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Root-zone CO_2 and root-zone temperature effects on photosynthesis and nitrogen metabolism of aeroponically grown lettuce (*Lactuca sativa* L.) in the tropics

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Abstract

Effects of elevated root-zone (RZ) CO_2 concentration (RZ [CO_2]) and RZ temperature (RZT) on photosynthesis, productivity, nitrate (NO_3^-), total reduced nitrogen (TRN), total leaf soluble and Rubisco proteins were studied in aeroponically grown lettuce plants in a tropical greenhouse. Three weeks after transplanting, four different RZ [CO_2] concentrations (ambient, 360 ppm, and elevated concentrations of 2,000; 10,000; and 50,000 ppm) were imposed on plants at 20ºC-RZT or ambient(A)-RZT (24–38ºC). Elevated RZ [CO_2] resulted in significantly higher light-saturated net photosynthetic rate, but lower light-saturated stomatal conductance. Higher elevated RZ [CO_2] also protected plants from both chronic and dynamic photoinhibition (measured by chlorophyll fluorescence F_v/F_m ratio) and reduced leaf water loss. Under each RZ [CO_2], all these variables were significantly higher in 20ºC-RZT plants than in A-RZT plants. All plants accumulated more biomass at elevated RZ [CO_2] than at ambient RZ [CO_2]. Greater increases of biomass in roots than in shoots were manifested by lower shoot/root ratios at elevated RZ [CO_2]. Although the total biomass was higher at 20ºC-RZT, the increase in biomass under elevated RZ [CO_2] was greater at A-RZT. Shoot NO_3^- and TRN concentrations, total leaf soluble and Rubisco protein concentrations were higher in all elevated RZ [CO_2] plants than in plants under ambient RZ [CO_2] at both RZTs. Under each RZ [CO_2], total leaf soluble and Rubisco protein concentrations were significantly higher at 20ºC-RZT than at A-RZT. Our results demonstrated that increased P_{Nmax} and productivity under elevated [CO_2] was partially due to the alleviation of midday water loss, both dynamic and chronic photoinhibition as well as higher turnover of Calvin cycle with higher Rubisco proteins.

Additional key words: elevated root-zone CO_2 concentration; photosynthesis; root-zone temperature; Rubisco; total reduced N.

Introduction

Plant roots are normally colonized by microorganisms and evolve more CO_2 than sterile roots growing among soil particles. It is not unusual for CO_2 to be up to more than 10-fold higher in the rhizosphere than the atmospheric CO_2 concentration (De Jong and Schappert 1972, Norstadt and Porter 1984). It has been reported that elevated RZ CO_2 could enhance growth of tomato (*Lycopersicon esculentum*) seedlings, especially under stress conditions, such as salinity and high temperature (Cramer and Richards 1999, Viktor and Cramer 2003). Our study with lettuce plants, which were grown under ambient temperature from 20–36°C under a maximal PPFD of 2,100 μmol m^{-2} s^{-1} in New Zealand, indicated that elevated RZ [CO_2] protected those plants from dynamic photoinhibition (He *et al.* 2007). We also found that elevated RZ [CO_2] reduced the negative impacts of high air temperature on photosynthesis, N metabolism, and the growth of lettuce plants, and thus enhanced photosynthesis and productivity in the walk-in room with controlled environment (He *et al.* 2004, 2010). It has been reported that, when plants are transpiring, a mixture of CO_2 released by respiration of local tissues and soil is transported from roots to shoot through xylem vessels (Teskey and McGuire 2002, 2007). It has been further

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Abbreviations: A-RZT – ambient root-zone temperature; DM – dry mass; F_o, F_m, – minimum and maximum chlorophyll fluorescence yield; F_v – variable fluorescence; F_v/F_m –maximum PSII quantum yield without actinic light; FM – fresh mass; g_{smax} – light-saturated stomatal conductance; NO_3^- – nitrate; NR – nitrate reductase; P_{Nmax} – light-saturated net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC – relative water content; RZ [CO_2] – root-zone CO_2 concentration; RZT – root-zone temperature; TRN – total reduced nitrogen; TM – turgid mass.

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confirmed by the same research team (McGuire *et al.* 2009) that internal respiratory CO_2, other than gas exchange from the atmosphere, could be used for the assimilation of carbon.

