

**PROMOTING NET PHOTOSYNTHESIS AND CO<sub>2</sub> UTILIZATION EFFICIENCY BY MODERATELY INCREASED CO<sub>2</sub> CONCENTRATION AND AIR CURRENT SPEED IN A GROWTH CHAMBER AND A VENTILATED GREENHOUSE**

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**ABSTRACT**

CO<sub>2</sub> gas was supplied at a rate of 0 or 3 mL min<sup>-1</sup> to the growth chamber with 2-3 air exchanges per hour, and an air current speed of 0.3 m s<sup>-1</sup> inside the growth chamber, in which tomato seedlings were placed. The CO<sub>2</sub> concentration in the chamber decreased from 790 on day 1 to 440 μmol mol<sup>-1</sup> on day 7 when CO<sub>2</sub> was supplied, whereas it decreased from 330 to 200 μmol mol<sup>-1</sup> in the control, where CO<sub>2</sub> was not supplied, and ambient CO<sub>2</sub> concentration was 410 μmol mol<sup>-1</sup>. On day 7, the net photosynthetic rate and leaf area index were 2.5 and 1.3 times, respectively, higher in the treated than in the control, and 96% of CO<sub>2</sub> supplied to the chamber was fixed by plants for photosynthesis. The net photosynthetic rate also increased significantly when the air current speed changed from 0.3 to 1.0 m s<sup>-1</sup> without supply of CO<sub>2</sub>. These results suggest that in a fully ventilated greenhouse in a hot climate region, the net photosynthetic rate may increase either by making the CO<sub>2</sub> concentration close to the ambient or by increasing the air current speed up to 1.0 m s<sup>-1</sup>.

**Key words:** air circulation, net assimilation rate, null balance CO<sub>2</sub> enrichment, plant growth, semi-closed plant production system

**INTRODUCTION**

Recently, greenhouses such as plastic house and net house have been widely adapted in Southeast Asian countries to protect crops from environmental pressures, such as severe weather and insects and birds, and for promoting plant growth (Bacusmo, 2009; Sharma, 2009). During the daytime, when the solar radiation and air temperature inside the greenhouse are high, the roof and/or side vents of the greenhouse need to be opened primarily to avoid the high temperature stress to plants (Adaros, 1984). Even though the vent opening also helps to supply ambient CO<sub>2</sub> into the greenhouse, the CO<sub>2</sub> concentration inside is still often 50–60 μmol mol<sup>-1</sup> lower than ambient (400 μmol mol<sup>-1</sup>) due to the photosynthetic activity of plants (Sanchez-Guerrero et al. 2005). This depletion of CO<sub>2</sub> concentration significantly reduces the net photosynthetic rate of the plants (Ohyama et al. 2005).

There have been many reports on the increased net photosynthetic rate by elevating the CO<sub>2</sub> concentration up to 700–1,000 μmol mol<sup>-1</sup> (Calvert, 1972; Enoch et al. 1976; Madsen, 1976; Besford et al. 1990; Stulen and den Hertog, 1993; Jacob et al. 1995; Sanchez-Guerrero et al. 2005). However, keeping the CO<sub>2</sub> concentration at 700–1,000 μmol mol<sup>-1</sup> is not practical for the greenhouse when the roof and/or side vents are open because its concentration is 300–600 μmol mol<sup>-1</sup> higher inside than outside and most supplied CO<sub>2</sub> escapes to the outside. Thus, a practical approach of CO<sub>2</sub> enrichment for the greenhouse with the vent opening is to increase and maintain the CO<sub>2</sub> concentration inside at

the same concentration as that outside. This means that supplied CO<sub>2</sub> does not escape to the outside, and thus all CO<sub>2</sub> supplied into the greenhouse is absorbed by the plants. By doing this, the plant photosynthesis can be promoted with minimum amount or cost for CO<sub>2</sub> enrichment and no emission of CO<sub>2</sub>, a global warming gas, to the atmosphere.

Natural ventilation is caused by pressure differences between inside and outside the greenhouse. The pressure differences are induced by two main forces, the buoyancy and wind forces (Boulard and Baille, 1995). The interior air current speed of the greenhouse is often lower than 0.5 m s<sup>-1</sup> in the daytime, even when the external wind speed is 3.0–5.0 m s<sup>-1</sup> (Wang et al. 1999). The low air current speed restricts the CO<sub>2</sub> gas exchange between the plant canopies and ambient air, resulting in the decrease of the net photosynthetic rate (Kim et al. 1996; Kitaya et al. 1998). Thus, the net photosynthetic rate can be increased by increasing the air current speed over the leaves up to the optimum level which would vary depending on the plant species, structure of plant community and other factors. (Wadsworth, 1959; Morse and Evans, 1962; Shibuya and Kozai, 1998; Kitaya et al. 2000). Many reports described that the net photosynthetic rate increases with increasing air current speed over the leaves in a range between 0 and 0.8 m s<sup>-1</sup> when the stomata are kept open, indicating no water stress (Kitaya et al. 2004; Yabuki, 2004). Thus, it is possible to increase the net photosynthetic rate of the plants in the greenhouse by increasing air current speed inside, for instance, using air mixing fans.

