MULTIFOREVER:

What has changed with it – scientific results, economic impact, communication with public and what should be next





MULTIFOREVER

ForestValue

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.

Presented by Ander Castander-Olarieta

MULTIFOREVER - Project Profile:

(original) participating partners:
- 7 experienced teams
- from 6 countries

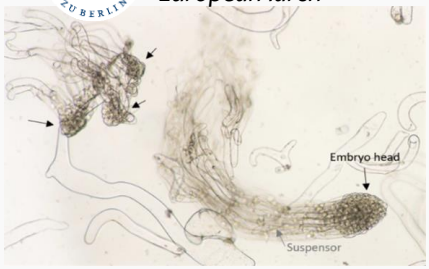
project duration:
- 01.04.2019 - 31.10.2022

no. of species in project focus:
- 8 important conifer species

(originally planned) total budget:
- 2,033,579 €

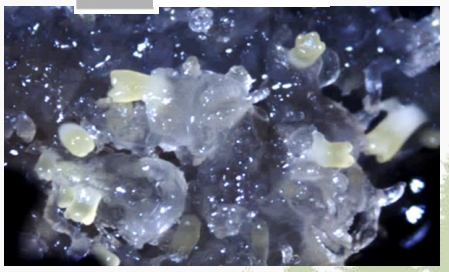
(originally planned) requested budget:
- 1,344,205 €

Douglas fir
Hybrid larch
European larch



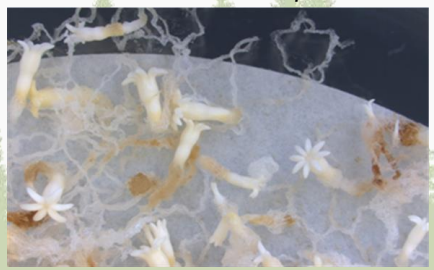
Humboldt-Universität zu Berlin, Germany
Contact: Andrea Rupps (coord.)

Loblolly pine
Hybrid pine



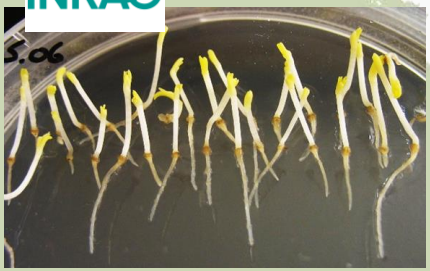
Instituto Nacional de Tecnología Agropecuaria, Argentina
Contact: Maria-Elena Gauchat

Radiata pine



Neiker-Tecnalia Basque Institute for Agricultural Research and Development, Spain
Contact: Paloma Moncaleán

Hybrid larch



Institut national de la recherche agronomique, France
Contact: Marie-Anne Lelu-Walter

Norway spruce

Natural Resources Institute Finland
Contact: Tuija Aronen

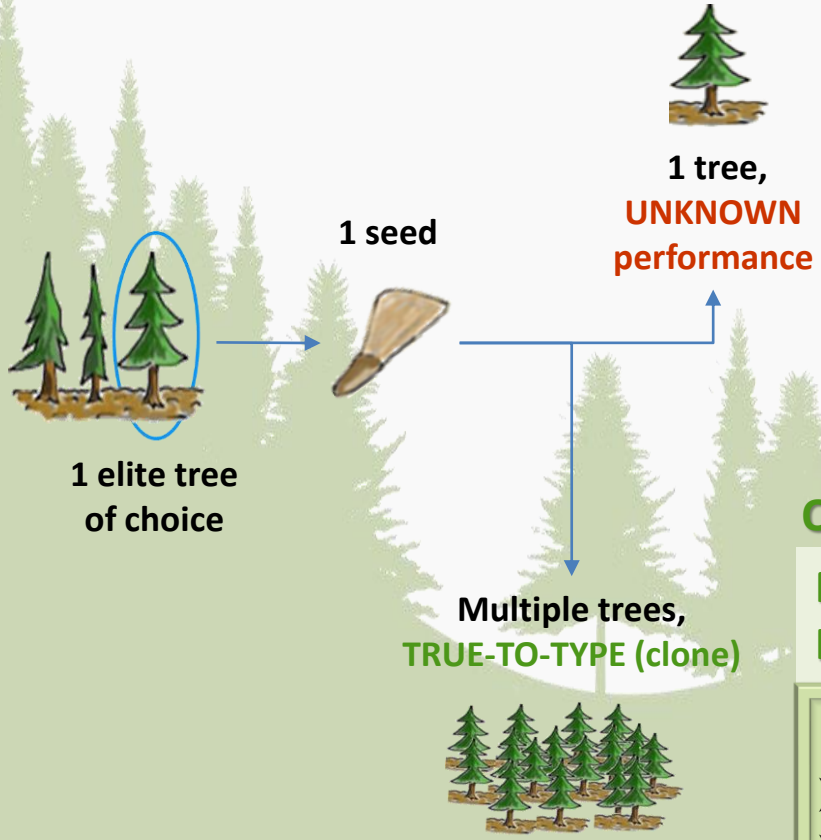
Swedish University of Agricultural Sciences
Contact: Ulrika Egertsdotter

