Somatic embryogenesis of Norway spruce in Finland

– on a way to practical applications

European Union
European Regional Development Fund

Leverage from the EU 2014–2020

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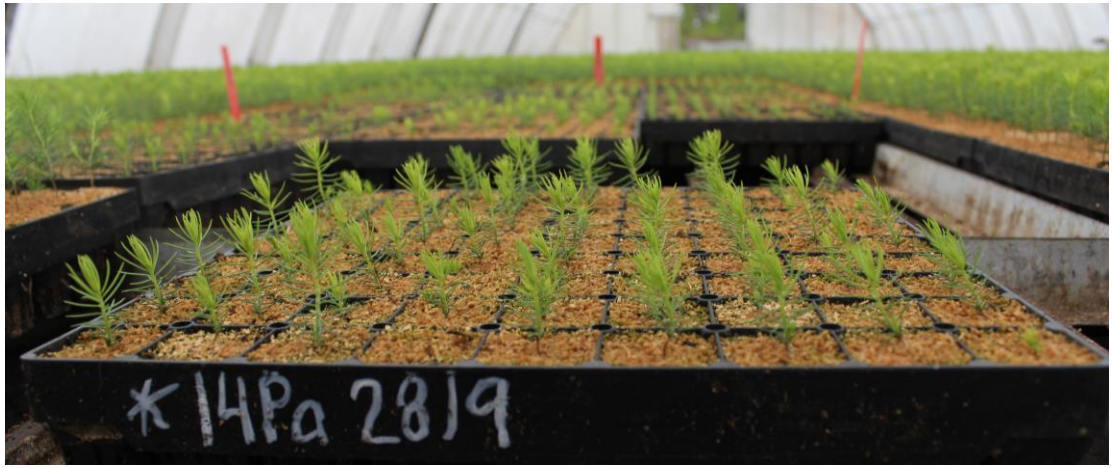
HealGenCAR conference
XX.6.2018

Luke
LUONNONVARAKESKUS

Somatic embryogenesis of Norway spruce in Finland – on a way to practical applications

Outline of the presentation

- Starting point of somatic embryogenesis (SE) research
- Methdology applied and studied
- Production of Norway spruce SE materials
- Regulatory issues & registration of SE materials
- Commercial SE propagation pilots
- Future views

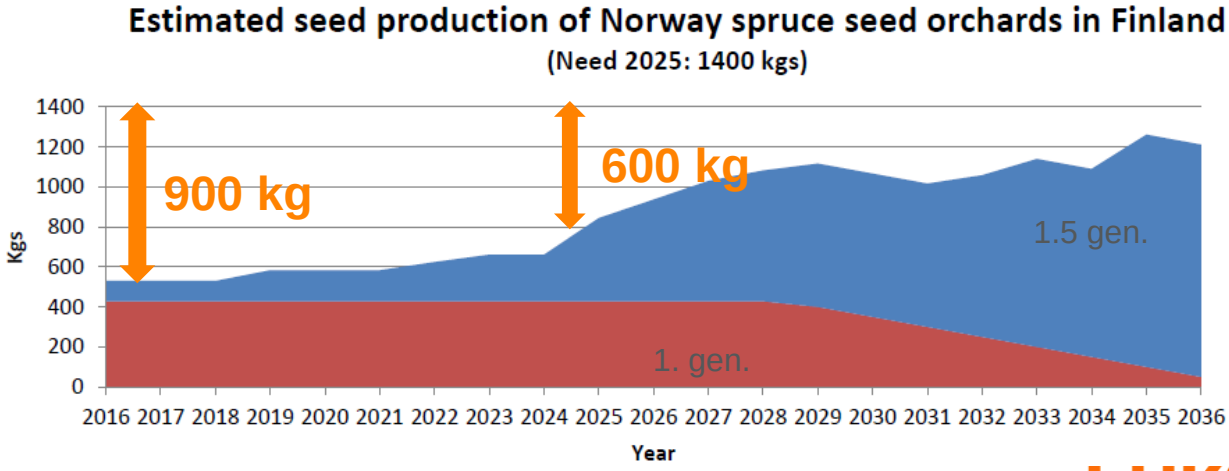


Starting point for SE research in 2011

Shortage of high-quality, bred forest regeneration material i.e. seed orchard seed

