CO₂ and air circulation effects on photosynthesis and transpiration of tomato seedlings

P. Thongbai [a,*], T. Kozai [b], K. Ohyama [a,b]

[a] Graduate School of Horticulture, Chiba University, 648 Matsudo, Chiba 271-8510, Japan
[b] Center for Environment, Health and Field Sciences, Chiba University, Kashiwa-no-ha 6-2-1, Kashiwa, Chiba 277-0882, Japan

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ABSTRACT

In the daytime, a CO_2 depletion of 10–15% and air circulation of less than $0.5\,m\,s^{-1}$ often occur in a naturally ventilated greenhouse during a sunny day with high wind speed ($3–5\,m\,s^{-1}$). We, therefore, investigated the effects of moderate increase of the CO_2 concentration above the atmospheric level ($500–600\,\mu mol\,mol^{-1}$) and air circulation up to $1.0\,m\,s^{-1}$ in a growth chamber on the net photosynthetic and transpiration rates of tomato seedlings as the first step. The average net photosynthetic rates were 2.1, 1.8, and 1.6 times higher in the growth chambers with increased CO_2 concentration ($500–600\,\mu mol\,mol^{-1}$) and air circulation ($1.0\,m\,s^{-1}$), increased CO_2 concentration, and increased air circulation, respectively, compared with those in the control (no increase in CO_2 concentration ($200–300\,\mu mol\,mol^{-1}$) or air circulation ($0.3\,m\,s^{-1}$). The transpiration rate increased with increased air circulation, while it decreased with increased CO_2 concentration regardless of air circulation. From the results, we consider that increasing the CO_2 concentration and/or air circulation in ventilated greenhouses up to the outside concentration ($350–450\,\mu mol\,mol^{-1}$) and $1.0\,m\,s^{-1}$, respectively, can significantly increase the net photosynthetic rate of greenhouse plants.

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1. Introduction

In general, net photosynthetic rate increases with increasing CO_2 concentration in a range between 0 and $1000\,\mu mol\,mol^{-1}$ (Allen and Amthor, 1995). Thus, the CO_2 concentration in greenhouses is often increased in the daytime up to about $1000\,\mu mol\,mol^{-1}$ ('CO₂ enrichment') to promote photosynthesis and plant growth in greenhouses with the vents closed (Hand et al., 1993; Nederhoff and Vegter, 1994; Aikman, 1996; Ceulemans et al., 1997). On the other hand, roof and/or side vents need to be opened ('natural ventilation') or fans need to be turned on ('forced ventilation') in the daytime to keep the air temperature or water vapor pressure deficit (VPD) at optimal values in the greenhouse when solar radiation and/or air temperature inside are high. Recently, however, there have been some reports on controlling the air temperature and VPD in the greenhouse by descending fog technology, which are useful to reduce the need for natural ventilation also, thus allowing for higher CO_2 concentrations to be maintained in the greenhouse (Ohyama et al., 2008; Stanghellini and Kempkes, 2008).

However, when the greenhouse is ventilated, CO_2 enrichment for keeping the CO_2 concentration inside higher than that outside

is not practical, because a considerable amount of CO_2 would be released to the outside, causing both a high CO_2 cost and emission of CO_2, a global-warming gas. Thus, CO_2 is usually enriched only when no ventilation is conducted in the early morning and late afternoon (except for many greenhouses using a co-generating system where much CO_2 is exhausted to the outside during heating and/or power generation) (Hand, 1984; Sanchez-Guerrero et al., 2005). On the other hand, CO_2 concentration during the midday in a ventilated greenhouse with fully-grown plants is approximately $50–60\,\mu mol\,mol^{-1}$ lower than that outside (Sanchez-Guerrero et al., 2005), even though CO_2 gas from the outside flows into the inside through the greenhouse vents. It indicates that the depletion of CO_2 in ventilated greenhouses limits the net photosynthetic rate of the plants. Thus, CO_2 gas should be supplied into the ventilated greenhouse and maintained at the similar concentration as that outside ($350–450\,\mu mol\,mol^{-1}$) ('zero or null balance CO_2 enrichment'). This approach is practical when ventilation in the greenhouse is needed.

