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Research Report

Effects of Photosynthetic Photon Flux and Carbon Dioxide Concentration on the Photosynthesis and Growth of Grafted Pepper Transplants during Healing and Acclimatization

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Abstract. In the production of grafted transplants, healing and acclimatization are the most critical processes for survival. We investigated the influence of the photosynthetic photon flux (PPF) and the carbon dioxide (CO_2) concentration during healing and acclimatization on the photosynthetic characteristics and growth of grafted pepper transplants to determine the optimum environmental conditions for healing and acclimatization in a healing chamber with artificial lighting source. Grafted pepper transplants were healed and acclimatized under two levels of CO_2 (374 or 1,013 μmol·mol^{-1}) and four levels of PPF (dark, 50, 98 or 147 μmol·m^{-2}·s^{-1}) for six days. The CO_2 exchange rates of the grafted pepper transplants significantly increased with increasing PPF during healing and acclimatization. The CO_2 exchange rates were higher under elevated CO_2 concentrations than ambient CO_2 concentration. The effect of CO_2 enrichment was greater in low light intensity. The CO_2 exchange rates at 50, 98 or 147 μmol·m^{-2}·s^{-1} under elevated CO_2 concentrations were 511, 261, and 172%, respectively, compared to the ambient CO_2 concentrations. The increase of photosynthesis led to an improvement in growth. The SPAD value, dry weight and leaf area were greater under higher PPF and CO_2 concentrations. PPF also influenced the anatomical structures of the leaves, and the palisade and spongy tissue cells of the leaves irradiated with higher PPF were aligned more densely, with more chloroplasts and small empty space. When compared to the tunnel in the greenhouse with natural light, healing and acclimatization under high CO_2 (1,000 μmol·mol^{-1}) and PPF (150 μmol·m^{-2}·s^{-1}) conditions in the healing chamber promoted the growth and graft union formation of grafted pepper transplants. The results suggested that high-quality grafted pepper seedlings could be achieved by healing and acclimatization in a healing chamber where optimal conditions such as high PPF and CO_2 were maintained within the range evaluated in this experiment.

Additional key words: *Capsicum annuum* L., CO_2, CO_2 exchange rate, grafting, PPF

Introduction

Vegetable grafting is widely practiced over the world (Lee et al., 2010) for a variety of purposes, including the reduction of soil-borne diseases (Louws et al., 2010) and the enhancement of tolerance against abiotic stresses such as low (Venema et al., 2008) and high temperatures (Rivero et al., 2003), flooding (Yetisir et al., 2006), drought (Rouphael et al., 2008), salt (Colla et al., 2006a, 2006b; Edelstein et al., 2011), alkalinity (Colla et al., 2010), and heavy metals (Savvas et al., 2010). Grafting is mostly practiced on fruit vegetables of the family Cucurbitaceae and Solanaceae.

In the production of grafted transplants, healing and acclimatization are critical for them to survive and grow as healthy plants, which involve the healing of the cut surface, connection of new vascular bundles in the graft union, and hardening for field or greenhouse survival (Lee and Oda, 2003). Healing and acclimatization have been practiced by focusing on the survival of grafted transplants rather than growth. After grafting, grafted transplants are usually healed and acclimatized in a tunnel covered with double-layered plastic film and shade cloth on a growing bench in greenhouse.

The tunnel is closed during three or four-day healing

period to prevent the grafted plants from wilting through excessive transpiration and to promote healing. When the tunnel is closed, light intensity inside the tunnel is very low (near the light compensation point), the air humidity is saturated (relative humidity > 90%), and the air current speed is near 0 $m \cdot s^{-1}$ (Kim and Park, 2001; Lee and Oda, 2003; Shibuya et al., 2003). Shibuya et al. (2003) reported that the net photosynthesis rate of grafted seedlings in the closed tunnel was almost 0 mg $CO_2 \cdot m^{-2} \cdot s^{-1}$. The temperature inside the closed tunnel during healing and acclimatization processes could often be higher than the outside temperature. It often exceeds a threshold high temperature at midday, sometimes resulting in the death of plants. The temperature fluctuation makes it difficult to maintain air humidity inside the tunnel. Under these environmental conditions, plants are in danger of heat and water stress, leading to lower the plant quality and weaken the plants (Wahid et al., 2007).

Environmental management during healing and acclimatization is usually performed based on the empirical knowledge of the grower, depending on the season or weather. A grower decides on the opening and closing of the tunnel based on the condition of the grafted transplants and the weather (Jang et al., 2011). Recently, the increase in unpredictable abnormal weather has caused greater problems in maintaining uniform and optimal environmental conditions for the healing and acclimatization of grafted transplants.

High-performance production systems unconstrained by weather conditions have recently been developed to produce high-quality transplants under artificial light (Kozai, 2005, 2009). Optimizing the temperature, relative humidity, photosynthetic photon flux (PPF), and carbon dioxide (CO_2) concentration in these systems makes it possible to achieve rapid and uniform growth of high-quality transplants throughout the year. This optimization can also be applied to healing and acclimatization for production of grafted transplants.

Recent reports have suggested that there is a higher survival rate, faster growth, and a higher quality of grafted plants under highly controlled healing conditions (Jang et al., 2011; Nobuoka et al., 2005). Reports have primarily focused on the increase of photosynthesis, which has been so far overlooked during healing and acclimatization. The increase of photosynthesis in grafted transplants during healing and acclimatization was confirmed by higher PPF conditions using artificial lights such as fluorescent lamps under highly controlled healing conditions (Jang et al., 2011, 2013). These conditions resulted in an improvement in the growth and quality of grafted transplants.

