Evaluation of the Effect of Photosynthesis on Biomass Production with Simultaneous Analysis of Growth and Continuous Monitoring of CO₂ Exchange in the Whole Plants of Radish, cv Kosena under Ambient and Elevated CO₂

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Abstract: The effects of elevated CO₂ (approximate doubling of atmospheric CO₂ concentration) on the rate of photosynthesis estimated from continuous monitoring of CO₂ exchange in whole plants were investigated in radish cv. Kosena accompanied with simultaneous analysis of growth for 6 days from 15 to 21 days after planting (DAP). The elevated CO₂ increased the dry weights of hydroponically grown radish plants by 59% at 21 DAP. The increase in dry weight was due to a combined effect of increased leaf area and increased photosynthetic rate per unit leaf area. Leaf area and the photosynthetic rate were increased by elevated CO₂ by 18-43% and 9-20%, respectively, during 15 to 21 DAP. Namely, an increase in the rate of photosynthesis is accompanied by an increase in leaf area, both having a significant effect on biomass production.

Key words: Biomass, CO₂ Fixation, Elevated CO₂, Growth Analysis, Radish.

A very basic and important correlation of the rate of photosynthesis per unit leaf area (LA) with productivity, and/or biomass of plants, is still not clear and is a controversial issue. Positive and negative correlations and even the absence of a correlation have been reported. Reynolds et al. (1994) reported a positive relationship between the average rate of photosynthesis in flag leaves and average grain yield using 16 modern spring wheat varieties. Evans and Dunstone (1970) found a negative relationship between the rate of photosynthesis (per unit LA) in flag leaves and grain weight, and a positive relationship between the area of the largest leaf blade and grain weight among wild and cultivated wheat. They concluded that a larger LA of cultivated wheat caused a high yield. On the other hand, Evans (1985) found no relationship between the rate of photosynthesis (per unit LA) in flag leaves and flag-leaf area among wild and cultivated wheat. Recently Murchie et al. (2002) reported that there was no association between the grain filling rate and the rate of flag leaf photosynthesis (per unit LA) among new plant-type varieties and indica variety of rice. These studies attempted to relate the rate of photosynthesis in a certain leaf at a certain time with biomass and/or productivity of the plants. The rate of photosynthesis changes very much during development (see, e.g., the accompanying paper, Usuda, 2004). It is crucial to know the integrated rate of photosynthesis in the whole plant during certain periods to evaluate the relationship between photosynthesis and biomass production.

Studies on the growth under elevated CO₂ should help to elucidate the effect of enhanced rate of photosynthesis per unit LA on biomass production. Several decades have already passed for evaluation of the effect of growth under elevated CO₂ on photosynthesis and biomass. Many studies have been done to clarify the effect of growth under elevated CO₂ on the rate of photosynthesis in a specific single leaf at a certain time (see, e.g., Bowes, 1991; Long and Drake, 1992). Still many questions remain to be answered (see, e.g., the accompanying paper, Usuda 2004). Recent development of facilities for free-air CO₂ enrichment has provided an opportunity to evaluate the effect of elevated CO₂ on canopy photosynthesis and biomass (Kimball et al., 2002). In these studies the rates of canopy photosynthesis were usually expressed on a soil surface area basis and not on a unit LA basis. The rates of daily canopy photosynthesis under elevated CO₂ (about 200 µmol mol⁻¹ higher than atmospheric concentration) (measured every few hours) have been determined 3 to 9 times during the whole growing season in cotton and wheat. There was a 21 to 40% increase in the rate in cotton (Hileman et al., 1994), and about a 16% season-long increase in wheat...
The effects of elevated CO₂ on the biomass of shoot, and peak LA index, were also determined in these and similar studies. In cotton, the biomass of shoot, and peak LA index, increased by 34% and from -15.6 to 3.8%, respectively, under elevated CO₂ (Mauney et al. 1992; Mauney et al. 1994). In wheat, the biomass of shoot, and peak LA index were increased by elevated CO₂ by 12% (Brooks et al., 2000) and 24% (Kimball et al., 2002), respectively. To my knowledge, only a few studies have been made on the rate of photosynthesis per unit LA in whole plant throughout the growth period under elevated CO₂ in relation to biomass production. Christ and Körner (1995) measured the rate of photosynthesis in the whole plants of wheat during the initial growth stage under ambient (around 275 µmol mol⁻¹) and elevated (around 500 µmol mol⁻¹) CO₂. They examined the carry-over effect of rather short-term exposure to elevated CO₂ in the early vegetative growth period on grain filling because they thought that increased tillering induced by elevated CO₂ in a very early stage was important for grain filling. The rate of photosynthesis per unit LA increased by 2 to 3 fold, but the dry mass of shoot increased only 20-75%. Gifford (1995) also measured the rates of photosynthesis in the whole plants of wheat for one day, and measured the total dry weight (DW) and LA after a certain period which was not specified in the report. He found that an approximate doubling of atmospheric CO₂ concentration resulted in a 25, 9 and 10% increase in DW, LA and the rate of photosynthesis per unit LA, respectively. Vivin et al. (1995) measured the rate of whole plant photosynthesis and DW in oak seedlings twice in the first year under elevated CO₂ (first, at an early growth stage, 77 days after germination, and the second, at the end of the growing season). The rate of photosynthesis and LA in the whole plant at the early growth stage were increased by ca. 100 and 30 to 40%, respectively, by approximate doubling of the atmospheric CO₂ concentrations. The enhancement by elevated CO₂, however, disappeared at the end of the growing season. The total DW of plants grown under elevated CO₂ was increased by 17% at the end of growing season. These results do not show the quantitative relationship between the increase in the rate of photosynthesis in whole plant and the increase in biomass production under elevated CO₂. Elevated CO₂ may accelerate plant ontogeny and result in an early decline in the photosynthetic capacity during growth (see, accompanying paper, Usuda, 2004). Considering the uncertainty of the effect of elevated CO₂ on the rate of photosynthesis per unit LA, and that on LA in relation to biomass production, this research was undertaken.

