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REVIEW

A comparative evaluation of the application of somatic embryogenesis, rooting of cuttings, and organogenesis of conifers

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Abstract: Vegetative propagation of conifers has found large-scale industrial application via somatic embryogenesis (SE), rooting of cuttings, and organogenesis. Genetic gain is achieved with all of these methods but is the highest with SE, primarily because SE cultures can be cryopreserved. This allows for plants derived from part of each cell line to be field tested over a long period while the rest of each cell line is kept in a juvenile state by cryopreservation for later use. This makes it possible to select the best performers within the best families. For rooting of cuttings and organogenesis, genetic gain is generally based on family average, which is less powerful. However, SE has its limitations, primarily because its initiation, maturation, or germination rates are too low to be effective for many species. Consequently, for many species, the preferred clonal propagation option is still rooting of cuttings or organogenesis. If better methods can be developed to keep ortets used for rooting of cuttings and organogenesis in a prolonged juvenile state, or if future developments in marker technology reach a point where within-family selection becomes possible without the aid of cryopreservation, rooting of cuttings and organogenesis will achieve the same genetic gain as SE.

Key words: cryopreservation, genetic gain, juvenility, ortet, ramet.

Résumé : La propagation végétative des conifères a trouvé une application industrielle à grande échelle via l'embryogenèse somatique (ES), l'enracinement de boutures et l'organogenèse. Toutes ces méthodes permettent d'obtenir un gain génétique mais l'ES procure le gain maximum, surtout parce que les cultures d'ES peuvent être congelées. Cela permet de tester au champ sur une longue période les plants dérivés d'une partie de chaque lignée cellulaire tandis que le reste de chaque lignée est conservé dans un état juvénile par cryoconservation pour usage ultérieur. De cette façon il est possible de sélectionner les plants qui performent le mieux au sein des meilleures familles. Dans le cas des boutures racinées et de l'organogenèse, le gain génétique est généralement basé sur la moyenne de la famille, ce qui est moins performant. Cependant, l'ES a ses limites, principalement parce qu'avec plusieurs espèces l'initiation, la maturation ou le taux de germination sont trop lents pour être efficaces. Par conséquent, dans le cas de plusieurs espèces l'option de propagation clonale préférée demeure l'enracinement de boutures ou l'organogenèse. Si on arrive à développer des méthodes pour conserver dans un état juvénile prolongé les ortets utilisés pour l'enracinement de boutures ou l'organogenèse, ou si les progrès dans la technologie des marqueurs atteignent un point où la sélection intrafamiliale devient possible sans avoir recours à la cryoconservation, l'enracinement de boutures et l'organogenèse vont procurer le même gain génétique que l'ES. [Traduit par la Rédaction]

Mots-clés : cryoconservation, gain génétique, état juvénile, ortet, ramet.

Introduction

There are several means of clonal propagation of conifers, most notably somatic embryogenesis (SE), rooting of cuttings, and organogenesis. For conifer species, where these methods have been effective in mass cloning, they all have resulted in obtaining genetically improved planting stock. There are other clonal propagation techniques, for example, grafting. However, grafting is used primarily for conifer seed orchard establishment and is rarely used for mass cloning. It is labor intensive and can result in graft rejection, which is high in some conifers (Miller and DeBell 2013) and sometimes delayed for many years (Sweet and Thulin 1973). Coppicing, which is very effective for many hardwood species (Wendling et al. 2014), is not an option for conifers because root or stump sprouts are not available for most species. This review will not deal with grafting and coppicing but is restricted to those methods that allow both mass cloning and genetic improvement, i.e., SE, rooting of cuttings, and organogenesis.

The objectives of this review are to show that (*i*) SE is not likely to completely replace rooting of cuttings and organogenesis, (*ii*) these technologies can sometimes be used effectively in conjunction with each other, and (*iii*) with improved cryopreservation and genetic marker technology, rooting of cuttings and organogenesis may eventually acquire some of the attributes that have made SE so effective in obtaining genetic gain.

Somatic embryogenesis

Somatic embryogenesis (SE) of conifers has found extensive commercial application, with companies using large numbers of somatic embryos annually in their plantations (Cyr and Klimaszewska 2002; Celestino et al. 2013; Lelu-Walter et al. 2013). In particular, it has been effective because embryogenic cultures can be cryopreserved and reactivated after cryopreservation. This has allowed long-term field testing of the clonal lines and, therefore, selection of superior lines prior to mass production of the selected lines.