The primary effects on plants of enriching CO_2 have been well documented and include reduction in stomatal conductance and transpiration, improved water-use efficiency, higher rates of photosynthesis, and increased light-use efficiency (Ainsworth and Long, 2005; Drake et al., 1997). Atmospheric CO_2 concentration is around 400 $\mu mol \cdot mol^{-1}$, while the CO_2 concentration in the closed-type plant production system using artificial lights is usually managed by more than 1,000 $\mu mol \cdot mol^{-1}$ for the promotion of photosynthesis.

This study aimed to investigate the photosynthetic characteristics, graft-take, and growth of grafted pepper transplants affected by PPF and CO_2 concentration during healing and acclimatization, in order to determine the optimal environmental conditions for healing and acclimatization in the healing chamber with artificial lighting source.

Materials and Methods

Plant Material and Growth Conditions

***Plant material and growing scions and rootstocks*:** The peppers (*Capsicum annuum* L.) 'Nokkwang' (Seminis Vegetable Seeds, Inc., Seoul, Korea) and 'Tantan' (Nongwoo Bio Co., Ltd., Suwon, Korea) were used as scions and rootstocks, respectively, for producing grafted transplants. Pepper seeds were sown into 72-cell plug trays (W 280 mm × L 540 mm × H 45 mm, Bumnong Co., Ltd., Jeongeup, Korea) filled with commercial growing substrate (BM 2, Berger Group Ltd., St. Modeste, QC, Canada). Seeds of rootstocks were sown two days before sowing the seeds of scions to obtain scions and rootstock with similar stem diameter. To promote germination, the plug trays were wrapped with vinyl chloride resin film and placed in the germination chamber maintained at 28°C.

After four days, the germinated seedlings were watered by overhead watering and moved to the growth chamber with artificial light (Hanbaek Co., Ltd., Bucheon, Korea), where the temperature was set at 25/18°C (light / dark period). The light period was 14 $h \cdot d^{-1}$, and PPF was approximately 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and provided by high pressure sodium, metal halide, and fluorescent lamps. These were bottom-irrigated twice a week with water and once a week with a nutrient solution (EC 1.4 $dS \cdot m^{-1}$, 'Hanbang' for seedling, Coseal Co., Ltd., Seoul, Korea).

The dry weight, number of true leaves, leaf area, and stem diameter of the scion and rootstock before grafting were 119.7 ± 3.9 mg, 4.98 ± 0.09, 30.16 ± 0.95 cm^2, 1.53 ± 0.02 mm, and 118.8 ± 3.9 mg, 5.32 ± 0.08, 30.27 ± 1.16 cm^2, 1.62 ± 0.02 mm, respectively.

***Grafting*:** Grafting was performed at four weeks after sowing the rootstocks. The epicotyls of the scions and rootstocks were cut below 1 cm from the first true leaf using a razor blade. After placing the scion on the rootstock, the grafted position was fixed tightly together with an ordinary grafting clip using the slice grafting method (Lee and Oda, 2003).

***Healing and acclimatization of grafted pepper transplants*:** The continuous CO_2 measurement system using a semi-open multi-chamber was used for the healing and acclimatization of grafted pepper transplants and the measurement of the whole-canopy CO_2 exchange rate (Jang et al., 2011; Mun et al., 2011; van Iersel and Bugbee, 2000;). Four light-transmitting healing chambers (W 350 mm × L 780 mm × H 220 mm inside dimension and 60 L volume) made of 10 mm-thick acryl plastic were placed in the growth chamber (Hanbaek Co. Ltd., Bucheon, Korea) at 22°C.

Each healing chamber had air inlets, outlets and an air drawing tube. Atmospheric air was drawn in through the inlet of each healing chamber at an airflow rate of 13 $L \cdot min^{-1}$ using air pumps (LP80VC, Youngnam Air Pump Inc., Busan, Korea) and flow meters (15 $L \cdot min^{-1}$, Kofloc, Kojima Instruments Inc., Kyoto, Japan), and air flowed out through the outlet (Mun et al., 2011; Shibuya et al., 2006).

A thermocouple (T-types), a humidity sensor (CHS-UPS, TDK, Japan), a heater (hair dryer heater, Kaiser KHD-5207i, My Friend Co., Ltd., Goyang, Korea) and a humidifier (nebulizer, CT-24, Techsin Electronic Co., Ltd., Foshan, China) were equipped to measure and to maintain the temperature and relative humidity inside the healing chamber at set point. The sensors were connected to a data logger (CR23X, Campbell Scientific Inc., Logan, UT, USA) with a switching power relay (SDM-CD16AC 16-channel AC/DC controller, Campbell Scientific Inc., Logan, UT, USA) that switched the heaters and humidifiers. The temperature and relative humidity data inside the healing chamber were collected every hour. The air temperature in the healing chamber was kept at 27°C, and the relative humidity at 90%.

Eleven fluorescent lamps (FL30SSD/29, Dooyoung Lighting Industrial Co., Ltd., Seoul, Korea) were installed approximately at 20 cm above the healing chamber, and the distance between two lamps was approximately 1 cm. The light levels were adjusted by the number of lamps and were measured on top of each healing chamber using a light meter with 6 quantum light sensor bars (Field Scout external light senor meter, Spectrum Technologies, Inc., Plainfield, IL, USA). The light period was 12 $h \cdot d^{-1}$.

Healing and acclimatization of grafted pepper transplants were conducted in the healing chamber for 6 days. Irrigation was not applied during healing and acclimatization.

Treatments

***Experiment 1. Effects of PPF and CO_2 during healing and acclimatization in a healing chamber with artificial lighting source*:** The experiment was conducted in the semi-open healing chamber. Six treatments were designed with the combination of two levels of CO_2 and four levels of PPF during healing and acclimatization (Table 1). For coding the treatments, ambient and elevated CO_2 were coded as A and E, respectively, whereas the dark condition and low, medium, and high PPF were coded as D, L, M, and H, respectively. The experimental design was a split-plot with CO_2 as the main plot and PPF as the sub plot. The experiment was repeated twice. In each replication, one 72-cell plug tray with 48 plants was measured.

