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Cryopreservation of Dormant Buds of Raspberry (*Rubus idaeus* L.)

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INTRODUCTION & AIM

Cryoconservation provides the possibility of long-term preservation of genetic resources of vegetatively propagated plants. For frost resistant woody plants, cryobanking of dormant buds is a cost-effective option, which allows the plant to be effectively regenerated by grafting. The aim of this study was to develop a method of cryopreservation of dormant buds by pre-dehydration and two-step freezing for raspberry (*Rubus idaeus* L.), which cannot be grafted but can be regenerated using *in vitro*.

METHOD

Sampling of plant material

Rubus idaeus L.

Varieties:
- **Sanibelle**
- **Willamette**

Raspberry twigs were harvested in the winter period 2021-2022 and 2022-2023 in the orchard of Crop Research Institute (Prague, Czech Republic).

Dehydration of dormant buds

- Uniform cuttings of uninodal segments (length 35 mm)
- Frost dehydration (-3.5 °C), open air

Study of water state of dormant buds during dehydration and cooling

Water activity of nodal segments

Water Activity Meter HP23-AW-A (Rotronic)

Low-temperature phase transitions (temperatures, percentage of crystallized water) of dormant buds.

Discovery X3(TA Instruments), Q2000 DSC (TA Instruments). Cooling and heating rates: 10 °C/min

Water content of nodal segments

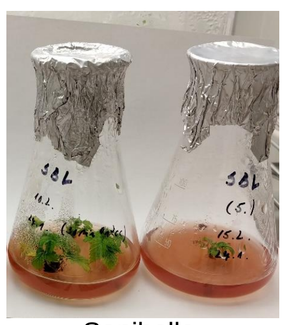
Gravimetrically (based on the weight change between fresh and dried nodal segments)

Cryopreservation of dormant buds

Freezing in 50 ml tubes covered with aluminium foil using a two-step cryoprotocol:
1. From -4 ° C to -30 ° C (cooling rate 1 ° C h^{-1}) in a computer-controlled freezer and after equilibration for 24 hours;
2. Immersing the tubes into liquid nitrogen

Thawing and regeneration of dormant buds

- Slowly thawing at +4 °C
- Rehydration in moist white peat for 14 days at +4 °C
- Regrowth in perlit

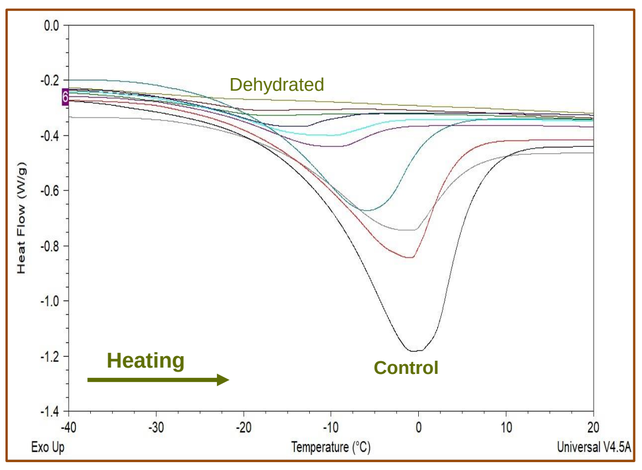


Sanibelle

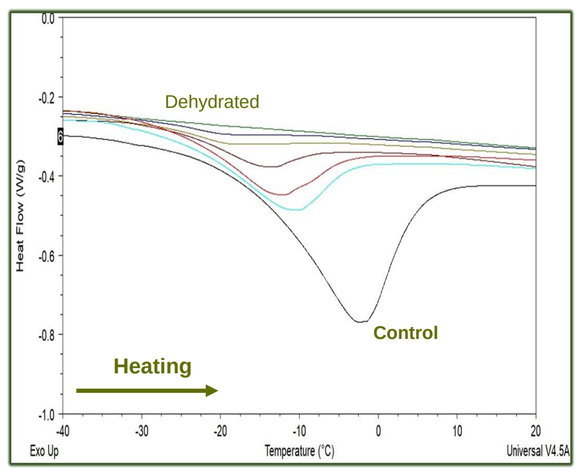
RESULTS & DISCUSSION

State of water in raspberries dormant buds during dehydration and cooling (DSC)

