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Integration of biotechnology, robot technology and visualisation technology for development of methods for automated mass production of elite trees

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Clonal propagation of elite trees by somatic embryogenesis can shorten periods needed for breeding of trees, and can ensure a stable production of high quality plants for the forestry sector. It will furthermore allow for relative fast market oriented breeding and the production of trees 'fit for purpose' and guarantee a consumer oriented and tailor made wood supply. However, commercial application of the technology has until now been hampered by two essential problems: 1) the production costs per plant must be reduced, 2) improved methods must be developed for transfer and acclimatisation of plants from sterile *in vitro* conditions to non sterile (*ex vitro*/*in vivo*) conditions in the nursery.
To solve these problems, a Danish based project has been established to combine clonal propagation by somatic embryogenesis (SE) with biotechnological breeding tools, and with robot – and visualisation technologies. The present project takes advantage of effective methods developed at the University of Copenhagen for SE in nordmanns fir and sitka spruce. These methods are used as a model system for development of biotechnological breeding tools in combination with automated plant production of plants for the forestry industry.

1 Introduction

Biotechnology has become an integrated part of plant breeding, and in recent years new methods have been developed for breeding and propagation of important plants in the agricultural-, ornamental- and forestry sector.
One of the promising methods is somatic embryogenesis (SE), where plants are produced from single cells without sexual reproduction. SE has some particular advantages for the development of cost effective methods for clonal mass propagation of elite plants:

- It is a very effective and fast method for clonal propagation.
- The method is suitable for automatisation and robot technology.
- The method is, for several plant species, the preferred basis for development of additional biotechnological breeding technologies as e.g. genetic transformation.
- Elite clones can be stored over extended periods in liquid nitrogen at -196°C

However, commercial application of the technology has until now been hampered by two essential problems:

- The production costs per plant must be reduced. Labour costs are low in the early steps of the process whereas they increase dramatically during the later stages (Fig 1).

- Improved methods must be developed for transfer and acclimatisation of plants from sterile *in vitro* conditions to non sterile (*ex vitro*/*in vivo*) conditions in the nursery.

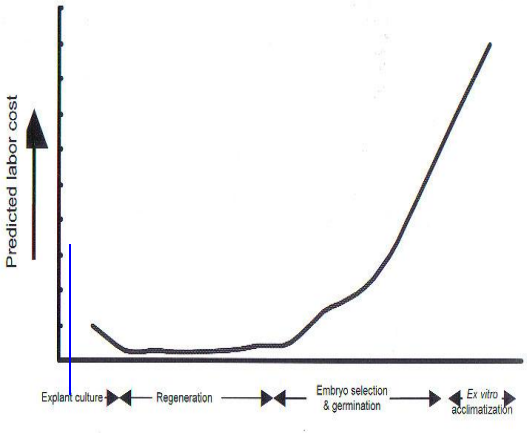

Fig. 1. Predicted labour cost by clonal propagation through somatic embryogenesis.
The labour costs are very low in the early steps of the production, whereas they increase dramatically during the later stages: 'embryo selection & germination' and 'ex-vitro acclimatization'). The aim of the present project is to reduce labour costs associated with the late stages of the production of cloned plants through development of robot- and visualisation technologies. (From Afreen & Zobayed, p. 96, 2005)

2 Model species

This study is based on effective methods for SE in nordmanns fir (*Abies nordmanniana*) and sitka spruce (*Picea sitchensis*) developed at the University of Copenhagen. The two species is of special interest for Danish forestry:

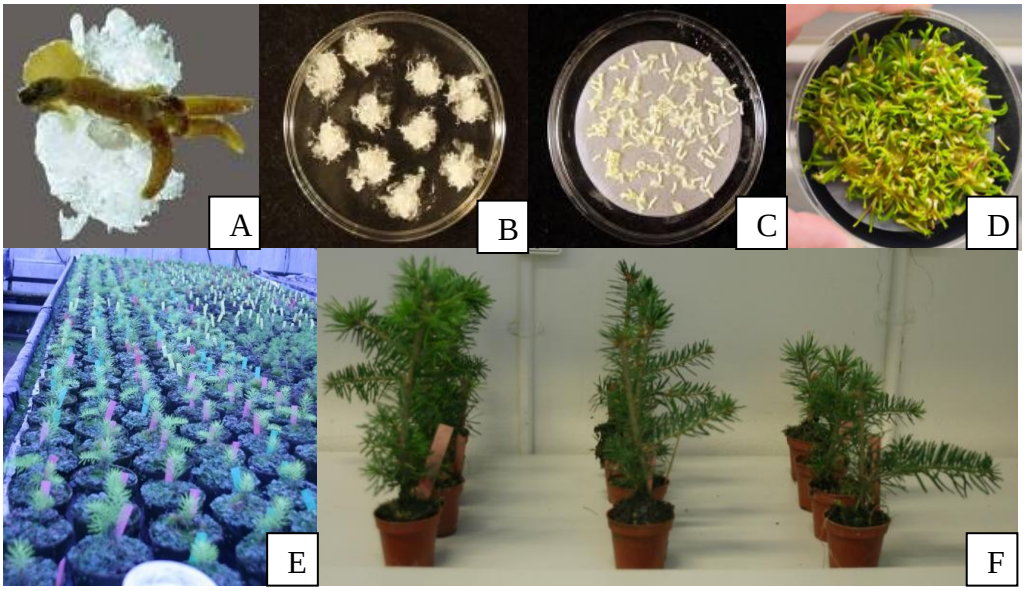

Fig. 2: Clonal propagation of conifers by somatic embryogenesis (Nordmanns fir):

