Cryoconservation of apices of sugar cane

Florence Paulet, Florent Engelmann

The development of *in vitro* microcutting techniques enabled CIRAD to set up an *in vitro* germ bank for sugar cane (*Saccharum* sp.). But the germ bank requires more and more space and an increasing number of personnel and the problem still remains of how to protect the biological stock from infection and genetic variation, the risk of which increases with the storage time. Freezing the apex in liquid nitrogen (–196° C) allows long-term storage of the clones in optimal conditions.

Materials and methods

The standard technique using chemical cryoprotectors (DMSO) has proved ineffective for sugar cane. Consequently, we tried the encapsulation/dehydration technique (DEREUDDRE *et al.*, 1990). This new form of cryoconservation was developed from techniques used for artificial seeds. The protective encapsulation alginate, polymerized after contact with calcium, allows cryoconservation of large apices which are easy to remove and no longer fragile. The technique is carried out in several stages: protective covering, pre-treatment, dehydration, cooling, immersion in liquid nitrogen

and post-treatment to stimulate renewed growth of the apex. These stages were developed successively on a commercial variety of sugar cane.

The procedure that produced the best results is the following: removal of the apex (0.5-1 mm) of vitroplants in full growth (Plate I, 3); storage on a Fuji MS-vitamin macro-element medium; encapsulation 24 hours later on the same medium; osmotic pre-treatment for two days in an agitated liquid medium in the presence of 0.75 M sucrose; dehydration of the beads (to 5-10% water content) for six hours at ambient temperature under a laminar flow hood; direct immersion of cryotubes in liquid nitrogen; slow reheating to ambient temperature; culture in the dark for one week on a solid MS medium in the presence of BAP and kinetin; transfer to the light in the presence of activated charcoal.

Results

Renewed growth of the apex was observed in the form of leaf emergence after one week (Plate II, 1). The regenerated plantlets had rooted after five weeks. With the variety Co 6415, the survival rate of the apices after cryoconservation ranged from 60-90%. The same method was used for four other varieties with a survival rate of between 40 and 90%.

No morphological difference was observed between cryoconserved apices and controls. Cryoconservation did not modify electrophoretic patterns of the leucin, aminopeptidase and amylase systems.

Prospects

Management of a cryoconserved collection necessitates carrying out successive cycles of freezing, since apices stored in liquid nitrogen and used subsequently can only be renewed from the proliferation cultures that they have produced. The procedure we developed has been carefully checked to make sure that this is possible. Plants produced from cryoconserved apices were frozen and thawed with identical survival rates to those obtained in the first freezing cycle.

Further trials are needed before a large-scale collection is undertaken following this procedure: histological analysis to check renewed growth starts directly from the apical meristem; application of the method to new varieties to confirm it is reproducible; and finally, more precise techniques (RFLP) to make sure that regenerated plants are true-to-type and to check the effects of several cycles of freezing-thawing and of long term storage in liquid nitrogen.

Bibliography

DEREUDDRE J., SCOTTEZ C., ARNAUD Y., DURON M., 1990. Résistance d'apex caulinaires de vitroplants de poirier (*Pyrus communis* L. cv. Beurré Hardy), enrobés dans l'alginate, à une déshydratation puis à une congélation dans l'azote liquide : effet d'un endurcissement préalable au froid. Comptes rendus de l'Académie des sciences, série III, 310 (7) : 317-323.

Paulet F., Engelmann Florent. (1994).

Cryoconservation of apices of sugar cane.

In : Teisson C. (ed.) In vitro culture of tropical plants. Montpellier : CIRAD, 11-13.

(Repères). ISBN 2-87614-162-0