- age structure of seed orchards (some growing old, others not yet productive)
- irregular flowering of species
- pest & pathogen problems
- open pollination -> lower genetic gain

Jukka Antola, 2/2015:



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Starting point for SE research in 2011




Need for hardy, ornamental conifers in Northern Europe

- to replace imported, less hardy plants with higher disease risk

- Luke has a collection of well-adapted special forms of Norway spruce (natural mutants, material produced via controlled crossings) suitable for landscaping business

→ How to propagate these ? Via rooted cuttings or SE?

Starting point for SE research in 2011

→ Decision to adapt SE in Norway spruce for propagation of both

1) Ornamental forms as ”first practical application”
- smaller markets → smaller number plants needed
- high price per a plant is not a problem; handwork ok

2) **Forest regeneration material**
- to fully exploit breeding gains
- to mitigate shortage of seed-orchard seed
- requires true mass-propagation (e.g. 5-10 % of 100 mill./year)

A few years later, Cristmas tree producers expressed great interest towards varieties having desired crown form without repeated shoot excision

→ 3) **Christmas tree forms**
- appr. 1 million planted every year

Starting point for SE research - ERDF financing

European Regional Development Fund –projects:

- **Vegetative propagation - knowhow and technology to support bioeconomy** 2011-2014
- **Vegetative propagation of spruce – towards future plant production** 2015-2016
- **Testing novel technologies for somatic embryogenesis of Norway spruce** 2017
- **SmartTree – Propagation technology for elite tree production** 2017-2019

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Leverage from the EU 2007–2013

Leverage from the EU 2014–2020

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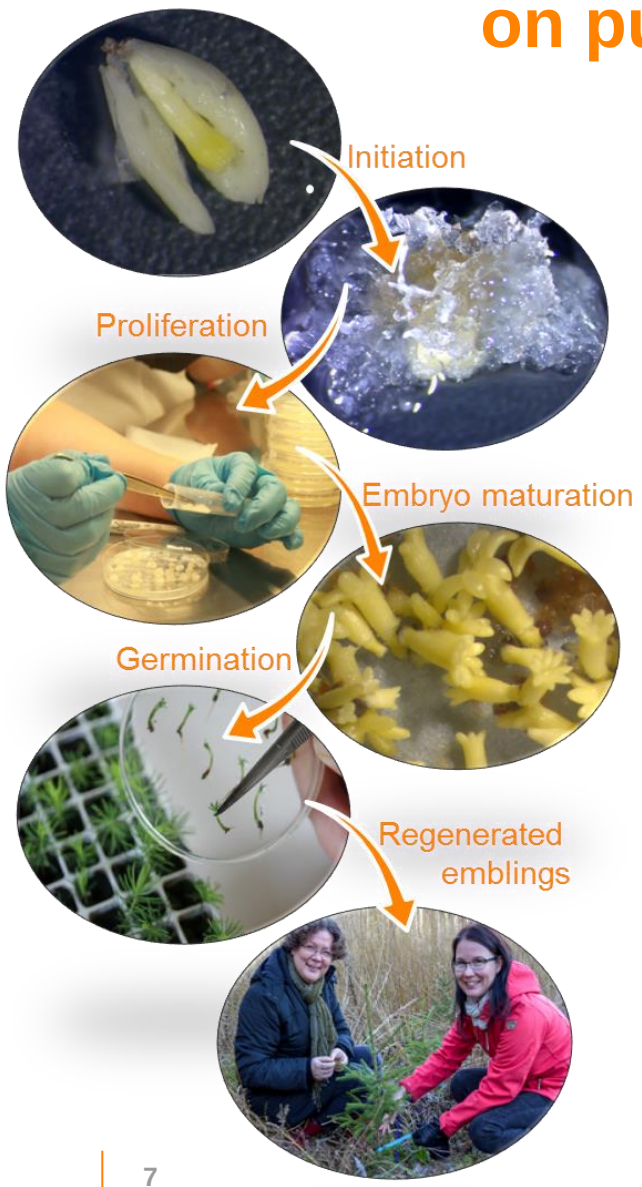
SE-methdology applied mostly based on published protocols and media