Douglas fir
Loblolly pine
Maritime pine

French Institute of Technology for Forest-based and Furniture Sectors
Contact: Jean-François Trontin

How to sustain productivity in plantation forests?

Required: High-performance system for the deployment of selected varieties
Solution: Multi-varietal forestry (MVF) based on clonal propagation of selected varieties



Classical:

SEED-BASED FORESTRY

OUR APPROACH:

MULTI-VARIETAL FORESTRY (MVF):

Benefits of MVF:

- More genetic gain per cycle
- More flexibility and diversity
- 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

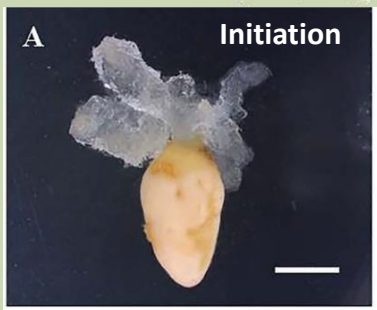
Multiple trees, TRUE-TO-TYPE (clone)

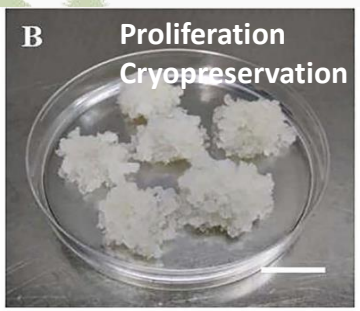
MULTI-VARIETAL FORESTRY (MVF)

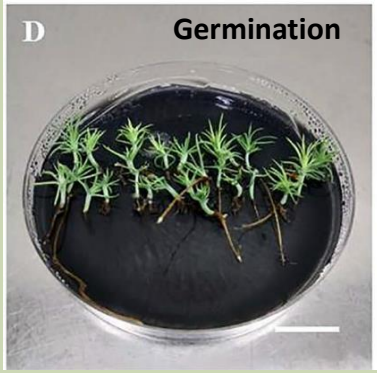
Based on SE in conifers

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

Still challenging from vegetative explants of trees







MULTIFOREVER intends to improve the status on all fronts of the *in vitro* process somatic embryogenesis and multi-varietal forestry:

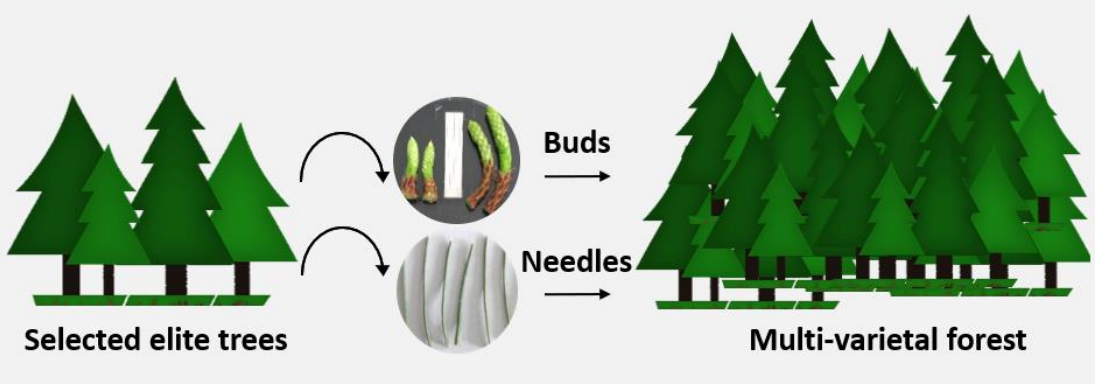
WP1	WP2	WP3	WP4
SE from trees	SE from seeds	Field trials	Up scaling

