

Кріоконсервування верхівок пагонів *Valeriana jatamansi*, цінної лікарської рослини Індії, що перебуває під загрозою зникнення

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Cryopreservation of Shoot Tips of *Valeriana jatamansi*, a High Value Threatened Medicinal Plant of India

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Valeriana jatamansi Jones, a medicinal plant native to the Himalayan region, holds significant trade value. However, due to overexploitation from the wild, it has become threatened and requires a comprehensive conservation approach. Cryopreservation emerges as a valuable adjunct to traditional conservation techniques, offering a promising solution to safeguard this species effectively.

A total of 18 accessions of this valuable plant are conserved in the In Vitro Genebank of Indian Council of Agricultural Research National Bureau of Plant Genetic Resources, New Delhi, India. Shoot tips (about 1 to 1.5 mm in length) were excised from 6-week-old stock cultures pregrown on Murashige and Skoog (MS) medium supplemented with 3 mg/l 6-Benzylaminopurine, shoot tips were precultured on MS + 0.3 M sucrose for 2 days. Thereafter, shoot tips were treated with loading solution for 20 min, dehydrated with Plant Vitrification Solution 2 (PVS2) (10 to 60 min) and cryopreserved using vitrification, droplet-vitrification, and V-cryoplate techniques.

Shoot tips subjected to PVS2 solution for 40 min reported high post-thaw regeneration using vitrification (73.33 %), droplet-vitrification (86.67 %), V-cryoplate (100%). Owing to the ease of the technique, droplet-vitrification protocol was verified in ten accessions, revealing post-thaw regeneration rates between 86.67 to 93.33 % across these accessions. This study highlights a highly efficient protocol for *V. jatamansi* germplasm conservation, demonstrating that droplet-vitrification is a reliable and sustainable method for long-term conservation.

Протокол кріоконсервування методом краплинної вітрифікації з широким спектром дії для збереження хмелю (*Humulus lupulus*)

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A Wide Spectrum Droplet Vitrification Cryopreservation Protocol for Conservation of Hops (*Humulus lupulus*)

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Hops (*Humulus lupulus* L., family: *Cannabaceae*) is a high value commercial crop of the brewing industry due to the flavour and aroma contributing resins and essential oils. However, field maintained hops germplasm collections are constantly exposed to diseases, pests and pathogens. Cryopreservation is an ideal alternative for the long-term conservation of germplasm, which can be used as a backup to active germplasm collections. Conventional cryopreservation methods using encapsulation-dehydration have been used for hops but, to our knowledge, newer cryopreservation techniques have not been applied for its conservation.

In this study, Droplet Vitrification (DV) was tested for development of a conservation protocol for hops. Shoot tips (2 mm) excised from one-month-old cultures were precultured on 0.3 M sucrose for 16 h, followed by treatment with a loading solution, containing 2 M glycerol and 0.4 M sucrose for 20 min and Plant Vitrification Solution 2 (PVS2) for different time durations. Shoot tips were then immersed in liquid nitrogen. For post-thaw recovery, shoot tip growth was recorded on regeneration medium containing MS salts with 4.4 µM BAP and 0.28 µM GA3.

Optimal cryoprotection (50% survival) was achieved with a 30 min of PVS2 treatment. Genetic fidelity of the regenerated plants was confirmed using 30 ISSR markers. The developed protocol was successfully tested on seven other accessions resulting in post thaw regeneration ranging from 43 to 70%.

This study presents a highly efficient protocol for conservation of hops. To the best of our knowledge, this is the first report on using DV for cryopreservation of hops shoot tips with high success rates. The developed protocol can be applied for large-scale cryobanking of valuable hops germplasm.

