LOW TEMPERATURE STORAGE OF TRANSPLANTS UNDER DIM LIGHT

Chieri KUBOTA
Department of Bioproduction Science
Faculty of Horticulture
Chiba University
Matsudo, Chiba 271, Japan
E-mail: ckubota@midori.h.chiba-u.ac.jp

1. Introduction

Storage methods to suppress growth of transplants and maintain their quality are required for successful commercial transplant production, since peak demand for transplants of flower or vegetable species falls during limited periods in spring and fall, due to the seasonal nature of the horticulture industry. Plug seedlings, probably due to their high planting density and limited rhizosphere, easily elongated or overgrown during the holding period before the market or greenhouse space is available. Transplant propagators or growers are thus often faced on the difficulties to have their transplants accepted in the market or in greenhouse/field when the transplants are ready for transplanting. One alternative to avoid shortages or overproduction of transplants is to produce them a few weeks early and store them until markets or greenhouse space are available. Traditionally, plant growth regulators are used extensively in the industry to reduce stem elongation and maintain visual quality of transplants and bedding plants during postproduction stages. However, restrictions on the use of plant growth regulators on horticultural crops have tremendously increased the interest in the use of nonchemical methods. Low temperature has been widely used for extending the life of harvested horticultural products, but it is not so commonly used as a means for restricting growth of transplants. In recent years however, low-temperature storage has been used as an alternative method to suppress the growth and to maintain the quality of transplants. This article will discuss the effects of dim light as an environmental factor affecting growth and quality of transplants during storage. Transplants in this article include seedlings, rooted or unrooted cutting, and in vitro micropropagated plantlets.

2. Quality degradation during low temperature storage of transplants

A successful storage must suppress the growth and development of the transplants during storage period, maintain their photosynthetic and regrowth potential while at the same time maintaining visual quality (morphology). Table 1 lists growth and quality characteristics of transplants which we have to maintain unchanged or in an acceptable range during storage.

Table 1. Growth and Quality characteristics of transplant (Kozai and Okawa, 1995).

1) Dry weight of shoots and roots
2) Fresh weight of shoots and roots
3) Leaf area, leaf thickness, and etiolated leaf area percentage
4) Stem length, internode length, and petiole length
5) Chlorophyll a and b concentrations and their ratio
6) Carbohydrate, protein, and lipid concentrations
7) Ethylene production rate
8) Microorganism concentration and its growth rate

The aim of lowering temperature in storage is to suppress the growth by reducing the respiration rate or the whole metabolic process of transplants. In dark storage however, dry weight decreases as increasing duration of storage period according to the respiration rates at the given temperature. Unfavorable dark storage environment induces loss of chlorophyll (Conover, 1967), leaf abscission (Curtis and Rodney, 1952), and susceptibility to pathogens (van Doesburg, 1962). Those changes are probably due to reduction of dry weight. To maintain dry weight unchanged, combination of low temperature to reduce respiration rate and dim light to compensate the carbon loss by the low respiration rate would be effective. Heins et al. (1992) showed that light at 1 μmol m^{-2} s^{-1} PPFD during low temperature storage averted undesirable shoot elongation and decreased the percent mortality of the plug transplants. Rajapakse et al. (1996) showed that rooted cuttings of chrysanthemum stored under light were darker green and had a higher chlorophyll concentration. They confirmed that provision of light was beneficial in delaying the development of leaf necrosis and maintaining quality of cultivars with short storage life at low temperatures. Illumination at such a low PPFD during storage would contribute to 1) suppressing the reduction of dry weight by photosynthesis, 2) preserving active chlorophyll in the leaves, and 3) suppressing elongation of stem or petiole.

In recent studies of our group, we focused on light compensation points, PPFD where plants have null CO_2 exchange rates (gross photosynthetic rates are equal to respiration rates), and examined the effects of storage under PPFD near light compensation points on the growth and quality of transplants.