To elevate the CO_2 concentration, a buffer chamber (W 320 mm × L 320 mm × H 850 mm inside dimension and 87 L volume) was made of 10 mm-thick acryl plastic. Atmospheric air was drawn in through the inlet of the buffer chamber using an oil-less piston pump (100RND, G&M Tech. Inc., Gunpo, Korea) and flow meters (100 $L \cdot min^{-1}$, Dwyer Instruments, Inc., Michigan City, IN, USA). The CO_2 level in the buffer chamber was controlled using flow meter and needle valves (100 $mL \cdot min^{-1}$, Dwyer Instruments, Inc., Michigan City, IN, USA) with additional CO_2 from a CO_2 bombe. Four micro fans (Suntronix fan SJ1238HA2, Sanju Electric Machinery, Co., Ltd., Shenzhen, Guangdong, China) inside the buffer chamber mixed the air to maintain a stable

Table 1. Carbon dioxide (CO_2) concentration and photosynthetic photon flux (PPF) during healing and acclimatization in each treatment.

Treatment code	CO_2 concentration ($\mu mol \cdot mol^{-1}$)	PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
AD[z]	373.7 ± 1.9[y]	-
AL	373.7 ± 1.9	50.0 ± 4.7
AM	373.7 ± 1.9	97.5 ± 4.7
AH	373.7 ± 1.9	148.6 ± 4.3
ED	1,013.0 ± 11.3	-
EL	1,013.0 ± 11.3	50.0 ± 4.7
EM	1,013.0 ± 11.3	97.5 ± 4.7
EH	1,013.0 ± 11.3	148.6 ± 4.3

[z]For treatment codes, A and E on the left represent the ambient and elevated CO_2 concentration, respectively; and D, L, M, and H on the right represent the dark condition and low, medium, high PPF, respectively.
[y]Mean ± standard error.

CO_2 concentration. The air in the buffer chamber flowed out through the outlet into the healing chamber.

***Experiment 2. Comparison of the growth of grafted pepper transplants that were healed and acclimatized in tunnel in the greenhouse vs. in healing chamber with artificial lighting source*:** The grafted pepper transplants were healed and acclimatized in tunnel in the greenhouse with natural light or in a healing chamber with artificial lighting for 6 days. The experiment had three treatments: (i) control of normal atmospheric CO_2 and light conditions, where healing and acclimatization were performed in a tunnel on a greenhouse bench; (ii) high CO_2 (1,000 μmol·mol^{-1} CO_2 concentration) and medium PPF treatment (100 μmol·m^{-2}·s^{-1} PPF) in the healing chamber; and (iii) a high CO_2 (1,000 μmol·mol^{-1} CO_2 concentration) and PPF treatment (150 μmol·m^{-2}·s^{-1} PPF) in the healing chamber. Each treatment had two replications. In each replication, one 72-cell plug tray with 48 plants was measured.

The healing and acclimatization of the control were performed in a tunnel made of double-layered plastic film on the greenhouse bench. Shade cloth was installed on the tunnel. For the first three days, the humidity inside the tunnel was kept high by closing the tunnel and using a humidifier (H-650C, LG Electronics, Seoul, Korea). Then, the tunnel was gradually opened during the following three days to acclimate the grafted transplants to normal conditions. Temperature, relative humidity, and PPF in the tunnel and greenhouse were measured using a temperature/humidity data logger (TR-75U Thermo Recorder, T and D Corp., Matsumoto, Japan) and a data logger (WatchDog 1000 Series, Spectrum Technologies, Inc., Plainfield, IL, USA) with a quantum light sensor (LightScout Quantum light sensor, Spectrum Technologies, Inc., Plainfield, IL, USA).

Data Collection and Analysis

***Measurement of whole-canopy CO_2 exchange rate*:** Individual gas from each healing chamber was pulled by a 3-way solenoid valve (VD3, Korcon, Seoul, Korea) every two minutes with a switching power relay (SDM-CD16AC 16-channel AC/DC controller, Campbell Scientific Inc., Logan, UT, USA). Two flow meters (1.0 L·min^{-1}, Kofloc, Kojima Instruments Inc., Kyoto, Japan) were also used at 0.5 L·min^{-1} for the sample and reference gas measurement, respectively. The CO_2 concentrations of the air at the inlet and outlet of the healing chamber were measured using the differential gas analyzer (LI-7000, Li-Cor Bioscience, St. Lincoln, NE, USA) after the moisture in the air was removed with a dehumidifying tube (SWG-A01-18/PP, Asahi Glass Engineering Co., Ltd., Chiba, Japan). The CO_2 concentration of the air in each chamber was measured for 2 minutes during a 10-minute cycle. The data were recorded for the last five seconds.

The CO_2 exchange rate of each healing chamber was estimated using the equation below with the following parameters: 1) CO_2 concentration; 2) air flow rate into the chamber; 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 transplants (Shibuya and Kozai, 1998).

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

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

***Microscopic observation*:** Three plants of each treatment were sampled for microscopic observation on the second and sixth day after grafting. Microscope cross sections were made according to the method described by Luft (1973). For anatomy measurements, graft unions from the plants on the sixth day after grafting were cut off. Leaf pieces from the scion on the sixth day after grafting were also cut off. These were infiltrated and fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer at pH 7.2 for 2 hours at 4°C. Then, these were rinsed and post-fixed in 1% osmium tetroxide for 2 hours at 4°C and held overnight in phosphate buffer. After fixation, these 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 Epon (EMS, Hatfield, PA, USA) embedding media to ensure complete dehydration. These were held overnight in 100% Epon before polymerization for 72 hours at 60°C. These were sectioned (1,500 nm), stained with periodic acid staining, and viewed under a light microscope (Axioskop 2, Carl Zeiss AG, Oberkochen, Germany).

