Short Communication Plant Tissue Culture Letters, 7(3), 199-201 (1987)

Plant Regeneration from Dried Carrot Callus Preserved in Liquid Nitrogen

Noboru KAIMORI and Toshiaki KAMEYA*

The Second High School Attached to Hachinohe Institute of Technology, Hachinohe, Aomori, Japan, 031
** Institute of Genetic Ecology, Tohoku University, Sendai, Japan 980*

(Received June 12, 1990)
(Accepted August 24, 1990)

During repeated subculturing, many plant tissue cultures show changes in morphogenetic potential,[2] productivity of useful chemicals,[3] and genetic characters.[4] Cryopreservation of cells in liquid nitrogen is one suitable method to resolve these problems.[5,6] One of the most important points for successful cryopreservation is the exclusion of freezable water from the cell before cryopreservation. Amorphous freezing substances like DMSO, glycerol, enthyleneglycol and others, and drying methods have been used for this purpose. However, there are only a limited number of papers on the drying methods.[7,8] Kaimori and Takahashi[9] showed the successful retention of differentiation potentials in carrot callus dried to equilibrium at room temperature. In this paper, we report that the dried callus can be preserved by direct immersion in liquid nitrogen.

Materials and Methods

Callus was induced from hypocotyls of domestic carrot, *Daucus carota* L. cv. Super Ogata Gosun Ninjin, on Murashige and Skoog's agar medium containing 5×10^{-6} M 2, 4-dichlorophenoxyacetic acid (2, 4-D). The pH of the medium was adjusted to 5.7 before the addition of agar (9 g/*l*). Cultures were maintained at 27℃ in continuous light (about 200 lux) from cool-white fluorescent tubes. They were subcultured every 4 weeks in the same medium for 3 months. Actively growing embryogenic calli were used for the experiments 2 to 3 weeks after subculture.

Calli of approximately 15 mg fresh weight were placed on a filter paper disc (Toyo Roshi No. 2) in a 15×15 mm petri dish, which was set in a larger (60×60 mm), tall cylindrical dish containing 15 mg of activated silica gel. The petri dish, silica gel, and filter paper were sterilized by heating at 160℃ for 1 hr. Calli were dried for 6 hr at 27℃ and stored in this state for 6 hr by sealing the dish with parafilm (American Can Co.). Water content of dried calli was calculated from the difference in weight before and ater drying for 24 hr at 80℃.

The dried calli were put into the cryotubes, and fresh calli were also attached to the inside of the cryotubes. They were immersed directly into liquid nitrogen. After 30 min of preservation, they were pulled out and placed in a room at 25℃ for 1 hr, and then they were transferred to the Murashige and Skoog's agar medium[1] in 18×130 mm test tubes and incubated under maintenance conditions described earlier. Regrowth and the differentiation of embryos and plantlets were examined under a stereoscope.

Results and Discussion

Table 1 shows survival rate of calli as determined by embryo and plantlet differentiation. The fresh calli, not dried, preserved in liquid nitrogen were pale yellow but none of them produced any embryos

Table 1. Survival rate of calli as determined by embryo and plantlet differentiation.

Treatment	No. of calli examined	Calli regenerated embryos and plantlets								
		7 days			10 days			14 days		
		+	++	+++	+	++	+++	+	++	+++[a]
No drying and LN[b]	50	0	0	0	0	0	0	0	0	0
Drying and LN[b]	60	0	0	0	20	23	15	0	0	60

[a] +, 1-3 embryos or plantlets; ++, 4-10 embryos or plantlets; +++, more than 10 embryos or plantlets.
[b] liquid nitrogen.

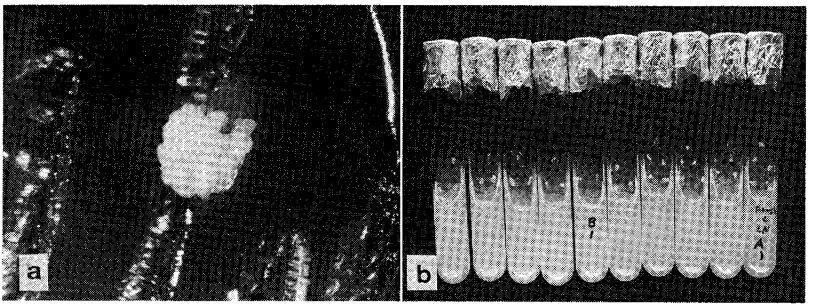

Fig. 1. No embryo formation of fresh callus preserved in liquid nitrogen at 14 days (a) and 21 days (b) after culture.

