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USE OF THERMAL ANALYSIS IN THE EVALUATION OF CRYOPRESERVATION PROTOCOLS FOR *RIBES NIGRUM* L. GERMPLASM.

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SUMMARY

Shoot-tips of *Ribes nigrum* cultivars 'Ben Tron' and 'Ben More' were capable of surviving and regenerating new shoots following cryopreservation. Recovery responses were significantly enhanced when vitrification methods of cryopreservation were used. Differential scanning calorimetry revealed that vitrification phenomena were reproducible and that it was possible to stabilise the glassy state on rewarming. The potential for *Ribes* germplasm cryopreservation to be utilised in large scale conservation initiatives is evaluated in this study.

Key Words.
Genetic resources, tissue culture, vitrification, encapsulation, differential scanning calorimetry.

Abbreviation.
DSC = differential scanning calorimetry/LN liquid nitrogen
PVS2 = plant vitrification solution #2

INTRODUC ION

The development of effective cryopreservation protocols for small fruit species is of key importance to the maintenance of effective germplasm collections that can provide a representative sample of the available genetic resources in a viable and secure manner. The preser-

vation of clonal germplasm is problematic, since whole plants or their tissues need to be conserved rather than seeds. For the most part, small fruit collections such as *Ribes* are held in field genebanks, where they are vulnerable to biotic and abiotic stresses; this method of conservation is also very expensive in terms of resources, including labour.

For *Ribes*, extensive genetic resources are held at both the Scottish Crop Research Institute (SCRI) and the US National Clonal Germplasm Repository (NCGR). At SCRI, the collection feeds directly into an active breeding programme. Recent advances in evaluating alternative methods of germplasm storage have shown that there is considerable potential for the long term cryogenic storage of soft fruit germplasm (1,11,15).

The blackcurrant (*Ribes nigrum* L.) is a member of the *Eucoreosma* subgenus of *Ribes*, and is the most widely grown bush fruit in Europe (2). Commercial cultivars fall into distinct groups, including those from Scandinavia (a main centre of diversity for the genus) and those from more southerly parts of Europe. Cultivars from the SCRI programme have successfully combined the major positive attributes from these previous two groups, and the 'Ben' series have been commercially successful due to their combination of good fruiting and agronomic performance, mainly due to improved frost tolerance during the flowering period. Of the cultivars used in this study, 'Ben More', is a hybrid between the Swedish cultivar 'Ojebyn' and the southern cultivar 'Goliath', and shows excellent cold tolerance at most stages of development, together with good vigour (3), while 'Ben Tron' is more typical of the Scandinavian group.

The storage of shoot tips at ultra-low temperatures in liquid nitrogen has been reported for several woody genera (13,14,15), and cryopreservation of shoot tips derived from micropropagated small fruit plants has been shown to be dependant upon cold acclimation and freezing method (12). The investigation presented here concentrated on two main methods of freezing, vitrification using cryoprotectants and encapsulation/dehydration, in comparison to controlled freezing. Cryoprotective methods employing vitrification and encapsulation/dehydration are dependant upon the circumvention of ice formation during extreme cooling. Ice nucleation is inhibited and water forms an amorphous glassy state. However, the glasses so formed, can be unstable and care must be taken during re-warming, to avoid devitrification and ice growth. Thus, one of the objectives of this study was to assess, using differential scanning calorimetry (DSC), the phase change behaviour of water when vitrification strategies were applied to *Ribes* shoot apices. The cryoprotective protocols used in this study incorporate cold-hardening and dimethyl sulphoxide (DMSO) pre-growth-treatments. DSC was further used to evaluate whether cold acclimation and pre-growth in DMSO alter the water phase characteristics of the shoot-tips during cooling and warming. The overall objective of these studies was to define and characterise potential methods for the large-scale cryopreservation of *Ribes* germplasm, applicable between sites and collections.

MATERIALS AND METHODS

General growth conditions and plant materials: Micropropagated plantlets of *Ribes nigrum* cvs Ben More and Ben Tron were multiplied and shoot tip meristems recovered on NCGR-*Ribes* medium (RIB), which contains the mineral salts and vitamins of Murashige and Skoog (9) but with only 30% of the normal ammonium and potassium nitrate concentrations, and (per

litre): 50 mg ascorbic acid, 20 g glucose, 0.1 mg N6-benzyladenine, 0.2 mg gibberellic acid, 6 g agar (Sigma, Poole, Dorset, UK), at pH 5.7. Plants were grown at 25°C with 16-hr days (25 $mol \cdot m^{-2} \cdot s^{-1}$). All plants were cold acclimated in a controlled environment chamber (Roma, Liverpool) for one week for 8 hr days at 22°C and 16 hr nights at -1°C, before 0.8mm shoot-tip meristems were excised (15).

Vitrification: A modification of a technique for white clover was used (18). Shoot tip meristems were pretreated *in vitro* for two days in the incubator under the cold acclimating conditions described above on RIB medium containing 5% DMSO. PVS2 cryoprotectant (v/v 30% glycerol, 15% ethylene glycol and 15% DMSO in liquid RIB medium with 0.4 M sucrose) was dispensed into cryovials on ice and shoot tip meristems added and stirred. After 20 minutes, vials were submerged in LN. Samples were rewarmed for one minute in a 45°C water bath then transferred to a 22°C water bath for 2 minutes. The shoot tips were rinsed in liquid RIB medium with 1.2 M sucrose, and transferred to RIB medium for recovery.

Encapsulation-dehydration: A method developed for pear was used (5). Shoot tip meristems were dissected onto agar plates, encased in alginate beads (3% low viscosity alginic acid with 0.75 M sucrose) and pretreated for 18 hr in liquid RIB medium with 0.75 M sucrose. Following pretreatment, the beads were separated out on sterile Petri dishes, air dried in the laminar flow hood for 3 or 4 hours, placed in cryovials, and plunged into LN. Vials were rewarmed at room temperature for 15 min, encapsulated meristems were then plated on RIB recovery medium. For moisture content determination, beads were weighed, dried in an oven at 104°C for 16 hr and reweighed.

