Cryopreservation of black, red and white currant using dormant buds and in vitro recovery

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Luke

Luke in brief

85 M€ State budget funding

151 M€ Turnover

66 M€ External funding

22 **Locations** head office in Helsinki, Viikki

1334 employees

648 Scientists

639 Experts*

47 Research professors

Vision

A sustainable future and well-being from renewable natural resources.

Mission

Through research, we create value and solutions for our customers by solving local and global challenges

*Employees in service groups and research infrastructure servicesas well as directors

Figures updated: 31 December 2023

22 locations

Our head office is located in Helsinki, Viikki. We have operations in 12 campuses with universities, research institutes and polytechnics

Locations

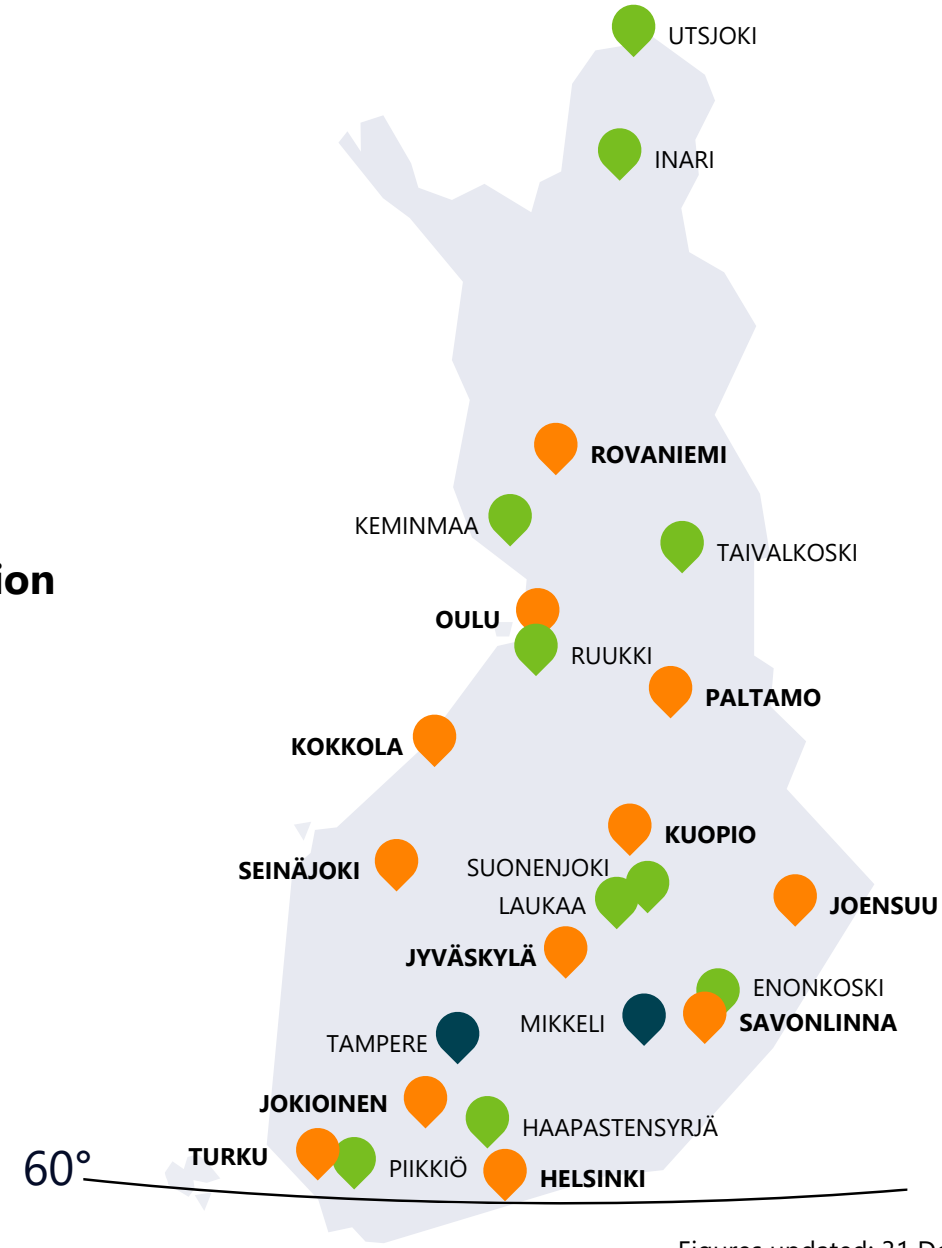
Helsinki
Jokioinen
Joensuu
Oulu
Jyväskylä
Kokkola
Kuopio (Maaninka)
Paltamo
Rovaniemi
Savonlinna
Seinäjoki
Turku