In the previous study, we found that a similar phenomenon, i.e., the depletion of CO<sub>2</sub> concentration and the insufficiency of air current speed occurred in a small CO<sub>2</sub> non-enriched growth chamber with artificial light, just like in the naturally ventilated greenhouse (Thongbai et al. 2010). Therefore, in the present experiment, we used growth chambers in order to develop an efficient CO<sub>2</sub> enrichment method with high CO<sub>2</sub> utilization efficiency, a method which can be applied for promoting the net photosynthetic rate and growth of the plants in a naturally ventilated greenhouse with vents open, prior to an experiment using an actual naturally ventilated greenhouse with vents open.

## **MATERIALS AND METHODS**

### **Plant material preparation**

Tomato seeds (*Solanum lycopersicum* cv. Momotaro) were sown in 72-cell plug seedling trays (W 270 mm × L 550 mm) filled with a soil mixture of peat moss (50%) and vermiculite (50%) and kept at an air temperature of 28°C for germination for 4 days. Four days after sowing (DAS), the trays with germinated seeds were moved to a closed transplant production chamber and kept at 250 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux (PPF) provided by white fluorescent lamps, 14 h d<sup>-1</sup> photoperiod, and 25 and 17°C air temperature during photo- and dark-periods, respectively.

### **Treatments, experimental setup and conditions**

The experiment was carried out in the growth chambers (MIR-153, Sanyo Electric Biomedical Co., Ltd., Japan). There were three treatments: increased CO<sub>2</sub> concentration and no increase in air current speed (0.3 m s<sup>-1</sup>) (treatment HL), increased air current speed at 1.0 m s<sup>-1</sup> and no increase in CO<sub>2</sub> concentration (treatment LH), and no increase in CO<sub>2</sub> concentration and air current speed as a control (treatment LL) (Table 1). During the photoperiod, CO<sub>2</sub> gas was supplied into the growth chamber at a flow rate of 3.0 ml min<sup>-1</sup> (2.4 and 2.8 gCO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> on days 1–3 and days 4–7 after treatment, respectively) in treatment HL. The gas flow rate was controlled by a mass flow controller (TC-1100 Tokyo Keiso Co., Ltd., Japan). Extra fans (DC 12 V 1.56 W, PWM fan CFY-90P, AINEX) were installed to increase the air current speed in the growth chamber in treatment LH.

**Table 1.** CO<sub>2</sub> concentration and air current speed in each treatment.

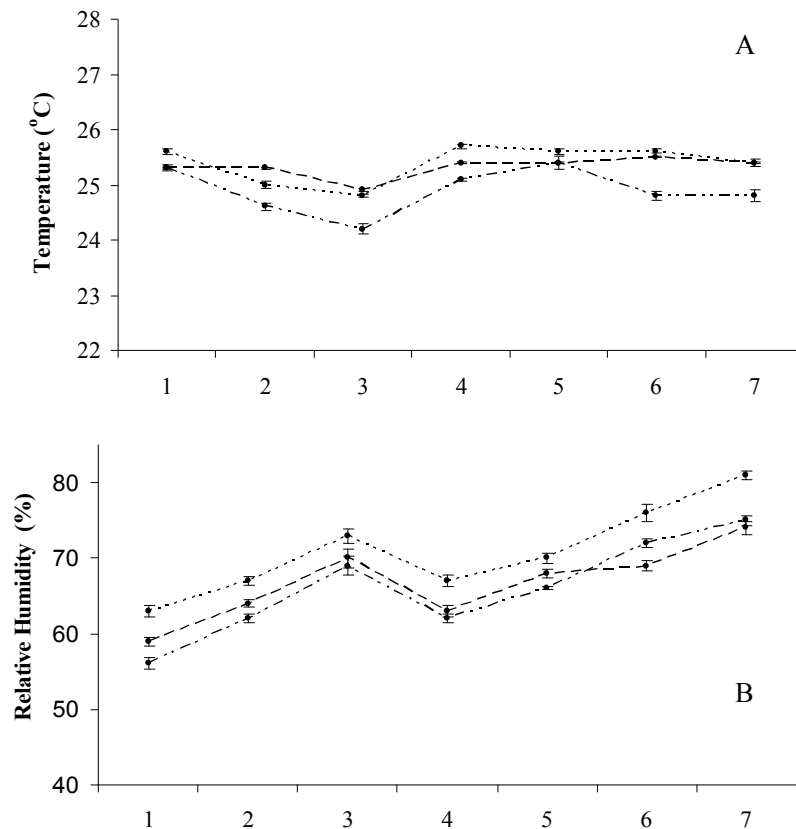
Treatment code	CO <sub>2</sub> concentration <sup>z</sup> during photoperiod	Air current speed (m s <sup>-1</sup> )
LL <sup>y</sup> (control)	Low	Low (0.3)
LH	Low	High (1.0)
HL	High <sup>x</sup>	Low (0.3)

