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Effect of Cryoprotectants on Cryopreservation of Sugarcane Cells

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ABSTRACT

Cell suspension of sugarcane cultivar ROC3 treated with different concentration and combination of cryoprotectant were cryopreserved. Before cryopreservation, temperature of cell suspension was reduced from room temperature to O°C/ min. and from O°C to -40°C at the rate of 0.5°C/min.. Then, cell suspension were storaged in liquid nitrogen for 30 minutes. After storage, sugarcane cells were recultured again on solid media. Sugarcane cells treated with 15% dimethysulphoxide (DMSO) and 1.OM sucrose showed the best regrowth among the cryoprotectants studied.

Keywords: cryoprotectant, cryopreservation, sugarcane, cell suspension

INTRODUCTION

Preservation of germplasm of vegetative-propagated crops such as sugarcane have to be maintained in the field in which the germplasm may be lost by the hazards of pests and pathogens. *In vitro* preservation by reducing growth of tissue is the alternative methods for preserving sugarcane germplasm. Recently, cryopreservation was developed to preserve plant tissue in which metabolism of preserved tissue is ceased, but still alive, and tissue can be storaged for long period. Cryopreservation was studied to preserve shoot tips of strawberry (Kartha *et al.*, 1908), shoot tips of potato (G rout and Henshaw, 1978 and Towill, 1983), embryos of large-seeded temperate trees (Pence, 1990) and cell suspension of various crops. Cryoprotectant, a substance to protect cell from the damage by freezing, is important for preserving tissue in liquid nitrogen. In this experiment, concentration and combination of cryoprotectant were studied to cryopreserve sugarcane cells.

MATERIALS AND METHODS

1. Cell suspension culture of sugarcane

Callus of sugarcane cultivar ROC3 derived from culture its shoot tip on MS media supplemented with 15% coconut water, 20g/l sucrose and 2,4-D 3 mg/l was subcultured on the same media every 2 weeks by mixing 2ml of cell suspension and 50ml fresh media. Cell suspension after subcultured for 5 days was used for cryopreservation experiment.

2. Cryoprotectant treatment

Cryoprotectan treatments in this study were 2 levels of dimethysulfoxide (DMSO) at 10% and 15% combined with 0.5M or 1.0M of sucrose or without sucrose. Cryoprotectant solution was prepared at twice the concentration used in the experiment. The same volume of cell suspension was gradually added into the cryopreservation tubes gradually within 40 minutes. The mixing solution was incubated under room condition for 40 minutes before freezing.

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3. Freezing method

Cell suspension with cryoprotectant in cryopreservation tubes were put in the programmable freezing chamber. The temperature was reduced from room temperature to O°C by 1°C/min. and kept at temperature at O°C for 15 minutes. From O°C to -10°C, the temperature was reduced by 0.5°C/min, kept at -10°C for 15 minutes and reduced to -40°C by 0.5°C/min.. The temperature was kept at -40°C for 20 minutes and then cryopreservation tubes were put in liquid nitrogen tank. After storage in liquid nitrogen for 30 minutes, cryopreveration tubes were thawed in 35°C water until ice in the cells became solution.

4. Cell washing and reculture

The solution of cell suspension and cryoprotectant in cryopreservation tubes was transferred to centrifuge tubes. Eight m/l of liquid media was added into each centrifuge tube by adding 1 ml every minute. The tubes were centrifuged in the rate of 500rpm for 5 minutes and the clear solution was removed by using pipette. The same washing method was repeated one time and then cell was cultured into the petri dish containing liquid media. solid media whose formulation was the same as the

RESULTS AND DISCUSSION

Sugarcane cells treated with cryoprotectant after storage in liquid nitrogen could regrow, while sugarcane cells without cryoprotectant could not survied (Table 1). Nevertheless, not all of sugarcane cells treated with cryoprotectant could be survived. The sugarcane cell treated with the low concentration of cryoprotectant (10%DMSO and 10% DMSO+ 0.5M sucrose) could not survive. Among the survived cell, sugarcane cells treated with high concentration (15% DMSO + 1.0M sucrose) tended to have better growth after storage in liquid nitrogen. It was noticed that the survival of sugarcane cells in this experiment was not high. The level of temperature during treating cryoprotectant may be the important factor. In this experiment, treatment was conducted under room temperature. Finkle *et al.* (1985) reported that low temperature was required during treating of cryoprotectant to decrease the exchanging rate of water in the cells and cryoprotectant which affected the vigor of cell under freezing during storage in liquid nitrogen.

The combination of the suitable concentration and kinds of cryoprotectant is important. Finkle and

Table 1 Growth and lag phase of cell suspension treated with different cryoprotectants with/without storage in liquid nitrogen.

Croprotectant	Without storage in LN_2		Storage in LN_2	
	Growth	Lag phase (days)[1/]	Growth	Lag phase (days)
Without cryoprotectant	+++++[2/]	5	-	-
10% DMSO	++	8	-	-
15% DMSO	++	11	+	32
10% DMSO + 0.5M Sucrose	++	9	-	-
10% DMSO + 1.0M Sucrose	+++	9	+	28
15% DMSO + 0.5M Sucrose	+++	10	+	35
15% DMSO + 1.0M Sucrose	+++	8	++	35

1/ days from the first day of culture cell on solid media to the days that microcalli could be observed

2/ +++++ = Good (same as growth of cell without cryoportectant and storage in liquid nitrogen)
++++ = Moderate good
+++ = Moderate
++ = Moderate poor
+ = Poor
- = No growth

Ulrich (1979) suggested that using more than one kind of cryoprotectant can dilute the concentration of one cryoprotectant and then reduce the toxicity of cryoprotectant to the cells. In this experiment, sugarcane cells treated with the combination of DMSO and sucrose showed better growth than the ones treated with only DMSO and the suitable concentration was 15% DMSO + 1.0M sucrose. Cryoprotectant not only protected cells from freezing injury but also reduced growth and extended lag phase period of cells. This lag phase was observed in cells without storage in liquid nitrogen. The extension of lag phase was longer in cells storaged in liquid nitrogen.

CONCLUSION

Sugarcane cells could be storaged in liquid nitrogen using slow freezing method with cryoprotectant The best combination of cryoprotectant in this study was 15% DMSO and 1.0M sucrose. Nevertheless, vigor of sugarcane cells when recultured was low.

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