CARBOHYDRATE STATUS AND POST STORAGE RECOVERY OF MICROPROPAGATED HOSTA PLANTLETS STORED AT VARYING TEMPERATURES IN LIGHT OR DARKNESS

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Abstract

Hosta (*Hosta tokudama* Makeawa 'Newberry Gold') plantlets were micropropagated photoautotrophically (without sugar in media) or photomixotrophically (with sugar in media) for 3 weeks at 23 °C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (*PPF*) prior to long term storage. Light compensation points were determined prior to each long-term storage experiment so that plants could be stored without excessive growth. Plantlets were stored for 4, 8, or 12 weeks in darkness or under white light (400-800 nm) and then transferred to the greenhouse for 60 d. Three independent experiments were performed at 5-, 10-, or 22 °C. Illumination during storage was necessary to maintain total soluble sugars (TSS), starch, and regrowth potentials of in vitro plantlets. All photoautotrophic plantlets stored in darkness were of poor quality and died when transferred to the greenhouse. Dark-stored photomixotrophic plantlets survived storage for 12 weeks at 5 °C but declined in appearance when storage temperature increased to 10 and 22 °C. The visual quality and greenhouse acclimatization of illuminated plants was best when plants were stored at 22 °C. After 60 d in the greenhouse, the dry weight of these plantlets was similar for 0, 4, 8, and 12 week storage periods. Supplying 2% sucrose in the culture medium increased leaf TSS and maintained overall plantlet quality during illuminated storage.

1. Introduction

Hosta plants are routinely propagated from division of lateral shoots. However, because only a few shoots can be obtained from each plant, introduction of a new cultivar can take several years. Tissue culture offers potential for rapid clonal multiplication (Paek and Ma, 1996). The rapid availability of many new and exciting hosta cultivars is in part due to the use of tissue culture as a means of commercial propagation. However, advantages of tissue culture such as rapid production, greater uniformity, phenotypic improvement, and disease elimination are often counteracted by high production costs and low profits. It is sometimes difficult to predict the ever-changing consumer demand of certain hosta species and equally difficult to distribute labor costs for the seasonal production of hosta. It would be highly significant to the horticultural industry if micropropagated plantlets could be stored for extended time periods in large quantities per unit space. In addition to ensuring availability of seasonal ornamental crops and distributing labor costs, techniques of holding micropropagated plantlets could

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significantly impact the globalization of markets where preserving plantlet quality during extended shipping periods is crucial.

Storage systems that minimize growth without sacrificing quality require the manipulation of light, temperature, and medium composition.Storage of plants at their light compensation point (where photosynthesis balances respiration) has proven effective in minimizing excessive elongation. Kubota *et al.*, (1995) have shown that low temperature in conjunction with fluorescent lighting at PPF as low as 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ maintained photosynthetic ability and dry weight of photoautotrophic (PA) broccoli seedlings stored for 6 weeks. In the photoautotrophic system, the micropropagated plant is dependent on its own production of photosynthates rather than using an exogenous carbon source in the form of sugar. Kubota and Kozai (1995) showed that light compensation points of stored plants vary with air temperature and sugar level in the medium. Addition of sucrose to the medium has been shown to increase dry weight, soluble sugars, and visual quality of plantlets (Wilson *et al.*, 1998a,b). However, decreasing or omitting sucrose in the medium can facilitate growth by promoting autotrophy, decreasing expense of materials, and reducing biological contamination (Kozai, 1991).

Low temperature storage has been used widely for preserving post-harvest quality of horticultural commodities. Provision of light in low temperature storage has gained interest in recent years to improve the quality during storage of plantlets. Recently, researchers found that red light irradiation during cool temperature storage of strawberry plants increased leaf area, chlorophyll and photosynthetic rates (Nishizawa *et al.*, 1997). However, few reports exist on low temperature storage of in vitro plantlets. It has been shown that in vitro broccoli seedlings can survive illuminated storage at 5 °C for up to 6 weeks (Kubota *et al.*, 1996), but not past 8 weeks (Wilson *et al.*, 1998a). Storage of in vitro broccoli seedlings at a lower temperature of 1 °C improved plantlet quality and regrowth potential, with red light being particularly effective in increasing dry mass and photosynthetic capacity of stored seedlings (Wilson *et al.*, 1999). However, it is clear that various plant species respond differently to low temperature stress (Lange *et al.*, 1991). Therefore, for each new species stored in vitro, varying temperatures must be explored to pinpoint optimum conditions for high quality plants. In the current research, we investigated the influence of light (provided by white fluorescent tubes at intensities near the light compensation point) and temperature (5-, 10-, and 22 °C) on storability and post-storage recovery of micropropagated hosta plantlets.