The net photosynthetic rate also increases with increasing air circulation over leaves in a range between 0 and $0.8\,m\,s^{-1}$ when the stomata are kept open (facing no water stress) (Kitaya et al., 2004; Yabuki, 2004). Also, the transpiration rate increases with increasing air circulation within the range of $0–1.0\,m\,s^{-1}$ (Kitaya et al., 2003). This is because the air circulation reduces the leaf boundary layer resistance of CO_2 and H_2O (water vapor) fluxes. The net photosynthetic and transpiration rates can increase with

* Corresponding author. Tel.: +81 47 137 8114; fax: +81 47 137 8114.
E-mail address: purnthongbai@graduate.chiba-u.jp (P. Thongbai).

Table 1
CO_2 concentration and air circulation in each treatment.

Treatment code	CO_2 concentration (μmol mol^{-1})[a] during photoperiod	Air circulation (m s^{-1})
LL[b] (control)	Low (200–300)	Low (0.3)
LH	Low (200–300)	High (1.0)
HL	High (500–600)	Low (0.3)
HH	High (500–600)	High (1.0)

[a] For the CO_2 concentration in each treatment during the photoperiod, see Fig. 1(a).
[b] For treatment code, L and H on the left represent low and high CO_2 concentration, respectively; L and H on the right represent low and high air circulation, respectively.

increasing air circulation until reaching the optimum level (Shibuya and Kozai, 1998; Kitaya et al., 2003). The optimum air circulation for the net photosynthetic and transpiration rates depends on the plant species, structure of plant community, plant canopy depth, and wind direction with plant position in the greenhouses, etc. (Wadsworth, 1959; Morse and Evans, 1962; Shibuya and Kozai, 1998; Kitaya et al., 2000; Sase, 2006). On the other hand, insufficient air circulation above the plant canopy causes limited gas exchange because of increased leaf boundary layer resistance (Kim et al., 1996; Kitaya et al., 1998).

Despite the fact that the main purpose of vent opening is to optimize the air temperature and relative humidity, ventilation still increases air circulation in a greenhouse. Many greenhouses with roof and/or side vents are naturally ventilated driven by pressure differences created at the vent openings either by the wind or by temperature differences (Mistriotis et al., 1997). Thus, the air circulation in naturally ventilated greenhouses is related to the degree of air exchange between the interior air of the greenhouse and its external environment due to the wind and temperature effects (Wang et al., 1999). Moreover, the air circulation in the greenhouses declines because of its reduction with canopy depth and distance from the vents (Sase, 2006). Thus, in order to increase the air circulation uniformly in the greenhouses, air mixing fans are needed.

The objective of this study was to investigate the effects of moderately increased CO_2 concentration and air circulation in ventilated greenhouses on net photosynthetic and transpiration rates. As the first step, the experiment was conducted using tomato seedlings in small plant growth chambers in a laboratory.

2. Materials and methods

2.1. Plant material preparation

Seeds of tomato (*Solanum lycopersicum* cv. Momotaro) were sown and kept at the air temperature of 28 °C for germination for 4 days in 72-cell plug seedling trays (W 270 mm × L 550 mm, Takii Co., Ltd., Japan) containing a mixture of peat moss and vermiculite (Napura Yodo, Yanmar Agricultural Equipment Co., Ltd., Tokyo, Japan).

Four days after sowing (DAS), the trays with germinated seeds were moved to a closed transplant production chamber kept at 250 μmol m^{-2} s^{-1} PPF provided by white fluorescent lamps, 14 h d^{-1} photoperiod, and 25 and 17 °C air temperatures during photo- and dark-periods, respectively. Sub-irrigation was applied once a day automatically with a commercial nutrient solution (Enshi Standard, Otsuka Chemical Co., Japan).

2.2. Experimental design, setup and conditions

Four treatments were designed by a combination of two levels of CO_2 concentration and air circulation (Table 1). Low CO_2 con-

centration (200–300 μmol mol^{-1}) and air circulation (0.3 m s^{-1}) were designed to simulate those conditions in a greenhouse. High CO_2 concentration (500–600 μmol mol^{-1}) and air circulation (1.0 m s^{-1}) were designed to simulate those conditions in a greenhouse with increased CO_2 concentration and increased air circulation by air mixing fans. For treatment code abbreviation, high and low CO_2 concentrations were abbreviated to H and L, respectively on the first letter, whereas high and low air circulations were abbreviated to H and L, respectively on the second letter.

During the photoperiod, CO_2 gas was supplied into the growth chamber at a flow rate of 2.8 ml min^{-1} (14.0 μmol CO_2 m^{-2} s^{-1}) in treatments HL and HH. Fans (DC 12 V 1.56 W, PWM fan CFY-90P, AINEX) were installed to increase the air circulation in the growth chamber in treatments LH and HH.

