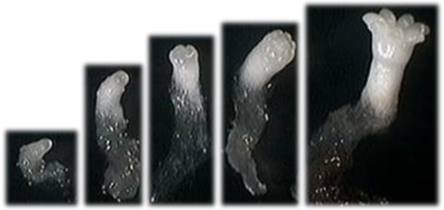
Midterm online Seminar, 17-18 Nov. 2020

Session 1 - Advanced forest management strategies



Developing somatic embryo

MULTIFOREVER (36 months, 2019-2022)

Towards intensification of conifer production through multi-varietal forestry based on somatic embryogenesis

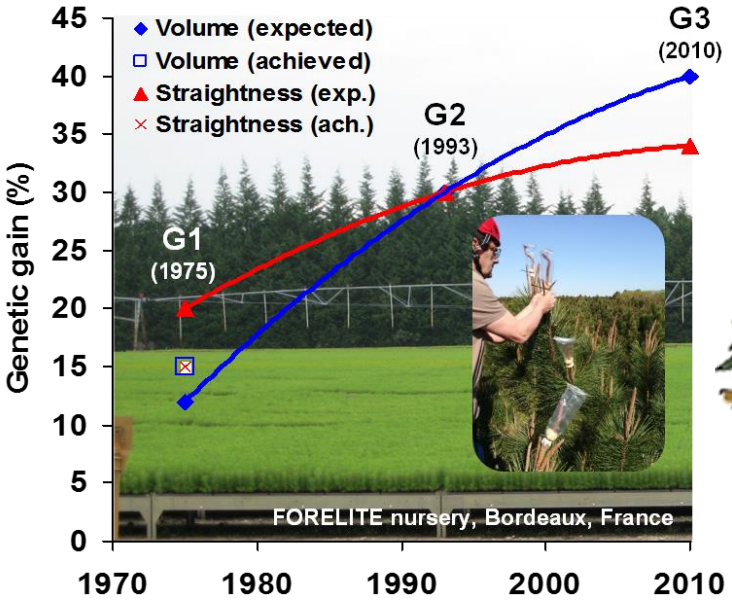
Coord. team | **J.-F. TRONTIN,** FCBA technological institute, France
A. RUPPS, J. RASCHKE, Humboldt Univ. Berlin, Germany

Wood biomass in intensively managed conifer plantations

Project MULTIFOREVER is supported under the umbrella of ERA-NET Cofund ForestValue by ANR (FR), FNR (DE), MINCyT (AR), MINECO-AEI (ES), MMM (FI) and VINNOVA (SE). ForestValue has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773324.

How to sustain productivity in plantation forests?

Investment in genetic breeding can pay off on multiple levels



Up to 10-20% genetic gain/cycle

Growth, form, disease resistance climate adaptability …

But a slow process: 1 cycle/10-15 years!

Y.S. Park 1998
(MVF concept in conifers)

It is essential to have a high-performance system for the deployment of selected varieties

1 elite tree of choice

1 seed

1 tree, UNKNOWN performance

SEED-BASED FORESTRY

Multiple trees, TRUE-TO-TYPE (clone)

Benefits of MVF:
- More genetic gain per cycle
- More flexibility

MULTI-VARIETAL FORESTRY (MVF)

➔ See MULTIFOREVER stakeholder-oriented article N°1, Mai 2020

How to sustain productivity in plantation forests?

Multi-varietal forestry based on clonal propagation of selected varieties

1 elite tree of choice

1 seed

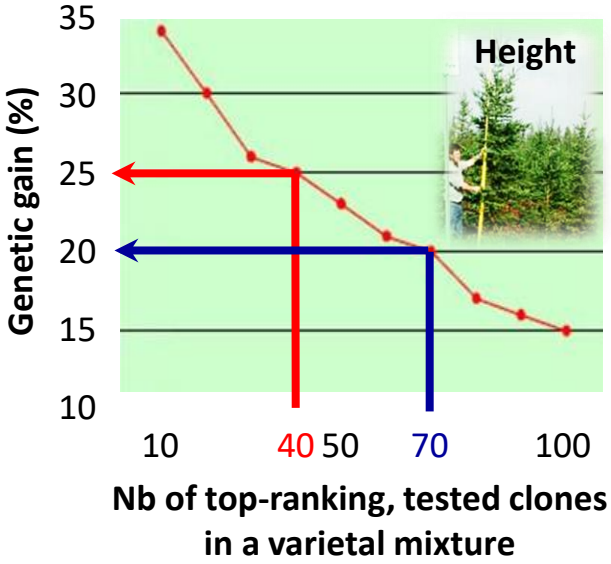
Multiple trees, TRUE-TO-TYPE (clone)