Experimental stations

Enonkoski
Haapastensyrjä (Loppi)
Inari
Keminmaa
Laukaa
Piikkiö (Kaarina)
Ruukki (Siikajoki)
Suonenjoki
Taivalkoski
Utsjoki

Research cooperation sites

Mikkeli
Tampere



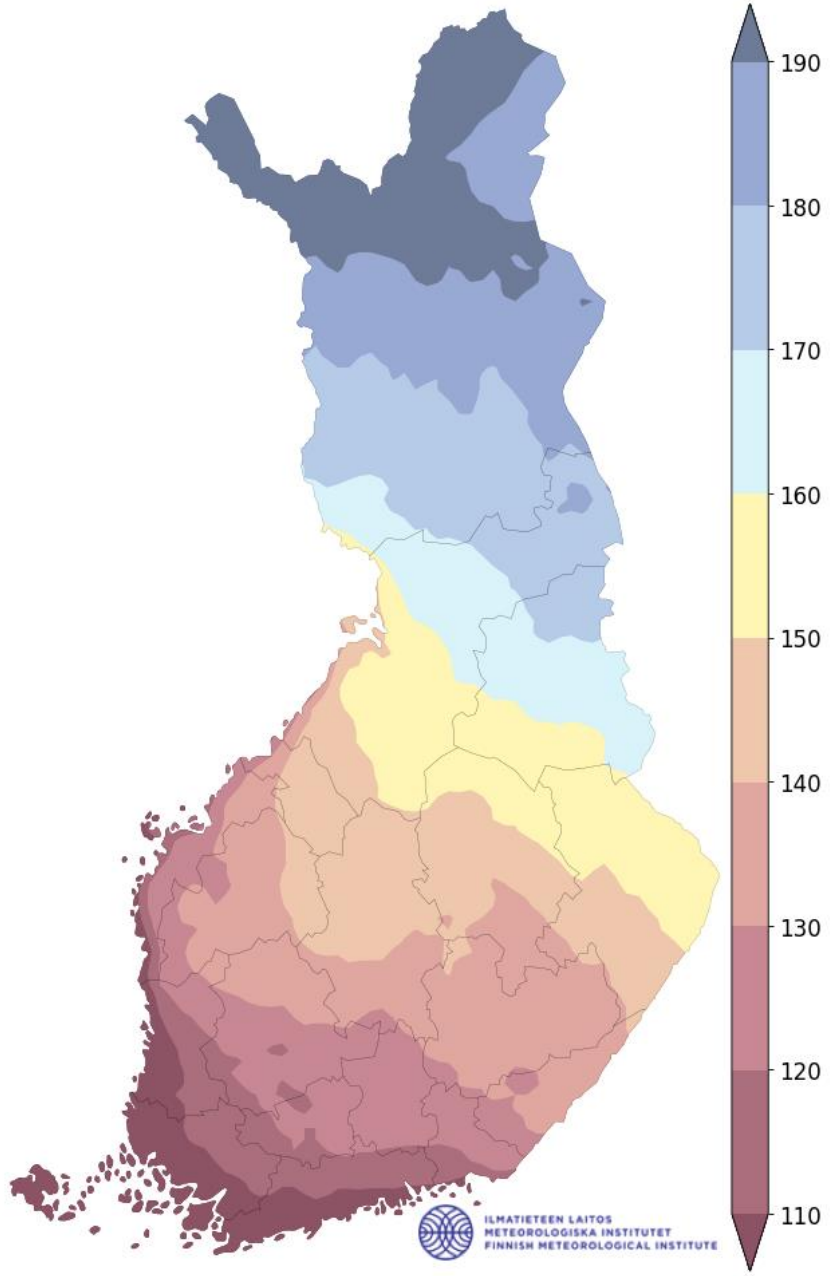
Figures updated: 31 December 2023

Backround

- Natural Resources Institute Finland (Luke)
  - Majority of our work is scientific research, 72%
  - **Statutory and expert services 21%**
  - Customer services 4%
  - Statistical services 3%

  - **The long-term conservation and sustainable use of the genetic diversity of crops in Finland**
    Finnish National Genetic Resources Programme for Agriculture, Forestry and Fishery

    Special adaptation to environmental conditions with
    - long day
    - short growing season
    - acidic soil
    - cold winter



Length of thermal winter
range from 110 to 190 days
Thermal winter = Average daily temperature below zero.

