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Original Research Article

Cryopreservation of *in Vitro* Grown Shoot Tips of Sweet Potato (*Ipomoea batatas* L.) by the Encapsulation-Vitrification Method

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Abstract - Sweet potato (*Ipomoea batatas* L.) shoot tips grown *in vitro* were successfully cryopreserved by encapsulation-vitrification. Encapsulated explants are very easily manipulated, due to the relatively large size of the alginate beads, and a large number of samples can be treated simultaneously. In this study, the effects of sucrose preculture, cryoprotectant preculture, and post-warm recovery media on regrowth, following liquid nitrogen (LN) exposure, were investigated to establish an efficient encapsulation-vitrification protocol for sweet potato. Shoot tips of plants grown *in vitro* were precultured in 0.3 M sucrose for 2 d before encapsulation. Encapsulated shoot tips were pre-incubated in liquid MS (Murashige and Skoog) medium containing 0.5 M sucrose for 16 h, before preculturing in sucrose-enriched medium (0.7 M sucrose) for 8 h. Shoot tips were osmoprotected with 35% plant vitrification solution 3 (PVS3) for 3 h, before being dehydrated with PVS3 for 2 h at 25°C. The encapsulated and dehydrated shoot tips were transferred to 2 mL cryotubes, suspended in 0.5 mL PVS3, and plunged directly into liquid N. High levels of shoot formation were obtained for the cv. Yeulmi (65.7%) and Yeonwhangmi (80.3%). The regrowth rates of cryopreserved samples in Yeulmi (78.9%) and Yeonwhangmi (91.3%), following culture on ammonium-free MS medium for 5 d, were much higher than those cultured on standard MS medium (65.7% and 80.3%, respectively). This encapsulation-vitrification is a promising method for the long-term preservation of sweet potato.

Key words - Cryopreservation, Sweet potato, Shoot tip, Encapsulation-vitrification, Cryoprotectant

Introduction

Sweet potato (*Ipomoea batatas* L.) ranks as the seventh most important staple crop globally, and the fifth most important in developing countries after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and cassava (*Manihot esculenta* Crantz; Loebenstein, 2009). Because sweet potato germplasm is usually vegetatively propagated, gene banks generally maintain clones *in vitro* or in a combination of field and *in vitro* culture. However, these methods are not desirable for long-term preservation, as they are labor-intensive and costly (Yi *et al*., 2015).

Cryopreservation is useful for the long-term storage of sweet potato, and for most vegetatively propagated plant germplasm (Towill, 1990). However, cryopreservation of sweet potato has been difficult because of its low tolerance to dehydration with plant vitrification solution 2 (PVS2) and its sensitivity to low temperature during cold-hardening (Takagi *et al*., 1998).

The encapsulation-vitrification procedure, developed by Tannoury *et al*. (1991) for cryopreservation of carnation (*Dianthus caryophyllus* L.) shoot tips, involves a combination of alginate encapsulation and PVS2 vitrification. In this technique, alginate-encapsulated shoot tips are precultured with sucrose-rich medium and loading solution, followed by dehydration with vitrification solution, and then are cultured on post-culture medium for survival and regrowth (Benson, 2008; Sakai and Engelmann, 2007; Sakai *et al*., 2008; Wang and Perl, 2006). With encapsulation-dehydration, encapsulation-vitrification is easy to handle and can accommodate the simultaneous treatment of a large number of samples (Feng *et al*., 2011). Hirai and Sakai (2003) were the first to describe an encapsulation-vitrification protocol for the successful cryopreservation of three Japanese sweet potato cultivars. This procedure was successfully applied to 13 sweet potato cultivars,

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with some modifications (Jenderek *et al*., 2008). Twenty sweet potato genotypes are preserved in cryostorage at Fort Collins, which contains a collection of sweet potato germplasm (Gaba and Singer, 2009). In this study, the effects of sucrose preculture, cryoprotectant preculture (loading), vitrification, and post-warming recovery media on regrowth rates, following liquid nitrogen (LN) exposure, were investigated to establish an efficient cryopreservation protocol for sweet potato, using an encapsulated-vitrification method.