In addition to genetic gain, MVF allows to support genetic diversity of clonally propagated, elite varieties

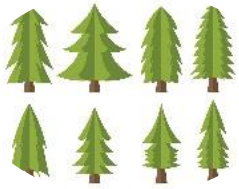
MULTI-VARIETAL FORESTRY (MVF)

= Our goal: productive AND sustainable plantation forestry

1- Define the genetic gain associated with mixtures of tested clones for a trait of interest



2- Set the necessary diversity in a clonal mix of elite, top-ranking clones



70 genotypes

20% gain

3- Optimize genetic gain by choosing the best combination of clones

Park et al. 2019 IUFRO 2.09.02 Proc. p. 230

Benefits: sustainable management of productive plantation forests!

Somatic embryogenesis (SE)

A promising technology to enable multi-varietal forestry in conifers

1 elite tree of choice

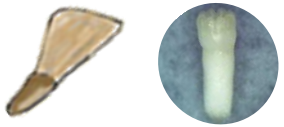
1 seed

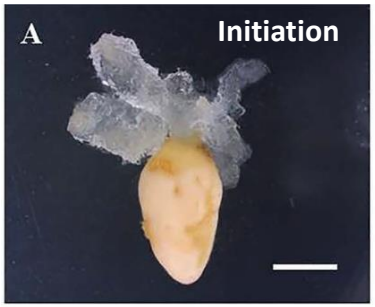
Multiple trees, TRUE-TO-TYPE (clone)

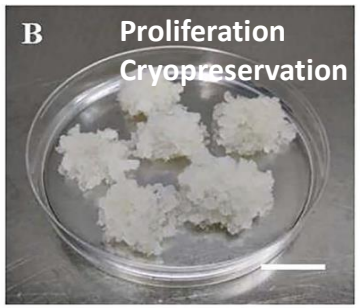
MULTI-VARIETAL FORESTRY (MVF)

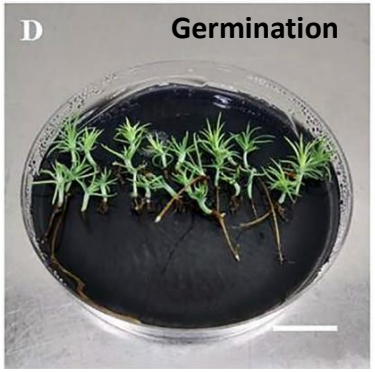
Based on SE in conifers

The *in vitro* process is currently starting from immature seeds









Still challenging from vegetative explants of trees

That's where our project starts!
Selected example/WP to follow …

WP1	WP2	WP3	WP4
SE from Trees	SE from seeds	Field trials	Up scaling

Castander-Olarieta et al. (2020). Tree Physiology (in press)

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WP1: towards SE from trees – '*The Holy Grail*'

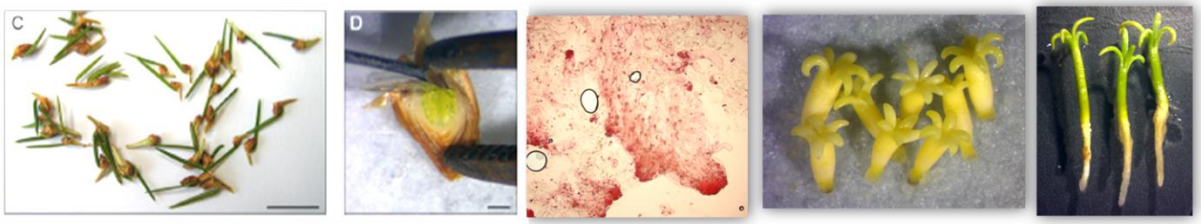
Understanding how and why initiation of SE is possible

Usually, **SE cultures are initiated from seed embryos**

It would make a big difference, **if cultures could be initiated from older trees**, **using e.g. their buds** as explants.