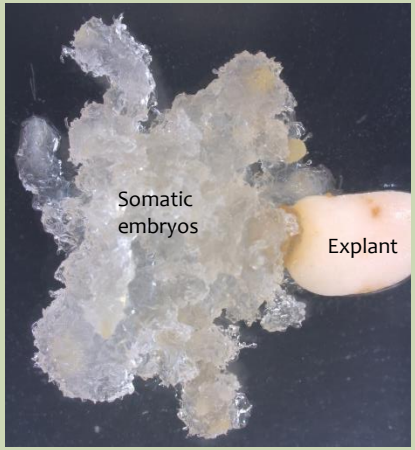
➢ Castander-Olarieta et al. (2020). Tree Physiology

WP1: Somatic embryogenesis from trees

Holy grail in conifer propagation…
… direct cloning of parental trees

- 3 ways to approach subject of initiation:
  - Identify mechanism
  - Initiate embryogenesis on "adult" material
  - Test effect of certain target genes

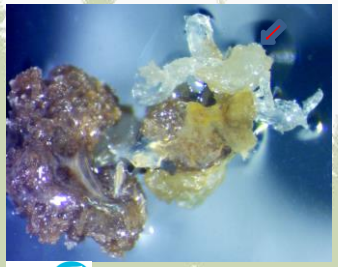




Pinus radiata

WP1: Somatic embryogenesis from trees

Results from Spanish and Finnish partners show hints that mechanism is fundamentally available



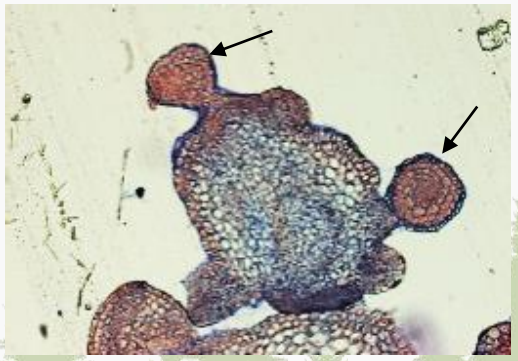
Picea abies : Somatic embryogenesis initiated from bud explants of somatic trees and regenerated to somatic seedlings

Pinus radiata: Shoots and roots coming from organogenesis from adult buds

- Pereira C, et al. (2021) Regeneration of *Pinus halepensis* (Mill.) through organogenesis from apical shoot buds. Forests
- Montalbán IA, et al. (2020). Use of biotechnology in forestry breeding programs for natural resources and biodiversity conservation: creating super trees for the future. *In* Chong P.A., Newman D.J., Steinmacher D. (Eds.) Agricultural, Forestry and Bioindustry Biotechnology and Biodiscovery.
- Multiforever ForestValue Newsletter No. 7

WP1: Somatic embryogenesis from trees

Using transgenesis to study the role of target genes for initiation



- Overexpression of conifer embryogenesis related gene *WOX2* (*WUSCHEL-related HOMEOBOX 2*) in Arabidopsis triggers somatic embryogenesis
- Plants are able to develop into plantlets in the next generation

➢ Hassani B, et al. (2022). Constitutive Overexpression of a Conifer *WOX2* Homolog Affects Somatic Embryo Development in *Pinus pinaster* and Promotes Somatic Embryogenesis and Organogenesis in *Arabidopsis* Seedlings. Frontiers in Plant science

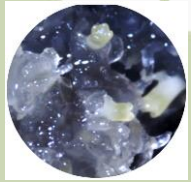
WP2: Somatic embryogenesis from seeds

Reducing *in vitro* bottlenecks

Tackling issues such as:

- Initiate somatic embryogenesis & recalcitrant tissues
- Offering medium and long term storage options - at reduced cost
- Stabilise maturation results
- Target parameters influencing yield and quality
- Improve conversion into plantlets