Seedlings, each with four true leaves (fresh weight: 2.25 ± 0.14 g, dry weight: 0.23 ± 0.014 g, height: 10 ± 0.2 cm, LAI (leaf area index): 2.8 ± 0.1), were selected 16 DAS and kept for 3 days in the growth chambers (MIR-153, Sanyo Electric Biomedical Co., Ltd., Japan). The air movement in growth chambers with and without fans is moved in the horizontal direction. Each growth chamber holding one tray with 72 seedlings was maintained at 25 °C air temperature, 300 μmol m^{-2} s^{-1} PPF measured at the tray surface, and 16 h d^{-1} photoperiod. The air temperature of the laboratory room, where the growth chambers were placed, was set at 25 °C. The CO_2 concentration in the laboratory room ranged between 400 and 500 μmol mol^{-1}. A commercial nutrient solution (N:P:K = 6:10:5) was supplied at a fixed volume to each tray prior to the photoperiod.

2.3. Measurements

Air circulation in the growth chamber was measured by using a hot-wire anemometer (Climomaster 6522, Kanomax Japan Inc., Japan) and expressed as an average of 10 measured points. Air temperature and relative humidity inside and outside the growth chambers were measured with thermo recorders (RS-12, Espec Mic Corp. Aichi, Japan). CO_2 concentrations inside and outside the growth chambers were measured with infra-red gas analyzers (model GMP 222, Vaisala Oyj, Helsinki, Finland). The data were recorded every minute by a data logger (midi logger GL800, Graphtec Corp., Yokohama, Japan). The thermo recorders and the gas analyzers were calibrated before the experiment.

2.4. Estimation

2.4.1. Net photosynthetic rate
Net photosynthetic rate, P_n (μmol CO_2 m^{-2} s^{-1}), was estimated according to the equation developed by Fujiwara et al. (1987):

$$P_n = \frac{\{kNV(C_{out} - C_{in}) + S\}}{A} \tag{1}$$

where k is the conversion factor of CO_2 from volume to molecular weight (40.9 mol m^{-3} at 25 °C); N is the number of air exchanges (s^{-1}) of the growth chamber; V is the air volume (0.106 m^3) of the growth chamber; C_{in} and C_{out} are the CO_2 concentration (μmol mol^{-1}) inside and outside the growth chamber under steady state conditions during the photoperiod, respectively; S is the supply rate of CO_2 (mol s^{-1}); and A is the area of the tray (0.149 m^2).

2.4.2. Transpiration rate
Transpiration rate, T_r (mmol H_2O m^{-2} s^{-1}), was estimated by the following equation:

$$T_r = \frac{[iNV(AH_{in} - AH_{out})]}{M_w A} + DW \tag{2}$$

where i is the volume of 1 kg of dry air (0.83 m^3 at 20 °C and 1 atm); AH_{in} and AH_{out} are the absolute humidity (g kg^{-1} dry air) inside and

outside the growth chamber under steady state conditions during the photoperiod; M_w is the molecular weight of water (g mol^{-1}); and DW is the weight of water drained from the growth chamber during the photoperiod (mol m^{-2} s^{-1}).

2.4.3. Diffusive resistances of CO_2 and H_2O gases

Diffusive resistances of CO_2 and H_2O gases, R_p and R_t (h m^{-1}), respectively, were estimated using the following equations (Yabuki, 2004):

$$R_p = k \frac{C_{out} - C_{in}}{P_n} \tag{3}$$

$$R_t = i \frac{AH_{out} - AH_{in}}{T} \tag{4}$$

where R_p is the total resistance of the leaf boundary layer, stomata and mesophyll and R_t is the total resistance of the leaf boundary layer and stomata.

2.4.4. Number of air exchanges per hour

Number of air exchanges per hour, N (h^{-1}), was estimated according to Kozai et al. (1986). The number of air exchanges in treatments HH, HL, LH and LL were 2.1, 3.7, 6.1 and 3.2 h^{-1}, respectively.

2.5. Statistical analysis

The entire experiment was repeated 3 times. In each replication, one tray with 72 seedlings (483 seedlings/m^2) was measured. The 30 min running average of the net photosynthetic and transpiration rates during the photoperiod was tested by analysis of variance (ANOVA). The Tukey–Kramer test at $p = 0.05$ was used to test for significant differences among treatments.

