A hybrid tissue culture protocol that combines conifer somatic embryogenesis with organogenesis as an alternative propagation platform for specialist applications

Cathie Reeves, Cathy Hargreaves, Marie-Anne Lelu-Walter, Jean-François Trontin, Paloma Moncaleán, Itziar Aurora Montalbán

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Reeves C [1*]., Hargreaves C [1]., Lelu-Walter M-A[2], Trontin J-F[3], Moncaleán P[4], Montalbán I[4]

[1] Scion, Private Bag 3020, Rotorua, New Zealand, catherine.reeves@scionresearch.com
[2] INRA, UR 0588 Unité Amélioration, Génétique et Physiologie Forestières, 2163 Avenue de la Pomme de Pin, CS 4001, Ardon, F-45075 Orléans Cedex 2, France.
[3] FCBA, Pôle Biotechnologie et Sylviculture Avancée, Equipe Génétique et Biotechnologie, Campus Forêt-Bois de Pierroton, 71 route d'Arcachon, F-33610 Cestas, France.
[4]Neiker-Tecnalia, Instituto Vasco de Investigación y Sesarrollo Agrario. Campus Agroalimentario de Arkaute, Apdo 46, 01080 Vitoria-Gasteiz, Spain.

Current somatic embryogenesis (SE) protocols for many pine species remain suboptimal for the purpose of reforestation. Some of the bottlenecks include losses of lines through the SE process due to lack of continued proliferation, poor embryo maturation or poor conversion of somatic embryos to *ex vitro* conditions. There is often significant variation among genotypes with respect to productivity and SE protocol improvements do not always benefit all genotypes.

There is often the desire to propagate specific cell lines, for example from top-ranked families. Selected lines may show desirable characteristics such as disease resistance or a high ability to be genetically engineered. There is also a need to propagate cell lines from species that are in general recalcitrant to somatic embryogenesis (e.g. *Pinus densiflora*). Cell lines from hybrid crosses, where there can be incompatibility issues between parent species, also present several challenges. In particular there are no protocols available for these new hybrid crosses which are often less responsive to all steps in the SE process.

An example of this recalcitrance could be a cell line which is highly transformable but only produces a limited number of good quality mature somatic embryos. This makes direct planting of somatic embryos to *ex vitro* conditions an unviable option. If the desired cell line will not respond positively to modification of the standard SE protocols or media for the species then the options for bulking up this material are limited. A hybrid tissue culture system combining germinated somatic embryos with organogenesis allows the flexibility to work with these desirable cell lines and allows the best aspects of the component propagation methods to be exploited.

In conclusion, we have a propagation protocol that takes advantage of the benefits of SE, which include cryopreservation to ensure juvenility while field testing takes place and the potential for genetic engineering. These features combined with organogenesis, which provides the advantages of increased early multiplication, uniformity of shoots and high rooting and conversion to planting stock/stoolbeds is a winning strategy for some conifer species.

This talk will highlight where Scion has used this hybrid system to circumvent issues around certain protocol stages, cell lines and conifer species.

Keywords: Somatic Embryogenesis, organogenesis, conversion, recalcitrance, hybrid protocol.