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This has had important implications in producing superior planting stock for reforestation (Park 2002; Lelu-Walter et al. 2013). However, even though SE is very effective in producing genetically superior planting stock and can, for many species, produce a large number of plants, it has its limitations. For several commercially important species, the SE initiation rates are low, and proper maturation of the somatic embryos is often a problem. For example, for *Pinus banksiana* Lamb., the initiation rate is only about 3% (Bonga 2012), and for *Pinus pinea* L., it is even lower (0.4%) (Celestino et al. 2013). Such low rates are far from practical; therefore, for many conifer species, the more traditional mass-propagation methods, primarily rooting of cuttings and organogenesis, are still being used extensively. It is not an objective of this review to describe methodologies of SE, rooted cuttings, and organogenesis, because many extensive reviews on that subject are available for consultation (Klimaszewska et al. 2007; Celestino et al. 2013). Instead, the effectiveness of SE, rooting of cuttings, and organogenesis will be compared with respect to their effectiveness in producing improved planting stock for reforestation.

Rooting of cuttings

Clonal propagation by using rooted cuttings has been highly successful. For example, in Brazil, large-scale planting with clonal *Eucalyptus*, obtained from juvenile stump sprouts of superior selected trees, has resulted in plantations ready for harvesting after 7 years (Ondro et al. 1995). This procedure cannot be followed for species for which juvenile stump sprouts are not available from adult trees, as is the case for most Coniferales. Conifer stem cuttings suitable for rooting are generally available only from plants that are too young to have demonstrated what kind of growth they are capable of over the long term. Because of the often poor correlation between juvenile and mature traits, proper testing generally requires at least about one-third to one-half the rotation age (Zobel 1981; Hodgetts et al. 2001; Weng et al. 2008). Juvenility can be prolonged a few years by repetitive pruning (hedging) or repeated cutting cycles (Bentzer 1993; Mason et al. 2002; Mitchell et al. 2004) but often not long enough to allow long-term field testing of the ortets to determine their suitability for clonal planting. Only for a few conifers has longer term clonal testing been possible. For example, *Picea abies* (L.) Karst. ramets have been maintained in a rooting-capable state for up to 20 years by serial rooting of cuttings every 3 years (Bentzer 1993). In a study with *Picea sitchensis* (Bong.) Carrière, serial rooting of cuttings provided material capable of rooting for up to 18 years, whereas hedges did so only for 11 years (Mason et al. 2002). These procedures have provided upward of 2.5 million plants per year for planting in Great Britain. Without hedging or serial propagation, the rooting ability of cuttings and the growth vigor of the rooted cuttings diminish after the ortet reaches about 2 years (Mason et al. 2002). For a few conifer species, reliable rooting of cuttings is possible at a more advanced age of the ortet. Such is the case for *Pinus radiata* D. Don, which can be cheaply mass-produced by the rooting of cuttings from trees up to 15 years old (Thulin and Faulds 1968).

Despite the fact that, for many conifers, long-term clonal testing prior to mass clonal propagation by rooted cuttings is not possible, cloning of young plants by rooting of cuttings can be highly effective in providing genetically improved planting stock. Mass cloning of young plants obtained from seed that was improved by breeding provides a clonal population that reflects the family average in performance and thus a population that is improved over their wild counterpart. For example, a gain of 10%–20% in height growth is possible for *P. abies* (Bentzer 1993), and a gain greater than 30% is possible for *Picea glauca* (Moench) Voss (Weng et al. 2010). Rooting of cuttings of young plants is also important when only a small quantity of improved seed is available, i.e., a quantity too low to be useful for direct seeding (Libby and Ahuja 1993). Rooted cuttings obtained from young plants generally perform as well as seedlings of the same family. They may be slower initially, but after a number of years, the difference often disappears, e.g., after 10 years for *Pinus taeda* L. (Stelzer et al. 1998). *Callitropsis nootkatensis* (D. Don) D.P. Little 1-year-old rooted cuttings performed better than their seedling counterparts (Russell 1993), whereas *P. glauca* rooted cuttings have shown a slightly lower growth rate than seedlings over a 5-year period (Beaulieu and Bernier-Cardou 2006). In Australia and New Zealand, production of rooted cuttings of *P. radiata* reached 3.3 million in 1992, with trees produced that way showing the benefit of having fewer and smaller branches and less stem taper than trees obtained from seed (Ritchie 1997). By 2010, 25% of the planting stock of this species was provided by rooting of cuttings and 4%–5% by SE (Find et al. 2014).

