Cryopreservation of oil-palm embryogenic suspensions

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Introduction

Oil-palm (*Elaeis guineensis* Jacq.) embryogenic cell suspensions produced from leaf calli were cultivated on liquid medium supplemented with 2,4–dichlorophenoxyacetic acid (2,4–D) (de Touchet *et al.* 1991; Duval *et al.* 1995). In this work, a cryopreservation protocol was developed to conserve the embryogenic capacities of the suspensions and to limit the risks of losing the material due to contamination (Engelmann 1997). A classical freezing was employed for freezing these oil-palm embryogenic cell suspensions. The effects of glucose and dimethylsulfoxide on cell survival were investigated and the optimal duration of post-treatment was determined.

Materials and methods

Four different cell lines (Nos. 121, 123, 221 and 341) were sampled during their proliferation phase. The pretreatment was carried out by incubating 0.3 ml of suspension per ml of cryoprotective medium for 1 h at 4°C. The cryoprotective media tested contained a range of four concentrations of glucose (0.1, 0.5, 1, 1.5M) combined with four concentrations of DMSO (0, 5, 10, 15%). After the pretreatment, cells were cooled at 0.5°C/min to –40°C, then immersed in liquid nitrogen. After rapid thawing (2 min at 40°C), the viability was estimated using staining with triphenyl tetrazolium. Results were expressed as percentage of the untreated control. Cells were then placed on semi-solid medium for regrowth before their transfer to liquid medium. The influence of the duration of post-treatment on semi-solid medium was tested from 0 to 28 d, and evaluated by measurement of subsequent growth rate of the cultures in liquid medium. Three replicates were used for each treatment. Results were analyzed by one-way ANOVA. Newman and Keul's tests were applied when significant effects were observed. The relative importance of each effect was then measured.

Results

Pretreatment

The effect of glucose and DMSO on survival after cryopreservation varied with the cell line (Fig. 1). Clone effects represented 36% of the total variance (data not shown). For all cell lines, DMSO had the main effect on survival after cryopreservation compared with glucose. DMSO explained 38–81% of the total variance (Table 1). Glucose had a significant effect on three of the four cell lines (Table 1). Statistical analyses allowed identification of the optimal concentration of DMSO as being 10% and for glucose, as 1M. Though glucose-DMSO

interaction was found to be significant in two lines, its importance was always lower than that of the separate effects of each compound alone.

Post-treatment

After a post-treatment of 14 d on semi-solid medium, the growth rate of cryopreserved cell suspensions was 70% during the first subculture in liquid medium, and 240% during the second subculture. Extending the post-treatment duration did not significantly improve the growth rate of cryopreserved cells (Fig. 2). Regeneration of somatic embryos from all cryopreserved cell lines was successful (data not shown). Thus, the cryopreservation protocol has no effect on embryogenic capacities.