This would allow **propagation of trees with known characteristics**.

This has been successfull in Norway spruce ...



... and now we are studying **genetic factors** affecting SE initiation to be able to better understand what triggers the process and enhance it!

We are especially investigating **gene expression at the very start of SE (cell level).**

Benefits: New, adapted varieties available more quickly.

Varis S, Klimaszewska K, Aronen T (2018) Somatic embryogenesis and plant regeneration from primordial shoot explants of *Picea abies* (L.) H. Karst. somatic trees. *Front. Plant Sci.* 9:1551.

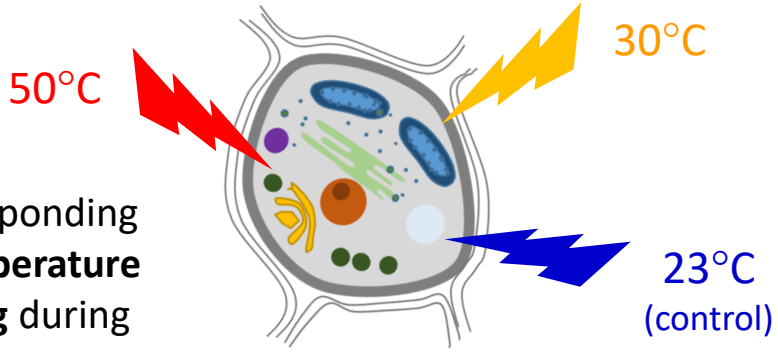
WP2: optimizing SE from seeds – *'Streamline the process!'*

Early SE as a target for temperature-induced priming effects

Priming a cell with temperature can lead to <u>delayed effects</u> on drought stress tolerance (radiata pine)

⇨ Trees can express some kind of <u>memory of stress</u>!

Potential benefits to breeders:
- Priming trees to induce new traits!
- Useful to cope with climate change.

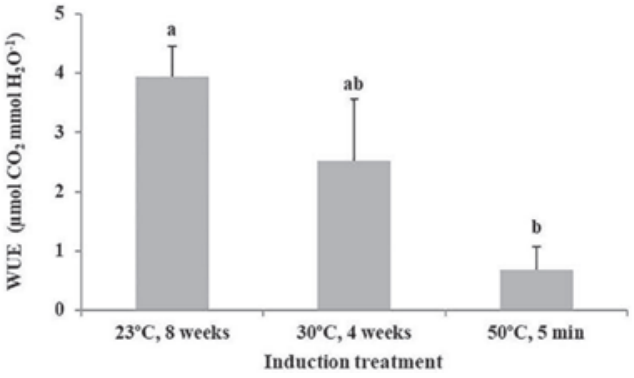


Cell responding to **temperature priming** during SE initiation

Cross-talk between plant hormone and epigenetics
➔ Cytokinins involved

Altered tolerance of SE plants to drought stress months/years later

Temperature priming affects Water Use Efficiency of regenerated SE plants (2-yr-old)



<u>MULTIFOREVER scientific production</u>

Priming during SE initiation:
- Castander-Olarieta et al. (2020) Tree Physiol (in press)
- Castander-Olarieta et al. (2020) Trees Struct Func (in press)
- Trontin JF, Raschke J, Rupps A (2020) Tree Physiol (in press)

Priming during SE maturation:
- Marques do Nascimento et al. (2020) 11, 1181

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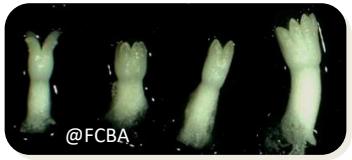
Castander-Olarieta et al. (2019) Front Plant Sci 9:1–16

WP2: optimizing SE from seeds – *'Streamline the process!'*

Somatic embryo vs zygotic embryo: biochemical analysis

When is the best time to germinate a somatic embryo in regard to its storage compounds?