Controlled Freezing: The method used was adapted for *Ribes* (15). Meristems were acclimated as above for two days on RIB medium with 5% DMSO, transferred to 0.25 ml liquid RIB medium in 1.2 ml plastic cryovials and 1 ml of the cryoprotectant PGD (w/v 10% each polyethylene glycol (MW 8000), glucose and DMSO in RIB medium) was added over 30 min. A further 30 min equilibrium at 4°C was followed by cooling at -0.5°C/min to -40°C, the vials were then plunged in LN. Samples were thawed for one minute in a 45°C water bath, transferred to a 22°C water bath for 2 minutes, rinsed in liquid RIB medium and plated on RIB medium for recovery.

DSC Techniques: DSC analysis was performed using a Perkin Elmer DSC 7 and a TAC 7 PC. The instrument was calibrated using zinc and indium standards. DSC profiles were constructed using a scanning rate of ±10°C.min-1. Samples were placed in aluminium pans sealed with the aid of a Perkin Elemer crimper. Scans were performed from +5°C to -150°C.

Data Analysis: Data from each freezing experiment comprise a mean of 3 cryovials, error terms are standard deviations (s.ds). Assessment of the recovery of the shoot tip meristems was made weekly for 6 weeks, and the phenological stage reached in each case was recorded. Total recovery encompassed greening, leaf expansion and shoot production. Cooling and warming thermal profiles were constructed for each sample. Three to six sample replicates were performed for each treatment system. As appropriate, DSC data are tabulated as means of nucleation, and/or melt events, glass transitions and devitrification phenomena, error terms are standard deviations. Representative thermal profiles are presented for each treatment.

RESULTS AND DISCUSSION

Comparison of the three methods of cryopreservation on 'Ben More' showed that encapsulation/dehydration was easily the most successful in terms of survival and shoot production (Figs. 1,2). Of the other methods investigated, vitrification outperformed the controlled freezing, and therefore the two most successful methods, viz. encapsulation/dehydration and vitrification were applied to the second cultivar, 'Ben Tron'.

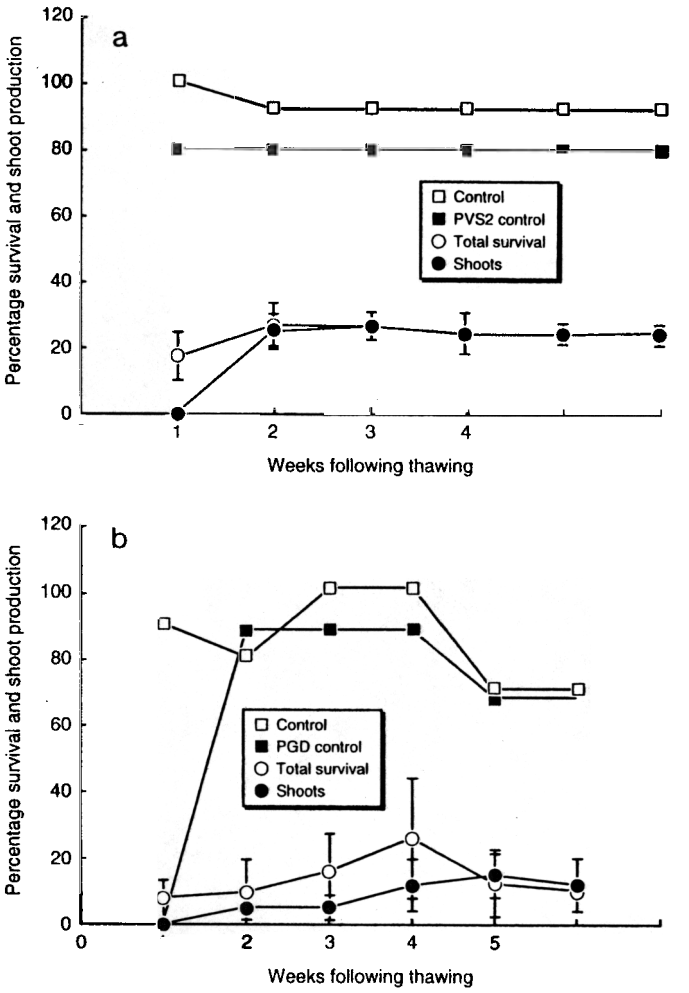

Figure Time course of recovery (%) for apical shoot-tips of *R. nigrum* L. cultivar Ben More following cryoprotection and cryopreservation using:

a. the PVS2 vitrification method (□pre-grown, cold-acclimated control, ■PVS2 cryoprotectant control and ○cryopreserved tissues)

b. controlled cooling using a Planar Cryo 10 Programmable Freezer (□ pre-grown, cold acclimated control, ■PGD cryoprotectant control and ○cryopreserved tissues)

Control data are derived from one replicate sample of 5-10 shoot-tips; cryopreservation data comprise the mean % recovery values for populations of 18-30 shoot-tips recovered from three replicate cryovials, error terms are standard deviations. Recovery is assessed as % shoot production for control treatments (■□) and total survival (○) and shoot production (●) for cryopreservation treatments.

