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Plant Regeneration from Shoot Tips of *Dianthus hybrida* Cryopreserved in Liquid Nitrogen up to 2 Years

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Attempts are in progress to cryopreserve the germplasm of various useful plants, but there is little information on long term storage of the germplasm in liquid nitrogen (LN_2) with subsequent plant regeneration.

Seibert[1] and Uemura and Sakai[2] reported the possibility of cryopreservation of shoot tips in *Dianthus caryophyllus*. The author reported a high survival rate of shoot tips cryopreserved up to 210 days in *Dianthus hybrida*.[3]

In this paper, *Dianthus hybrida* shoot tips cryopreserved for various time periods in LN_2 were thawed, recultured, and tested for viability. The results confirmed that cryopreservation of shoot tips would be useful for maintenance of base germplasm collections of *Dianthus*.

Shoot tips (approx. 0.7 mm) excised from mother plants of *Dianthus hybrida* cv. Sakuranadesiko growing in a greenhouse were put into 0.5 ml plastic straws (bull semen straws, 3 mmϕ×125 mm, manufactured by Fujihira Industry Co. Ltd. were used) with cryoprotectant solution (10% dimethyl sulfoxide and 3% glucose). After heat sealing, the straws were incubated for 1 hr at 20°C.

The straws were slowly cooled at a rate of 0.5°C/min from room temperature to −40°C and automatically seeded at −3.5°C, and then quickly cooled at a rate of 33°C/min down to −160°C with the program-freezer FFP-190 (Osaka Sanso Industry Co. Ltd). Finally the straws were immersed and stored in LN_2. Further details of the above procedure can be obtained in previous publication.[3]

After various storage times in LN_2, the frozen straws were rapidly thawed in warm water (25-30°C). Shoot tips were rinsed 6 times with sterilized water and cultured on half strength of MS medium[4] supplemented with 0.1 mg/*l* 6-benzylaminopurine (BA), 0.5 mg/*l* 1-naphthaleneacetic acid (NAA), 20 g/*l* sucrose and 0.8 g/*l* agar for 40 days under continuous illumination of 1,500 lux at 25°C.

After thawing, the survival rates of shoot tips cryopreserved in LN_2 for 1 day, 1 week, 1 month, 1 year, 2 years were 100%, 95%, 100%, 94.5%, 100%, respectively (**Table 1**). Eighty-nine to 100% surviving shoot tips regenerated shoots within 40 days of culture (**Fig. 1**). The terminal part of shoots (approx. 20 mm) was cut off and transferred onto growth regulator-free MS medium. After rooting, plantlets were transplanted into soil in a greenhouse (**Fig. 2**), after which they flowered. The flowers of plants derived from cryopreserved shoot tips had no symptoms of virus infection. This suggested that the use of shoot tips as a material of cryopreservation would be expected to store the germplasm as a pathogen-free state.

Kartha *et al.*[5,6] reported that regeneration rates of pea and strawberry shoot tips gradually decreased as storage periods increased. On the other hand, Bajaj[7] reported that no significant difference in the percentage viability of potato and cassava shoot tips cryopreserved for periods ranging from 3 months to 4 years. In this study, the viability of *Dianthus* shoot tips cryopreserved in LN_2 was maintained up to 2 years. It is not clear whether this difference depends on the species or freezing methods (preculture, cryoprotectant solution, cooling rate, terminal temperature of pre-freezing, thawing rate, *etc.*). These results suggest that the long term storage studies are required in each crop in order to establish the *in vitro* gene bank.

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Table 1. Viability of Dianthus shoot tips cryopreserved in liquid nitrogen (LN_2) for vairous time periods.

Time in LN_2	No. of shoot tips cultured	Surviving cultures no. (%)	No. of cultures with shoots[a)]
1 day	20	20 (100)	20
1 week	20	19 (95)	19
1 month	20	20 (100)	19
1 year	19	18 (94.7)	16
2 years	22	22 (100)	20
Cont. (unfrozen)	40	40 (100)	40

[a)] After 40 days of culture.

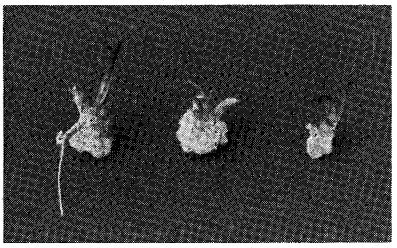

Fig. 1. Shoots regeneration from *D. hybrida* shoot tips following cryopreservation for 2 years.

Fig. 2. *D. hybrida* plants obtained from the shoot tip culture following cryopreservation for 2 years.

References

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≪和文要約≫

2年間液体窒素下で保存したナデシコ茎頂からの植物体再生

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温室栽培の *Dianthus hybrida*（品種サクラナデシコ）より茎頂をとり出し，10% ジメチルスルホキシド＋3% グルコース溶液とともに，0.5℃/min の速度で −40℃ まで予備凍結を行った後，液体窒素下に保存した．2年後，温水により急速解凍した茎頂は100% 生存しており，再生したシュートは正常に生育・開花した．