

Encapsulation, cold storage, and growth of *Hibiscus moscheutos* nodal segments

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Received: 8 July 2005 / Accepted: 18 July 2006 / Published online: 10 November 2006
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Abstract Nodal segments of *Hibiscus moscheutos* (hardy hibiscus) were excised from proliferating axillary shoot cultures and encapsulated in high density sodium alginate hardened by 50 mM CaCl₂. Nodal segments 4 mm long grew as well as and were easier to encapsulate than 8 mm long nodal segments. Although nodal segments grew regardless of the concentration of sodium alginate, 2.75% was determined to produce the highest quality encapsulated nodal segments beads (sufficient alginate coating and ease of use) because of the viscosity produced by the 2.75% sodium alginate solution. When encapsulated segments were stored at 5°C they did not grow in light or darkness. During the first month on fresh proliferation medium under normal incubation conditions following 5°C storage in the dark for up to 24 weeks, root

number and root and shoot elongation were inhibited linearly as storage time increased. All encapsulated nodal segments survived 24 weeks of 5°C storage in two separate experiments. In fact, 80% of encapsulated hardy hibiscus nodal segments survived refrigerated storage for 1½ years (78 weeks) and after 3 months on proliferation medium, the nodal segments produced nearly the same length axillary shoots with the same number of axillary nodes per shoot as compared to encapsulated segments either not stored at 5°C or stored for 24 weeks at 5°C. Growth from encapsulated and cold-stored ‘Lord Baltimore’ nodal segments was more vigorous than from ‘Southern Belle’ nodal segments.

Keywords Alginate · Hardy hibiscus · In vitro conservation · Microencapsulation · Micropropagation · Preservation

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Introduction

Alginate encapsulation is a technique that can be used in conjunction with micropropagation for in vitro conservation. It can be used for germplasm storage or as a means to reduce the need for transferring and subculturing during off-season periods. During cold storage, encapsulated nodal segments require no transfers to fresh medium.

Cold storage has the potential to reduce the cost of maintaining germplasm cultures because of the reduced need for manual labor because of less frequent subculturing. Nearly half of the production costs of micropropagation are attributable to labor (Standardi and Piccioni 1998). Miaja et al. (2000) reported using encapsulation of *Vitis vinifera* L. to reduce high labor requirements of maintaining germplasm cultures. Cold storage also lessened the possibility of genetic instability from frequent subculturing and adventitious regeneration. Encapsulation can also provide a source of aseptic explant material that can be used if stock plants or proliferation cultures become infested with bacteria, fungi, or arthropods (West and Preece 2006).

For cold storage to be successful, tissue dehydration must be prevented and growth must return to normal rates when placed back under standard incubation conditions. An alginate-gelled matrix surrounding an explant slows the process of desiccation and provides the mechanical pressure needed to retain the tissue within the encapsulation medium during long-term storage. The matrix, however, will dry slowly during storage unless it is maintained in a high humidity environment. Typically this is accomplished in sealed petri dishes (Fig. 1). The cold, dark, humid conditions of storage have proven to be successful for storage of encapsulated tissues of several plant

species (Ballester et al. 1997; Monette 1987; Orlikowska 1992; Piccioni and Standardi 1995).

The purpose of this study was to develop a cold storage protocol for alginate encapsulated vegetative explants of hardy hibiscus by evaluating nodal segment sizes, sodium alginate concentrations, presence of light, storage temperatures, and storage times on nodal segment survival and growth.

Materials and methods

Source of explant material for proliferation cultures

Two *H. moscheutos* cultivars, ‘Lord Baltimore’ (asexually propagated) and ‘Southern Belle’ (sexually propagated) were greenhouse-grown in 3.8 l pots containing a 2 sphagnum peat:1 vermiculite:1 perlite (by volume) medium and placed under natural light conditions with 15 g/3.8 l pot of supplemental fertilization using 14N–4.2P–11.6K Osmocote® slow-release fertilizer. Irrigation water was applied by hose to the growing medium with care taken to avoid wetting the shoots. Both cultivars were evaluated and compared in each of the subsequent experiments.