***Growth parameters*:** After healing and acclimatization, graft-take and growth parameters such as shoot length, number of true leaves, chlorophyll content (by SPAD 502 Plus Chlorophyll meter, Spectrum Technologies, Inc., Plainfield, IL, USA), leaf area (by LI-3100 area meter, Li-Cor Bioscience, St. Lincoln, NE, USA), and fresh mass were measured. Dry mass was measured after drying the samples at 80°C for at least three days using the dry oven (DS-80-3, Dasol Scientific Co., Ltd., Hwaseong, Korea). Percent dry matter in root, stem, and leaf were calculated from the ratio between dry mass and fresh mass (g·g^{-1}), and specific leaf area (SLA) was expressed as the ratio of leaf area to leaf dry mass (cm^2·g^{-1}). Ten plants in each treatment were sampled. Data were analyzed using SAS v.9.1 software (SAS Institute,

Cary, NC, USA).

Results and Discussion

Environmental Condition during Healing and Acclimatization

The successful production of grafted transplants depends on healing and acclimatization. Healing and acclimatization of the grafted vegetable transplants are generally performed in a tunnel in the greenhouse bench. During healing and acclimatization, keeping the temperature, relative humidity, and light intensity at optimal levels is essential to prevent wilting, to promote graft union formation, and to harden transplants for field conditions. However, such optimization is difficult because environmental conditions inside the tunnel are often influenced by external environmental and exceed the optima, especially at midday and in summer.

In the greenhouse with only roof vents on the ridge line and an exhaust fan on the sidewall side without extra devices for cooling, the air temperature, relative humidity, PPF, and photoperiod fluctuated and varied with time and season (Fig. 1). The maximum air temperature in the greenhouse ranged from 33 to 38°C from July to September. The temperature inside the tunnel was approximately 10°C higher than the greenhouse. The increase in the temperature may simultaneously cause reduction of the relative humidity. At the highest temperature in the tunnel, the relative humidity decreased. Accordingly, grafted transplants in a tunnel in a greenhouse are in danger of heat and water stress, especially at daytime when the temperature is high and evapotranspiration

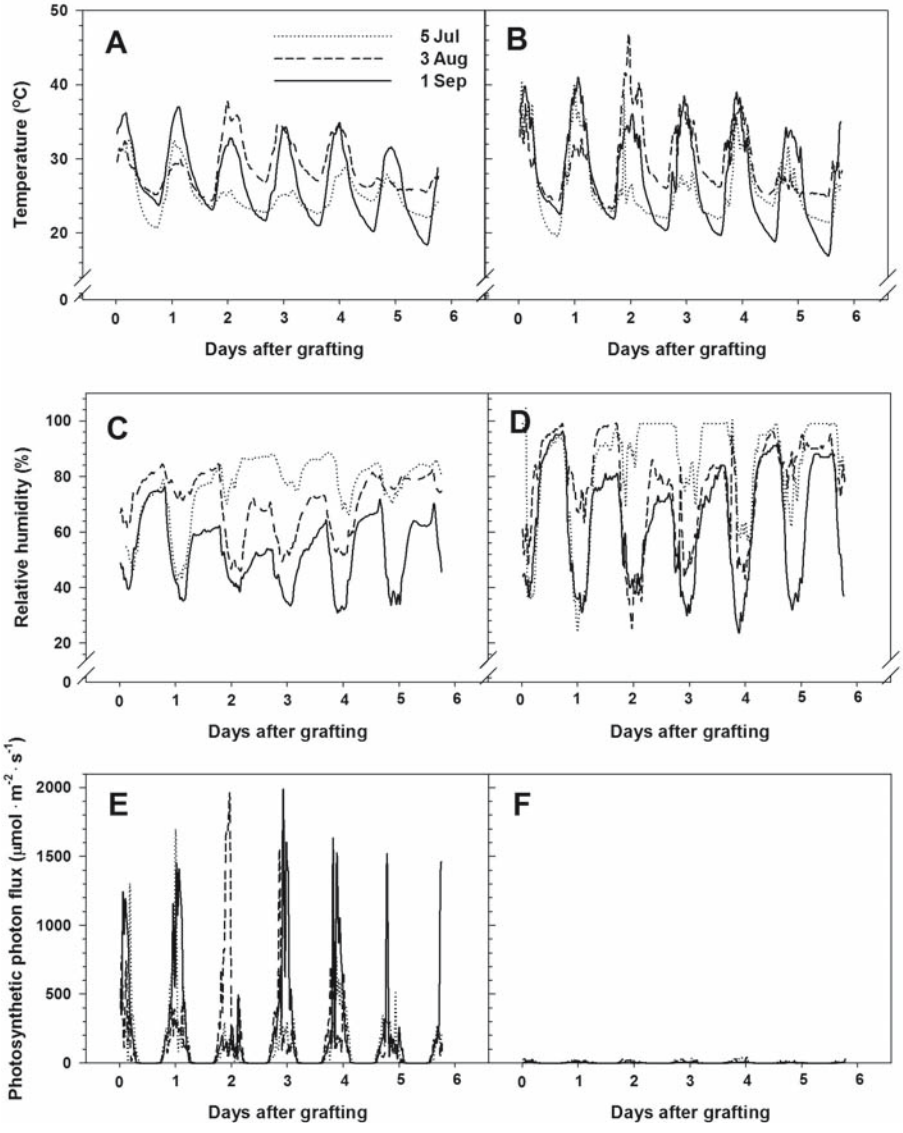

Fig. 1. Time course of air temperature, relative humidity, and photosynthetic photon flux in a tunnel (B, D, and F) in a greenhouse (A, C, and E) during the healing and acclimatization of grafted pepper transplants.

