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In Vitro Storage of Hosta Micropropagules - Effect of Media Sucrose on Post-Storage Recovery

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Nature of work: In vitro storage under growth retarding conditions delays the necessity for frequent transfers to fresh media and allows flexibility in meeting market demand leading to efficient management of labor. Storage conditions should preserve the post-storage quality and regrowth potential of in vitro plants. Wilson et al. (1998) reported that low light [photosynthetic photon flux (PPF) of 5-7 mmol m^{-2} s^{-1}] in storage improved post-storage quality and recovery potential of in vitro plantlets. In our research, increasing media sucrose to 5% or 7% during the multiplication phase (stage II) increased the internal sugar levels, biomass, and quality in Hosta micropropagules. When these cultures were transferred to rooting phase (stage III) in a media containing 3% sucrose and subsequently stored for 5 weeks at 10∞ C, under a PPF of 5 mmol m^{-2} s^{-1}, plantlets from the 5% or 7% sucrose media were of better quality than the plantlets from 1% or 3% sucrose (unpublished data). Data suggests that sucrose loading during multiplication phase had positive influence on post-storage plant quality (unpublished).

Sugar-free micropropagation holds significance in commercial micropropagation because sucrose-free medium reduces media contamination and consequent loss (Kozai, 1991). Therefore, the objective of this investigation was to examine if sucrose loading during multiplication phase allows in vitro rooting and storage in sucrose-free medium.

This study was conducted with two cultivars of Hosta: *Hosta tokudama* Tratt. 'Newberry Gold' and Hosta 'Striptease'. Stage II Hosta buds cultured in 5% media sucrose were procured from Southern Sun Propagation Systems, Norris, SC. Plants were transferred to stage III for rooting (on sorbarod plugs) (Ilacon Industries, UK) in magenta boxes (Magenta Corp. Chicago IL) containing modified Murashige and Skoog (1962) liquid medium. During stage III plants were cultured in media with 3% sucrose (photomixotrophic cultures) or without sucrose (photoautotrophic cultures) for four weeks at 25±2 °C under a PPF of 150 mmol $m^{-2}s^{-1}$. Nine buds of 'Newberry Gold' were cultured in each culture vessel while six buds of 'Striptease' (due to larger bud size) were cultured per

vessel. Following Stage III, cultures were stored, in the same culture vessel with residual medium, for 7 or 14 weeks under 5 mmol m^{-2} s^{-1} at 10° C, with and without a 2-week intermittent period of darkness during the final phase of storage to simulate shipping. Plantlets (four vessels from each treatment) were harvested for dry weight at the end of stage III (prior to storage) and after storage. Post-storage plantlets, from five culture vessels of each treatment, were transferred (after removal of necrotic leaves) to 606-cell packs containing a commercial potting mix (Superfine germinating mix, Fafard, Anderson, SC) and grown under mist for 4 weeks for acclimatization. After 4 weeks under the mist, mortality of the plantlets was recorded. Data were analyzed by ANOVA and treatment differences were separated using LSD at P = 0.05. Efficacy of sucrose loading on sucrose-free storage was assessed by comparing post storage survival and plant quality in both photoautotrophic and photomixotrophic cultures.

Results and Discussion: Presence of media sucrose led to significantly higher shoot and root biomass in both the cultivars at the end of stage III (Fig.1 A & B). In ‘Striptease’, the root biomass of photomixotrophic cultures increased by 70% during 7 weeks of storage, while in photomixotrophic cultures under continuous illumination, the root biomass remained unchanged thereafter. But in ‘Striptease’ photomixotrophic cultures that were stored for 14 weeks dark period had a negative influence on the root biomass (Fig 2A). However, in ‘Newberry Gold’, no change in root biomass occurred during 7 weeks of storage but a significant root growth occurred between 7 and 14 weeks of storage (Fig 2B). Incidence of shoot apex necrosis was higher in photoautotrophic cultures of both the cultivars, consequently, reflected by poorer percentage of survival in the greenhouse compared to the photomixotrophic plantlets (Table 1). Both photoautotrophic and photomixotrophic ‘Striptease’ cultures stored for 7 or 14 weeks recovered in the greenhouse but quality of photomixotrophic cultures were better than photoautotrophic cultures (data not shown). Photoautotrophic ‘Striptease’ cultures had a significantly greater percentage of mortality (about 25%) compared to photomixotrophic cultures (0%) after 7 weeks of storage. Photoautotrophic and photomixotrophic ‘Newberry Gold’ cultures stored for 7 weeks recovered in the greenhouse but extending the storage duration to 14 weeks led to further decline in the greenhouse survival of the photoautotrophic plantlets reflected by high percentage of mortality (Table 1). Overall, sucrose-free medium during rooting and storage led to poor post-storage recovery in both the cultivars, while extending storage duration led to further deterioration in ‘Newberry Gold’. Results indicate between-cultivar differences in rooting and post storage recovery; ‘Striptease’ can survive better in photoautotrophic cultures than ‘Newberry Gold’. Media sucrose during rooting stage and storage

