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Research Report

Effects of Light Intensity and Relative Humidity on Photosynthesis, Growth and Graft-take of Grafted Cucumber Seedlings during Healing and Acclimatization

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Abstract. Healing and acclimatization are key processes for the survival of grafted plants. This study evaluated the influence of light intensity (photosynthetic photon flux, PPF) and relative humidity during the healing and acclimatization period on the photosynthetic characteristics, graft-take, and growth of grafted cucumber (*Cucumis sativus* L.) seedlings, using a system for the continuous measurement of the CO_2 exchange rate, in order to establish optimum environmental conditions for the healing and acclimatization of grafted cucumbers seedlings. Cucumbers (*Cucumis sativus* L. cv. Joeun Baekdadaki) were grafted onto rootstocks (*Cucurbita maxima* D. × *C. moshata* D. cv. New Shintozwa). Six combinations of two levels of relative humidity (95 and 90%) and three levels (0, 142, and 237 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of light intensity were set up during healing and acclimatization. Increasing light intensity significantly increased CO_2 exchange rates during healing and acclimatization. At 95 and 90% relative humidity, the CO_2 exchange rates at 237 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity were 1.5 and 1.8 times higher than those at 142 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, respectively. The light intensity during healing and acclimatization also affected the amount and distribution of chloroplasts in scion cotyledon. The amount of chloroplasts increased with the increase of PPF during healing and acclimatization, which covered most of cell wall with little open space left, compared with that of dark condition. As PPF increased, the shoot length, ratio of shoot to root, and specific leaf area decreased but the hypocotyl diameter, leaf area, dry weight, and percent dry matter increased. On the other hand, the relative humidity ranging from 90 to 95% did not significantly affect the CO_2 exchange rates during healing, acclimatization, and growth of grafted cucumber seedlings. As a result, PPF during healing and acclimatization affected the growth and quality of grafted cucumber seedlings. This showed that higher PPF condition may improve the growth and quality of grafted cucumber seedlings.

***Additional key words*:** CO_2 exchange rate, *Cucumis sativus* L., *Cucurbita maxima* D. × *C. moshata* D., graft

Introduction

Interest in the use of grafted fruit vegetables has increased in Korea, Japan, and throughout Asia and Europe, under intensive cropping systems. The purpose of vegetable grafting is to improve resistance to soil-borne pests and pathogens, adaptation to abiotic stresses, and growth and yield by promoting the absorption of nutrients (Lee and Oda, 2003; Rivero et al., 2003). Almost all cucurbits such as cucumbers, melons, and watermelons for greenhouse cultivation are being grafted in Korea. There is also an increasing trend in the grafting of solanaceous crops such as eggplants, peppers, and tomatoes (Lee and Oda, 2003).

The successful production of grafted transplants requires highly technical grafting skills and environmental control during healing and acclimatization period. Grafted transplants are produced by 1) raising scions and rootstocks; 2) grafting; 3) healing and acclimatization; and 4) raising the grafted seedlings before transplanting. Healing and acclimatization are very important processes that are necessary for grafted plants to survive (Lee and Oda, 2003). The grafted seedlings form new vascular bundles as part of the graft union, during the healing and acclimatization period, and become acclimatized to the outer surroundings.

Generally, healing and acclimatization of the grafted plants are done in a tunnel, made of double-layered plastic film and shade cloth, in a greenhouse. Environmental management during healing and acclimatization is usually done by the

empirical knowledge of a grower depending on the season or weather. To prevent grafted plants from wilting by excessive transpiration and to promote healing, the tunnel is closed during the three- to four-day healing period. The opening and closing of the tunnel are controlled based on the condition of the grafted plants and the weather. When the tunnel is closed, the air in the tunnel is saturated (Relative humidity > 90%) and light intensity is slightly higher than the light compensation point (below photosynthetic photon flux (PPF) of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Kim and Park, 2001; Lee and Oda, 2003). When the air current speed in the tunnel is near 0 $m \cdot s^{-1}$, the net photosynthesis rate of the grafted plants is almost 0 mg $CO_2 \cdot m^{-2} \cdot s^{-1}$ (Shibuya et al., 2003). Under these environmental conditions, the grafted seedlings scarcely grow and are in danger of heat stress, infection by pathogens and overgrowth wherein roots are arising from the hypocotyls of the scion.