3. Results

3.1. CO_2 concentration

The CO_2 concentrations in the growth chambers during the photoperiod in treatments of low CO_2 concentration with high or low air circulation (LH and LL, respectively) were approximately 160–220 μmol mol^{-1} lower than those in the laboratory where the growth chambers were (460 μmol mol^{-1}), whereas those in treatments of high CO_2 concentration with high or low air circulation (HH and HL, respectively) were approximately 90–110 μmol mol^{-1} higher than those in the laboratory (Fig. 1(a)). The average CO_2 concentrations for 3 days were 243 in treatment LL, 273 in LH, 545 in HL and 569 μmol mol^{-1} in HH; the difference in average CO_2 concentration between high and low CO_2 concentration treatments was about 300 (=(545 + 569)/2 − (243 + 273)/2) μmol mol^{-1}.

3.2. Net photosynthetic rate (P_n)

Table 2 shows the daily significant effects of CO_2 concentration, air circulation and their interaction on P_n. The daily effect of CO_2 concentration on P_n was more significant than that of air circulation and the interaction, respectively. The result of P_n shows that P_n was significantly higher in treatments HH, HL and LH than in treatment LL (Fig. 1(b)). The average P_n for 3 days in treatment HH was 1.2, 1.3, and 2.1 times higher than that in treatments HL, LH, and LL, respectively. P_n in treatment HL was 1.1 and 1.8 times higher than that in treatment LH and LL, respectively. P_n in treatment LH was 1.6 times higher than that in treatment LL. P_n on the third day in treatments LL, LH, HL and HH were 1.5, 1.6, 1.5 and 1.2 times, respectively, compared with P_n on the first day.
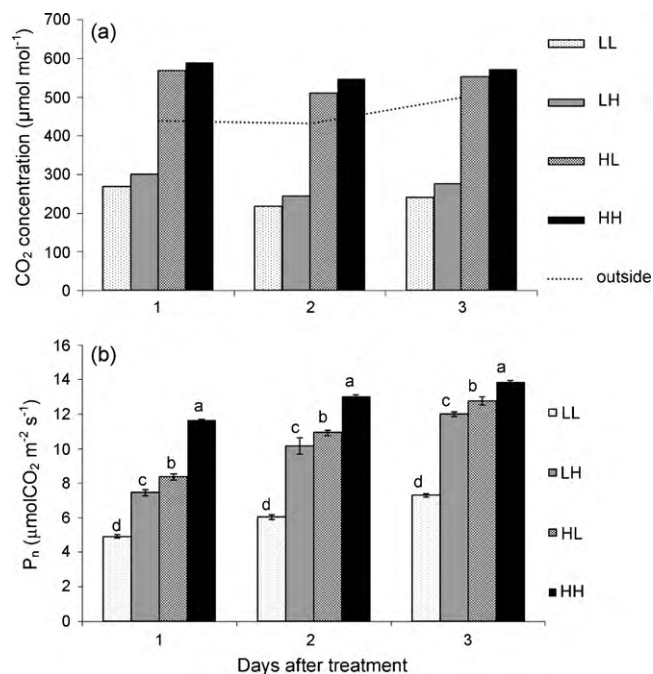

Fig. 1. CO_2 concentration inside the growth chamber (a) and net photosynthetic rate (P_n) (b) of tomato plants during the photoperiod. For treatment codes, see Table 1. Each bar represents mean ± standard deviation. P_n with different letters are significantly different at $p = 0.05$ by the Tukey–Kramer test.

3.3. Air temperature (TEMP), relative humidity (RH), absolute humidity (AH) and vapor pressure deficit (VPD)

TEMP in each treatment was approximately 0.3 °C different from that in other treatments throughout the experiment (data not shown). The RH and AH in treatment LH were higher than those in the other treatments, whereas VPD in treatment LH was lower than that in the other treatments. On the other hand, the RH and AH in treatment HH were lower than those in the other treatments, whereas VPD in treatment HH was higher than that in the other treatments. In treatments LL and HL, the RH, AH and VPD were almost similar to each other.

3.4. Transpiration rate (T_r)

The daily significant effects of CO_2 concentration, air circulation and their interaction on T_r shown in Table 3 indicate that the daily effect of CO_2 concentration on T_r was more significant than that of the interaction and air circulation, respectively. Fig. 3 shows T_r as affected by CO_2 concentration and air circulation that the daily average T_r was significantly different among treatments. T_r was considerably higher in treatments LH and LL than in treatments HL and HH. However, T_r on the third day in all treatments was about 1.4 times compared with that on the first day.