3. Growth suppression and quality preservation of eggplant plug seedlings by low temperature storage under dim light

Storage of plug seedlings under light compensation points were examined by Kozai et al. (1996). Eggplant (*Solanum melongena* L.) plug seedlings were grown at 30C/20C with photo-/dark period temperature photosynthetic photon flux density (PPFD) of 350 μmol m^{-2} s^{-1}. On 20th day after germination, the seedlings were stored for 3 weeks at 9C air temperature and PPFD of 0 (darkness), 2, 8, or 16 μmol m^{-2} s^{-1}. The results showed that the seedlings stored for 3 weeks in darkness or under 2 μmol m^{-2} s^{-1} PPFD reduced dry weight, while those under 8 or 16 μmol m^{-2} s^{-1} PPFD increased dry weight (Fig. 1).

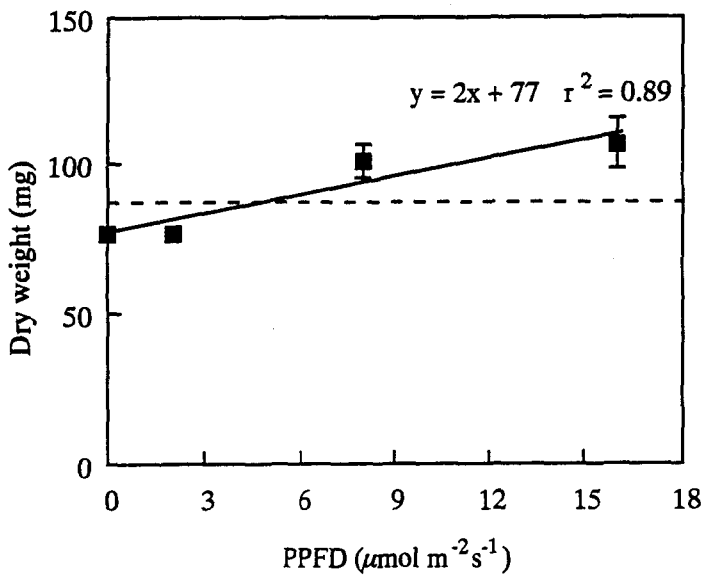

Fig. 1 Dry weight per seedling after 3 weeks in storage as affected by PPFD during storage. Horizontal dotted line represents the pre-storage level. Vertical bars represent ±S.D. of the means (Kozai et al., 1996).

Table 2 Percent survival, fresh and dry weights, leaf area, and number of leaves per seedling 5 days after transplanting as affected by PPFD during storage (Kozai et al., 1996).

PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	Survival (%)	FW (g)	DW (mg)	Leaf area (cm^2)	Leaf no. (-)
0 (Darkness)	44	1.1	101	16	2
2	100	3.2	300	73	4
8	100	2.6	241	59	4
16	100	3.0	283	73	4
ANOVA	-	*	*	*	*

*Significant at $P \leq 0.05$.

The light compensation point of seedling measured before storage was 8 $\mu mol\ m^{-2}\ s^{-1}$ PPFD and it decreased to 4 or 5 $\mu mol\ m^{-2}\ s^{-1}$ PPFD after storage regardless of PPFD during storage. Table 2 shows the percent survival, fresh and dry weights, leaf area, and number of leaves per seedling 5 days after transplanting. All the plantlets stored under light survived at transplanting and grew successfully during the post-storage culture period. Dark storage lowered the photosynthetic capacity and, thus, reduced percent survival and post-storage growth rate. For keeping seedlings at no-growth status and preventing quality degradation, it would be required to store the seedlings under conditions where the seedlings have null CO_2 exchange rates throughout the storage period.