Early phases (initiation, proleferation, maturation) of SE **succeed well** using published protocols and modified Litvay's medium

- average initiation frequency > 70 %

Soon observed that **cryopreservation** of SE materials, as well as **laboratory-nursery interface** in SE production **require further development**

→ **Modification of protocols**

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Cryopreservation of embryogenic cultures of Norway spruce

Slow-cooling of SE samples in programmable Planer device and in Mr. Frosty was compared

- Comparison of pretreatments, cryoprotectants and slow-cooling devices: 12 treatments
- Tested with 136 spruce SE-lines

→ Pretreatment on semi-solid medium with increasing sucrose concentration, PGD mixture as cryoprotectant and a programmable freezer (0.17˚ C/min)

→ 87 % (up to 100 %) recovered

- Varis, Ahola, Jaakola & Aronen (2017) Reliable and practical methods for cryopreservation of embryogenic cultures and cold storage of somatic embryos of Norway spruce. Cryobiology 76: 8–17

Improving SE maturation & storage

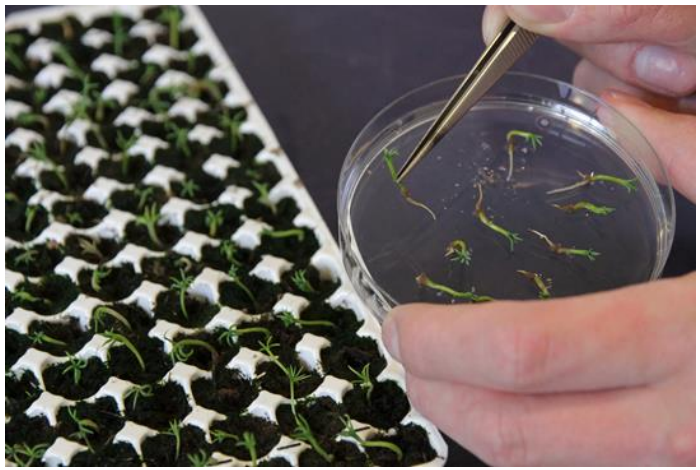
Tikkinen M, Varis S, Peltola H & Aronen T (2018) Improved germination conditions for Norway spruce somatic cotyledonary embryos increased survival and height growth of emblings. Submitted to Trees

Tikkinen M, Varis S & Aronen T (2018) Development of somatic embryo maturation and growing techniques of Norway spruce emblings towards large scale field testing. Forests 9(6) 325:https://doi.org/10.3390/f9060325

- Reduction of ABA 60→ 30 uM resulted in increased embryo yield

- Mature SE can be safely stored at +4C for months without effect on their germination ability
- On filter paper (on maturation medium), stable temperature

Improving SE germination and acclimatization



Diffrent germination media, duration of *in vitro* germination (1-5 w) and growing techniques tested

→ Germination medium without inorganic

→ Lowered lightning in germi

→ Short *in vit* , then directly t in Plantek 81f cont peat

80 % survival (as tested with 11 full-sib families, 121 lines, < 4000 emblings)

→ Also possible to use mini-plugs & growth room with led-lights for 1st growing season

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Production of SE material for forest regeneration

- SE -initiations made in 2011, 2012, 2014
- Controlled crossings among elite trees (61) from the southernmost breeding zones (I and II)