Materials and Methods

Preparation of plant material

In vitro grown sweet potato cultivars Yeulmi and Yeonwhangmi were used in this study. We used the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 3% sucrose and adjusted to a pH of 5.7 prior to autoclaving at 121°C for 15 min. For sample preparation, nodal segments (about 8 ㎜ long) were removed from *in vitro* stock cultures and transferred to MS basal medium supplemented with 0.5 ㎎/L 6-benzylaminopurine (BA). Following a 14-day culture, excised shoot tips (1 ㎜ in length with 3-5 leaf primordia) were dipped in 0.3 M sucrose MS liquid medium under white fluorescent light (2000 lux), with a 16 h daylight photoperiod at 25°C.

Encapsulation, preculture, and osmoprotection

Excised shoot tips were pre-incubated in liquid MS medium supplemented with 0.3 M sucrose at 25°C on a rotary shaker. Then, pre-incubated shoot tips were encapsulated with sodium alginate (100-300 cP; Sigma-Aldrich, St. Louis, Missouri) into beads, according to Dereuddre *et al*. (1990). Beads were precultured in liquid MS medium, and supplemented with 0.5M sucrose MS liquid medium at 25°C on a rotary shaker (100 rpm) before being transferred to a sucrose-enriched (0.7 M) liquid MS basal medium and precultured. Osmoprotection was accomplished by incubating precultured beads in liquid MS medium, and supplemented with 35% PVS3 (17.5% glycerol+17.5% sucrose) and 40% PVS3 (20% glycerol+20% sucrose) on a rotary shaker (100 rpm) at 25°C for various periods of time (0-5 h). As shown in Table 1, PVS2 contains 30% glycerol, 15% dimethyl sulfoxide (DMSO), 15% ethyleneglycol, and 13.7% sucrose in MS medium (pH 5.7), whereas PVS3 contains 50% (w/v) glycerol and 50% (w/v) sucrose in MS medium (pH 5.7).

Vitrification and freezing

Precultured and osmoprotected shoot tips were dehydrated in 20 ㎖ PVS2 or PVS3, in a 100 mL magenta vessel and on a rotary shaker (100 rpm) at 25°C for various time periods (30-180 min). After dehydration, beads were transferred to 2 ㎖ cryotubes and suspended in 0.5 ㎖ PVS2 or PVS3. Cryotubes were plunged directly into liquid nitrogen (LN) for at least 1 h (Fig. 1-B).

Thawing and plant recovery

After being preserved in LN, cryotubes were rapidly warmed in a water bath at 40°C for 2 min. PVS3 was drained from the cryotubes and enhanced twice at 10 min intervals with 1 ㎖ 0.8 M sucrose solution at 25°C. The LN-treated beads were placed on recovery medium I (Fig. 1-A) consisting of basal MS medium and supplemented with 0.5 ㎎/L BA and 1 ㎎/L gibberellic acid 3 (GA_3), for 5 d in the dark. The shoot tips were removed from the beads for recovery, transferred to recovery medium II consisting of basal MS medium supplemented with 0.5 ㎎/L GA_3, and cultured in the dark for 3 days. Then they were cultured under dim light for 5 days, and cultured under white fluorescent

Table 1. Compositions of the vitrification and loading solutions used in this study

Solution	Composition	Reference
PVS2	30%G[z]+15%DMSO[y]+15%EG[x]+13.7%Suc[w] in MS	Sakai *et al*. 1990
PVS3	50% glycerol+50% sucrose in MS	Nishizawa *et al*. 1993
LD1[v)]	35% PVS3 (17.5% glycerol+17.5% sucrose in MS)	-
LD2[u)]	40% PVS3% (20% glycerol+20% sucrose in MS)	-

[z]G, glycerol; [y]DMSO, dimethyl sulfoxide; [x]EG, ethylene glycol; [w]Suc, sucrose; [v]LD1, loading solution 1; [u]LD2, loading solution 2.