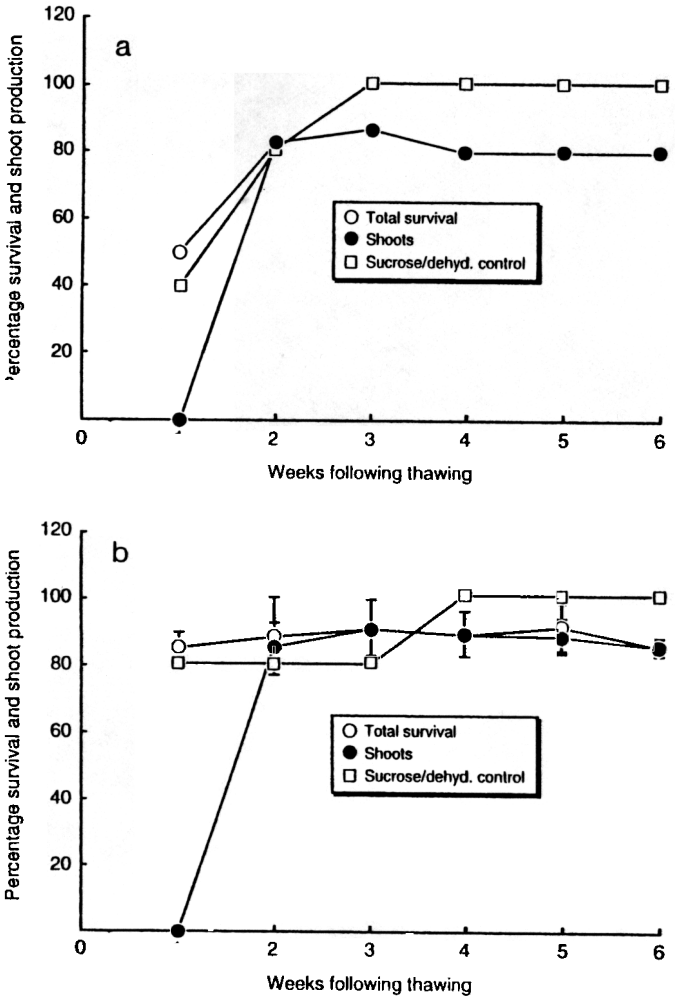

Figure 2. Time course of recovery (%) for apical shoot-tips of *R. nigrum* L. cultivar Ben More following cryopreservation using the encapsulation/dehydration method and air drying treatments of:

a. 3 hours (cryopreservation data comprise a % recovery value for a population of 28 shoot-tips recovered from one cryovial).

b. 4 hours (cryopreservation data comprise the mean % recovery values for populations of 15-21 shoot-tips recovered from three replicate cryovials).

Error terms are standard deviations. Recovery is assessed as % shoot production for control treatments (□) and total survival (○) and shoot production (●) for cryopreservation treatments.

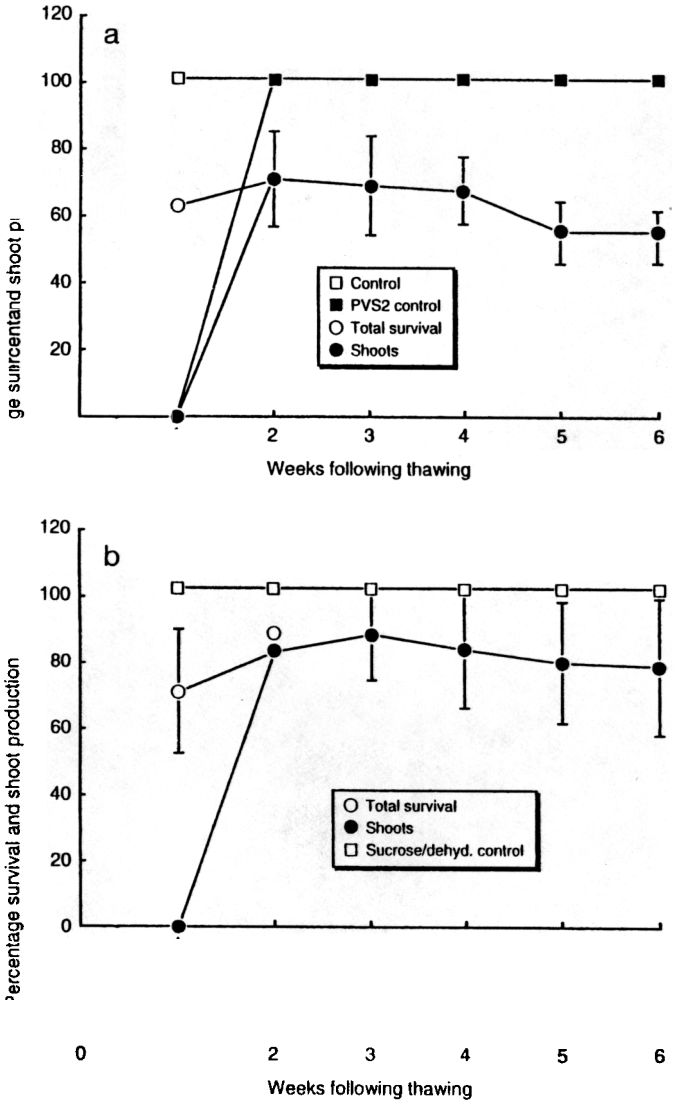

Figure 3. Time course of recovery (%) for apical shoot-tips of *R. nigrum* L. cultivar Ben Tron following cryoprotection and cryopreservation using:

a. the PVS2 vitrification method (□pre-grown, cold acclimated control, ■PVS2 cryoprotectant control and ○cryopreserved tissues.

b. encapsulation/dehydration (□sucrose/dehydration (4 hour air drying) control, ○cryopreserved tissues.

Control data are derived from one replicate sample of 5-10 shoot-tips; cryopreservation data comprise the mean % recovery values for populations of 10-20 shoot-tips recovered from 3-4 replicate cryovials, error terms are standard deviations. Recovery is assessed as % shoot production for control treatments (□■) and total survival (○) and shoot production (●) for cryopreservation treatments.