→ Over 3000 lines cryopreserved

→ 853 tested for their performance, testing continued all the time

  * embryo production capacity / gFW
  * germination & conversion frequency
  * growth at nursery

  → field trials with other breeding materials

→ **SE-lines with good embryo production capacity (over 200/gFW) found in all families**

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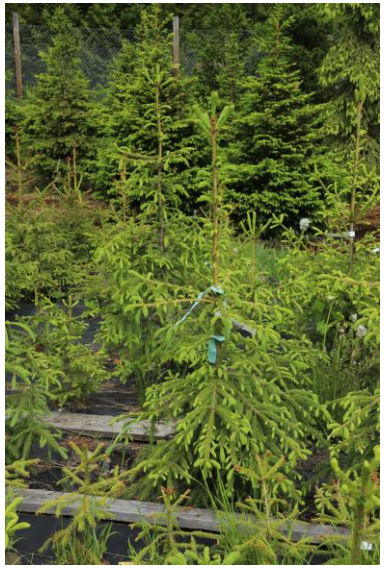
Production of ornamental SE-lines for landscaping

- Over 300 SE –lines originating from crosses between spruce special forms cryostored
- Around 20 lines confirmed having ornamental value; these are available for commercial use

Picea abies f. *cruenta*

Picea abies f. *aurea*



Picea abies f. *pendula*

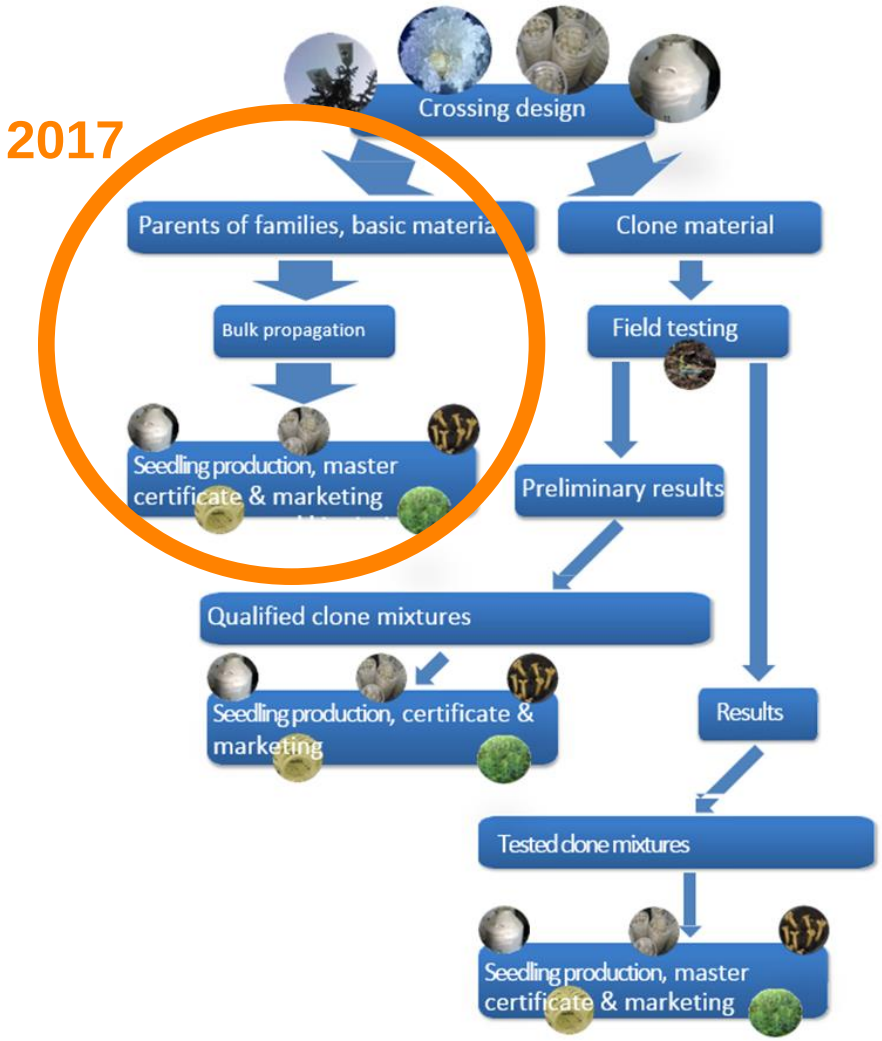
Red-needled pendulous

Picea abies f. *virgata*

SE lines for Christmas tree production ?