When older ortets are used, differences in volume, form, and shape can occur (Mitchell et al. 2004). For *P. radiata*, the ortet age that delivers the best ramets is 3–4 years (Menzies et al. 2000). Similarly, Foster et al. (1987) concluded that *P. taeda* ortets older than 5 years pass on mature traits to their ramets. Sometimes, somewhat older ortets are used to obtain ramets that are sufficiently mature to reduce some of the defects associated with the juvenile growth habit while retaining the fast juvenile growth rate (Sweet 1973). Ramets of young *P. radiata* plants show growth characteristics that are better than those of seedlings (Frampton and Foster 1993). On the other hand, rooted cuttings from juvenile *Pseudotsuga menziesii* (Mirb.) Franco stock plants exhibited traits of mature trees during and after 5 years in the field (Ritchie et al. 1994). In comparing the field performance of plants obtained by rooting of cuttings and by SE, the latter may show reduced initial growth rates compared with those of seedlings, but this can often be remediated by improved culture practices (Högberg et al. 2003). Rooting of cuttings is useful in propagating plants produced from selected SE lines in a cost-effective manner. Forest Genetics Ltd and ArborGen New Zealand produce about 50 000 somatic seedlings annually of *P. radiata* that are established as stool-bed plants. Forest Genetics Ltd annually produces and sells 2.5–3 million rooted cuttings in New Zealand. The annual market for *P. radiata* in New Zealand is about 50 million, half of which are seedlings and the rest are rooted cuttings from hedges (M. Carson, personal communication, October 2014). In this example, SE is primarily used to provide genetically improved clonal lines that are subsequently mass-produced by the rooting of cuttings. A similar procedure is used for *P. sitchensis* (Lelu-Walter et al. 2013). Other companies produce somatic seedlings in numbers large enough for direct planting, sometimes totalling millions (Cyr and Klimaszewska 2002; Celestino et al. 2013). As a note of caution, it is generally assumed that if one takes several scions from a plant, these are all genetically identical. However, genetic variation has been observed within trees. A study of *Populus trichocarpa* Torr. & A. Gray ex Hook. clones has shown that leaves are genetically different from roots and that the crowns of clones arising from root sprouts are, therefore, genetically different from the crowns of the parent tree (Yong 2012). It has also been postulated that genetic variation occurs among the shoot meristems within the crowns of highly branched trees (Cherfas 1985; Gill et al. 1995). To what extent such variation occurs in conifers is not known.

Organogenesis

There is a long tradition of trying to obtain clonal propagation by in vitro means. Prior to the development of somatic embryogenesis, attempts were made to use organogenesis as a means of clonally propagating conifer species. Success was first achieved with *Pinus palustris* Mill. by Sommer et al. (1975) using excised, mature zygotic embryos as explants. In organogenesis, adventitious shoots are formed directly on the zygotic embryo, on parts of the zygotic embryo, or from meristematic nodules. These shoots are subsequently rooted to form plantlets. Although organogene-

sis has been very effective for some conifer species, in particular *P. radiata* (Aitken-Christie et al. 1988; Montalbán et al. 2011), for many, this technology never reached a practical application stage primarily because of low plantlet formation rates, poor rooting, and excessive handling and costs.

One reason that organogenesis has never become as popular as the later invented SE procedure is the difficulty in keeping organogenic cultures in a juvenile state by cryopreservation. It has been possible to store adventitious shoots of *P. radiata* for up to 5.5 years at 4 °C, but only shoots that had been stored for 17 months were capable of rooting (Aitken-Christie and Singh 1987). This is not long enough for a proper field test. The ability to cryopreserve material for propagation by organogenesis is desired for species for which organogenesis provides plantlets for a large number of genotypes per family, whereas SE does so for only a limited number of genotypes within the family (Hargreaves et al. 2004, 2011). It was found that partially desiccated cotyledons of *P. radiata*, detached from mature zygotic embryos, can be cryopreserved without cryoprotectant pretreatment with one-step cooling. After up to 28 days in cryopreservation, the thawed cotyledons produced the same number of adventitious shoots and plants as the noncryopreserved control cotyledons, but plant height was 8% lower in the cryopreserved material after 21 months in the field (Hargreaves et al. 2004). Subsequently, it was found that more genotypes were captured by culture of cryopreserved cotyledons than were obtained from axillary shoots arising from epicotyls of the noncryopreserved parts of the same zygotic embryo (Hargreaves et al. 2005).

A well-responding seed of *P. radiata* can form about 260 000 clonal trees in 2.5 years from pieces of meristematic tissue (Aitken-Christie et al. 1988). Unfortunately, cloning by organogenesis is not always true to type. Propagules derived from cotyledons of *Pseudotsuga menziesii* (Mirb.) Franco seedlings showed mature characteristics during a 5-year field test (Ritchie et al. 1994). However, the incidence of such abnormalities may be an indication of suboptimal culture conditions and may be reduced by protocol improvements.

