process of conifer somatic embryogenesis in multi-varietal forestry

stored seeds in relatively high frequencies. Although SE has been achieved from bud explants of 10-year-old SE-derived trees, the frequency of such initiation is still low at this time (Klimaszewska et al. 2011). Thus, an early stage of zygotic embryo explants such as megagametophytes containing zygotic embryos is preferred, particularly for pine species.

Several formulations of initiation media are used, for example, DCR (Gupta and Durzan 1986), MS (Murashige and Skoog 1962), LV (Litvay et al. 1985), and their modifications. The initiation media typically contain plant growth regulators (PGR), most frequently auxin (2,4-D) and cytokinin (BA). The explants are placed on the initiation medium, which is usually solidified with agar or gellan gum. Responsive explants typically initiate embryonal mass (EM) in 4–6 weeks. Once EM is obtained, it can be proliferated in liquid suspension culture or in a bioreactor. EM is continuously proliferated as long as fresh medium is supplied. The proliferation medium is essentially the same as initiation medium or a slightly modified formulation for further growth. The initiation and proliferation of EM are carried out in darkness at 23–25 °C. Initiation of SE is affected by the level of PGR, genotype, and the stage of zygotic embryo development (particularly for pines).

Maturation of somatic embryos is achieved by withdrawing the auxin and cytokinin and adding abscisic acid (ABA), and the inorganic and organic medium composition is usually the same as for initiation. A critical factor that promotes

development of a large number of mature somatic embryos is the restriction of water availability by physical means, or use of osmotic means, or combination of both. Accordingly, control of gel strength, polyethylene glycol, and a combination of the two are frequently used. To improve mature embryo production, the EM is first suspended in PGR-free liquid medium before culture on a filter disk placed on the maturation medium. Improved somatic embryo quality is an important factor because the embryos must be converted to vigorously growing plants.

Germination of somatic embryos is usually carried out on semi-solid medium without PGR. It has been found that it is beneficial to culture the somatic embryos in darkness for first 7 days before exposure to light. This usually ensures the elongation of hypocotyls. At present, the germinants are transplanted into the substrate used in the greenhouse and cultured in the greenhouse or in the nursery. However, it is also possible to germinate somatic embryos directly in micro-plugs filled with vermiculite saturated in liquid media. Initially, the somatic plantlets require immediate fertilization and high relative humidity, often requiring a fine misting system in the greenhouse. Thereafter, fertilization and pesticide application are the same as the regular schedule.

3 Cryopreservation and Thawing

The most important advantage of plant propagation by SE that the EM can be stored at ultra-low temperatures (−140°C to −196 °C) without changing genetic make-up or losing viability. This allows for the development of high-value tree varieties usually involved in lengthy field tests, while the corresponding varietal lines are cryogenically stored. Once those varietal lines with desirable attributes have been identified, the corresponding EM can be thawed and mass produced for deployment. Thus, cryopreservation ensures the production of the same varietal lines consistently over time.

Since the first publication on cryopreservation of white spruce (Kartha 1985), the protocol has been modified for use with several conifer species. The current protocol entails suspending 2 g (fresh mass) of EM in 7 ml of liquid maintenance medium supplemented with 0.4 M sorbitol for 18–24 h, and then, just before freezing, cold DMSO solution is added to the cell suspension on ice. The cell suspension is kept on ice for 1–2 h and then dispensed to cryo-vials. The cryo-vials are placed in alcohol-insulated containers (“Frosty” Nalgene™ container) that are pre-cooled for 2 h at −80 °C. The container with cryo-vials is then placed in a freezer at −80 °C for 1–2 h, during which time slow cooling of the cell suspension takes place (approximately −1 °C/min). Subsequently, the container is plunged in a liquid nitrogen cryo-tank and stored.

For thawing and regrowth of EM, the cryo-vials are rapidly thawed in water bath at 37 °C for 1–2 min, and the cell suspension is poured over a filter-paper disk placed on a thick pad of sterile blotting paper, allowing the storage solution to drain off. The top-most filter paper with cells is transferred to semi-solid initiation medium. Culture growth typically occurs in 1–2 weeks.