In this study, radish cultivar Kosena was grown hydroponically. It grows rapidly and can be harvested at 21 days after planting (DAP). The shoot of Kosenna is edible and the DW of shoot is more than 85% of total DW at 21DAP (Usuda et al., 1999). Analysis of growth and continuous monitoring of CO₂ exchange in the whole plant were done simultaneously for 6 days from 15 to 21 DAP. In addition, the amount of water transpired per day was also estimated to evaluate the effect of elevated CO₂ on water use efficiency. The dry matter accumulated during this period was more than 80% of the final total DW at 21DAP (Usuda et al., 1999 and see also Fig. 2 in this study). Therefore, the analyses of the rate of photosynthesis per unit LA in the whole plants estimated from CO₂ exchange in whole plant and the increase in biomass in this study should provide us a realistic and rational insight into the effect of elevated CO₂ on the rate of photosynthesis and biomass production.

Material and Methods

1. **Plant material and growth conditions**

*Raphanus sativus* L. cv Kosena was used. In the experiments to measure the rate of photosynthesis and transpiration in the first leaf of plants at various ages, plants were grown as described in the accompanying paper (Usuda, 2004). In the experiments to measure the rates of photosynthesis and transpiration in the whole plants with growth analysis, plants were grown as described in the accompanying paper (Usuda, 2004) until 15 DAP but with the following exception. Six plants were grown in a container with ca. 3.5 L of aerated culture medium from 9 to 15 DAP. At 15 DAP, seven relatively uniformly growing plants were selected from 18 plants. Four of them were harvested and three were held in the container with ca. 3.5 L of aerated (100 mL min⁻¹) culture solution. The plants (n=3-1) were grown in a controlled growth chamber (Eyelatron FL301NH, Tokyo-Rikakiki, Tokyo, Japan) with a 14.5-h light/9.5-h dark cycle. Light intensities were 46, 130 and 570 µmol m⁻² s⁻¹ at plant height during (0-1st h and 13.5-14.5th h), (1-2nd h and 12.5-13.5th h), and (2-12.5th h) of the light period, respectively. The temperatures were 20 ± 0.5, 23 ± 0.5, 24 ± 0.5 and 25 ± 0.5°C during the dark period, and, (0-1st h and 13.5-14.5th h), (1-2nd h and 12.5-13.5th h), and (2-12.5th h) of the light period, respectively. Humidity was kept at 60 ± 10%. Light was provided from fluorescent lamps (Neolumi-super FL40S-W Mitsubishi Electric, Tokyo, Japan) during the whole light period and a metal-halide lamp (MT400DL/BUD Iwasaki Electric, Tokyo, Japan) was added during 2-12.5th h of the light period.

2. **Measurements of the rates of photosynthesis and transpiration using a single leaf**

The rates of photosynthesis and transpiration in the first leaf of plants at various ages were measured as described in the accompanying paper (Usuda, 2004).
3. Continuous measurements of CO\(_2\) exchange in the whole plants

The rates of CO\(_2\) exchange in the whole plants (n=1-3) grown in the above mentioned growth chamber (volume of the chamber was about 300 L) were measured continuously with an open system using an infrared gas analyzer from 15 to 21 DAP. Air was taken into a big container of 600 L from outside and supplied to the growth chamber after passing through another container of 300 L. Flow rates in the experiments with ambient and elevated CO\(_2\) were 70 and 90 L min\(^{-1}\), respectively. This kept the air pressure in the chamber always at a slightly elevated atmospheric pressure. A preliminary check by supplying pure nitrogen into the chamber showed that the gas flow at the rate of 50 L min\(^{-1}\) guaranteed no entry of air from outside the chamber. An air pump (DA-241S, Ulvac-Kiko, Yokohama, Japan) and mass flow controller (CMQ0200J/K, Yamatake, Tokyo, Japan) were used to provide a constant air flow, and the rate of air flow was logged in a data logger (K8DL-G16, Omron, Tokyo Japan) every 30s. Air circulation in the chamber was maintained with the equipped fan and an additional fan at a wind volume of 18 m\(^3\) min\(^{-1}\). The CO\(_2\) concentration in the inlet air and the differences in the concentration of CO\(_2\) between the inlet and the outlet air were monitored with an infrared gas analyzer having two channels, one for the absolute mode and another for the differential mode (MLT3.2 Emerson Process Management, Hasselroth, Germany) after passing through a cooling trap and an air dryer of membrane type (Sunsep SWG-A01-18/PP, Asahi Glass Engineering Co, Chiba, Japan). The data were logged in the data logger every 30s. Zero adjustment in the differential mode of measurement, was made every day. Span adjustment in the differential mode of measurement and zero and span adjustment in the absolute mode of measurements were made once a week. Elevated CO\(_2\) (375 \(\mu\)mol mol\(^{-1}\)) was supplied by adding pure CO\(_2\) into the inlet air in the first container using a mass flow controller (MC-2100NC, Lintec, Shiga, Japan). The actual CO\(_2\) concentration in the inlet air fluctuated considerably (see Fig. 4). The average concentrations of CO\(_2\), in the inlet air of ambient and elevated CO\(_2\) were 388 ± 16, and 752 ± 17 \(\mu\)mol mol\(^{-1}\) (mean ± SD, n>50,00), respectively. The concentrations of CO\(_2\) in the chamber during the light periods were 340-384 and 692-758 \(\mu\)mol mol\(^{-1}\) in ambient and elevated CO\(_2\), respectively. The following control experiments were conducted in each experiment to check the rate of CO\(_2\) exchange in microorganisms in the culture solution. After the final harvest at 21 DAP the rates of CO\(_2\) exchange in aerated culture solution without plants were monitored for at least one day as described above. These rates were very small and within the noise levels. Therefore I assumed that CO\(_2\) exchange by microorganisms in the culture solution was negligible in this study.