3.5. Diffusive resistances of H2O gas (Rt, leaf boundary layer resistance plus stomata resistance) for transpiration rate and CO2 gas (Rp, Rt plus mesophyll resistance) for net photosynthetic rate

R_p was almost equal to R_t in treatments LL and LH throughout the experiment, whereas the average R_p for 3 days was 3.4 and 5.8 times lower than the average R_t for 3 days in treatments HL and HH, respectively (Fig. 4). R_t in treatment HH was 1.8 times higher than that in treatment HL, whereas R_p in treatment HH was almost equal to that in treatment HL throughout the experiment. R_p in treatments HH and HL decreased with time, whereas R_p in treat-

Table 2
The 30 min running average of net photosynthetic rate (P_n) during the photoperiod on days 1–3 after treatment as affected by CO_2 concentration (μmol mol^{-1}) and air circulation (m s^{-1}).

ANOVA					
Days after treatment	Factor	d.f.	SS	MS	F
1	CO_2	1	7.78×10^7	7.78×10^7	1.66×10^{5}[***]
	Air circulation	1	4.53×10^7	4.53×10^7	9.64×10^{4}[***]
	$CO_2 \times$ air circulation	1	7.30×10^5	7.30×10^5	1.55×10^{3}[***]
	Error	844	3.96×10^5	4.70×10^2	
	Total	847	1.24×10^8		
2	CO_2	1	7.99×10^7	7.99×10^7	4.53×10^{4}[***]
	Air circulation	1	5.17×10^7	5.17×10^7	2.93×10^{4}[***]
	$CO_2 \times$ air circulation	1	5.52×10^6	5.52×10^6	3.14×10^{3}[***]
	Error	844	1.49×10^6	1.76×10^3	
	Total	847	1.39×10^8		
3	CO_2	1	7.17×10^7	7.17×10^7	1.27×10^{5}[***]
	Air circulation	1	4.47×10^7	4.47×10^7	7.91×10^{4}[***]
	$CO_2 \times$ air circulation	1	1.72×10^7	1.72×10^7	3.05×10^{4}[***]
	Error	844	4.77×10^5	5.65×10^2	
	Total	847	1.34×10^8		

d.f., degree of freedom.
[***] Two-way analysis of variance (ANOVA) ($p < 0.001$).

ments LL and LH, and R_t in all treatments were constant throughout the experiment.

4. Discussion

4.1. CO_2 concentration

The depletion of CO_2 concentration in treatments LH and LL (Fig. 1(a)) indicates that P_n of the plants in the growth chamber is limited by the low CO_2 concentration. Also, P_n of the plants in the ventilated greenhouse is limited by the CO_2 depletion, even though the vent opening helps to supply CO_2 into the greenhouse (Sanchez-Guerrero et al., 2005). Additionally, the CO_2 within the plant canopy can also be a source for photosynthesis (Tartachnyk and Blanke, 2007). The concentration varies from large at the bottom, where the growing substrate respires day and night, to low

at the top, where the vents replenish the CO_2. However, a suitable method still needs to be developed to increase P_n in ventilated greenhouses as well as in the growth chamber, with a minimum CO_2 supply rate.

In this study, the CO_2 concentrations in treatments HH and HL higher than those outside are not practical for a naturally ventilated greenhouse due to the excess release of CO_2 into the outside. Thus, a CO_2 gas supply system is significantly needed to use for maintaining the CO_2 concentration inside the naturally ventilated greenhouse at the similar concentration as that outside with a suitable method in order to increase P_n.

4.2. Net photosynthetic rate (P_n)

Ziska et al. (1991) reported that all C_3 species show significant increases in P_n under increased CO_2 concentrations compared with

Table 3
The 30 min running average of transpiration rate (T_r) during the photoperiod on days 1–3 after treatment as affected by CO_2 concentration (μmol mol^{-1}) and air circulation (m s^{-1}).

ANOVA					
Days after treatment	Factor	d.f.	SS	MS	F
1	CO_2	1	8.44×10^5	8.44×10^5	1.74×10^{6}[***]
	Air circulation	1	4.47×10^4	4.47×10^4	9.21×10^{4}[***]
	$CO_2 \times$ air circulation	1	1.26×10^5	1.26×10^5	2.59×10^{5}[***]
	Error	844	4.10×10^2	4.86×10^{-1}	
	Total	847	1.02×10^6		
2	CO_2	1	2.24×10^6	2.24×10^6	9.83×10^{5}[***]
	Air circulation	1	3.25×10^3	3.25×10^3	1.43×10^{3}[***]
	$CO_2 \times$ air circulation	1	1.14×10^5	1.14×10^5	5.02×10^{4}[***]
	Error	844	1.92×10^3	2.28	
	Total	847	2.35×10^6		
3	CO_2	1	2.50×10^6	2.50×10^6	1.03×10^{7}[***]
	Air circulation	1	2.14×10^3	2.14×10^3	8.84×10^{3}[***]
	$CO_2 \times$ air circulation	1	9.55×10^3	9.55×10^3	3.94×10^{4}[***]
	Error	844	2.05×10^2	2.42×10^{-1}	
	Total	847	2.51×10^6		