Aseptic nutrient media and proliferation stock cultures

Nodal explants of the two cultivars were placed in vitro using the methods described by West and Preece (2004). All media contained Driver and Kuniyuki Walnut (DKW) minerals and vitamins (Driver and Kuniyuki 1984), 3% sucrose, and 10^{-7} M thiazuron (TDZ). The pH was adjusted to 5.8 ± 0.1 with 1.0 N KOH or 1.0 N HCl prior to adding 6.5 g l^{-1} plant tissue culture grade agar (PhytoTechnology Laboratories), dispensing into 25×150 mm borosilicate glass culture tubes capped with autoclavable plastic lids, and autoclaving for 20 min at 121°C and 1.0 kg cm^{-2} . The proliferation cultures were transferred every 2 weeks to fresh DKW medium with the same ingredients. Proliferation cultures were incubated approximately 30 cm beneath cool white fluorescent lamps that provided a photon flux of approximately $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and a 16-h photoperiod at 25°C .

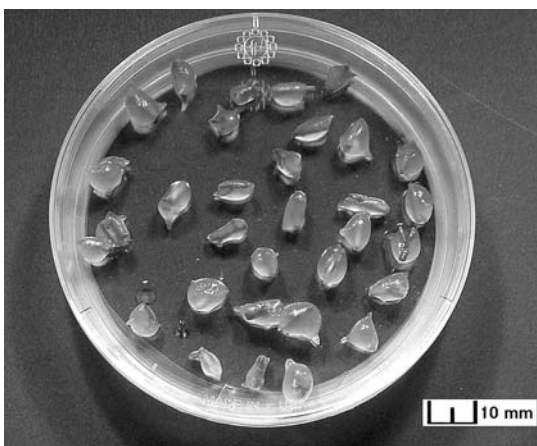


Fig. 1 Multiple alginate encapsulated nodal segments of *Hibiscus moscheutos* stored in 100×15 mm petri dish sealed with parafilm to maintain high humidity environment

Alginate encapsulation, cold storage, and subsequent culture incubation

Nodal segments (4 ± 1 mm long, except for the nodal segment size experiment) were excised from proliferating microshoots and had their subtending leaves removed. The nodal segments were then coated with high viscosity sodium alginate (Product Number A7128, Sigma Chemical Co.) and placed into a sterile 50 mM CaCl_2 solution for 30 min. The encapsulated nodal segments were transferred to two 5-min sterile deionized water rinses then placed in 100×15 mm petri dishes with the edges wrapped with parafilm. The petri dishes were then placed into refrigerated storage ($5 \pm 1^\circ\text{C}$.) for 4 weeks in darkness (unless otherwise noted). Encapsulated nodal segments were placed individually in 25×150 mm borosilicate glass culture tubes containing proliferation medium. The nodal segments were then incubated for 4 weeks, unless otherwise noted, under cool white fluorescent lamps that provided a photon flux of approximately $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a 16-h photoperiod at 25°C .

Data collection and statistical analysis

All experiments were arranged as completely random designs and conducted twice. A minimum of 10 explants per cultivar were used in each run of every experiment for replications. After 4 weeks of incubation under cool white fluorescent lamps and a 16-h photoperiod at 25°C , data were taken on shoot and root number, shoot and root length, and number of nodes containing at least one axillary bud with at least 5 mm of internodal tissue above and below the node (nodes suitable for subculturing or encapsulation) (unless otherwise noted). Only shoots >5 mm long and roots >1 mm long where counted and used for data analysis. All data were analyzed using analysis of variance with the General Linear Model (GLM) of SAS (SAS Institute, Inc., 1999). Data set columns having 50% or more zeros, were normalized using a square root transformation $(y+0.5)^{0.5}$ (Steele and Torrie 1980).

