

# Use of Low Temperature to Improve Storage of *in vitro* Broccoli Seedlings Under Various Light Qualities

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**ABSTRACT.** Broccoli (*Brassica oleracea* L. Botrytis Group 'Green Duke') seeds were germinated *in vitro* photoautotrophically (without sugar in medium) or photomixotrophically (with sugar in medium) for 3 weeks at 23°C and  $150 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  photosynthetic photon flux (PPF). Vessels were then stored at  $1 \pm 0.35^\circ\text{C}$  under  $1.6 \pm 0.20$ ,  $4.1 \pm 0.35$ , or  $8.6 \pm 0.50 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  constant PPF each of white (400-800 nm), red (600-700 nm), or blue (400-500 nm) light. Concentrations of CO<sub>2</sub> inside the vessels were monitored until equilibrium was reached. Light compensation point was reached at  $\approx 2.0 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  for photoautotrophic seedlings and at  $\approx 4.0 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  for photomixotrophic seedlings.

Therefore, in the long-term storage experiment, seedlings were

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The authors would like to thank Dr. William Bridges of the Department of Experimental Statistics at Clemson University for his help in statistical analysis of the data. This research was supported by a grant from the U.S. Department of Agriculture, National Research Initiative Competitive Grants Program (Project No. 9500909).

stored for 4, 8, or 12 weeks at  $1 \pm 0.35^\circ\text{C}$  in darkness or under  $3 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  constant PPF (average light compensation point) of white, red, or blue light. Variable to maximal chlorophyll fluorescence (Fv/Fm) of leaves decreased as storage time increased, regardless of media composition. Illumination during storage was necessary to maintain dry weight and regrowth potentials of *in vitro* seedlings. All photoautotrophic seedlings stored in darkness were of poor quality and died when transferred to the greenhouse. Seventy-five percent of dark-stored photomixotrophic seedlings survived storage for 12 weeks but declined in appearance, dry weight, total soluble sugars (TSS), and chlorophyll fluorescence. Red light during storage increased seedling dry weight, TSS, and regrowth potential. Supplying 2% sucrose in the culture medium increased dry weight and maintained overall seedling quality during irradiated storage. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]

**KEYWORDS.** *Brassica oleracea*, postharvest physiology, low temperature storage, *in vitro* culture, spectral quality

## INTRODUCTION

Micropropagated vegetable plant sales are increasing due to short production times, uniformity, and phenotypic improvement. Plant tissue culture is currently used in many fields of plant biotechnology such as breeding and maintenance of hybrid lines for seed production, propagation, genetic engineering, secondary metabolite production, embryogenesis, and cryopreservation (Kozai and Smith, 1995). Demand for micropropagated plants is sometimes difficult to predict and balance with labor needed for frequent subculturing. One way to ensure availability of quality plantlets is the use of effective holding or storage techniques. Storage of micropropagated plants can be used to (1) reduce production costs by extending intervals between subcultures, (2) meet seasonal and sporadic market demands, (3) facilitate long distance shipping, and (4) synchronize laboratory production with availability of greenhouse space for transplanting. Storage systems that minimize growth without sacrificing quality require the manipulation of light, temperature, and medium composition. Storage of plants at their light compensation points has proven effective in minimizing excessive elongation. Kubota et al. (1995) have shown

that low temperature in conjunction with fluorescent lighting at intensities as low as  $2 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  maintains photosynthetic ability and dry weight of broccoli seedlings stored for 6 weeks without sucrose. Light compensation points of stored plants vary with air temperature and medium sugar level (Kubota and Kozai, 1995).

Sucrose is provided routinely in the medium as a respiratory substrate during *in vitro* storage (Kubota and Kozai, 1995). Addition of sucrose to the medium increases dry weight, soluble sugars, and maintains overall seedling quality during illuminated storage at  $5^\circ\text{C}$  (Wilson et al., 1998a,b). Alternatively, sucrose can be reduced substantially or omitted from the medium to facilitate growth by promotion of autotrophy, decrease expense of materials, and reduce biological contamination (Kozai, 1991). These benefits have been recognized for many plant species grown *in vitro* under enriched  $\text{CO}_2$  conditions including red raspberry (Deng and Donnelly, 1993), eucalyptus (Kirdmanee et al., 1995) and potato (Nakayama et al., 1991).