Some papers have reported higher survival rate, faster growth, and higher quality of grafted plants under highly controlled healing conditions (Kim et al., 2001; Nobuoka et al., 2005; Shibuya et al., 2003). These have mainly focused on the increase in the net photosynthesis rate in grafted plants during healing and acclimatization, by increasing air current speed and light intensity. It may result in an improvement of the graft-take, growth, and quality of grafted plants.

Generally, 75-85% of relative humidity accelerates the photosynthesis rate, though relative humidity may influence the photosynthesis differently in other environmental conditions. High relative humidity, of over 90%, decreases the photosynthesis rate due to reduced stomata aperture. Under lower relative humidity conditions, the photosynthesis is apt to decrease due to water stress induced by excess transpiration (Kitaya, 2005). Accordingly, lowering relative humidity levels, rather than the general healing and acclimatization conditions in which relative humidity is maintained nearly saturated, may promote photosynthesis by preventing the stomata apertures from closing.

Some data on the continuous photosynthesis rate and transpiration rate of the entire plant during healing and acclimatization period is available. Determining the timing of the connection of the vascular bundles and understanding the characteristics of the photosynthesis and transpiration rates, before and after the vascular bundle connections are made, can lead to more precise and optimal control of environmental factors such as temperature, humidity, and light intensity during healing and acclimatization period. Faster growth with higher survival rate under optimum environmental conditions may shorten or even do away with the period of acclimatization, after healing.

The objective of this study was to investigate the photosynthetic characteristics, graft-take, and growth of grafted cucumbers during healing and acclimatization and to determine the optimum environmental conditions for the healing and acclimatization. Specifically, it was conducted to examine the effects of light intensity and relative humidity on the rate of photosynthesis, the growth and graft-take of grafted cucumbers during healing and acclimatization, using a curing box in which the photosynthesis and transpiration rates were measured continuously.

Materials and Methods

Plant Material and Growing Scions and Rootstocks

Cucumbers (*Cucumis sativus* L. cv. Joeun Baekdadaki) were used as scions while pumpkins (*Cucurbita maxima* D. × *C. moshata* D. cv. New Shintozwa) were used as rootstocks for producing grafted plants. They were sown into 105-cell plug trays (W 280 mm × L 540 mm × H 48 mm, Bumnong co., LTD.) and 50-cell plug trays (W 280 mm × L 540 mm × H 50 mm, Bumnong Co., LTD., Korea), respectively, filled with commercial growing substrate (BM2, Berger Group LTD., Canada). The planting densities of scions and rootstocks were approximately 694 plants$\cdot m^{-2}$ and 330 plants$\cdot m^{-2}$, respectively. They were placed in a germination room maintained at 28℃ for 2 and 3 days, respectively. After germination, the seedlings were grown in a growth chamber with artificial light (Hanbaek Co. LTD., Korea), where temperature was set at 25/18℃ (light / dark period), light period was 14 hours$\cdot d^{-1}$, and PPF was approximately 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by high pressure sodium, metal halide, and fluorescent lamps. They were bottom-irrigated twice with water or a nutrient solution (EC 1.4 $dS \cdot m^{-1}$, 'Hanbang' for seedling, Coseal Co., LTD., Korea), respectively. The dry weight, leaf area, and stem diameter of the scion and rootstock before grafting were respectively 39.4 ± 1.1 mg, 11.67 ± 0.23 cm^2, 1.36 ± 0.04 mm, and 170.1 ± 3.3 mg, 27.05 ± 0.76 cm^2, 2.51 ± 0.06 mm.

Grafting

A week after sowing, grafting was done by splice grafting method when the cotyledons of the scions and rootstocks were completely unfolded. One cotyledon and the growing point of the rootstock were removed for grafting. The scion was cut 5 mm below cotyledon. After placing the scion on the rootstock, ordinary grafting clips were used to fix the grafted position tightly together (Lee and Oda, 2003).