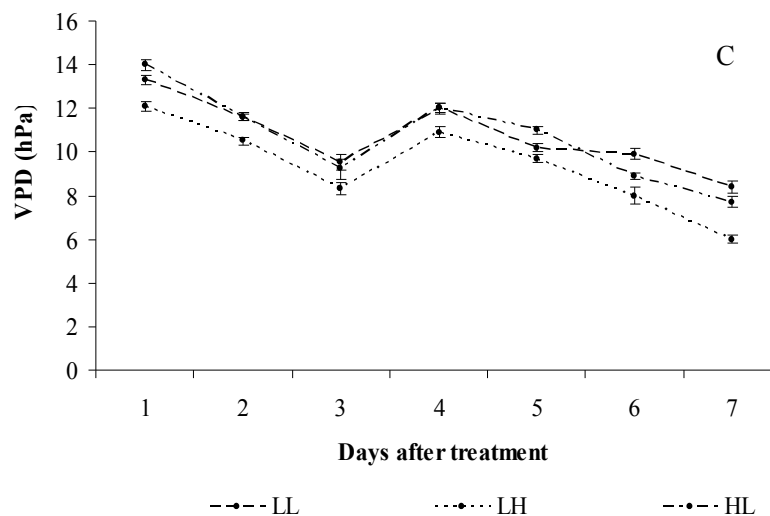
<sup>z</sup> For the CO<sub>2</sub> concentration (μmol mol<sup>-1</sup>) after treatment during the photoperiod, see Fig. 2.

<sup>y</sup> For treatment code, L and H on the left represent low and high CO<sub>2</sub> concentration, respectively; L and H on the right represent low and high air current speed, respectively.

<sup>x</sup> The CO<sub>2</sub> gas supply rate in treatment HL was constant at 3.0 ml min<sup>-1</sup>.

The seedlings at four true leaves stage (fresh weight: 297 ± 13 mg, dry weight: 23 ± 1.6 mg, height: 5.7 cm, leaf area index (LAI): 0.4 ± 0.02 m<sup>2</sup> m<sup>-2</sup>) were selected on 12 DAS (days after sowing) and kept for 7 days in the growth chambers. The condition of growth chamber holding 72 seedlings in one tray (483 seedlings/m<sup>2</sup>) was set at 25°C air temperature, 300 μmol m<sup>-2</sup> s<sup>-1</sup> PPF on average measured at the tray surface, and 16 h d<sup>-1</sup> photoperiod. The environmental conditions during the experiment are shown in Fig. 1. The air temperature of the laboratory room where the growth chambers were placed was set at 25°C. The CO<sub>2</sub> concentration in the laboratory room ranged between 400 and 500 μmol mol<sup>-1</sup>. A commercial nutrient solution (Enshi Standard, Otsuka Chemical Co., Japan) was supplied at a fixed volume to each tray prior to the photoperiod.





**Fig. 1.** Experimental conditions: (a) air temperature (TEMP), (b) relative humidity (RH) and (c) vapor pressure deficit (VPD) inside the growth chambers under steady state conditions during the photoperiod. The numbers of plants placed in each growth chamber on days 1–3 and days 4–7 after treatment were 72 and 62 plants, respectively. For treatment codes, see Table 1. Each bar represents mean  $\pm$  standard deviation. Dashed line: LL, Dotted line: LH and Chain line: HL.

### Environmental measurements

Air current speed in the growth chamber was measured by using a hot-wire anemometer (Climomaster 6522, Kanomax Japan Inc., Japan) and expressed by the 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<sub>2</sub> concentrations inside and outside the growth chambers were measured with infra-red gas analyzers (GMP 222, Vaisala Oyj, Helsinki, Finland). The data were recorded every minute by a data logger (midi logger GL800, Graphtec Corp., Yokohama, Japan).

### Growth parameter measurements and growth analyses

On days 3 and 7 (15 and 19 DAS, respectively), ten seedlings were randomly sampled from each treatment for measurement of shoot and root dry weights and leaf area. Growth analyses of the plants revealed relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), and leaf area index (LAI).

