Survival and recovery of yam (*Dioscorea* spp.) apices after cryopreservation using encapsulation-dehydration

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Introduction

Yams, which are edible or medicinal tuber crops, are very important crops in many developing countries. Efficient conservation methods are needed for these crops. Medium- and long-term conservation have been explored with *Dioscorea* (Malaurie *et al.* 1998a, 1998b, 1998c, 1998d). In addition to previous works by Mandal *et al.* (1996), Malaurie *et al.* (1998b) have investigated the effect of three factors on the survival of encapsulated apical shoot-tips of *in vitro* plantlets of two yam (*Dioscorea bulbifera* L. and *D. alata* L.) genotypes after cryopreservation. The factors studied were: (i) the pretreatment duration in sucrose liquid medium, (ii) the sucrose concentration, and (iii) the duration of desiccation with silica gel. Techniques and the most important points of the note are presented here.

Material and methods

Two clones – Brazo fuerte for *D. alata*, and Nouméa Imboro for *D. bulbifera* – from the *in vitro* collection of yam germplasm maintained at ORSTOM Montpellier (Malaurie *et al.* 1993) were used. Nodal cuttings were subcultured on MS standard medium. Mother microplants, 5–8 months old, were used for massive shoot production; after 18 ± 4 d in culture, apices were excised from the young growing shoots. All cultures were maintained under the following standard environmental conditions: at 27 ± 1°C, under a light intensity of 36 µE m^{-2} s^{-1} (PAR), with a 12-h light/12-h dark photoperiod. Excised apical shoot-tips (2–5 mm long) were placed overnight in Petri dishes on solidified medium containing 5% (w/v) sucrose. Apices were encapsulated in 3% (w/v) calcium alginate. Encapsulated apices were pretreated for 3–10 d (*D. alata*) or 13 d (*D. bulbifera*) in liquid MS standard medium with various sucrose concentrations (0.75 to 1.1M), in 125-ml Erlenmeyer flasks, on a rotary shaker (91 rpm) under standard environmental conditions. After sucrose pretreatment, beads were dehydrated for 4–23 h in 125-ml airtight boxes filled with 40 g silica gel. Twenty beads were used per treatment: 10 dried beads were placed in a 2-ml polypropylene sterile cryotube and frozen directly in liquid nitrogen where they were kept for at least 2 h; the 10 remaining beads were placed in Petri dishes onto 2GG medium (Malaurie *et al.* 1993) without activated charcoal and maintained on 30 g/L sucrose for dehydration treatments up to 12 h, and supplemented with 50 g/L sucrose, 1 mg/L benzylaminopurine, 0.01 mg/L naphtalene acetic acid for longer desiccation periods. The residual water content of the alginate beads was expressed in g water per g dry weight. Dry matter was determined after desiccating 20–50 beads in 125-ml airtight boxes containing 40 g of dry silica gel, for up to 30 d (DW_{30}) (Table 1).

Table 1. Dry mass and water content of sucrose-pretreated alginate beads, determined after 30 d of drying with silica gel in airtight boxes at room temperature

Sucrose concentration	DW30 (% FW) estimated by linear regression[†]	Water content before dehydration (g/g DW)
0.75M	28.8	2.47
0.9M	33.3	2.00
1.0M	36.3	1.76
1.1M	39.3	1.54

[†] From mean values over 13–15 replicates for each of the four sucrose concentrations (y= 6.4319 + 29.872x; N= 4; r= 0.999). Similar results were obtained from replicate data (y= 6.4177 + 29.883x; N= 55; r= 0.960); data not shown. (Adapted from Malaurie *et al.* 1998b, with permission).

Results

The duration of sucrose pretreatment had a significant effect on the survival of cryopreserved *D. alata* apices only, and optimal pretreatment durations were between 3 and 7 d. The sucrose concentration employed during pretreatment had a strong effect on both genotypes. With *D. alata*, the highest survival was noted with 0.9M sucrose, and with 0.9 up to 1.1M sucrose in the case of *D. bulbifera*. With both genotypes, high survival rates after cryopreservation were obtained for bead water contents lower than 0.15 g H_2O/g DW only. With *D. alata*, the highest survival rate (67%) was achieved after pretreatment with 0.9M sucrose followed by 11–12 h of dehydration, and survival rates were not significantly different after 9–23 h of dehydration (Fig. 1). With *D. bulbifera*, the highest survival rate (65%) was achieved after pretreatment with 1M sucrose followed by 14–16 h of dehydration. Survival was not significantly different for desiccation periods between 11 and 23 h. After 3 months of culture on medium without growth regulators, 60% of cryopreserved *D. bulbifera* shoot-tips had developed into plantlets, whereas this was the case with 19% of frozen *D. alata* apices only, owing to browning and/or callusing of numerous shoot-tips.

