Modified droplet-vitrification cryopreservation of arctic bramble (*Rubus arcticus*) and hybrid arctic bramble

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The goal of the study was to find a modification of droplet-vitrification method for valuable Nordic arctic bramble (*Rubus arcticus*) and hybrid arctic bramble (*Rubus a.* ssp. × *stellarcticus*) and to proceed to the long-term cryopreservation of the mandate variety collections of *Rubus* at Luke.

Plant material and Methods

In vitro cultures of the Finnish arctic brambles ‘Pima’ and ‘Susanna’ and the Swedish hybrid arctic brambles ‘Beata’ and ‘Sofia’ were established from the nuclear plants of certified production (Fig. 1). Cultures were grown at 22° C and multiplied at ~6 week intervals on fructose-based medium containing 0.5 mg/L BAP and 0.25 mg/L IBA.

Figure 1. Blooming arctic bramble (*Rubus arcticus*) ‘Susanna’

Pretreatment Last multiplication media contained MS mineral salts and vitamins with 0.5 mg/L BAP, 0.05 mg/L IBA, 30 g/L sucrose, 0.1g/L myo-inositol, pH 5.8. Plants, 3-4 weeks after the last subculture, were cold acclimated (CA) for 1 week at 22° C with 8h light (50 µE × m−2 × s−1)/+4° C 16h dark; last two days before apex excision +4° C 24h dark. Shoot tips and lateral buds (1.5 - 2 mm) were excised from CA plants and grown for 24 h on MS medium containing 0.25 M sucrose and active charcoal following 24h on MS medium containing 0.5 M sucrose under the CA conditions.

Vitrification procedure Shoot tips were treated in strainer with loading solution (LS) for 30 min. Strainer was wiped and placed in the cryoprotectant PVS2 (Sakai et al., 1990), held for 30 min altogether. In the end of the PVS2 treatment, 10 shoot tips were placed on aluminum foil strip in three 10µl drops of PVS2. The cryo vials were immersed in liquid nitrogen (LN) and held for minimum 1 hour.

We express our gratitude to the Finnish Ministry of Agriculture and Forestry for financial support. We thank The Nordic Genetic Resource Centre (NordGen) for the support for the preceding cryoprotocol studies on *Rubus*.

Thawing and regrowth assessment Three vials were thawed (2 min water bath at 38° C), and 5-8 vials were long-term preserved (two clonal lines with two repetitions). The shoot tips were rinsed 20 min in 2 ml liquid 1M sucrose MS medium. Shoot tips were planted on a solid regeneration medium (in vitro medium described). Recovery showing meristems were transferred onto a fresh medium. Recovery was recorded as survival and regeneration rates.

Results and Discussion

The results showed survival and regeneration after cryopreservation with all cultivars studied (Table 1, Fig. 2). The two studied hybrid arctic brambles responded to this cryopreservation protocol with higher regeneration rates compared to the two arctic brambles studied. As Reed (2008) highlights that cold acclimation increases the recovery of cryopreserved *Rubus* meristems, even longer cold acclimation pretreatment might result as higher regenerations.

Table 1. Survival and regeneration rates post LN on studied four *Rubus* cultivars.

Cultivar	Survival rate (%)	Regeneration rate (%)
Susanna	30…40	23…40
Pima	20…40	14…40
Beata	43…53	37…50
Sofia	40…77	33…73

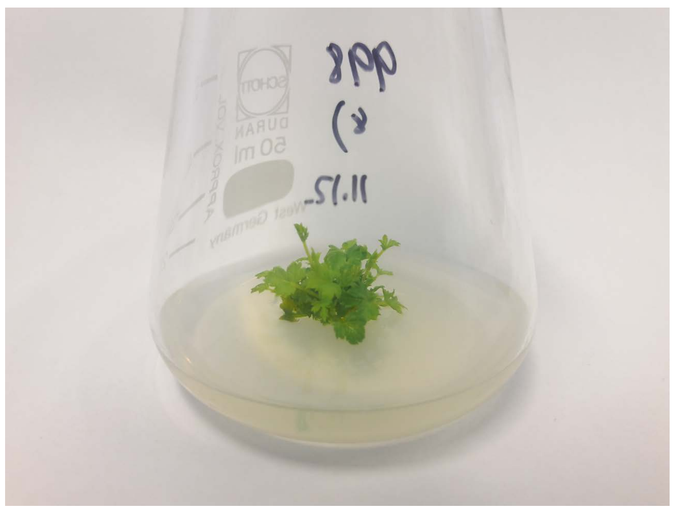

Figure 2. Individual *Rubus arcticus* ‘Pima’ apex has regenerated into a normal microplantlet 7,5 week post LN. All studied cultivars regenerated with good quality and formed dividable cultures post LN. No excess callus formation occurred nor hyperhydricity.

Conclusions

- Studied cryopreservation method was shown applicable for cryopreservation of arctic brambles, and long-term cryopreservation for two clonal lines for each cultivar with two repetitions was performed.
- The *Rubus* cryopreservation studies at Luke will continue with cloudberry (*Rubus chamaemorus*) to long-term preserve the cloudberry genetic resources.

References

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