Fig. 1. Cryopreservation of sweet potato shoot tips using encapsulation-vitrification. A, Establishment on recovery medium I of encapsulated shoot tips grown *in vitro* after thawing; B, Cryopreservation of sweet potato shoot tips in liquid nitrogen tank; and C, Plant regrowth from cryopreserved sweet potato 'Yeulmi' shoot tips 8 weeks after thawing.

Table 2. Effect of preculture on the regrowth percentage of the control (-LN) and cryopreserved (+LN) sweet potato shoot tips in two cultivars cooled to -196°C

Preculture before encapsulation (h)	Preculture after encapsulation (h)	Regrowth rate (%)			
		Yeulmi		Yeonwhangmi	
		-LN	+LN	-LN	+LN
0.3M (24)	0.5M (16)	99.8	46.2 c[z]	100	54.3 c
	0.5M (16) → 0.7M (8)	100	49.5 c	100	58.8 c
0.3M (48)	0.5M (16)	100	58.3 b	97.7	75.3 b
	0.5M (16) → 0.7M (8)	98.1	65.7 a	99.3	80.3 a

[z]Means within columns followed by the same letter are not significantly different at 5% level.

light at an intensity of 2000 lux with a 16 h photoperiod. The culture environment was the same as that for stock cultures.

Assessment of regrowth rates and data analysis

Approximately 10 shoot tips were used per treatment, and each experiment was replicated three times. Regrowth rates were estimated at 8 weeks after thawing, by counting the number of shoot tips that were differentially swollen and green (Fig. 1-C). The data were analyzed according to an ANOVA model, and the means were separated using the Duncan's multiple-range test ($p<0.05$).

Results and Discussion

Effects of preculture with sucrose-rich solution on recovery

Table 2 shows the effects of preculture on recovery of sweet potato shoot tips cooled to -196°C. The effects of preculture on shoot tips, before and after encapsulation, were investigated using liquid MS medium containing 0.3, 0.5, and 0.7 M sucrose. As shown in Table 2, at 2 d, 0.3 M sucrose preculture produced a significantly higher level of recovery growth when followed by preculture with 0.5 M for 16 h and 0.7 M sucrose for 8 h. The 1-day preculture with 0.3 M sucrose gave low recovery rates (Table 2). It is understood that preculture before encapsulation is very critical for the cryopreservation of sweet potato by encapsulation-vitrification. Several researchers have reported the importance of preculture. Matsumoto *et al*. (1994) reported that the absence of preculture in 0.3 M sucrose results in a reduced recovery rate in wasabi, which is similar to the results reported for cassava (Charoensub *et al*., 1999). Even in sweet potato, there was no survival observed without preculture (Pennycooke and Towill, 2000). Sucrose preculture provides some protection against dehydration from PVS2 exposure, even when no cryoprotectant preculture is used (Pennycooke and Towill, 2001). Blakesley *et al*. (1997) also reported that sucrose alone is sufficient to

provide some cryoprotection, with or without evaporative dehydration, for some sweet potato clones.

Effects of preculture with osmoprotectants on recovery

The kinetics of the exposure of explants to vitrification solutions are critical for explant recovery (Towill and Jarret, 1992). Thus, exposure of shoot tips or beads, with shoot tips, to the vitrification solution is usually performed in two steps. First, they are placed in a low concentration of cryoprotectants to facilitate permeation of individual solutes, and then they are subsequently transferred to higher concentrations to provide the desired level of dehydration (Pennycooke and Towill, 2000). The effects of loading solution and application time on the shoot formation rates of vitirified shoot tips are shown in Fig. 2. Precultured shoot tips were osmoprotected in a loading solution for 1-5 h at room temperature. The loading solutions, LD1 and LD2, contained MS basal medium supplemented with 35% and 40% PVS3, respectively. As shown in Fig. 2, LD1 (35% PVS3) treatments for 3 h produced the highest regrowth rates, 65.7% and 80.3% of vitrified shoot tips cooled to -196°C in the Yeulmi and Yeonwhangmi cultivars, respectively. In this study, no loading (0 h), which was the treatment of only preculture with sucrose-rich solution, did not produce regenerants (Fig. 2). These results indicate an optimal osmoprotectant treatment after preculture is necessary. Individuals without osmoprotectant preculture (loading) did not produce regenerants.