4. Plant harvest and measurement of plant fresh weigh during continuous monitoring of CO\(_2\) exchange

Every day between 15 to 21 DAP, fresh weight (FW) of each plant was measured. From 16 to 20 DAP the growth chamber was opened once a day at the 6.5-7th h in the dark period for about 10-15 min to measure the FW of the plants and weight of culture medium. At 17, 19 and 21 DAP one plant was harvested. Therefore for the measurements of CO\(_2\) exchange, three, two and one plant(s) were used during 15-17, 17-19 and 19-21 DAP, respectively. After harvest the plants were separated into leaves without midrib, midrib plus stem, fibrous roots and storage roots. The LA of each leaf without midrib was determined with a leaf area meter (AM100, Analytical Development Company, Hoddesdon, U.K). DW of each leaf without midrib, all midribs plus stem, fibrous roots and storage root were determined after drying at 80 °C for at least 2 weeks.

5. Determination of the amount of water transpired

The amount of water transpired from plants was determined as follows. The weights of culture medium and FW of plants were determined every day from 15 to 21 DAP, around the 6.5-7th h in the dark period. Plants were taken from the container and kept in measuring cylinders to collect water withdrawn by the roots. The weights of the culture medium in the container, and the culture medium collected from roots, as described above, were determined. From these measurements the weights of total water lost from culture medium per day (referred to as \(W_{lm}\)) were obtained. The amounts of water lost from the aerated culture medium without plants per day were also determined separately after 21 DAP, as described above. These amounts were referred to as \(W_{lc}\). The amounts of water absorbed by plants and retained within plants per day were obtained from the differences of the weights between the increase in FW and DW of plants per day. These amounts will be referred to as \(W_p\). The amount of water transpired from plants per day (\(W_t\)) was obtained from \(W_{t}=W_{lm}-W_{lc}-W_p\). The actual amounts of \(W_{lm}\), \(W_{lc}\) and \(W_p\) were 174-377, ca. 8-32, and around 15 g day\(^{-1}\), respectively. Therefore the major portion of water lost from the culture medium was due to transpiration by plants.

6. Determination of growth characters

Growth characters of relative growth rate (RGR) (g g\(^{-1}\) day\(^{-1}\)), net assimilation rate (NAR) (g m\(^{-2}\) day\(^{-1}\)), leaf area ratio (LAR) (LA total DW\(^{-2}\)) (m\(^2\) kg\(^{-1}\)), leaf weight ratio (LWR) (DW of leaf total DW\(^{-1}\)) (g g\(^{-1}\)), and specific leaf area (SLA) (LA leaf DW\(^{-1}\)) (m\(^2\) kg\(^{-1}\)) were obtained from the following equations (1) to (5),
Photosynthesis and Biomass

Fig. 1. Correlation of FW of the whole plant with total LA (a), DW of the whole plant (b), and DW of total leaves (c). Plants were grown under ambient (O) and elevated CO\(_2\) (X). Equations for regression for (a), (b), and (c) were as follows: (a) in ambient CO\(_2\), \(y = -0.0492x^2 + 17.5x + 0.47\). (b) in elevated CO\(_2\), \(y = 0.00012x^2 + 0.0885x - 0.183\). (c) in ambient CO\(_2\), \(y = 0.000049x^2 + 0.0625x - 0.169\). (c) in elevated CO\(_2\), \(y = 0.000197x^2 + 0.0920x - 0.591\).

where \(W_1\) and \(W_2\) are the total DW of plant at \(t_1\) and \(t_2\), respectively and \(L_1\) and \(L_2\) are total LA at \(t_1\) and \(t_2\), respectively and \(LW_1\) and \(LW_2\) are total DW of leaves at \(t_1\) and \(t_2\), respectively.

7. Statistics

For the data analysis, \(t\)-test and analysis of variance in two regression lines were done using Excel 2001 (Microsoft Corporation, Redmond, USA) and Multivariate Analysis Ver.4.0 (Esumi, Tokyo, Japan), respectively. For regressions Kaleida Graph Ver.5.0 (Synergy Software, Reading, USA) was used.

Results

1. Correlation of FW of the whole plant with total LA, DW of the whole plant and DW of total leaves

Every two days from 15 to 21 DAP, one plant was harvested under ambient and elevated CO\(_2\), and FW and DW of the whole plant, LA and DW of each leaf were determined. FW of the whole plant was significantly correlated with total LA, DW of the whole plant and DW of total leaves (Fig. 1). These relationships were used for the estimation of these values of the plants not harvested but for which FW of the whole plants were determined. These estimated values were used for the analysis of the rate of photosynthesis per unit LA and others.

2. Effect of elevated CO\(_2\) on the total LA, DW of the whole plant and shoot to root ratio

Elevated CO\(_2\) accelerated the increases in total LA and
and DW of the whole plant during the growth from 15 to 21 DAP (Fig. 2). Total LA of the plants grown under elevated CO$_2$ was 18-43% larger than that under ambient CO$_2$ during this period. DW of the plants under elevated CO$_2$ was 59-82% heavier than that under ambient CO$_2$. At 21 DAP, LA and DW of the plants grown under elevated CO$_2$ were 43% and 59% greater than those under ambient CO$_2$. Expanded leaves had nearly the same LA under ambient and elevated CO$_2$, but leaf initiation and expansion were accelerated by elevated CO$_2$ (Fig. 3 and data not shown). Increase in total LA was due to early initiation and expansion of leaves but not due to larger area of fully-expanded leaves. At 21 DAP, the ratio of shoot to root in terms of DW under ambient and elevated CO$_2$ was 6.89 ± 0.36 and 6.58 ± 0.80 (mean ± SD, n=3), respectively. There was no significant effect of elevated CO$_2$ on this ratio.