4. Low temperature storage of transplant at the light compensation point: air temperature and light intensity for growth suppression and quality preservation

A method for storing transplants in vitro was developed using light compensation points in conjunction with low temperatures (Kubota and Kozai, 1995). Broccoli (*Brassica oleracea* L., cv 'Ryokurei') plantlets, aseptically germinated and cultured for 3 weeks in vitro, were used as model transplants. Culture conditions were: 23C air temperature, 160 $\mu mol\ m^{-2}\ s^{-1}$PPFD, and 3.6 air exchanges per hour of the vessel. Prior to storage, CO_2 exchange rates of the

plantlets were measured at 3, 5, 10, 15, and 25C air temperatures under 0 (darkness), 2, and 4 μmol m^{-2} s^{-1}PPFD to determine light compensation points of the plantlets cultured in the medium with or without 20 g l^{-1} sucrose. Plantlets were stored for 6 weeks at 5, 10, and 15C under either 0 or 2 μmol m^{-2} s^{-1} continuous PPFD, which was near their light compensation points at these temperatures.

Results from the measurements of CO_2 exchange rates showed that the light compensation point varied with air temperature and with medium sugar level (Fig. 2). When the plantlets were cultured without sugar in the medium, light compensation points were ≃2 μmol m^{-2} s^{-1} PPFD at 3, 5, and 10C, and ≃4 μmol m^{-2} s^{-1} PPFD at 15C. When the plantlets were cultured with sugar in the medium, light compensation points were ≃2 μmol m^{-2} s^{-1} PPFD at 3 and 5C, and ≃4 μmol m^{-2} s^{-1} PPFD at 10 and 15C. During 6-week storage, dry weight of the plantlets cultured without sugar in the medium was maintained unchanged under conditions where CO_2 exchange rates were kept at almost zero (5 and 10C under 2μmol m^{-2} s^{-1} PPFD). However, dry weight of the plantlets cultured with sugar in the medium was maintained almost unchanged under all the conditions except for darkness at 15C (Fig. 3). High transplant qualities were successfully preserved at light compensation points: 2 μmol m^{-2} s^{-1} PPFD at 5 to 10C without sugar, and 5C with sugar in the medium.

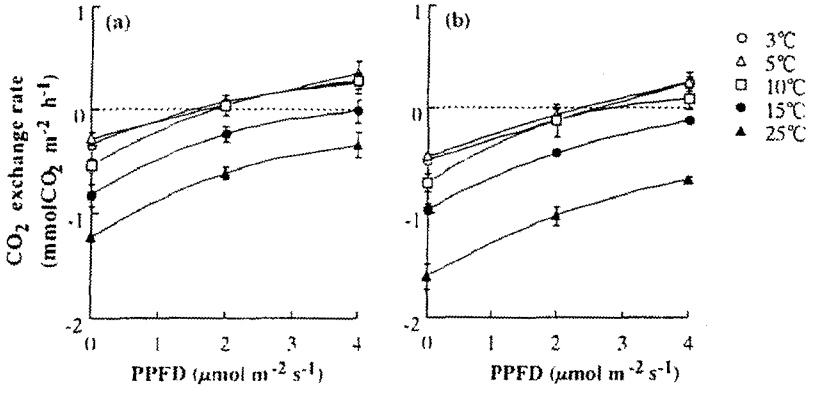

Fig. 2 Carbon dioxide exchange rates per leaf area of broccoli plantlets cultured (a) photoautotrophically (without sugar in the medium) and (b) photomixotrophically (with sugar in the medium) as affected by air temperature and PPFD (Kubota and Kozai, 1995). Vertical lines represent standard deviations.