According to Pennycooke and Towill (2001), treatment with 20% PVS2 or 2 M glycerol+0.4 M sucrose also provided dehydration protection, compared to the treatment lacking cryoprotectant preculture. Volk *et al*. (2004) reported that osmotic loading treatment increases osmolarity of the cell and minimizes osmotic damage caused by the vitrification solution. The purported role of the cryoprotectant preculture, which is called loading, is to allow permeation by solutes, such that subsequent desiccation in the presence of the concentrated vitrification solution might create conditions for glass formation during cooling (Benson, 2008; Pennycooke and Towill, 2000; Towill and Jarret, 1992). Direct exposure without osmoprotectant preculture did not yield viable shoot tips in sweet potato (Towill and Jarret, 1992). Results from several studies have indicated that exposure of less tolerant shoot tips directly to the vitrification solution result in mortal damage, due to osmotic stress and/or chemical toxicity (Pennycooke and Towill, 2000; Volk *et al*., 2006; Wang *et al*., 2005). Adequate preconditioning by cryoprotective loading could be applied to mitigate these harmful effects (Langis and Steponkus, 1990).

Effects of vitrification solution and application time on recovery

To determine the optimal vitrification solution and application time, shoot tips precultured with sucrose-rich and osmoprotectant solution shoot tips were dehydrated using PVS2 or PVS3, for 30-180 min prior to being plunged into LN. The highest rates of shoot formation were obtained from shoot tips of Yeulmi (65.7%) and Yeonwhangmi (80.3%) treated with PVS3 for 120 min at 25°C (Table 3). The reasons why cryopreservation by encapsulation-vitrification is successful is because it facilitates the control of dehydration procedures

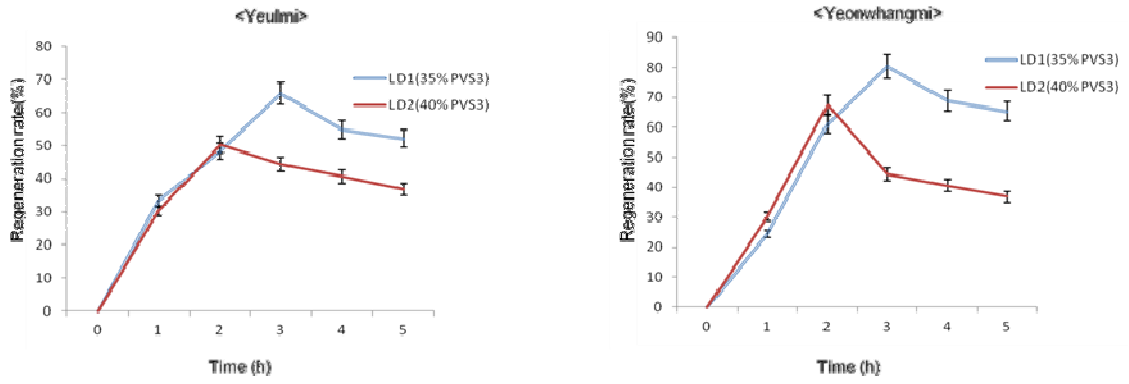

Fig. 2. Effects of incubation time in 35% PVS3 and 40% PVS3 at 25°C on percentage of shoot formation of encapsulated sweet potato shoot tips cooled to -196°C. PVS3; 50% glycerol+50% sucrose in MS. Bars; standard deviation.