Initiation
Multiplication



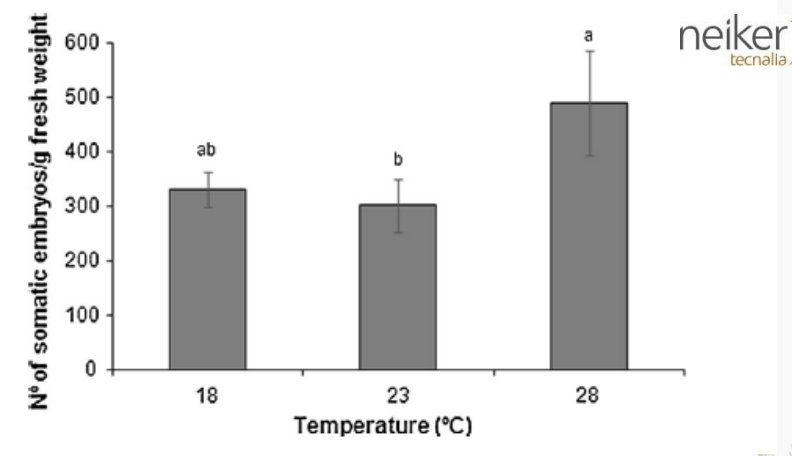
Maintenance &
Cryopreservation

Maturation

Germination & Acclimatization

WP2: Somatic embryogenesis from seeds

Analysis of factors impacting on initiation and subsequent development
- temperature -



Pinus radiata

- Initiation percentage lowest at highest temperature
- But number of somatic embryos highest, when initiated in high temperature

- do Nascimento A.M.M., Polesi L.G., Back F.P., Steiner N., Guerra M.P., Castander-Olarieta A., Moncaleán P., Montalbán I.A. 2021. The chemical environment at maturation Stage in Pinus spp. Somatic Embryogenesis: Implications in the Polyamine profile of somatic embryos and morphological characteristics of the developed plantlets. Frontiers in Plant Science
- Pereira C., Castander-Olarieta A., Sales E., Montalbán I.A., Canhoto J., Moncaleán P. 2021. Heat stress in Pinus halepensis somatic embryogenesis induction: effect in DNA methylation and differential expression of stress-related genes. Plants
- Castander-Olarieta A., Pereira C., Mendes V., Correia S., Manadas B., Canhoto J., Montalbán I.A., Moncaleán P. 2022.Thermopriming-associated proteome and sugar content responses in Pinus radiata embryogenic tissue. Plant Science
- do Nascimento A.M.M., Montalbán I.A., Llamazares D., Goicoa T., Ugarte M.D., Moncaleán P. 2022. High temperature and water deficit cause epigenetic changes in somatic plants of Pinus radiata D. Don. 2022. Plant Cell Tissue and Organ culture
- Castander-Olarieta A., Pereira C., Montalbán I.A., Mendes V.M., Correia S., Suárez-Alvárez S., Manadas B., Canhoto J., Moncaleán P. (2021). Proteome-wide analysis of heat-stress in Pinus radiata somatic embryos reveals a combined response of sugar metabolism and translational regulation mechanisms. Frontiers in Plant Science
- Castander-Olarieta A, Pereira C, Sales E, Mendes VM, Manadas B, Correia S, Arrillaga I, Canhoto J, Goicoa T, Ugarte MD, Montalbán IA, Moncaleán P (2020). Induction of radiata pine somatic embryogenesis at high temperatures provokes a long-term decrease in DNA methylation/hydroxymethylation and differential expression of stress-related genes. Plants
- Trontin JF, Raschke J, Rupps A (2021). Tree "memory": new insights on temperature-induced priming effects during early embryogenesis. Tree Physiology 41(6): 906-911. Invited Commentary on the Castander-Olarieta et al. paper accepted in Tree Physiology
- do Nascimento AMM, Alves Barroso P, Ferreira do Nascimento NF, Goicoa T, Ugarte MD, Montalbán IA, Moncaleán P (2020). Pinus spp. somatic embryo conversion under high temperature: effect on the morphological and physiological characteristics of plantlets. Forests

WP2: Somatic embryogenesis from seeds

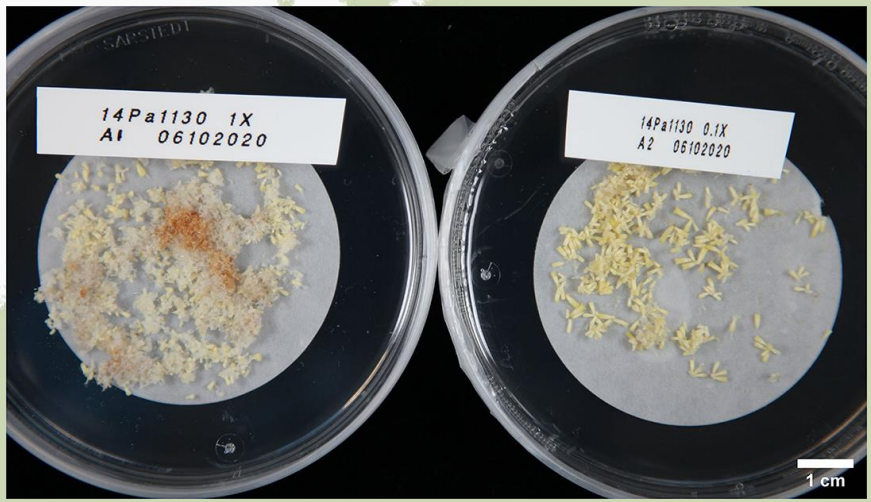
Analysis of factors impacting on multiplication, maturation and subsequent development