Encapsulated nodal segment size and alginate concentration experiment

The focus of this experiment was to compare the two cultivars; 4 ± 1 m or 8 ± 1 mm long nodal segments; and 0 (control, non-encapsulated), 2.0, 2.5, 3.0, or 3.5% sodium alginate for encapsulation, resulting in a $2 \times 2 \times 5$ factorial combination of cultivar, nodal segment size and sodium alginate concentration treatments. Further refining of the range of sodium alginate concentrations was performed by comparing 2.5, 2.75, or 3.0% alginate on nodal segments of the two cultivars, resulting in a 3×2 factorial combination of sodium alginate concentration and cultivar treatments.

Light and temperature experiment

Encapsulated nodal segments from both cultivars were stored at $5 \pm 1^\circ\text{C}$ or $25 \pm 1^\circ\text{C}$ in darkness or with $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ cool white fluorescent light (16-h photoperiod) resulting in a $2 \times 2 \times 2$ factorial combination of cultivar, temperature and light treatments.

Six month storage experiment

Encapsulated nodal segments of from both cultivars were stored in darkness at $5 \pm 1^\circ\text{C}$ for 0–24 weeks. Every 2 weeks for 24 weeks (13 storage time treatments counting a non-cold stored control) a petri dish including 10 encapsulated nodal segments of each cultivar was taken out of storage and encapsulated nodal explants were placed singly in 25×150 mm borosilicate glass culture tubes containing proliferation medium for subsequent incubation. This experiment resulted in a 2×13 factorial combination of cultivar and storage time treatments.

Long-term storage experiment

Encapsulated ‘Lord Baltimore’ nodal segments were stored in dark refrigeration ($5 \pm 1^\circ\text{C}$) for 0, 24, or 78 weeks. Encapsulated nodal segments were produced at different times for storage. The encapsulated nodal segments that were stored for 78 weeks were produced first. After 64 weeks

from the date of the first encapsulation, nodal segments were encapsulated and stored for 24 weeks. After 78 weeks from the date of the first encapsulation, nodal segments were encapsulated and not stored. All encapsulated nodal segments (stored or not stored) were simultaneously placed individually into 25 × 150 mm borosilicate glass culture tubes containing proliferation medium for subsequent incubation. At this same time, shoots from the 0 and 24 week treatments were subdivided into 10 mm long nodal segments, with only the most apical segment placed back into culture. Encapsulated nodal segments that had been stored for 78 weeks did not produce nodes with 5 mm of internode to each side and therefore, intact encapsulated nodal segments plus any newly developing shoots were transferred at the first 4 week transfer time for this treatment. After 8 weeks in proliferation conditions, shoots and roots were evaluated again from the subcultured nodal segments (produced from the 0 and 24 week encapsulated nodal segments) and the transferred encapsulated nodal segments (78 week encapsulated nodal segment). Nodal segments from all three treatments produced shoots that were long enough to subculture, therefore the most apical 10 mm long nodal segments were excised and subcultured for an additional 4 weeks. At this time (12 weeks under proliferation conditions), shoots and roots were evaluated for a final time. Survival of nodal explants was evaluated by the nodal segment's ability to produce shoots and roots and nodes not producing shoots and roots were considered to not have survived storage. This experiment resulted in a 2 × 3 × 3 factorial combination of cultivar, storage time and subsequent proliferation culture time treatments.

Results and discussion

Encapsulated nodal segment size and alginate concentration experiment

Although nodal segment size (4 mm or 8 mm long) did not significantly affect subsequent production of axillary shoots or shoot length, the larger segments were more difficult to

encapsulate because the internode ends tended to protrude out of the encapsulation beads, thus drying out the nodal segments. Ballester et al. (1997) reported that encapsulated shoot tips of *Camellia japonica* L. that were 5–6 mm or 10–11 mm long had a higher survival rate and produced more new shoots than 1–2 mm long encapsulated shoot tips. As growth differences between the sizes of the nodal segments was minimal and because the smaller sized segments were easier to obtain from proliferating axillary shoot cultures and to encapsulate, we chose the 4 mm size for all subsequent experiments.

