Advances on somatic embryogenesis in forest trees

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▶ **To cite this version:**

Marie-Anne Lelu-Walter, Daniel Cornu. Advances on somatic embryogenesis in forest trees. Cost Eurosilva Working Group I : Growth and Development. Workshop "Advances on somatic embryogenesis in forest trees", Dec 1998, Orléans, France. 1998. hal-02840964

HAL Id: hal-02840964
https://hal.inrae.fr/hal-02840964v1

Submitted on 7 Jun 2020

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M.A. LELU

COST " EUROSILVA " Working Group I : Growth and Development

Workshop :

" Advances on somatic embryogenesis in forest trees "

December 3rd-4th, 1998
in Orléans, France

Organising committee

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Time Table

Wednesday, December 2.

6: 00 PM- 21: 00 PM — Registration Hôtel Ibis, Orléans

Thursday, December 3.

8: 00 AM Registration — Hôtel Ibis, Orléans

8: 30 AM Welcome : MA Lelu

Introduction : **Björn Sundberg** : Chairman of the Cost-Eurosilva Working Group I

Jean-Charles Bastien : Director of INRA Station of Orléans

I. Physiological aspects of somatic embryogenesis in forest trees

I1. Induction of somatic embryogenesis :

9: 00 AM **Helly Häggman** : Factors affecting the induction of somatic embryogenesis in pine

9: 20 **Marc Pâques** : Somatic embryogenesis from old conifers : a way to speed up the best clones availability for fibre-culture.

9: 40 Maria do Rosario Alvelos : induction of somatic embryogenesis in *Pinus pinaster*.

9: 45 Discussion Part I1.

10: 05 COFFEE

I2. Proteins, markers of somatic embryogenesis :

10: 35 **Hélène David** : Germin-like proteins are apoplastic markers of somatic and zygotic embryos of pine.

10: 55 **Ulrika Egertsdotter** : Extracellular proteins in embryogenic suspension cultures of Norway spruce.

11: 15 Discussion Part I2.

11: 40 General Discussion (parts I1-I2)

12: 40 LUNCH

I3. Medium, hormonal analysis :

2: 00 PM **Krystyna Klimaszewska** : Manipulation of embryogenic tissue water potential during somatic embryo maturation in conifers using *Pinus strobus* as an example.

2: 20 **Philippe Label** : Plant Hormones during hybrid larch and walnut somatic embryogenesis.

2: 40 Helena Lipavska : Carbohydrate status of Norway spruce somatic embryos during maturation- the effect of PEG.

2: 45 Martin Vagner : Endogenous phytohormones during Norway spruce somatic embryo development.

2: 50 Karoliina Niemi : Ectomycorrhizal fungi as growth regulators in somatic embryogenesis of conifers.

2: 55 Discussion part I3.

3: 30 BREAK

I4. Informations from zygotic embryogenesis:

4: 00 **Inger Hakman** : Aquaporins in Norway spruce - their putative functions during embryo formation and plant establishment.

4: 20 **Nicole Michaux-Ferriere** : Zygotic embryogenesis as a model for somatic embryogenesis ? Some examples from tropical trees.

4: 40 Jana Albrechtova : Anatomical and histochemical studies of Norway spruce somatic embryo maturation- the effect of PEG.

4: 45 Discussion part I4.

5: 15 General Discussions (parts I3-I4)

Friday, December 4.

II Towards an integration of somatic embryogenesis in a breeding program and other applications.

II.1. Genetic transformation :

8: 30 **Josef Schmidt** : Aspect of genetic transformation in *Quercus.*

8: 50 **David Clapham** : Transformation of conifers by particle bombardment.

9: 10 **Gilles Pilate** : An overview of conifers transformation from embryogenic cultures with special emphasis on *Agrobacterium* transformation.

9: 30 Jens Find : *Abies nordmanniana*: Transformation and regeneration of transgenic plants

9: 35 Discussion part II.1

10: 00 COFFEE

II.2. Desiccation, cryopreservation :

10: 30 **Bruno Florin** : Recent advances in long-term preservation of coffee and cocoa genetic resources.

10: 50 **Marie-Anne Lelu** : desiccation and cryopreservation in conifers.

11: 10 Pawel Chmielarz : somatic embryogenesis in *Quercus robur* L.

11 : 15 Discussion part II.2

11: 35 General discussions (parts II1- II2)

12: 35 LUNCH

II.3 Conformity:

2: 00 PM **Jean-Luc Fourré** : Somaclonal variation and somatic embryogenesis in forest trees.

2: 20 **Jean-Luc Verdeil** : The mantled floral abnormality in oil palm clones : hormonal and molecular approach.

2: 40 Discussion Part II.3

3: 00 BREAK

II. 4. Clonal propagation of forest trees via somatic embryogenesis ?

3: 30 **Krystyna Szczygiel** : Role of somatic embryogenesis in conservation of genetic diversity.

3: 50 **Mike Becwar** : Clonal propagation of pines via somatic embryogenesis.

4: 10 **D. Thompson** : Are we ready to integrate somatic embryogenesis into European forestry ?

4: 30 Mariano Toribio : Bottlenecks in somatic embryogenesis in *Quercus suber*.