Black currant *Ribes nigrum L.*
Red and white currant *R. rubrum group*

- Woody shrubs or small bushes
- Cultivated in gardens and for commercial fruit production
- Clonally propagated varieties and landraces, high phenotypic diversity between genotypes
- In Finland both usually very frost hardy, hardiness can vary between cultivars
- Differences between varieties in vitro cultivation, black currant is usually easier than red or white currants

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Gene bank collection of black currant

- Gene bank collection is maintained in field
- New field collection was established because of Black currant reversion disease
  - ➢ Plants were produced via micropropagation

- Safety duplicate collection in cryo
  - In vitro shoot tips >modified droplet vitrification

Plants in cool (+2 - +6°C) greenhouse

- Mother plants tested according to legislation for certified plant production
- Young gene bank plants produced for new field collection tested for black currant reversion disease
    - Extra plants not needed for field

Dormant bud cryopreservation?

Plant material should be

- **well cold-acclimated**
- **fully dormant**
- **as healthy** as possible

In winter months, greenhouse temperature above +2 °C

Why *in vitro* recovery?

PROS

- Revival of material possible any period of a year
- Multiplication of plant material possible from small number of regenerated buds
- Requires only meristematic part of bud to be alive

CONS

- Requires sterile laboratory facilities
- Needs staff accustomed to sterile work
- Risk of contaminations
- Requires suitable growing medium
- Requires functional regeneration chain

Cryoprotocol for black currant

Cryoprotocol used for black currant

- **No pre-dehydration** of plant material
- Cooling at the **rate of 0.17°C / min from 0°C to -38°C**
  - > Hold at -38°C for about 30 mins
  - > Immersion of cryoboxes in liquid nitrogen
- **Fast thawing** in water bath
- Recovery via **in vitro** culture

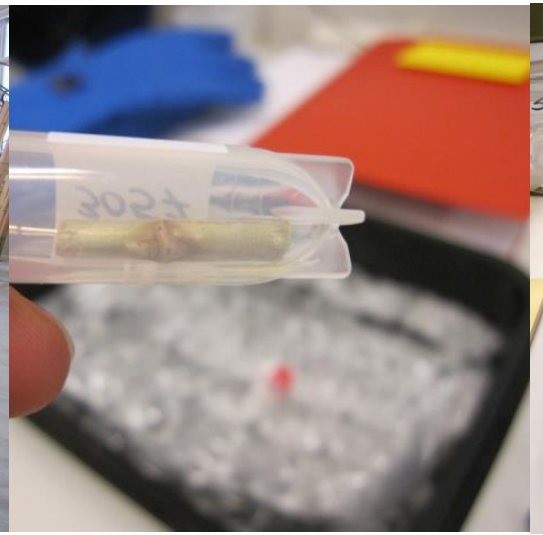
Protocol: Ryynänen, L. Jokipii, S. and Häggman, H. 2008. Controlled Rate cooling of Silver Birch and Aspen Dormant buds In: B. Reed (Ed.), Plant Cryopreservation: A practical Guide. p. 432-435. Springer, New York

Experiment with *Ribes nigrum* cv. "Mortti" using non-dehydrated buds and in vitro recovery

Steps of the protocol: Collection and sample preparation

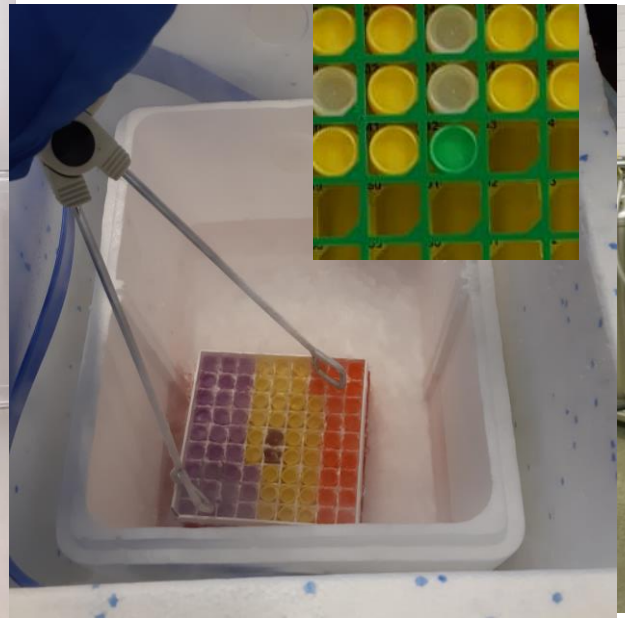
1. Bud material collected from field (-5°C) or cool (+4°C) greenhouse in January
2. Branches stored in a cold room (+2°C) overnight
3. Approx. 2 cm stem segments with bud cut from branches and sealed in cryotube
4. Cryotubes placed in cryoboxes and kept at 0°C overnight