Efforts to make cryopreservation applicable to rooting of cuttings and organogenesis

For many hardwood species, cryopreservation of shoot tips and dormant buds has been achieved, primarily for gene-pool preservation. New plants are regenerated in vitro from this gene pool when needed (Millar 1993; Engelmann 2011; Pijut et al. 2011). With regard to gymnosperms, cryopreservation of shoot tips of *Tetraclinis articulata* (Vahl) Mast. (Cupressaceae) has been reported (Serrano-Martinez and Casas 2011). Micropropagation of conifers from shoot explants has been rare (Bonga et al. 2010; Bonga 2012) and is, at present, not commercially practical. However, if effective methods for micropropagation from shoot tips could be developed for conifers, cryopreservation could possibly become as useful for rooting of cuttings as it is for SE in the selection of superior genotypes.

As mentioned earlier, cotyledons of *P. radiata* can be cryopreserved for up to 28 days. If the excised cotyledons could be cryopreserved for longer than that, it could possibly allow long-term testing prior to mass cloning. A part of the cotyledons of each donor zygotic embryo could be cryopreserved, with the remaining cotyledons being used for the production of plants by organogenesis for field testing. After the field test has identified superior genotypes, the corresponding cryopreserved cotyledons could be thawed and used for mass production of plants by organogenesis. This would provide the same ability to select superior genotypes as can be achieved with cryopreserved SE cell lines and would be useful for those species for which organogenesis is more effective than SE.

Deployment

Conifers have a long life cycle, which adds to the risks of using clonal populations. Disastrous effects due to lack of diversity have occurred, for example, in clonal poplar populations (Bishir and Roberds 1999). However, although risk can decrease with a larger number of different clones being employed, it can also increase. The level of risk is unlikely to be reduced if the number of clones exceeds 30–40 (Bishir and Roberds 1999). Another model suggests that approximately 18 genotypes are optimal under many conditions and that, for merchantable volume, no more than 30 clones are needed for risk protection and near-optimal timber yield (Yanchuck et al. 2006). This model also indicates that planting blocks with a mixture of clones has advantages over planting a mosaic of blocks, with each block containing a different single superior clone. These deployment rules would apply to all clonal populations no matter whether of SE, rooted cutting, or organogenesis origin.

Cost comparison of rooting of cuttings, organogenesis, and SE

The cost of producing clonal propagules is an important factor in determining the practical viability of the different clonal propagation methods. Producing seedlings from seed is always the cheapest method, and clonal propagation is only warranted when a gain in genetic makeup overrides the extra cost associated with such cloning. Rooting of cuttings is cheaper than producing propagules by organogenesis or by SE. For *P. abies*, the cost of a rooted cutting approaches that of a seedling (Mikola 2009), and the use of rooted cuttings is, therefore, economically feasible. Organogenesis has been cost effective for *P. radiata*, where the expense of a tissue culture produced plantlet is close to that of a seedling (Nairn 1993).

Whether the increased cost of SE is outweighed by savings due to increased production depends on many factors (Sorensson 2006). For example, weed control by herbicide application can boost volume growth considerably at a much cheaper cost than is incurred by planting somatic embryos. However, the improved disease resistance and stem quality that can be obtained by using selected SE clonal lines may still make use of somatic embryos the preferred option. For southern pines, a cost almost 10 times higher has been quoted for somatic seedlings than for seedlings obtained from open-pollinated seed, but use of the former is still economical because of their superior performance (Sorensson 2006).

Genetic profiling

Conifers have large, complex genomes and a long life cycle that frequently delays expression of desirable traits. A further complication is that these traits often are under polygenic control. This causes problems in traditional breeding programs, making the application of marker-assisted selection desirable (Ritland et al. 2011; Chhatre et al. 2013). Rapid progress in DNA technology has occurred over the last few decades, reaching the point where determining the total genome of conifers, despite its size and complexity, is now possible, e.g., *P. abies* (Sederoff 2013), *P. taeda*, and *P. glauca* (Isik 2014). Of the various molecular technologies that have been developed over the last few decades, the most promising for selection purposes for breeding appears to be "genomic selection". It has been highly effective in cattle breeding and is finding application elsewhere. In conifer tree breeding, it is still in its infancy due to the large and complex conifer genome and the cost of the procedure, although the cost is expected to decrease in the future. Genomic selection is based on dense marker coverage, where all of the loci causing phenotype variation among individuals in the population are traced (Isik 2014). With regard to the efficacy of rooting of cuttings and organogenesis vs. SE, it is possible that with genomic selection the advantage

of SE, i.e., its cultures can be cryopreserved, may diminish. It is feasible that cryopreservation may eventually no longer be needed for within-family selection. In that case, it may become possible to derive the same genetic gain advantages with rooting of cuttings and organogenesis as with SE.