4 : 35 Discussion part II.4.

5: 00 General Discussions

6:30 **ADJOURN**

Factors affecting induction of somatic embryogenesis in Scots pine.

Hely M. Häggman, Tuija S. Aronen and Jana Krajnakova
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Scots pine (*Pinus sylvestris* L.) is one of the most common conifers in Nordic countries. The traditional breeding programs have been focused on the species, and during the last few years also biotechnological studies (i.e. vegetative propagation, genetic transformation and molecular biology) have been emphasized. An efficient vegetative propagation technique is necessary both for biotechnological applications as well as for more effective transfer of genetic gain achieved through tree breeding to practical forestry. In Scots pine, regeneration has succeeded through somatic embryogenesis but the method is still under development. In this presentation we have focused on factors affecting induction and proliferation of embryogenic cultures.

One-year-old immature seed cones were collected from elite clones of Scots pine growing in Punkaharju clone collection (61°48'N; 29°17'E). Embryogenic cultures were established on immature female gametophytes including immature zygotic embryos with suspensor tissues. The effect of the following factors on induction was tested: time of collection, developmental phase of the explant tissues at the time of collection, the growth regulators and gelling agent concentration in the induction media, cold-treatment before inoculations, and the response of different seed families on induction of embryogenic cultures.

The optimal stage for cone collections in southern part of Finland is usually in July, when the day degrees are between 400 and 650. Anatomical studies have shown that the immature zygotic embryos have the ability to initiate embryogenic cultures shortly after fertilization. Most of the Scots pine elite plus trees have been able to produce embryogenic cultures. Generally, the initiation frequencies have been relatively low and depending on the collection year as well as mother tree. Storing immature cones in cold room before inoculation is as good as immediate inoculation at the collection date or even increases, but not siginificantly, the number of embryogenic cell lines. For successful initiation and proliferation of embryogenic cultures auxin supplement has been necessary in the proliferation medium. The two auxin containing media DCR1 (13.6 µM 2,4-D and 2.2 µM BA) and DCR3 (9.05 µM 2,4-D and 2.2 µM BA) tested did not differ statistically from each other. Also the gelrite concentration affects the number of embryogenic cell lines but due to the low initiation frequency the difference is not significant.

Somatic embryogenesis from old conifers: a way to speed up the best clones availability for fibre-culture.

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AFOCEL, a French company founded by the wood industries, is involved in Somatic embryogenesis since 1986 in order to be able to scale up the process at an industrial level. The demonstration of the possibility to produce currently a thousand quality plants of Norway spruce has been made by AFOCEL. The plantations now older than 5 years are indicating that the somatic seedlings quality is good and that the process could be used at an industrial level for the clonal propagation of elite clones. The estimated cost of a somatic seedling ready for acclimatization and for field plantation is respectively estimated around 2.5 and 5 Francs. A substantial reduction of those costs is still expected because the possibilities of reducing the hand labor both in the lab and in the nursery.

The interest of the technology by itself is relatively low if it is used to amplify untested very juvenile genotypes. The fact that the technology can be successfully combined with the cryopreservation is interesting : it allows to maintain juvenile the embryogenic tissues as long as requested for the field trials evaluation. However the valorization of somatic embryogenesis would be much more higher if this technology is applicable to old selected genotypes

As a consequence AFOCEL is focusing from years on the possibility to recover somatic embryos from " old " selected conifers.

In order to progress in that way, the research has been focused on the possibility to recover somatic embryos from more and more older plants starting on one hand from juvenile to mature trees and on the other hand from somatic to zygotic plants.

As results from those investigations :

- we made the demonstration that it is possible to recover somatic plantlets from old conifers via somatic embryogenesis as recently described in a patent application.
- we have learned a little more on the factors involved in the control of the initiation embryos from relatively old conifers.

Some of those information's will be presented in order to stimulate the discussion on the scientific investigations required to increase the level of embryogenesis initiation rate from juvenile and mature conifers.

Germin-like proteins are apoplastic markers of somatic and zygotic embryos of pine.

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In coniferous species, preglobular somatic embryos consist of embryonic heads composed of small cells rich in cytoplasm attached to long highly vacuolated suspensorial cells. These embryos are maintained as callus-like embryogenic tissues on a 2.4-D containing medium *(Domon et al., 1994)*. We previously characterized this early stage of pine development by the presence of germin-like proteins (GLPs) ionically bound to the cell surfaces *(Domon et al. 1995)*.

Germins and GLPs are proteins that belong to a multigenic family present in numerous species *(Membré et al 1997)* and expressed in different physiological processes including development and stress tolerance. No precise function has been disclosed for them except that cereal germins express an oxalate oxidase activity *(Dumas et al., 1993; Lane et al., 1993)*.
In order to understand the involvement of GLPs in early embryo development, a cDNA library was constructed from pine preglobular somatic embryos. A 926bp full length cDNA named *PcGER1* (Genbank AF039201) encoding a predicted 220 amino acid protein precursor was isolated and further characterized. Expression of the recombinant PcGER1 in *E. coli* confirmed the relationships between PcGER1 and germins *(Neutelings et al., 1998)*.