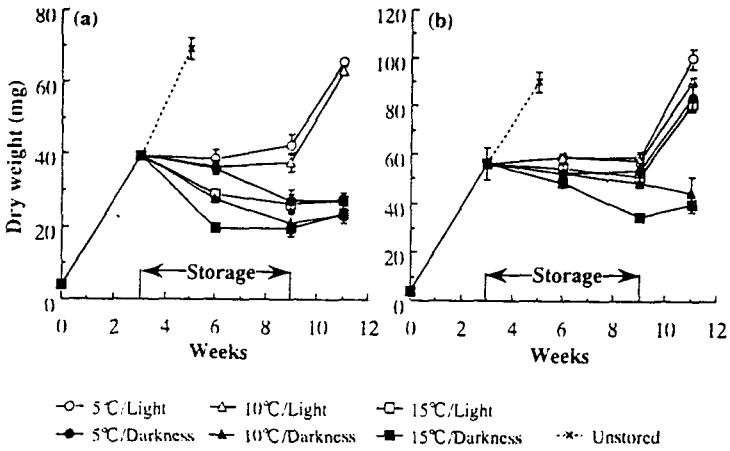

Fig. 3 Changes in dry weight of broccoli plantlets cultured (a) photoautotrophically (without sugar in the medium) and (b) photomixotrophically (with sugar in the medium) as affected by air temperature and PPFD during storage (Kubota and Kozai, 1995). Plantlets were cultured for 3 weeks and stored for 6 weeks at 5, 10, or 15C under 0 (darkness) or 2 μmol m^{-2} s^{-1} PPFD. After storage, plantlets were cultured subsequently under the same culture conditions before storage. Vertical lines represent standard deviations. Dashed lines indicate the dry weight of the unstored plantlets.

Effects of PPFD higher than light compensation points were examined for stored broccoli plantlets in vitro (Kubota et al., 1995). The plantlets cultured photoautotrophically at 23C air temperature and 160 μmol m^{-2} s^{-1} PPFD were stored at 5, 10, or 15C under continuous illumination at 2 or 5 μmol m^{-2} s^{-1} PPFD. Dry weight, photosynthetic and regrowth potential of plantlets were best preserved at 5C under 2 μmol m^{-2} s^{-1} PPFD. At 10 and 15C, photosynthetic and regrowth potential of plantlets after storage were higher under 5 than 2 μmol m^{-2} s^{-1} PPFD, and decreased as air temperature or duration of storage increased. Under 5 μmol m^{-2} s^{-1} PPFD, plantlets elongated at 10C and 15C during storage (Table 3). Dry weight of plantlets at 10C under 5 μmol m^{-2} s^{-1} PPFD increased during storage. The CO_2 exchange rates of the plantlets were positive (higher photosynthetic rate than respiration rate) and were close to zero at 5C throughout the 6-week storage

period and at 15C under 5 μmol m^{-2} s^{-1} PPFD for the 3-week storage period (Table 4).

Table 3. Dry weight, quality score, height, and chlorophyll fluorescence value f_{max}/f_0 at 730 nm of the plantlets before and after 6-week storage (From Kubota et al., 1995). Means±S.D. shown.

Treatment Temp	PPFD	Dry weight (mg)	Quality score	Height (mm)	f_{max}/f_0
Before storage					
		60±2.8	5.0	36±0.3	3.7±0.09
After storage					
5	2	62±3.8^{NS}	4.8±0.0**	45±6.9^{NS}	3.5±0.09^{NS}
10	2	49±0.5**	2.1±0.2**	43±5.1^{NS}	2.7±0.19**
10	5	70±3.8*	4.5±0.0**	50±1.5**	3.2±0.56^{NS}
15	2	32±1.7**	1.2±0.2**	-^{Z}	-^{Z}
15	5	54±2.2^{NS}	2.2±0.3**	56±2.8**	3.0±0.29*

^{Z}Not measured.

$^{NS, *, **}$Nonsignificantly or significantly different from those before storage by t-test at $P < 0.05$ or 0.01, respectively.

Table 4. CO_2 exchange rates per plantlet (nmol CO_2 h^{-1}) during storage (From Kubota et al., 1995). Means ± standard deviations.

Treatment Temp	PPFD	Weeks in storage 0^{Z}	1	3	6
5	2	4600±120	64±33	58±30	33±30
10	2		-54±44	-87±30	-53±31
10	5		101± 5	92±10	96± 1
15	2		-77±55	-197±67	-40±18
15	5		32± 6	8±52	-39±21

^{Z}Net photosynthetic rate per plantlet before storage under culture conditions.

This means that the light compensation point of the plantlets examined was ≃2 μmol m^{-2} s^{-1} PPFD at 5C and was ≃5 μmol m^{-2} s^{-1} PPFD at 15C. Higher PPFD than light compensation points maintained the photosynthetic potential of the plantlet; however, caused undesirable shoot elongation, dry weight increase, and consequently, the quality degradation of the plantlets. It should be emphasized that the small PPFD difference caused considerable differences in plantlet growth and quality during storage. These results suggest that proper combinations of air temperature and PPFD during storage would contribute to minimizing increase/decrease in dry weight and preserving photosynthetic and regrowth potential of plantlets.