In the control treatment (0% alginate), the nodal segments had been placed in petri dishes without being encapsulated or in the presence of supplemental water or any nutrient medium. After 4 weeks of dark storage at 5°C, these control nodal segments were dead and had completely dried up. Because of this, the control nodal segments were omitted from the data analysis. There was no significant difference between cultivars.

Alginate concentration affected root growth (Table 1). When alginate concentration of 3.5% was used, root length was significantly less than with the other alginate concentrations. There are two factors that may have caused this effect: the actual physical barrier of the denser matrix may have delayed the emergence of the root or the higher concentration of sodium from the sodium alginate caused a change in water potential, resulting in less water for root growth. Timbert et al. (1995) reported that 2.5–3.0% alginate increased bead density and had a negative effect on germination of carrot somatic embryos because they were unable to rupture and emerge from the encapsulated bead as compared to 1.5–2.0% alginate having higher germination frequency (%).

We found that high concentrations (3.0% and 3.5%) of high viscosity sodium alginate were difficult to dissolve in water and were so highly viscous that the solution was difficult to decant and use for encapsulation of nodal segments. However, the low concentrations (2.0% and 2.5%) of high viscosity sodium alginate had lower viscosity and coated the nodal segments poorly. Because both the higher (3.0% and 3.5%) and lower (2.0%

Table 1 Effects of alginate concentration on root growth from encapsulated *Hibiscus moscheutos* nodal segments that were first stored in darkness at $5 \pm 1^\circ\text{C}$ for 4 weeks then cultured in vitro on proliferation medium under cool white fluorescent lamps and 25°C for an additional 4 weeks

% Alginate	n^c	Mean root length (mm)
2.0	80	30.2
2.5	80	30.1
3.0	80	30.1
3.5	80	25.8
Significance ^a		**
T -test ^b	5%	3.0
	1%	4.0

^a***Significant at the 1% level according to the F -test with 3 and 303 df

^b T -test for paired comparisons

^cNumber of single encapsulated nodal segment replications in each mean

and 2.5%) concentrations had encapsulation limitations related to the viscosity of the matrix medium, another experiment was developed to compare an intermediate alginate concentration (2.75%). Although there were no differences in shoot or root number or growth, there were differences in handling of the matrix medium. The 2.75% concentration was determined to be the

best because it coated the nodal segments very well and was the easiest to drop into the calcium chloride solution. The resulting encapsulation beads held the nodal segments in place and still provided enough resistance to external mechanical pressure for ease of handling. Therefore, 2.75% high density sodium alginate was used in all subsequent experiments.

Light and temperature experiment

There was a significant interaction among light, storage temperature and cultivar on shoot and root growth of stored encapsulated nodes (Table 2). Encapsulated nodal segments that were stored cold (5°C) did not develop any shoots or roots during storage regardless if light was present (Fig. 2). At 25°C , ‘Lord Baltimore’ shoots grew longer than ‘Southern Belle’ in both darkness and under fluorescent lamps.

Growth from encapsulated nodal segments stored at 25°C was dependent on light (Fig. 2). When exposed to light, the nodal segments developed shoots and roots, similar to their in vitro response on proliferation medium. When placed in darkness, the encapsulated nodal segments developed longer, etiolated shoots with no roots. As growth during storage is not desirable

Table 2 Effects of cultivar, presence of light and storage temperature on shoot and root growth from encapsulated *Hibiscus moscheutos* nodal segments after 4 weeks under each storage condition^a