Very recently *(Dunwell and Gane, 1998)* it was emphasized on the absolute conservation of residues involved in structure determination of several procaryotic and eucaryotic proteins including germins. The two germin conserved motifs described by the authors are found in PcGER1. We also PCR-amplified a pine genomic fragment of 852 bp named *gf*-0.8. The alignments with *PcGER1* cDNA confirmed the absence of introns in the corresponding genomic region, as it is the case for wheat germins. Southern blot analysis was performed to estimate the number of *PcGER1* genes in the Carribean pine genome which appears to possess only a single copy of *PcGER1* while another related gene might be present.

PcGER1 was highly expressed in the four embryogenic lines analysed as shown by the unique band corresponding to a 0.9 kb transcript. A strong signal was also obtained in quiescent zygotic embryos. Conversely, *PcGER1*was never expressed in nonembryogenic calli nor in haploid female megagametophytes and during germination.

In conclusion, we have cloned a GLP cDNA, *PcGER1*, from pine which is expressed specifically in embryogenic lines and in quiescent embryos. Our objectives are now to study (1) the regulation of this GLP, (2) and the biochemical properties of the recombinant glycosylated polypeptide PcGER1 produced in tobacco. Our aim is also to disclose whether the expression of GLPs is correlated with the bipolar structure of the conifer preglobular somatic embryos (small embryo head and large suspensor).

Domon et al.- Plant Physiol Biochem 32: 137-147 (1994).
Domon et al. - Plant Physiol 108: 141-148 (1995).
Dumas et al. - C R Acad Sci Paris 316: 793-798 (1993).
Dunwell J, Gane P - J Mol Evol 46: 147-154 (1998).
Lane et al. - J Biol Chem 268: 12239-12242 (1993).
Membré et al. - Plant Mol Biol 35: 459-469 (1997).
Neutelings G.et al.- Plant Mol Biol, sous presse (1998).

Extracellular proteins in embryogenic suspension cultures of Norway spruce.

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Different cell lines of Norway spruce contain somatic embryos that reach different developmental stages. The cell line-specific differences in embryo morphology can be linked to the embryos capacity to enter the maturation phase, and subsequently form normal-looking plantlets. We have divided our cell lines into two groups, A and B. Somatic embryos in the group A cell lines have a high maturation frequency and are characterised by embryos with a large embryonic region (> 400 Êm). Group B embryos seldom forms mature embryos and characteristically have a small embryonic region (<200Êm). The size of the embryonic region, and the capacity to mature, is to some extent regulated by auxin and cytokinin. The morphology of the somatic embryos are also influenced by protein-related compounds of embryo origin, in the extracellular environment. We have been using well established suspension cultures of group A and B cell lines, to study how extracellular proteins regulate the development of somatic embryos. The effects of different compounds on embryo morphology have been studied in bioassays. The total secretion of proteins to the suspension culture media reflects the embryo morphology as shown by in vivo labelling techniques. Proteins and proteoglycans that differ in the extracellular environment of group A and B include zeamatin, chitinases, peroxidases and arabinogalactaproteins (AGPs).

The zeamatin protein is specifically found in an A cell line, and an immunologically related protein only appear in the control B cell line when cultured with cytokinin alone. A 28 kDa chitinase is specifically secreted by group A cell lines, and in group B cell lines cultured with cytokinin alone. A related chitinase isolated from sugarbeet promote the formation of embryos in bioassays. However, an endogenous spruce chitinase has a detrimental effect on the growth of the embryos. The activity of peroxidases is higher in group A cultures than in group B cultures. Inreasing the level of peroxidases in the culture medium is however not influencing the morphology of the group B embryos, indicating isoform specificity in a similar way to what appear to be the case for chitinases. Group A and B cell lines contain different amounts and species of extracellular AGPs. Isolated extracellular AGPs from group A cell lines, or from the crude extracts of seeds, can enhance the size of the embryonic regions of group B embryos. Additionally, the aggregation of embryos and the expansion of cells are influenced. Extracellular proteins in the suspension culture medium are also present in seeds. Extracts of whole seeds strongly stimuate growth rate and embryo morphology in both group A and B cell lines, and seed extract treated B embryos are forming mature somatic embryos in response to an ABA treatment. Proteins have been shown to be crucial components of the seed extract for its stimulatory effect, supporting the hyphothesis that proteins play an important role in regulating embryo development.

Manipulation of embryogenic tissue water potential during somatic embryo maturation in conifers using *Pinus strobus* as an example.

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In conifers, the osmotic water stress to which the embryogenic tissue is exposed either initially (such as in the case of low molecular weight solutes) or continuously (such as in the case of high molecular weight solutes) triggers and/or maintains the maturation process of somatic embryos. Since in *P. strobus* high numbers of somatic embryos mature on medium with no significant changes in the solute composition, except for increased gellan gum concentration (Klimaszewska and Smith 1997), it represents a model to study the dynamics in the water status of the tissue during the maturation process.