The recovery rate of cryopreserved genotypes has been high at about 95 % for many spruce and pine species; however, some species may require special treatment, such as a nurse culture (Hargreaves et al. 2002). It was generally observed that successful cryogenic storage is somewhat dependent on EM vigor. At this time, no published reports of the long-term recovery rate of cryopreserved EM are available; however, it was observed that cryostorage of five lines of *P. strobus* up to 7 years did not show any adverse effect on the recovery of cultures (K. Klimaszewska, personal communication). Park et al. (1998a) compared the genetic stability of a set of embryogenic lines after 3 and 4 years of cryopreservation, respectively, for in vitro and ex vitro characters and found highly consistent results between two thawing dates. Cryopreservation maintains juvenility and minimizes undesirable genetic change caused by prolonged subculture because ultra-low temperatures stop cellular metabolic functions (Kartha 1985). Therefore, it is prudent to use cryogenic storage as a means of minimizing any potential genetic change. There is, however, a possibility that the initial freezing may cause alterations. Therefore, it is recommended to cryopreserve EM lines first, then thaw a part of stored EM and propagate candidate lines for field testing and subsequent deployment.

4 Breeding and Variety Deployment

Among the many applications of SE, the most important application is its use in MVF. There are many advantages of MVF (Libby and Rauter 1984) including: (1) the capture of much greater genetic gain than is possible through conventional seed orchard breeding by exploiting both additive and non-additive genetic variation (Park et al. 1998b); (2) the flexibility to rapidly deploy suitable varieties in line with changing product goals, climate, or environment; and (3) the ability to design and manage genetic gain and plantation diversity according to need.

It is likely that MVF will be practiced on high productivity sites with intensive forest management in connection with long-term tree improvement programs, where seed orchards produce improved seeds for general reforestation. Therefore, with an existing long-term program, MVF may be used as a complementary strategy. The complementary function of MVF in connection with traditional seed orchard breeding is schematically illustrated in Fig. 2. Typical clonal seed orchard (CSO) breeding uses some form of recurrent selection and maintains a breeding population (BP) consisting of genetically selected individuals. Grafts of these are planted in seed orchards for the production of improved seeds. To obtain genetic improvement for the next generation, the individuals in the BP are mated, often by positive assortative mating (PAM), to produce material for next-generation selection and genetic testing and so form the next-generation breeding population. Based on the genetic testing, the initial CSO can be rogued for further improvement. Seeds from the seed orchards are genetically improved as they capture the additive genetic variability among the parents and are well suited for extensive reforestation. Complementary MVF may begin with the elite individuals selected from the BP. The elite parents