contributed towards enhanced post storage survival, by offering nutritional support for improved rooting and maintenance of growing shoot apex during storage.

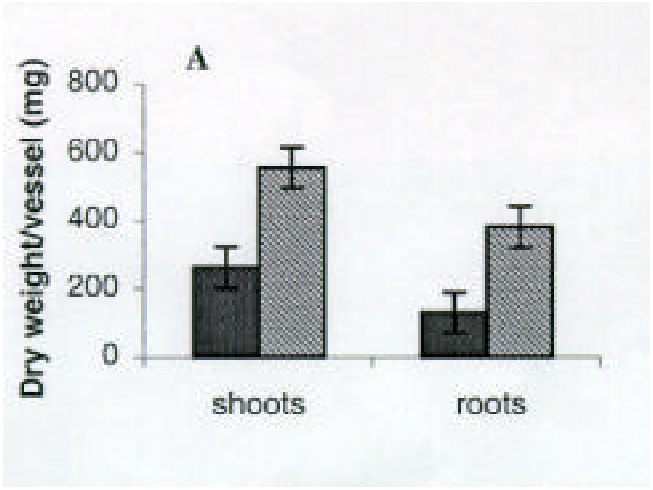
Significance to Industry: In vitro techniques are being increasingly used in the large-scale production of uniform disease free propagation material. Demand for propagation materials is often seasonal and therefore production peaks strive to match demand peaks. Employing sufficient labor exclusively during production peaks is impractical, because micropropagation involves expensive trained labor. Developing techniques for in vitro storage and subsequent shipping enables efficient utilization of labor, thereby, bringing down production cost in commercial micropropagation. Our study demonstrates that supplementing media with sucrose improves rooting and provides sustenance through out low temperature storage, enabling prolonged storage, as well as ensuring enhanced post storage survival.

Literature Cited:

1. Wilson, S.B., K. Iwabuchi, N.C. Rajapakse, and R.E. Young. 1998. Responses of Broccoli seedlings to light quality during low-temperature storage in vitro: II. Sugar content and photosynthetic ability. HortScience, Vol. 33:1258-1261

2. Kozai, T. 1991. Autotrophic micropropagation In: Bajaj Y.P.S (ed) Biotechnology in agriculture and forestry Vol.17. Hi-Tech and Micropropagation I. Springer, Berlin Heidelberg. 1991. pp.313-343.

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



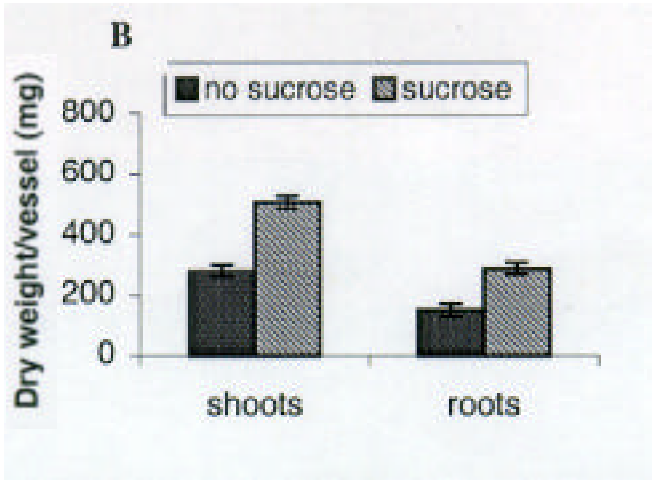


Figure 1. Shoot and root biomass per vessel in the photoautotrophic (no sucrose) and photomixotrophic (3% sucrose) cultures of Hosta 'Striptease' (A) and *Hosta tokudama* Tratt. 'Newberry Gold' (B) at the end of stage III (prior to storage). Means ± S.E. are shown

