Working Papers of the Finnish Forest Research Institute 114: 64–67

Production of Norway spruce somatic embryos − a practical point of view

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Somatic embryogenesis is a sequence of operations with technical, biological and logistic aspects. The technical challenges are encountered mainly in the automation of the processes, most important the steps where somatic embryos and plantlets/plants are handled one-by-one, i.e. partial desiccation, germination and acclimation. Biologically, the maturation-desiccation-germination sequence is a very critical issue, and lack of root formation seems to be a major problem. A protocol that produces somatic embryos with high quality and a high germination rate from many cell lines is desirable but not so easy to achieve. Sorting procedures will probably be necessary, of embryos before germination and of plantlets after germination. Furthermore, cryopreservation is experienced as a bottleneck and methods for preserving more tissue samples per time unit is wanted.

Keywords: automation, plant production, somatic embryogenesis

Background

Somatic embryogenesis is a vegetative propagation method with attractive features and during the two decades that has passed since the process was described the first time for Norway spruce a lot of research and development have been made. The two most important advantages of somatic embryogenesis is the cryopreservation option and the rapid multiplication of genotypes.

In principal, there are two propagation alternatives at hand when applying vegetative propagation: 1. propagation of many genotypes and few plants per genotype, typically for clonal testing, 2. propagation of few genotypes and many plants per genotype, typically for mass propagation of tested clones. Bulk propagation, i.e. propagation of untested clones, takes a position in between but is closer to alternative 2 than alternative 1. The two alternatives provides to some extent different requirements for the practical propagation work.

Procedure bottlenecks and their nature

The very first activity in a somatic embryogenesis propagation is excision of the zygotic embryo. If the protocol involves immature embryos the excision is preceded by dissection of cones. Regardless of the age of the zygotic embryos, it is hard to see any automation of this work. This position holds also for the next step, initiation, when the excised embryos are put on initiation medium. But the next step, proliferation of successfully initiated cell lines, can be performed in vessels with liquid media, s.c. bioreactors, that already are in use by e.g. CellFor. Proliferation is the growth phase of the propagation, and a rapid growing culture grows exponentially, which means that the workload increases dramatically in late stages of the propagation. Using bioreactors thus reduces the manpower needed for propagating big amounts of tissue.

To automate maturation is more difficult but not impossible, e.g. can tissue be maturated on membranes on liquid maturation media. However, when somatic embryos have matured the propagation moves into handling embryos one-by-one, in partial desiccation and germination. This is where automation is most beneficial as it reduces time-consuming manual labour, but it is also a big challenge. The key is how to find a technique that speeds up the handling without taking risks in damaging the small embryos. The idea of an artificial seed shell to protect the embryo and making it easier to handle has been proposed but so far no product has entered the market. Unprotected embryos can be handled by properly designed robots but also in this case no commercial product has been presented. Another aspect of the maturation-desiccation-germination steps is that there is a need of sorting. Either sorting of the mature somatic embryos to get a high probability of each embryo to produce a plant, or sorting after germination combined with transplanting of germinating plants. Root formation during germination appears to be an important issue in this context.

Finally, automatic handling would be beneficial for cryopreservation as well. However, a shortening of the time for preparation of samples that will go into storage would also be an important step forward.

Generally, propagation for clonal testing is more tolerant to manual operations and the resulting higher costs, as they will be diluted by a high number of plants per clone once the clone is selected. Mass propagation of selected clones will however be dependent upon an automatic procedure that can bring the costs down to reasonable levels. Similarly, propagation of a bulk material needs automatic handling.

Multiplication and deployment aspects

One restriction of somatic embryogenesis is that genotypes react differently with different protocols. To maximise the number of cell lines that will be produced in a propagation event, several protocols should be used. But for practical reasons normally only one standard protocol is followed. This results in very skewed distributions of clones per family and plants per clone (Fig. 1). The number of rooted cuttings varies considerably among clones and often a minority of the propagated clones contribute with a majority of the produced plants.

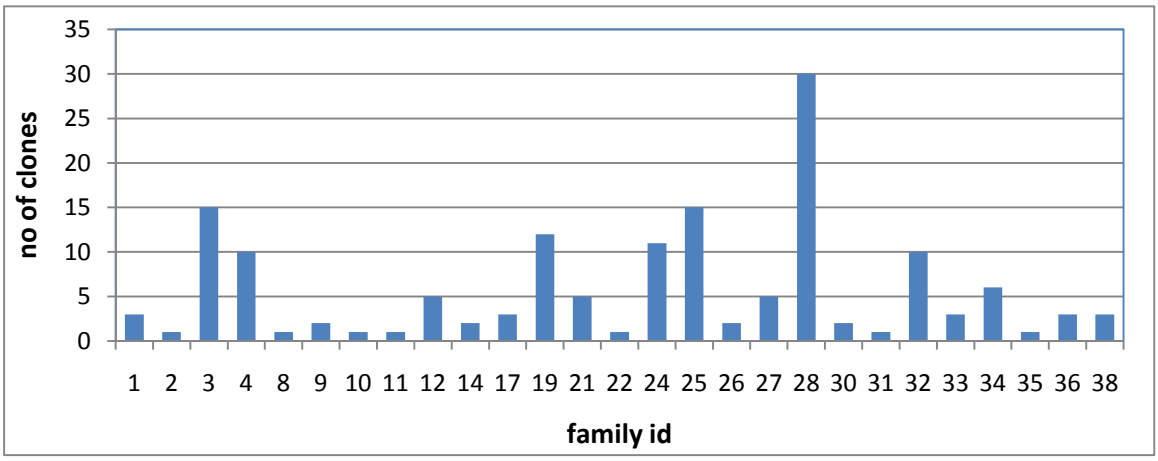

Fig. 1. Example of uneven distribution of clones among families.