Future prospects

For species for which SE is effective, it will probably remain the preferred propagation method, especially if the process can be automated and made cheaper. Somatic embryogenesis will remain attractive especially because of its high propagation rate and because it has applications not available otherwise. To mention just a few of these, an important application is genetic transformation, a process that is still largely experimental for forest tree species (Ruotsalainen 2014). Even though various conifer tissues have been used for genetic transformation (Aronen et al. 1995), it is most often achieved through SE (Trontin et al. 2002; Klimaszewska et al. 2010). Another application of conifer SE that could be important is rescue of embryos resulting from wide crosses that abort prematurely due to megagametophyte and embryo incompatibility. By excising the immature embryos before they abort and by their culture in vitro, plants with novel genotypes can be obtained (Ho 1987). For conifers, immature zygotic embryos are well suited for SE, generally performing better than mature ones. To my knowledge, rescue of embryos that will abort if not removed from the seed and grown to maturity in vitro has not been reported for conifers but has been achieved for some forest species, for example, for embryos resulting from interspecific and intergeneric crosses within the Salicaceae (Payamnour et al. 2013). Another potentially useful application uniquely suited for conifer SE is for embryogenesis in cultures arising from haploid megagametophytes. Dihaploid trees could be created, although lethal and semi-lethal recessives could make the process difficult, requiring selection for genotypes low in these recessives. More promising is the possibility of fusing haploid protoplasts of related species, thus creating somatic hybrids (Rohr 2004).

Conclusion

In conclusion, SE is desired for its flexibility, but rooting of cuttings and organogenesis still have their place. The advantage of being able to cryopreserve SE cultures may eventually lose some of its significance if cryopreservation is extended for use in the rooting of cuttings and organogenesis process. Furthermore, the usefulness of the latter two may improve if molecular selection techniques are developed to the point where selection of superior genotypes can occur without requiring long-term testing.

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References

Aitken-Christie, J., and Singh, A.P. 1987. Cold storage of tissue cultures. *In* Cell and tissue culture in forestry. Vol. 2. Specific principles and methods: growth and developments. *Edited by* J.M. Bonga and D.J. Durzan. Martinus Nijhoff Publishers, Dordrecht, the Netherlands. pp. 285–304.

Aitken-Christie, J., Singh, A.P., and Davies, H. 1988. Multiplication of meristematic tissue: a new tissue culture system for radiate pine. *In* Genetic manipulation of woody plants. *Edited by* J.W. Hanover and D.E. Keathley. Plenum Press, New York and London. pp. 413–432.

Aronen, T., Hohtola, A., Laukkanen, H., and Häggman, H. 1995. Seasonal changes in the transient expression of a 35S CaMV-GUS gene construct introduced into scots pine buds. Tree Physiol. **15**: 65–70. doi:10.1093/treephys/15.1.65.

Beaulieu, J., and Bernier-Cardou, M. 2006. Comparison of early height growth between white spruce seedlings and rooted cuttings. Can. J. For. Res. **36**(12): 3246–3250. doi:10.1139/x06-170.

Bentzer, B.G. 1993. Strategies for clonal forestry with Norway spruce. *In* Clonal forestry II, conservation and application. *Edited by* M.R. Ahuja and W.J. Libby. Springer-Verlag, Berlin. pp. 120–138.

Bishir, J., and Roberds, J.H. 1999. On numbers of clones needed for managing risks in clonal forestry. For. Genet. **6**: 149–155.

Bonga, J.M. 2012. Recalcitrance in the *in vitro* propagation of trees. *In* Proceedings of the IUFRO Working Party 2.09.02 conference on "Integrating vegetative propagation, biotechnologies and genetic improvement for tree production and sustainable forest management", Brno, Czech Republic, 25–28 June 2012[online]. *Edited by* Y.-S. Park and J.M. Bonga. Available from http://www.iufro20902.org/ [accessed October 2014]. pp. 37–46.

Bonga, J.M., Klimaszewska, K.K., and von Aderkas, P. 2010. Recalcitrance in clonal propagation, in particular of conifers. Plant Cell Tissue Organ Cult. **100**: 241–254. doi:10.1007/s11240-009-9647-2.