Description of a Device for the Healing and Acclimatization and Measurement of CO_2 Exchange Rate of Grafted Cucumbers

A semi-open multi-chamber system was used to measure CO_2 exchange rate (van Iersel and Bugbee, 2000). A light-transmitting box (inside dimension of W 350 mm × L 780

mm × H 220 mm, volume of 60 L) made of 10 mm-thick acryl plastic was used for the healing and acclimatization and measurement of CO_2 exchange rate of the grafted cucumbers (Fig. 1). Four healing boxes were placed in the growth chamber (Hanbaek Co. LTD., Korea) where the temperature was set at 22℃. The healing box had air-inlets and outlets, air pumps, and air drawing tube. It was also equipped with a heater (hair dryer heater, Kaiser KHD-5207i, My Friend Co., LTD, Korea) and a humidifier (nebulizer, CT-24, Techsin Electronic Co. Ltd., China) to maintain temperature and relative humidity inside the box.

Atmospheric air was drawn in through the inlet of the healing box at an airflow rate of 13.5 L·min^{-1} using the air pumps (LP80VC, Youngnam Air Pump Inc., Korea) and flow meters (15 L·min^{-1}, Kofloc, Japan), and flowed out through the outlet (Shibuya et al., 2006). The CO_2 concentrations of the air at the inlet and the outlet were measured using the infrared gas analyzer (LI-6400, Li-Cor Bioscience, USA) (Long et al., 1996) after moisture in the air was removed with dehumidifying tube (SWG-A01-18/PP, Asahi Glass Engineering Co., Ltd., Japan). Gas exchange in each chamber was measured for 2 minutes during a 10-minute cycle. The data were recorded every 20 seconds.

All sensors were attached to a data logger (CR23X, Campbell Scientific Inc., USA) with a power relay (SDM-CD16AC 16-channnel AC/DC controller, Campbell Scientific Inc., USA) switching heaters, humidifiers, fluorescent lamps, and solenoid valves. Each solenoid valve for gas exchange was, in turn opened for 2 minutes and closed. The temperature and relative humidity data inside the box were also collected every hour using thermocouple (T-types) and a humidity sensor (CHS-UPS, TDK, Japan), respectively (Fig. 2). Air temperature in the box was kept at 27℃. The air in the box was humidified up to set value by switching a humidifier.

Eleven fluorescent lamps (FL30SSD/29, Dooyoung Lighting Industrial Co., Ltd., Korea) were installed about 20 cm above the box and the distance between two lamps was approximately 1cm. Light levels were adjusted by the number of lamps and measured at the end of experiment above the top of each healing box using a light meter with quantum light 6 sensor bars (Field Scout external light senor meter, Spectrum Technologies, Inc., USA). Light period was 12 hours·d^{-1}.

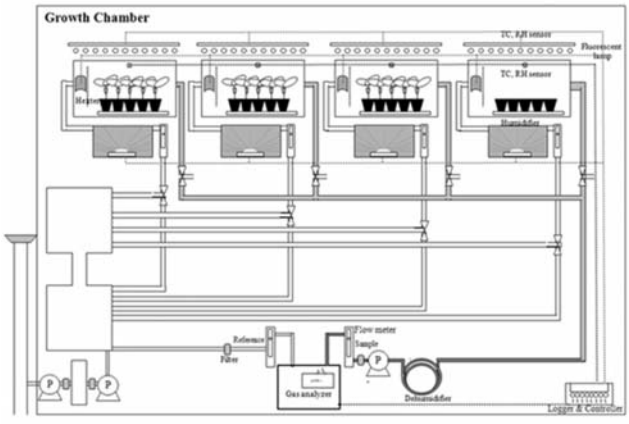

Fig. 1. Schematic diagram of the device and healing boxes used for environmental control and measurement during healing and acclimatization of grafted cucumbers seedlings.

Treatment

Healing and acclimatization of the grafted cucumbers were conducted in the healing box for 6 days. Irrigation was not applied during healing and acclimatization period.