3. CO$_2$ exchange in the whole plants grown under ambient and elevated CO$_2$

The exchange of CO$_2$ in whole plants was monitored continuously for 6 days from 15 to 21 DAP at 10 to 15 min intervals during the dark period in a day. The rate of CO$_2$ exchange was calculated from the rate of the inlet air flow, and differences in CO$_2$ concentration between the inlet and the outlet air. Fig. 4 shows the recordings of CO$_2$ exchange under ambient and elevated CO$_2$. Although the CO$_2$ concentration in the inlet air fluctuated considerably, the rate of CO$_2$ exchange in the whole plants could be calculated. From this type of experiment the rate of CO$_2$ uptake (per plant and per unit LA) and dark respiration (per plant and per FW of plant) were obtained. LAs of plants in each day (6.5-7th h of the dark period) were estimated as described above (see, Fig. 2). LA and FW of plants at a certain time were estimated using these values and assuming that LA and FW increased linearly during the period where two values were obtained at the beginning and the end of the period. These values were used for the determination of the rates of CO$_2$ fixation per unit LA and respiration per g FW.

4. Effect of elevated CO$_2$ on growth characters

The increase in LA and DW during 15 to 21 DAP was accelerated by elevated CO$_2$ (Fig. 2). Table 1 shows RGR, NAR, LAR, LWR and SLA of the plants grown under ambient and elevated CO$_2$ during 15-17, 17-19 and 19-21 DAP. RGR decreased during 15-21 DAP but was not significantly affected by elevated CO$_2$. RGR, however, plotted against DW was higher in the plants grown under elevated CO$_2$ than under ambient CO$_2$ (Fig. 5). NAR stayed almost constant during 15 to 21 DAP, but was slightly increased by elevated CO$_2$ during
Fig. 5. Relationship between relative growth rate (RGR) and mean DW of the plants grown under ambient (○) and elevated (x) CO₂. RGRs were obtained from DWs at t₁ (W₁) and at t₂ (W₂). RGRs were plotted against mean values of DW (0.5x(W₁+W₂)). Level of significance between two regression lines was p<0.05.

Table 1. Effects of elevated CO₂ on the growth characteristics. Plants were grown under ambient or elevated CO₂ from 15 to 21 DAP and growth analyses were done using the values of total DW, LA and DW of leaves measured and estimated every two days. Triplicate experiments of Exp1-3 for ambient CO₂ and Exp 4-6 for elevated CO₂ were done. For details, see text.

|                | Ambient CO₂ grown | Elevated CO₂ grown |
|----------------|-------------------|-------------------|
|                | Exp 1 | Exp 2 | Exp 3 | Mean±SD (1) | Exp 4 | Exp 5 | Exp 6 | Mean±SD (2) | (2)/(1) | Sig¹ |
| RGR³(15-17)    | 0.380 | 0.350 | 0.320 | 0.350±0.030 | 0.408 | 0.365 | 0.438 | 0.401±0.037 | 1.150 | NS   |
| RGR³(17-19)    | 0.313 | 0.308 | 0.312 | 0.311±0.003 | 0.237 | 0.266 | 0.360 | 0.288±0.065 | 0.93  | NS   |
| RGR³(19-21)    | 0.255 | 0.294 | 0.307 | 0.285±0.027 | 0.271 | 0.210 | 0.289 | 0.257±0.042 | 0.90  | NS   |
| NAR³(15-17)    | 18.8  | 17.1  | 15.5  | 17.1±1.7    | 23.9  | 22.0  | 22.4  | 22.8±1.0    | 1.33  | *    |
| NAR³(17-19)    | 16.9  | 17.1  | 17.5  | 17.2±0.3    | 16.5  | 18.6  | 25.9  | 20.3±4.9    | 1.18  | NS   |
| NAR³(19-21)    | 16.4  | 18.6  | 18.6  | 17.9±1.3    | 21.6  | 14.5  | 23.1  | 19.7±4.6    | 1.10  | NS   |
| LAR³(15-17)    | 20.2  | 20.5  | 20.6  | 20.4±0.2    | 16.8  | 16.5  | 19.6  | 17.6±1.7    | 0.86  | **   |
| LAR³(17-19)    | 18.5  | 18.0  | 17.8  | 18.1±0.4    | 14.4  | 14.3  | 13.9  | 14.2±0.2    | 0.78  | **   |
| LAR³(19-21)    | 15.5  | 15.8  | 16.5  | 15.9±0.5    | 12.6  | 14.4  | 12.5  | 13.2±1.1    | 0.83  | **   |
| LWR³(15-17)    | 0.678 | 0.691 | 0.681 | 0.683±0.007 | 0.680 | 0.671 | 0.675 | 0.675±0.005 | 0.99  | NS   |
| LWR³(17-19)    | 0.684 | 0.691 | 0.703 | 0.693±0.010 | 0.688 | 0.688 | 0.681 | 0.686±0.004 | 0.99  | NS   |
| LWR³(19-21)    | 0.700 | 0.678 | 0.672 | 0.683±0.015 | 0.693 | 0.677 | 0.661 | 0.677±0.016 | 0.99  | NS   |
| SLA³(15-17)    | 29.8  | 29.7  | 30.2  | 29.9±0.3    | 24.7  | 24.7  | 29.0  | 26.1±2.6    | 0.87  | *    |
| SLA³(17-19)    | 27.0  | 26.0  | 25.3  | 26.1±0.9    | 20.9  | 20.8  | 20.5  | 20.7±0.2    | 0.79  | ***  |
| SLA³(19-21)    | 22.1  | 23.3  | 24.6  | 23.3±1.3    | 18.2  | 21.3  | 19.0  | 19.5±1.6    | 0.83  | *    |

¹ *** P<0.01, ** P<0.05, * P<0.1, NS, not significant
² relative growth rate (g g⁻¹ day⁻¹)
³ net assimilation rate (g m⁻² day⁻¹)
⁴ leaf area ratio (m² kg⁻¹)
⁵ leaf weight ratio (g g⁻¹)
⁶ specific leaf area (m² kg⁻¹)

15-21 DAP and significantly increased by 1.33 fold during 15-17 DAP. LAR and SLA decreased from 15 to 21 DAP and was significantly decreased by elevated CO₂. LWR stayed almost constant during 15 to 21 DAP and was not affected by elevated CO₂. Lower LAR under elevated CO₂ was due to lower SLA but not LWR.