Vitrification: Clear genotypic differences were observed in the relative performance of 'Ben More' and 'Ben Tron', with the latter showing a better rate of survival and shoot production (60%) compared to 'Ben More' (20%). Evidence of survival was apparent within the first week after thawing for both cultivars, rising to its maximum level by week 2. All survivors regenerated shoots (Figs. 1-3). The cryoprotectant PVS2 solution is highly phytotoxic, and it is suggested that genotypic differences in response may explain the poorer performance of 'Ben More'. It is therefore essential that procedures are optimised to take account of these differences.

Encapsulation/dehydration: An initial examination of dehydration duration was made using 'Ben More' shoot tips (Fig.2). Differences between 3 h and 4 h dehydration were marginal, with the latter showing slightly better post-thaw development. Subsequent experiments therefore used only the 4 h dehydration treatment. Genotypic differences using this technique were far smaller than those found in the vitrification experiments, with shoot production of *ca.* 80% for both genotypes. As found previously using vitrification all surviving meristems proved capable of initiating subsequent shoot production (Figs. 2,3). Recovery of encapsulated meristems showed a similar time course to the vitrification experiments, with evidence of survival after 1 week and shoot production after 2 weeks. Maximum shoot production occurred between 2 and 3 weeks in both genotypes.

Controlled freezing: Comparative studies using this technique were made using 'Ben More' only (Fig.1). Both survival and recovery remained <20% throughout the 6 week time course. Evidence of both survival and shoot production was slower to emerge compared to the other two methods, appearing at 2 weeks, and maximising at 4 weeks.

Three months after thawing, shoot-tip meristems from the two *Ribes* genotypes showed growth following all three cryopreservation methods (Fig.4). In the case of 'Ben More', the greatest number of plants were regenerated from shoot-tips cryopreserved using the

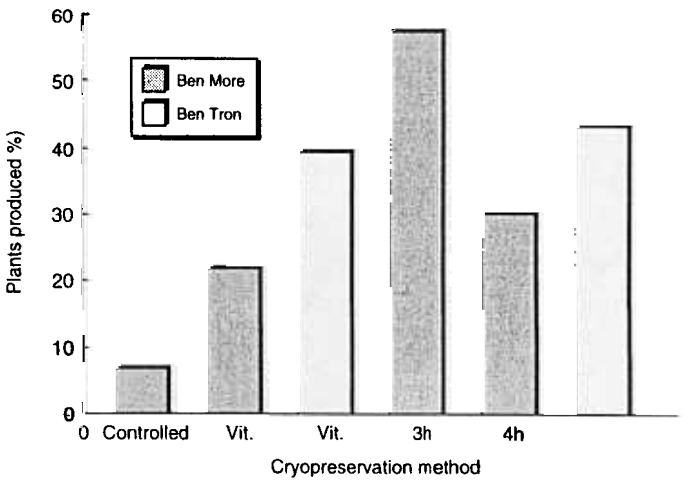

Figure 4. Long term recovery assessment of plant regeneration from cryopreserved shoot-tips of *R. nigrum* L. cultivars Ben More and Ben Tron. Data comprise the total % plants regenerated from populations of shoot-tips cryopreserved using the three freezing methods and are designated: controlled (the controlled cooling, Planar, Cryo 10 Programmable Freezer method, total shoot-tips frozen = 60), Vit (PVS2 vitrification, total shoot-tips frozen = 81), 3h, 4h (Encapsulation/dehydration/ 3 hours/4 hours air drying, total shoot-tips frozen = 28 and 56 respectively). Assessments were performed 3 months after thawing.