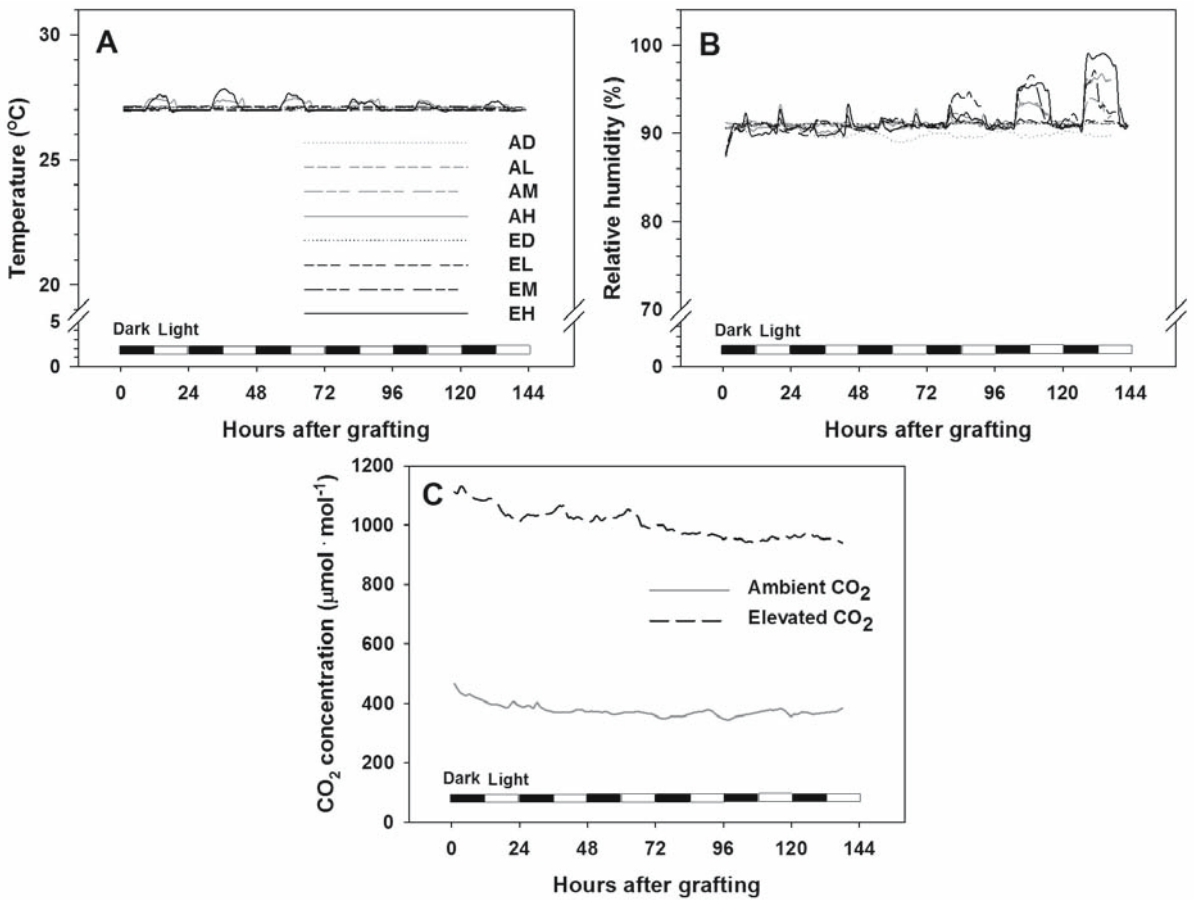

Fig. 2. Time course of air temperature, relative humidity, and carbon dioxide (CO_2) concentration in a healing chamber with artificial lighting source during healing and acclimatization of grafted pepper transplants.

is accelerated. The PPF in the tunnel was below 40 μmol·m^{-2}·s^{-1}, fluctuating with day and season.

Recently, high-performance production systems such as closed-type transplant production systems with artificial lighting have been developed as a way to produce high quality transplants regardless of the weather (Kozai, 2005, 2007; Kozai et al., 2000). The air temperature, relative humidity, PPF, and CO_2 concentration in these systems can be controlled easily and accurately, leading to rapid and uniform growth of high-quality transplants throughout the year, regardless of the outside weather. These systems can also be applied to the production of grafted transplants. The air temperature and relative humidity in the healing chamber in the study were maintained within the optimal range during healing and acclimatization (Fig. 2). The CO_2 concentration, PPF, and photoperiod in the healing chamber were also controlled and constant during healing and acclimatization.

PPF and CO_2 Effects in the Healing Chamber with Artificial Lighting Source

Healing and acclimatization have been performed under very low light condition near the light compensation point. However, photosynthesis was promoted by the improvement of light intensity under highly controlled conditions during healing and acclimatization, resulting in a higher survival rate, faster growth, and higher quality on grafted plants (Jang et al., 2011, 2013; Nobuoka et al., 2005).

The stable maintenance of environmental conditions at the optimal range in the healing chamber reduces the danger of heat and water stress where the grafted transplants are exposed in a tunnel in the greenhouse. Further, in the healing chamber with artificial lighting source, the photosynthesis of grafted pepper transplants during healing and acclimatization increased because of the increased PPF (Fig. 3). This result concurred with the previous finding that the photosynthesis of grafted cucumber transplants during healing and acclimatization increased due to increased PPF (Jang et al., 2011).