- phytohormones, stress response and liquid culture -

Picea abies

- Suitability of amount of phytohormones in liquid culture (in comparison to semi solid media) analyzed on:
  - Growth rate
  - Good-quality cotyledonary embryo yield
  - Somatic plantlet survival in a greenhouse
  - Indicators of stress conditions:
    - In suspension culture:
      - hydrogen peroxide (H_2O_2) content elevated
      - guaiacol peroxidase activity increased

Suspension culture prior maturation treated with phytohormones



Full phytohormones (1 x) vs (0.1 x)

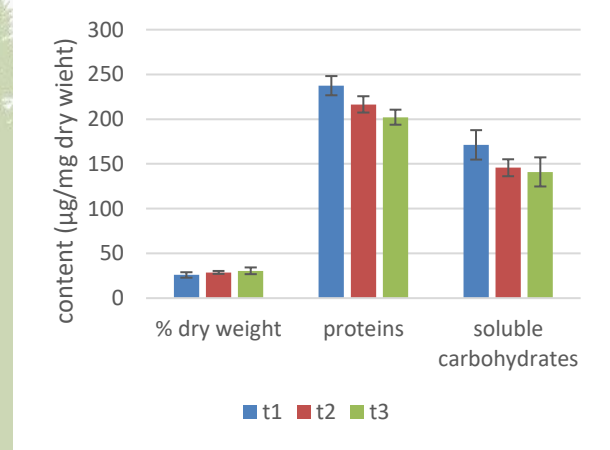
WP2: Somatic embryogenesis from seeds

What is the optimal time to acclimatize somatic embryos according to their storage compound composition ?

- The duration of maturation for somatic embryos influences the quality and the potential vigour of resulting seedlings.
- The quality is determined in terms of storage compound analyses.

Protein content	Df	SumSq	Fvalue	Pr(>F)
maturation	4	64133	42.9	7.07e-16 ***
genotype	2	8550	11.4	7.63e-05 ***

Pinus radiata
- storage compounds: analysis of proteins and carbohydrates



WP2: Somatic embryogenesis from seeds

Is cold storage a possibility to preserve embryos and improve their quality / quantity ?

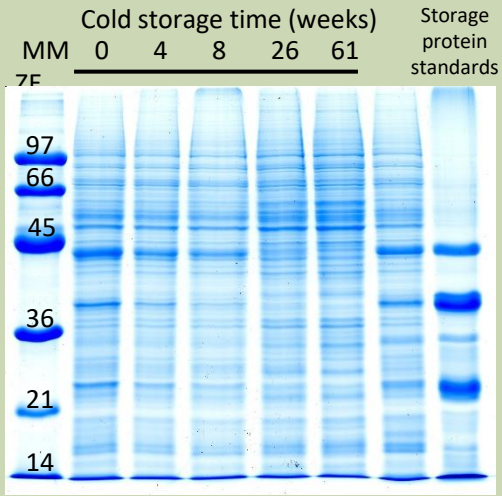
The ability to store mature embryos makes large-scale production more flexible and calculable - and can predict the germination capacity in greenhouse.

- What are the consequences of cold storage on the embryo quality?

  Cold storage also improves SE yield, due to post-maturation development taking place in non-synchronized cultures.

- Which storage time is acceptable for more production flexibility?
  - 4-8w : the storage compound composition of SE is closer to that of ZE, **improving the greenhouse survival** of SE.
  - 26-61w : **the storage is not detrimental** and can be used if needed to even workload in mass-propagation.