In the tropics, the combination of high light and high temperature causes photoinhibition in plants (He *et al.* 1996, He and Lee 2004). In our study, with temperate lettuce plants grown in a tropical greenhouse, it was found that midday F_v/F_m ratios were much lower in plants grown at high A-RZT than at 20ºC-RZT (He *et al.* 2001). Under high A-RZT and high light, low internal CO_2 concentration reduced the photosynthetic utilization of radiant energy (He and Lee 2004). We also found that high A-RZT caused shoot water deficit by altering the balance between water uptake by the root system and water loss from the shoot of lettuce plants (He *et al.* 2001). Stomata closure induced by water deficit depletes CO_2 in the intercellular spaces and at the chloroplast level reduces photosynthetic CO_2 assimilation (Chaves and Oliveira 2004). Cooling the RZ of lettuce plants alleviated not only stomatal limitations of photosynthesis, measured by leaf relative water content (RWC) and photosynthetic gas exchange, but also mitigated nonstomatal limitations of photosynthesis such as photosystem II (PSII) functionality, Rubisco protein and activity even though their aerial parts were exposed to ambient temperature from 24 to 38ºC (He and Lee 2001, 2004). In our research, NO_3^- is the only N source supplied into the nutrient solution to aeroponically grown vegetables (He 2010). NO_3^- must be converted to NH_4^+ in the plant before N can be incorporated into amino acids and other organic N compounds such as Rubisco proteins. Nonstomatal limitation of photosynthesis could be partly due to poor root development in plants grown at A-RZT, which resulted in N deficiency (He *et al.* 2001). Hence, higher NO_3^- assimilation rate is required to support faster growth of temperate lettuce plants growing at cool RZTs (Tan *et al.* 2002, He *et al.* 2008).

In the present study, our experimental design using aeroponically grown lettuce plants in a tropical greenhouse in Singapore, enabled us to test the following hypotheses: (*1*) elevated RZ [CO_2] and RZT did not only affect photosynthetic gas exchange and photosynthetic utilization of radiant energy, but also the plant growth, especially the root growth associated with the uptake and accumulation of NO_3^-; (*2*) the total product of N metabolism, such as the total reduced N (TRN), total soluble and Rubisco protein, was also affected by elevated RZ [CO_2] and RZT. This research could have a practical significance to aeroponic vegetable production by growing plants under elevated RZ [CO_2] to enhance productivity under high A-RZT.

Materials and methods

Plants and cultivation: Crisphead-type lettuce plants (*Lactuca sativa* L. cv. 'Wintergreen', *South Pacific Seeds Ltd*., New Zealand) was used as the plant material. After germination, seedlings were transplanted into polyurethane cubes soaked in water and placed in trays. These trays were then transferred to the greenhouse, where they were allowed to establish seedlings for two days. The seedlings were then transplanted to the aeroponic system previously described by Lee (1993). The nutrient solution used was Netherlands Standard Solution (Douglas 1985). At full strength, solution pH was maintained at *ca*. 6.5 and an electrical conductivity was 2.2 mS. The aerial parts of plants were subjected to the fluctuations of ambient temperature ranging from 24–38ºC under 100% prevailing solar radiation. The maximum PPFD at the plant canopy level was about 1,200 μmol m^{-2} s^{-1} on sunny days. Relative humidity was between 65–95%. Roots were grown in either 20ºC-RZT or A-RZT.

RZ [CO_2] treatments: Three weeks after transplanting, four different RZ [CO_2] (ambient, 360 ppm, and elevated concentrations of 2,000; 10,000; 50,000 ppm) were imposed on plants at each of two RZTs. During the photoperiod, between 10:30–11:30 h, when photosynthetic rate was the highest in the greenhouse, ambient [CO_2] was between 360 and 380 ± 5 ppm. We defined ambient [CO_2] as 360 ppm. Different elevated RZ [CO_2] concentrations were controlled by using premixed CO_2-air mixtures (*SOXAL*, *Singapore Oxygen Air Liquide Pte Ltd.*, Singapore), supplied from compressed air cylinders at ~ 0.5 L min^{-1}, with separate venting to different aeroponic troughs in the greenhouse. [CO_2] at the shoot base was measured with *LI-COR 6400* photosynthesis system (*LI-6400*, *Biosciences*, Lincoln, NE, USA). No significant increase in atmospheric [CO_2] was found, indicating that elevated CO_2 supplied to the RZ was in the form of CO_2 dissolved in the nutrient mist sprayed to the roots.