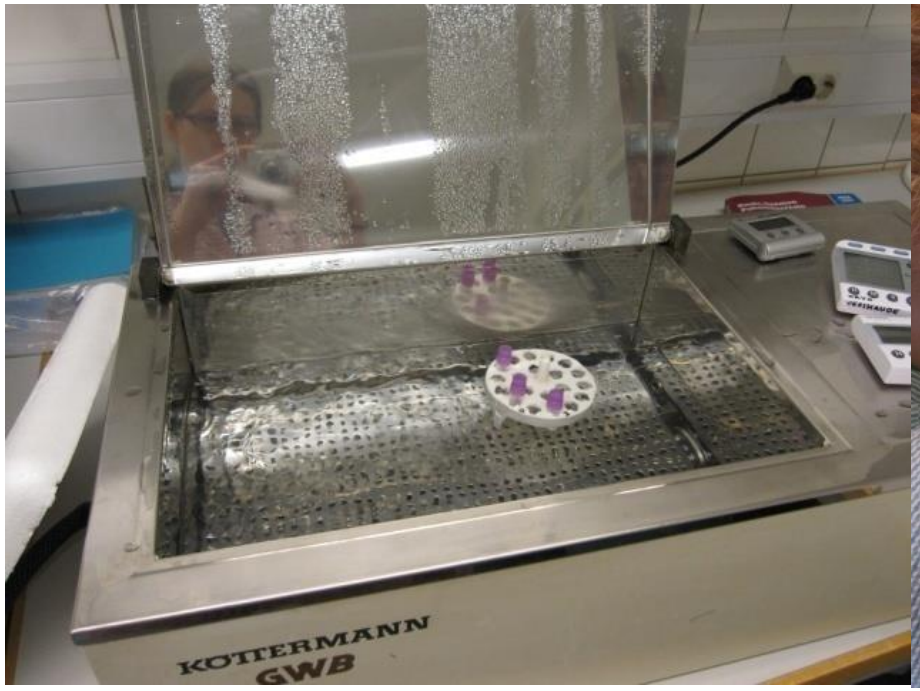

Steps of the protocol: Cooling and storage to cryotank

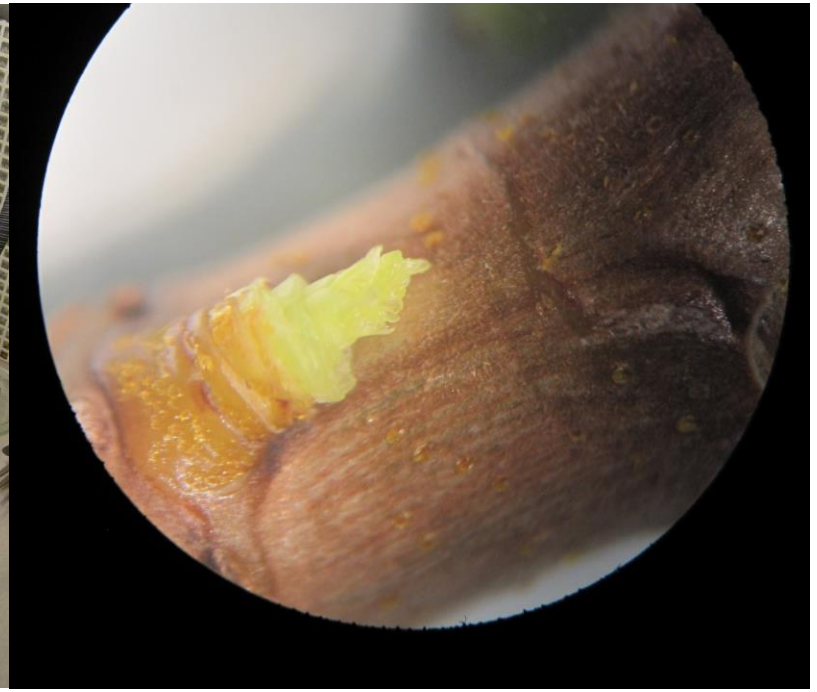
5. Cryotubes cooled at 0.17°C / min from 0°C to -38°C in controlled-rate freezer (Planer kryo 10-16) and held at that temperature for about 30 mins.
6. Cryotubes immersed in liquid nitrogen and stored in cryotank



Steps of the protocol: Thawing and excision of shoot apex

7. Cryotubes thawed in 37°C water bath for 3 mins, (then kept on ice for 30 secs)
8. Twigs surface sterilized in 75 % ethanol approx. for 20-30 secs. + quick dip in 100 % ethanol
9. Shoot apex (1-2 mm) excised from buds and transferred to culture tubes (1 shoot apex / tube) containing recovery medium.