Six treatments were designed by a combination of two (2) levels of relative humidity and three (3) levels of light

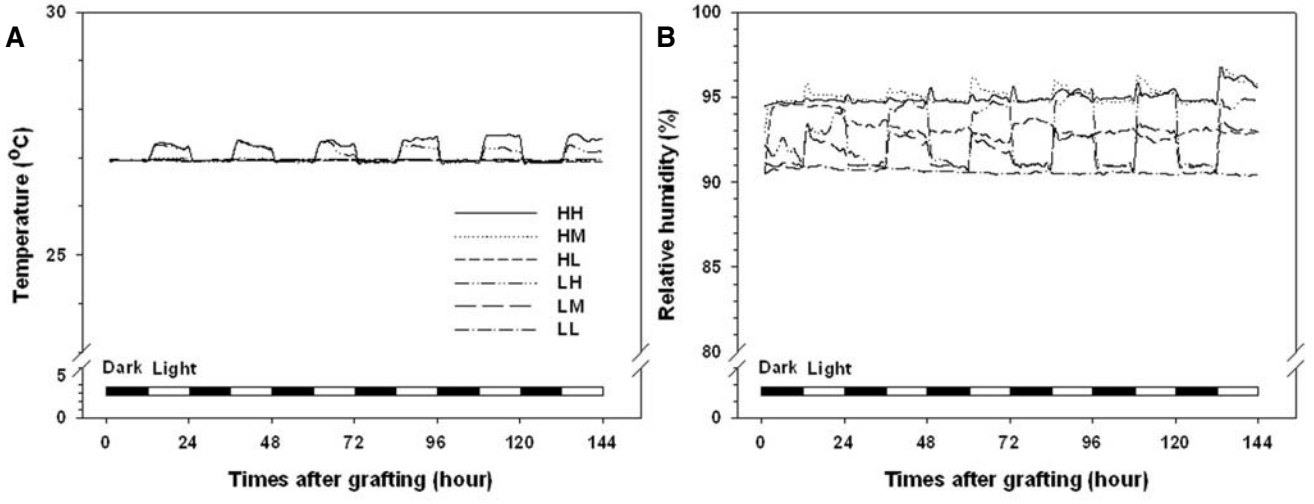

Fig. 2. Time course of air temperature and relative humidity conditions in each treatment during healing and acclimatization of grafted cucumbers seedlings.

intensity during healing and acclimatization (Table 1). For treatment code abbreviation, high and low relative humidity were abbreviated to H and L, respectively on the first letter, whereas high, medium, and low light intensity (PPF) were abbreviated to H, M, and L, respectively on the second letter. The experimental design was a split-plot with relative humidity as the main plot and light intensity as the sub plot. The experiment was repeated twice. In each replication, one 50-cell plug tray with 40 plants (330 plants·m^{-2}) was measured.

CO_2 Exchange Rate Measurement

The CO_2 exchange rate of each healing box was estimated using the equation below with the following parameters; 1) CO_2 concentrations; 2) air flow rate to the box; and 3) area of the plug tray. The CO_2 generation rate from the growing media and roots was neglected because it was small when compared with the exchange rate of the seedlings (Shibuya and Kozai, 1998).

$$CER = F (C_i - C_o) / A$$

where CER is the CO_2 exchange rate in the healing box (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), F is the air flow rate in the healing box ($mol \cdot s^{-1}$), C_i and C_o are the CO_2 concentrations in the inlet and outlet of the healing box (μmol $CO_2 \cdot mol^{-1}$), and A is the area of the plug tray (m^2).

Microscopic Observation

Cross sections of specimens for microscopic observation were prepared as described by Luft (1973). Leaf pieces for measurement of anatomy were cut off from the cotyledon of scions at 6 and 13 days after grafting. They were infiltrated and fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer (pH 7.2) for 2 hours at 4℃. Then they were rinsed and post-fixed in 1% osmium tetroxide for 2 hours at 4℃, and held overnight in phosphate buffer. After fixation, they were dehydrated in a graded series of ethyl alcohol (40, 60, 80, 90, 95, and 100% in distilled water [v/v]). The tissues were further processed with three changes of propylene oxide, for 15, 15, and 30 minutes per change, and gradually infiltrated (3 hours each at 30, 50, and 100% embedding media in propylene oxide) with embedding media, Epon to ensure complete dehydration. They were held overnight in 100% Epon before polymerization at 60℃ for 72 hours. They were sectioned (1500 nm), stained with periodic acid staining (P.A.S), and viewed with light microscope (Axioskop 2, Carl Zeiss AG, Germany).