5. Effect of elevated CO₂ on photosynthesis, transpiration, water use efficiency and respiration

Table 2 shows the amounts of carbon gained (mg plant⁻¹ 2 days⁻¹) (C gained), the apparent rate of photosynthesis (APS) per unit LA during 2-12.5th h of the light period, the ratio of C gained per DW increase (C/DW) during 2 days, the amounts of water transpired (W lost) (g plant⁻¹ 2 days⁻¹), the values of water use efficiency (WUE) (µmol CO₂ fixed per mmol H₂O transpired) during 2 days, the amounts of carbon respired during dark period (C dr) (mg C plant⁻¹ 2 days⁻¹), the rate of dark respiration (DR) (nmol CO₂ g FW⁻¹ 2 days⁻¹) and the ratio of photosynthesis to respiration (PS/Res) under ambient and elevated CO₂ from 15 to 21 DAP. The amount of C gained increased during this period, and was increased by elevated CO₂.
APS was estimated from the amount of CO₂ fixed during 2-12.5th h of the light period, neglecting the amount of carbon respired in roots, stems and midribs. Assuming that the rate of respiration in roots, stems and midribs was the same as that of dark respiration and was proportional to their DW, the absolute values of the rates of respiration in roots, stems and midribs during this period were less than 5% of the rate of CO₂ fixation. Therefore, the estimated values of APS may be very close to the real values. Under ambient and elevated CO₂, the APS per unit LA was around 18 and 20-22 µmol CO₂ m⁻² s⁻¹, respectively. Thus, elevated CO₂ increased the APS by 9-20%. C/DW and DR stayed almost constant during 15-21 DAP and were not significantly influenced by elevated CO₂. W lost and C DR increased during 15 to 21 DAP and were significantly increased by elevated CO₂ only during 19-21 DAP but not during 15-19 DAP. WUE stayed

### Table 2. Effects of elevated CO₂ on the amounts of C gained (C gained), the rate of apparent photosynthesis (APS), the ratios of C gained to DW increased (C/DW), the amounts of transpired water (W lost), water use efficiency (WUE), the amounts of respired carbon during dark period (C resp), the rates of dark respiration in whole plants (DR) and the ratio of photosynthesis to respiration (PS/Res). Plants were grown under ambient or elevated CO₂ from 15 to 21 DAP and exchange of CO₂, H₂O transpired, DW, LA and FW were determined during the period. For details, see text.

|                      | Ambient CO₂ grown | Elevated CO₂ grown | (2)/(1) Sig. |
|----------------------|-------------------|---------------------|--------------|
|                      | Exp1  | Exp2  | Exp3  | Mean±SD (1) | Exp4  | Exp5  | Exp6  | Mean±SD (2) | (2)/(1) Sig. |
| C gained² (15-17)    | 456.3 | 503.1 | 469.9 | 476.4 ± 24.1 | 643.5 | 735.1 | 698.4 | 692.3 ± 46.1 | 1.45 ***     |
| C gained² (17-19)    | 850.4 | 843.9 | 785.2 | 817.5 ± 51.5 | 1234 | 1373 | 1199 | 1269 ± 92.1 | 1.55 ***     |
| C gained² (19-21)    | 1335 | 1215 | 1113 | 1221 ± 111 | 1794 | 2169 | 2057.0 | 2007 ± 192.5 | 1.64 ***     |
| APS³ (15-17)         | 18.2  | 18.4  | 18.6 | 18.4 ± 0.2  | 19.3 | 19.4 | 21.2 | 20.0 ± 1.1  | 1.09 *       |
| APS³ (17-19)         | 17.5  | 17.9  | 18.5 | 17.9 ± 0.5  | 21.2 | 21.4 | 22.0 | 21.6 ± 0.6  | 1.20 ***     |
| APS³ (19-21)         | 18.7  | 17.6  | 17.4 | 17.9 ± 0.7  | 20.5 | 19.1 | 21.1 | 20.5 ± 1.6  | 1.14 **      |
| C/DW⁴ (%) (15-17)    | 37.7  | 43.0  | 49.3 | 43.3 ± 5.8  | 32.0 | 39.0 | 42.8 | 38.0 ± 5.5  | 0.88 NS      |
| C/DW⁴ (%) (17-19)    | 39.9  | 44.2  | 44.4 | 42.8 ± 2.5  | 53.2 | 48.7 | 37.6 | 46.5 ± 8.0  | 1.09 NS      |
| C/DW⁴ (%) (19-21)    | 51.9  | 39.1  | 38.5 | 43.2 ± 7.9  | 41.5 | 55.0 | 44.4 | 47.1 ± 7.1  | 1.09 NS      |
| W lost⁵ (15-17)      | 152.3 | 162.4 | 152.1 | 155.6 ± 5.9 | 160.1 | 143.7 | 131.2 | 145.0 ± 14.5 | 0.93 NS      |
| W lost⁵ (17-19)      | 260.8 | 264.0 | 246.3 | 260.0 ± 12.2 | 268.3 | 292.0 | 229.8 | 263.4 ± 31.4 | 1.01 NS      |
| W lost⁵ (19-21)      | 378.4 | 380.6 | 333.4 | 364.1 ± 26.6 | 399.5 | 469.2 | 427.5 | 432.1 ± 35.1 | 1.19 *       |
| WUE⁶ (15-17)         | 4.49  | 4.64  | 4.63 | 4.59 ± 0.08 | 6.03 | 7.67 | 7.98 | 7.23 ± 1.06 | 1.57 **      |
| WUE⁶ (17-19)         | 4.73  | 4.79  | 4.62 | 4.71 ± 0.09 | 6.90 | 7.05 | 7.83 | 7.26 ± 0.50 | 1.54 ***     |
| WUE⁶ (19-21)         | 5.29  | 4.79  | 5.01 | 5.03 ± 0.25 | 6.74 | 6.93 | 7.22 | 6.96 ± 0.24 | 1.38 ***     |
| C DR⁷ (15-17)        | 65.3  | 69.0  | 54.0 | 62.8 ± 7.8  | 94.5 | 62.7 | 58.9 | 72.0 ± 19.5 | 1.15 NS      |
| C DR⁷ (17-19)        | 137   | 109   | 96.0 | 114 ± 21    | 177  | 117  | 114 | 136 ± 35.5 | 1.19 NS      |
| C DR⁷ (19-21)        | 173   | 182   | 152 | 169 ± 15.4  | 206  | 242  | 202 | 217 ± 22.0 | 1.28 **      |
| DR⁸ (15-17)          | 4.26  | 4.24  | 3.61 | 4.04 ± 0.37 | 4.36 | 3.29 | 2.81 | 3.49 ± 0.79 | 0.86 NS      |
| DR⁸ (17-19)          | 4.53  | 3.78  | 3.85 | 4.05 ± 0.41 | 4.44 | 3.47 | 3.53 | 3.81 ± 0.54 | 0.94 NS      |
| DR⁸ (19-21)          | 3.65  | 3.93  | 3.62 | 3.73 ± 0.17 | 3.52 | 3.23 | 3.75 | 3.75 ± 0.26 | 0.94 NS      |
| PS/Res⁹ (15-17)      | 3.16  | 3.28  | 3.84 | 3.43 ± 0.36 | 3.09 | 5.04 | 5.09 | 4.41 ± 1.14 | 1.29 NS      |
| PS/Res⁹ (17-19)      | 2.85  | 3.46  | 3.52 | 3.28 ± 0.37 | 3.16 | 5.04 | 4.56 | 4.25 ± 0.98 | 1.30 NS      |
| PS/Res⁹ (19-21)      | 3.45  | 3.03  | 3.29 | 3.26 ± 0.21 | 3.84 | 3.94 | 4.43 | 4.07 ± 0.32 | 1.25 **      |

