”Clonal Forestry – Who Are You Kidding”
Meeting in Scotland, September 2002

A review

Clonal propagation of Scots pine - experiences in all the methods tried

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Project: Application of biotechnology for genetic research and gene conservation of forest trees

Vegetative propagation techniques developed for Scots pine

1) Rooted cuttings
2) Organogenesis
3) Somatic embryogenesis

- Material / explants ?
- Propagation efficiency ?
- Quality of cloned plants ?
- Specific problems ?
- Potential solutions / new developments ?

Scots pine cuttings

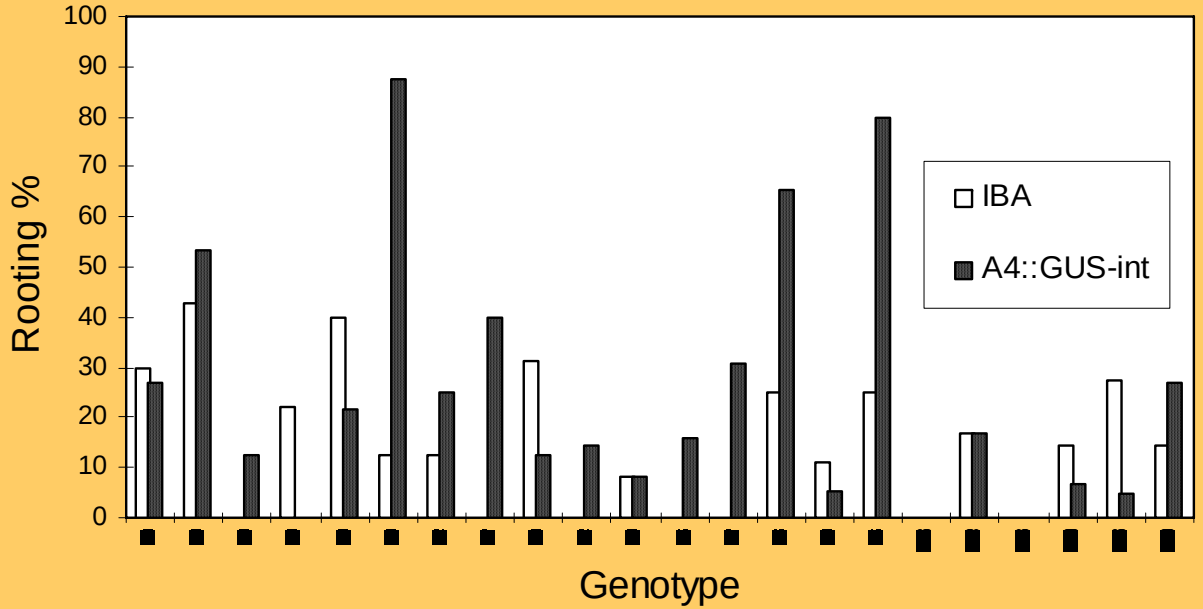
- long shoots
- needle fasicles as such,
  or after pruning the branches,
  or after chemical treatment of stock plants

- rooted: 0-100 % depending on stock plant genotype, age, and growth regulator treatments

Material at FFRI

- fascicular shoots induced in 2-year-old seedlings by cytokinin spraying
- auxin (IBA) / *Agrobacterium rhizogenes* / mycorrhizal treatments to induce rooting

IBA / Agrobacterium treatments:



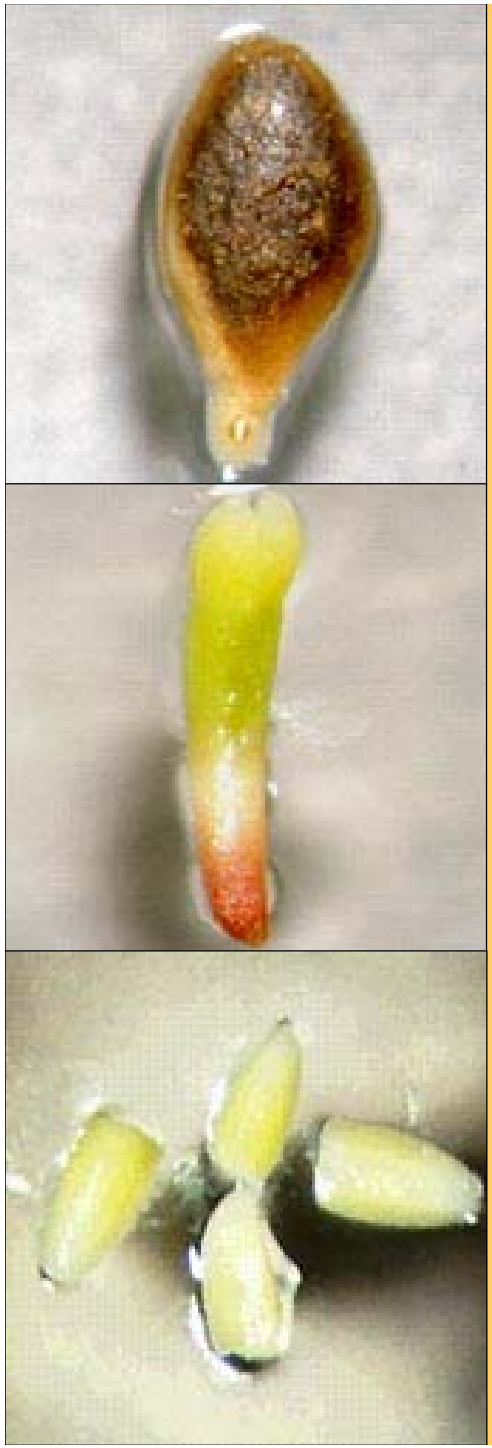
- Rooting enhanced by agrobacteria, different strains affected pine genotypes differently
- Roots were not transformed – transient expression of *aux* genes or soil modification by bacteria ?

Niemi, Salonen, Ernstsen, Heinonen-Tanski & Häggman,
Can. J. For. Res. 30:1221-1230 (2000):

Application of ectomycorrhizal fungi (*Pisolithus tinctorius* and *Paxillus involutus*) enhanced rooting of Scots pine fascicular shoots

Rooted cuttings

- With cytokinin spraying, up to 150 fascicular shoots / seedling; average yield 40-60
- Auxin treatment needed, agrobacteria or mycorrhiza can enhance rooting
- On an average, 30-50 % of cuttings rooted, in the best genotypes over 80 %
- Ouality of plants produced is not good: 40 % orthotrophic



Tissue culture of Scots pine through organogenesis

- Explants: cotyledons excised from germinating embryos
- ½GD medium, gelled with agar/Gelrite during shoot formation and with agar during rooting
- 5 µM BA and 0.05 µM NAA for shoot induction
- Repeated 2.7 µM NAA pulses for rooting

Plant regeneration through organogenesis

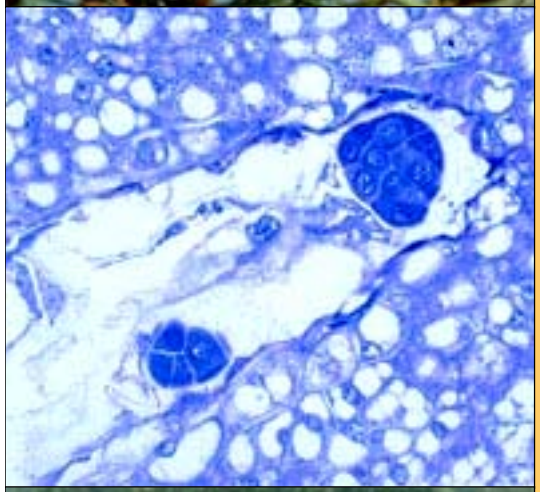
- 13-60 % of embryos can be induced to form adventitious shoots
- Average multiplication rate: 3-15 shoots per embryo, the maximum was 35
- On an average, only 6 % of shoots rooted (0-100 % depending on genotype)
- Quality of the plants produced was poor: majority were plagiotrophic and ramified