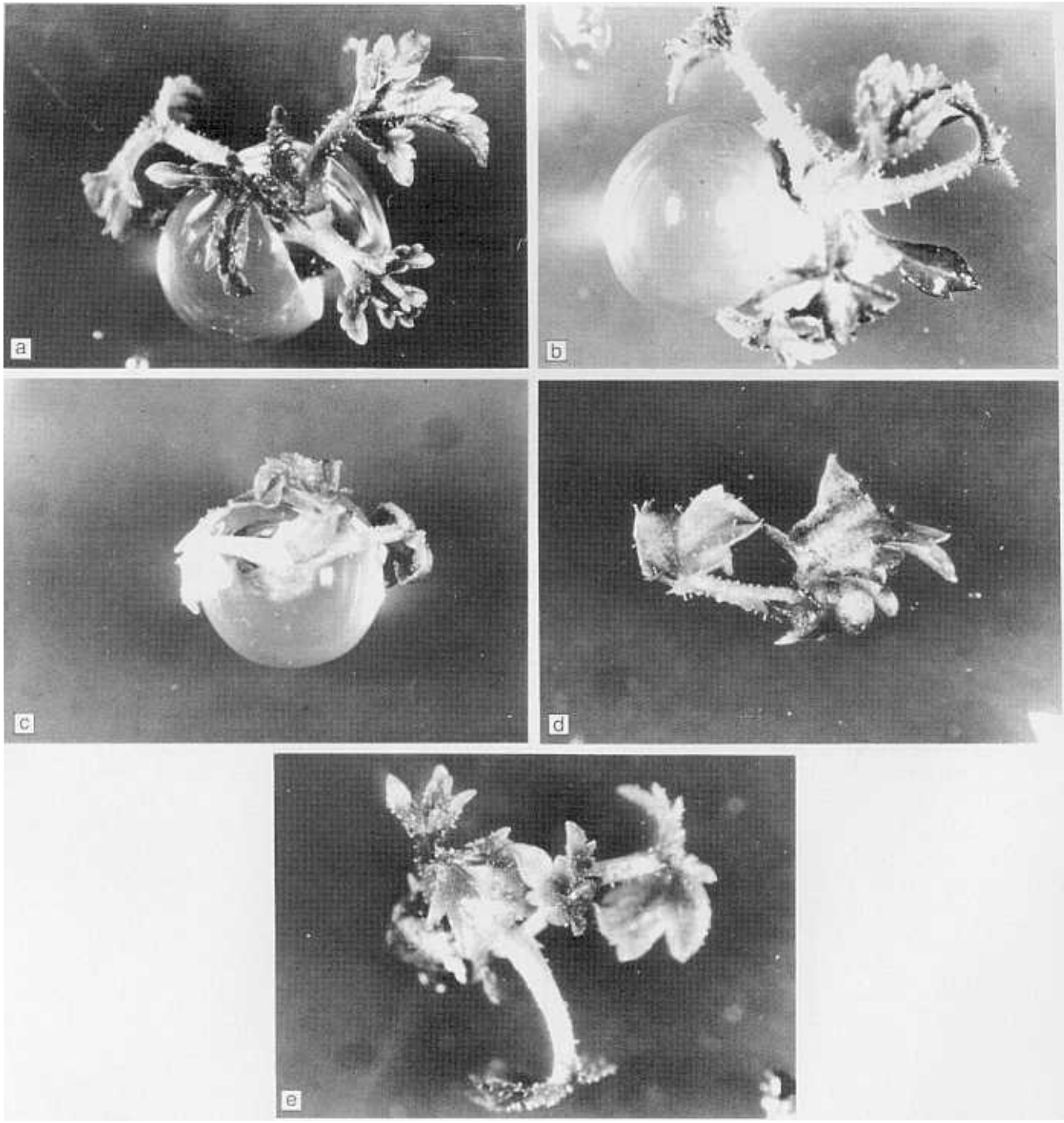

Fig. 5. Cryopreserved plantlets of *Ribes nigrum* after 2 weeks' recovery
a. plantlet of cv. Ben More after 3h encapsulation
b. plantlet of cv. Ben More after 4h encapsulation
c. plantlet of cv. Ben Tron after 4h encapsulation
d. plantlet of cv. Ben Tron after vitrification
e. plantlet of cv. Ben More after vitrification

encapsulation/dehydration method, with a 3 h dehydration proving the most effective. Fewer plants were regenerated from vitrified shoot tips. 'Ben More' showed very little difference in the number of plants regenerated from vitrified or encapsulated shoot tips. However, it must be recognised that this longer-term recovery assessment is also dependent upon post-thaw tissue culture manipulations, and differences in tissue culture performance for *Ribes* have been reported (4). Figure 5 demonstrates the progression of post-cryopreservation survival and regeneration responses in shoot-tips recovered from liquid nitrogen.

Differential Scanning Calorimetry Studies: Differential scanning calorimetry profiles constructed for non-acclimated and acclimated *Ribes* shoot-tips and acclimated shoot-tips pre-grown in the presence of DMSO show highly reproducible ice nucleation and melt phenomena (Table 1). Thermal profiles indicate that the plant tissues undergo one major nucleation and ice melt event respectively (Figure 6.). Cold acclimation and DMSO pre-growth treatments did not influence the nucleation and melt characteristics of the apices.

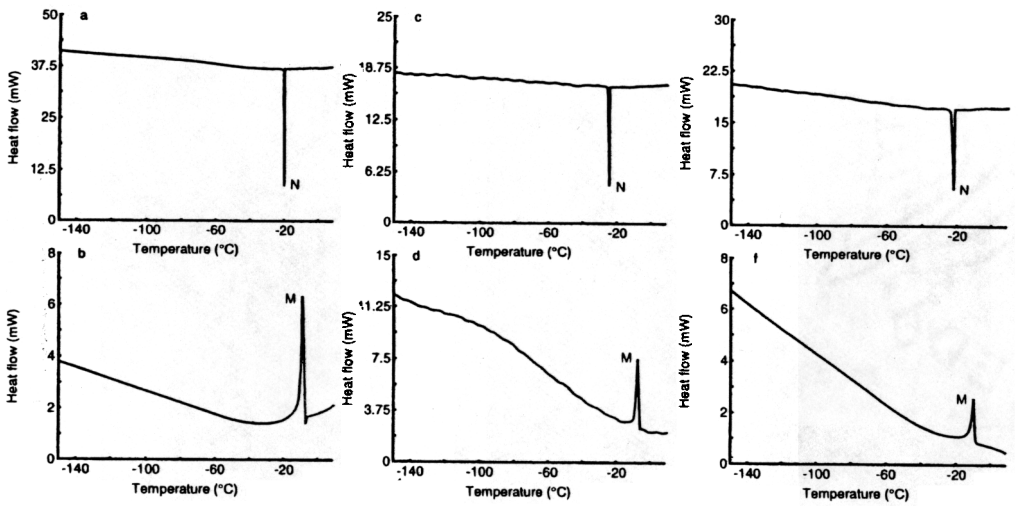

Figure 6. Representative Differential Scanning Calorimetry profiles of thermal transitions in non-acclimated, acclimated and DMSO-treated shoot-tips of *Ribes nigrum* cv Ben More.

Profiles are representative scans from a non-acclimated shoot-tip during cooling (a) and warming (b) an acclimated shoot-tip during cooling (c) and warming (d) and an acclimated DMSO-treated shoot-tip during cooling (e) and warming (f). Profiles were constructed using a scanning rate of ± 10°C min^{-1}.

Notation of thermal characteristics: N = nucleation
M = melt
Tg = glass transition

In order to determine the characteristics of the cryoprotectant PVS2, it is important to consider the thermal behaviour of two different components: the PVS2 solution and the plant tissue. Table 2 and Figure 7 summarise the cooling- and warming-cycle thermal behaviour of each component, alone, and in combination. Assessments of PVS2 alone demonstrate an absence of ice nucleation and evidence of a glass transition on cooling. This cooling behaviour was reproducible for all replicates. However, on warming, differences between replicate samples were observed and the two representative scans shown in Figure 7 indicate that the glassy state can either be stable or unstable. Thus, the possibility exists that devitrification of PVS2 can occur on warming.