1 *** P<0.01, **, P<0.05, *, P<0.1, NS, not significant
2 mg Carbon gained plant⁻¹ 2 days⁻¹
3 apparent photosynthesis, µmol CO₂ m⁻² s⁻¹
4 100x g C gained g DW⁻¹ increase during 2 days
5 g water lost plant⁻¹ 2 days⁻¹
6 water use efficiency, mmol CO₂ gained per mol⁻¹ H₂O transpired during 2 days
7 mg respired C plant⁻¹ 2 days⁻¹ during dark period
8 dark respiration, nmol CO₂ g fw⁻¹ s⁻¹
9 PS was the amount of carbon gained during the light period of 2 days with a plant and Res was the amount of carbon lost during 2 days with a plant assuming the rate of respiration during light period was the same as that during dark period.
Table 3. Effects of elevated CO2 on the rates of apparent photosynthesis and water use efficiency with the first leaf of plants at various ages grown under ambient or elevated CO2. The rates of photosynthesis and water use efficiencies were obtained under 500 µmol photon m-2 s-1 and 25 ± 1°C. The rates of photosynthesis were measured under the concentrations of CO2 in the inlet air of 350 (P 350) or 750 µmol mol-1 (P 750). When the rate of apparent photosynthesis was 27.6 µmol CO2 m-2 s-1, the concentration of CO2 in the leaf chamber decreased about 39 µmol mol-1.

|                   | Ambient CO2 Grown | Elevated CO2 Grown |
|-------------------|-------------------|--------------------|
|                   | (µmol mol-1)      | (µmol mol-1)       |
| PS350             |                  |                    |
| (1)               | 12.7 ± 4.7 (4.0-17.9, n=12) | 10.5 ± 4.0 (2.3-16.5, n=11) |
| PS750             |                  |                    |
| (2)               | 18.5 ± 7.0 (6.0-27.6, n=12) | 18.5 ± 6.4 (6.6-26.6, n=11) |
| (2)/(1)           | 1.46 ± 0.12 (n=12) | 1.85 ± 0.37 (n=12) |
| WUE               | 7.19 ± 0.74 (n=12) | 12.18 ± 3.06 (n=11) |

Each value is mean ± SD. For units, see Table 2.

Values in the parenthesis were the highest and lowest values obtained with the first leaf of various ages.

a The rates of apparent photosynthesis were determined under a CO2 concentration in the inlet air of 350 µmol mol-1
b The rates of apparent photosynthesis were determined under a CO2 concentration in the inlet air of 750 µmol mol-1
c WUE determined under a CO2 concentration in the inlet air of 350 µmol mol-1
d WUE determined under a CO2 concentration in the inlet air of 750 µmol mol-1

almost constant during 15-21 DAP and was significantly increased by elevated CO2. The effect of elevated CO2 on the ratio of the amount of carbon fixed during the light period to that respired during the whole day (PS/Res) was assessed by assuming that the rate of respiration during the day was the same as that in the dark period. There was a significant positive effect of elevated CO2 on this ratio during 19 to 21 DAP (Table 3).

6. Effect of elevated CO2 on the rate of photosynthesis and water use efficiency in the first leaf of plants at various ages

The APS and WUE in the first leaf of the plants at various ages (11 to 25 DAP) grown under ambient and elevated CO2 were determined under the similar condition to the growth condition with the highest light intensity (Table 3). The rate of photosynthesis and transpiration in the first leaf decreased with aging (see Fig. 1 in the accompanying paper, Usuda 2004). The rate of APS in the first leaf of ambient CO2 grown plants at various ages under ambient CO2 condition were 17.9 to 4.0 µmol CO2 m-2 s-1. The rates increased by 46% on an average by elevated CO2. On the other hand, the rates of APS in the first leaf of elevated CO2 grown plants at various ages under elevated CO2 were 26.6 to 6.6 µmol CO2 m-2 s-1. The rates under ambient CO2 were 16.5 to 2.3 µmol CO2 m-2 s-1. Therefore the rates increased by 85% on average by elevated CO2. WUE of the first leaf of the plants grown under ambient and elevated CO2 were 7.19 and 12.2, respectively.