Picea abies
embryo protein profile (SDS page)



WP3: Field trials

Having something to show for … in 10 years time

Approach this by:

- Exchanging clones of different species amongst partners to test transferability of production line
- Plan and plant field trials with and of clonal / standard mixtures
- Evaluate these and existing fields

WP3: Field trials

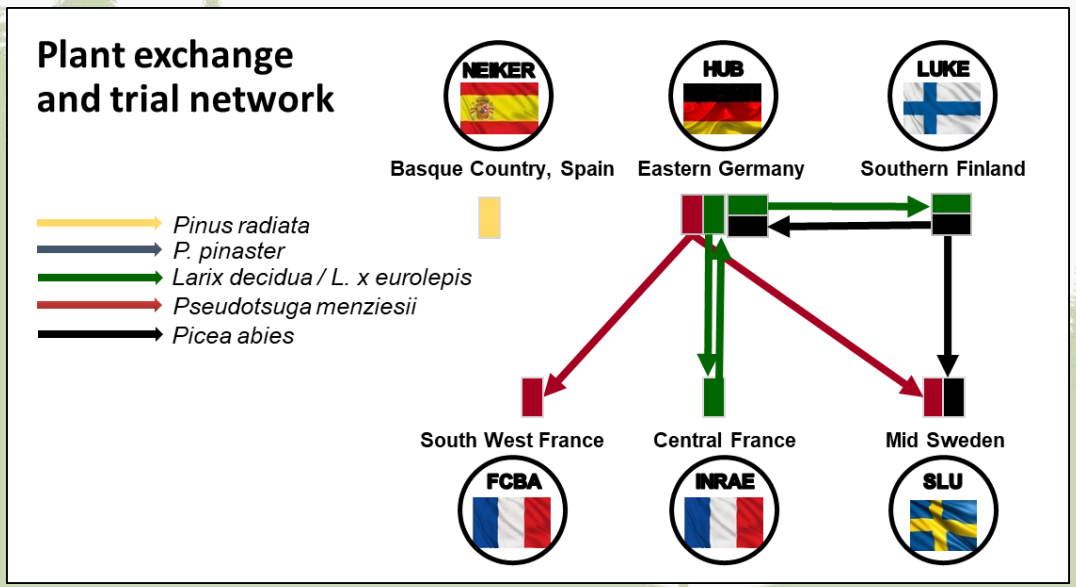
Planning for demonstration

Existing somatic embryogenesis plantations in France and Finland

Project partners (HUB, FCBA, LUKE) gathering at an experimental reforestation site of somatic trees in Finland, 2019.

WP3: Field trials

Planning for demonstration



3 years of MULTIFOREVER – adapted exchange plan (5 species; 11 demo sites)

Picea abies

Pinus radiata

Pinus pinaster

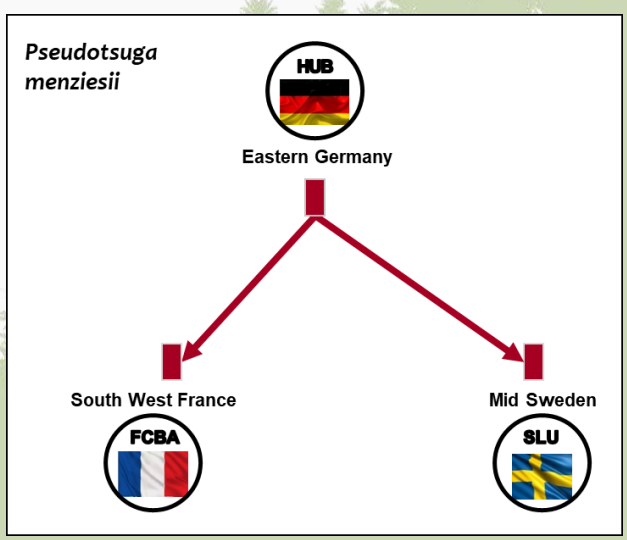
Ready to plant somatic plantlets

WP3: Field trials

Spring 2020

Corona and organizing a European ready-to-plant exchange

Planting of Douglas-fir somatic seedlings on the French site near Peyrat-le-Château (with the assistance of ONF-PNRGF).



WP4: Up-scaling

How to scale from research sized lots up into production lines

– Upscaling and approaching the market –

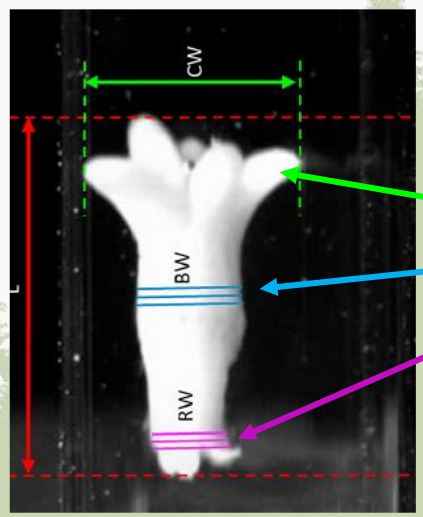
What are the major obstacles :

