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Survival cryopreservation of hop shoot tips monitored by differential scanning calorimetry

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Abstract

Differential scanning calorimetry is used for monitoring the survival conditions of hop (*Humulus lupulus* L.) shoot tips under cryopreservation. The survival is related to the absence of formation and growing of ice crystals during the cooling process as shown by the absence of heat effect from ice formation. Samples with different degrees of humidity were obtained through a controlled process of dehydration. © 1998 Elsevier Science B.V.

Keywords: Cryopreservation; Differential scanning calorimetry; *Humulus lupulus* L.

1. Introduction

Thermal analysis techniques provide information in several research fields [1,2]. The main applications are characterization of a wide variety of materials, such as synthetic compounds [3,4], natural rocks [5,6] and foods [7–9].

Cryopreservation methods [10] are widely used for storage of germplasm of plant species, but previous to storage, it is necessary to prepare the material to optimize survival. Determination of the degree of survival as a function of conditions is usually carried out by means of cultures in adequate media [11]. This system gives very good results but is inconveniently slow (20–30 days). In this paper, differential scanning calorimetry (DSC) is used as a rapid and reliable method for monitoring the survival capacity of cryopreserved plant systems.

2. Experimental

Plants of *Humulus lupulus* L. cv Nugget were surface sterilized with 1% sodium hypochlorite for 20 min, followed by three rinses with sterile distilled water. Nodal segments (1 cm) were in vitro cultured on Adams medium [12] which contained 3% glucose, 4.4 mM benzylaminopurine (BAP) and 0.5 mM indolebutyric acid (IBA), solidified with agar (0.75%, pH 5.2). The micropropagation chain was maintained for one year by transferring nodal segments onto fresh medium at six-week intervals. Growth conditions were 25°C and 16 h photoperiod (40 $\mu m\ m^{-2}\ s^{-1}$).

For cryopreservation experiments, in vitro shoots (6 weeks after the last transfer) were cold acclimated in a controlled environment chamber for 21 days (8 h

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photoperiod at 12°C and 16 h dark at 6°C). Shoot tips, comprising the meristematic dome with two or three pairs of foliar primordia (0.5–2.0), were excised from apical and axillary buds using forceps and scalpel under a stereo-microscope. After excision, cryopreservation of the shoot tips were performed as previously described [13] by trapping the shoot tips in an alginate matrix (shoot tips suspended in a liquid culture medium with 0.5 M sucrose, 4.4 mM BAP, 0.28 mM GA_3, and 3% alginic acid but free of calcium, then dropped with a Pasteur pipet into the same liquid medium supplemented with 100 mM calcium chloride but without alginic acid, to form beads of calcium alginate each having one shoot tip), and preculturing the beads for two days in solid medium with 0.75 M sucrose. Dehydration was undertaken by placing 10 beads on top of a filter paper in an open Petri dish containing 30 g of silica-gel (previously activated at 110°C for 2 days), and placing the dish in the air current of a flow cabinet at 20°C for different times (1–8 h). Beads with different moisture contents were cooled from 20°C to −40°C (at 5°C min^{-1}), and immersed in liquid nitrogen, 10 beads per 2 ml-cryovial (Nalgene). Vials were rewarmed in a flow cabinet at room temperature for 5–15 min, and beads cultured on solidified Adams medium with the hormonal supplement used for encapsulation. After 30 days of culture, shoots were recovered and transferred to the medium used for shoot multiplication to determine the best dehydration times for shoot-tip survival.

For water-content determinations, alginate beads containing seven shoot tips each were placed for 8 h under similar conditions as those described above for dehydration in the cryopreservation procedure, and finally dried in an oven at 110°C for 16 h and reweighed. DSC was performed with a Mettler TA 4000 system (DSC 30; rate of cooling 5°C min^{-1}) on beads after 0, 1, 2 and 3 h of dehydration. Sample masses were between 15 and 30 mg, as a function of the water content. One bead was placed in an aluminum pan, and each analysis was repeated at least six times to ensure reproducibility.

3. Results and discussion

Fig. 1 shows water content of the alginate beads trapping shoot tips as a function of the dehydration time in the air of a flow cabinet at 20°C. The sample mass remains constant from the third hour of dehydration (12% water content). Survival after applying the cryopreservation procedure [14] to samples with different water contents (Fig. 2) shows that dehydration is necessary for shoot-tip survival after immersion in liquid nitrogen. Only for water contents of 8–12% is survival high enough (85–90%). For water contents >20% there is no survival of the samples.

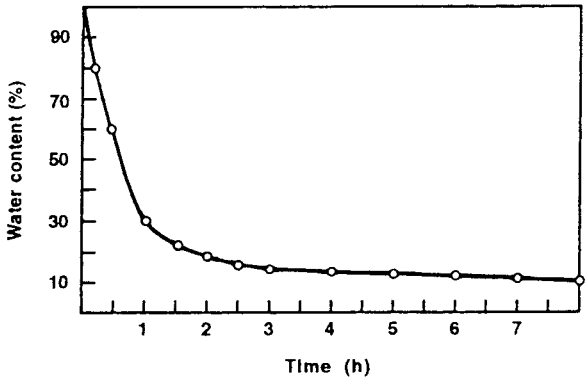

Fig. 1. Changes in water content of encapsulated shoot tips (seven per bead) after two-day preculture with 0.75 M sucrose, expressed as a function of air-drying time. Three replicate batches of 10 shoot tips each were prepared.