After grafting, water supply from the root to cut scions was limited, and grafted pepper transplants wilted for the first 2 days after grafting. These transplants were gradually recovered as their healing and acclimatization were processed. Under light conditions, grafted transplants withered for the first 2 days after grafting. During this period, the CO_2 exchange rates of grafted pepper transplants were low, approximately

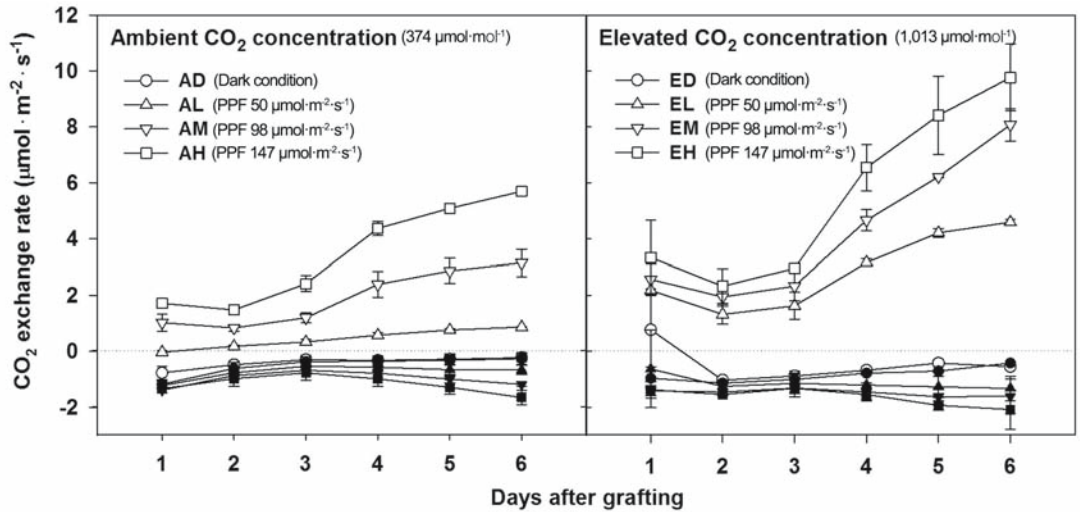

Fig. 3. The carbon dioxide exchange rates of grafted pepper transplants during healing and acclimatization as affected by carbon dioxide concentration (CO_2) and photosynthetic photon flux (PPF).

2 μmol·m^{-2}·s^{-1}, although positive. From the third day after grafting, the CO_2 exchange rates increased more rapidly with time. At this transition point, it is assumed that water transport from rootstock to scion had begun (Mun et al., 2011). Meanwhile, under dark condition, the CO_2 exchange rate was below zero throughout the healing and acclimatization, and the assimilation product was assumed to be consumed by respiration without photosynthesis. With the PPF range evaluated in the study, the CO_2 exchange rates of grafted pepper transplants significantly increased with increasing PPF during healing and acclimatization. The CO_2 exchange rates were higher under elevated CO_2 concentrations than ambient CO_2 concentration. Under ambient CO_2 concentration, the CO_2 exchange rate of grafted pepper transplants at 50 μmol·m^{-2}·s^{-1} (AL) was negative even at daytime on the first day of treatment, and increased by 0.9 μmol·m^{-2}·s^{-1} on the sixth day after grafting. The CO_2 exchange rates on the sixth day at 98 μmol·m^{-2}·s^{-1} (AM) and 147 μmol·m^{-2}·s^{-1} (AH) were 344% (3.1 μmol·m^{-2}·s^{-1}) and 633% (5.7 μmol·m^{-2}·s^{-1}), respectively, compared to 50 μmol·m^{-2}·s^{-1} (AL). Under elevated CO_2 concentrations (1,013 μmol·mol^{-1}), the CO_2 exchange rate at 50 μmol·m^{-2}·s^{-1} (EL) was 4.6 μmol·m^{-2}·s^{-1} on the sixth day after grafting. The CO_2 exchange rates on the sixth day at 98 μmol·m^{-2}·s^{-1} (EM) and 147 μmol·m^{-2}·s^{-1} (EH) were 176% (8.1 μmol·m^{-2}·s^{-1}) and 213% (9.8 μmol·m^{-2}·s^{-1}), respectively, compared to 50 μmol·m^{-2}·s^{-1} (EL).

The effect of CO_2 enrichment on the photosynthesis was greater in low light intensity. The CO_2 exchange rates at 50 (EL), 98 (EM), and 147 μmol·m^{-2}·s^{-1} (EH) under elevated CO_2 concentrations were 511, 261, and 172%, respectively, compared to those under the same PPF conditions at ambient CO_2 concentrations. Many studies have reported that elevated CO_2 concentrations increase photosynthesis. Drake et al. (1997) reported that growth in elevated CO_2 increased photosynthesis 58% compared with the rate of plants grown in normal ambient CO_2, due to increased carboxylation rate of Rubisco and the competitive inhibition of the oxygenation of ribulose-1,5-bisphophate. Harnos et al. (2002) reported that photosynthetic activity was significantly increased in the lower canopy layer where irradiance was very poor, particularly within the canopy of plants under elevated CO_2.

Moreover, it is reported that elevated CO_2 increases water use efficiency by decreasing transpiration (Jianlin et al., 2008). During healing and acclimatization, it is important to decrease transpiration because there is no additional irrigation, and there is poor water transport from roots to shoots. Therefore, higher PPF and CO_2 are necessary for promoting photosynthesis and reducing transpiration during healing and acclimatization, although the CO_2 exchange rates of grafted transplants during healing and acclimatization are smaller than the of non-grafted transplants (Mun et al., 2011).

The increased photosynthesis led to the improvement in growth. On the sixth day after grafting, the shoot length, number of true leaves, SPAD value, leaf area, and dry weight of roots, stems, and leaves increased with increasing PPF during healing and acclimatization (Table 2). The percent dry matter also increased, but the specific leaf area decreased as the PPF increased. The SPAD value, leaf area, and dry weight under elevated CO_2 concentrations were greater than the ambient CO_2 concentrations. The effect of CO_2 enrichment on the growth was also greater in low light intensity. Dry weights of grafted pepper transplants at 50 (EL), 98 (EM), and 147 μmol·m^{-2}·s^{-1} (EH) under elevated CO_2 concentrations were 167, 144, and 138%, respectively, compared to those under the same PPF conditions at ambient CO_2 concentrations. This result concurred with the

Table 2. Growth of grafted pepper transplants as affected by the carbon dioxide (CO_2) concentration and photosynthetic photon flux (PPF) during healing and acclimatization on the sixth day after grafting.