- SE-lines originating from crosses and self-pollinations of *P. abies* f. *pyramidata* under testing → observations on growth habit /crown form takes several years
- Seed embryos from *P. abies* f. *globosa* would be appreciated !

Commersialization: Regulatory issues



Two possible ways to get approval to SE material as forest regeneration material:

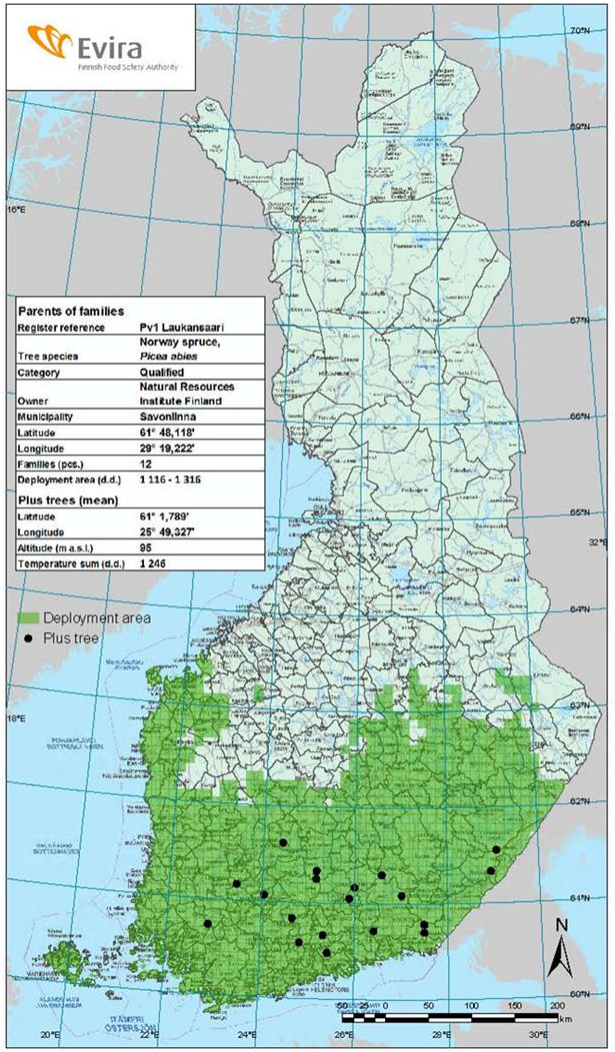
I) **Bulk propagation** using material from parents of full-sib families (controlled crossing of the best parents)

II) **Registered clones** are clone mixtures after field testing (testing takes years)

Registration of SE material for commercial use

Parents of families

- Pv1 Laukansaari
- Norway spruce, 12 families, not related to each other
- *Qualified* category
- The laboratory of Natural Research Institute is located in Savonlinna Eastern Finland
- Parents (plus trees) are located in Southern Finland at the mean height of 95 meters
- The recommended utilization area shown in green



Pilot propagations of forest regeneration material

- *Master certificates* issued to Luke
- Luke is selling germinated emblings ready for greenhouse cultivation to several customers
- Bulk propagation of 12 registered full-sib families , 10 lines/family (alltogether 120 genotypes) thawed from cryo for production
- Genotypes mixed in containers during transplanting to greenhouse
- Production numbers: tens of thousands

Cost analysis of SE propagation

	Salaries, %	Materials, %	Share of costs, %
Management	100	0	6
Thawing from cryo	92	8	4
Proliferation of cultures	88	12	10
Maturation of SE	83	17	20
Selection & germination of SE	98	2	60

Tikkinen 2018, PhD thesis in prep.