Figure 7, profiles C and D are representative scans assessing the combined thermal behaviour of 3 *Ribes* shoot-tips in the presence of PVS2. A very broad ice nucleation event was observed

Table 1. Summary of Differential Scanning Calorimetry assessments of thermal transitions in non-acclimated, acclimated and DMSO-treated shoot-tips of *Ribes nigrum* CV Ben More.

	Cooling Cycle				Warming Cycle					
	Ice nucleation	Onset of nucleation	Tg	Onset of Tg	Ice nucleation	Onset of nucleation	Ice melt	Onset of melt	Tg	Onset of Tg
Non cold-acclimated shoot-tips	-19.60 ±0.68	-19.35 ±0.57	—	—	—	—	-2.51 ±0.29	-4.25 ±0.42	—	—
Cold acclimated shoot-tips	-19.53 ±0.69	-19.28 ±0.67	—	—	—	—	-2.42 ±0.08	-3.88 ±0.22	—	—
DMSO pretreated shoot-tips	-20.32 ±1.35	-20.01 ±1.18	—	—	—	—	-3.29 ±1.22	-5.74 ±2.07	—	—

Data are means (n=6, errors=s.ds) of DSC cooling/warming profiles performed on six apical shoot-tips

Table 2. Summary of Differential Scanning Calorimetry assessments of thermal transitions in PVS2 solutions and acclimated DMSO-treated shoot-tips if *Ribes nigrum* CV Ben More cryopreserved with PVS2.

	Cooling Cycle				Warming Cycle					
	Ice nucleation	Onset of nucleation	Tg	Onset of Tg	Ice nucleation	Onset of nucleation	Ice melt	Onset of melt	Tg	Onset of Tg
PVS2 solution	—	—	-124.96 ±6.39	-120.03 ±7.24	Evidence of glass instability				-119.06 ±0.58	-123.73 ±1.95
Shoot-tips and PVS2	-114.69 ±4.80	-95.89 ±6.39	—	—	-133.59 ±1.37	-136.46 ±1.46	-56.64 ±1.53	-75.47 ±1.13	—	—
PVS2 treated shoot-tips. PVS2 removed	—	—	-76.30 ±4.30	-71.48 ±4.49	—	—	—	—	Evidence of glass stability	

Data are means (n=6, errors=s.ds) of DSC cooling/warming profiles performed on six apical shoot-tips

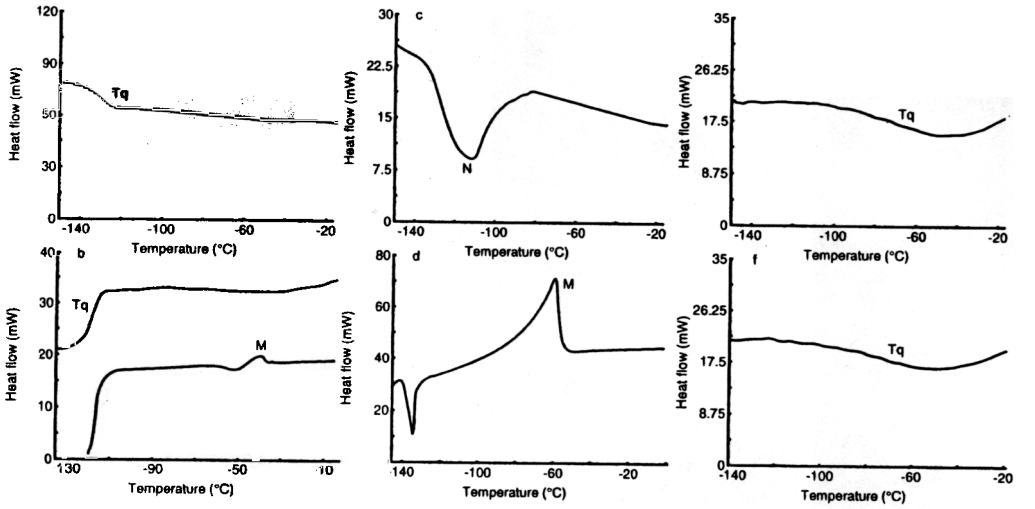

Figure 7. Representative Differential Scanning Calorimetry profiles of thermal transitions in PVS2 solutions and acclimated, DMSO-treated shoot-tips of *Ribes nigrum* cv Ben More cryoprotected with PVS2.

Profiles are representative scans for PVS2 only, during cooling (a) and warming (B1 and2) and PVS2 in combination with 3 shoot-tips during cooling (c) and warming (d). Profiles e and f are cooling and warming scans respectively for a shoot-tip which has been cryoprotected with PVS2 and the solution removed immediately before DSC analysis. Profiles were constructed using a scanning rate of ± 10°C min^{-1}.

Notation of thermal characteristics: N = nucleation
M = melt
Tq = glass transition

on cooling and on warming both nucleation and melt phenomena occurred. To determine if these events were associated with the extracellular PVS2 solution and/or the plant tissue, *Ribes* shoot-tips were first cryopreserved with PVS2 which was then removed immediately before performing DSC. Figure 7, profiles E and F, show no evidence for ice nucleation in PVS2 treated shoot-tips, and this was reproducible for all replicate samples (Table 2). It would therefore appear that the shoot-tips undergo vitrification on cooling and that the glass is stable on warming.