Table 1. Relative humidity and light intensity (PPF) during healing and acclimatization in each treatment.

Treatment code	Relative humidity (%)[z]	PPF (μmol·m^{-2}·s^{-1})
HH[y]	95	237 ± 8
HM	95	142 ± 8
HL	95	0
LH	90	237 ± 8
LM	90	142 ± 8
LL	90	0

[z]For the relative humidity in each treatment during healing and acclimatization, see Fig. 2B.
[y]For treatment code, H and L on the left represent high and low relative humidity, respectively; H, M, and L on the right represent high, medium, and low light intensity (PPF), respectively.

Growth Parameter

Grafted cucumber seedlings were grown in a glasshouse after six-day healing and acclimatization. Graft-take and growth parameters, such as fresh and dry weight, and stem diameter were measured on day 6 and 13 after grafting. Ten plants in each treatment were sampled. Data were analyzed using SAS v.9.1 software (SAS Institute, Cary, NC).

Results

CO_2 Exchange Rate

Fig. 3 shows the CO_2 exchange rates of grafted cucumber seedlings during healing and acclimatization under different light intensities (PPF) and relative humidity conditions. Increasing PPF significantly increased CO_2 exchange rates during healing and acclimatization. During light period, the CO_2 exchange rates in treatments HH and LH were 1.5 and 1.8 times higher than those of treatments HM and LM, respectively. The CO_2 exchange rates on the sixth day in treatments HH, HM, LH and LM increased more than 2 times, compared

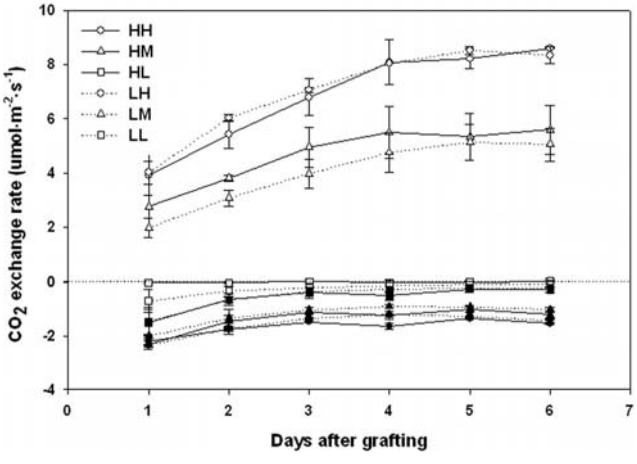

Fig. 3. CO_2 exchange rates of grafted cucumber seedlings during healing and acclimatization affected by light intensity (PPF) and relative humidity. For treatment codes, see Table 1. Open circles represent the CO_2 exchange rates during light period and closed circles represent the CO_2 exchange rates during dark period. Results are means ± SE.

with those on the first day. The CO_2 exchange rates during dark period were negatively correlated with PPF. However, the relative humidity at 90 to 95% did not significantly affect the CO_2 exchange rates during healing and acclimatization.

Leaf Anatomical Differences

Microscopic observation of scion cotyledons showed the difference in the distribution of chloroplast in the cell (Fig. 4). More chloroplasts in the scion cotyledon healed and acclimatized in high light condition (HH) (Fig. 4A) were observed, compared with the cotyledon healed and acclimatized in medium light (HM) (Fig. 4B) or dark condition (HL) (Fig. 4C). At day 13 after grafting, chloroplasts in the cell had increased in all treatments and those in high light condition (HH) (Fig. 4D) were more than those in medium light (HM) (Fig. 4E) or dark condition (HL) (Fig. 4F).