Gellan gum is a bacterial (*Pseudomonas elodea*) polysaccharide composed of glucuronic acid, rhamnose and glucose. Agars are derived from seaweed (agarophytes) and represent a spectrum of closely related polysaccharides belonging to the family of galactans. These qualitative differences between the two gelling agent types are significant enough to pose a question concerning the possibility of stimulatory effects of certain gellan gum components on the maturation of somatic embryos of *P. strobus*. To determine if this was a viable hypothesis, *P. strobus* somatic embryo maturation was carried out on filter paper discs placed on media gelled with gellan gum or several types of agar. In order to make the comparison meaningful, gel strength of all the maturation media was measured and the embryonal masses' response compared at similar gel strength values. The results showed that the medium gel strength was a critical factor in exerting the maturation response regardless of the type of solidifying agent used and that both water availability and tissue water potential were negatively correlated with the gel strength of the maturation medium. There was a reduction in the water content of approximately 50 mg per 227 mg filter paper disc between medium gelled with 0.4 and 1.2% gellan gum and 70 mg between medium gelled with 0.8 and 2.8 % agar Difco-Noble. It is concluded that the tissue was exposed to varying amounts of water during the culture period and this in turn affected the water status of the tissue and the maturation response. Initially, the water potential of the embryogenic tissue was in equilibrium with the water potential of the maturation medium (-043±0.01 MPa) but after two weeks of culture, a clear trend was established. The embryogenic tissue cultured on maturation medium with 0.4 and 0.6% gellan gum had a higher (less negative) water potential than that of the medium and the embryogenic tissue cultured on medium with 0.8 and 1.0% gellan gum had a lower water potential. This trend was maintained throughout week 12. The mature somatic embryos from medium with 1.0 % gellan gum had water potential of –0.70±0.00 MPa and germinated at a high frequency. In contrast, somatic embryos matured on medium with 0.6 % gellan gum had a water potential of –0.32±0.02 MPa and developed precociously after weeks 6 and 7.

In conclusion, embryogenic tissue water potential of *P. strobus* and other conifer species can be manipulated, without changing the solute composition of the medium, to procure production of somatic embryos that display high germination frequency. These somatic embryos germinate without any post-maturation treatment and are also amenable to desiccation.

Plant Hormones during hybrid walnut and hybrid larch somatic embryogenesis.

Philippe Label, Sandrine Dronne, Markus Gutmann, Isabelle Lucas, Isabelle Jourdain, Sabine Saly, Claude Joseph, Daniel Cornu and Marie-Anne Lelu.

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Vegetative propagation of hybrid walnut (*Juglans regia* X *J. nigra*) is difficult through traditional techniques like cuttings but more powerful techniques like somatic embryogenesis offer a good solution to produce vegetatively selected trees. Production of somatic embryos is performed through an adventive secondary SE and secondary somatic embryos (SSE) produced from the "primary" somatic embryo (PSE) are isolated, thus becoming PSE and subcultured iteratively. These steps occur without any exogenous plant growth regulator complementation. The effect of liquid endosperm on SSE production will be developed and correlations between endogenous hormonal levels and SSE formation will be outlined.

Among the conifers, the hybrid between European and Japanese larches has a great potential for reforestation programmes due to its fast growth and good wood quality. Somatic embryogenesis offers a solution to produce large quantities of propagules especially in hybrid larch (*Larix* x *leptoeuropaea*) species. The hormonal status at different stages of somatic embryo development will be presented, either during embryogenic induction, embryonal mass growth or somatic embryo maturation. Correlations between internal hormones and germination will also be discussed with a special emphasis on the role of dessication.

The presentation will aim to draw general lines of hormonal regulation of somatic embryogenesis and will tentatively formulate fundamental questions on that subject that could be adressed and discussed during the present COST meeting.

Aquaporins in Norway spruce - their putative functions during embryo formation and plant establishment.

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Aquaporins, water channel proteins that are members to the large MIP (major intrinsic protein) family of transmembrane channel proteins, have now bee identified in several plant species. These proteins were named aquaporins (AQP) because of their high efficiency and selectivity to conduct water in the direction of an osmotic gradient. This capacity to transport water, is often demonstrated by increased water permeability of Xenopus oocytes expressing heterologous mRNA. The discovery of aquaporins in plants have suggested their involvement in a number of processes from long-distance transport of both water and assimilates to single cell expansion and osmoregulation, that requires the transport of water across cellular membranes, and, have directed new attention to the dynamics of water transport in plants particularly under water limiting conditions.

Plant MIPs are encoded by a relatively large gene family, and these genes display differences in their tissue and developmental expression patterns as well as in their response to various environmental conditions. In an earlier investigation we identified a protein isolated from mature seed of *Picea abies* (Norway spruce) as an a-TIP homologue by its biochemical characteristics such as molecular mass, partial amino acid sequence, and its location in protein storage vacuolar (PSV) membranes. We also identified similar proteins in *P. abies* female gametophytes of seeds prior to embryo development, in mature somatic embryos and in young seedlings where it was particularly abundant in the root.

In the biotechnology program somatic embryogenesis is generally regarded as the most important method to mass-produce selected clones. During this process an important step is the conversion of somatic embryos into emblings and their further establishment as soil-growing plants. Here root formation and growth is often a problem. For these reasons we are interested in processes involved in germination and seedling growth, and a better knowledge about the regulation of aquaporins may therefore be an advantage. Such knowledge may also be useful for improving plant production in the more conventional breeding programs such as through seedlings or cuttings.

