Amino Acids Differ in their Effect on the Post-thaw Recovery of Cryopreserved *Lavandula vera* Cells

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Cryogenic storage of meristem tips or cultured cells is a suitable method for long-term preservation of horticultural plants (Kantha, 1985). Successful storage of cultured *Lavandula vera* DC. cells and regeneration of plantlets from the stored cells has been previously reported (Watanabe et al., 1983). Recently, we have shown that *L. vera* cells at the initial stage of post-thaw culture are very sensitive to ammonium (Kuriyama et al., 1996). Ammonium as a major element of a culture medium is toxic to freeze-damaged cells, although it is required for the growth of freeze-recovered cells or unfrozen control cells (Kuriyama et al., 1989). This result suggests that other nitrogenous compounds also affect the survival rate of cryopreserved cells. In this study, the effect of amino acids on the post-thaw recovery was examined in cryopreserved *L. vera* cells.

Lavandula vera cells were routinely subcultured at 27 °C every 3 weeks on Linsmaier and Skoog's (1965) medium supplemented with 3% sucrose, 10 µM IBA, 1 µM BA, and 0.2% Gelrite (Wako Pure Chemical Ind., Osaka, Japan) (LS medium) (Watanabe et al., 1983). Cells cultured for 7 d were transferred to glass tubes containing NH₄NO₃-free liquid LS medium and were cooled in an ice bath. A cryoprotectant solution (20% glucose and 10% DMSO in distilled water) was added gradually to an equal volume of the cell suspension over 30 min. Cryoprotected cells were transferred to polypropylene tubes (10 × 35 mm) on ice, then cooled in a programmed freezer (Cryo-Med, Mt. Clemens, Mich.) at 1 °C·min⁻¹ to -40 °C, and finally immersed in liquid N. The

stored cells were thawed rapidly by swirling in a 60 °C water bath for 40–60 s until the ice in the tube had just disappeared, then the tube was chilled on ice to prevent an excessive rise in temperature (Watanabe et al., 1983). Thawed cell suspensions were diluted with 10 volumes of liquid culture medium over 10 min and washed. Washing was repeated 3 times. Frozen-thawed cells at a packed cell volume of 0.25 mL were placed in petri dishes (9 cm in diameter) containing 25 mL of solid medium and cultured 7 d at 27 °C. Various amino acids (20 mM) were individually added to NH₄NO₃-free LS medium that contained KNO₃ (20 mM) as a principal source of N.

The effect of various amino acids on the post-thaw recovery of cryopreserved cells was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) according to the method of Steponkus and Lanphear (1967). This method provides the assessment of cell viability by measuring the efficiency of the mitochondrial electron transport chain, as suggested by Towill and Mazur (1975). We examined the viability of cryopreserved rice (*Oryza sativa* L.) and *L. vera* cells by this method and showed correspondence between the results of the TTC test and post-thaw regrowth (Kuriyama et al., 1989, 1996). The amino acids we tested differed in their effect on the post-thaw recovery (Fig. 1). Alanine and glutamic acid appeared to be suitable for the post-thaw recovery because the cells cultured with these amino acids were more viable than the control cells (Fig. 1). In a previous study, we reported that freezing injury of cryopreserved *L. vera* cells is repairable, but the repair is inhibited by ammonium (Kuriyama et al., 1996). Here, we report that the repair of freezing injury also depends on the amino acid species.

To make cryopreservation widely useful, it is important to enhance the survival rate of difficult-to-preserve materials. Use of suitable amino acids may help to enhance the survival rate of preserved cells or tissues of *L. vera* and, possibly, other species.

Fig. 1. Effect of various amino acids on post-thaw recovery of cryopreserved *Lavandula vera* cells. TTC assay values were measured in unfrozen cells and in post-thaw cultured cells, then each value (the mean of three measurements) was shown as the percentage of the TTC assay value of unfrozen cells. Control: Ammonium-free LS medium.

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