Scots pines produced through organogenesis are early-flowering

- megasporangiate strobili at the age of 3 years, but plants are too small for successful cone development

- microsporangiate strobili at the age of 4 years producing viable pollen

- embryo rescue enables conservation of the original juvenile genotype; only part of the cotyledons are used as explants for organogenesis



Somatic embryogenesis of Scots pine

- Explants: immature female megagametophytes including zygotic embryos at proembryo or early embryo stage

- Cones including the explants can be stored at +5°C up to two months before SE initiation

- DCR-medium, with
  I) 9.1/13.6 µM 2,4-D and 2.2 µM BA for induction and proliferation of cell masses
  II) activated charcoal, 32 µM ABA, PEG8000 and 60 g/l of sucrose for embryo maturation
  III) 20 g/l of sucrose for embryo germination

Plant regeneration through somatic embryogenesis

- 28 % of seed families (n = 25) showed potential for somatic embryogenesis; within these 3 % of the explants produced embryogenic cultures
- Of the selected seed families, 75 % produced SE cultures, frequency of proliferating explants varying 0.2-4% depending on the family
- Average multiplication rate: 50 (10-200) somatic embryos per line per maturation cycle
- Problems in germination of cotylenodary somatic embryos

Karoliina Niemi & Hely Häggman (2002):

Pisolithus tinctorius promotes root development and further forms myccorrhizal structures in Scots pine somatic embryos *in vitro*

Improvement in germination frequencies of somatic embryos in different lines :

50 → 83 %
41 → 58 %
23 → 48 %
0 → 60 %

For comparison, SE results of Scots pine achieved by other research groups:

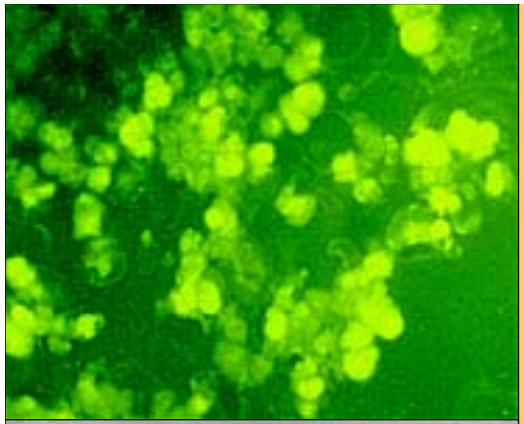
Keinonen-Mettälä et al. (1996)
Scand. J. For. Res. 11:242-250

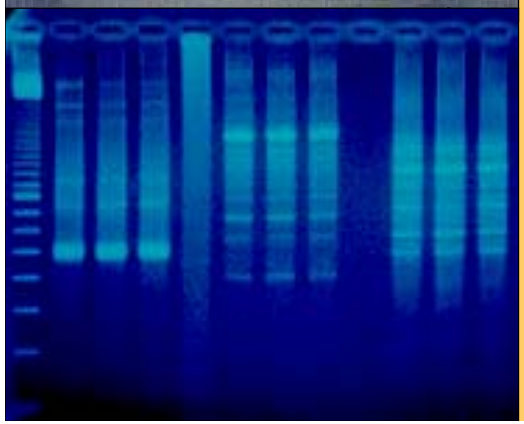
→ 33 % of seed families responsive (n = 138)
→ 0.2-9 % of explants proliferating

Lelu et al. (1999) Phys. Plant.105:719-728

→ 91 % of seed families responsive (n = 11)
→ 1-23 % of explants proliferating
→ 65-72 % of embryos germinated and
40-48 % of them developed into plants

The system is still under development…





Cryopreservation of embryogenic cultures of Scots pine

- 75 % of the embryogenic lines tested survived cryostorage

- PGD mixture (PEG6000, glucose & DMSO) was the best cryoprotectant
  → better regrowth than with other treatments or in the controls

- Morphological appearance and RAPD profiles were the same as in the non-frozen cultures

Clonal forestry in the case of Scots pine

- we are kidding ?

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