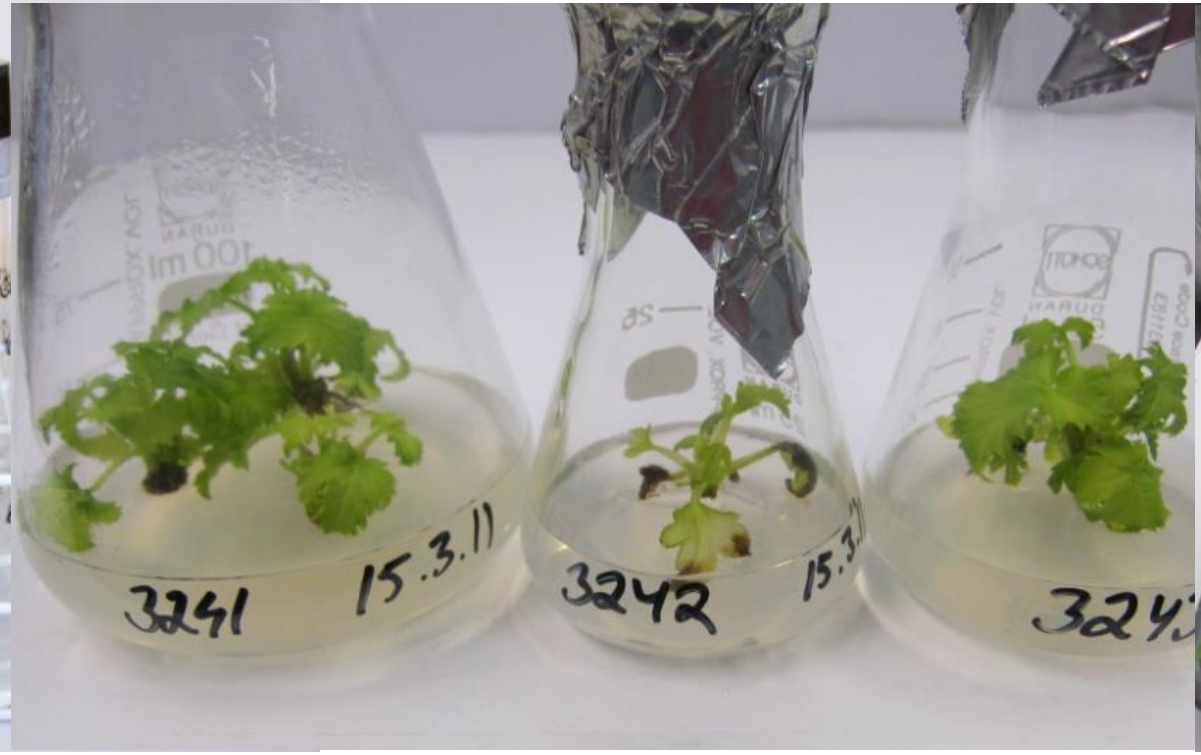


Steps of the protocol: In vitro recovery

10. Subculture done every 2 or 3 weeks, shoot apex or growing shoots cleaned under stereomicroscope and transferred into new media

    Experiment with black currant cv. Mortti: regenerated shoots were transplanted in vivo



Results from experiment with black currant cv. Mortti

Buds cryopreserved from greenhouse: Recovery 66 % CI [55 – 76 %]

Number of flower buds: 39 / 80

Bud size range 1 – 4 mm

MC of twigs 55 – 58 %

Contaminations: 0

Buds cryopreserved from field: **Recovery 86 %** CI [76 – 93 %]

Number of flower buds: 77/80

Bud size range 2 – 6 mm

MC of twigs 56 – 58 %

Contaminations: 1

40 field buds thawed after 4 years cryostorage: recovery 58 % CI [41 – 73 %]

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Long term cryopreservation of black currant accessions

- Protocol tested with cv. Mortti has been used for 23 accessions
- Dormant buds collected from cool greenhouse
  - Mother plants used for certified plant production
  - Extra gene bank plants produced for new field collection
  - ➢ Collection, cutting and sealing twigs in cryovials was usually done during the same day