- Up-scaling from research purposes to production line
- Reducing cost – by reducing manual labour
- Approaching a market – Identifying readiness and obstacles to developing pilot facilities

WP4: Up-scaling

Reducing cost – by reducing manual labour

developing an automation unit



Measuring width of:

Cotyledons

Body

Root

- Selecting well developed embryos for germination

WP4: Up-scaling

Reducing cost

– By reducing manual labour developing an automation unit

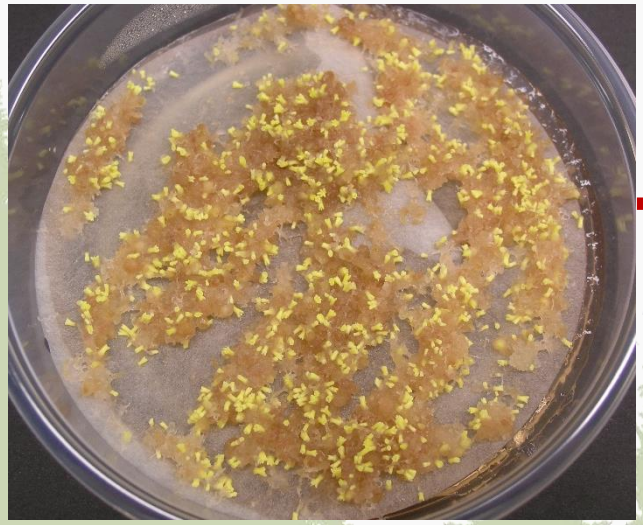


Plate with mature somatic embryos (Douglas Fir)

Plate with germinated somatic seedlings (Radiata Pine)

- Germination is the most expensive step of in vitro production

- Le K-C et al. (2021. Evaluation of parameters to characterize germination-competent mature somatic embryos of Norway spruce (*Picea abies*). Biosystems Engineering
- MULTIFOREVER upcoming ForestValue Newsletter No. 9; Young researcher exchange – HUB → SLU

WP4: Approaching a market

Identifying readiness and obstacles to developing pilot facilities

Finnish survey to assess perception of forest owners and professionals towards tree breeding and vegetative propagation

→ Response mostly by forest owners

→ Low response rate, but still nearly 3 000 responses

- respondents were asked about risks of vegetative propagation, and which characteristics should be improved

→ Some willingness to pay more for improved forest reproductive material recognized

Table 4. Risks related to vegetative propagation asked from the respondents in the survey in the survey of perceptions towards tree breeding and vegetative propagation, the mean values of answers on scale from one (not at all important) to four (highly important) overall presented with a standard deviation, and the proportion of respondents answering 'Cannot say'.

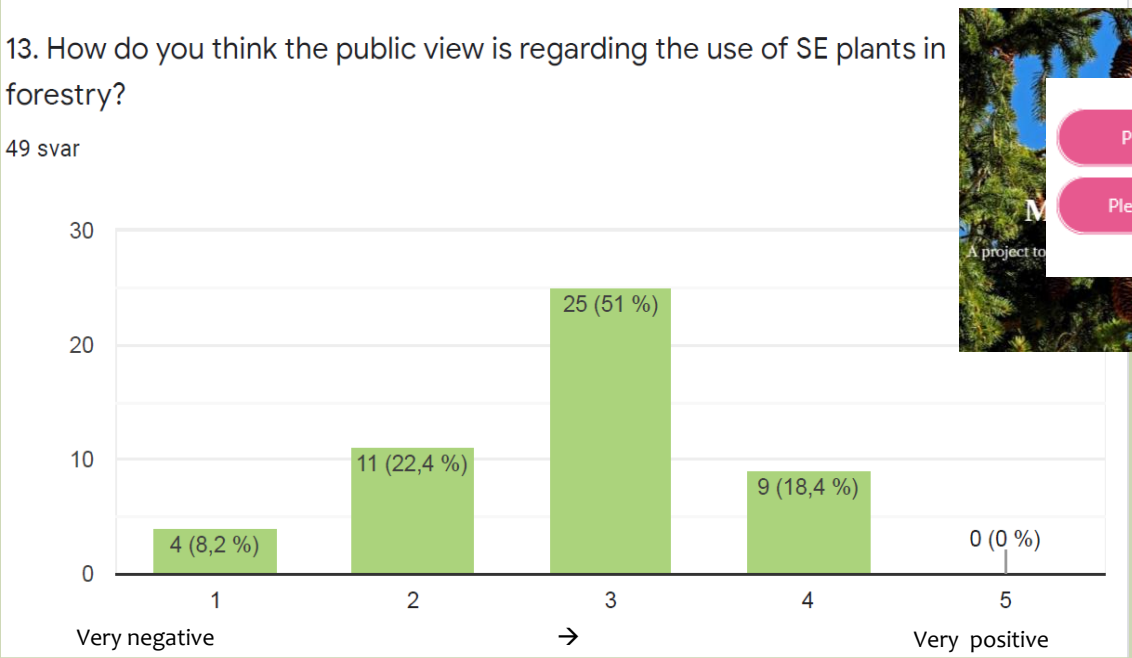
Risk description	Mean	Std. Dev.	Cannot say, %
Depletion in genetic diversity	3.01	0.76	5.90
Unknown risks related to vegetative propagation	2.95	0.80	12.70
Prejudices and false perceptions related to vegetative propagation	2.89	0.75	8.90
Too limited an amount of information available on vegetative propagation	2.88	0.76	9.70
Negative perception towards vegetative propagation in society	2.72	0.81	8.00
Relatively small gain when contrasted to the cost of vegetative propagation	2.70	0.79	23.30
Technology is not considered reliable on an industrial scale	2.66	0.70	13.40
Prejudices towards vegetative propagation in my industry sector	2.59	0.76	12.00