2. Materials and Methods

2.1. Plant material and culture condition

Hosta tokudama 'Newberry Gold' plantlets were subcultured in vitro photoautotrophically (PA, no sugar in the medium) or photomixotrophically (PM, 2% sucrose in the medium) for 3 weeks in Murashige and Skoog (1962) liquid media (10 mL per plantlet) supplemented with vitamins (Gamborg, 1970). Four plantlets were cultured on Sorbarod cellulose support plugs (Sorbarod, Baumgartner Papiers SA, Switzerland) in 375 mL GA-7 vessels (Magenta, Chicago, Ill.). Two holes (10 mm in diameter) in opposite sides of the vessel were covered with 0.5 µm membrane filter disks (Milli-Seal, Millipore K.K., Tokyo) to provide ≈ 3.2 air exchanges per hour (Kozai *et al.*, 1986). Culture room temperature was 23 ± 2 °C during the 16 h photoperiod and 19 ± 1 °C during the 8 h dark period. *PPF* was 80 ± 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the culture shelf.

2.2. Storage treatments

In three consecutive but independent experiments, vessels containing in vitro plantlets were stored for 4, 8, or 12 weeks at 5-, 10-, or 22 °C. Determination of light compensation points for storage experiments has been previously reported (Wilson *et al.*, 1999b). At 5 °C, plantlets were stored in darkness or under 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white

(average light compensation point from previous experiment). At 10 °C, plantlets were stored in darkness or under 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light (average light compensation point from previous experiment). At 22 °C, plantlets were stored in darkness or under 11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light (an estimated light compensation point based on previous experiments). At the start of storage, the air diffusive filters of the vessel were covered with plastic tape to minimize the number of air exchanges and to avoid excessive water loss.

2.3. Carbohydrate analysis

Leaves were frozen in liquid nitrogen and freeze-dried. Sugar content was determined for 5 °C and 10 °C experiments only. To obtain sufficient tissue for carbohydrate analysis, two seedlings were pooled to generate a sample. Leaves were ground, and soluble sugars from 50 mg of leaf tissue was extracted overnight with methanol : chloroform : water (12:5:3 by volume) as described by Miller and Langhans (1989). Sucrose, glucose, and fructose were separated and detected using an HPLC with a refractive index detector (Waters Associates, Milford, MA) and a Bio-Rad HPX-87C column (Bio-Rad, Richmond, CA) maintained at 85°C. Quantification of individual sugars was based on an internal standard of mannitol (1 mg). Combined concentrations of sucrose, glucose, and fructose were reported as total soluble sugars. As described by Miller and Langhans (1989), starch was determined via a glucose oxidase assay on dried residue left in the pasteur pipets following soluble carbohydrate extraction.

2.4. Post storage recovery in greenhouse

Upon removal from storage at 4, 8, or 12 weeks, plantlets from two vessels were transferred to soilless media (Metro Mix 360, The Scotts Co., Marysville, Ohio) in 804 grow packs and placed under mist in the greenhouse for 4 weeks. Plantlets were removed from mist and grown in a different greenhouse location without mist for an additional 4 weeks. Visual quality of plantlets (based on color and form) in the greenhouse was assessed bi-weekly for 8 weeks by three people and an average rating was recorded. Visual quality evaluations were based on a scale from 1 to 5, whereby 1=very poor quality, severe leaf necrosis, leaf yellowing, not marketable; 2=poor quality, large areas of leaf necrosis, leaf yellowing, not marketable; 3=fair quality, marginally marketable; 4=good quality, no yellowing, marketable; and 5=excellent quality, no leaf necrosis, no yellowing, highly marketable.

2.5. Experimental design, data collection, and statistical analysis

Experiments were conducted similarly but independently (due to limited # of low temperature incubators) at 5-, 10-, and 22 °C. Vessels (photoautotrophic and photomixotrophic) were randomized in each incubator. Five photoautotrophic and five photomixotrophic vessels were harvested from each treatment after 0, 4, 8, or 12 weeks of storage. Each vessel with four plantlets was considered a replication. Three vessels from each treatment were used for dry weight and carbohydrate measurements. Two vessels from each treatment were used for post-storage recovery analysis in the greenhouse. Data were analyzed by ANOVA and treatment differences were separated by LSD at P=0.05.