Results from viability assessments: about 20 buds / accession

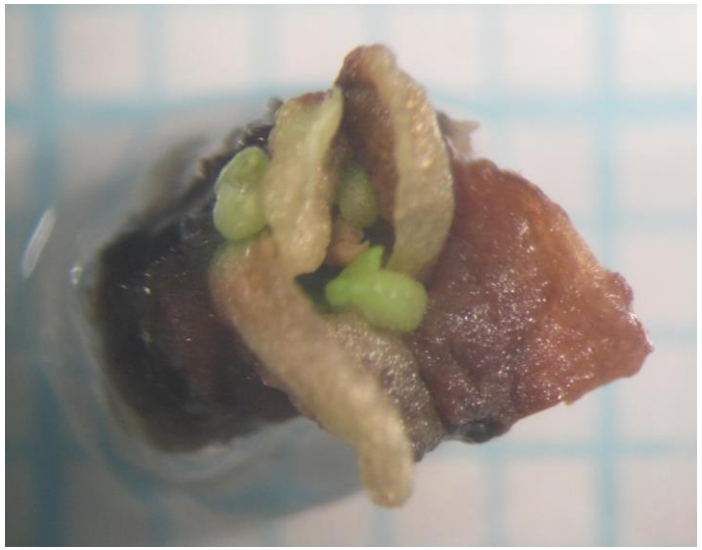
- Estimated recovery rate of buds ranged from 9 % to 90 %,
  - between 60 – 90 % for 15 accessions
  - between 42 – 59 % for 7 accessions
  - 83 % for vegetative bud
  - 43 % for flower bud

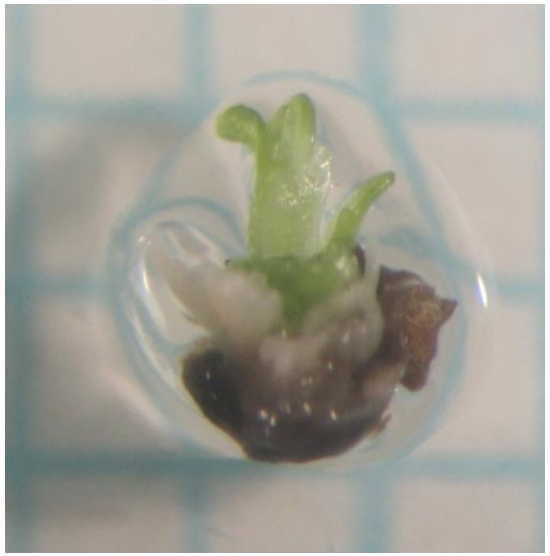
Black currant cv. Kangosfors 4 weeks from initiation

Black currant cv. Nikkala

Buds cryopreserved with their natural MC + in vitro recovery What should be noted?

- Avoid contaminations >change clean tweezers and knives often when peeling the bud
- Cleaning of dead tissue from explant is important in initial stage of recovery Remove dead tissues> rescue regenerating shoot
- Regeneration takes time





- Flower buds should be avoided, identifying the bud type may be difficult from intact buds

Vegetative bud, black currant

Flower bud, black currant

- Recovery of shoot may start from axillary vegetative buds alongside flower primordia

Flower bud from field

Flower bud from greenhouse

Cryoprotocol for red and white currants

Protocol tested for red and white currants

- How to improve cryosuccess of dormant buds collected from greenhouse?
  - Is artificial cold acclimation needed?

    >Storage at **- 5°C** or at **+ 4°C** after collection

    Experiment with 5 accessions (2 red and 3 white)

  - **Dehydration of twigs** prior cryopreservation at - 5°C
  - **Cooling rate 1°C / h from - 5°C to -30°C**

    >Hold at 30°C for 24 h

    >Immersion in liquid nitrogen
  - **Slow thawing** at + 2°C - + 4°C overnight
  - **Rehydration** in moist cotton for 6 to 10 days
  - Recovery via **in vitro** culture

Mother plants of red and white currants maintained in greenhouse

Experimental set up:
Storage of branches prior cryopreservation at - 5°C or at + 4°C