d.f., degree of freedom.
[***] Two-way analysis of variance (ANOVA) ($p < 0.001$).

those under the atmospheric CO_2 concentration. Kitaya et al. (2003) demonstrated that P_n of tomato seedling canopies increases with increasing air circulation from 0.01 to 1.0 m s^{-1}. The higher P_n in treatments HH, HL and LH was obtained as shown in Fig. 1(b). These results can be explained by the fact that the resistance values R_p are lower in treatments HH, HL and LH than in treatment LL (Fig. 4).

An interesting point is that the increased air circulation from 0.3 to 1.0 m s^{-1} in treatment LH provides a similar effect of increased CO_2 concentration from 273 to 545 μmol mol^{-1} on P_n in treatment HL. These results suggest that an increase in air circulation is an alternative method to increase P_n, and that increases in CO_2 concentration and air circulation at the same time very effectively increase P_n.

Application to the greenhouse, a method to increase the air circulation (air speed) affecting the uniformity of greenhouse environment and consequently, the uniformity of plant growth and quality is considerable. The air circulation in naturally ventilated greenhouses is affected by the shape, size (area) and arrangement of vent opening, distance from then vents as well as external wind speed and direction (Wang et al., 1999; Sase, 2006; Baeza et al., 2008). Moreover, the plant arrangement including plant density and canopy structure in the greenhouse also affects the interior airflow and the consequent ventilation performance (Sase, 2006). Generally, during the daytime the interior air circulation of naturally ventilated greenhouse is less than 0.5 m s^{-1}, even if the external wind speed is high (Wang et al., 1999). This restricts the gas exchange between the plants and ambient air. Thus, it is possible to increase the air circulation in greenhouses and keep it in uniformity by using air mixing fans.

4.3. Transpiration rate (T_r)

T_r increased with increased air circulation in treatment LH (Fig. 3). This agrees with the results of Kitaya et al. (2003) that T_r of sweet potato increases with increasing air circulation ranging from 0.01 to 1.0 m s^{-1}. This is because an increase in the air circulation decreases the diffusive resistances, especially the leaf boundary layer resistance.

On the contrary, T_r decreased with increased CO_2 concentration in treatments HH and HL (Fig. 3). This is because R_t is higher than R_p in treatments HH and HL (Fig. 4). These results demonstrate that the plants in treatments HH and HL allow CO_2 gas to diffuse into the leaves, while they prevent H_2O gas from being released in order to protect themselves from dehydration (Sharkey, 1984; Field et al., 1995; Drake et al., 1997).

Note that at high CO_2 concentration, the higher the air circulation is, the less the plants transpire as shown in Fig. 3. This means that stomatal resistance plays an important part in T_r at high CO_2 concentration, even though the leaf boundary layer resistance is decreased by increasing the air circulation.