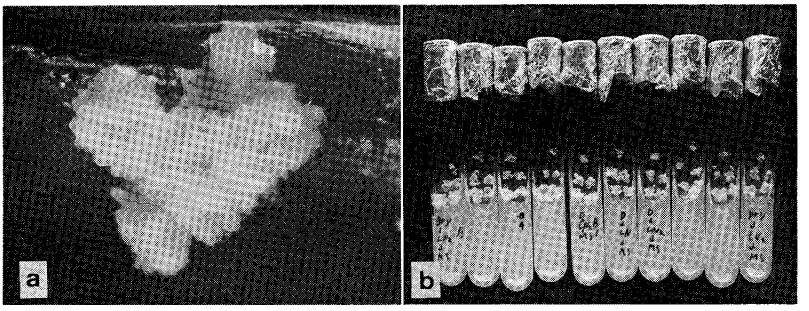

Fig. 2. Many embryos differentiated from dried callus preserved in liquid nitrogen at 14 days (a) and 21 days (b) after culture.

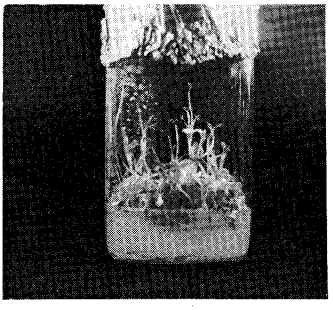

Fig. 3. Many plantlets grown from dried callus preserved in liquid nitrogen at about 2 months after culture.

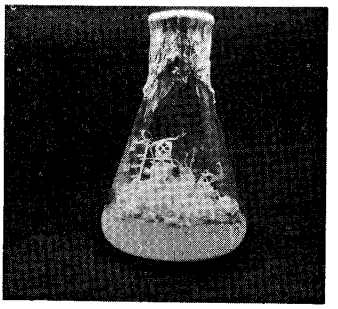

Fig. 4. Many plantlets grown from dried callus not preserved in liquid nitrogen at about 2 months after culture.

in 14 days of culture (**Fig. 1**), and they did not show any growth even after 6 months, although one callus did produce embryos after about 2 months. On the other hand, all dried calli were yellow and differentiated many embryos after 14-21 days (**Fig. 2**). These embryos developed into plantlets after 2 months (**Fig. 3**). There was no difference between dried calli preserved and not preserved in liquid nitrogen on the differentiation of plantlets (**Fig. 4**), which was similar to that of fresh calli without treatment of drying and preservation in liquid nitrogen.

The water content of the dried calli used here was 3%. The present results demonstrate that dried calli with water content as low as 3% could be frozen directly in liquid nitrogen as suggested by Kaimori and Takahashi.[9]

Nitzsche[8] showed that dried carrot callus survived at 15, −40, and −80℃ for one year. However, it was not clear whether the callus maintained the differentiation potential or not, because embryo or plant formation was not observed. In the present experiments, all dried calli survived and retained totipotency even after direct immersion in liquid nitrogen. The calli dried by the present simple method should be preserved in liquid nitrogen for a long time like seeds of many plant species.[10]

We are grateful to Prof. Aiya Ishihara (Iwate University) for his valuable suggestion.

References

1) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
2) Halperin, W., 1986. In "Cell Culture and Somatic Cell Genetics of Plants" (ed. by Vasil, I. K.), Vol. 3: p. 3-47, Academic Press, New York.
3) Yamada, Y., 1984. In "Cell Culture and Somatic Cell Genetics of Plants" (ed. by Vasil, I. K.), Vol. 1: p. 629-636, Academic Press, New York.
4) Larkin, P. J., W. R. Scowcroft, 1981. Theor. Appl. Genet., **60**: 197-214.
5) Bajaj, Y. P. S., J Reinert, 1977. In "Plant Cell, Tissue, and Organ Culture" (ed. by Reinert, J., Y. P. S. Bajaj), p. 757-789, Springer-Verlag, Berlin.
6) Withers, L. A., 1985. In "Cell Culture and Somatic Cell Genetics of Plants" (ed. by Vasil, I. K.), Vol. 2: p. 253-316, Academic Press, New York.
7) Nitzsche, W., 1978. Z. Pflanzenphysiol., **87**: 469-472.
8) Nitzsche, W., 1980. Z. Pflanzenphysiol., **100**: 269-271.
9) Kaimori, N., N. Takahashi, 1989. Jpn. J. Breed., **39**: 379-382.
10) Sakai, A., M. Noshiro, 1975. In "Crop Genetic Resources for Today and Tomorrow" (ed. by Hawkes, Int. Bot. Programme, Vol. 2: 317-326, Cambridge Univ. Press, London.

≪和文要約≫

液体窒素に貯蔵した人参乾燥カルスの植物体再生

貝守　昇，亀谷寿昭*

八戸工業大学第二高等学校
* 東北大学遺伝生態研究センター

人参の乾燥処理カルスと乾燥無処理カルスを液体窒素に貯蔵した．無処理カルスの14日後の生存率は，0% であったが，処理カルスの生存率は 100% であり，すべてのカルスが多数の不定胚や幼植物体を分化した．これらの結果は，人参カルスは乾燥後，直接液体窒素に入れて長期貯蔵できることを示している．