### Estimation

#### *Net photosynthetic rate*

Net photosynthetic rate,  $P_n$  (gCO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>), was estimated according to the equation developed by Fujiwara et al. (1987):

$$P_n = \{S - [k \cdot N \cdot V \cdot (C_{in} - C_{out})]\} / A \quad [1]$$

where  $k$  is the conversion factor of CO<sub>2</sub> from volume to molecular weight ( $1.96 \times 10^{-3}$  gCO<sub>2</sub> m<sup>-3</sup> at 25°C);  $N$ , the number of air exchanges per hour (h<sup>-1</sup>) of the growth chamber;  $V$ , the air volume (0.106 m<sup>3</sup>) of the growth chamber;  $C_{in}$  and  $C_{out}$ , the CO<sub>2</sub> concentrations ( $\mu\text{mol mol}^{-1}$ ) inside and outside the

growth chamber under steady state conditions during the photoperiod; S, the supply rate of CO<sub>2</sub> (g h<sup>-1</sup>); and A, the total leaf area (leaf area per plant × the number of plants in tray).

*CO<sub>2</sub> utilization efficiency (CUE)*

CO<sub>2</sub> utilization efficiency, CUE, of the growth chamber was estimated according to the equation developed by Yoshinaga et al. (2000):

$$CUE = 1 - \{ [k \cdot N \cdot V \cdot (C_{in} - C_{out})] / S \} = A \cdot P_n / S \quad [2]$$

CUE can be estimated hourly, daily or weekly. In case of daily CUE, hourly P<sub>n</sub> is summed up and divided by S.

*Number of air exchanges*

Number of air exchanges, N (h<sup>-1</sup>), was estimated according to the method described by Kozai et al. (1986). The number of air exchanges in treatments LL, LH and HL were 3.4, 4.5 and 2.1 h<sup>-1</sup>, respectively.

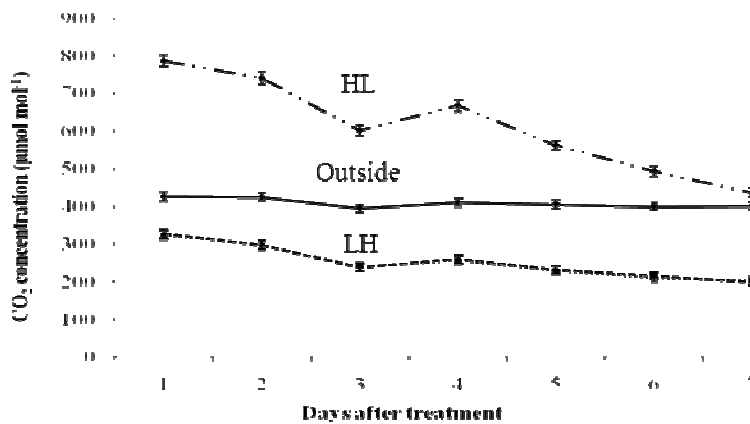
**Statistical analysis**

The entire experiment was repeated three times. In each replication, one tray with 72 seedlings was used for measurement. The data of growth parameters and growth were analyzed by analysis of variance (ANOVA). The Tukey-Kramer test at *p* = 0.05 was used to test for significant differences among treatments.

**RESULTS AND DISCUSSION**

**CO<sub>2</sub> concentration**

The CO<sub>2</sub> concentration (μmol mol<sup>-1</sup>) inside and outside the growth chambers during the photoperiod is presented in Fig. 2. The daily average of CO<sub>2</sub> concentration outside the growth chamber, C<sub>out</sub>, remained almost constant at around 410 μmol mol<sup>-1</sup>.



**Fig. 2.** CO<sub>2</sub> concentrations (μmol mol<sup>-1</sup>) inside and outside (C out) the growth chambers during the photoperiod. Chain, dashed and solid lines denote, CO<sub>2</sub> concentrations in treatment HL, LH and outside, respectively. The CO<sub>2</sub> gas supply rate in treatment HL was constant at 3.0 ml min<sup>-1</sup>. In treatment LH, CO<sub>2</sub> was not supplied. The numbers of plants placed in each growth chamber on days

1–3 and days 4–7 after treatment were 72 and 62 plants, respectively. For treatment codes, see Table 1. Each bar represents mean  $\pm$  standard deviation.

In treatment HL, the daily average of CO<sub>2</sub> concentration during the photoperiod inside the growth chamber, C<sub>HL</sub>, tended to decrease during the study period. The difference in daily average of CO<sub>2</sub> concentration during the photoperiod between C<sub>HL</sub> and C<sub>out</sub>,  $\Delta C_{HL}$ , also tended to decrease. In treatments LH and LL, compared with those in treatment HL, the daily averages of C<sub>LH</sub> and C<sub>LL</sub> tended to decrease gradually, and were almost the same as each other. On the other hand,  $\Delta C_{LH}$  and  $\Delta C_{LL}$  tended to increase gradually.