Sanibelle



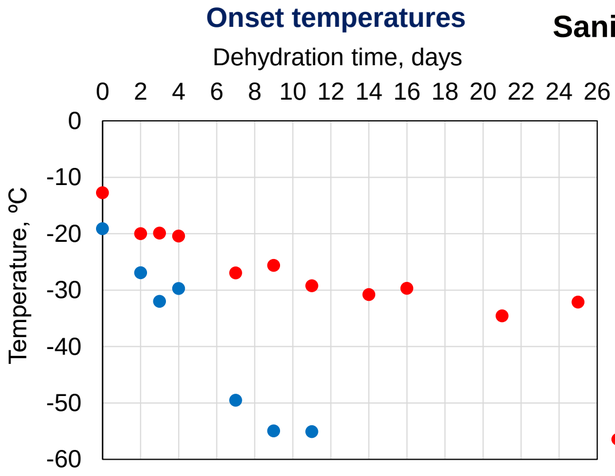
Willamette



Dehydration – melting peaks disappearance

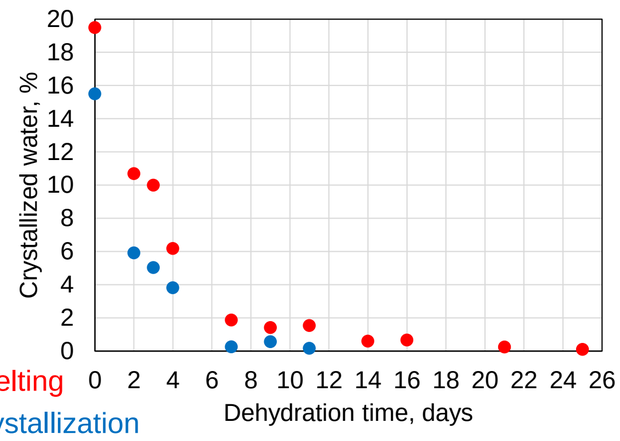
Changes in onset temperatures and enthalpies of crystallization and melting in dormant raspberry buds during dehydration

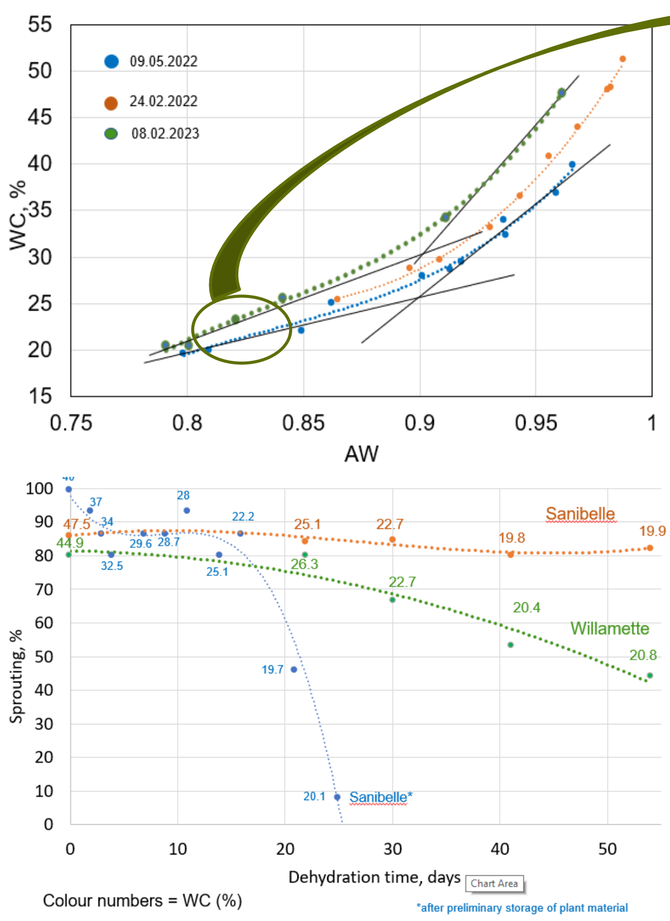
Onset temperatures

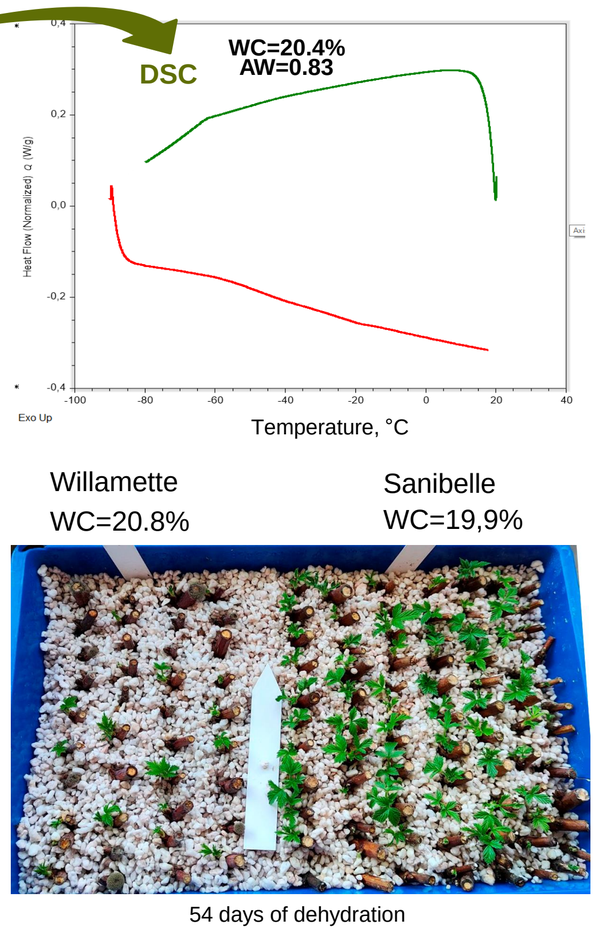


Sanibelle

Enthalpies







Willamette
WC=20.8%

Sanibelle
WC=19,9%

54 days of dehydration

CONCLUSION

This water activity below 0.9, where crystallization no longer occurred in the cooling phase, only an insignificant melting peak was recorded in the heating phase, and the rate of decrease of water activity in the buds was significantly reduced, should be used for cryopreservation of raspberry buds. This approach makes it possible to obtain up to 74% of viable raspberry buds after cryopreservation.

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