In order to further our studies regarding aquaporin-related proteins in *P. abies* we have cloned a gene expressed in the female gametophyte during seed maturation and another isoform expressed in seedling roots. Probes from these clones have been used in Northern experiments and for *in situ* hybridization in order to identify the expression pattern of aquaporin homologues in various tissues of *P. abies*.

Zygotic embryogenesis as a model for somatic embryogenesis ? Some examples from tropical trees.

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When induction conditions of somatic embryogenesis are well-known for a species, one of the keys for an efficient micropopagation method is to control development of the embryos obtained. A percentage of embryos as high as possible has to be well-formed, to be able to germinate and then converted to plantlets before transferring to the green-house and then in the field.
For many species, and specially for woody species, blockages during ontogenesis or germination of the embryos were noticed (Oil Palm, Rubber tree, Cocoa, Coconut and Grapevine). To remove these blockages it is necessary first to identify them (structural, biochemical or physiological anomalies) by comparing somatic embryo development with zygotic embryos "in ovulo" considered as a model (Rangaswamy, 1986; Carman, 1989) Then, taking into account the differences registered between both the evolutions, new culture conditions can be defined to improve the quality of the embryos. Such an approach has been followed by our team to improve embryogenesis process in Rubber tree (Etienne et al. 1993 a et b), Oil Palm (Morcillo et al.1998) Cocoa (Alemanno et al. 1997) among others.
For the three considered species, the study of water parameters, of accumulation of reserves (protein, starch and lipids) of sugar content or ABA concentration throughout the zygotic embryo evolution have pointed out the importance of a "maturation " phase taking place after ontogenesis and growth were completed. It is during this phase (at the beginning or end) that embryos were dehydrating (more or less according to their recalcitrance level) and accumulating reserves.
At the end of the somatic embryogenesis process, studies of some parameters demonstrated that embryos formed were more often non maturing even those morphologically well-formed. An additional phase of culture in conditions allowing a progressive dehydration and a synthesis of specifi reserves (increasing sucrose or amino acid concentrations in the culture medium or adding ABA) has improved percentages of germinated embryos for the three considered species.
The application of molecular biology technology to investigate the genes concerned with embryogenesis and their expression has provided interesting results in model plants (Goldberg et al. 1994; Vroemen et al.1996; Komamine et al. 1998). "Differential Display" used to examine gene expression throughout the development of zygotic and somatic embryos could provide a better knowledge of both systems (Pullmann et al.1998) and therefore improve the efficiency of somatic embryogenesis regeneration for the species we are studying.

Alemanno L. et al. 1997 - In vitro Cell Dev.Biol-Plant 33: 163-172
Carman J.G 1989 - In vitro Cell Dev. Biol-Plant 25 : 1155-1162
Etienne H. et al .1993a- Plant Sci.92: 111-119
Etienne H. et al 1993b-J.Exp.Bot.44 :1613-1619
Goldberg R.B et al 1994- Science 266: 605-614
Komamine A. et al.1998- IX Congress on Plant Tissue Culture. Jerusalem Israel June 14-19
Morcillo F. et al. 1998- Plant Physiol.Biochemistry
Pullman G. et al. 1998- IX Congress on Plant Tissue Culture. Jerusalem Israel June 14-19
Rangaswamy N.S 1986- Proc. Indian Acad.Sci 96 : 247-271
Vroemen C.W et al. 1996- Plant Cell 8:783-791

Aspects of genetic transformation in *Quercus.*

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Immature zygotic embryos were used as explant source to induce somatic embryogenesis throughout several years from several open pollinated trees at different sites. The suitable developmental window lasted from 3 to 18 weeks after anthesis with the highest initiation rate of nearly 60% occuring approximately 13 weeks after anthesis on 5 (M 2-4D plus 0.5 (M BAP. Embryogenic competence of the callus lines has been maintained by regular subculturing on 1 (M BAP or hormone free media for four years. Maturation media with increased agar concentration (1%) proved to enhance germination rate significantly. Conversion rate of matured somatic embryos into plantlets could be increased to 80% by a partial desiccation treatment.

Somatic embryogenic callus was shown to be infectable by *Agrobacterium tumefaciens*. Additional wounding of embryogenic tissue caused significant decreases in regeneration. A cocultivation period of two days seemed to enhance the regeneration capacity of oak somatic embryogenic callus more than four days cocultivation. An optimum of 70% regeneration could be observed after two passages on 50 mg/l kanamycin + 300 mg/l cefotaxime. More than 100 lines produced initially have been subcultured on 50 and 100 mg/l kanamycin for two consecutive years, respectively. Samples were repeatedly tested with selected PCR primers for the presence of the CAT (chloramphenicol acetyltransferase) gene. More than 50% of the highly embryogenic cultures exhibited the CAT fragment for up to 15 months, whereas *in vitro* plantlets germinated from four kanamycin tolerant cultures surviving up to two years did not contain the insert.