The CO<sub>2</sub> concentration in all treatments decreased from day 1 to day 3 and from day 4 to day 7 but increased from day 3 to day 4 (Fig. 2). This increase was probably caused by the decrease in the number of plants in the chamber between day 3 (72 plants) and day 4 (62 plants) since ten plants were randomly selected and used for growth analysis on day 3.

The weekly averages of C<sub>LH</sub> and C<sub>LL</sub> were approximately 150  $\mu\text{mol mol}^{-1}$  lower than the weekly averages of C<sub>out</sub> (409  $\mu\text{mol mol}^{-1}$ ), whereas the weekly average of C<sub>HL</sub> was approximately 200  $\mu\text{mol mol}^{-1}$  higher than that of C<sub>out</sub> (Fig. 2). The depletion of CO<sub>2</sub> concentration in treatments LH and LL indicates that P<sub>n</sub> increased with passage of days or the increase in LAI. On the contrary, the increase in P<sub>n</sub> was limited by the lowering CO<sub>2</sub> concentration. Similarly, P<sub>n</sub> of the plants is limited by the CO<sub>2</sub> depletion in a naturally ventilated greenhouse as LAI increases, even though the CO<sub>2</sub> gas from the outside air can flow into the inside through the vents (Sanchez-Guerrero et al. 2005). Thus, it can be said that P<sub>n</sub> of plants in naturally ventilated greenhouses is often limited by the low CO<sub>2</sub> concentration, as in growth chambers with the number of air exchanges of 3.4–4.5 h<sup>-1</sup>.

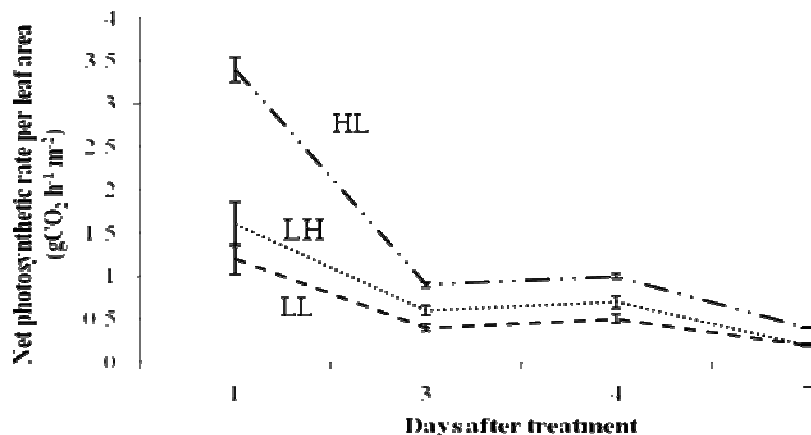
On the other hand, the higher C<sub>HL</sub> and decrease in  $\Delta C_{HL}$  during the study period are due to the constant CO<sub>2</sub> supply at the rate of 3.0 mL min<sup>-1</sup> throughout the experiment. This means that the CO<sub>2</sub> supply rate needs to be increased as the plants grow or as P<sub>n</sub> increases to keep the CO<sub>2</sub> concentration at a constant level.

### **Net photosynthetic rate (P<sub>n</sub>)**

The P<sub>n</sub> per leaf area was higher in treatments HL and LH than in treatment LL (Fig. 3). This agrees with the results of Ziska et al. (1991), Shibuya and Kozai (1998), and Thongbai et al. (2010) that P<sub>n</sub> of the plants increases with increasing the CO<sub>2</sub> concentration and/or air current speed.

The P<sub>n</sub> per leaf area in all treatments decreased from day 1 to day 3 and from day 4 to day 7 but increased slightly from day 3 to day 4 (Fig. 3). This slight increase is due to the decrease in total leaf area on day 3. On the other hand, the P<sub>n</sub> per plant and P<sub>n</sub> per floor area tended to increase with time during the study period (Yamada et al. 2000, Chintakovid and Kozai, 2000, Thongbai et al. 2010). This is because the number of plants and floor area were constant during the study period. The decrease of the P<sub>n</sub> per leaf area on days 1-3 and 4-7 can be explained by the fact that LAI increased with time during the study period (Tables 2 and 3).

The decrease in P<sub>n</sub> per leaf area with time indicates that excess LAI at a later stage of plant production is detrimental in the photosynthetic efficiency (Papadopoulos and Pararajasingham, 1997).