Light quality is another important factor affecting plant growth (Economou and Read, 1987; Sergeeva et al., 1994). Warrington and Mitchell (1976) reported highest leaf carbohydrate concentration under red-biased and highest leaf protein concentration under blue-biased light treatments. Eichhorn et al. (1990) showed that phytochrome modulates the rate of photosynthetic electron transport in isolated chloroplast suspensions of duckweed. While different artificial lighting systems (i.e., metal halide, sodium vapor, and fluorescent lamps) are used for various plant research, light emitting diodes (LEDs) have been used recently for practical application in micropropagation. LEDs provide a monochromatic, photosynthetic efficient, radiation source that uses less space and generates less heat than conventional light sources (Tennessen et al., 1994).

Low temperature storage has been used widely for postharvest applications, but few reports exist on low temperature storage of *in vitro* plantlets. Effects of low temperature and light quality have been reported for photoautotrophic *in vitro* broccoli seedlings stored for 6 weeks (Kubota et al., 1996) and for photoautotrophic and photomixotrophic *in vitro* broccoli seedlings stored for 12 weeks (Wilson et al., 1998a,b). It has been shown that *in vitro* broccoli seedlings can survive storage at  $5^\circ\text{C}$  for up to 6 weeks (Kubota et al., 1996), but not past 8 weeks (Wilson et al., 1998a). Storage of plants at less than  $5^\circ\text{C}$  may improve plantlet quality and regrowth potential and further pin-

point the optimum temperature for storage. Therefore, in the current research, we investigated the light compensation points for photoautotrophic and photomixotrophic *in vitro* broccoli seedlings and the influence of light quality (provided by fluorescent tubes or LEDs at the light compensation point) on morphology and regrowth potential of *in vitro* broccoli seedlings stored at 1°C for up to 12 weeks.

## MATERIALS AND METHODS

### Plant Material and Culture Conditions

'Green Duke' broccoli seeds were surface-disinfected with 1% sodium hypochlorite for 10 min and germinated *in vitro* photoautotrophically (no sugar in the medium) or photomixotrophically (2% sucrose in the medium) for 3 weeks in Murashige and Skoog (1962) liquid media (10 ml per seedling) supplemented with vitamins (Gamborg, 1970). Four seedlings were cultured in 375 ml GA-7 vessels (Magenta, Chicago, IL). To obtain uniform growth, three seeds were placed on each Sorbarod cellulose support plug (Sorbarod, Baumgartner Papiers SA, Switzerland), and the seedlings were thinned to one per plug when the cotyledons were fully expanded. 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  $\approx 3.2$  air exchanges per hour (Kozai et al., 1986). Culture room temperature was  $23 \pm 2^\circ\text{C}$  during the 16 h photoperiod and  $19 \pm 1^\circ\text{C}$  during the 8 h dark period; PPF was  $150 \pm 20 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  on the culture shelf.

### Determination of Light Compensation Point

Three weeks after culture initiation, *in vitro* seedlings were transferred to smaller GA-7-3 vessels (Magenta, Chicago, IL) that did not have membrane filters. The vessels were capped with Magenta lids and the closure was sealed with sculpting clay and Parafilm® to prevent gas from escaping. Vessels were stored at 1°C in separate low temperature incubators (Precision 815, Precision Scientific, Chicago, IL) equipped with overhead lighting from 15-W cool-white fluorescent tubes (General Electric F15T12-CW), red LED's or 20-W blue

fluorescent tubes (General Electric F20T12-B) at 1.6, 4.1, or 8.6  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  PPF. Light intensities within the incubator were adjusted by application of mesh screening or positioning of vessels. Light intensity was measured on the culture shelf in an empty vessel with lid. Spectral distributions of the three light sources in storage have been reported (Wilson et al., 1998a). A 0.5 ml gas sample was taken from the head space of each vessel at 12 h intervals during storage, and CO<sub>2</sub> concentration inside the vessels was measured using a gas chromatograph with a thermal conductivity detector (Model 8A, Shimadzu Co., Kyoto, Japan). Gas sampling was terminated after 3 d when CO<sub>2</sub> concentrations inside the vessels reached a steady state.