Transformation of conifers by particle bombardment

David Clapham, Sara von Arnold

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Transgenic plantlets of *Picea glauca* were produced by Ellis and coworkers in 1993 (Bio/Technology 11, 84-89) using particle bombardment and the method has subsequently been applied to other conifer species. In our department, a particle inflow gun enabled efficient production of transgenic plantlets of *Picea abies* from embryogenic suspension cultures. In studies of transient expression of the *gusA* reporter gene driven by various promoters, the *Zea* ubiquitin promoter was three times as active as the *Helianthus* ubiquitin promoter, four times as active as the *Eucalyptus* CAD promoter and 12-16 times as active as the 35S promoter. In four cell lines, placing the cells 1-3 h before bombardment on proliferation medium supplemented with 0.125-0.25 M myoinositol enhanced transient expression 15-40-fold. The procedure for stable transformation was developed using the plasmid pAHC25 which contains the *bar* gene and the *gusA* gene, both driven by the *Zea* ubiquitin promoter. Cells were maintained on proliferation medium supplemented with 0.25 M myoinositol and, from day 8 after bombardment, supplemented with Basta as selective agent. Callus resistant to Basta appeared from two months after bombardment. Of over 100 independent Basta-resistant sublines, 65% expressed the cotransformed reporter gene, even when it was not linked to the selectable marker. Over 80% of the sublines retained their embryogenic potential. Of eleven transformants analyzed, four contained transgenes in low copy number (1-3). Over 200 Basta-resistant sublines from four cell lines have been established, of which 138 are confirmed as transformed. Plantlets have been regenerated and potted.

Transformation of conifers via *Agrobacterium tumefaciens*

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For a long time, conifers were thought to be recalcitrant to *Agrobacterium* infection. However, the first conifer species - larch - was genetically transformed through cocultivation with *Agrobacterium rhizogenes* (Huang et al., 1991, In Vitro Cell Dev. Biol., 27, 201-207). We have developped an efficient procedure for the genetic transformation of hybrid larch (*Larix kaempferi* x *L. decidua*) using *Agrobacterium tumefaciens* as vector for DNA transfer (Levée *et al.* 1997, Plant Cell Reports, 16, 680-685). After cocultivation, embryogenic masses (EM) resistant to the selection agent were isolated. Using somatic embryogenesis numerous plantlets can easily be regenerated from each of antibiotic-resistant EM and only seven months are required from the cocultivation step to the acclimatisation of plantlets in the greenhouse. Since this first report, the procedure has been optimized in order to increase the yield of antibiotic-resistant EM. These improvements allow us to regularly obtain up to 50 different EM resistant to the selective agent per g FW. All of the lines analyzed by molecular techniques exhibited stable integration of the transgene. Copy number remains fairly low as 4 of 12 transgenic lines analyzed seems to have only one copy inserted. The high transformation yield obtained probably results, at least in part, from the high growth rate of the embryogenic lines used in transformation experiments. To be efficient, this method also requires a careful adjustment of selection pressure (timing of antibiotic application, antibiotic concentration). This procedure has been shown to be efficient on different hybrid larch genotypes. It still needs to demonstrate that it can be transferred to other larch species and further to other *Pinaceae* species.

This presentation will outline a description of the transformation procedure and detail the first results on the molecular characterisation of transgenic hybrid larch.

Recent advances in long-term preservation of coffee and cocoa genetic resources

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Coffee and Cocoa are of great economical importance for many countries. In vitro techniques present a real interest in the development of new improved varieties but also for the preservation of genetic resources of these tropical species because they produce non-orthodox seeds, which are unsuitable for germplasm storage. Cryopreservation has proven to be reliable for long-term storage of biological materials. Cryopreservation of plant somatic embryos and/or embryogenic cells may therefore be used for germplasm storage and exchange, but also for a constant supply of embryogenic strains for propagation of elite clones or genetic modification experiments. Because of both the labour intensive and time consuming issues linked to the establishment of these cultures and the decline of their embryogenic potential with the subculture number, cryopreservation appears to be a key tool for clonal propagation of elite plants. Based on results obtained for somatic embryos and embryogenic strains of coffee and cocoa, cryopreservation approaches will be discussed.

Desiccation and cryopreservation in conifers.

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Most seeds undergo desiccation during later stages of zygotic embryo development. This process has a role in the transition between embryo maturation and germination. In contrast, somatic embryo development is continuous without desiccation such as in zygotic embryos. To produce somatic embryos capable of synchronized germination, appropriate methodology has been developed. The rate of drying is a critical factor in acquisition of desiccation tolerance and generally slow drying is applied to somatic embryos (i.e. desiccation realized under high relative humidities 80-98 %). Desiccation tolerance has also been enhanced by different treatments such as exogenous application of abscisic acid (ABA) and osmotic stress (PEG). In many conifers (*Picea glauca, P. glauca engelmannii, P. mariana, P. sitka, Larix leptoeuropaea*), partial drying of somatic embryos promotes rapid and synchronous germination and plantlet development.

The use of dried somatic embryos might also constitute a method for storage of selected genotypes. For this purpose, desiccation treatments were tested under low relative humidities (43-59%) in order to reduce somatic embryos water content (close to stored seed embryos). In hybrid larch, survival of somatic embryos to these severe desiccations was depending on their maturation duration (Lelu *et al.* 1995).