Branches collected in December 2018 and 2021

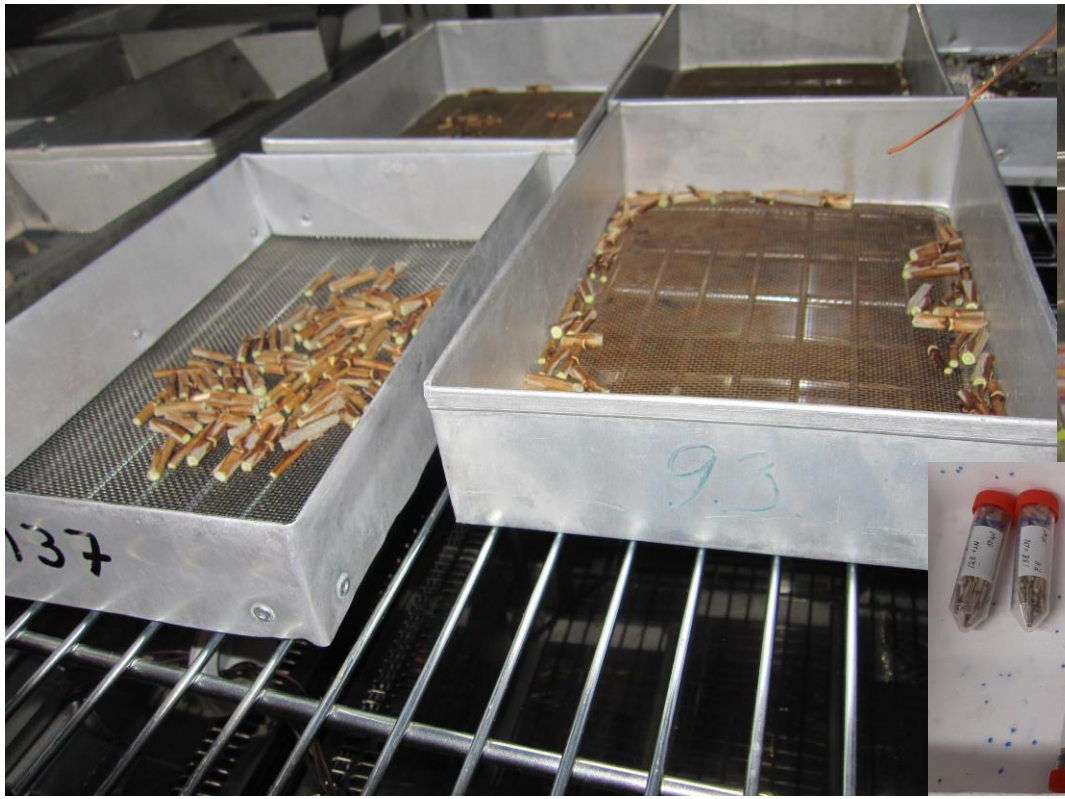
Controlled rate freezer

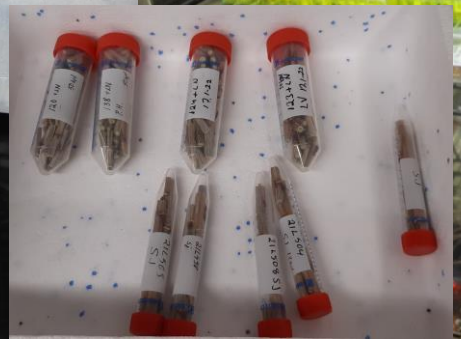
Brushwood wrapped in plastic bags, 2018-9 for 17 days, 2021-22 for 12 days

Red and white currants, steps of the protocol: Dehydration of twigs, >cooling >immersion in LN