### Storage Treatments

In a separate experiment, vessels containing *in vitro* seedlings (approximately 4 cm tall) were stored for 4, 8, or 12 weeks at  $1 \pm .35^\circ\text{C}$  in darkness or under  $3 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  of white, red or blue light (average light compensation point selected from previous experiment). At the start of storage, the air diffusive filters of the vessels were covered with plastic tape to minimize the number of air exchanges and to avoid excessive water loss. Concentrations of CO<sub>2</sub> inside and outside of three representative vessels per treatment were measured weekly during storage by gas chromatography.

### Chlorophyll Fluorescence

At the beginning and after 4, 8, and 12 weeks of storage, chlorophyll fluorescence of seedlings was nondestructively measured using an OS-500 modulated fluorometer equipped with a trifurcated fiberoptic system (Opti-Sciences, Haverhill, MA) to evaluate photosynthetic efficiency of seedlings. Variable to maximal fluorescence measurements (Fv/Fm) were performed using an excitation light intensity of  $75 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  and a modulation intensity of  $40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  on leaves that were dark adapted for 15 min.

### Carbohydrate Analysis

Following chlorophyll fluorescence measurements, leaves and stems were frozen in liquid nitrogen and freeze-dried for dry weight

measurements. To obtain sufficient tissue for carbohydrate analysis, two seedlings were pooled to generate a sample. Leaves and stems were ground separately, and soluble sugars from 50 mg of leaf tissue and 25 mg of stem tissue were 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.

### Experimental Design and Statistical Analysis

Vessels (photoautotrophic and photomixotrophic) were arranged in a completely randomized design under each light treatment. Five photoautotrophic and five photomixotrophic vessels were harvested from each light treatment after 0, 4, 8, or 12 weeks of storage. Each vessel with four seedlings was considered a replication. Three vessels from each treatment were used for laboratory measurements. Data were subjected to analysis of variance (ANOVA) and treatment differences were separated by LSD at  $P = 0.05$ .

### Acclimatization to Greenhouse

Upon removal from storage at 4, 8, or 12 weeks (in March, April, and May, respectively) seedlings were transferred to soilless media (Metro Mix 360, The Scotts Co., Marysville, OH) in 804 grow packs and placed on a greenhouse bench (Clemson, SC) under mist for 4 weeks. Seedlings were then transferred to 1 gallon pots. After an additional week under mist, seedlings were grown in a greenhouse until flowering. At the edible flower stage, leaf and stem dry weights were determined and plant survival rates were surveyed.

## RESULTS

### Light Compensation Point

At 1°C, light compensation point was reached at about 2 and 4  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  in photoautotrophic and photomixotrophic seedlings,

respectively (data not presented). Therefore, 3  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  PPF (average) was chosen for long-term storage at 1°C because both photoautotrophic and photomixotrophic cultures were stored in the same incubator.

### Dry Weight

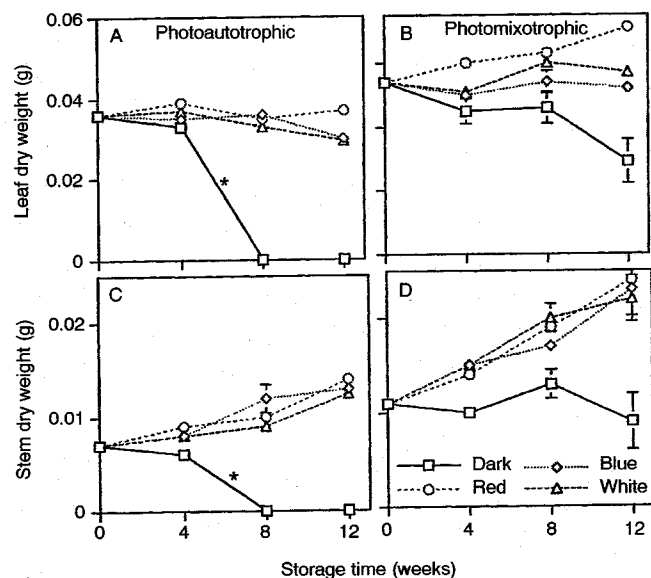
Light quality, storage time, and media composition affected seedling dry weight (Table 1). Photoautotrophic seedlings stored in darkness declined dramatically in appearance (color and form) after 4 weeks of storage and were considered dead after 8 weeks (Figure 1 A). Leaf dry weight of photomixotrophic seedlings gradually decreased during dark storage (Figure 1 B). During 12 weeks of storage, red light maintained leaf dry weight in photoautotrophic seedlings and increased that of photomixotrophic seedlings, whereas white or blue light slightly decreased leaf dry weight of photoautotrophic seedlings and maintained that of photomixotrophic seedlings (Figure 1 A and B). Stem dry weight of photomixotrophic seedlings remained relatively unchanged during dark storage (Figure 1 D). Light during storage increased stem dry weight of both photoautotrophic and photomixotrophic seedlings; however, the increase was greater in the latter (Figure 1 C and D).