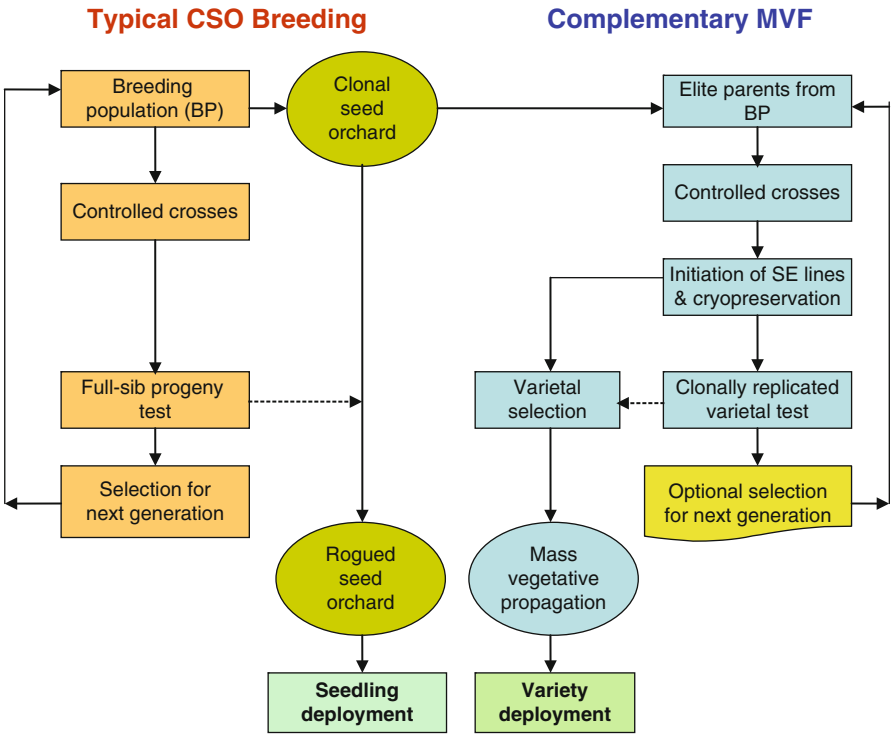

Fig. 2 Schematic illustration of a typical seed orchard breeding with complementary multi-varietal forestry

can be control pollinated (CP), open pollinated (OP), or supplementary mass pollinated (SMP) to produce an offspring population. The resulting seeds from these crosses are subjected to SE initiation and, once SE is initiated, the embryogenic tissue (ET) lines are cryogenically stored. Parts of the ET from each line are then thawed, propagated, and planted in a clonally replicated varietal test (CVT). Based on the CVT, high-value embryogenic varieties are retrieved from cryostorage and mass propagated for planting on the productive sites. The deployment of tested varieties offers much greater genetic gain than CSO seeds as it captures both additive and non-additive genetic variation.

MVF is adaptable to new and small-scale breeding programs. For example, a new program may be initiated by collecting OP seeds from phenotypically selected trees from wild stands, followed by the development of embryogenic varieties by SE. For shorter term, small-scale programs, a breeder may opt out of establishing a seed orchard. In other words, the cost for seed orchard establishment, management, cone collection, and seed processing are replaced by that of cryogenic storage. Assembly of a next-generation breeding population may, normally, not be carried

out, but an optional selection may be made. In this case, a breeder may have to rely on the use of molecular markers to control and monitor relatedness within the selected population.

5 Varietal Testing

The main advantage of MVF is the ability to obtain greater genetic gain by using all available genetic variances including non-additive variances (Park 2002). This benefit can be realized only through varietal selection based on varietal testing (VT), which is focused on selection and deployment of suitable varieties rather than genetic testing aimed at estimating genetic parameters. Thus, genetic gain calculated from selected varieties of the VT is the actual realized gain, as the same varieties will be deployed in the plantations.

In many established long-term breeding programs, the BP is often structured into sublines, which are groups of 10–20 parents within which breeding is carried out. The top tier of these sublines is referred to "nucleus" or elite subline, which contains the best parents of the BP. This strategy is designed to deliver fast genetic gain from elite crosses (Coterill et al. 1989). Thus, the development of varietal lines can begin by making elite crosses with the nucleus subline. If the breeding program is new, it can be started with open-pollinated seeds of phenotypic selections from wild stands.

In seed orchard breeding, the selected parents from these sublines are planted in a seed orchard and allowed to produce wind-pollinated seeds; however, the strategy is often inefficient due to pollen contamination, asynchronous flowering leading to unequal parental contribution, etc. In MVF, the development of varietal lines requires controlled crossing of the best parents and the propagation of several individuals within each cross by SE. For example, with a set of 20 parents, a breeder can produce up to 380 possible crosses without selfing, but, these are unrealistic numbers. In reality, the number of crosses to make and the number of individuals to propagate within each cross depend on the availability of resources and logistics. Typically in eastern Canada, VT is established using 200–300 varietal lines developed from 20 to 30 crosses, e.g., 200 candidate varieties may consist of 10 genotypes each from 20 crosses. A series of tests are planted in multiple years to ensure that large numbers of candidate varietal lines are available for selection, and the tests are conducted at multiple locations.