Discussion and conclusion

This study demonstrated that apices of *D. alata* cv. Brazo fuerte and *D. bulbifera* cv. Nouméa Imboro could be successfully cryopreserved using the encapsulation-dehydration technique. For the two species, the water content of encapsulated apices had to be decreased down to 0.15 g H_2O/g DW in order to obtain high survival after freezing. The percentage of water loss was of 67, 62, 58 and 55% FW (± 1%) for 0.75, 0.9, 1 and 1.1M sucrose pretreatments, respectively.

Our results demonstrated that, in most cases, survival increased when dehydration was extended to a defined threshold, around 0.13–0.15 g H_2O/g DW, which was obtained after desiccation periods from 10 to 18 h. It seemed that, with this soft desiccation process, we could rub out differences in residual water, which certainly exist between apices from a same plot.

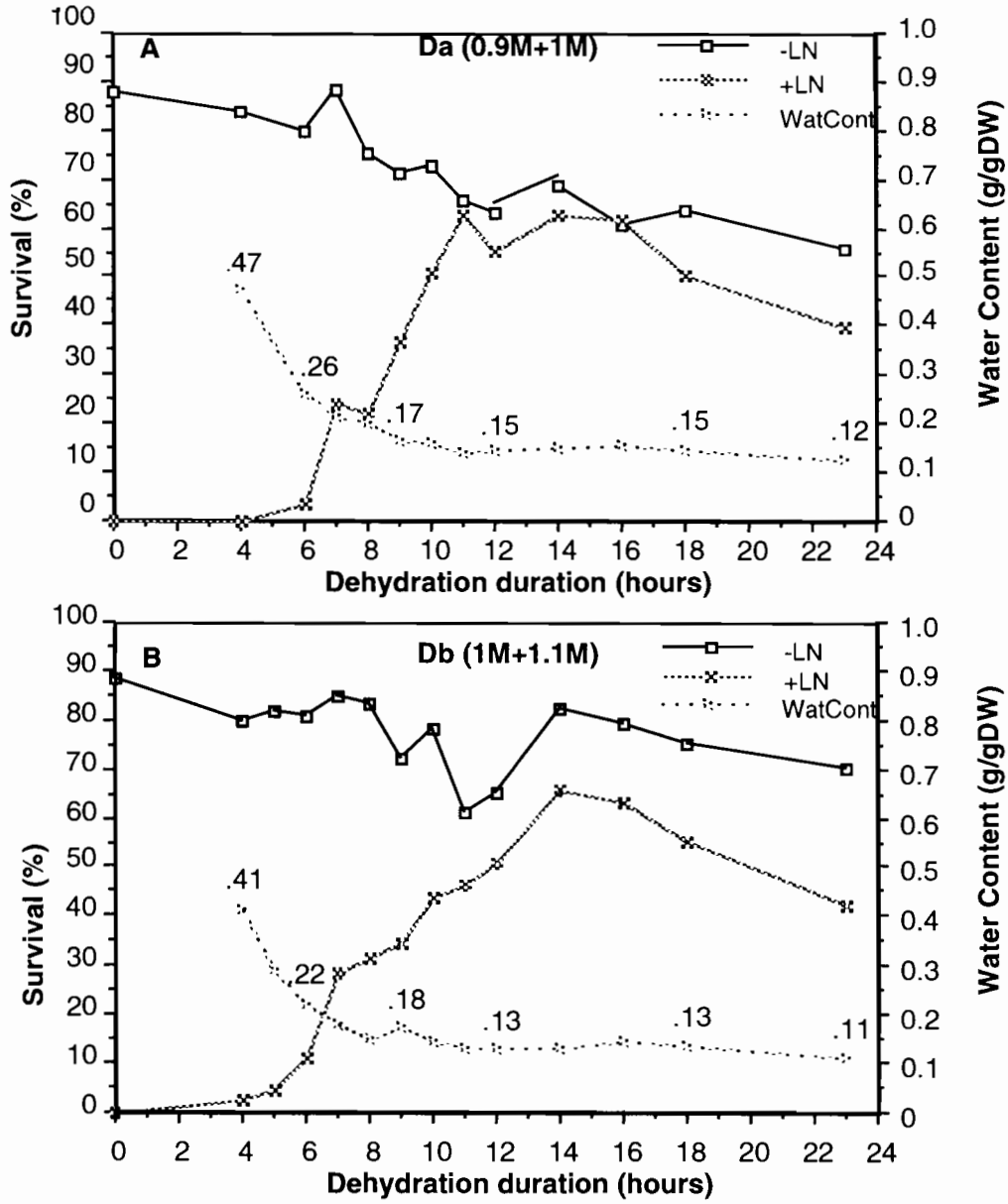

Fig. 1. Effect of dehydration duration on the water content of beads and survival rate of control (–LN) and cryopreserved (+LN) encapsulated apices of *D. alata* Brazo Fuerte after pretreatment with sucrose (0.9M+1M) (**A**), and *D. bulbifera* Nouméa Imboro after pretreatment with sucrose (1M+1.1M) (**B**). Each point corresponds to a mean over two sucrose concentrations and all the 6 or 8 pretreatment durations used depending on the clone. (Reprinted from Malaurie *et al.* 1998b, with permission).

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