TABLE 1. Abbreviated analysis of variance for the main effects and interactions of sucrose, light quality, and time on the growth of broccoli seedlings during *in vitro* storage at 1°C.

Treatment	Significance of F value				
	Fv/Fm	Leaf dry weight	Stem dry weight	Leaf TSS <sup>2</sup>	Stem TSS
Sucrose (S)	**	**	**	**	**
Light quality (L)	**	**	**	**	**
Week (T)	**	**	**	*	NS
S*L	**	**	*	**	**
S*T	**	**	**	**	NS
L*T	**	**	**	NS	NS
S*L*T	**	**	NS	NS	NS

NS, \*, \*\* Indicates nonsignificance or significance at alpha = 0.05 and 0.01, respectively.  
<sup>2</sup>TSS = total soluble sugars

FIGURE 1. Leaf and stem dry weight of broccoli seedlings grown photoautotrophically (no sucrose) or photomixotrophically (with sucrose) during storage in dark or light at 1°C. Means  $\pm$  SE are shown. \* indicates seedlings were dying.

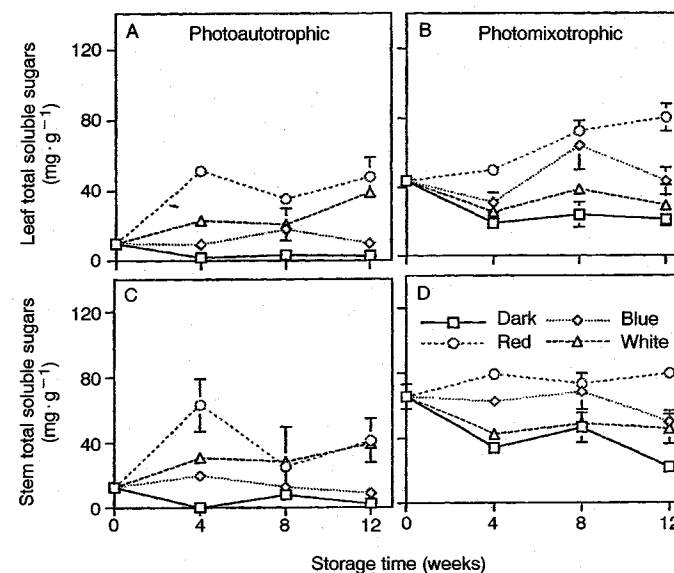


### Soluble Sugars

Before storage, photomixotrophic seedlings had approximately four and five times more leaf and stem total soluble sugars (TSS), respectively, than did photoautotrophic seedlings (Figure 2 A, B, C, and D). Both leaf and stem TSS decreased in darkness. In photoautotrophic seedlings, leaf and stem TSS decreased rapidly to almost undetectable levels during 4 weeks of dark storage while leaf TSS of photomixotrophic seedlings decreased to  $\approx 55\%$  of the original level and then remained relatively constant.

Light in storage generally increased both leaf and stem TSS in photoautotrophic seedlings, except for blue light where TSS remained relatively constant (Figure 2 A and C). However, in photomixotrophic seedlings stored under red or blue light, leaf TSS increased between 4 and 8 weeks and either continued to increase (red light) or decreased (blue light) close to the original level (Figure 2 B). Stem TSS of

FIGURE 2. Effects of light of leaf and stem total soluble sugar concentration in photoautotrophic (no sucrose) and photomixotrophic (with sucrose) broccoli seedlings stored at 1°C. Means  $\pm$  SE are shown.



photomixotrophic seedlings slightly increased under red light or slightly decreased under white or blue light (Figure 2 D). After 4 weeks, leaf and stem TSS were highest under red light, regardless of media composition. The changes in individual sugars during storage were significantly affected by light and, after 8 weeks of storage, decreased sucrose levels in photoautotrophic and photomixotrophic seedlings generally corresponded to increased hexose levels (glucose and fructose), particularly for seedlings stored under red light (data not presented).