4.4. Effects of increased CO_2 concentration on P_n and T_r

An increase in the CO_2 concentration increases P_n, but decreases T_r (Figs. 1(b) and 3). This result contrasts with the study of Tartachnyk and Blanke (2007), in which the increased P_n with increasing the light intensity at the CO_2 concentration of 500 μmol mol^{-1} was associated with a concomitant increased T_r. The results of the increased P_n but decreased T_r under the high CO_2 concentration of this study can be explained by the fact that the intercellular CO_2 concentration (C_i) of the plants grown in the present atmosphere increases when the atmospheric CO_2 concentration (C_a) increases, and that C_i is maintained at 0.7 C_a ($C_i = 0.7 C_a$), even when C_a is varied (Drake et al., 1997). This means that C_i rises due to an increase in C_a, and then increases P_n (Drake et al., 1997; Aphalo and Jarvis, 1993) despite the fact that the stomatal
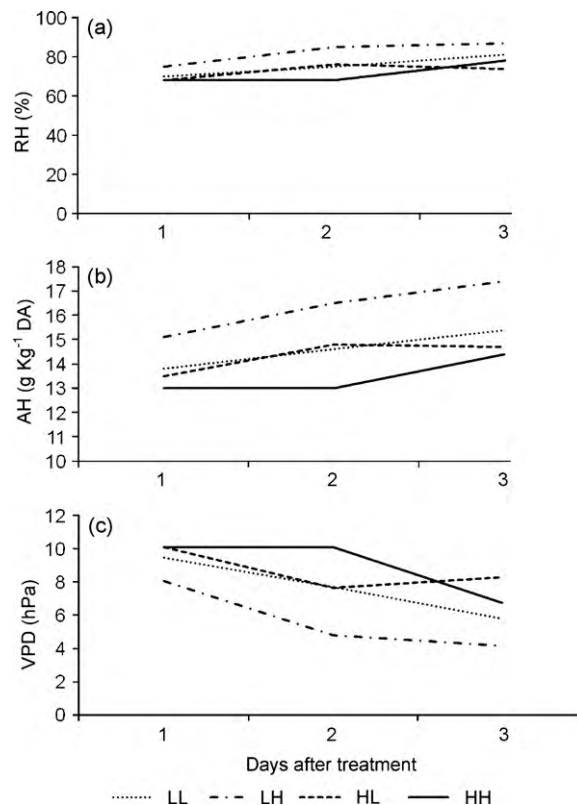

Fig. 2. Time courses of (a) relative humidity (RH), (b) absolute humidity (AH) and (c) vapor pressure difference (VPD) inside the growth chambers under steady state conditions during the photoperiod. Air temperature in each treatment was 25 ± 0.3 °C. For treatment codes, see Table 1.

resistances increase due to reductions in the stomatal apertures (Morison, 1987; Field et al., 1995; Drake et al., 1997). Field et al. (1995) observed the stomatal apertures of 28 plant species and found that the apertures reduced by 20% with increased CO_2 concentration. Thus, an increase in the stomatal resistance causes a reduction in T_r (Jarvis et al., 1999). Additionally, at low C_i, the mesophyll capacity is insensitive to changes in T_r, whereas the mesophyll capacity is sensitive to changes in T_r at high C_i (Sharkey, 1984).

4.5. Effects of increased air circulation on P_n and T_r

When CO_2 level is not increased, P_n and T_r increase with increased air circulation as shown in treatment LH (Figs. 1(b) and 3). This agrees with the results of Shibuya and Kozai (1998) that P_n and the evapotranspiration rate of a canopy of tomato seedlings under an air circulation of 0.6 m s^{-1} were, respectively, 1.9 and 1.4 times those under 0.1 m s^{-1}. Kitaya et al. (2003) also found that P_n and T_r increase significantly as the air circulation increased from 0.01 to 0.2 m s^{-1} in a controlled ecological life support system (CELSS). An appropriate air circulation promotes P_n and T_r by increasing the gas exchanges between the plants and the ambient air (Yabuki and Miyagawa, 1970; Monteith and Unsworth, 1990; Jones, 1992; Yabuki, 2004), which is important to decrease the leaf boundary layer resistance (Martin et al., 1999; Kitaya, 2005). Additionally, the total diffusive resistance of cucumber leaf to water vapor and CO_2 also decreases at lower VPD and higher air circulation (Yabuki and Miyagawa, 1970). This is similar to the conditions in treatment LH as shown in Fig. 2(c).