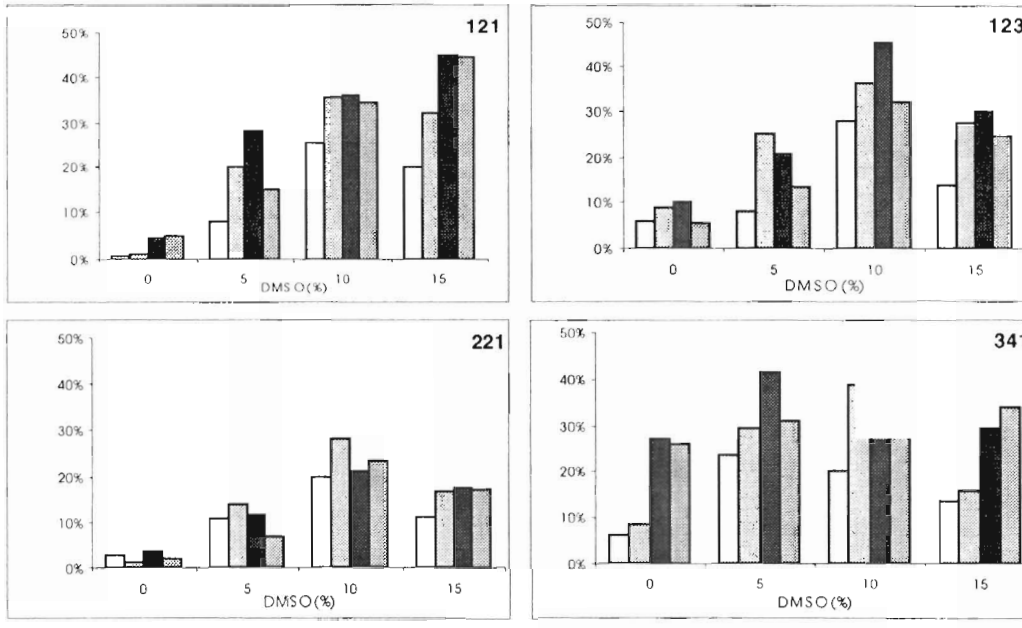

Fig. 1. Effect of the composition of cryoprotective medium on survival of four different cryopreserved cell lines. Cryprotectant was a combination of four concentrations of DMSO and four concentrations of glucose (0.1, 0.5, 1, 1.5M – bars, left to right).

Table 1. Relative importance of glucose and DMSO effects on survival after liquid nitrogen treatment for four cell lines. Values represent % of the total variance.

	Cell line number			
	121	**123**	**221**	**341**
Glucose	13.68	17.54	NS	44.01
DMSO	78.88	81.45	58.14	38.16
Interaction	7.44	NS	NS	17.83

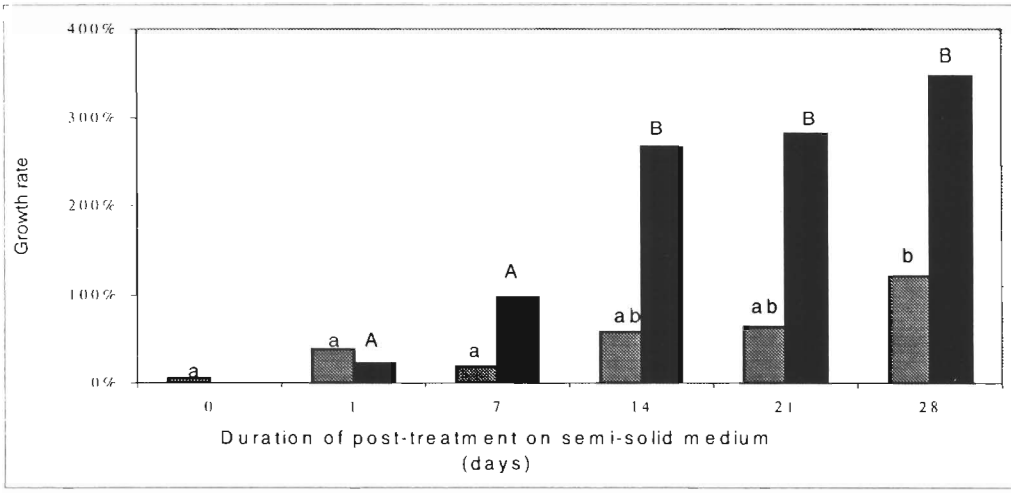

Fig. 2. Post-treatment of cryopreserved cells. Growth rate during the first (▩) and the second (■) subculture in liquid medium. Same letters (small letters for the 1st cycle and capital letters for the 2nd cycle) indicate results not significantly different at the 0.05 level according to the Newman and Keul's tests.

Conclusion

A protocol including pretreatment of cell suspensions with 10% DMSO and 1M glucose, two-step freezing, rapid rewarming and a 14-d post-treatment on semi-solid medium is now routinely employed in our laboratory for the cryopreservation of oil-palm embryogenic cell lines.

References

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Chabrillange Nathalie, Aberlenc Bertossi Frédérique, Noirot Michel, Duval Yves, Engelmann Florent. (2000).

Cryopreservation of oil-palm embryogenic suspensions.

In : Takagi H. (ed.). Cryopreservation of tropical plant germplasm : current research progress and application.

Rome (ITA) ; Tsukuba : IPGRI ; JIRCAS, p. 341-343.

JIRCAS International Agriculture Series ; 8). Cryopreservation of Tropical Plant Germplasm : Current Research Progress and Application : International Workshop, Tsukuba (JPN), 1998/10/20-23.

ISBN 92-9043-428-7.