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PLANT REGENERATION FROM SUGARCANE EMBRYOGENIC CALLI CRYOPRESERVED IN LIQUID NITROGEN

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Research for setting up a technique for the cryopreservation of sugarcane embryogenic calli started in 1990 at IRAT/CIRAD (Institut de Recherches Agronomiques Tropicales et des cultures vivrières / Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier) in cooperation with ORSTOM (French Scientific Research Institute for Development through Cooperation, Montpellier). The first part of the experiments consisted in testing various parameters using a single variety; its results are reported here.

The variety used was Co6415. Embryogenic calli of this variety were obtained from the rolled young leaves of in vitro grown plantlets as described by GUIDERDONI and DEMARLY (1988, Plant Cell, Tissue and Organ Culture, 14: 71-88). The explants were cultured on a MS solid callus induction medium (MURASHIGE and SKOOG, 1962, Physiol. Plant, 15 : 473-497), supplemented with 3 mg/l of 2,4-D, at 27°C and under dark condition. Embryogenic calli were subcultured every four weeks on a standard MS solid medium containing 1 mg/l of 2,4,-D, under the same conditions.

For cryopreservation experiments, small pieces of 9-10 week old embryogenic calli (about 3-4 mm of diameter) were used. We tried to set up optimal conditions for the following steps of a cryopreservation process : preculture, pretreatment, freezing (cooling rate and prefreezing temperature). Preculture (24 to 48 hrs in liquid medium) was found to be unnecessary for callus growth recovery after cryopreservation. Among the various connditions tested, a mixture of sucrose (0.3 to 0.5M) and DMSO (5 to 15%) ensured the highest survival rate. The optimal freezing conditions included freezing at 0.5°C/min to - 40, -45 or -50°C. After rapid thawing, the calli were transferred to the same standard medium and under the same conditions for regrowth. After 6-7 weeks, the regrown calli were transferred to a solid MS medium, lacking 2,4-D, at 27°C and under light (1200 lux) for shoot regeneration. With these conditions, we obtained up to 100% calli surviving and regrowing after a stay in liquid nitrogen. The growth rate and the regeneration potential were comparable to those of the unfrozen controls.

At present, a large number of plantlets has been produced from cryopreserved calli of Co6415. These plantlets are now multiplied in vitro and their development is similar to plants of unfrozen material. Their trueness to type is being tested. The same technique will soon be applied to other genotypes of the IRAT in vitro collection.

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