Cultivar	Storage temp ($^\circ\text{C}$)	Light ^d condition	Shoot number	Mean shoot length (mm)	Root number	Mean Root length (mm)
Lord Baltimore	5	Dark	0.0	0.0	0.0	0.0
		Light	0.0	0.0	0.0	0.0
	25	Dark	1.0	32.4	0.0	0.0
		Light	1.0	26.2	3.0	12.6
Southern Belle	5	Dark	0.0	0.0	0.0	0.0
		Light	0.0	0.0	0.0	0.0
	25	Dark	1.0	23.2	0.0	0.0
		Light	1.0	19.8	2.7	13.4
Significance ^b		**	**	**	**	
T -test ^c	5%		0.01	0.11	0.06	0.12
	1%		0.01	0.15	0.09	0.16

^aData were transformed for analysis using $(y + \frac{1}{2})^{1/2}$, non-transformed data are presented

^b***Significant at the 1% level, according to the F -test with 1 and 151 df

^c T -test for paired comparisons

^dCool white fluorescent lamps provided $40 \mu\text{M m}^{-2} \text{s}^{-1}$ photon flux and a 16 h photoperiod

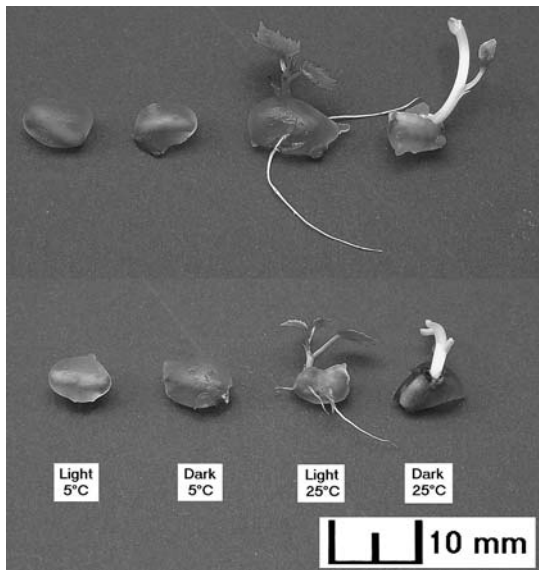


Fig. 2 Encapsulated *Hibiscus moscheutos* nodal segments stored in light or dark at 5 ± 1 or $25 \pm 1^\circ\text{C}$ for 4 weeks. Top row is ‘Lord Baltimore,’ bottom row is ‘Southern Belle’

and because darkness is easy to obtain during refrigeration, all subsequent storage treatments were in darkness at $5 \pm 1^\circ\text{C}$.

Six month storage experiment

As storage time increased the number of adventitious roots that were produced from encapsulated nodal segments of both cultivars decreased in a linear manner after 4 weeks on proliferation medium at 25°C (Fig. 3). Shoot number was unaffected by the refrigerated storage conditions because all nodes produced one shoot after 1 month of proliferation culture regardless of the storage time, indicating that all buds survived cold storage.

There was a significant interaction between cultivar and storage time on shoot and root length (Fig. 4). As storage time increased, shoot and root length decreased in a linear manner for both cultivars after 4 weeks of incubation on proliferation medium at room temperature. Therefore, the mechanisms that control adventitious root formation and root and shoot elongation were negatively affected by the refrigerated conditions. However, as we show below, repair appears to

occur because shoot length recovers with increasing time under proliferation conditions. In this experiment, ‘Southern Belle’ roots and shoots grew more slowly compared to ‘Lord Baltimore’ (Fig. 3, and 4)

Encapsulated nodal segments of both cultivars were still 100% viable after 6 months of cold-dark storage. In contrast, Ballester et al. (1997) reported only 10% of encapsulated shoot tips of *Camellia japonica* survived after 75 days of storage at $2\text{--}4^\circ\text{C}$ and only 1% survived 6 months of cold storage. Therefore, encapsulated nodal segments of hibiscus survive cold storage better than encapsulated shoot tips of *C. japonica*.