Celestino, C., Carneros, E., Ruiz-Galea, M., Alonso-Blázquez, N., Alegre, J., and Toribio, M. 2013. Cloning stone pine (*Pinus pinea* L.) by somatic embryogenesis. *In* Options Méditerranéennes, A, no 105, Mediterranean Stone Pine for Agroforestry. *Edited by* S. Mutke, M. Piqué, and R. Calama. International Centre for Advanced Mediterranean Agronomic Studies, Zaragoza, Spain. pp. 89–96.

Cherfas, J. 1985. When is a tree more than a tree? New Sci. **106**: 42–45.

Chhatre, V.E., Byram, T.D., Neale, D.B., Wegrzyn, J.L., and Krutovsky, K.V. 2013. Genetic structure and association mapping of adaptive and selective traits in the east Texas pine (*Pinus taeda* L.) breeding populations. Tree Genet. Genomes, **9**: 1161–1178. doi:10.1007/s11295-013-0624-x.

Cyr, D.R., and Klimaszewska, K. 2002. Conifer somatic embryogenesis: II. Applications. Dendrobiology, **48**: 41–49.

Engelmann, F. 2011. Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cell. Dev. Biol. Plant, **47**: 5–16. doi:10.1007/s11627-010-9327-2.

Find, J.I., Hargreaves, C.L., and Reeves, C.B. 2014. Progress towards initiation of somatic embryogenesis from different tissues of radiata pine (*Pinus radiata* D. Don) using cotyledonary embryos. In Vitro Cell. Dev. Biol. Plant, **50**: 190–198. doi:10.1007/s11627-013-9581-1.

Foster, G.S., Lambeth, C.C., and Greenwood, M.S. 1987. Growth of loblolly pine rooted cuttings compared with seedlings. Can. J. For. Res. **17**(2): 157–164. doi:10.1139/x87-028.

Frampton, L.J., and Foster, G.S. 1993. Field testing vegetative propagules. *In* Clonal forestry I: genetics and biotechnology. *Edited by* M.R. Ahuja and W.J. Libby. Springer-Verlag, Berlin. pp. 110–134.

Gill, D.E., Chao, L., Perkins, S.L., and Wolf, J.B. 1995. Genetic mosaicism in plants and clonal animals. Annu. Rev. Ecol. Syst. **26**: 423–444. doi:10.1146/annurev.es.26.110195.002231.

Hargreaves, C.L., Grace, L., van der Maas, S., Reeves, C., Holden, G., Menzies, M., Kumar, S., and Foggo, M. 2004. Cryopreservation of *Pinus radiata* zygotic embryo cotyledons: effect of storage duration on adventitious shoot formation and plant growth after 2 years in the field. Can. J. For. Res. **34**(3): 600–608. doi:10.1139/x03-226.

Hargreaves, C.L., Grace, L.J., van der Maas, S.A., Menzies, M.I., Kumar, S., Holden, D.G., Foggo, M.N., Low, C.B., and Dible, M.J. 2005. Comparative in vitro and early nursery performance of adventitious shoots from cryopreserved cotyledons and axillary shoots from epicotyls of the same zygotic embryo of control-pollinated *Pinus radiata*. Can. J. For. Res. **35**(11): 2629–2641. doi:10.1139/x05-178.

Hargreaves, C.L., Reeves, C.B., Find, J.I., Gough, K., Menzies, M.I., Low, C.B., and Mullin, T. 2011. Overcoming the challenges of family and genotype representation and early cell line proliferation in somatic embryogenesis from control-pollinated seeds of *Pinus radiata*. N. Z. J. For. Res. **41**: 97–114.

Högberg, K.A., Bozhkov, P.V., and von Arnold, S. 2003. Early selection improves clonal performance and reduces intraclonal variation of Norway spruce plants propagated by somatic embryogenesis. Tree Physiol. **23**: 211–216. doi:10.1093/treephys/23.3.211.

Ho, R.H. 1987. Embryo Culture. *In* Cell and tissue culture in forestry. Vol. 2. Specific principles and methods: growth and developments. *Edited by* J.M. Bonga and D.J. Durzan. Martinus Nijhoff Publishers, Dordrecht, the Netherlands. pp. 137–167.

Hodgetts, R.B., Aleksiuk, M.A., Brown, A., Clarke, C., Macdonald, E., Nadeem, S., and Khasa, D. 2001. Development of microsatellite markers for white spruce (*Picea glauca*) and related species. Theor. Appl. Genet. **102**: 1252–1258. doi:10.1007/s00122-001-0546-0.

Isik, F. 2014. Genomic selection in tree breeding: the concept and an outlook to the future. New For. **45**: 379–401. doi:10.1007/s11056-014-9422-z.