Discussion

1. Effect of elevated CO2 on LA and DW

Elevated CO2, approximate doubling of atmospheric CO2 concentration, increased DW accumulation by 59% in hydroponically grown Kosena at 21 DAP, when it reached a reasonable size for harvest. Kimball (1983) analyzed the effect of elevated CO2 (elevated by approximately 300 µmol mol-1) on productivity in a number of species and showed a 30% increase on an average of productivity by elevated CO2. In the present study, however, elevated CO2 increased DW by 59%. Such a difference in the magnitude of the increase in productivity or DW may be attributed to the following three factors. 1) The concentration of CO2 was elevated by about 350 µmol mol-1 in the present study. 2) Kosena was grown hydroponically with ample water and nutrient. 3) Plants in the present study were harvested at a relatively early stage during vegetative growth (21 DAP).

In this study, elevated CO2 increased LA at 21 DAP, but the effect of elevated CO2 on LA varies with the studies. For example, a 35, 20 and –7% increase in total LA was reported in hybrid poplar (Curtis et al. 1995), Desmodium paniculatum (Wulff and Strain, 1982) and rice (Ziska and Teramura, 1992), respectively. Pett (1986) reported that LA of cucumber was increased by elevated CO2 (1000 µmol mol-1) during initial seedling growth stage but not thereafter. In rice, the area of the fully-expanded 12th leaf of the plants grown under elevated CO2 of 1000 µmol mol-1 was about 25% smaller than that of the plants grown under ambient CO2 (Makino et al., 2000). Recently, Kimball et al. (2002) summarized the results of the analysis of the effect of free-air CO2 enrichment on agricultural crops. According to their analysis, the peak LA index was increased by –15.6 to 24% by elevated CO2 (~600 µmol mol-1) in C3 plants, again showing a large variation in the effect of elevated CO2 on LA. Such a large variation might be due to the difference in plant species or growth conditions. The positive effect of elevated CO2 on total LA found in this study was due to early initiation and expansion of the leaves but not due to the larger size of fully-expanded leaves (Fig. 3).
This is basically consistent with the previous finding mentioned above.

2. Effects of elevated CO$_2$ on the growth characteristics

RGR of Kosena hydroponically grown under ambient and elevated CO$_2$ was between 0.257 and 0.401 g g$^{-1}$ day$^{-1}$ (Table 1). According to Lambers et al. (1990), RGR of herbaceous plants grown in growth rooms with optimum nutrient supply was 0.1-0.3 g g$^{-1}$ day$^{-1}$. This indicates that Kosena grew very rapidly under the conditions used in this study. RGR decreased with increasing DW of the plants, and RGR relative to DW was increased by elevated CO$_2$ (Fig. 5). In other species, positive and negative effects and even absence of effect of elevated CO$_2$ on RGR have been reported (Wulff and Strain, 1982; Poorter et al., 1988; Musgrave and Strain, 1988; Atkin et al., 1999; Makino et al., 2000). Generally speaking, elevated CO$_2$ increased RGR during the initial growth stage, but thereafter it had no effect or a rather suppressive effect on RGR (see e.g., Poorter et al., 1988). NAR of 17-23 g m$^{-2}$ day$^{-1}$ obtained in this study was within the range of the reported values of 7-20 g m$^{-2}$ day$^{-1}$ in C3 plants grown under optimum nitrogen and moderate to high light intensity (Lambers et al., 1990). Elevated CO$_2$ enhanced NAR slightly (Table 1). It seemed to be due to increased rate of photosynthesis per unit LA, which was increased by 9 to 20% by elevated CO$_2$ (Table 2). A positive effect and absence of the effect of elevated CO$_2$ on NAR have been observed in previous studies (Ford and Thorne, 1967; Porter and Grodzinski, 1984; Patterson et al., 1988; Poorter et al., 1988; Musgrave and Strain, 1988; Atkin et al., 1999; Makino et al., 2000). NAR, however, was usually stimulated by elevated CO$_2$ treatments at the beginning of the growth and not thereafter (see, e.g., Poorter et al., 1988). LAR of 13.2 to 20.4 m$^{-2}$ kg$^{-1}$ were obtained in this study. The values of LAR vary widely with the species and the environmental condition (Lambers et al., 1990), but it was decreased by elevated CO$_2$ in this study. This is similar to previous findings (Ford and Thorne, 1967; Jolliffe and Ehret, 1985; Makino et al., 2000). The LWR of ca 0.68 g g$^{-1}$ obtained in this study is within the values of 0.3 to 0.8 g g$^{-1}$ observed in the plants grown at optimum nitrogen supply and moderate to high light intensity (Lambers et al., 1990). LWR was not influenced by elevated CO$_2$ in this study, as reported for Acassia species by Atkin et al. (1999). LWR was, however, slightly increased by elevated CO$_2$ in bush bean (Jolliffe and Ehret, 1985), cotton (Patterson et al., 1988) and Plantago major (Poorter et al., 1988).

The SLA values of 20 to 30 m$^2$ kg$^{-1}$ observed in this study were within the range of the values of 10 to 50 m$^2$ kg$^{-1}$, reported for the plants grown under optimum nitrogen supply and moderate to high light intensity (Lambers et al., 1990). SLA was decreased by 13-21% by elevated CO$_2$ in this study. This is consistent with previous findings (Ford and Thorne, 1967; Porter and Grodzinski, 1984; Hrubec et al., 1985; Jolliffe and Ehret, 1985; Patterson et al., 1988; Ziska and Teramura, 1992; Rufty et al., 1994). The decrease in LAR under elevated CO$_2$ in radish was, therefore, caused by the decrease in SLA but not LWR. Both increased leaf thickness and increased dry matter content resulted in lower SLA. The mechanisms responsible for the lower SLA under elevated CO$_2$ are not yet clear.