Differential SE success rate could have an impact on capturing potential genetic gain for MVF; however, a simulation study demonstrated that embryogenic propensity among families had no significant impact on gain (Lstibůrek et al. 2006). It also indicated that, even though there is reduced variation among families due to differential SE success, the variation within family is not changed. Thus, it is reasonable to expect much of the genetic gain will be derived from within-family variation. In establishing VT, one would generate more embryogenic candidate lines within a family than by using more families with smaller number of individuals within a family.

However, a balancing act is necessary to obtain optimal gains from both among- and within-family selection as well as for diversity concerns.

The VT is a key part of MVF because it will provide appropriate data for varietal selection and for the management of plantations. The flexibility of MVF is primarily derived from VT because it is intended that VT will continuously provide relevant data on growth, quality, insect and disease resistance, adaptation to changing environments and climate, and other traits throughout the rotation age and beyond. The final assessments of candidate varieties in the VT may take a long time; however, whenever the relevant and updated data become available, the breeders are offered the flexibility to rapidly adapt to the change by simply thawing and deploying appropriate varieties from the cryopreservation as environmental sensitivities emerge or goals are changed. Thus, it is important to establish VT with large numbers of candidate varieties across a wide range of sites. This may require planting tests in several successive years.

The use of clonal replicates, as in VT, is essential for disease and insect resistance screening experiments, especially when the objective of the experiment is to develop resistant varieties. Challenge tests based on a family or provenance level lack the precision to identify resistant individual genotypes because these tests include several different individuals comprising family or provenance, and thus, they only provide the resistance levels at the family or provenance average. The removal of the "genotype effect," which is achieved by the use of clonal replicates that provide genetic uniformity of candidate varieties in the challenge test, is essential for capturing individual genotypes that are truly resistant. Similar to VT, clonally replicated genetic testing (CRT) has been used in tree breeding experiments designed to obtain additional genetic parameters. For example, using control-pollinated families, Mullin and Park (1992) further demonstrated the partitioning of epistatic variance. Thus, CRT provides additional genetic information for breeding programs and offers efficiency and precision of parameter estimation for quantitative traits (Foster and Shaw 1988).

6 Balancing Genetic Gain and Diversity

In breeding, an increased genetic gain is usually achieved through a reduction in genetic variability. The major concern in deployment of cloned varieties is that a narrow genetic base may make clonal varieties vulnerable to disease and insects, and this may lead to plantation failure. For known diseases and insects, MVF has an advantage because resistant varieties may be developed or identified, but, for unknown or introduced diseases and insects, protection against them is less predictable despite the large genetic variability with a tree species, as entire tree species can on occasion succumb to novel pests or diseases (as discussed in various other chapters). It is difficult, if not impossible, to design a protection scheme against unknown diseases and insects. However, it is generally assumed that the more varieties are deployed in a plantation, the lower the risk will be. The increased number of