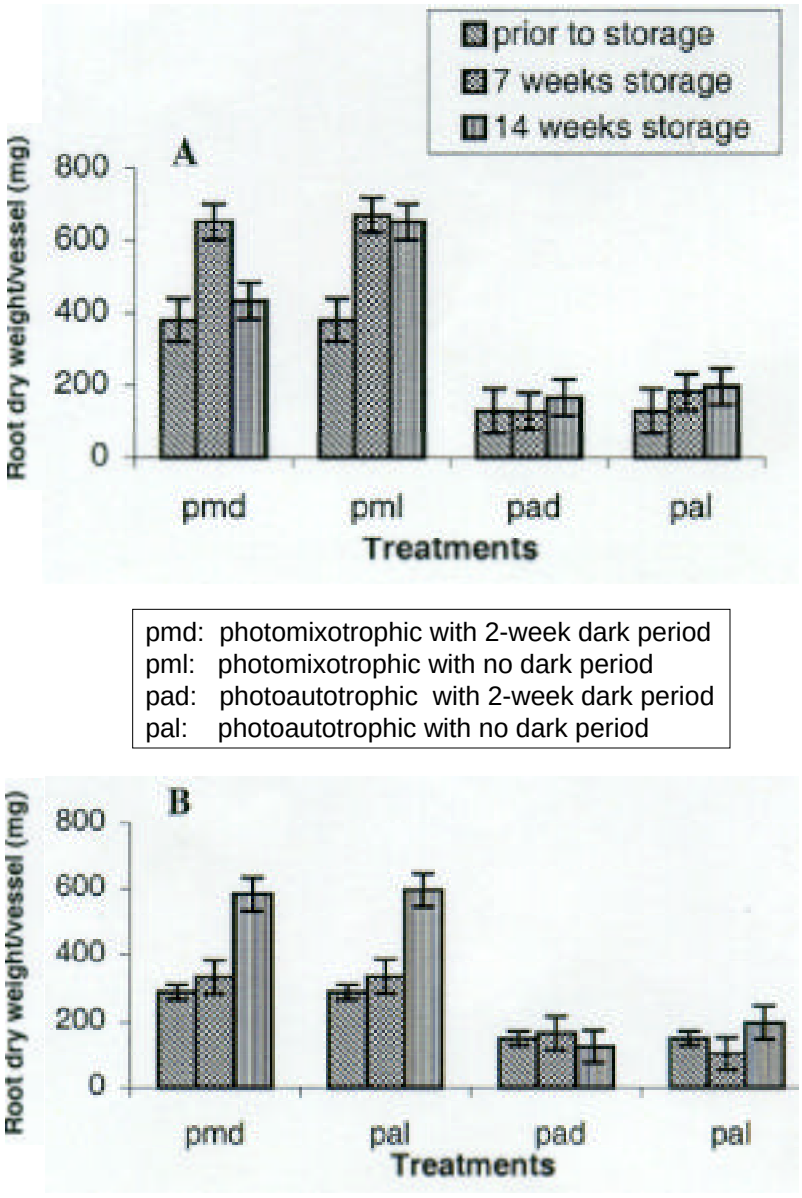


Figure 2. Post-storage root biomass in Hosta 'Striptease' (A) and *Hosta tokudama* Tratt. 'Newberry Gold' (B). Cultures were transferred to rooting in photoautotrophic (no sucrose) or photomixotrophic (3% sucrose) media and subsequently taken to storage for 7 or 14 weeks at 10 ^{o}C under 5 µmol m^{-2} s^{-1} of PPF with or without an intermittent dark period during storage. Means $\pm$ S.E. are shown

Table 1. Percentage of plantlet mortality in the photoautotrophic and photomixotrophic cultures of *Hosta tokudama* Tratt.'Newberry Gold' and Hosta 'Striptease' following 4 weeks post-storage recovery in greenhouse. Means with the same letter are not significantly different within each cultivar.

Treatments	% Post acclimatization mortality			
	Newberry Gold		Striptease	
	7 weeks	14 weeks	7 weeks	14 weeks
Photomixotrophy with 2-week dark period	0% c	2.2% c	0% b	20.8% ab
Photomixotrophy with no dark period	0% c	0% c	0% b	10% ab
Photoautotrophy with 2-week dark period	35.5% b	88.9% a	26.7% a	33.3% a
Photoautotrophy with no dark period	35.5% b	84.4% a	25.0% ab	20.0% ab