Note

Differences in Viability after Freeze-preservation in Liquid Nitrogen among Rice Callus Lines

Katsumi WATANABE, Saburo UENO
and Hisateru MITSUDA*Interdisciplinary Research Institute of Environmental
Sciences, Hichihon-matsu, Itsutsuji,
Kyoto 602, Japan*

Received October 17, 1989

Freeze-preservation in LN is one means available for preserving plant cells. Many studies are made on the freeze-preservation of plant cells.¹⁾ We have reported that lavender callus cells differ in cryoprotection, and that the frequency of colony formation of the freeze-preserved cells rose with the repetition of the freeze-thaw procedure and highly cryoprotectable cells were obtained.²⁾ These results raise the possibility that plant callus cells generally differ in cryoprotectability, thus the change in viability after freeze-preservation by repeating freeze-thawing may be a general characteristic of the freeze-preservation of plant callus cells. However, little is known about such changes in viability. Therefore, we freeze-preserved callus cells of rice, which is an important cereal, to examine it.

Rice callus cells were induced from seeds on Linsmaier-Skoog agar medium³⁾ with 10^{-5} M 2,4-dichlorophenoxyacetic acid at 27°C under light and were subcultured every 2 weeks under the same conditions.

Rice callus cells were frozen in LN, then thawed and cultured by a method similar to that used for lavender callus cells.²⁾ Cell suspensions containing single cells to small aggregates (≤ 1 mm) of rice callus cultures at a logarithmic growth phase were chilled on ice. An ice-chilled cryoprotectant solution (a mixture of 10% (v/v) dimethylsulfoxide and 20% (w/v) D-glucose in distilled water) was added gradually to an equal volume of the cell suspension over a period of 30 min, after which the resulting cell suspension was kept on ice for 1 hr and was cooled at 1°C/min to -30°C , then transferred into LN as described elsewhere.⁴⁾ After storage in LN, the cell suspension was thawed rapidly and spread on a semi-solidified medium. The spread cells were cultured for up to 3 months under the conditions described above and the numbers of colonies formed were counted. Formed colonies were transferred onto fresh solidified medium and were cultured under the same conditions. The cell aggregates that developed from those colonies were frozen again, and then

thawed and cultured according to the same freeze-thaw procedure as described above.

The number of colonies formed of freeze-thawed rice callus cells increased with the repetition of the freeze-thaw procedure (Fig. 1). The average value at the eighth procedure was thirty times higher than that at the first procedure.

On the other hand, fragile callus cell-aggregates occurred in the unfrozen, rigid originally induced callus cell-aggregates during subcultures of many generations. Consequently, the unfrozen control callus culture after the subcultures became a mixture of rigid callus cells and fragile callus cells. This suggested the possibility that the viability of callus cells before and after the subcultures might differ. Therefore, the callus cells after the subcultures were freeze-preserved. The number of colonies formed of the callus cells after the subcultures (Fig. 2B) was higher than that of the callus cells before the subcultures (Fig. 2A). This high value of the callus cells after the

Fig. 1. The Change of the Frequency of Colony Formation of Rice Callus Cells by Repeating the Freeze-thaw Procedure.

One-half ml of settled cells were cultured on a semi-solidified medium and the numbers of colonies formed were counted. (A) The first procedure (original induced callus cells); (B) the fourth procedure; (C) the sixth procedure; (D) the eighth procedure. $\bar{x}$, average value; n , number of petri dishes.

Fig. 2. The Change of the Frequency of Colony Formation of Unfrozen Control Rice Callus Cells after Subcultures of Many Generations without Freeze-thawing.

One-half ml of settled cells were cultured on a semi-solidified medium and the numbers of colonies formed were counted. (A) Callus cells before the subcultures (original induced callus cells); (B) a mixture of rigid callus cells and fragile callus cells after the subcultures; (C) fragile callus cells separated after the subcultures; (D) rigid callus cells similar to originally induced ones. $\bar{x}$, average value; n , number of petri dishes.

subcultures might be due to the fragile callus cells. To examine it, fragile callus cells and rigid callus cells were separated, then cultured individually and were freeze-preserved. The frequencies of colony formation of the two types of callus cells are shown in Figs. 2C and 2D. The fragile callus cells formed many colonies, but the rigid callus cells formed only a few colonies. Thus, it was concluded that the number of colonies formed of the callus cells after the subcultures was increased by the fragile callus cells.

Highly cryoprotectable rice callus cells were obtained by repeated freeze-thawing in the same manner as lavender callus cells.²⁾ However, the callus cells selected by repeated freeze-thawing resembled the separated fragile callus cells in the frequency of colony formation (Figs. 1D and 2C). The selected callus cells were fragile and the suspension of the cells contained a large number of small cell aggregates in a similar manner as the separated fragile callus cells. We need further studies to make clear whether the rice callus line selected by repeated freeze-thawing is a highly freezing-tolerant line or a fragile line such as that occurring during subculture.

These results suggested that the viability after freeze-preservation differed among rice callus lines and that the increase of viability by repeating freeze-thawing might be one of characteristics of the freeze-preservation of plant callus cells.

References

- 1) K. K. Kartha, *IAPTC Newsletter*, No. 45, 2 (1985).
- 2) K. Watanabe, Y. Yamada, S. Ueno and H. Mitsuda, *Agric. Biol. Chem.*, **49**, 1727 (1985).
- 3) E. M. Linsmaier and F. Skoog, *Physiol. Plant.*, **18**, 100 (1965).
- 4) K. Watanabe, H. Yoshikawa, S. Ueno and H. Mitsuda, *Annual Report of Interdisciplinary Research Institute of Environmental Sciences*, **6**, 63 (1987).