EFFECTS OF LOADING AND VITRIFICATION SOLUTIONS ON SURVIVAL OF ZYGOTIC EMBRYOS OF *HEVEA BRASILIENSIS* MUEL. ARG. IN LIQUID NITROGEN

Y.L. Hor

Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Keywords: vitrification, loading, zygotic embryos, *Hevea*, liquid nitrogen.

Introduction

Hevea bralisiensis is an important plantation crop in Malaysia and its continued improvement is highly dependent on an effective plant breeding programme. The success of such programmes hinges on the availability of broad genetic base and their long-term conservation.

Conservation of *Hevea* germplasm is difficult owing to the recalcitrant nature of its seeds. In recent years, alternative in vitro techniques have been demonstrated to be successful for *Hevea* where 61-71% survival have been achieved (Normah et al. 1987; Yap, 1997 More recently, vitrification technique was successfully demonstrated as a valuable cryogenic protocol for cryopreservation of many plant parts. The objective of this study was to evaluate the potential of the vitrification technique for rubber zygotic embryos and to compare its effectiveness with other techniques.

Materials and Methods

Zygotic embryos were excised aseptically from rubber seeds of clones PB260, PBIG 6, 7 and 8. To evaluate the effectiveness of various solutions, excised zygotic embryos were precultured on a solidified Murashige and Skoog (MS) medium supplemented with 0.3M sucrose for 16 hours at 25°. After preculture, the embryos were loaded in 2.0M glycerol and 0.4M sucrose for 40 minutes. The embryos were then exposed to three vitrification solutions for 20, 40 and 60 minutes. The vitrification solutions were PVS {22% glycerol + 15% ethylene glycol (EG) + 15% propylene glycol + 7% dimethyl sulphoxide (DMSO) + 0.4M sucrose} (Uragami et al. 1989); PVS2 {30% glycerol + 15% DMSO + 0.4M sucrose} (Sakai et al. 1990) and L solution {20% glycerol + 30% EG + 10% DMSO + 15% sucrose + 10mM $CaCl_2$} (Ishikawa, 1994). After exposure, the viability and survival of the embryos with and without LN exposure were assessed.

To establish the exposure time for vitrification, *Hevea* embryos were also immersed in the best vitrification solution based on the above study for up to 120 minutes. All procedures were carried out as described above.

A third study was also carried out to compare the above optimum vitrification procedure with other cryopreservation techniques.

Results and Discussion

The PVS2 and L solutions were more effective than PVS solution in cryopreserving embryos. However, PVS2 gave higher percentage viability compared to L solution. After 8 weeks culture, only embryos treated with PVS2 could survive but exposure up to 60 minutes was insufficient to result in high survival suggesting that PVS2 was more effective.

For optimum time of exposure to PVS2, longer exposure resulted in better viability and survival. Shoot formation was observed in embryos exposed for 70-120 minutes, indicating more tissues were vitrified. The highest viability obtained was with embryos exposed for 80 minutes. Longer exposure to PVS2 for 140 minutes and above was too toxic for the embryos to survive.

The moisture content of embryos after PVS2 exposure stabilised to around 43.3-46.6%. Moderate survival of the vitrified *Hevea* embryos confirmed the potential of PVS2 for cryopreservation even when they have relatively high moisture. This is especially so when compared with naked desiccation (Normah et al. 1986) and encapsulation dehydration (Yap, 1997) where high survival was only obtained at a low moisture of 16%-18%.

Comparison of the vitrification technique with other cryopreservation methods suggests that the use of PVS2 was superior with 75.2% viability and 57.8% survival after LN exposure Desiccation of naked embryos, naked embryos with sucrose preculture and encapsulated embryos were relatively less effective in cryopreserving rubber embryos with viability and survival ranging from 344-50.0% and 21.7-31.8% respectively.

Conclusions

The vitrification cocktail PVS2 was more effective than PVS and L solution for survival of *Hevea* xygotic embryos in liquid nitrogen (LN). The best time of exposure PVS2 was found to be 80 minutes.

Comparison of the vitrification technique with other methods of cryopreservation suggests that the use of PVS2 was superior with 75.2% viability and 57.8% survival after LN exposure. Desiccation of naked embryos, naked embryos with sucrose pre-treatment and encapsulated embryos with sucrose pre-treatment were relatively ineffective.

References

Ishikawa M. (1994). National Institute of Agrobiological Resources, Japan. Personal Communication.

Normah M.N., Chin, H.F., and Hor. Y.L. 1987. Desiccation and cryopreservation of embryonic axes of *Hevea brasiliensis*. *Pertanika*. 9: 229-303.

Sakai A., Kobayashi S. and Oiyama I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. Var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Report*. 9: 30-33.

Uragami A., Sakai, A., Nagai M. and Takahashi T. 1989. Survival of cultured cells and somatic embryos of *Asparagus officinalis* cryopreserved by vitrification. *Plant Cell Report*. 8: 418-421.

Yap L. V. 1997. The effects of desiccation, sucrose and abscisic acid preculture on survival of alginate-encapsulated zygotic embryos of rubber (*Hevea brasiliensis)* following liquid nitrogen exposure. M.Agric.Sc. Project. Universiti Putra Malaysia, Malaysia.

Supported by IRPA Grant 01-02-04-0001