Table 3. Effects of PVS2 and PVS3 at 25°C on regrowth rate of the control (-LN) and cryopreserved (+LN) sweet potato shoot tips in two cultivars cooled to -196°C

Vitrification solution (min.)	Regrowth rate (%)			
	Yeulmi		Yeonwhangmi	
	-LN	+LN	-LN	+LN
PVS2 (30)[z]	100	35.1 c[x]	99.5	46.1 c
PVS2 (60)[y]	100	43.1 b	100	65.6 b
PVS3 (120)	100	65.7 a	100	80.3 a
PVS3 (180)	98.6	49.3 b	98.1	70.3 b

[z]P VS2 : 30% glycerol+15% DMSO+15% ethylene glycol+13.7% sucrose in MS.
[y]P VS3 : 50% glycerol+50% sucrose in MS.
[x]Means within columns followed by the same letter are not significantly different at 5% level.

and prevents injury by chemical toxicity or excessive osmotic stress during treatment with the vitrification solution (Sakai and Engelmann, 2007).

Apical or axillary shoot tips cryopreserved by vitrification, with or without encapsulation, have been reported to produce shoots directly, without intermediary callus formation or genetic variants (Fukai *et al*., 1991; Hirai *et al*., 1998; Matsumoto *et al*., 1995; Yamada *et al*., 1991). Cryopreservation by complete vitrification of shoot tips eliminates concern for the potentially damaging effects of intra- or extracelluar crystallization (Rall, 1987). Complete vitrification requires the use of highly concentrated but less toxic cryoprotectants (Nishizawa *et al*., 1993). As previously mentioned, cryopreservation of sweet potato is difficult because of its low tolerance to dehydration in the presence of PVS2 (Takagi *et al*., 1998). Therefore, in this study, we used a glycerol-based, less toxic vitrification solution (PVS3), in addition to PVS2, for shoot recovery (Table 1). As a result, PVS3 produced significantly high regrowth rates, compared to PVS2.

Improvement in recovery by altering the post-warming medium

In this study, we investigated whether the ammonium ion concentration in MS medium affects viability of cryopreserved sweet potato shoot tips. The viability of cooled samples, following culture on ammonium-free MS medium for 5 days, was significantly increased compared to those cultured on MS medium. As shown in Table 4, regrowth rates of shoot tips in Yeulmi cultured on NH_4NO_3 free-MS medium (78.9%) were higher than those cultured on regular MS medium (65.7%). The regrowth rate of Yeonwhangmi cultured on NH_4NO_3 free-MS medium (91.3%) was higher than culturing on standard MS medium (80.3%). This means that damage from vitrification could be repairable, if cryogenic conditions are somewhat optimized (Pennycooke and Towill 2001). Studies on rice and lavender cultures demonstrated that composition of the recovery medium could influence regrowth and that ammonium is critical (Kuriyama *et al*., 1989; Kuriyama *et al*., 1996). Altering the recovery medium for the encapsulation-vitrification method improved recovery of the cryopreserved sweet potato shoot tips.

Vitirification facilitates freezing of explants within a short time period. However, it is difficult to simultaneously treat a large number of samples with this technique, as duration of the successive steps of the vitrification protocol is often very short (Matsumoto *et al*., 1995; Sakai and Engelmann, 2007). These steps require a very precise duration and small-sized explants are difficult to manipulate (Matsumoto *et al*., 1995; Sakai and Engelmann, 2007). Encapsulated explants are very easy to manipulate, due to the relatively large size of the alginate beads. According to Wang *et al*. (2005), the percentage of shoot formation of raspberry (*Rubus* spp.) shoot tips cryopreserved using encapsulation-vitrification is 30% higher than that of the shoot tips cryopreserved by the encapsulation-dehydration technique. The encapsulation-vitrification method is user-friendly and greatly reduces the time required for dehydration compared to the encapsulation-encapsulation method. Since its establishment, this method has been successfully applied to a wide range of plant species, from 22 genera (Sakai and Engelmann, 2007). Therefore, encapsulation-vitrification

Table 4. Effects of different regrowth media on the recovery rate of the control (-LN) and cryopreserved (+LN) sweet potato shoot tips in two cultivars cooled to -196°C