In conifer species, production of somatic embryos that can be stored dry has not been so much studied due to the development of more efficient storage method such as cryopreservation. Indeed cryopreservation in which embryonal masses are frozen under controlled conditions and stored in liquid nitrogen (LN) is a reliable method and has been an important tool for long term preservation of germplasm. In general, embryonal masses are precultured with high concentrations of sucrose or sorbitol (0.4 to 0.6 M) and pre-treated with cryoprotectants such as DMSO (5%). Then successful cryopreservation has been achieved by slow prefreezing from -30°C to -40°C prior immersion in LN. It has been demonstrated that embryonal masses are amenable to cryopreservation and plantlets have been regenerated from cryostored embryonal masses of numerous conifer species (*Abies, Larix, Picea, Pinus* sp.).

M.A. Lelu, K. Klimaszewska, G. Pflaum and C. Bastien, 1995.
Effect of maturation duration on desiccation tolerance in hybrid larch (*Larix x leptoeuropaea* Dengler) somatic embryos. In Vitro Cell. Dev. Biol., 31, 15-20.

Somaclonal variations and somatic embryogenesis in forest trees.

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Aside from traditional propagation techniques, somatic embryogenesis is often considered to be a very promising technique for vegetative multiplication of forest trees. Moreover, this method is already used as a regeneration model for genetic transformation experiments. However, plantlets derived from *in vitro* culture may exhibit phenotypical anomalies called somaclonal variations (Larkin and Scowcroft 1981). For the "Tissue Culture Association Terminology Committee", a somaclonal variation is a phenotypic variation, either genetic or epigenetic (i.e. non genetic) in origin, displayed among somaclones (Schaeffer 1990). Somaclonal variation is caused by the *in vitro* culture system itself or may be the result of pre-existing physiological or genetic variation in somatic. Virus elimination can also explain some cases of somaclonal variation. A variety of studies on somaclonal variation have been performed only on *in vitro* growing cells or tissues (calli, cellular suspensions, protoplasts, embryogenic tissues), rather than on acclimated regenerants. The diminution or the loss of regeneration capacities of embryogenic cell lines is often ascribed to somaclonal variations. For forest trees with a long life cycle there is a lack of information concerning the transmissibility of somaclonal variation to the offspring, but for herbaceous species with a short life cycle, it has been demonstrated that phenotypical anomalies produced by *in vitro* propagation systems can be heritable, partially heritable or not transmitted through meiosis. These last two cases of variation often called epigenetic or non-genetic variation, appear in regenerated plants as a result of physiological responses. For instance, shoot regeneration from dedifferentiated cultures can result in an immature form of the parental clone that may or may not be stable over time. Beside these epigenetic phenomena, somaclonal variation that is fixed as mutations may be essentially identical to sports and other somatic mutations. Especially in tissue culture, the combination of a high rate of mitotic division, and the occurrence of unusual combinations of nutrients and phytohormones active in both the production and repair of mutational events, may explain at least in part, the high rate of mutations. Many factors influence the level of somaclonal variation like the regeneration system used, the culture conditions and the number of subcultures or other specific procedures like protoplast isolation, gene transfer or cryopreservation. Some species or clones are also more susceptible than others. Morphogenetic, cytogenetic, cytometric, and molecular approaches have been used to study somaclonal variation. This talk will review advantages and drawbacks of these different approaches.

The mantled floral variation in oil palm clones : hormonal and molecular approach

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Clonal propagation of oil palm (*Elaeis guineensis* Jacq.) through somatic embryogenesis has led to the production of several million clonal plantlets to date. Evaluation in the field has revealed the occurrence of variant palms (below 5%) showing an abnormal flower development, called "mantled"(development of staminods or stamens in carpell-like organs). Furthermore, clonal oil palm plantlets originating from Nodular Compact Calli (NCC) have been shown to exhibit the "mantled" variant phenotype at an average level below 5%, whereas this rate has been found to attain 100% in plantlets derived from Fast Growing Calli (FGC).

Endogenous levels of cytokinins were measured in the two type of callus of oil palm by an HPLC-ELISA methodology. No significant amounts of cytokinins were detected in FGC whereas they accumulated in NCC. Furthermore, it has been demonstrated that an increase in the 2,4-D level produces a decrease in the NC endogenous cytokinins content and promote the evolution of NC into FGC. Analysis of inflorescences issued from abnormal and normal individuals confirm the disorder characterized by lower levels of cytokinins.

Studies of ploidy level and RAPD polymorphism have not revealed any genomic changes that could be linked with the variant phenotype. These results are consistent with the hypothesis of an epigenetic origin for the abnormality.

Levels of global DNA methylation were estimated after enzymatic hydrolysis of genomic DNA to nucleosides and HPLC quantification of 5-Methyl deoxyCytidine. Methylation rates of genomic DNA [(5mdC) / (5mdC+dC)] have been investigated in regenerated oil palms, with the aim of comparing normal and variant regenerants within the same clonal line. Global levels of genomic DNA methylation were found to range 20 to 25%. DNA methylation has been found to discriminate the variants at the adult age. In some clonal lines, analyses of leaf genomic DNA have revealed a substantial demethylation in severely "mantled" palms (20.6 % versus 22.2 %).

A significant hypomethylation in FGC (23.2 % *vs* 18.7%) has been found when compared to NCC from the same clonal line.