5. Low temperature storage of micropropagated plantlets under selected light environments

Cool white fluorescent lamps were used during most published studies of storage. Light quality has been observed to influence plant growth and morphology of plants ex vitro (Smith, 1982) and in vitro (Economou and Read, 1987) at ambient temperatures. Quality of light in low-temperature storage was examined by Kubota et al. (1996). Broccoli (cv. Green Duke) and *Hosta tokudama* F. Maekawa 'Newberry Gold' plantlets, which were ready for transplanting after photoautotrophic (sugar-free) culture, were stored for 4 to 6 weeks at 5C under various light qualities and PPFD.

The effects of light quality on broccoli plantlet characteristics before and after 3 or 6 weeks of storage at 5C, 2 μmol m^{-2} s^{-1}PPFD were shown in Table 5. Illumination during storage maintained quality, and regrowth potential of plantlets stored at low temperature. PPFD affected quality of broccoli and *Hosta* plantlets. Broccoli plantlets responded to the storage light quality, while *Hosta* did not. White light maintained the quality of broccoli plantlets better during 6 weeks of storage than did red or blue light. Red and blue light caused an increase in internode length and reduction in chlorophyll concentrations compared to white light. Photosynthetic and regrowth potentials of plantlets were not affected by spectral quality during storage. Considering changes in dry weight, stem length, and leaf yellowing, the quality of broccoli plantlets was best maintained under white light at 2 μmol m^{-2} s^{-1}PPFD. PPFD and light quality were shown to be important factors in the preservation of transplant quality and suppression of growth of the plantlets during low-temperature storage.

Table 5. Light quality effects on broccoli plantlet characteristics before and after 6 weeks of storage at 5C and 2 μmol m^{-2} s^{-1}PPFD (From Kubota et al., 1996). Means ± SE are shown.

Light	Dry wt (mg)	No. leaves	Stem length (mm)	Chlorophyll conc. (mg m^{-2})
Before storage				
	58±1.1	4.0±0.09	33±1.1	516±13
After 6 weeks of storage				
Darkness	42±3.4**	4.2±0.05^{NS}	31±1.6^{NS}	320±14**
Blue	64±1.6*	4.7±0.06*	46±2.3**	154±31**
Red	62±1.8^{NS}	4.3±0.07^{NS}	45±1.4**	254±38**
White	60±1.1^{NS}	4.5±0.10^{NS}	38±2.2^{NS}	322±40**
ANOVA[Z]	NS	NS	*	*

[Z]Significance among the blue, red, and white light treatments.

^{NS}, *, **Nonsignificantly or significantly different from those before storage at $P \leq 0.05$ or 0.01, respectively.

6. Effect of dim light during low temperature storage on the postharvest quality of radish sprouts

Provision of light can be applied in storage of leafy cuttings or leafy vegetables. Hosoda et al. (1981) examined the effects of illumination on komatsuna (*Brassica campestris* L. *var. komatsuna*) leaves and found that illumination in storage suppressed chlorophyll degradation and reduction of ascorbic acid concentration. In the experiment reported by Kobayashi et al. (1996), radish sprouts (*Raphanus sativus* L.) harvested in a commercial operation were kept for a day at 20C air temperature under 0 (darkness) or 50 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (pre-storage treatment) and stored for 16 days at 5C air temperature under 0 or 5 μmol m^{-2} s^{-1} PPFD (storage treatment). The aim of pre-storage treatment was to examine the effect of illumination during shipping conditions on the quality of spouts. For treatments under light, white light was provided continuously (24 h d^{-1} photoperiod) with cool white fluorescent lamps. The best quality was obtained under conditions where light was provided throughout the pre-storage and the storage period. Dark storage caused the shoot elongation and chlorophyll degradation (Fig. 5), although no significant

decrease was observed in dry weight of the sprouts. Light in low temperature storage was shown to contribute to keeping high postharvest quality of the radish sprouts.