Regrowth medium I	Regrowth medium II	Regrowth rate (%)			
		Yeulmi		Yeonwhangmi	
		-LN	+LN	-LN	+LN
MS(standard)	MS(standard)	100	65.7 b[z]	100	80.3 b
	MS(NH_4NO_3-free)	100	43.2 c	100	55.4 c
MS(NH_4NO_3-free)	MS(standard)	100	78.9 a	98.4	91.3 a
	MS(NH_4NO_3-free)	99.1	41.2 c	100	54.7 c

[z]Means within columns followed by the same letter are not significantly different at 5% level.

could be considered a promising method for long-term preservation of sweet potato. Further research is required, however, using cytological and molecular analyses to confirm the morphological and genetic stability of regenerated plantlets produced by this type of germplasm preservation.

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References

Benson, E.E. 2008. Cryopreservation of phytodiversity: A critical appraisal of theory & practice. Curr. Rev. Plant Sci. 27:141-219.

Blakesley, D., T. Percival, M.H. Bhatti and G.G. Henshaw. 1997. A simplified protocol for cryopreservation of embryogenic tissue of sweet potato [*Ipomoea batatas* (L.) Lam.] utilizing sucrose preculture only. CryoLetters 18:77-80.

Charoensub, R., S. Phansiri, A. Sakai and W. Yongmenitchai. 1999. Cryopreservation of cassava *in vitro* grown shoot tips cooled to -196°C by vitrification. CryoLetters 20:89-94.

Dereuddre, J., C. Scottez, Y. Amud and M. Duron. 1990. Resistance of alginate-coated axillary shoot tips of per tree (*Pyrus communis*: Beauty Hardy) *in vitro* plantlets to dehydration and subsequent freezing in liquid nitrogen: Effects of previous cold hardening. C.R. Acad. Sci. Paris. Ser III 310. pp. 317-321.

Feng, C., Z. Yin, Y. Ma, Z. Zhang, L. Chen, B. Wang, B. Li, Y. Huang and Q. Wang. 2011. Cryopreservation of sweet potato (*Ipomoea batatas*) and its pathogen eradication by cryotherapy. Biotechnology Advances 29:84-93.

Fukai, S., M. Goi and M. Tanaka. 1991. Cryopreservation of shoot tips of *Chrysanthemum morifolium* and related species native to Japan. Euphytica 54:201-204.

Gaba, V and S. Singer. 2009. Propagation of sweet potato, in situ germplasm preservation and preservation by tissue culture: *In* Loebenstein, G. and G. Thottaappilly (eds.), The Sweet Potato. Springer, NewYork, USA. pp. 65-80.

Hirai, D. and A. Sakai. 2003. Simplified cryopreservation of sweet potato [*Ipomoea batatas* (L.) Lam.] by optimizing conditions for osmoprotection. Plant Cell Rep. 21:961-966.

Hirai, D., K. Shirai, S. Shirai and A. Sakai. 1998. Cryopreservation of *in vitro*-grown meristems of strawberry (*Fragaria* × *ananassa* Duch.) by encapsulation-vitrification. Euphytica 101:109-115.

Jenderek, M.M., D. Skogerboe and D. Ellis. 2008. Improvement of cryopreservation technique for long-term storage of shoot tips of *Ipomoea batatas*. *In vitro* Cell Dev. Biol. Anim. 44:S69.

Kuriyama, A., K. Watanabe, K. Kawata, F. Kawi and M. Kanamori. 1996. Sensitivity of cryopreserved *Lavandula vera* cells to ammonium ion. J. Plant Physiol. 148:693-605.

Kuriyama A., K. Watanabe, S. Ueno and H. Mitsuda. 1989. Inhibitory effect of ammonium ion on recovery of cryopreserved rice cells. Plant Sci. 64:231-235.

Langis, R. and P.L. Steponkus. 1990. Cryopreservation of rye protoplasts by vitrification. Plant Physiol. 92:666-671.

Loebenstein, G. 2009. Origin, distribution and economic importance: *In* Loebenstein, G. and G. Thottappily (eds.), The Sweet Potato. Springer, NewYork, USA. pp. 9-12.