Long-term storage experiment

‘Lord Baltimore’ nodal segments responded differently during each month of culture on proliferation medium depending on how long they were stored under dark refrigerated conditions compared to those that were not stored (Table 3). Nodal segments that had not been refrigerated produced shoots that were significantly longer and had more nodal segments during the first month on proliferation medium as compared to nodal segments stored for 24 or 78 weeks. However, both non-refrigerated and nodal segments stored for 24 weeks produced shoots sufficiently long after 4 weeks under proliferation conditions to be subdivided and the most apical 10 mm long nodal segments from both of these treatments were transferred to fresh proliferation medium for further growth and analysis. Single node subdivision is used for commercial hardy hibiscus micropropagation. After the second month in vitro (week 8), new growth from recultured nodes from non-refrigerated and nodal segments stored for 24 weeks was not significantly different, indicating full recovery of the growth (shoot length and nodal segment number) from nodal segments that had been cold-stored for 24 weeks.

Nodal segments that were stored for $1\frac{1}{2}$ years (78 weeks) were slower to recover than those not refrigerated or stored for 24 weeks. New growth from these longest-term encapsulated nodal segments was retarded and the new shoots were not sufficiently long for subdivision after 4 weeks under proliferation conditions. However, after

Fig. 3 Effects of weeks in dark, cool storage on mean root number of *Hibiscus moscheutos* ‘Lord Baltimore’ (LB) and ‘Southern Belle’ (SB) nodal segments after 4 weeks in vitro on proliferation medium under cool white fluorescent lamps and 25°C

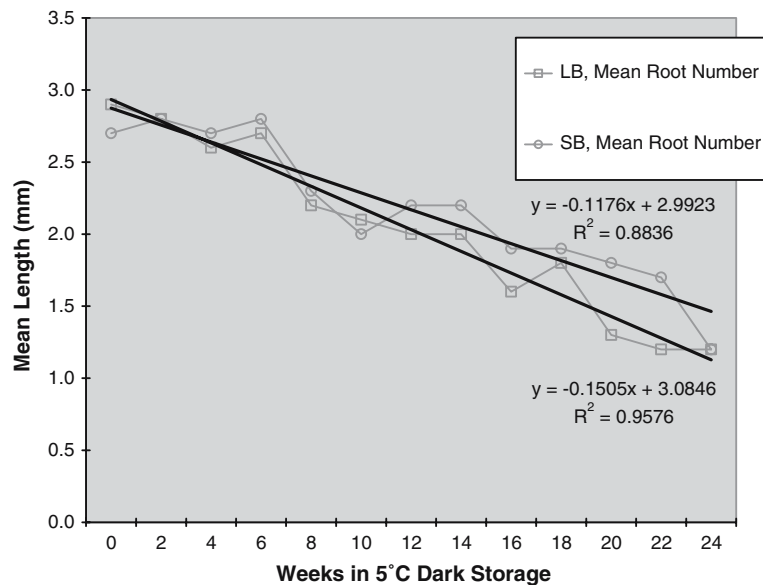
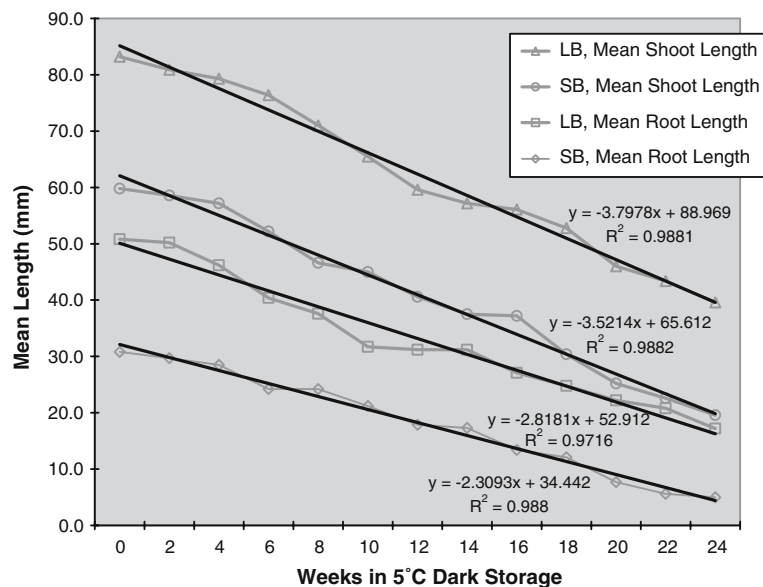