### Photosynthetic Ability

Prior to storage, media composition did not significantly affect chlorophyll fluorescence of seedlings (Figure 3 A and B). During dark storage, chlorophyll fluorescence decreased considerably after 4 weeks in photoautotrophic seedlings and after 8 weeks in photomixotrophic seedlings. During storage in light, the net photosynthetic rate (data not presented) and Fv/Fm of photoautotrophic and photomixotrophic

seedlings decreased gradually over time, regardless of light quality (Figure 3 A and B).

The levels of  $\text{CO}_2$  in dark-stored photoautotrophic vessels were higher than ambient levels outside the vessel ( $\approx 650 \mu\text{mol} \cdot \text{mol}^{-1}$ ) only during the first 4 weeks of storage (Figure 4 A). The  $\text{CO}_2$  levels in vessels stored in light were lower than ambient, regardless of light quality, and lowest under red light. Photomixotrophic cultures stored in darkness accumulated more  $\text{CO}_2$  than did photoautotrophic cultures

FIGURE 3. Effects of light on the ratio of variable to maximum chlorophyll fluorescence ( $F_v/F_m$ ) in photoautotrophic (no sucrose) and photomixotrophic (with sucrose) broccoli seedlings stored at  $1^\circ\text{C}$ . Means  $\pm$  SE are shown.

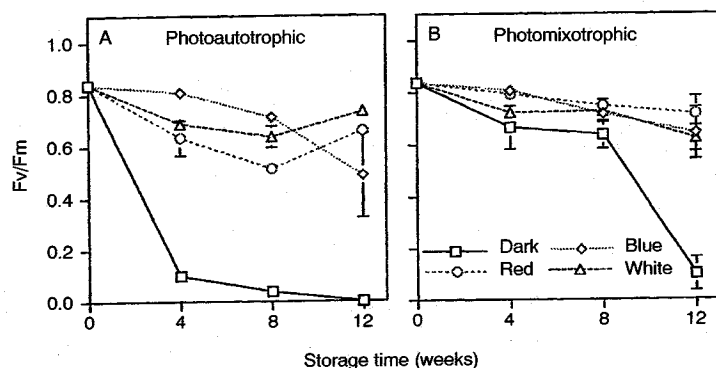
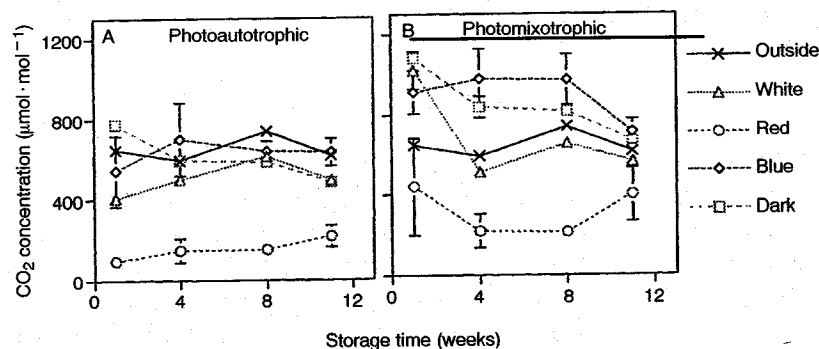


FIGURE 4. Effects of light on  $\text{CO}_2$  concentrations inside and outside vessels during storage of photoautotrophic (no sucrose) and photomixotrophic (with sucrose) broccoli seedlings stored at  $1^\circ\text{C}$ . Means  $\pm$  SE are shown.



(Figure 4 B). Unlike photoautotrophic seedlings, photomixotrophic seedlings stored in blue light or darkness continued to produce  $\text{CO}_2$  throughout 12 weeks of storage. However,  $\text{CO}_2$  accumulation decreased after 8 weeks. Under white light,  $\text{CO}_2$  levels in photomixotrophic cultures were appreciably higher than ambient after 1 week storage but decreased to levels equivalent to ambient by week 4.  $\text{CO}_2$  levels were lowest in photomixotrophic cultures stored under red light.

### Acclimatization to Greenhouse

Photoautotrophic seedlings did not survive acclimatization to the greenhouse when stored for up to 4 weeks in the dark (Figure 5 A). Dark-stored photomixotrophic seedlings survived acclimatization to greenhouse conditions, although survivability decreased by 25% after 12 weeks storage (Figure 5 B) and stem dry weight decreased more than that of seedlings stored under light (Figure 5 F, Table 2).