Klimaszewska, K., Trontin, J.-F., Becwar, M.R., DeVillard, C., Park, Y.-S., and Lelu-Walter, M.-A. 2007. Recent progress in somatic embryogenesis of four *Pinus* spp. Tree For. Sci. Biotechnol. **1**: 11–25.

Klimaszewska, K., Pelletier, G., Overton, C., Stewart, D., and Rutledge, R.G. 2010. Hormonally regulated overexpression of *Arabidopsis WUS* and conifer *LEC1* (*CHAP3A*) in transgenic white spruce: implications for somatic embryo development and somatic seedling growth. Plant Cell Rep. **29**: 723–734. doi:10.1007/s00299-010-0859-z.

Lelu-Walter, M.A., Thompson, D., Harvengt, L., Sanchez, L., Toribio, M., and Pâques, L.E. 2013. Somatic embryogenesis in forestry with a focus on Europe: state-of-the-art, benefits, challenges and future direction. Tree Genet. Genomes, **9**: 883–899. doi:10.1007/s11295-013-0620-1.

Libby, W.J., and Ahuja, M.R. 1993. Clonal forestry. *In* Clonal forestry II, conservation and application. *Edited by* M.R. Ahuja and W.J. Libby. Springer-Verlag, Berlin. pp. 1–8.

Mason, W.L., Menzies, M.I., and Biggin, P. 2002. A comparison of hedging and repeated cutting cycles for propagating clones of Sitka spruce. Forestry, **75**: 149–162. doi:10.1093/forestry/75.2.149.

Menzies, M.I., Dibley, M.J., Faulds, T., Aimers-Halliday, J., and Holden, D.G. 2000. Morphological markers of physiological age for *Pinus radiata*. N. Z. J. For. Sci. **30**: 359–364.

Mikola, J. 2009. Successes and failures in forest tree cutting production in Finland. Working Papers of the Finnish Forest Research Institute 114. pp. 39–43.

Millar, C.I. 1993. Conservation of germplasm in forest trees. *In* Clonal forestry II, conservation and application. *Edited by* M.R. Ahuja and W.J. Libby. Springer-Verlag, Berlin. pp. 42–65.

Miller, L.K., and DeBell, J. 2013. Current seed orchard techniques and innovations. *In* National proceedings: forest and conservation nursery associations — 2012. USDA Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado, Proceedings RMRS-P-69. pp. 80–86.

Mitchell, R.G., Zwolinski, J., and Jones, N.B. 2004. A review on the effects of donor maturation on rooting and field performance of conifer cuttings. South. Afric. For. J. **201**: 53–63. doi:10.1080/20702620.2004.10431774.

Montalbán, I.A., De Diego, N., and Moncaleán, P. 2011. Testing novel cytokinins for improved in vitro adventitious shoots formation and subsequent ex vitro performance in *Pinus radiata*. Forestry, **84**: 363–373. doi:10.1093/forestry/cpr022.

Nairn, B.J. 1993. Commercial micropropagation of radiata pine. *In* Micropropagation of woody plants. *Edited by* M.R. Ahuja. Kluwer Academic Publ., Dordrecht, the Netherlands. pp. 383–394.

Ondro, W.J., Couto, L., and Betters, D.R. 1995. The status and practice of forestry in Brazil in the early 1990s. For. Chron. **71**: 106–119. doi:10.5558/tfc71106-1.

Park, Y.S. 2002. Implementation of conifer somatic embryogenesis in clonal forestry: technical requirements and deployment considerations. Ann. For. Sci. **59**: 651–656. doi:10.1051/forest:2002051.

Payamnour, V., Hashemi, S., Mofidabadi, A.J., and Ahmadi, A. 2013. Inter-specific and inter-generic hybridization in Salicaceae (*Populus nigra* × *P. deltoids* and *P. nigra* × *Salix alba*) using an embryo rescue technique. Agric. For. **59**: 59–65.

Pijut, P.M., Lawson, S.S., and Michler, C.H. 2011. Biotechnological efforts for preserving and enhancing temperate hardwood tree biodiversity, health and productivity. In Vitro Cell. Dev. Biol. Plant, **47**: 123–147. doi:10.1007/s11627-010-9332-5.

Ritchie, G.A. 1997. Operational use of vegetative propagation in forestry: world overview of cloning and bulking. *In* National proceedings: forest and conservation nursery associations — 1996. USDA, Forest Service, Pacific Northwest Research Station, Portland, Oregon, Gen. Tech. Rep. PNW-GTR-389. pp. 192–196.