Matsumoto, T., A. Sakai, C. Takahashi and K. Yamada. 1995. Cryopreservation of *in vitro*-grown apical meristems of wasabi (*Wasabia japonica*) by encapsulation-vitrification method. CryoLetters 16:189-206.

Matsumoto, T., A. Sakai and K. Yamada. 1994. Cryopreservation of *in vitro* grown apical meristems of wasabi (*Wasabia japonica*) by vitrification and subsequent high plant regeneration. Plant Cell Rep. 13:442-446.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nishizawa, S., A. Sakai, Y. Amano and T. Matsuzawa. 1993. Cryopreservation of asparagus (*Asparagus officinalis* L.) embryogenic suspension cells and subsequent plant regeneration by vitrification. Plant Sci. 91:67-73.

Pennycooke, J.C. and L.E. Towill. 2000. Cryopreservation of shoot tips from *in vitro* plants of sweet potato [*Ipomoea batata* (L.) Lam.] by vitrification. Plant Cell Reports 19:733-737.

Pennycooke, J.C. and L.E. Towill. 2001. Medium alterations improve regrowth of sweet potato [*Ipomoea batatas* (L.) Lam.] shoot tips cryopreserved by vitrification and encapsulation-dehydration. CryoLetters 22:381-389.

Rall, W.F. 1987. Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology 24:367-402.

Sakai, A and F. Engelmann. 2007. Vitrification, encapsulation-vitrification and droplet-vitrification: A review. CryoLetters 28:151-172.

Sakai, A., D. Hirai and T. Niino. 2008. Development of PVS-based vitrification and encapsulation-vitrification protocols: *In* Reed, R.M. (ed.), Plant Cryopreservation: A practical guide. Springer, Berlin, Germany. pp. 33-57.

Takagi, H., N.T. Thinh and O.M. Kyesm. 1998. Cryopreservation of vegetatively propagated tropical crops by vitrification. Acta Hort. 461:485-495.

Tannoury, M., J. Ralambosoa, M. Kaminski and J. Dereuddre. 1991. Cryopreservation by vitrification of coated shoot tips of carnation (*Dianthus caryophyllus* L.) cultured *in vitro*. C. R. Acad. Sci., Paris, Ser III 313. pp. 633-638.

Towill, L.E. and R.L. Jarret. 1992. Cryopreservation of sweet potato shoot tips by vitrification. Plant Cell Rep. 11:175-178.

Towill, L.E. 1990. Cryopreservation: *In* Dodds, J.H. (ed.), *In vitro* methods for preservation of plant genetic resources. Chapman and Hall, London, UK. pp. 41-69.

Volk, G.M., J.L Harris and K.E. Rotindo. 2006. Survival of mint shoot tips after exposure to cryoprotectant solution components. Cryobiology 52:305-308.

Volk, G.M., N. Maness and K. Rotindo. 2004. Cryopreservation of garlic (*Allium sativum* L.) using plant vitrification solution 2. CryoLetters 15:219-226.

Wang, Q, J. Laamanen, M. Uosukainen and J.P.T. Valkonen. 2005. Cryopreservation of *in vitro*-grown shoot tips of raspberry (*Rubus idaeus* L.) by encapsulation-vitrification and encapsulation-dehydration. Plant Cell Rep. 24:280-288.

Wang, Q.C. and A. Perl. 2006. Cryopreservation in floricultural crops: *In* Silva, J.T. (ed.), Floricultural, ornamental and plant biotechnology: advances and topics. Global Science Books, London, UK. pp. 523-539.

Yamada, T., A. Sakai, T. Matsumura and S. Higuchi. 1991. Cryopreservation of apical meristems of white clover (*Trifolium repens* L.) by vitrification. Plant Sci. 78:81-87.

Yi, J.Y., G.A. Lee, J.W. Chung, Y.Y. Lee, J.G. Kwak and S.Y. Lee. 2015. Morphological and genetic stability of dormant apple winter buds after cryopreservation. Korean J. Plant Res. 28:697-703.

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