A: *Embryogenic cultures are initiated from zygotic embryos, which are produced from controlled crossings of elite material.* ***B:*** *The embryogenic culture. The somatic embryos proliferate continuously and one Petri dish contains several thousand somatic embryos. The number of embryos doubles in approx. two weeks. At this stage it is possible to store the cultures over extended periods in liquid nitrogen (-196°C) and/or to ad new traits by gene technology.* ***C:*** *Mature somatic embryos are identical to mature embryos from seeds.* ***D:*** *Germinated embryos* ***E:*** *Plants 12 month after transfer to soil* ***F:*** *Three different clones of nordmanns fir, phenotypically distinguishable after 2 years in soil.*

Nordmanns fir for the production of Christmas trees.
Forest trees are almost exclusively produced from seeds. For Nordmanns fir the seeds are collected in the natural forests in the Caucasus region in Georgia or from Danish seed orchards. The quality of the imported seeds are often unpredictable, and the production of high quality seeds are unstable from both Danish and foreign sources.

Nordmanns fir has a generation time of 25-30 years, and traditional breeding programmes is for this reason extremely time consuming. The extended generation period is a general problem in breeding programmes for forest trees, but it is particularly a problem in specialised industries that is dependent on fast breeding and development of new products. For this reason, the production of Christmas trees is hampered by plants with unpredictable genetic traits. The result is that only 15 % of the produced trees are of best quality. Approx. 60 % of the trees are of average or lower quality, and approx. 25 % of the produced trees in a stand are discarded because they are not suited for sale (Fig 3).

Sales price (D.kr.) for Christmass trees by use of clonal propagation

Category	%-distribution		Price per tree	Sales price per 100 trees	
	Seedlings	Cloned		Seedlings	Cloned
Superior	15	60	90	1350	5400
Standard	40	20	55	2200	1100
Class III	20	5	30	600	150
Discharged on form	20	10	0	0	0
Discharged on damage	5	5	0	0	0
Total	**100**	**100**		**4150**	**6650**

Average gain per tree by cloned material is approx. 25 D.kr. (approx. 3 €)

Fig. 3. Nordmanns fir. *An example of economical gain from integration of cloning techniques in breeding programmes of forest trees. Distribution of trees in categories of quality and estimated sales price for 100 trees for plants produced from seedlings or by clonal propagation of elite material.*

Clonal propagation of elite material will ensure that the proportion of high quality trees increases and that the average price per produce tree is improved (Fig 3). Therefore there is considerable interest in development of methods for clonal mass propagation of elite trees. Traditional methods such as propagation by cuttings have been difficult for nordmanns fir by poor rooting and plagiotropic growth. At present, SE is therefore the most promising method for enhancing gains from tree breeding programmes and for bulk propagation of elite trees.

Sitka spruce for production of biofuels.
Sitka spruce plantations is characterized by a low input forestry as regards management, harvest operations, application of herbicides, pesticides and fertilizers and the wood of Sitka spruce is of higher density compared to species as willow and poplar. Furthermore, emission of NOX , HCL, and dioxin from wood combustion is low compared to straw, cereals and grasses (Obernberger et al. 2006). Incentives are therefore high for optimizing the production of wood for combustion and ethanol production.

At present the wood from Sitka spruce is mainly valuable for combustion since the amount and composition of lignin of conifers is more difficult to separate from the cellulose compared to other genera (e.g. Saddler et al. 2006). Nevertheless, ongoing research (e.g. Xue Jun et al. 2005, Saddler et al. 2006) may make it economically feasible to make ethanol from conifers in the future.

Vegetative propagation of elite trees identified for bio fuel production will improve the output significantly. Especially when production is based on few clones as shown in a clonal field trial with 23-year-old Sitka spruce where the production of dry matter for one of the clones was twice the dry matter production of a standard Sitka spruce stand (Costa e Silva et al., 1994).

The results from the Danish breeding program (Roulund 1990, Costa e Silva et al. 1994, Hansen & Roulund 2001) can be combined with efficient biotechnological based methods for clonal mass propagation by SE (Fig.4) (Krogstrup et al. 1988, Find et al 1993, Kristensen et al. 1994). Sitka spruce has been emphasized by OECD as a 'target species' for implementation of biotechnology in breeding programs for forest trees (OECD 2002).