At increased CO_2 level, P_n increases with increased air circulation, whereas T_r decreases with increased air circulation as shown in treatment HH (Fig. 1(b) and Fig. 3). These will be described below.
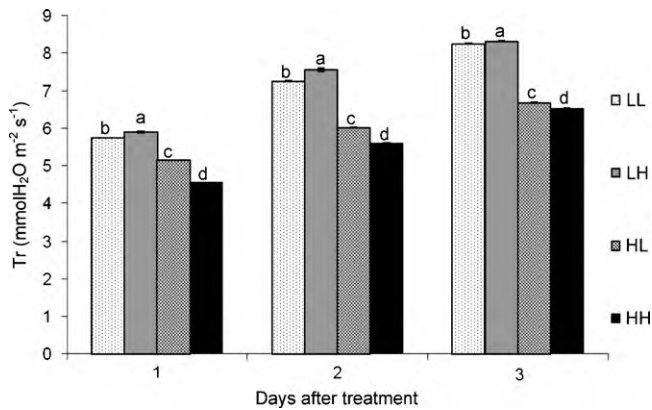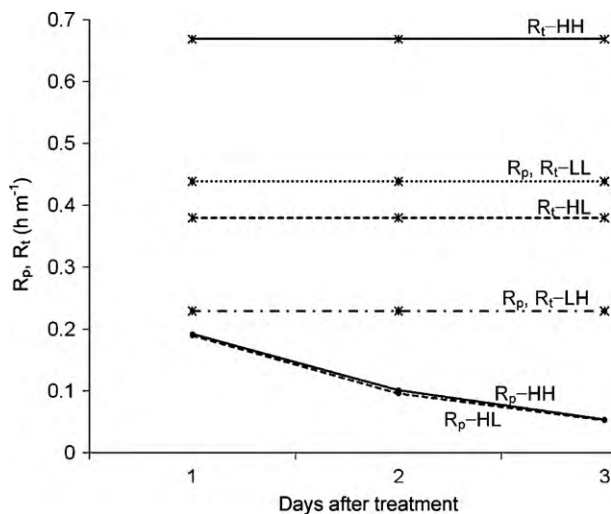
Fig. 3. Transpiration rate (T_r) during the photoperiod as affected by CO_2 concentration (μmol mol^{-1}) and air circulation (m s^{-1}). For treatment codes, see Table 1. Each bar represents mean $\pm$ standard deviation. T_r with different letters are significantly different at $p = 0.05$ by the Tukey–Kramer test.

4.6. Effects of increased CO_2 concentration and increased air circulation on P_n and T_r

Increases in the CO_2 concentration and air circulation, respectively, cause an increase in C_i and decrease in the leaf boundary layer resistance, thereby increasing P_n. This agrees with the results of Kitaya et al. (2004) that P_n under the CO_2 concentration of 800 μmol mol^{-1} was 1.2 times higher than that under 400 μmol mol^{-1} at air circulation ranging from 0.1 to 0.8 m s^{-1}.

On the other hand, combined increases in the CO_2 concentration and air circulation result in decreased T_r (Fig. 3). This is explained by the fact that although the air circulation significantly decreases the leaf boundary layer resistance more than the stomatal resistance (Aphalo and Jarvis, 1993), at increased CO_2 concentration, the stomata respond to a carbon fixing substrate pool and optimize the water loss of the carbon gain by keeping the water loss at a minimum (Cowan, 1977; Cowan and Farquhar, 1977; Andrew and William, 1998). Moreover, the stomatal responses to VPD allow them to guard against excessive water loss. T_r is lower at high VPD than at low VPD (Sharkey, 1984). Between the effects of increased VPD and increased CO_2 concentration, the stomatal resistance rises sufficiently to reduce T_r (Aphalo and Jarvis, 1993). This is similar to the conditions in treatment HH as shown in Fig. 2(c).

Fig. 4. Time courses of the total resistance of the leaf boundary layer, stomata and mesophyll (R_p) and the total resistance of the leaf boundary layer and stomata (R_t) during the photoperiod as affected by CO_2 concentration (μmol mol^{-1}) and air circulation (m s^{-1}). For treatment codes, see Table 1.

From this study, we observed that increased CO_2 concentration has the added advantage of water use efficiency (WUE; the ratio of P_n to T_r per unit leaf area) which is important for modern agricultural plant production, especially in semiarid areas and areas where there is a shortage of available water. This agrees with the finding of previous studies that increased CO_2 concentration increases WUE due to reduced T_r (Arp, 1991; Polley et al., 1993; Drake et al., 1997).

5. Conclusion

The present study showed that increasing the air circulation from 0.3 to 1.0 m s^{-1} had a similar effect as increasing the CO_2 concentration from 273 to 545 μmol mol^{-1} to raise the net photosynthetic rate by 62–76%. Increasing the CO_2 concentration from 273 to 569 μmol mol^{-1} and air circulation from 0.3 to 1.0 m s^{-1} at the same time increased the net photosynthetic rate by 111%. We can use these results to increase the net photosynthetic rate of plants by maintaining the CO_2 concentration inside a ventilated greenhouse at the similar concentration as that outside and/or increasing the air circulation to 1.0 m s^{-1} using air mixing fans. Moreover, the present study showed that increasing the CO_2 concentration increases the water use efficiency regardless of air circulation. Further study is needed to verify these findings using plants grown in an actual greenhouse. The study also showed that the net photosynthetic rate of plants in the plant growth chamber is limited due to the CO_2 concentration being about 160–220 μmol mol^{-1} lower than the outside CO_2 concentration (460 μmol mol^{-1}) and/or relatively low air circulation.

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