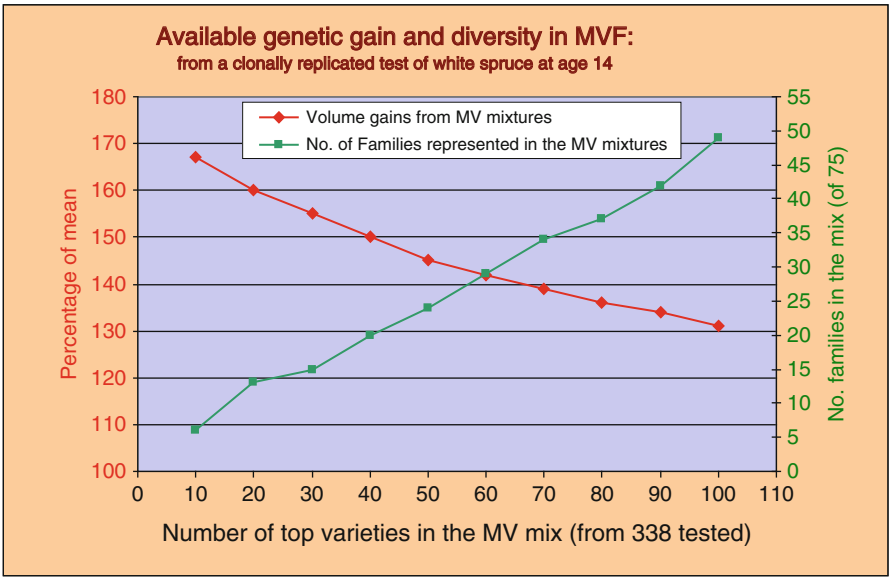

Fig. 3 Available genetic gains as percentage of test mean for multi-varietal mixes (–♦–) and corresponding diversity of MV mixes represented by the number of families in the mix (–■–)

varieties in the plantation will reduce genetic gain. Therefore, it is necessary to balance genetic gain and diversity. Lindgren (1993) discussed some basic principles for determining an appropriate number of varieties in a plantation: (1) if the species being deployed is short lived or short rotation, a lower number of varieties may be used because the exposure to potential risks is short; (2) a lower number of varieties may be used if plantation management is intensive and includes pest management; and (3) the more well known a variety, the more acceptable is its extensive use.

Once an appropriate number of varieties is selected, they may deployed in varietal blocks or random mixtures (Libby 1982). In eastern Canada, however, an approach called "desired gain and diversity" is used. This approach uses the best available VT data to select varieties while considering a predetermined level of genetic gain or diversity. For example, the available genetic gain and management of plantation diversity is illustrated in Fig. 3. The data shown in the graph were obtained from a 14-year-old clonally replicated test of white spruce (*Picea glauca*) that included 338 candidate varieties derived from 75 full-sib crosses using a disconnected diallel mating. In the graph, if we take the top ten varieties of the 338 in the test, the genetic gain for volume is 68 % better than the average of all varieties in the test. Even when we take the 100 best, the genetic gain is 30 % better than the average. This is still a large genetic gain compared with that obtained by seed orchard breeding. Incidentally, the average (100 %) represents an idealized seed orchard gain without inefficiencies. The number of families represented in a varietal mix may be considered as an indicator of diversity. If we take the ten best varieties, this varietal mix is represented

by only seven of the 75 full-sib families in the test. If we take the top 100, i.e., taking 30 % above the seed orchard gain, the varietal mix is represented by 48 of 75. In other words, we can balance a desired level of genetic gain and diversity based on the VT test results. For example, it is likely that a large number of varieties may be included in the MV mix at an early stage of VT; however, when the test is mature or varietal characterization is sufficient, a smaller number of elite lines may be used. Therefore, determining an appropriate number of varieties is a dynamic process based on best available data at a given time.

Another approach to manage genetic diversity in plantations is to mix in selected varietal lines with lower-cost seed orchard seedlings. This will increase the initial plantation diversity. Typically, in eastern Canada, about 40 % of plantation basal area will be commercially thinned at about half rotation age, leaving superior quality trees for final harvesting. Thus, it is likely that most final crop trees would consist of the tested varietal lines, and this reduces exposure to potential risks for the crop trees. The thinning will favor the best phenotypes regardless of genetic origin, and exceptional trees originating from seed orchard seeds will also become crop trees to fill gaps created when some varieties or ramets perform poorly and are removed during thinning. The highest quality trees, regardless of genetic origin, will remain in the plantations. This approach can be combined with the 'desired gain and diversity' strategy. Therefore, the diversity of plantations is dynamically managed spatially and over time, and selection of varieties is continuously revised based on VT throughout the testing period and as new varieties are introduced at each breeding cycle, resulting in different compositions of varietal mix.