Fig. 3 shows the typical DSC profiles for these systems. The starting material (100% water content) shows an exothermic peak related to ice formation centered at -10.9 ± 0.6°C, with $Q=163\pm2$ J/g (Fig. 3(a)) related to ice formation. Samples dehydrated for one hour (30% water content), (Fig. 3(b)), also show this signal but with less heat, $Q=89\pm7$ J/g, in accordance with a lower content of extracellular water, which also explains the lower nucleation temperature (-32 ± 4°C) [10]. For samples dehydrated for two hours (15% water content), a further decrease of the exothermic peak is recorded ($Q=3\pm3$ J/g), (Fig. 3(c)). A longer dehydration time, 3 h (12% water content), gives total loss of the ice formation signal (Fig. 3(d)).

The disappearance of the ice nucleation signal indicates that plant samples have been properly prepared for storage at supercooling temperatures (−196°C, liquid nitrogen) to achieve high rates of survival (shoot recovery) after thawing. Fig. 4 shows the correlation between the heat of ice formation and survival rate after cryopreservation. Small increases in

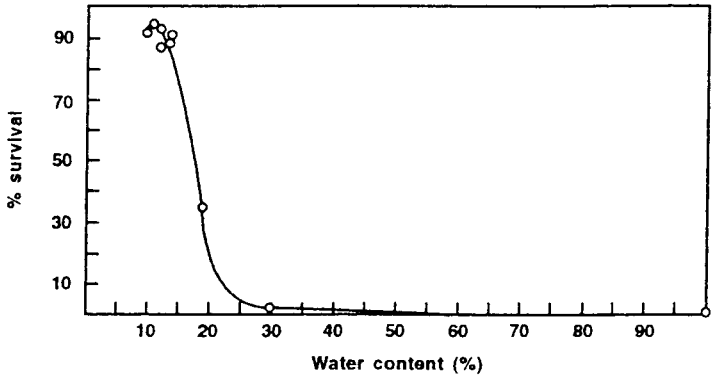

Fig. 2. Effect of water content on the percentage of survival from shoot-tip beads (one per bead) after preculture in sucrose, and subsequent immersion in liquid nitrogen. Five replicated batches (10 beads each) for each set of conditions were made.

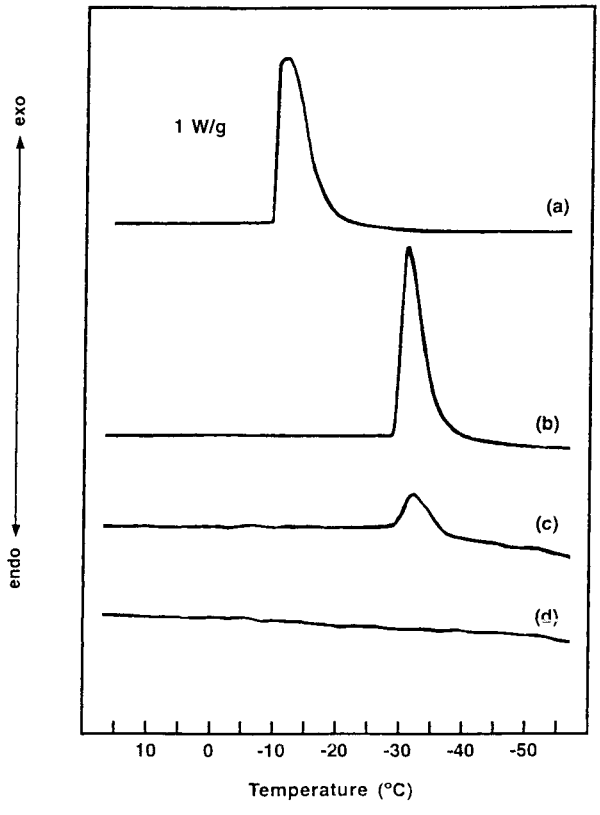

Fig. 3. DSC profiles of encapsulated hop shoot tips with different water contents: (a) 100%, (b) 15%, (c) 12%; and (d) 12%. Six different samples for each water content were analyzed.

the heat effects registered between 12 and 15% water content give rise to large decreases (60%) in survival of the plant material.

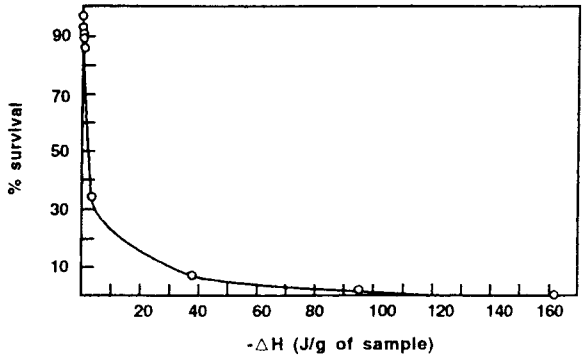

Fig. 4. Hop shoot-tip survival rate vs. ice formation heat.

4. Conclusion

Differential scanning calorimetry is a powerful tool for the control of water content of hop shoot tips before cryopreservation. The absence of heat effects from ice formation in partially dehydrated plant samples is correlated with high survival rates after immersion in liquid nitrogen. In contrast, ice formation is always related to insufficient dehydration and no survival of the plant material after cryopreservation.

Acknowledgements

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