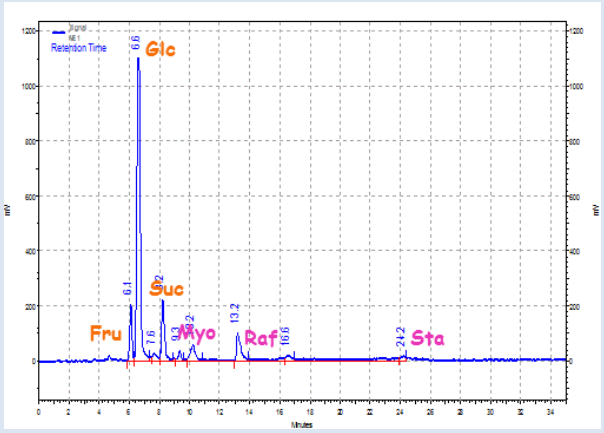
SE

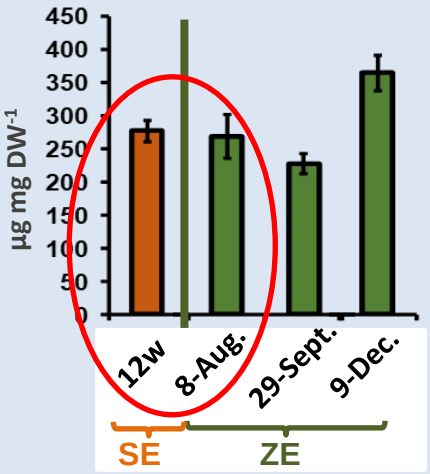


Maturity?

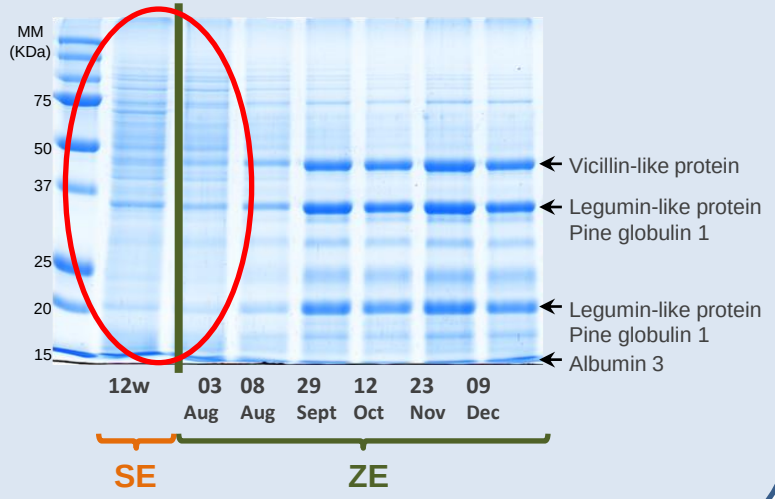
ZE from seed (reference)

Carbohydrate levels





Protein levels



Large cooperation among partners For different species

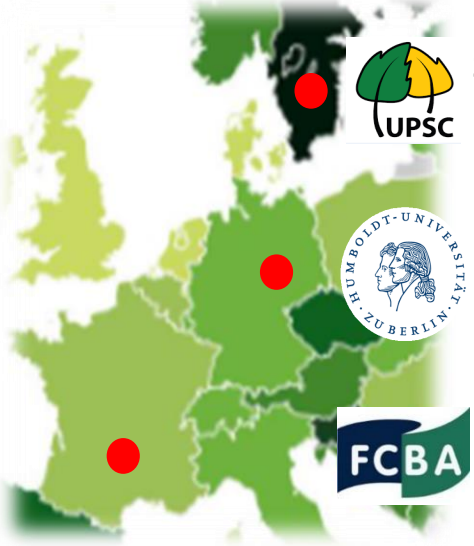
INRAE

HUMBOLDT-UNIVERSITÄT ZU BERLIN · INTA · Luke NATURAL RESOURCES INSTITUTE FINLAND · NEIKER MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

Benefits: Towards high-quality SE plants for new variety deployment.