3. Results and Discussion

3.1. Carbohydrates

Before storage, photomixotrophic plantlets had approximately 60% more leaf total soluble sugars (TSS) than did photoautotrophic plantlets (Fig. 1). In photoautotrophic plantlets, leaf TSS decreased rapidly to undetectable levels during 4 weeks of dark storage

at 5 °C and to almost undetectable levels during 4 weeks of dark storage at 10 °C. In photomixotrophic plantlets, leaf TSS slightly decreased during the 12 week storage duration. Light in storage increased TSS in photoautotrophic and photomixotrophic plantlets during the first 4 weeks, but then levels decreased as storage time increased. Reduced TSS during storage corresponded with decreased dry weights (Wilson *et al.*, 1999b). Starch decreased dramatically during the first 4 weeks of storage, regardless of media composition, light, or temperature (Figure 2 A-D). This correlated with increased TSS during the first 4 weeks of storage, thereby revealing carbohydrate metabolism changes before and during storage. Likewise, Piqueras *et al.* (1998) investigated the regulation of carbohydrate metabolism during acclimatization of tissue cultured Calathea plantlets and found higher starch contents in the roots and stems compared with leaves, while sucrose concentration was highest in stems, followed by leaves and roots. Hosta plantlets stored under white light had higher starch concentrations than plantlets stored in darkness. In photoautotrophic plantlets stored in darkness, starch concentrations decreased to almost undetectable levels after 4 weeks storage (Figure 2 A,C). The inability of these plants to regenerate in the greenhouse suggests that stored starch reserves in the crown tissue were also depleted (data not presented).

3.2. Visual quality and post storage recovery

Regardless of storage temperature, visual quality of plantlets at the time of removal from storage decreased as in vitro storage time increased from 4 to 12 weeks (data not presented). Therefore, only visual quality data from plantlets stored for 12 weeks (longest treatment) at 5 °C, 10 °C, and 22 °C is shown (Fig. 3). All dark-stored photoautotrophic plantlets never recovered in the greenhouse and were considered dead (Fig. 3 A and C). When stored under illumination, photoautotrophic plantlets had good visual quality when stored at 10 and 22 °C (Fig. 4 C and E), but poor visual quality when stored at 5 °C (Fig. 3 A).

Photomixotrophic plantlets stored in light or darkness had higher visual quality ratings in the greenhouse than did photoautotrophic plantlets (Fig. 3 A-F). The reduced visual quality of photoautotrophic plantlets was consistent with previous research (Wilson *et al.*, 1998a) and attributed to reduced dry weight and carbohydrate reserves. All photomixotrophic plantlets stored at 5 °C survived acclimatization to the greenhouse, and there were no visual quality differences between dark and light stored plantlets. At 10 °C, dark stored plantlets had lower visual quality ratings than did light stored plantlets (Fig. 3 B and D), but plantlets were still considered marketable. However, at 22 °C, dark stored plantlets were etiolated and never recovered after storage (Fig. 3 F). This indicates that the optimum temperature for dark storage of photomixotrophic hosta plantlets is 5 °C. For photomixotrophic plantlets stored under illumination, the initial visual quality (immediately prior to transfer to greenhouse) was best when plantlets were stored at 22 °C rather than 5 or 10 °C. However, regardless of storage temperature, as time in the greenhouse increased, visual quality increased. Initially (immediately after removal from storage), better quality hosta plantlets were obtained by increasing the storage temperature, thereby preventing the perennial plants from entering dormancy. This differs from previous research where lowering storage temperature from 5 °C to 1 °C improved storability of in vitro broccoli seedlings. Better survival rates of broccoli seedlings at 1 °C were attributed to the decreased metabolism and high carbohydrate reserves (Wilson *et al.*, 1999a).

4. Conclusions

Illumination is required for storage of photoautotrophic hosta plantlets between 5-22 °C, and recommended for storage of photomixotrophic plantlets at higher temperatures between 10-22 °C. Supplying sucrose in the medium (in addition to light) is necessary to maintain high quality plantlets during storage. Optimal temperature conditions for

illuminated storage of photomixotrophic hosta plantlets is 10-22 °C. Decreasing the storage temperature from 22 °C to 10- or 5 °C decreases the visual quality of the plantlets when initially transferred to the greenhouse, although plantlets recover after 4 weeks (increased visual quality) and are considered marketable. The survivability and strong regrowth potential of stored hosta plantlets indicate that carbohydrates were partitioned and stored in the crown tissue. Leaf TSS and starch reserves were depleted in photoautotrophic plants and reduced in photomixotrophic plants stored in darkness. Future research should be directed towards investigating carbohydrate status during the acclimatization of plantlets to the greenhouse.