Price estimate for germinated emblings produced by Luke, using manual SE propagation

- 2017: 0,74 - 4,55 € (+VAT)
- 2018: 0,14 - 4,55 € (+VAT)

Price depends on propagation characteristics of line (embryo production/gFW), number of lines, and amount of emblings to be produced

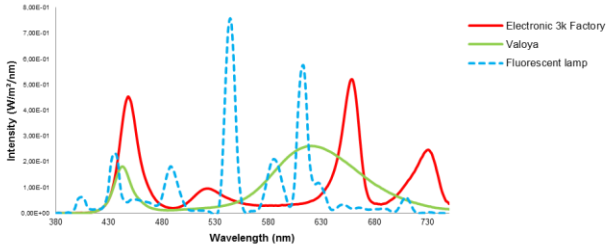
- special forms more expensive
- Luke's overheads included in price

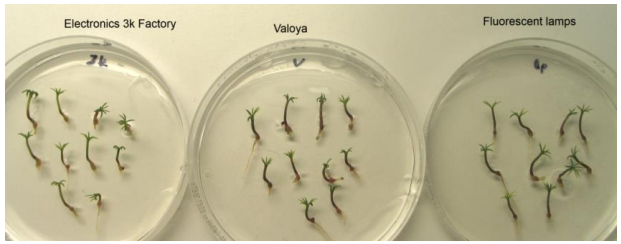
Research to improve cost-effectiveness & potential for mass-propagation

- **Database SOTKA**
- for controlling large amount of data on SE lines in cryostorage and production
- **LED lights**
- for saving energy (reducing costs)
- potential effects of varying light spectra and intensity
- **TIS bioreactors**
- **Automation & robotics**

Adjustable LED lights in SE propagation

..a possibility to change light spectrum (red, far-red, green, blue) and intensity





LEDs produce less heat, air-cooling still necessary.

Both standard spectrum LED lights and adjustable ones using Pulse-Width Modulation technique work; tested during SE:

Proliferation: no effect..

Maturation:

- no effect on embryo production but root:shoot ratio affected

Germination:

- emblings less curved under LEDs, root:shoot –ratios being studied

Early growth:

- Light intensity important!

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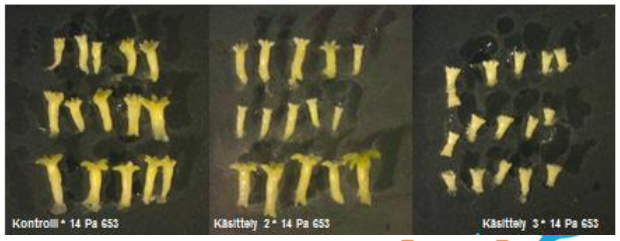
TIS bioreactors

- Several commercial models tested, further experiments with PlantForm
- Bioreactors not optimal:
  - sterilisation & maintenance requires a lot of manual work
  - space use is not efficient
  - plastic is not durable enough
- Proliferation works well, maturation requires a lot of optimization
- Conditions within bioreactor affect SE quality and germination



Paavilainen et al. Production of Norway spruce somatic embryos in TIS bioreactors. Ms in prep.