7 Industrial Production

Industrial production of varieties by SE is at an early stage of development, and it is known that several companies and organizations in Canada, Chile, France, New Zealand, USA, and the UK are actively involved in development. Most conifer SE applications have been developed using the Petri dish-based system and in vitro germination. These approaches are suitable for establishing VT and small-scale commercial production; however, they are viewed as more expensive for commercial production than seedlings. Improving the efficiency of SE for a mass propagation system that is amenable to automation is an important task for commercialization. Some promising developments for an efficient SE system include rapid proliferation of embryogenic tissue in liquid culture and photoautotrophic micro-propagation techniques (Kozai et al. 2005) applicable to SE, and a computer-aided robotic system for a transplant system (Find 2009). Despite the lack of reliable automated systems for SE production, the cost is similar to commercial rooting of cuttings production. Although planting stock production by SE is preferred, it is still complex and relatively expensive due to a lack of automation. Alternatively, mass propagation of varietal lines can be achieved by the use of serial rooting of cuttings, whereby a few juvenile plants of varietal lines thawed from cryogenic storage are used as donor plants (Park et al. 1998b). Mass vegetative propagation by rooting of cuttings

from juvenile donor plants is readily available for several conifers species, especially for spruce species. In this case, SE and cryopreservation, in conjunction with VT, are used as a tool for developing varietal lines. In addition to the lack of an automated SE system, another factor impeding industrial application is a shortage of the tested embryogenic varieties to deploy. Therefore, any breeding program that includes future MVF must start establishing well-designed VT now, because there will be at least about 5 years of lag time between the establishment of VT and the first deployment of MVF. Current productivity and quality improvement in MVF is achieved by careful exploitation of existing natural genetic variability; however, MVF is likely to be the delivery mechanism for value-added tree biotechnology products in the future.

8 Hybrid Varieties

In agriculture, hybridization usually refers to crossing of different strains (or homozygous lines) within a species, but in forestry, it refers to crossing between different species or distinctly different races within a species. The benefits of hybridization in forestry include the capture of hybrid vigor in growth traits and the combination of desirable traits. An example of hybrid vigor is demonstrated by the interspecific crosses between Japanese (*Larix kaempferi*) and European larches; an example of combining desirable traits is demonstrated by pitch (*P. rigida*) and loblolly (*P. taeda*) pine hybrids, which are successfully used in Korea to take advantage of the cold tolerance and fast growth in the respective species. Despite the huge potential benefits, hybridization in conifers has rarely been used as a modern breeding method, partly due to the labor intensiveness of hybrid seed production by mass controlled pollination and the inefficiencies of seed production in a bi-species orchard designed to produce hybrid seed. SE is an ideal tool for developing hybrid varieties as it can be used for mass production of hybrid plants from a relatively small number of interspecific crosses. Also, the use of SE enables the selection of elite individuals within the crosses, further improving hybrid characteristics. The development of blister-rust-resistant white pine varieties through interspecific hybridization between *P. strobus* and *P. wallichiana* (and backcrossing to *P. strobus*) is underway in Ontario, Canada, which adopted the use of SE for variety development (Lu 2008). The deployment considerations, including selection for variety mix, would be similar to those for MVF, as discussed earlier.

9 Other Applications of Somatic Embryogenesis

9.1 Embryo Rescue

Somatic embryogenesis can be used in embryo rescue. Crossing between distantly related species can be a useful technique for creating unusual genotypes, but such crosses usually result in abortion. Embryo rescue has been carried out with a large number of species, including tree species. A couple of recent examples of the latter

are citrus triploid hybrids (Aleza et al. 2010) and banana (Uma et al. 2011) but, to our knowledge, embryo rescue has never been attempted with conifers. It is generally believed that embryo abortion is due to a genetic incompatibility between the developing embryo and the maternal tissue surrounding it. Even though embryos may not develop fully within the megagametophyte tissue with the seed, these embryos can develop normally if they are removed from seeds before abortion occurs and can then be grown to maturity in vitro, or immature embryos may be induced to initiate SE. The latter is a possibility because SE often initiates during the initial cleavage polyembryony stage of the zygotic embryo. Thus, embryo rescue has the capacity to create genotypes that are not possible to obtain through breeding. Subsequent mass propagation of a rescued embryo can be highly effective if propagated by SE.