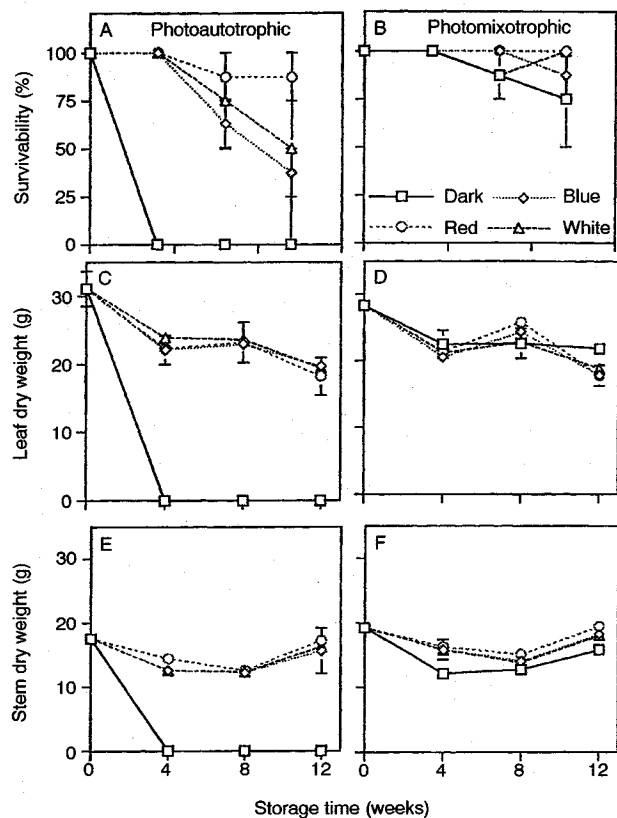
For photoautotrophic seedlings, increased duration of storage in the light correlated with decreased survivability and leaf dry weight after plants were transferred to the greenhouse, regardless of light quality (Figure 5 A and C). During acclimatization to the greenhouse after 12 weeks storage, 50% of seedlings stored under white or blue light survived, whereas  $\approx 90\%$  of seedlings stored under red light survived (Figure 5 A, Table 2). For photomixotrophic plants in the greenhouse, leaf dry weight decreased but survival rates remained high (Figure 5 B and D).

## DISCUSSION

### Influence of PPF

At  $1^\circ\text{C}$ ,  $3 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  PPF is sufficient to maintain seedlings of broccoli close to their light compensation point. Storage at (or near) light compensation point is particularly desirable for maintaining plants in a relatively "no growth" status without excessive shoot elongation and dry weight increase caused by storing plants under PPF higher than the light compensation point (Kubota et al., 1995). The overall poor quality of photoautotrophic seedlings was attributed to reduced dry weight, TSS, and photosynthetic efficiency after 4 weeks

FIGURE 5. Survivability (A, B), leaf dry weight (C, D), and stem dry weight (E, F) of broccoli plants after 90 d in greenhouse as affected by light treatments and media composition during *in vitro* storage at 1°C. Means  $\pm$  SE are shown.



of dark storage. Environmental stresses that reduce the quantum efficiency of photosystem II lead to a characteristic decrease in  $F_v/F_m$  (Krause and Weis, 1991). Supplying sucrose in the medium extended dark storage potential to 12 weeks; however, seedling quality suffered as dry weight, TSS, and  $F_v/F_m$  decreased, particularly after 8 weeks of dark storage. Consequently, only 75% of the dark-stored plants survived in the greenhouse upon removal from storage. However, this is significantly greater than 0% survivability of plants stored at 5°C in darkness (Wilson et al., 1998a).

CO<sub>2</sub> concentrations were higher than ambient only during the first

TABLE 2. Abbreviated analysis of variance for the main effects and interactions of sucrose, light quality, and time on the post-storage growth of broccoli seedlings in the greenhouse.

