

Somatic embryogenesis (SE) of Norway spruce (*Picea abies*) using primordial shoot explants

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SE from Norway spruce primordial shoot explants

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Photo Lassi Palmujoki



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Background and benefits

- Recalcitrance of adult conifers has prevented vegetative propagation of trees with known and desired characteristics
- This far conifer embryogenic cultures could have been initiated only from young material, in practice from **zygotic embryos**
- Method for the initiation of SE cultures from adult trees would benefit tree breeding
- Production of trees with elite characteristics which become evident after sexual maturation
- Special forms which are not flowering

Photo Lassi Palmujoki



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What's done

- Promising results in *Pinus sylvestris*: embryogenic tissue initiated from shoot bud explants, but no plant regeneration
- Successful initiations in white spruce
 - Mature SE trees as the source of explants
 - Primordial shoots as explants
 - Initiation and plant production protocol developed for the zygotic embryo explants

Trontin et al. (2016) In: Yill-Sung Park, Jan M Bonga, Heung-Kyu Moon (eds) Vegetative Propagation of Forest Trees. Korea Forest Research Institute. Seoul, Korea. p. 211-260.

Klimaszewska and Rutledge (2016) In: Yill-Sung Park, Jan M Bonga, Heung-Kyu Moon (eds) Vegetative Propagation of Forest Trees. Korea Forest Research Institute. Seoul, Korea. p. 211-260.

Klimaszewska et al. (2011) Planta 233: 635-647.



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What we have done?

- SE induction protocol for white spruce was applied to Norway spruce
- Collections of primordial shoots from SE plantation
 - March and April 2015, April 2016 and 2017
 - October 2016 and November 2017
- Original SE lines were initiated 2011, plants were produced winter/spring 2012 and planted spring 2014
- Shoots were collected from 40 genotypes

Photo Teijo Nikkanen



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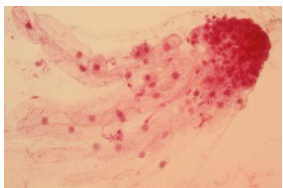
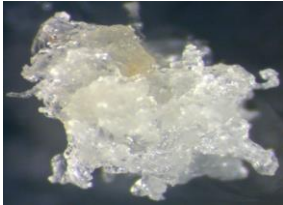
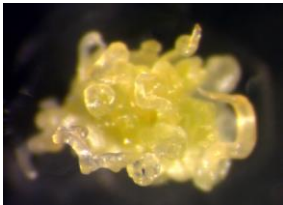
What we have done?



- At least 40 buds from each genotype
 - At first all trees/genotype were pooled
 - Later collections from parts of the trees/genotype
- Protocol includes:
 - Removing of the needles and base scales
 - Disinfection with ethanol and H₂O₂
 - Removing of the shoot from a halved bud
 - Halving the shoot again
 - Removing to mLM proliferation media
 - Waiting

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Results



- Two genotypes responded positively
 - Genotype A produced 5 and genotype B 23 well proliferating sublines
 - Both from spring and autumn collections
 - Genetic stability of sublines was detected using microsatellite markers
 - However, not all trees among genotype were responsive
- A lot of callus growth, covers embryogenic cells?
- Different embryo production capacity between genotypes
 - Although numerous early embryos shown in cell mass of both genotype



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Conclusions and what next?

- SE initiation protocol developed for primordial shoots of white spruce can be introduced to Norway spruce
- Have to screen a lot of genotypes to find embryogenic tissue growth
- Need for more studies on factors regulating explant's response to SE induction
 - Responsive and non-responsive trees among the same genotype gives new opportunities
 - Gene expression analysis without genotype-specific factors
- Other tissues as explants, artificial induction of rejuvenation?
 - Bonga (2017) Trees, 31:781-789, Bonga (2018) Can. J. For.Res, 48:1-5



Thank You!