Ritchie, G.A., Duke, S.D., and Timmis, R. 1994. Maturation in Douglas-fir: II. Maturation characteristics of genetically matched Douglas-fir seedlings, rooted cuttings and tissue culture plantlets during and after 5 years of field growth. Tree Physiol. **14**: 1261–1275. doi:10.1093/treephys/14.11.1261.

Ritland, K., Krutovsky, K.V., Tsumara, Y., Pelgas, B., Isabel, N., and Bousquet, J. 2011. Genetic mapping of conifers. *In* Genetics, genomics and breeding of conifers. *Edited by* C. Plomion, J. Bousquet, and C. Kole. CRC Press, Boca Raton, Florida. pp. 196–238.

Rohr, R. 2004. State of development of technology for haploid production from gymnosperm microgametophyte and megagametophytes culture. Propag. Ornam. Plants, **4**: 3–18.

Ruotsalainen, S. 2014. Increased forest production through forest tree breeding. Scand. J. For. Res. **29**: 333–344. doi:10.1080/02827581.2014.926100.

Russell, J.H. 1993. Clonal forestry with yellow-cedar. *In* Clonal forestry II, conservation and application. *Edited by* M.R. Ahuja and W.J. Libby. Springer-Verlag, Berlin. pp. 188–201.

Sederoff, R. 2013. Genomics: a spruce sequence. Nature, **497**: 569–570. doi:10.1038/nature12250.

Serrano-Martinez, F., and Casas, J.L. 2011. Cryopreservation of *Tetraclinis articulate* (Vahl.) Masters. Cryo Letters, **32**: 248–255.

Sommer, H.E., Brown, C.L., and Kormanik, P.P. 1975. Differentiation of plantlets in longleaf pine (*Pinus palustris* Mill.) tissue cultured in vitro. Bot. Gaz. **136**: 196–200. doi:10.1086/336802.

Sorensson, C. 2006. Varietal pines boom in the US south. N. Z. J. For. **51**: 34–40.

Stelzer, H.E., Foster, G.S., Shaw, V., and McRae, J.B. 1998. Ten-year growth comparison between rooted cuttings and seedlings of loblolly pine. Can. J. For. Res. **28**(1): 69–73. doi:10.1139/x97-193.

Sweet, G.B. 1973. The effect of maturation on the growth and form of vegetative propagules of radiata pine. N. Z. J. For. Sci. **3**: 191–210.

Sweet, G.B., and Thulin, I.J. 1973. Graft incompatibility in radiata pine in New Zealand. N. Z. J. For. Sci. **3**: 82–90.

Thulin, I.J., and Faulds, T. 1968. The use of cuttings in the breeding and afforestation of *Pinus radiata*. N. Z. J. For. **13**: 66–77.

Trontin, J.-F., Harvengt, L., Garin, E., Lopez-Vernaza, M., Arancio, L., Hoebeke, J., Canlet, F., and Pâques, M. 2002. Towards genetic engineering of maritime pine (*Pinus pinaster* Ait.). Ann. For. Sci. **59**: 687–697. doi:10.1051/forest:2002057.

Wendling, I., Trueman, S.J., and Xavier, A. 2014. Maturation and related aspects in clonal forestry — part II: reinvigoration, rejuvenation and juvenility maintenance. New For. **45**: 437–486. doi:10.1007/s11056-014-9415-y.

Weng, Y.H., Park, Y.S., Krasowski, M.J., Tosh, K.J., and Adams, G. 2008. Partitioning of genetic variance and selection efficiency for alternative vegetative deployment strategies for white spruce in eastern Canada. Tree Genet. Genomes, **4**: 809–819. doi:10.1007/s11295-008-0154-0.

Weng, Y., Park, Y.S., and Krasowski, M.J. 2010. Managing genetic gain and diversity in clonal deployment of white spruce in New Brunswick, Canada. Tree Genet. Genomes, **6**: 367–376. doi:10.1007/s11295-009-0255-4.

Yanchuck, A.D., Bishir, J., Russell, J.H., and Polsson, K.R. 2006. Variation in volume production through clonal deployment: results from a simulation model to minimize risk for both a currently known and unknown future pest. Silvae Genet. **55**: 25–37.

Yong, E. 2012. Tree's leaves genetically different from its roots [online]. Nature News, 10 August 2012. Available from http://www.nature.com/news/tree-s-leaves-genetically-different-from-its-roots-1.11156 [accessed October 2014].

Zobel, B. 1981. Vegetative propagation in forest management operations. *In* Proceedings of the 16th Southern Forest Tree Improvement Conference, Blacksburg, Virginia. pp. 149–159.