Automation & robotics

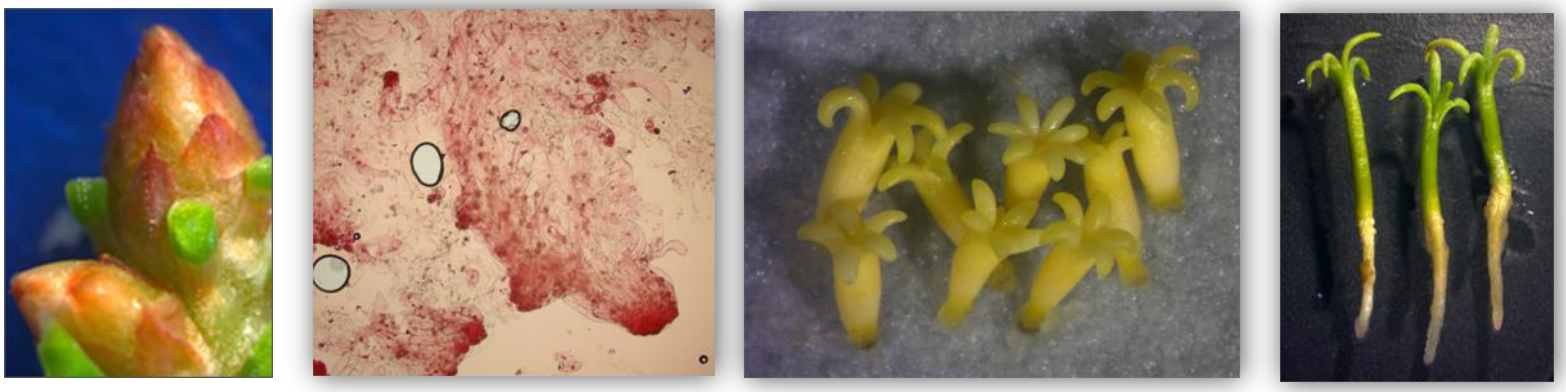
South-Eastern Finland
University of Applied Sciences

Project ”SmartTree – propagation technology for elite tree production” going on:

- XAMK develops technology for automated SE selection and transplanting, as well as designed biorector models
- Luke will test the devices, and study potential effect of automation on embling germination and growth

Would it be possible to propagate mature trees with known characteristics vis SE ?

- Promising results in *Pinus sylvestris*, no plant regeneration
- **Successfull, repeatable SE initiation, followed by plant regeneration from bud explants in *Picea abies***



- Trontin, Aronen, Hargreaves, Lelu-Walter, Montalban, Moncalean, Reeves, Quoniou, Klimaszewska (2016) International effort to induce somatic embryogenesis in adult pine trees. 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.
- Varis, Klimaszewska & Aronen (2018, ms in prep) Somatic embryogenesis and plant regeneration using primordial shoot explants of *Picea abies*

Summary & future views

- SE protocol based on manual labor works well, with developed protocol emblings can be grown in modern forest nurseries without extra adjustments
- Good amount of SE lines available, with wide genetic background
- First SE materials registered in 2017 as forest regeneration material; with first commercial pilot propagations running in 2018
- Close collaboration with breeding program, national authorities, and commercial plant producers is important
- Automation needed to reduce price of emblings
- Test plantations & wider genetic background in SE material needed to gain experience, and for proof of concept

Experimental plots followed

Openess & discussion needed

- Media presentations
- Visits & events for stakeholders
- Discussion within scientific community

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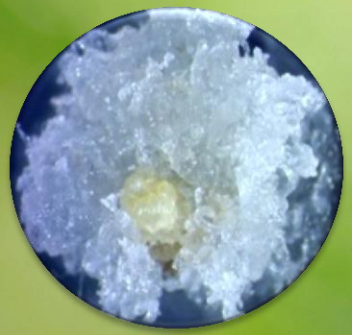
SE: Tuija Aronen, **Saila Varis, Mikko Tikkinen, Sakari Välimäki;** & Laura Paavilainen, Frida Lappalainen, Susanne Heiska, Laura Jaakola

Special forms: Teijo Nikkanen

Laboratory personnel: Airi Huttunen, Paula Matikainen, Kaija Porkka & Aila Viinanen

Luke' s field personnel

Collaborators: Taimityllilä, XAMK, UPM, UEF, SweTree, Valoya, Evira, Forest tree breeding program



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Thank You!

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