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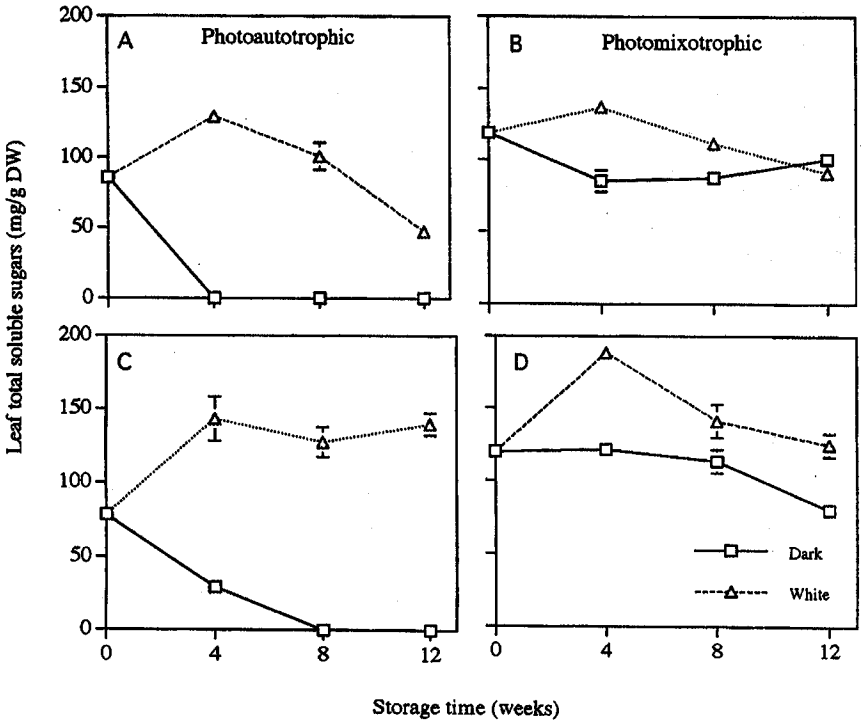
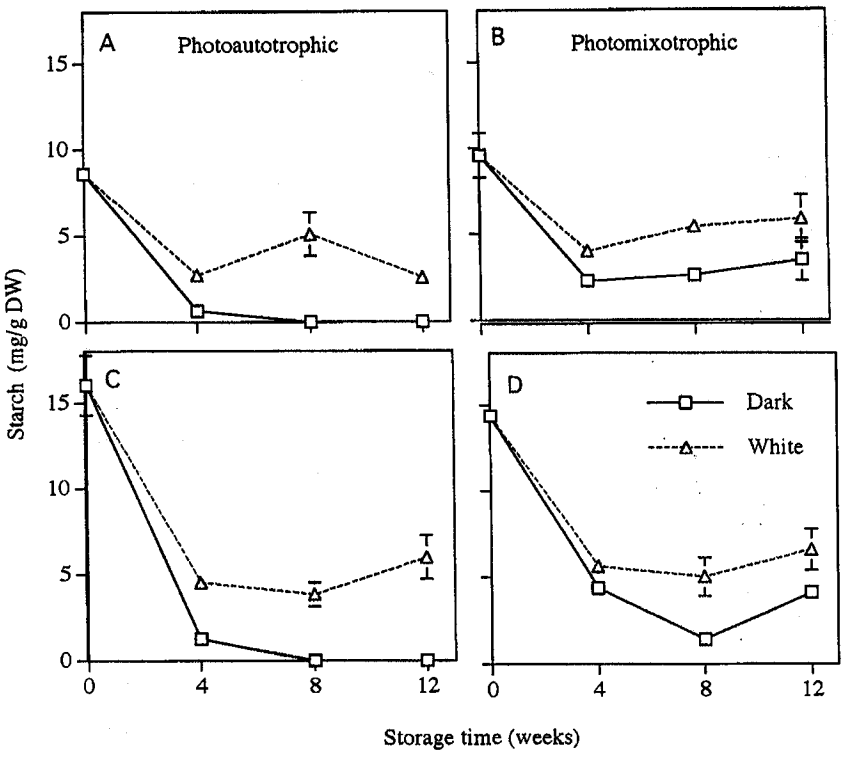
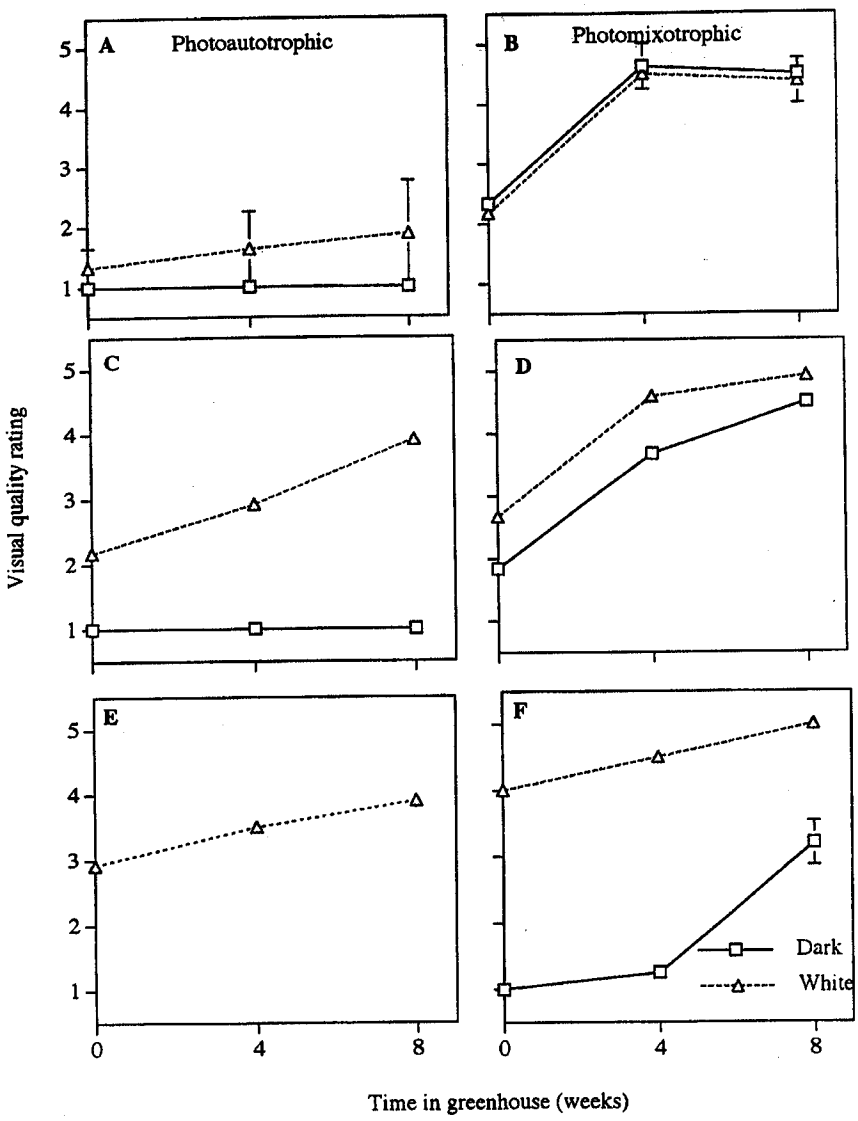
Figures



1. Effects of light and temperature on leaf total soluble sugar concentrations of photoautotrophic and photomixotrophic hosta plantlets after 0, 4, 8, and 12 weeks storage at 5 °C (A,B) and 10 °C (C,D). Means ± SE are shown.



2. Effects of light and temperature on starch concentrations of photoautotrophic and photomixotrophic hosta plantlets after 0, 4, 8, and 12 weeks storage at 5 °C (A,B) and 10 °C (C,D). Means ± SE are shown.



3. Visual quality (color and form) of hosta plantlets in greenhouse after photoautotrophic or photomixotrophic storage for 12 weeks at 5 °C (A,B), 10 °C (C,D), and 22 °C (E,F). Visual quality evaluations were based on a scale from 1 to 5, whereby 1=very poor quality, severe leaf necrosis, leaf yellowing, not marketable; 2=poor quality, large areas of leaf necrosis, leaf yellowing, not marketable; 3=fair quality, marginally marketable; 4=good quality, no yellowing, marketable; and 5=excellent quality, no leaf necrosis, no yellowing, highly marketable. Means ± SE are shown.