Measurements of light-saturated photosynthetic CO_2 assimilation (P_{Nmax}) and stomatal conductance (g_{smax}) in the greenhouse: Two weeks after different elevated RZ [CO_2] treatments, P_{Nmax} and g_{smax} of attached, fully expanded leaves (the 6th leaves from the base) were measured simultaneously during 09:00–11:00 h with an open infrared gas analysis system (*LI-6400*, *Biosciences*, Lincoln, NE, USA). Readings were taken with a LED light source which supplied 1,200 μmol m^{-2} s^{-1} of PPFD. The light source emitted in the wavelength range 660–675 nm. Average ambient [CO_2] and relative humidity in the chamber were 380 ± 2 μmol mol^{-1} and 70%, respectively. Leaf chamber temperature was set at 32ºC according to prevailing ambient conditions. Due to the large number of samples, measurements were made on two consecutive days. Measurements of P_{Nmax} and g_{smax} for plants treated with different RZ [CO_2] were carried out for plants grown at 20ºC-RZT on day 1, while those of A-RZT plants were completed on the next day. These measurements were repeated three times on the same leaves (total measurement period of 6 d).

Chlorophyll fluorescence F_v/F_m ratio: The chlorophyll fluorescence was measured with a *Plant Efficiency Analyser* (*Hansatech Instruments Ltd.*, England) on the same leaves from which P_{Nmax} and g_{smax} were recorded. The readings were carried out during 07:00–19:00 h. Attached leaves were predarkened with clips for 15 min prior to measurements. Dark-adapted leaves were placed under the light pipe and irradiated with the pulsed lower intensity-measuring beam to measure initial chlorophyll fluorescence (F_0). Maximum chlorophyll fluorescence (F_m) was assessed by 0.8 s of saturated pulse (> 6,000 µmol m^{-2} s^{-1}). The variable fluorescence yield (F_v) was determined by $F_m - F_0$. The efficiency of excitation energy captured by open PSII reaction centres in dark-adapted plant samples was estimated by F_v/F_m.

RWC: Fresh leaves were harvested from the same plants used for the measurements of P_{Nmax}, g_{smax}, and F_v/F_m ratio between 13:00–13:30h. RWC was determined using leaf discs as (FM – DM)/(TM – DM) × 100, where FM is the fresh mass, DM is the dry mass, and TM is the turgid mass of the leaf after floating on distilled water for 24 h.

FM and DM of shoots and roots: All plants were harvested after 3 weeks of elevated RZ [CO_2] treatments. At harvest, 6 weeks was the total duration of the plant growth. Following the removal from the troughs between 09:00 and 10:00 h, the plants were separated into shoots and roots. The shoots and the roots were blotted carefully and weighed separately. All tissues were wrapped in aluminum foil, dried at 80°C for 4 d and then reweighed.

NO_3^-: Dried tissue was ground using a pestle and mortar with deionised water and then incubated at 37°C for 2 h. Sample turbidity was removed by filtration through a 0.45 µm pore diameter membrane filter prior to analysis. The NO_3^- was determined using a *Flow Injection Analyser* (*QuikChem 8000*, *Lachat Instruments Inc.*, Milwaukee, WI, USA) by catalytical reduction of NO_3^- to NO_2^- by passage of the sample through a copperized cadmium column. NO_2^- was then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble dye had a magenta color, which was read at 520 nm.

TRN: Dried samples were placed into a digestion tube with a Kjeldahl tablet and 5 cm^3 of concentrated sulphuric acid. After the digestion was completed, the mixture was allowed to cool for 30 min and TRN concentration was determined by a *Kjeltec auto 1030 analyser* (*Tecator AB*, Höganäs, Sweden). TRN concentration was quantified through titration.

Total soluble protein: Thirty-six leaf discs, each of diameter 1 cm, were sampled from different leaves in the middle of the photoperiod and immediately stored in liquid nitrogen. Leaf samples were ground to fine powder in liquid nitrogen with pestle and mortar. Once the liquid nitrogen had evaporated, 100 mM Bicine [N,N-bis(2-hydroxyethyl)-glycine]-KOH (pH 8.1), 20 mM $MgCl_2$, and 2% PVP buffer was added. The mixture was placed in a *20.1 Ti* rotor and centrifuged at 100,000 × g for 30 min at 4°C in a *Beckman XL-100K* ultracentrifuge (*Beckman*, USA) to prepare a soluble protein extract. Aliquots of the soluble extract were mixed with 80% cold acetone and centrifuged at 25°C for 10 min at 2700 × g using a bench-top centrifuge. The precipitate was dissolved in 1 M NaOH and the total soluble and Rubisco proteins were determined as described by Jordan *et al.* (1992).