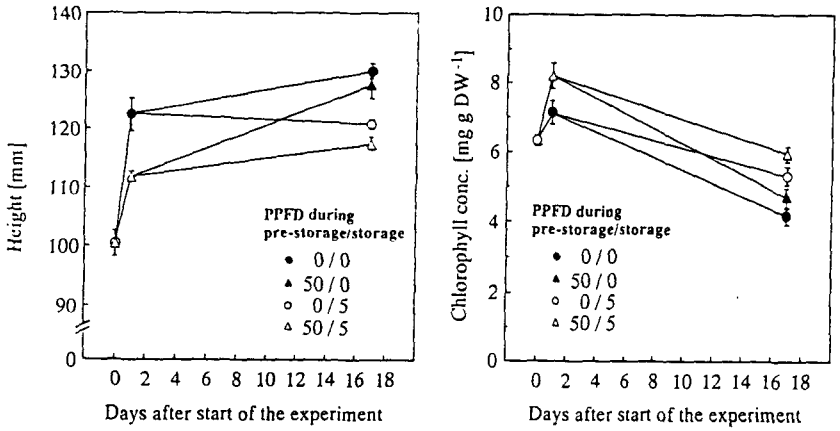

Fig. 5 Changes in plant height and chlorophyll concentration of the radish sprouts. The sprouts were kept for a day at 20C under 0 (darkness) or 50 μmol m^{-2} s^{-1} PPFD (pre-storage treatment) and stored for 16 days at 5C under 0 or 5 μmol m^{-2} s^{-1} PPFD (storage treatment) (Kobayashi et al., 1996). Means ±S.E. shown.

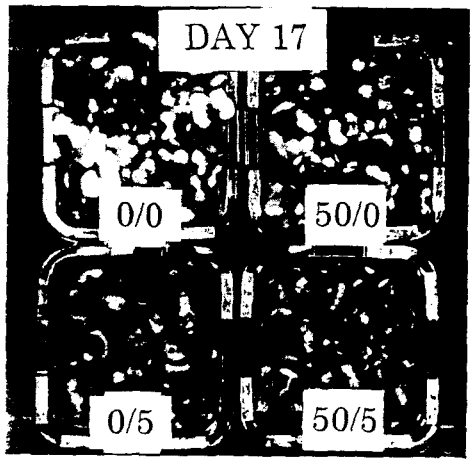

Fig. 6 Radish sprouts on day 17 (after 16 days in storage at 5C). The sprouts were kept for a day at 20C under 0 (darkness) or 50 μmol m^{-2} s^{-1} PPFD (pre-storage treatment) and stored for 16 days at 5C under 0 or 5 μmol m^{-2} s^{-1} PPFD (storage treatment) (Kobayashi et al., 1996). The PPFD in the pre-storage/storage treatment were shown.

6. Future research required for environment control in storage of tansplants

The following factors need to be controlled to extend the maximum storage period without any degradation in quality:

1) Aerial environment
   a. Temperature
   b. Light (intensity, quality, photo-/dark period, cycle, direction)
   c. Carbon dioxide concentration
   d. Humidity (relative humidity, absolute humidity, water vapor pressure deficit
   e. Air current speed
   f. Ethylene concentration
   g. Oxygen concentration
2) Medium/soil environment (nutrient component, water, physical or chemical characteristics of the medium/soil)
3) Biological environment (pathogen, microorganism, insect pest)

For each factor, more research would be needed in terms of environment control strategy, measurement, economic feasibility, etc.

Storage of transplants has not been investigated enough to give the fundamental data to understand the relationship between environment and the growth and quality of transplant in storage. Furthermore, as shown in radish sprout, data obtained from storage of transplants can be applied for postharvest technology of leafy vegetables. Postharvest technologies developed for vegetables or fruit (i.e. controlled or modified atmosphere storage) may be applicable for storage of transplants.

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