Encapsulation/dehydration offers an alternative approach to achieving vitrification in plant tissues. Cryoprotection is dependent upon the removal of water from plant tissues encapsulated

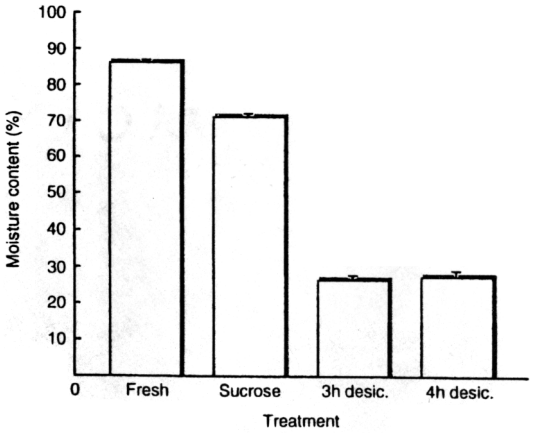

Figure 8. Moisture content profiles (% fresh weight basis) of alginate beads exposed to sucrose and air dehydration treatments. Data are designated:
freshly prepared beads (fresh), beads exposed to 0.75m sucrose for 18h (sucrose) and sucrose-treated beads, air-dehydrated for 3h and 4h. Dry weights were determined by oven-drying the beads for 20h at 110°C. Data are means of 10 replicate beads and error terms are standard deviations.

Table 3. Summary of Differential Scanning Calorimetry assessments of thermal transitions in calcium-alginate beads and beads containing encapsulated shoot-tips of *Ribes nigrum* CV Ben More.

	Cooling Cycle				Warming Cycle			
	Ice nucleation	Onset of nucleation	Tg	Onset of Tg	Ice Melt	Onset of melt	Tg	Onset of Tg
Ca^{2+} alginate bead	-17.68 ±1.14	-16.98 ±0.97	—	—	2.30 ±1.74	-5.11 ±2.85	—	—
0.75M sucrose treated Ca^{2+} alginate	-23.06 ±2.95	-22.21 ±3.05	—	—	0.93 ±0.68	-9.62 ±0.65	—	—
Treated Ca^{2+} alginate bead. 3hr air dry	—	—	-74.50 ±3.56	-69.32 ±3.01	-21.55 ±0.71	-28.42 ±1.95	-72.01 ±1.13	-76.52 ±3.55
Treated Ca^{2+} alginate bead. 4hr air dry	—	—	-72.95 ±5.04	-65.35 ±3.36	—	—	-58.57 ±3.39	-61.49 ±2.23
Ca^{2+} alginate encapsulated shoot-tip. 3hr air dry	—	—	-72.54 ±6.93	-66.44 ±6.90	-21.45 ±0.68	-29.74 ±2.51	-73.94 ±3.22	-77.66 ±2.53
Ca^{2+} alginate encapsulated shoot-tip. 4hr air dry	—	—	-67.97 ±1.36	-63.53 ±3.53	—	—	-59.52 ±4.09	-64.04 ±3.66

Data are means (n=6, errors=s.ds) of DSC cooling/warming profiles performed on individual beads and/or beads containing individual shoot-tips.

in a Ca2+-alginate matrix using osmotic dehydration and air desiccation. The success of the method is largely dependent upon desiccation tolerance and the ability to circumvent ice nucleation during cooling and warming. Moisture content profiles determined for 10 replicate Ca2+-alginate beads (Fig. 8) show that after 3-4 hours of drying in an air flow a bead moisture content of 25% (fresh weight basis) can be reproducibly achieved, with little difference between the moisture contents of beads which had been air dried for 3 and 4 hours respectively.

To determine the efficacy of dehydration treatments in producing stable and reproducible vitrification events, DSC analyses were performed at each stage of the protocol. Analyses of sucrose-treated and untreated Ca^{2+}-alginate beads demonstrate reproducible ice nucleation and melt events (Table 3). Both transitions were associated with a major thermal peak (Fig.9). It was not possible to remove encapsulated shoot-tips from dehydrated beads and the bead/plant tissue system could only be assessed in combination. However, a comparison of the thermal behaviour in beads and beads containing encapsulated shoot-tips was undertaken. It was not possible to assign specific thermal events to the encapsulated shoot-tips within the alginate matrix (Table 3), and therefore only representative DSC profiles of encapsulated shoot-tips are shown in Figures 9 and 10. Glass transitions could be identified in cooling profiles of both 3 and 4 hour air-dried beads (Fig. 10A and 10C). However, warming profiles of the 3 hour air-drying treatments indicated a devitrification event associated with a major melt peak (Table 3, Fig. 10B). In comparison, the glassy state of the 4 hour air-dried beads was reproducibly stable on warming (Table 3, Fig 10D). There may be some evidence of devitrification at *ca.* -60°C, but this did not however result in further destabilisation, as the major melt peak observed in the 3 hour air dried samples did not occur. The moisture content profiles of the 3 and 4 hour air-dried beads are very similar (Fig.8) and it would appear that stabilisation of the glassy state on warming (Figs. 10B and 10D) could be marginal for this system.

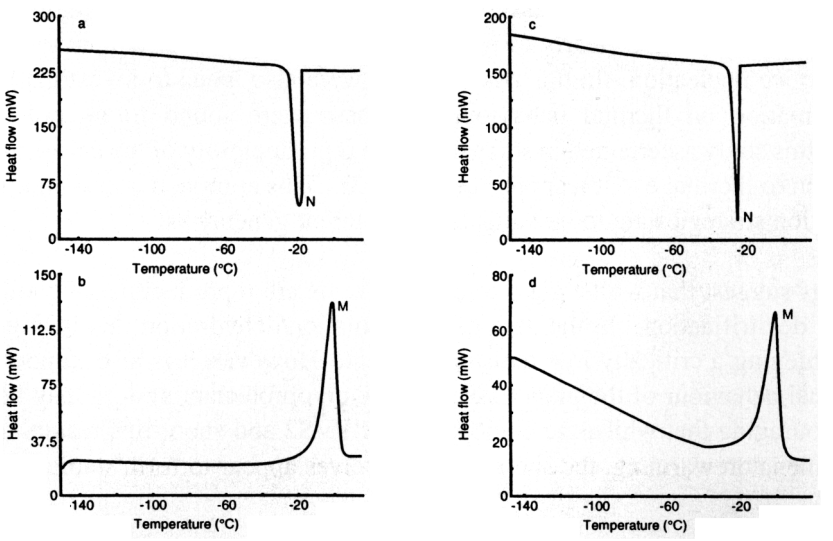

Figure 9. Representative Differential Scanning Calorimetry profiles of thermal transitions in calcium-alginate beads.