Treatment	Significance of F value		
	Leaf dry weight	Stem dry weight	Survivability
Sucrose (S)	**	**	**
Light quality (L)	**	**	**
Weak (W)	**	NS	*
S*L	**	**	**
S*W	NS	NS	NS
L*W	NS	NS	NS
S*L*W	NS	NS	NS

NS, \*, \*\* Indicates nonsignificance or significance at alpha = 0.05 and 0.01, respectively.

week in dark-stored photoautotrophic cultures. Rapid decline of plants and severely decreased TSS concentrations during this time indicated that sufficient substrate was not available for respiration. Kubota et al. (1997) reported that 6 weeks of dark storage reduced TSS in photoautotrophic seedlings by 60-80%. In dark stored photomixotrophic cultures, the higher than ambient CO<sub>2</sub> concentrations indicate that seedlings had higher respiration rates than photoautotrophic seedlings. The respiration rate declined during storage, probably due to loss of respiratory substrate. The availability of sugars appears to have played a key role in maintaining plantlet viability in dark storage.

### Influence of Light Quality

When light was supplied to photoautotrophic seedlings during storage, CO<sub>2</sub> concentrations inside the vessels were lower than ambient. This indicates that photosynthesis was occurring during storage, regardless of light quality. However, red light was more effective than blue or white light in maintaining photosynthesis. Light quality also influenced photosynthesis in photomixotrophic seedlings. However, vessels containing photomixotrophic seedlings under blue light had higher CO<sub>2</sub> levels than ambient suggesting that respiration was greater than photosynthesis during storage. Vessels stored under white light had higher than ambient CO<sub>2</sub> concentrations during early storage but decreased closely below ambient levels thereafter, suggesting that

seedlings were at their compensation point. CO<sub>2</sub> concentrations of seedlings stored under red light remained well below ambient levels regardless of storage time. This is in agreement with previous results of low temperature storage of broccoli seedlings at 5°C, where CO<sub>2</sub> concentrations were lowest in vessels stored under red light (Wilson et al., 1998b).

During storage, the quality of photomixotrophic broccoli seedlings was best maintained under red light, which increased TSS and dry matter accumulation into leaves in comparison with other light treatments. This is consistent with previous research where red light storage at 5°C for 12 weeks produced high quality seedlings having increased photosynthesis and carbohydrates (Wilson et al., 1998b). Positive effects of red light on plant physiology have been well documented. Wareing and Thompson (1976) showed that red light increases cytokinin and gibberellin levels in plants. Research indicates that the morphogenic effects of red and blue light actually may be mediated by changes in the phytohormone balance (Sergeeva et al., 1994). Tennesen et al. (1994) compared red LED's to white light and showed that at low light intensities, photosynthesis and stomatal conductance were greater in red light than in white light. Likewise, research by Warrington and Mitchell (1976) showed highest leaf carbohydrate concentration under red-biased and highest leaf protein concentration under blue-biased light treatments. Reduced stem TSS concentration of photoautotrophic seedlings under blue light agree with findings of Szasz and Barsi (1971) where blue light inhibited sugar and starch synthesis.

Illuminated seedlings responded differently to light quality in storage, but these developmental responses did not significantly affect the leaf and stem dry weight of the surviving plants once they reached maturity in the greenhouse. Similarly, Decoteau and Friend (1991) reported that end-of-day red or far-red light treatments affected tomato transplant growth but not subsequent fruit production when plants were transferred to the field. However, light quality did affect the post-storage survivability of plants in the greenhouse. The high survival rate of red light-stored seedlings after greenhouse acclimatization correlates with high TSS and photosynthesis during storage.

### ***Influence of Storage Duration***

Visual quality of seedlings diminished as storage duration increased. This correlated with decreased photochemical yield of photo-

system II measured as chlorophyll fluorescence and decreased quantum yield of CO<sub>2</sub> assimilation measured as net photosynthesis. Chilling injuries to the photosynthetic apparatus lead to a significant decrease in the variable fluorescence level (Karukstis, 1991). Regardless of light quality, seedlings stored photomixotrophically had higher survival rates in the greenhouse than did seedlings stored photoautotrophically. In a previous experiment, seedlings survived *in vivo* acclimatization only when stored for 4 weeks or less at 5°C (Wilson et al., 1998a). Better survival rates at a lower storage temperature is attributed to decreased metabolism (Reid, 1991) and sufficient carbohydrate reserves. Carbohydrate reserves at harvest influence the quality and post-storage growth potential of plants (Kunneman and Albers, 1992; Paton and Schwabe, 1987; Wilson et al., 1998b). Reducing the storage temperature to 1°C increased seedling quality; however, caution must be taken to avoid deleterious effects of freezing injury that may be incurred on plants if the temperature falls below the freezing point (Reid, 1991).

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