➢ Tikkinen M, et al. (2021). Interest in vegetatively propagated Norway spruce materials – a survey among Finnish forest owners and professionals. Silva Fennica

WP4: Approaching a market

Identifying readiness and obstacles to developing pilot facilities

Swedish survey developed to assess basic perception of European forest owners and forestry professionals



You are still warmly invited to participate!

WP5: Communication with public

Raising awareness of subject and for our research…

…to receive more commitment from forestry stakeholders and promote future consortium activities towards commercial application

- Present progress appropriately to research community
- Offer demonstration of material, plants and laboratories (technical tours)
- Delivering information to divers audiences
  - Webpage, brochure
  - Newsletter
  - Stakeholder-oriented articles

Please visit our project homepage at:

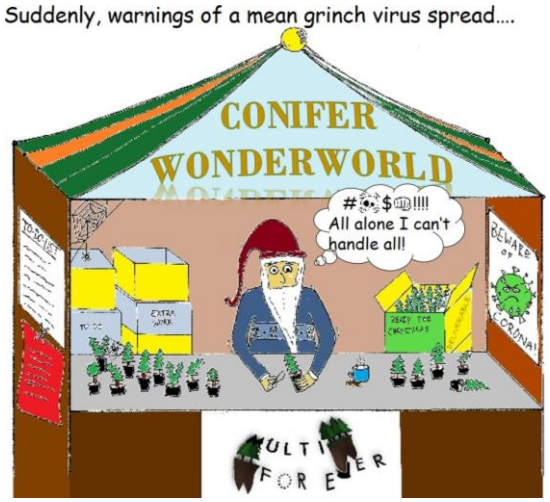
www.multiforever.com

Policy makers visiting NEIKER labs in May 2022.

Interacting with the public,
Long Night of Sciences, July 2022.

WP6: Networking

Develop a tight network



Share knowledge and expertise and solve problems together to further somatic embryogenesis in research and production

- Protocols shared
- Young researchers visited partners labs:

  → Countries with less open forestry (southern countries) towards somatic embryogenesis benefited from cooperation with rapidly advancing Nordic countries.

What should be next?

- Continue working together to combat global warming with expertise from and in our field
  - Elucidate regulatory mechanisms on initiation and embryogenicity
  - Narrow gap north-south: status and acceptance
  - Reduce bottlenecks in plant production
  - Keep building clone collections with defined material (stress tolerant, superior growth characteristics, …) for many species
  - Define site adapted clonal mixtures
- Increase transparency for public - to further understanding and acceptance of somatic embryogenesis, clones, multi-varietal forestry, …by
  - Raising awareness with demonstration fields
  - Communication with users, policy makers and public

Website: www.multiforever.com
Contact: acastander@neiker.eus
andrea.rupps@hu-berlin.de (coord.)

Thank you for your attention!

MULTIFOREVER team visiting larch plots at the final conference in Orléans, France, June 2022.

ForestValue

Website: https://forestvalue.org/
Twitter: https://twitter.com/ForestValue2017
LinkedIn: https://www.linkedin.com/groups/12110816/