Treatment code	Shoot length (cm)	Number of leaves	SPAD value	Leaf area (cm^2)	SLA ($cm^2 \cdot g^{-1}$)	Shoot to root ratio	Dry weight (mg)			Percent dry matter (%)		
							Root	Stem	Leaf	Root	Stem	Leaf
AD	6.7 d[z]	4.9 c	22.4 e	24.1 c	542 ab	4.7 c	16.3 cd	29.9 cd	44.6 e	8.0 ab	7.3 bc	8.4 fg
AL	7.3 c	5.5 b	24.9 d	24.7 c	573 a	5.4 a-c	14.2 d	30.4 cd	43.7 e	7.9 b	6.9 bc	8.7 ef
AM	7.3 c	5.8 a	26.4 cd	31.6 b	565 a	5.8 a-c	16.0 cd	31.7 c	57.2 d	8.8 a	6.8 c	9.2 de
AH	7.4 bc	6.1 a	26.3 cd	34.9 b	488 cd	6.5 a	18.5 c	36.0 b	72.8 c	7.9 b	7.6 b	10.0 bc
ED	7.0 d	4.6 c	27.2 bc	30.9 b	574 a	5.0 bc	18.2 c	26.7 d	54.4 d	6.6 c	6.0 d	7.7 g
EL	8.1 a	5.9 a	29.1 a	44.3 a	517 bc	5.6 a-c	22.6 b	37.7 b	86.8 b	6.8 c	7.2 bc	9.7 cd
EM	7.7 b	5.9 a	28.8 ab	41.0 a	461 de	6.3 ab	22.3 b	38.6 b	89.7 b	7.0 c	7.4 bc	10.5 ab
EH	7.7 b	6.2 a	29.5 a	42.8 a	425 e	5.9 a-c	26.3 a	45.7 a	103.3 a	7.9 ab	8.9 a	11.2 a

[z]Different letters correspond to significantly different values at $p \leq 0.05$ according to Duncan's multiple range test (DMRT).

Table 3. Growth of grafted pepper transplants healed and acclimatized in a tunnel in a greenhouse and a healing chamber with artificial lighting source on the sixth day after grafting.

Treatment code[z]	Shoot length (cm)	Number of leaves	SPAD value	Leaf area (cm^2)	SLA ($cm^2 \cdot g^{-1}$)	Shoot to root ratio	Dry weight (mg)			Percent dry matter (%)		
							Root	Stem	Leaf	Root	Stem	Leaf
C (control)	7.1 a[y]	5.9 b	32.1 b	35.2 a	447 a	6.9 a	18.1 a	39.3 b	79.5 b	11.4 a	8.6 b	10.9 b
EM	7.1 a	6.3 a	32.8 b	34.2 a	348 b	7.0 a	22.8 a	44.5 ab	103.7 a	12.0 a	9.1 b	14.1 a
EH	6.9 a	6.5 a	35.7 a	35.2 a	324 b	7.8 a	21.3 a	46.2 a	110.2 a	11.2 a	10.1 a	14.9 a

[z]For treatment codes, C (control) represents the treatment with normal CO^2 and light condition, in which healing and acclimatization of grafted transplants are performed in a tunnel on a greenhouse bench, and EM and EH represent a high CO^2 (1,000 μmol·mol^{-1}) and medium PPF (100 μmol·m^{-2}·s^{-1}) treatment and a high CO^2 and PPF (150 μmol·m^{-2}·s^{-1}) treatment in the healing chamber, respectively.
[y]Different letters correspond to significantly different values at $p \leq 0.05$ according to Fisher's least significant difference (LSD) test.

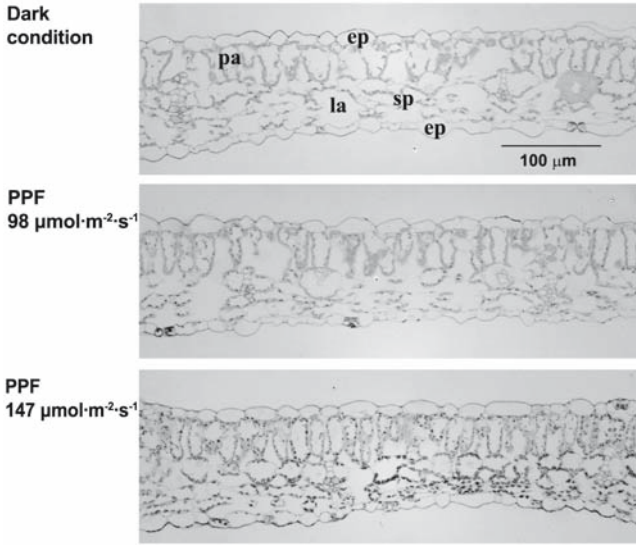

Fig. 4. Leaf cross-sections of scions healed and acclimatized at the dark and 98 or 147 μmol·m^{-2}·s^{-1} PPF on the sixth day after grafting.

report that the growth response to CO_2 is pronounced at very low light condition (Granados and Körner, 2002). In their report, increasing the atmospheric CO_2 concentration from 280 to 420 μmol·mol^{-1} increased the relative effect of CO_2-enrichment by 63% at low light (42 μmol·m^{-2}·s^{-1}), compared to 37% at high light (87 μmol·m^{-2}·s^{-1}). Curtis and Wang (1998) also reported that elevated CO_2, on the average, has a greater effect on the growth in the low light than the high light. The percentages of graft-take were close to 100% in all treatments (data not shown).