Fig. 4 Effects of weeks in dark, cool storage on mean shoot and root length of *Hibiscus moscheutos* ‘Lord Baltimore’ (LB) and ‘Southern Belle’ (SB) nodal segments after 4 weeks in vitro on proliferation medium under cool white fluorescent lamps and 25°C



8 weeks under proliferation conditions there was sufficient new shoot growth from these encapsulated nodal segments for subdivision.

During the last 4 weeks under proliferation conditions (by week 12), mean shoot length, mean root number and length produced from recultured nodal segments from encapsulated nodal segments stored for 78 weeks was nearly the same as new growth from recultured nodal segments

that were not refrigerated or stored for 24 weeks, indicating recovery.

All nodal segments that were either not refrigerated or were stored for 24 weeks survived to the end of the experiment. However, 20% of encapsulated nodal segments that were stored for 78 weeks died during storage or the first month under proliferation conditions. A survival rate of 80% is remarkable for 4 mm long encapsulated

Table 3 Effects of time in $5 \pm 1^\circ\text{C}$ dark storage shoot and root growth from encapsulated nodal segments of *Hibiscus moscheutos* ‘Lord Baltimore’ after 3 months in vitro incubation on proliferation medium under cool white fluorescent lamps and 25°C

Weeks in 5°C dark storage	Weeks in proliferation culture	Shoot number	Mean shoot length (mm)	Mean root number	Mean root length (mm)	Nodal Segment number ^d	Survival (%)
0	4	1.0	96.3	4.9	54.3	5.3	100
	8	1.0	88.8	5.1	54.2	5.2	100
	12	1.0	95.2	4.9	58.0	5.4	100
24	4	1.0	45.9	1.7	41.0	3.3	100
	8	1.0	87.6	4.8	52.2	4.9	100
	12	1.0	88.5	4.7	51.8	4.9	100
78	4	0.8	6.3	0.2	2.9	1.0	80
	8	0.8	26.3	1.4	27.4	2.6	80
	12	0.8	57.6	3.5	38.5	4.3	80
Significance ^a		ns	**	**	**	**	
T-test ^b	5%	–	19.5	1.1	12.8	0.8	–
	1%	–	25.7	1.5	16.8	1.1	–
Contrast							
	Linear ^c		**	**	**	**	

^ans, **Nonsignificant, significant at the 1% level, according to the *F*-test with 4 and 80 df

^bT-test for paired comparisons

^cLinear trend analysis was conducted on nodal segments stored for 78 weeks to determine whether their recovery from cold storage during proliferation culture was at a steady pace

^dMean number of nodal segments (containing at least one axillary bud and at least 10 mm long) that could be excised from axillary shoots for subculturing and using for encapsulation or micropropagation

nodal segments that were stored in a dark refrigerator for 1½ years (78 weeks) with no addition of water or fresh medium. Those that did survive for 1½ years grew more slowly during the first 3 months under proliferation conditions than those stored for 24 weeks or not refrigerated (Fig. 5). These longest-term stored nodal segments showed steady linear growth over the 3 months under proliferation conditions (Table 3). This indicates that these nodal segments slowly recovered from any damage that occurred during long-term storage. Others have also reported that it takes time for explants to recover from cold storage stress after return to proliferation culture conditions (Ballester et al. 1997).