Rubisco protein: Aliquots of known protein concentration (usually 40 µg) were mixed with equal amount of 20% glycerol, 0.02% bromophenol blue, 5% SDS, 0.125 M Tris (pH 6.6), and 10% mercaptoethanol. The proteins were then separated in 12.5% to 22% gradient polyacrylamide gel with a 3.4% stacking gel (Jordan *et al.* 1992). A running buffer of 192 mM glycine, 3.47 mM SDS, and 25 mM Tris-HCl (pH 8.6) was used to run the gels before they were stained with 0.2% Coomassie Brilliant Blue in 10% acetic acid, and 50% methanol. The gels were destained with 7% acetic acid and 25% ethanol. The separated proteins were analysed using an *EPSON FX-850* scanning densitometer (*Pharmacia LKB Ultrascan XL*, USA). Areas of large and small subunits were calculated in terms of Rubisco concentrations according to a standard.

Statistical analysis: A two-way analysis of variance (*ANOVA)* was used first to test the effect of RZ [CO_2] and RZT on all variables. A separate *ANOVA* was then used to discriminate means across all treatments. For F_v/F_m ratios, at each RZT, one-way *ANOVA* was used to test significant differences among different RZ [CO_2], with means discriminated using *Tukey*'s multiple comparison test, when main effects *ANOVA* tests were significant. Under each RZ [CO_2], a *t*-test was also used to test for differences of two RZT under the same RZ [CO_2]. All statistical analyses were carried out using *MINITAB* software (*MINITAB, Inc., Release 15, 2007*).

Results

P_{Nmax}, g_{smax}, F_v/F_m, and midday RWC: Two weeks after different elevated RZ [CO_2] treatments, P_{Nmax} and g_{smax} were determined in the attached leaves in the greenhouse. Data obtained from the first two days are shown in Fig. 1. Similar data were observed over the next 4 consecutive days (data not shown). The interaction term "RZ [CO_2] × RZT" of two-way *ANOVA* for P_{Nmax} and g_{smax} was not significant (Table 1). Separate *ANOVA* analysis showed that at both RZTs, P_{Nmax} was significantly higher at each of three different elevated RZ [CO_2] compared with those of plants grown at ambient RZ [CO_2] of 360 ppm (Fig. 1*A*). P_{Nmax} was similar at RZ [CO_2] of 10,000 and

Table 1. Two-way analysis of variance of physiological variables, with *p* values presented for each main effect and their interaction.

	RZ [CO_2]	RZT	RZ [CO_2] × RZT
P_{Nmax} (Fig. 1*A*)	< 0.001	< 0.001	0.45
g_{smax} (Fig. 1*B*)	< 0.001	< 0.001	0.89
Midday leaf RWC (Table 2)	< 0.001	< 0.001	0.19
Shoot FM (Fig. 3*A*)	< 0.001	< 0.001	0.44
Shoot DM (Fig. 3*B*)	< 0.001	< 0.001	0.29
Root FM (Fig. 3*C*)	< 0.001	< 0.001	0.08
Root DM (Fig. 3*D*)	< 0.001	< 0.001	0.73
Shoot/root FM Ratio (Fig. 3*E*)	< 0.001	< 0.001	0.39
Shoot/root DM Ratio (Fig. 3*F*)	< 0.001	< 0.001	0.82
Shoot NO_3^-concentration (Fig. 4*A*)	< 0.001	< 0.001	0.33
Root NO_3^-concentration (Fig. 4*B*)	< 0.001	< 0.001	0.61
Shoot TRN concentration (Fig. 4*C*)	< 0.001	< 0.001	0.95
Root TRN concentration (Fig. 4*D*)	< 0.001	< 0.001	0.16
Leaf total soluble protein concentration (Table 3)	< 0.001	< 0.001	0.21
Leaf Rubisco protein concentration (Table 3)	< 0.001	< 0.001	0.54