Fig 4. Sitka spruce field trial with trees produced by somatic embryogenesis. Established in 1991. (Photo Michel Kristensen, 2003)

3 Integration of biotechnology and robot technology in SE

3.1 Robots – visualisation – automatisation

The robot technology is integrated in two particularly labour consuming processes:

A) Isolation, selection and handling of mature somatic embryos (Fig. 5)

- Development of visualisation and handling technology for quality assessment, sorting and orientation of 3-5 mm long mature embryos.
- Development of technologies for transfer of embryos to germination medium.

Fig 4. Prototype of robot used in the project.

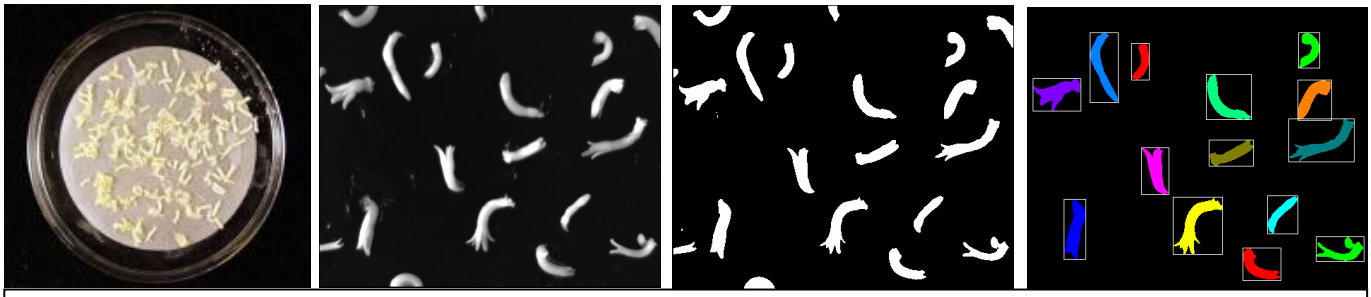

Fig. 5. Isolation and visualisation for quality assessment, sorting and orientation of 3-5 mm long mature embryos. *The automated quality assessment is based on digitalized photos of mature embryos. Physical isolation of each mature embryo is required for visualisation and automated handling because embryos that are situated too close will be perceived as one structure by the software. The software can then distinguish different classes of embryos and can find the correct orientation, in respect to root and top. The software is trained to focus on classes of embryos that have been identified as high quality by an experienced technician. The first prototype can handle one embryo every 4 seconds.*

B) Identification and transfer of germinated plants to soil (Fig 6)

- Development of visualisation technology for identification of germinated plants with root.
- Development of handling technologies for transfer of plants from sterile growth medium to non sterile growth plugs.

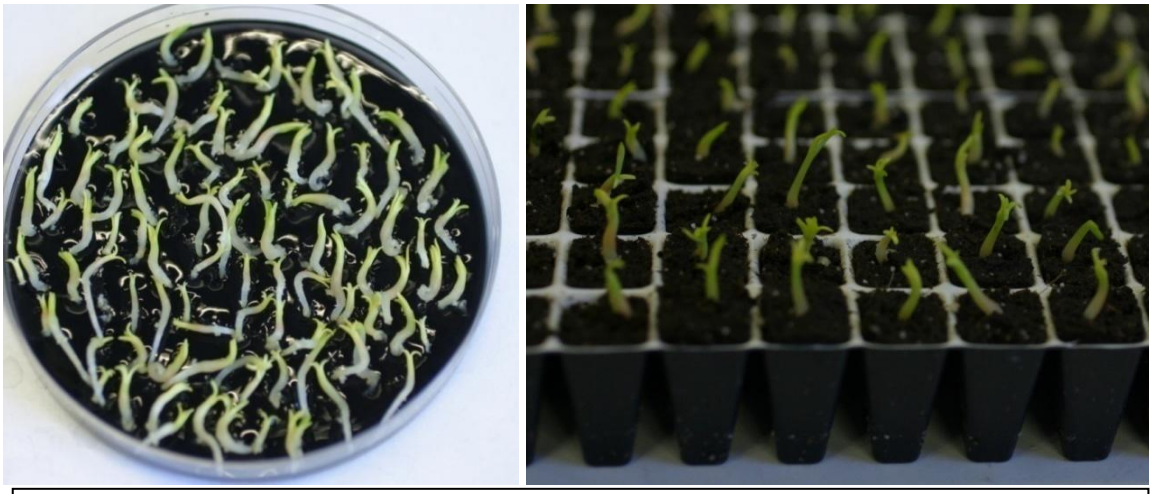

Fig. 6. Transfer of rooted embryos to growth plugs. *The second step in the automated process is the transfer of rooted embryos from germination medium into growth plugs. The software identifies orientation and a grip point based on recognition of hypocotyle and root, and a handling device has been developed, which can handle the one plant every 4 seconds.*

4 Conclusion

It has been possible to develop a prototype for automated handling of the two described processes in Somatic embryogenesis. From a biological point of view, the most challenging point has been to develop the biological methods, so that they are suited for automated processing, by e.g. development of methods that ensures synchronised plant development during the different steps of somatic embryogenesis.

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