Selection of superior clones after field testing is based on traits like growth, wood quality etc. in some kind of selection index. With somatic embryogenesis, a propagation trait will be added to the selection. Practically, this means that propagation ability will be a part of the index. Clones that are difficult to propagate in high numbers will not be selected even if the field performance is superior. Including one more trait will thus mean that more clones (zygotic embryos) needs to be included in the initiation to achieve the same genetic gain.

The other option of deployment, bulk propagation of superior families where a set of untested clones from selected and tested families is mass propagated, must also consider the restrictions mentioned above. Normally a larger number of cell lines is necessary to reach a high probability that the mean of propagated clones corresponds to the mean that can be calculated from the predicted family values. If the family response to somatic embryogenesis is unknown the insurance is, once again, to include more families.

Where will the breakthrough come?

Undoubtedly, the one-by-one handling of embryos and germinants is the most important issue in somatic embryogenesis development (Fig. 2).

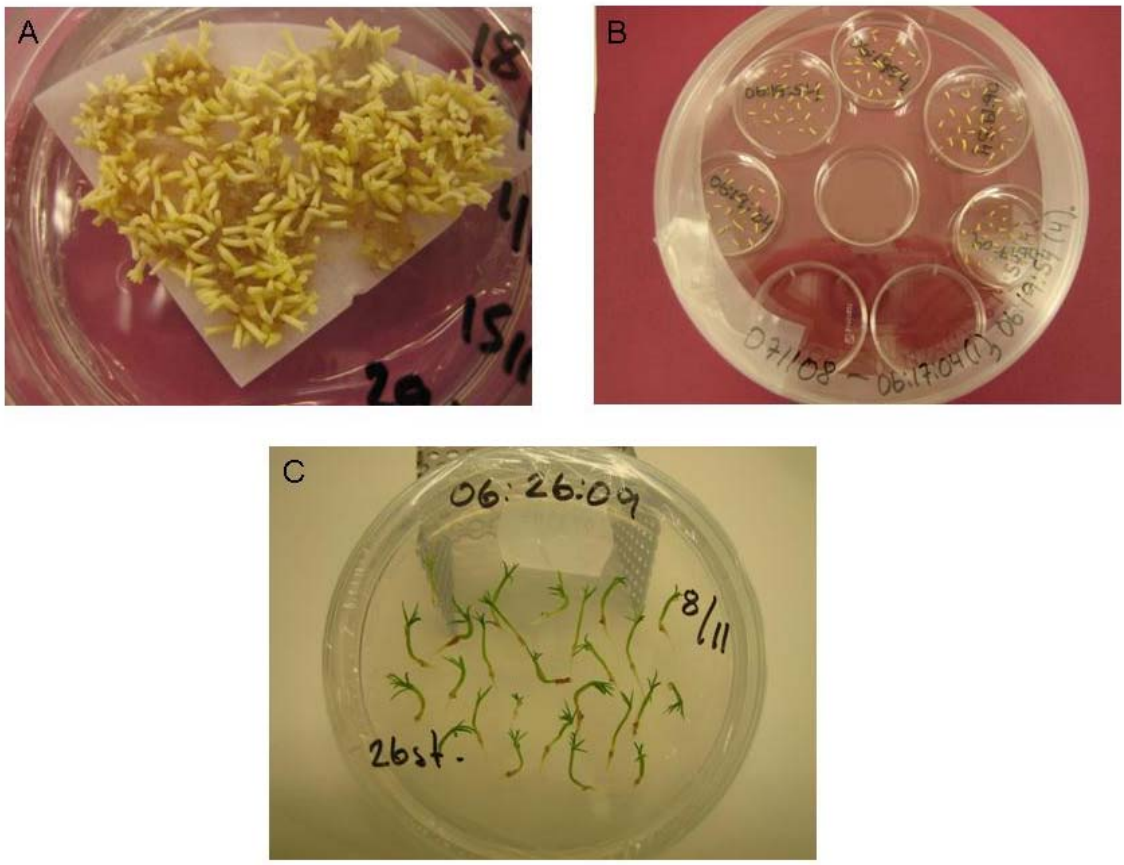

Fig. 2. Maturation **(A)**, partial desiccation **(B)** and germination **(C).** The stages in somatic embryogenesis where automation would be the breakthrough for large scale application of somatic embryogenesis. (Photos: Karl-Anders Högberg)

It is hard to see wide-spread large-scale applications without solution of this problem. Other important improvements may come as the biological understanding increases, like an improved standard protocol with higher probability of proper germination. More efficient cryopreservation procedures are also important to develop, but the crucial point is the automation of maturation-desiccation-germination stage and it is here we can expect the breakthrough in somatic embryogenesis to come.