Profiles are representative scans for freshly prepared untreated calcium-alginate beads during cooling (a) and warming (b) and calcium alginate beads treated for 18h in 0.75 sucrose followed by immediate cooling (c) and warming (d). Profiles were constructed using a scanning

Notation of thermal characteristics: N = nucleation
M = melt

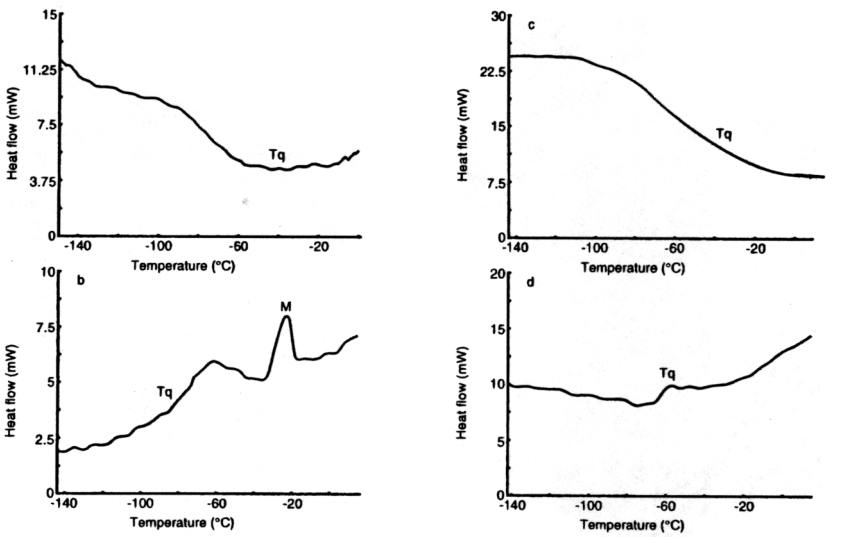

Figure 10. Representative Differential Scanning Calorimetry profiles of thermal transitions in calcium-alginate beads containing encapsulated shoot-tips of *Ribes nigrum* cv Ben More.

Profiles are representative scans for calcium-alginate beads containing one shoot-tip, treated for 18h in 0.75M sucrose followed by 3h and 4h air drying. Scans represent the 3h treatment, cooling (a) and warming (b) and the 4h treatment, cooling (c) and warming (d). Profiles were constructed using a scanning rate of ±10°C min^{-1}.

N = nucleation
M = melt
Tq = glass transition

These studies concur with those of other workers (6,16) who report that the cryoprotective mode of action of the PVS2 and encapsulation/dehydration methods can be attributed to ice avoidance and the formation of a glassy state during cooling. However, stabilisation of the glassy state on re-warming is clearly of major importance in terms of circumventing cellular damage due to ice nucleation. In this study, efforts were also made to investigate the affects of cold acclimation on thermal behaviour and these were found to be not significant. Furthermore, this study ascertained in some detail the reproducibility of the cryoprotective systems in relation to thermal event response (Tables 1-3). This approach is essential if common cryoconservation strategies are to be validated in different genebanks.

These findings suggest that whilst glass transition events are reproducible on cooling, warming can incur devitrification. In the case of encapsulation/dehydration this is largely dependent upon achieving a critically low moisture content. However, it is also important to note that the thermal behaviour of the plant tissue and the cryoprotectant system may be different. It is thus encouraging that whilst the combination of PVS2 and shoot-tips results in devitrification phenomena on warming, the shoot-tips themselves appear to form stable glasses.

CONCLUSIONS

Vitrification is based on the ability of water to form an amorphous glass in high viscosity solutions, and uses high concentrations of cryoprotectant chemicals such as glycerol, ethylene glycol and dimethyl sulphoxide (DMSO) to prevent ice formation within the plant tissues during ultra-rapid cooling; several genera have been cryopreserved using this method (7,10,18). In the case of the *Ribes* cultivars used in this study, the PVS2 cryoprotection method was more

effective than the controlled cooling technique. Encapsulation of meristems in calcium alginate beads, followed by bead dehydration using osmotica and air desiccation, and eventual freezing in LN has proved successful in the cryopreservation of *Ribes* cultivars 'Ben Tron' and 'Ben More'. The use of differential scanning calorimetry (DSC) to examine the physical phase changes occurring during cryogenic treatments has been described by several workers (6,16,17), and has proved an invaluable aid in characterising the stability of the cryoprotectant systems which are dependant upon vitrification.

The examination of *Ribes* cryopreservation protocols shows that all three methods resulted in regenerated plants. The most promising for the long-term storage of these two *Ribes* genotypes appears to be encapsulation/dehydration, in terms of both plant recovery and genotypic adaptability. Vitrification may also have some potential for some genotypes, and further studies are in progress. As a result of this work there now exists the potential for *Ribes* germplasm cryopreservation to be utilised in large scale conservation initiatives. It is thus essential that all aspects of the cryopreservation protocol are evaluated for reproducibility. Future method validations will be greatly assisted by combining studies of thermal behaviour with plant regeneration responses.

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