3. Effects of elevated CO$_2$ on the amount of carbon gained, the rates of photosynthesis and respiration, and water use efficiency

Gifford (1995) reported a 25% increase in carbon accumulation per plant under elevated CO$_2$ of 710 µmol mol$^{-1}$ in wheat. In radish elevated CO$_2$ enhanced the amount of carbon gained per plant by 45 to 64%. Therefore, the effect of elevated CO$_2$ on carbon accumulation on a plant basis seems to be high in radish compared with wheat. It may be due to the difference in the effect of elevated CO$_2$ on LA between wheat and radish (see above for details).

The rate of photosynthesis in the first leaf of plants at various ages varied substantially (Table 3, see also Fig. 1 in the accompanying paper, Usuda 2004). The rates of photosynthesis in these leaves were increased by 46 to 85% by increasing CO$_2$ concentration in the inlet air from 350 to 750 µmol mol$^{-1}$ (Table 3), but elevated CO$_2$ accelerated ontogeny (see accompanying paper Usuda 2004). Therefore, continuous measurement of the rate of photosynthesis in whole plant is crucial to assess the effect of photosynthesis on biomass production under elevated CO$_2$. The rate of photosynthesis per unit LA calculated from CO$_2$ exchange in the whole plants was increased by 9 to 20% by increasing the CO$_2$ concentration from 340-384 to 692-758 µmol mol$^{-1}$ (Table 2). Elevated CO$_2$, however, accelerates growth (Fig. 3, see also the accompanying paper, Usuda 2004), and therefore, increases the number of senescent leaves and also mutual shading. This may be why the increase in photosynthetic rate caused by elevated CO$_2$ in whole plants (9 to 20%) was lower than that in single leaves (the first leaf) measured under a constant light intensity of ca. 500 µmol m$^{-2}$ s$^{-1}$ (46 to 85%).

The rate of dark respiration per plant (C$_{\text{dark}}$) was slightly higher under the elevated CO$_2$ than under ambient CO$_2$ during 19 to 21 DAP, but the rate of dark respiration per g FW was not influenced by elevated CO$_2$ (Table 2). There was no effect of elevated CO$_2$ on the rate of dark respiration per unit LA in the first leaf of Kosena at various ages (Fig. 1b in the accompanying paper Usuda 2004). The effect of elevated CO$_2$ on respiration is an important issue in evaluating the effect of global increase in atmospheric CO$_2$ on growth and biomass production, and many studies have been
conducted. Reduction or no effect of elevated CO$_2$ on respiration were often reported, but even a promotive effect has been reported (Amthor 1997). Recently Jahnke and Krewitt (2002) pointed out the difficulty in accurate measurement of the rate of dark respiration under elevated CO$_2$. They improved the method of measurement of dark respiration under elevated CO$_2$ and concluded that elevated CO$_2$ had no effect on leaf respiration. The results obtained in this study support this conclusion. Since elevated CO$_2$ increased the rate of photosynthesis (Table 2), this means that elevated CO$_2$ increased the ratio of the rate of photosynthesis to that of respiration per plant. Gifford (1995), however, reported that elevated CO$_2$ had no effect on the ratio of photosynthesis to respiration in wheat. The rates of dark respiration were low compared with that of photosynthesis and fluctuations of the concentration of CO$_2$ in the inlet air might hamper accurate measurement of the rate of dark respiration (Fig. 4). More precise measurement is necessary to draw a final conclusion on these subjects.

WUE of a single leaf at various ages under a light intensity of 500 μmol m$^{-2}$ s$^{-1}$ under ambient CO$_2$ was 7.2 mmol CO$_2$ mol$^{-1}$ H$_2$O and was increased to 12.2 mmol CO$_2$ mol$^{-1}$ H$_2$O by elevated CO$_2$ (Table 3). On the other hand total WUE of the whole plants during the day and night was increased from 4.6-5.0 to 7.0-7.3 mmol CO$_2$ mol$^{-1}$ H$_2$O by elevated CO$_2$ (Table 2). The lower values in the whole plant compared with the single leaf seemed to be due to CO$_2$ evolution and transpiration during the dark period which were included in the values measured with the whole plants but not in the values measured with the single leaf. WUE increased by 70, and 38 to 57%, with the single leaf and the whole plant, respectively. Many studies have revealed an increase in instantaneous transpiration efficiency for leaves exposed to atmospheric CO$_2$ enrichment, primarily attributed to the reduced stomatal conductance, enhanced photosynthesis, or both factors in combination. Increases in the instantaneous transpiration efficiency due to elevated CO$_2$ ranged from 25 to 229% (Wullschleger et al., 2002). The values obtained in this study fall in the range confirming that WUE is improved under elevated CO$_2$.

**Conclusion**

Approximate doubling of atmospheric CO$_2$ concentration enhanced DW by 59% in hydroponically grown radish cv. Kosena after 21 DAP. LA was increased by 18-43% by elevated CO$_2$ during 15 to 21 DAP. The rate of photosynthesis in the first leaf of plants at various ages was increased by 46 to 85% by elevating CO$_2$. But the rates of photosynthesis changed very much during leaf development and elevated CO$_2$ accelerated ontogeny (see, e.g., the accompanying paper, Usuda, 2004). Therefore integration of the rate of photosynthesis by continuous measurement of CO$_2$ exchange in the whole plants is essential to evaluate the effects of elevated CO$_2$ on photosynthesis. The rate of photosynthesis per unit LA determined from CO$_2$ exchange in whole plants was increased by 9 to 20% by elevated CO$_2$. The difference between the effects of elevated CO$_2$ measured with the single leaf and the whole plants was attributed to the acceleration of senescence and mutual shading caused by elevated CO$_2$. In this study, I quantitatively assessed the effect of elevated CO$_2$ on LA, photosynthetic rate per unit LA and dry-matter production. All results shown here indicate that the elevated CO$_2$ increases both photosynthetic rate per unit LA and LA, resulting in higher biomass productivity.

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