Plant Growth

Tables 2 and 3 present the growth of cucumber seedlings on day 6 and 13 after grafting, respectively. The shoot length decreased, while the number of unfolded leaves, hypocotyl diameter of rootstock, leaf area, and dry weight of root and shoot increased with an increase in PPF during healing and acclimatization period. At day 6 after grafting, the percent dry matter increased but specific leaf area (SLA) and ratio of shoot to root decreased as PPF increased (Fig. 5). Moreover, the relative humidity at 90 to 95% did not significantly affect the growth of grafted cucumber seedlings. There were no differences in the case of graft-take among treatments. The percentages of graft-take were over 95% in all treatments (data not shown).

Discussion

Healing and acclimatization are critical for grafted plants to survive. They involve healing of the cut surface and hardening for field or greenhouse survival (Lee and Oda, 2003). Light intensity, relative humidity, and temperature are the key environmental factors influencing the healing and acclimatization of grafted seedlings.

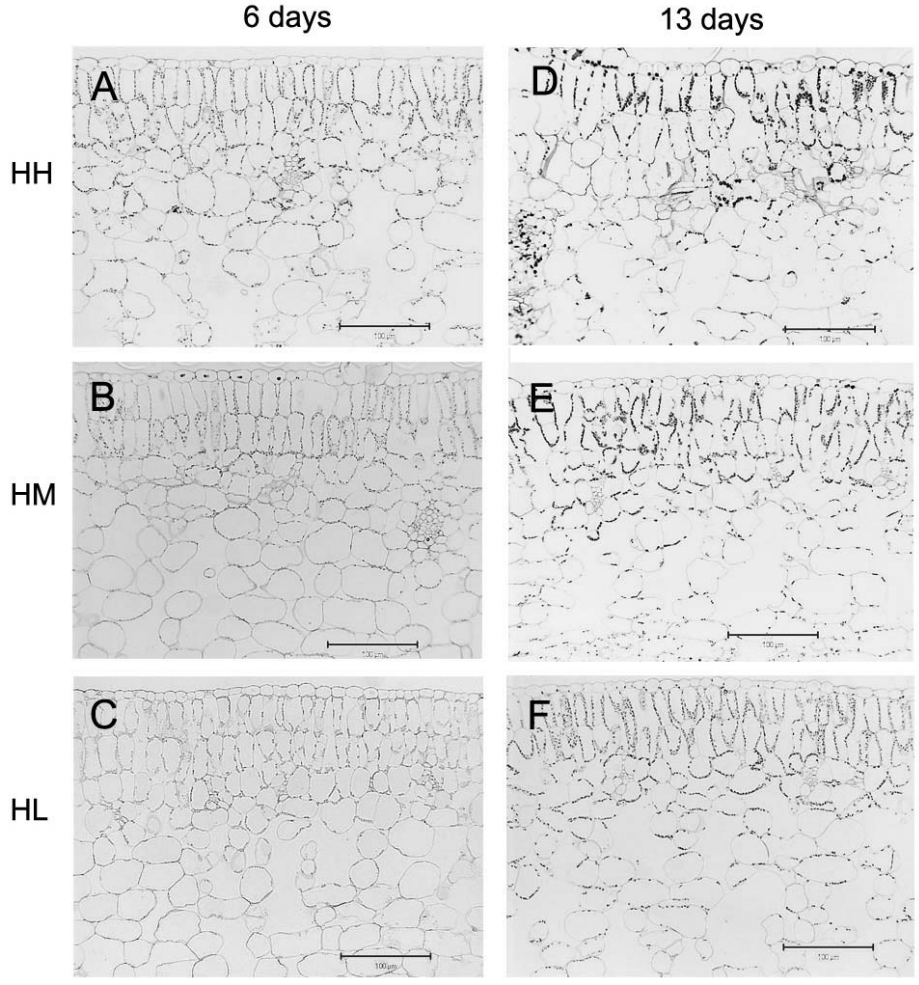

Fig. 4. Cross-sections of scion cotyledons healed and acclimatized in high light (HH) (A), medium light (HM) (B), and dark condition (HL) (C) for 6 days. D, E, and F show the leaf cross-sections of scion cotyledons in each treatment on day 13 after grafting.

Table 2. Growth of grafted cucumbers affected by light intensity (PPF) and relative humidity (RH) conditions during healing and acclimatization on day 6 after grafting.