9.2 Species Conservation and Restoration

Somatic embryogenesis combined with cryopreservation can have an important impact on species conservation and restoration. For example, whitebark pine (*P. albicaulis*) is a keystone species growing in the subalpine regions of Alberta and British Columbia and is threatened in its natural range. No seeds are available for reforestation as they are a food source for birds and animals, and its wingless seed is dispersed only by birds. The serious threats come from white pine blister rust (*Cronartium ribicola*), white pine weevil (*Pissodes strobi*), mountain pine beetle (*Dendroctonus ponderosae*), prolonged fire suppression preventing natural regeneration, and projected climate change. In this case, SE can be used as an alternative means of producing planting stock as the seed availability is very limited. Through SE, valuable genotypes can be cryogenically stored over the long term, and restoration of the sites with better adapted and pest-resistant genotypes can be accomplished at a later date. This is a strategy adopted by the Alberta Sustainable Resource Development in Canada (Park et al. 2010). Similar efforts are also in progress for limber pine (*P. flexilis*) in Alberta.

9.3 Genetic Engineering

Genetic transformation allows integration of valuable genes or traits that are absent from even the elite breeding material into selected genotypes. For example, genetic engineering for pest resistance, or genes providing tolerance to salt, heavy metal, or drought in trees, can be extremely valuable. Obtaining disease and insect resistance by traditional selection and breeding is very difficult for forest trees, primarily due to the long generation time and difficulty in finding resistant genotypes in the wild. Merkle et al. (2007) examined the possibilities of restoring American chestnut (*Castanea dentata*) devastated by the chestnut blight fungus (*Cryphonectria parasitica*)

which was accidentally introduced into North America from China early in the 20th century, through introducing antifungal genes using embryogenic culture. SE is the primary enabling technology for both transformation and subsequent propagation. Among the available transgenic technologies, transformation of embryogenic culture is the most common one. It is generally achieved through co-cultivation with *Agrobacterium* carrying the transgene. The advantages of using SE in genetic engineering are: the process can be carried out in a strictly confined environment; the transformed and non-transformed cells can be separated by inclusion of an antibiotic resistance gene; and, as SE in most species starts from single cells, one can avoid ending up with chimera, i.e., individuals with both transformed and non-transformed cells. However, the stability and containment of transgenes are important issues that have to be dealt with before genetically modified trees are deployed. Engineering of sexual sterility genes into SEs is a means of achieving containment, but it is not yet widely available. Due to a potentially adverse environmental impact and bio-safety issues, most jurisdictions around the world regulate testing and deployment of transgenic trees (Trontin et al. 2007; and also see the chapter of Häggman et al. in this book for an overview of this area).

9.4 Epigenetic Memory

It is presently generally accepted that epigenetics refers to changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence (Meehan et al. 2005). Epigenetic memory effects in Norway spruce during zygotic embryogenesis and seed maturation have been reported by Johnsen et al. (2005), who found that adaptive traits in progenies were influenced by the temperature that the maternal trees were exposed to during seed development. von Aderkas et al. (2007), working with interior spruce (*P. glauca* x *P. engelmannii*) SE, reported that somatic embryos, matured at a lower temperature (5 °C), showed significantly higher cold tolerance than those matured at 20 °C. However, it is not known whether such epigenetic memory effects will continue during the seedling and adult tree stages; however, Johnsen et al. (2005) found that epigenetic memory effects persist for many years in the filial generation. This is an important research area for plant adaptation especially under climate change scenarios. SE can be used for studying epigenetic memory effects as well as embryo development.

10 Concluding Remarks

Somatic embryogenesis is the first conifer biotechnology to be applied in tree improvement for the implementation of MVF and has opened new commercial opportunities for the forest industry. Several companies around the world are in the process of implementing MVF to take advantage of the increased productivity,

production of wood with desired attributes, and flexibility in meeting future demands. Currently, the system of producing plants by SE is slightly more expensive than the traditional seedling production system due to the lack of an automated production system, but this might change as SE is highly amenable to automation. In addition to the economic benefits of implementing MVF, SE can be used as an important research tool for conifer development, ecophysiology, pest resistance, functional genomics, among other things.

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