**Fig. 3.** Net photosynthetic rate ( $P_n$ ) per leaf area of tomato plants in each treatment during the photoperiod. Chain, dotted and dashed lines denote,  $\text{CO}_2$  concentrations in treatment HL, LH and LL, respectively. The  $\text{CO}_2$  gas supply rate in treatment HL was constant at  $3.0 \text{ ml min}^{-1}$  ( $2.4$  and  $2.8 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  on days 1–3 and days 4–7 after treatment, respectively). In treatment LH,  $\text{CO}_2$  was not supplied. For treatment codes, see Table 1. Each bar represents mean  $\pm$  standard deviation.

In treatment HL, the decrease in  $P_n$  per leaf area from day 1 to day 3 was sharper than that from day 4 to day 7. This can be explained by the fact that, from day 1 to day 3, LAI was lower than 2 so the leaves were not considerably overlapped. On the other hand, on day 4 to day 7, LAI was greater than 4, indicating that inter-shading within the canopy was intense (Asrar et al., 1984; Leverenz and Hinckley, 1990). This causes a decrease in  $P_n$  per leaf area due to a reduction in light transmission into the plant canopies (Norman and Arkebauer, 1991). Papadopoulos and Ormrod (1988) have also reported that LAI significantly relates to the light penetration into the plant canopies. Moreover, the difference in  $P_n$  per leaf area between treatments LH and LL on day 7 was close to each other compared with that on the other days. This is also due to the high LAI (approximately 5.3) in treatment LH as the same reasons as mentioned previously. Therefore, to increase the  $P_n$  of the plants in a naturally ventilated greenhouse and plant production system, the plant spacing needs to be carefully designed.

The weekly average of  $P_n$  was 1.3 times higher in treatment LH than in treatment LL (Fig. 3), even though the  $\text{CO}_2$  concentrations in these treatments were almost the same (Fig. 2). This is because an increase in the air current speed decreases the leaf boundary layer resistance, promoting gas exchange between the plant canopies and the ambient air (Yabuki and Fukui, 1981; Martin et al. 1999; Kitaya et al. 2003; Kitaya, 2005). A possible reason for the increase in  $P_n$  in treatment LH compared with  $P_n$  in treatment LL is that the number of air exchanges per hour of the growth chamber,  $N$ , in treatment LH ( $4.5 \text{ h}^{-1}$ ) was higher than that in treatment LL ( $3.4 \text{ h}^{-1}$ ). One possible explanation for the higher  $N$  in treatment LH is the increased air current speed in the growth chamber where the extra fans were installed.

Since  $P_n$  can be estimated by Eq. [1], if the  $\text{CO}_2$  concentration is lower in a greenhouse than outside, with/without a  $\text{CO}_2$  supply system,  $P_n$  can be increased by increasing the  $N$ . The same applies for a greenhouse.

**Table 2.** Effects of CO<sub>2</sub> concentration and air current speed on relative growth rate and net assimilation rate between days 3 and 7 after treatment and leaf area ratio, specific leaf area, leaf area index, dry weights of shoot and root and leaf area of tomato plants on day 7<sup>z,y</sup>.

	Treatment code			Significance <sup>w</sup>
	LL	LH	HL	
Relative growth rate (RGR, gDW g <sup>-1</sup> d <sup>-1</sup> )	0.26 ± 0.02	0.34 ± 0.02	0.35 ± 0.03	NS
Net assimilation rate (NAR, gDW m <sup>-2</sup> d <sup>-1</sup> )	6.8 ± 0.6 b	9.2 ± 0.8 ab	11.2 ± 1.1 a	*
Leaf area ratio (LAR, m <sup>2</sup> g <sup>-1</sup> )	0.041 ± 0.002 a	0.036 ± 0.001 a	0.030 ± 0.001 b	*
Specific leaf area (SLA, m <sup>2</sup> g <sup>-1</sup> )	0.058 ± 0.003 a	0.051 ± 0.002 a	0.042 ± 0.002 b	*
Leaf area index (LAI, m <sup>2</sup> m <sup>-2</sup> )	4.4 ± 0.18 b	5.3 ± 0.20 a	5.7 ± 0.20 a	*
Shoot dry weight per plant (mg)	240 ± 16.9 c	320 ± 21.9 b	420 ± 22.6 a	*
Root dry weight per plant (mg)	26 ± 2.4 c	37 ± 2.7 b	47 ± 2.4 a	*
Leaf area per plant (m <sup>2</sup> )	0.011 ± 0.0004 b	0.013 ± 0.0005 a	0.014 ± 0.0005 a	*

<sup>z</sup> Means within a row followed by different letters are significantly different at  $P \leq 0.05$  by the Tukey-Kramer test.

<sup>y</sup> Each value represents mean ± SE of 10 plants.