Evidence for a direct relationship in oil palm between hypomethylation of genomic DNA and the determinism of the "mantled" somaclonal variant phenotype is still yet to be obtained, but our studies on DNA methylation have already opened promising lines of research.

Role of somatic embrygenesis in conservation of genetic diversity.

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Mass decline of forest trees populations in Poland was first observed in the eighties at the higher elevation (800 m.) of Western Sudety mountains. This forest population decline resulted in increased necessity of it's geneticall diversity protection. This disaster caused by air pollution resulted at first in Norway spruce population decline. Total deforestation area was estimated at the level of 15,000 hectares. Near half of Norway spruce stands in Poland are under influence of industrial emissions. Additionally, silver fir and oak populations decline is observed. Other species as larch, or beech produce seeds irregularly. On the other hand, oak, chestnut and sycamore recalcitrant seeds storage is difficult for a long time. All these reasons resulted in Program of Forest Genetic Resources Conservation. This program includes establishment of gene banks as protection plots *in-situ* and *ex-situ* for long storage of seeds, pollens and plant tissues.

For genetic resources and geneticall diversity conservation, storage of tissues or organs at the temperatures of 0 - 10° C or in liquid nitrogen can be used. More efficient method of trees tissue and organs long term storage is use of cryopreservation. This method is useful also in respect to recalcitrant seeds. Embryogenic callus can be stored for a long time in liquid nitrogen and it can be use in future for production of planting material for reforestation and conservation of forest trees genetic resources in gene bank. For this reason methods of somatic embryogenesis and cryopreservation need more work to be improved. Wide range of genotypes have to be used for induction of somatic embyogenesis which should protect genetic diversity. Additionally use of this method should avoid mutations and adaptation of emblings to natural conditions have to be improved. Very important will be also possibility of embryogenic tissue induction on organs collected form old trees. At present in Forest Research Institute in Warsaw, we study of somatic embryogenesis for long term storage in liquid nitrogen and propagation of Norway spruce, European larch and silver fir Polish provenances.

Clonal propagation of pines via somatic embryogenesis.

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The development and use of somatic embryogenesis (SE) for the clonal propagation of pines will be discussed. Primary advantages of SE over other conventional propagation systems include (1) the ability to cryo-preserve cell lines to overcome problems associated with maturation of stock plants, (2) the potential to automate embryo production and synthetic seed manufacture, and (3) the flexibility associated with having an indefinite long-term cryogenic storage bank to use in clonal testing. We have developed an efficient SE system for use in clonal testing of loblolly (*Pinus taeda*) and other pine species. Components of the system will be described, including (1) improved culture initiation, enabling a higher genotype capture frequency, (2) increased somatic embryo production, and (3) methods to enhance embryo quality that translates into better germination and conversion to vigorous somatic embryo-derived planting stock. Establishment of clonal field tests of pine somatic embryo-plants will enable the selection of desirable genotypes for plantation deployment and use in conjunction with genetic engineering. Even with the above improvements, pine species remain recalcitrant relative to some conifers. SE in pines appears to be under strong genetic control. This high degree of genotype specificity makes the application of SE to clonal propagation challenging. Fundamental genetic and biochemical research is needed to elucidate mechanisms that control induction of SE and completion of the regeneration process. Results will be presented on factors that may contribute to genotype specificity during the culture induction step in pine species.

Are We Ready to Integrate Somatic Embryogenesis in European Forestry ?

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Among forest tree species, conifer somatic embryogenesis is the most well developed and nearest to integration in tree improvement programmes. Although this process was originally reported from three European research laboratories, presently it is at the "pilot-scale" in parts of the world outside of Europe. Therefore, the most pressing question for people at this Workshop is not "Should we integrate somatic embryogenesis with conventional tree improvement? (the answer is clearly yes) or "How can we integrate this technology?" (we will work this out when we begin to integrate) but rather the more important "What do we need to do to advance the application of this technology in Europe?" or How can we catch up with the others ?.

Research over the past 15 years on conifer somatic embryogenesis first concentrated on developing an understanding and then attempting to overcome the technical bottlenecks in the process. This is where we are at present. We have in some species such as the spruces, a process, while not completely understood, controllable or predictable, can, nevertheless, produce enough plants to demonstrate the potential of the technology. What we need to do now (like others elsewhere), is to create interest among potential customers and to develop a market for this technology.

Conifer somatic embryogenesis is not yet "market driven" because very few foresters and tree breeders are demanding emblings. This technology is actually "technology driven" where we need to find an application, a market and a customer for emblings. This is mainly due to the fact that most customers (foresters and tree breeders) do not fully understand and appreciate both the applications and the implications of this technology. The challenge now is to create and develop this market.

We need to continue to work on improving our basic understanding of the process as well as overcoming the bottlenecks such as low initiation rates, maturation/germination problems and the production of high quality emblings. At the same time we need to stimulate interest on the part of tree breeders and foresters in the process. To accomplish this we need to start work on scale-up of the technology, establishment of both field demonstrations and scientific trials and to illustrate and document the biological and economic aspects of emblings. Development of the application of this technology is essential if interest and support for further basic research in this technology is to continue.

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