Conclusion

Hibiscus moscheutos is well adapted to alginate encapsulation of small, 4 mm, nodal segments utilizing 2.75% high viscosity sodium alginate. These can be stored at 5°C for up to 1½ years with no maintenance with reduced shoot and root

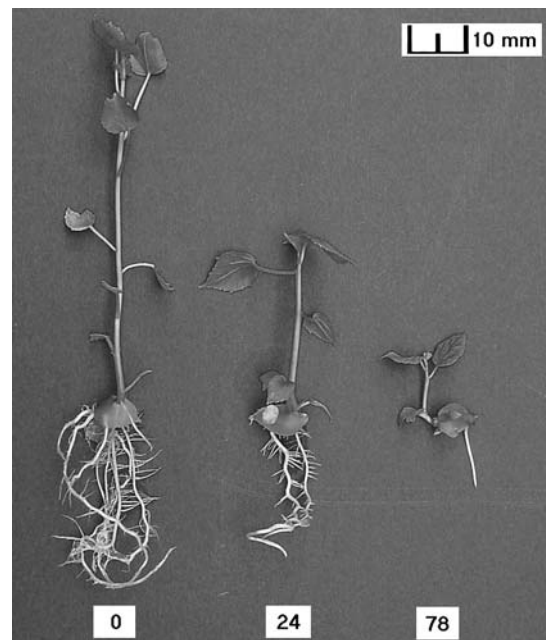


Fig. 5 Effects of number of weeks in dark, cool storage (5°C) on mean root number of *Hibiscus moscheutos* ‘Lord Baltimore’ nodal segments after 4 weeks in vitro on proliferation medium under cool white fluorescent lamps and 25°C

vigor. Recovery from the stress of storage may take one to 3 months under proliferation conditions, depending on the length of the storage time. Long-term storage under commonly available refrigerated conditions (5°C) is easier and less costly than cryopreservation that requires deep freezing or vitrification. Common refrigeration at 5°C for storage of small, light weight propagules may be useful for micropropagation laboratories to avoid maintaining cultures during slow times of the year.

References

- Ballester A, Janeiro LV, Vieitez AM (1997) Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica* L. and *Camellia reticulata* Lindley. *Sci Hort* 71:67–78
- Driver JA, Kuniyuki AH (1984) In vitro propagation of paradox walnut rootstock. *Hortscience* 19:507–509
- Miaja ML, Gribaudo I, Vallania R, Fernandez LF (2000) Low temperature storage and cryopreservation of a *Vitis vinifera* L. germplasm collection: first results. *Acta Hort* 538:177–181
- Monette PL (1987) Organogenesis and plantlet regeneration following in vitro cold storage of kiwifruit shoot tip cultures. *Sci Hortic* 31:101–106
- Orlikowska T (1992) Effect of in vitro storage at 4°C on surviving and proliferation of two apple rootstocks. *Plant Cell Tiss Org Cult* 31:1–7
- Piccioni E, Standardi A (1995) Encapsulation of micro-propagated buds of six woody species. *Plant Cell Tiss Org Cult* 42:221–226
- Standardi A, Piccioni E (1998) Recent perspectives on synthetic seed technology using nonembryogenic in vitro-derived explants. *Int J Plant Sci* 159(6):968–978
- Steel RGD, Torrie JH (1980) Principles and Procedures of Statistics. In: A biometrical approach, 2nd edn. McGraw-Hill, New York
- Timbert R, Barbotin J-N, Kersulec A, Bazinet C, Thomas D (1995) Physico-chemical properties of the encapsulation matrix and germination of carrot somatic embryos. *Biotechnol Bioeng* 46:573–578
- West TP, Preece JE (2004) Effects of thidiazuron and nutrient salt formulations on micropropagation of hardy hibiscus (*Hibiscus moscheutos* L.). *Acta Hort* 630:293–297
- West TP, Preece JE (2006) Use of acephate, benomyl and alginate encapsulation for eliminating culture mites and fungal contamination from in vitro cultures of hardy hibiscus (*Hibiscus moscheutos* L.). *In Vitro Cell Dev Biol Plant* 42:301–304