<sup>w</sup> NS and \* indicate non-significant and significant difference, respectively, at  $P \leq 0.05$  by the Tukey-Kramer test

### Plant growth and analysis

On day 7, dry weight significantly varied among treatments, while leaf area and LAI were not significantly different between treatments HL and LH (Table 2). These findings agree with the result that increased CO<sub>2</sub> concentration significantly increased dry weight more than leaf area (Radoglou and Jarvis, 1992). Furthermore, increased CO<sub>2</sub> concentration and increased air current speed generally increased P<sub>n</sub> resulting in increased dry weight and promoted plant growth (Knight and Mitchell, 1988; Wheeler and Tibbitts, 1997; Kitaya et al. 2003; Shibuya et al. 2006). Moreover, increase of dry weight in response to increased CO<sub>2</sub> concentration and increased air current speed has been reported for various plant varieties, i.e. tomato, cucumber, bean, etc. (Porter and Grodzinski, 1984; Mortensen, 1987; Eamus and Jarvis, 1989).

RGR was not significantly different among treatments between days 3 and 7 (Table 2). NAR was higher in treatment HL, whereas LAR and SLA were lower in treatment HL. These suggest that at increased CO<sub>2</sub> concentration, the higher values of NAR were offset by lower values of LAR which is the result of lower values of SLA (Pettersson and McDonald, 1992). A reduction in SLA with increased CO<sub>2</sub> concentration is the result of changes in leaf anatomy and leaf thickness (Radoglou and Jarvis, 1992; Farrar and Williams, 1991). An increase in leaf thickness is the result of greater cell enlargement, which is sensitive to CO<sub>2</sub> concentration (Radoglou and Jarvis, 1992). Cell enlargement affects the internal leaf surface available for absorption of CO<sub>2</sub> gas which is likely to have consequences for the photosynthesis and growth of plants (Nobel et al. 1975; Farrar and Williams, 1991; Pettersson and McDonald, 1992).



**Table 3.** Effects of CO<sub>2</sub> concentration and air current speed on relative growth rate and net assimilation rate between days 0 and 3 after treatment and leaf area ratio, specific leaf area, leaf area index, dry weights of shoot and root and leaf area of tomato plants on day 3<sup>z</sup>.

	Treatment code			Significance <sup>y</sup>
	LL	LH	HL	
Relative growth rate (RGR, gDW g <sup>-1</sup> d <sup>-1</sup> )	0.46 ± 0.02	0.46 ± 0.02	0.54 ± 0.03	NS
Net assimilation rate (NAR, gDW m <sup>-2</sup> d <sup>-1</sup> )	13 ± 0.7	13 ± 0.8	16 ± 1.4	NS
Leaf area ratio (LAR, m <sup>2</sup> g <sup>-1</sup> )	0.038 ± 0.001	0.038 ± 0.001	0.034 ± 0.002	NS
Specific leaf area (SLA, m <sup>2</sup> g <sup>-1</sup> )	0.052 ± 0.001	0.052 ± 0.002	0.047 ± 0.003	NS
Leaf area index (LAI, m <sup>2</sup> m <sup>-2</sup> )	1.7 ± 0.07	1.7 ± 0.08	1.9 ± 0.10	NS
Shoot dry weight per plant (mg)	85 ± 4.4	84 ± 5.2	109 ± 10.0	NS
Root dry weight per plant (mg)	7.5 ± 1.0	7.8 ± 0.8	9.2 ± 0.8	NS
Leaf area per plant (m <sup>2</sup> )	0.003 ± 0.0001	0.003 ± 0.0002	0.004 ± 0.0002	NS

<sup>z</sup> Each value represents mean ± SE of 10 plants.

<sup>y</sup> NS indicates non-significantly different at  $P \leq 0.05$  by the Tukey-Kramer test.

### CO<sub>2</sub> utilization efficiency (CUE)

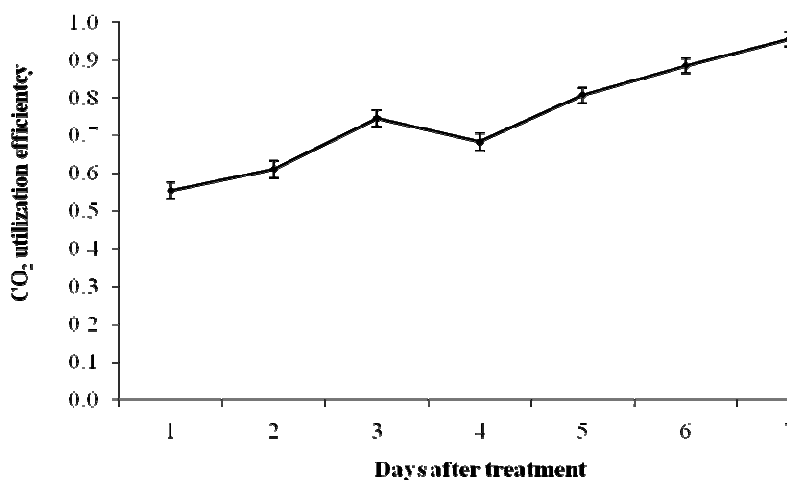
Daily average of CO<sub>2</sub> utilization efficiency in treatment HL is presented in Fig. 4. The daily average of CUE tended to increase during the study period. This can be explained by the fact that the daily average of C<sub>HL</sub> decreased with time due to the increased absorption of CO<sub>2</sub> by the plants with time, whereas the daily average of C<sub>out</sub> was almost constant and the CO<sub>2</sub> gas supply rate was fixed at 3.0 ml min<sup>-1</sup> throughout the experiment. Thus, CO<sub>2</sub> escaped to the outside decreased with time.