The increased PPF influenced also the leaf anatomical structures (Fig. 4). The palisade and spongy mesophyll cells of the leaves irradiated with higher PPF were aligned more densely, had a small empty space and more chloroplasts, compared with the medium PPF or dark conditions. The leaf anatomical structures under higher PPF during healing and acclimatization were close to the sun leaf type. This similarity is expected to increase the transplants' adaptability to the outside environment of high PPF.

Comparison of Growth of Grafted Pepper Transplants Healed and Acclimatized in a Tunnel in the Greenhouse and Healing Chamber

In comparing the healing chamber with artificial lighting source and the tunnel in the greenhouse with natural light, healing and acclimatization under high CO_2 (1,000 μmol·mol^{-1}) and medium (100 μmol·m^{-2}·s^{-1}) (EM) or high PPF (150 μmol·m^{-2}·s^{-1}) (EH) condition in the healing chamber improved growth (Table 3). This result can be attributed to the increase

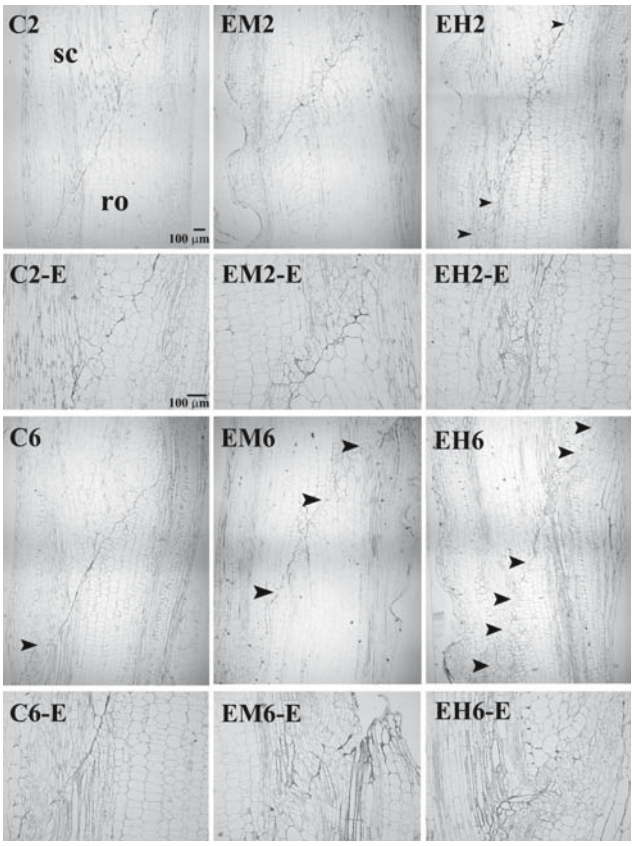

Fig. 5. Longitudinal sections through the graft union of the grafted pepper transplants healed and acclimatized in a tunnel on a greenhouse bench (C2-C6), and under high CO_2 (1,000 μmol·mol^{-1}) and medium photosynthetic photon flux (PPF) (100 μmol·m^{-2}·s^{-1}) conditions (EM2-EM6), or high CO_2 and PPF (150 μmol·m^{-2}·s^{-1}) conditions (EH2-EH6) in the healing chamber. The 2 and 6 means the cross-sections of graft union on the second and sixth day after grafting. The C-E, EM-E, and EH-E show the magnified image of the vascular bundles of graft-union. The arrow points the juncture where scion and rootstock were united.

of photosynthesis and decrease of exposure to stressful conditions.

Graft union formation was also influenced by environmental conditions during healing and acclimatization. The graft union is the part of a grafted plant where the scion is joined to the rootstock. The graft union is formed through several developmental stages: (1) development of a necrotic layer, (2) callus proliferation, (3) differentiation of new vascular tissues, and (4) a full vascular graft union formation between the scion and rootstock (Fernández-García et al., 2004; Flaishman et al., 2008; Jeffree and Yeoman, 1983).

In tomatoes, the major hydraulic connections within the graft union were reported to be functional on day 5 after grafting (Turquois and Malone, 1996). Fernández-García et al. (2004) also reported that the differentiation of the callus parenchyma to form new cambial initials and the subsequent union of the newly formed vascular strand with the original vascular bundle both in rootstock and scion begins between day 4 and 8 and is fully developed by day 15 after grafting.

In this study, graft unions healed and acclimatized under different environmental conditions were observed under a microscope on the second and sixth day after grafting. On the second day after grafting, the graft union under high CO_2 and medium PPF conditions (Fig. 5 EM2) or the tunnel in the greenhouse (Fig. 5 C2) had not formed, although the graft union under high CO_2 and PPF condition (Fig. 5 EH2) was starting to partly form.

On the sixth day after grafting, the connection of the graft union surrounding the vascular bundle was processed. The graft union that was healed and acclimatized under high CO_2 and PPF conditions (Fig. 5 EH6) had a closer connection between the scion and rootstock, compared with medium light conditions (Fig. 5 EM6) or the tunnel in the greenhouse (Fig. 5 C6).

Therefore, management of the healing and acclimatization conditions influences graft union formation, and such formation can be accelerated by improving the healing and acclimatization conditions through an increase in photosynthesis and decrease exposure to stressful conditions.

In conclusion, PPF and CO_2 concentration during healing and acclimatization affect the photosynthesis and growth of the grafted pepper transplants, and higher PPF and CO_2 conditions improved photosynthesis, growth of the transplants, and the graft union formation. The healing chamber enables the grower to control and to maintain more optimal conditions for grafted transplants than the tunnel in the greenhouse, resulting in the production of high-quality grafted transplants.

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