WP3: field trials based on SE – *'It's the mix that matters!'*

Exchange of Douglas-fir clones: from Germany to France and Sweden



Southern Sweden

April 20

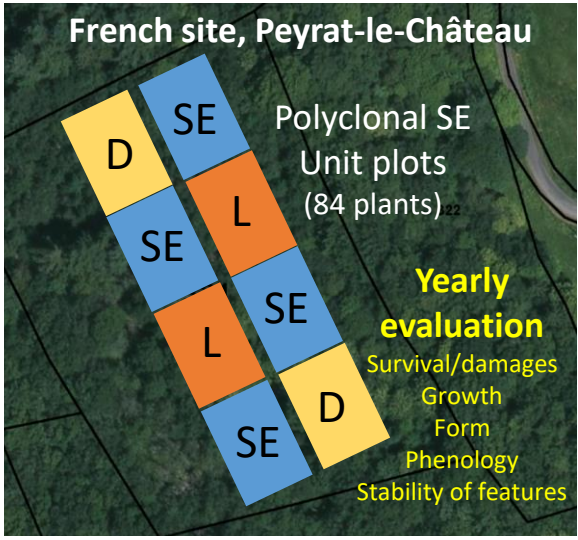
Germany

March 20

Central France

14 SE clones in mixture
From directed crossings
(2-3 –yr-old plants)

Seedling ref.
German: Daun (D) –all 3 sites
French: Luzette (L) – all 3 sites
Finnish: Kouvola – Sweden



08 April 2020: Arrival of the German ready-to-plant Douglas-fir SE plants and seedling standard (Daun) in Peyrat-le-Château

22-23 April 2020: Planting by FCBA

25 June 2020: Trial in Peyrat-le-Château, 2 months after planting (high survival following a rainy spring)

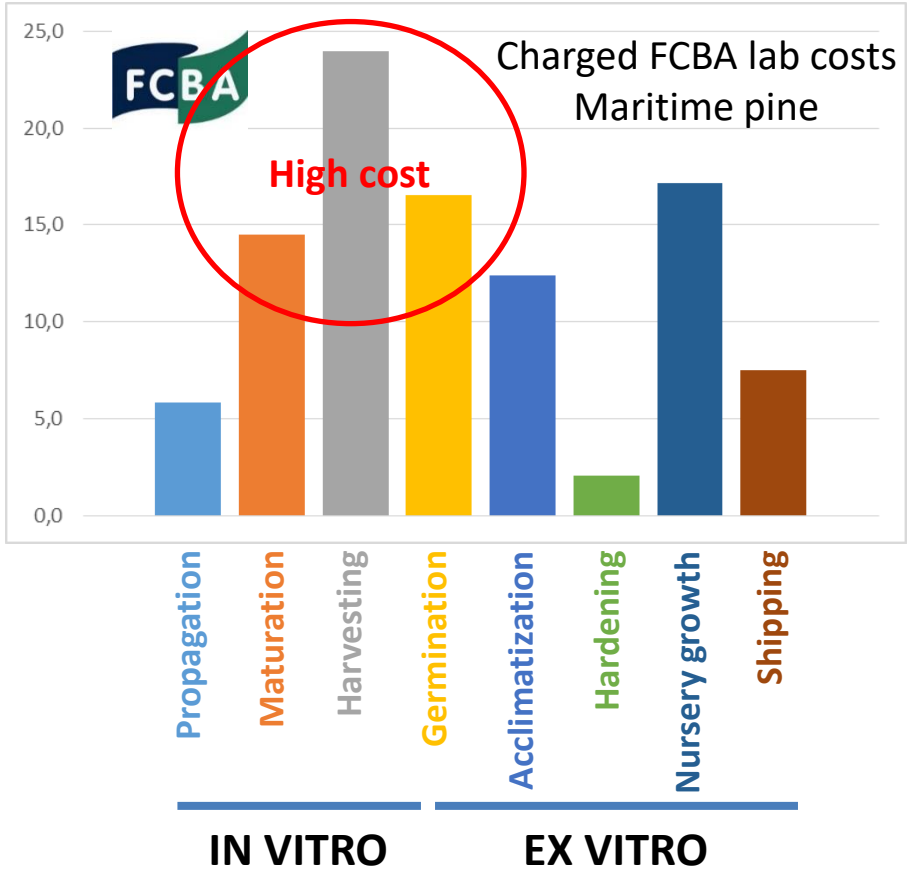
Benefits: Somatic seedlings at field as the first cross-European perspective for conifer multi-varietal forestry!