CUE also increases with time when the daily amount of supplied CO<sub>2</sub> is increased with increasing P<sub>n</sub> to keep the CO<sub>2</sub> concentration inside a growth chamber at a constant level throughout the experiment (Yoshinaga et al. 2000). In HL on day 7, CUE was nearly 100% because C<sub>in</sub> was almost equal to C<sub>out</sub>.

It is clear from the results that CUE in a naturally ventilated greenhouse will be nearly 100% when the CO<sub>2</sub> concentration inside is maintained at the same concentration as ambient, as is indicated by Ohyama et al. (2005). Also, in a plant production system in which the CO<sub>2</sub> concentration is maintained at a higher concentration than ambient CO<sub>2</sub> concentration, CUE can be increased by keeping the N at the minimum value.

Note that CUE increased from day 1 to day 3 and from day 4 to day 7 but decreased from day 3 to day 4. This result is related to the fact that C<sub>HL</sub> decreased from day 1 to day 3 and from day 4 to day 7 but increased from day 3 to day 4 (Fig. 2) as mentioned in section 3.1 (CO<sub>2</sub> concentration).

If C<sub>in</sub> is kept at a constant level higher than outside by increasing the CO<sub>2</sub> supply rate with time, CUE increases as P<sub>n</sub> per floor area increases, because the amount of CO<sub>2</sub> released to the outside is almost constant at the constant C<sub>in</sub>. Thus, an optimal CO<sub>2</sub> concentration must be determined by considering all the changes in CUE, P<sub>n</sub>, and cost performance of CO<sub>2</sub> supply.



**Fig. 4.** CO<sub>2</sub> utilization efficiency (CUE) of the growth chamber with a CO<sub>2</sub> supply rate of 3.0 ml min<sup>-1</sup> and the number of air exchanges of 2.1 h<sup>-1</sup>. The numbers of plants placed in the growth chamber on days 1–3 and days 4–7 after treatment were 72 and 62 plants, respectively. Each bar represents mean ± standard deviation. For definition of CUE, see Eq. (2) in the text.

## CONCLUSION

In a plant growth chamber, with 3-5 air exchanges per hour, CO<sub>2</sub> concentration in the chamber during the photoperiod was 100-200 μmol mol<sup>-1</sup> lower than that outside. This difference in CO<sub>2</sub> concentration between inside and outside the plant growth chamber increased when the number of air exchanges per hour was decreased, resulting in increased net photosynthetic rate of plants. The net photosynthetic rate of plants likewise, increased with increased air current speed and CO<sub>2</sub> concentration.

The increase in CO<sub>2</sub> concentration in the chamber, by supplying CO<sub>2</sub> or enrichment higher than levels outside, increased the net photosynthetic rate and consequently plant growth. However, the CO<sub>2</sub> utilization efficiency (CUE), i.e., amount of CO<sub>2</sub> fixed by plants divided by amount of CO<sub>2</sub> supplied, decreased with increasing the CO<sub>2</sub> concentration in the growth chamber. On the other hand, when CO<sub>2</sub> was supplied to keep the CO<sub>2</sub> concentration at the same level as outside, all the CO<sub>2</sub> supplied was fixed by the plants, resulting in no release of CO<sub>2</sub> to the outside, namely CUE=1. It is considered that similar results are obtained in the greenhouse.

The present study demonstrates that CO<sub>2</sub> concentration in a plant growth chamber and a greenhouse with a limited number of air exchanges per hour is considerably lower than that outside during the photoperiod, when plants are grown in it. This depletion of CO<sub>2</sub> concentration limits the net photosynthesis and thus growth of plants. Lastly, CO<sub>2</sub> supply or enrichment, to keep its concentration at the same level as outside, is an efficient way of promoting the net photosynthesis without losing the supplied CO<sub>2</sub> to the outside. This null balance CO<sub>2</sub> supply method can be applied for any growth chamber and greenhouses, even with a high number of air exchanges per hour.

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