Treatment code	Shoot length (cm)	Number of leaves	Hypocotyl diameter[z] (mm)	Leaf area (cm^2)	Dry weight (mg)	
					Root	Shoot
HH	8.09	0.95	4.08	50.31	39.9	228.9
HM	8.71	1.00	3.85	47.97	30.3	188.0
HL	9.59	-	3.51	36.28	20.0	128.5
LH	7.45	1.47	4.25	48.10	46.2	271.9
LM	7.46	1.57	3.92	45.47	36.8	240.7
LL	9.65	0.55	3.79	38.50	24.8	198.4
PPF (A)	***[y]	***	***	***	***	***
RH (B)	ns	ns	ns	ns	ns	ns
A × B	*	*	ns	ns	ns	*

[z]Hypocotyl diameter was measured at the middle of rootstock hypocotyls.
[y]ns indicates nonsignificant; *significant at $P \leq 0.05$; ***significant at $P \leq 0.001$.

Table 3. Growth of grafted cucumbers affected by light intensity (PPF) and relative humidity (RH) conditions during healing and acclimatization on day 13 after grafting.

Treatment code	Shoot length (cm)	Number of leaves	Hypocotyl diameter[z] (mm)	Leaf area (cm^2)	Dry weight (mg)	
					Root	Shoot
HH	8.40	2.35	5.15	87.57	49.8	434.0
HM	8.66	2.35	4.85	75.45	42.3	372.7
HL	10.00	0.95	4.20	51.37	27.8	290.9
LH	7.08	1.95	4.79	69.16	51.5	356.0
LM	7.85	2.20	4.76	71.13	40.8	304.6
LL	9.36	1.15	4.14	46.72	27.6	249.3
PPF (A)	***	***	***	***	***	***
RH (B)	ns	ns	ns	ns	ns	ns
A × B	ns	*	ns	*	ns	ns

[z]Hypocotyl diameter was measured at the middle of rootstock hypocotyls.
[y]ns indicates nonsignificant; *significant at $P \leq 0.05$; ***significant at $P \leq 0.001$.

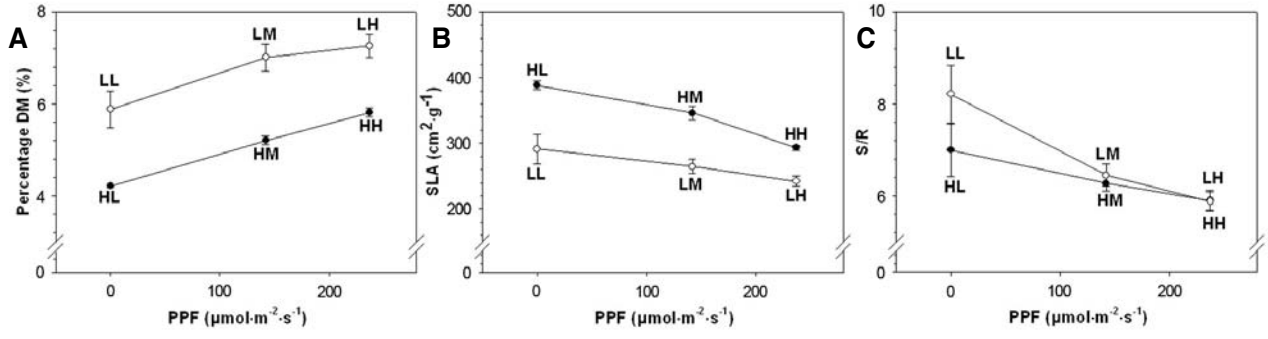

Fig. 5. Percent dry matter (DM) (A), specific leaf area (SLA) (B), and ratio of shoot to root (S/R) (C) of grafted cucumber seedlings affected by light intensity (PPF) and relative humidity during healing and acclimatization on day 6 after grafting. For treatment codes, see Table 1. Open circles represent 90% relative humidity and closed circles represent 95% relative humidity. Results are means ± SE.