Dehydration at - 5°C
for 17 to 22 days in 2019, for 15 to 18 days in 2022

Cooling at the rate of 1°C / h
from - 5°C to -30°C , hold at 30°C for 24 h





Red and white currants, steps of the protocol: Thawing and rehydration

Slow thawing at + 2°C - + 4°C overnight

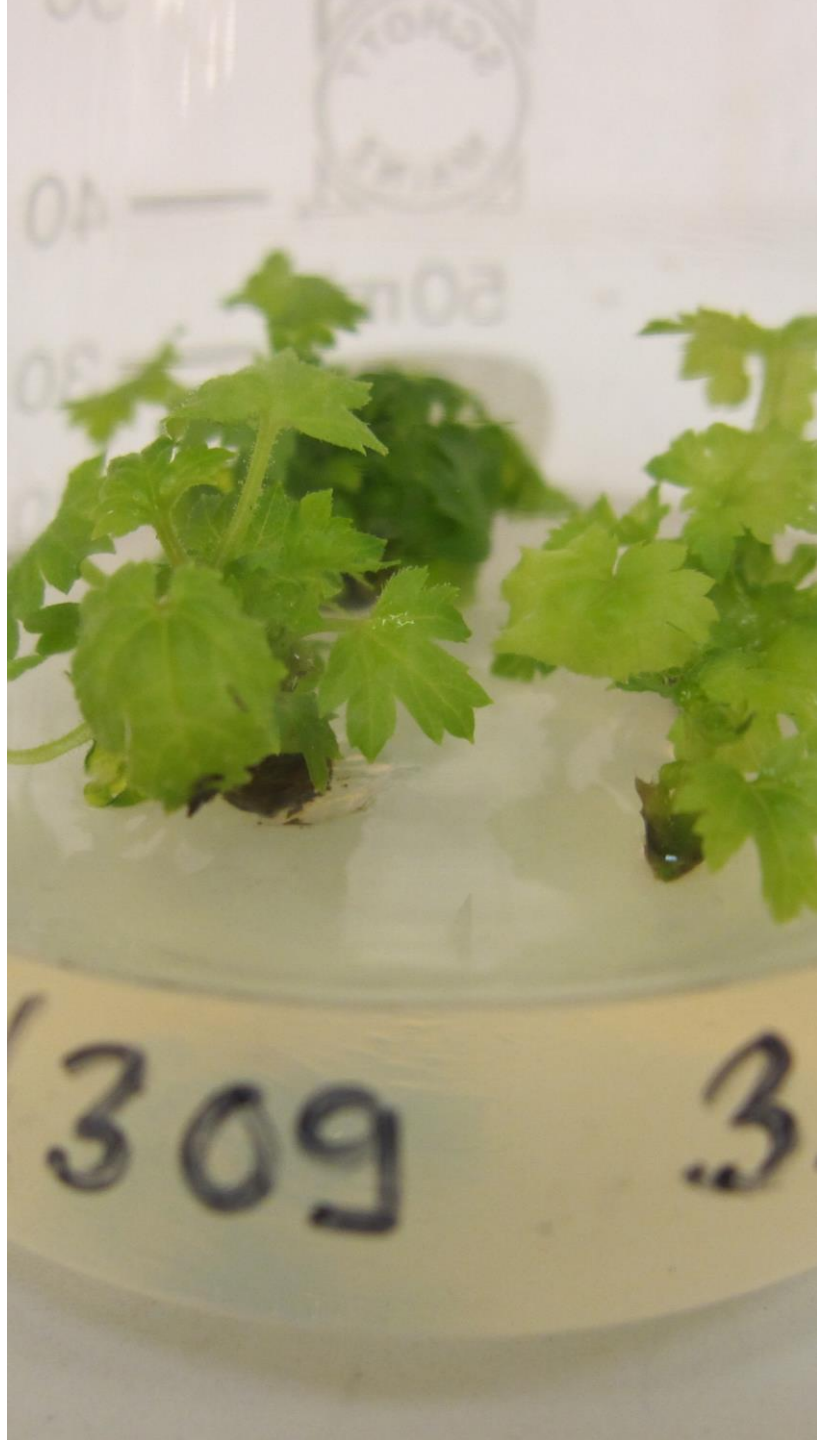
Rehydration in moist cotton for 6 to 10 days, surface sterilization in 75% ethanol for about 30 to 50 secs + dipped in 100 % ethanol

Results from experiments with red and white currant

- The storage temperature of branches (+4 °C or -5 °C) was significant (α=0.05) with one white currant accession
  - Recovery of 85 % for buds stored at +4 °C prior dehydration
  - Recovery of 72 % for buds stored at -5 °C prior dehydration

- The actual recovery rate of buds varied by accession
  - 33% - 85% for buds stored at +4 °C prior dehydration
  - 43% - 96% for bud stored at -5 °C prior dehydration

- The results of the study highlighted
  - the variation in moisture content of dehydrated twigs
    - Ranged from 31 % to 47 %, when dehydrated 17 to 22 days in 2019
    - Ranged from 26 % to 35 % when dehydrated 15 to 18 days in 2022



Rehydration of dehydrated buds was important; 10 days was better than 6 days. However, long rehydration time increases the risk of contaminations.

Control bud, red currant cv. Punahilkka

Dehydrated + Cryopreserved + rehydrated for 10 days
white currant cv. Piikkiön Helmi

Conclusions

Conclusions

based on our experiences with dormant buds collected from the cool greenhouse

Black currant

- With in vitro recovery, pre-dehydration of twigs may be omitted
- In vitro recovery of buds cryopreserved at their natural moisture content may require some effort
- Subzero temperatures before collection may not be necessary

Red and white currant

- The storage temperature of branches (at - 5°C or at +4°C) prior dehydration of twigs at - 5°C may not have a significant effect on the recovery of buds in vitro

Luke

Thank you for your attention!

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