➔ See MULTIFOREVER stakeholder-oriented article N°2, Aug. 2020

WP4: Scaling-up & automation – *'Who will make the big bucks?'*

Towards cost-effective SE plant production for MVF

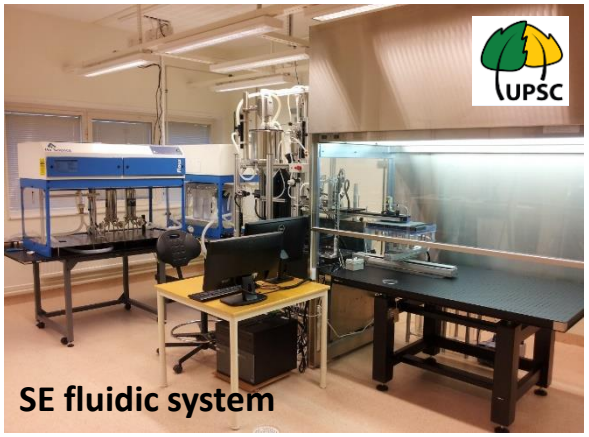
Contribution (%) of each SE step to the production cost of a deliverable somatic plant



Need for improved *in vitro* culture techniques and automated process for conifers

Bioreactors make the SE process more effective and up-scaled (e.g. **maturation**, **germination** steps)

Automation reduces the need for manual labor (e.g. **embryo harvesting & selection**)



Benefits: Significant reduction in the production costs of somatic plants to make it competitive with that of a conventional seedling

Does Covid19 interfere with our plans?

Force Majeure initiated (Cons. Agreement #5.4): March/April 2020

WP1 SE from trees | WP2 SE from seeds | WP3 MVF trials | WP4 Up scaling | WP5 Communication | WP6 Management

<u>Consequences</u>: Lock-down of the project; not overcome within 6 weeks after notification:

- **Culture regime and management interrupted** – start from anew (WP1,2,3)
- **Evaluation impossible** – repetition of experiments (WP2)
- Personal communication missing (WP5)
- Borders closed, **transport restricted** (WP3)
- **Dissemination delays** (technical/field days, workshops, WP5)
- Home office limitations (publications, WP5)
- **Canceled researcher exchange** (WP1,2,4)
- Some **budget cuts** (WP6)

Best-practices implemented to minimize its effects:

- ✓ Asap **repetition** of affected experiments (WP1,2,3)
- ✓ **Transatlantic cooperation** for seasonal experiments (WP2)
- ✓ Tele meetings (WP5)
- ✓ **Successful plant transfer** despite lock-down limitations (WP3)
- ✓ Home work (microscopy, evaluation) (WP1,2)

Extension of 6 months granted by ANR to the French Parties

Some project extension now advisable for the whole Consortium

Current end date (Consortium): 31 march 2022

WP4: Exploitation of results – *'Who will make the big bucks?'*

Exploring and addressing the market

Cost-effective, automated SE plant production

FACTORY SE

- **Affordable plants with tailored characteristics**
  - Improved productivity
  - Site adaptability
  - Pest resistance
  - Drought tolerance
- **Reliable year-around plant production**
  - Independent of environmental conditions

Our SE system is already well advanced!

High **benefits** to breeders, plant producers & forest growers!

We aim to address the conifer tree market

SE TREE MARKET

- Detailed **market analysis**
- Development of a **business plan**
- Conceptualisation of a **pilot SE plant production facility**

Tools (under way)

- **Surveys** to understand stakeholders needs
  Ongoing: Swedish survey; **thereupon**: EU survey
- **Expanding our stakeholders group** to strengthen our efforts towards an SE factory

So far our transnational, transatlantic support (6 countries) consists of:

Welcome to join our stakeholder group! Your participation is valuable for our future forest systems!

11 Supporters

6 Funding agencies

Thank you for your attention!

Thank you to the whole MULTIFOREVER team!

FCBA - Trontin JF, Gallou A
HUB - Rupps A, Raschke J
INRAE - Lelu-Walter MA, Teyssier C, Poitelon C
INTA - Gauchat ME, Vera Bravo C, Boleso MA
LUKE - Aronen T, Varis S, Tikkinen M
NEIKER - Moncaleán P, Montalbán I, Ziluaga Amigó I
UPSC - Egertsdotter U, Dedicova B, Dobrowolska I, Ranade S., Street N, Strömberg A-K

If you're interested in further details or support - please contact us!

We are looking for both scientific and strong, practical-oriented collaborations and support for implementation of multi-varietal forestry in conifers based on somatic embryogenesis!

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