In the case of the healing and acclimatization of grafted plants done in a tunnel inside a greenhouse, natural sunlight may lead to temperature rise, and fall of relative humidity inside the tunnel, as well as increase of evapotranspiration

of grafted seedlings (Kitaya et al., 1996). Thus, shading materials are usually used to keep light intensity at low level (PPF below 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and provide high relative humidity (more than 90% or near 100%) to avoid excessive heat build-up (Kim, 2000). Even though low light intensity enhances the graft-take of seedlings, it may inhibit the increase of dry matter. Under PPF below 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the net photosynthesis rate of the grafted plants is almost 0 mg $CO_2 \cdot m^{-2} \cdot s^{-1}$ (Shibuya et al., 2003).

On the other hand, increasing PPF during healing and acclimatization resulted to increased CO_2 exchange rates of grafted cucumber seedlings under artificial lights (Fig. 3). The temperature inside the healing box increased with an increase in PPF during healing and acclimatization period, but the temperature difference among treatments was within 0.5℃ only (Fig. 2).

In the previous experiment of the healing and acclimatization of grafted cucumber seedlings using rootstocks without cotyledon, it was observed that CO_2 exchange rates were negative even at 2 days after grafting but gradually increased to positive values from day 3. For the first 2 days after grafting, the cotyledons of scions had withered during which, the net photosynthesis of grafted cucumber seedlings was below zero. At the transition period when CO_2 exchange rates turned to positive, the vascular bundles between scion and rootstock were supposed to be connected together. However, with single-cotyledon rootstock, the CO_2 exchange rates were positive from the first day after grafting and gradually increased to more than 2 times on day 6. The positive CO_2 exchange rate was attributed to the photosynthesis of rootstock cotyledon which could enhance the faster union process of the vascular bundles between scion and rootstock.

Light intensity influences not only the photosynthetic characteristics but also the morphology of plants such as leaf thickness and arrangement of leaf cell (Lee et al., 1985; Li et al., 2009; McMillen and McClendon, 1983; Oguchi et al., 2003). Plants grown under high light intensity condition have higher photosynthetic capacity, greater leaf thickness, and more chloroplasts in mesophyll cells. The light intensity during healing and acclimatization affected the amount and distribution of chloroplasts in scion cotyledon. The amount of chloroplasts increased with the increase of PPF during healing and acclimatization and they covered most of cell wall with little open space left, compared with that of dark condition (Fig. 4).

With the PPF range evaluated in this experiment, the shoot length, ratio of shoot to root, and specific leaf area decreased but the hypocotyl diameter, leaf area, dry weight, and percent dry matter increased as PPF increased (Tables 2 and 3). Generally, higher quality plug transplants have higher percent dry matter and lower ratio of shoot to root, specific leaf area, and hypocotyl length (Kitaya et al., 1998). The increase of PPF during healing and acclimatization led to the improvement of growth and quality of grafted cucumber seedlings. These results concurred with that reported by Nobuoka et al. (2005) in which the graft-take and growth of grafted tomatoes were enhanced by light during healing and acclimatization.

The relative humidity during healing and acclimatization is critical for the survival and growth of grafted seedlings. Under the range of 75-85% relative humidity in which there is accelerated plant photosynthetic process, the grafted seedlings that are excised (root-removed) and have difficulty in absorbing water, are apt to wilt or wither due to water stress induced by excess transpiration. Accordingly, in a greenhouse, grafted seedlings are usually healed and acclimatized in nearly 100% relative humidity. Nobuoka et al. (1996) and Kim et al. (2001) have reported that it is necessary to control the relative humidity at higher than 90% for suppressing the evapotranspiration of grafted seedlings and thus enhancing the graft-taking of grafted seedlings. Under the range of PPF and relative humidity in this experiment, the percentages of graft-take were over 95% in all treatments (data not shown). However, the relative humidity ranging from 90 to 95% did not significantly affect the CO_2 exchange rates during healing and acclimatization as well as the growth of grafted cucumber seedlings.

In summary, PPF during healing and acclimatization affects the growth and quality of grafted cucumber seedlings and higher PPF condition may improve the growth and quality of grafted cucumber seedlings. The control of light intensity and relative humidity during healing and acclimatization may be particularly useful for a closed-type plant culture system where environmental conditions can be controlled accurately and may be better than a greenhouse.

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