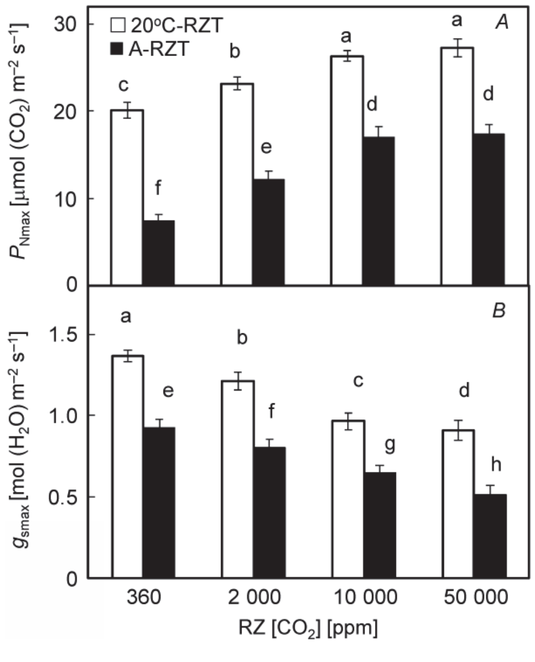

Fig. 1. P_{Nmax} (*A*) and g_{smax} (*B*) of lettuce plants grown under different levels of elevated RZ [CO_2] at 20°C-RZT (*open bars*) and A-RZT (*closed bars*) for 2 weeks. Each value is the mean of 5 measurements of 5 different leaves (*n* = *5*). Vertical bars represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

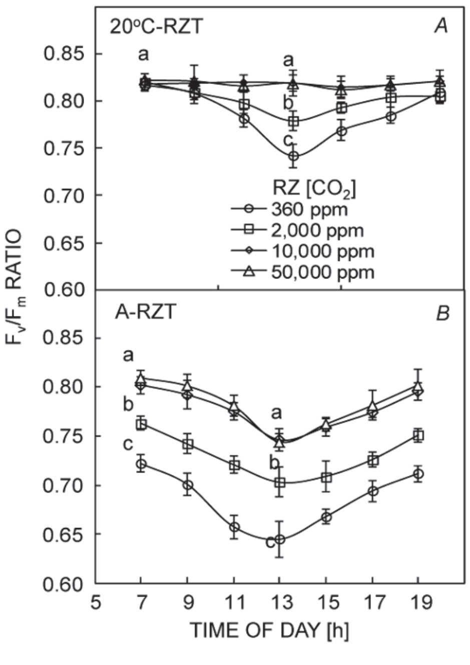

Fig. 2. Diurnal changes of F_v/F_m ratio of lettuce plants grown under different levels of elevated RZ [CO_2] at 20°C-RZT (*A*) and A-RZT (*B*) for 2 weeks. Each value is the mean of 5 measurements of 5 different leaves (*n* = *5*). Vertical bars represent the standard errors. Means with *different letters* above the curves are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

Table 2. Midday leaf RWC [%] of lettuce plants grown under different levels of elevated RZ [CO_2] at 20°C-RZT and A-RZT for 2 weeks. Each value is the mean of 5 measurements of 5 different leaves ($n = 5$). Means with *different letters* are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

	RZ [CO_2] [ppm] 360	2,000	10,000	50,000
20°C-RZT	82.2 ± 1.21^{c}	87.2 ± 0.70^{b}	$94.\pm 0.88^{a}$	94.6 ± 0.61^{a}
A-RZT	65.2 ± 0.89^{f}	68.2 ± 1.03^{e}	75.9 ± 0.76^{d}	76.3 ± 0.87^{d}

50,000 ppm, but it was much higher than RZ [CO_2] of 360 and 2,000 ppm. It was interesting to note that at both RZTs, g_{smax} was significantly lower in all plants grown at elevated RZ [CO_2] than at ambient RZ [CO_2] of 360 ppm, but g_{smax} values of plants grown at 10,000 and 50,000 ppm RZ [CO_2] did not differ significantly (Fig. 1*B*).

Diurnal changes in F_v/F_m were determined in the attached leaves during 6 sunny days. Similar data were observed during any of those 6 days and only data obtained from the 2nd day are shown in Fig. 2. At 20°C-RZT, no significant differences in F_v/F_m ratios were observed among different RZ [CO_2] treatments (Fig. 2*A*) at 07:00 h and they were all greater than 0.8. Similar values of F_v/F_m ratios were observed for plants grown under RZ [CO_2] of 10,000 and 50,000 ppm throughout the day at 20°C-RZT. However, reduced F_v/F_m ratios were observed in plants grown under RZ [CO_2] of 360 and 2,000 ppm from 11:00 h and the lowest F_v/F_m ratios were observed at 13:00 h with greater decrease in F_v/F_m ratio under RZ [CO_2] of 360 ppm than under RZ [CO_2] of 2,000 ppm. For these plants, recovery of F_v/F_m ratios were seen from 15:00 h onwards and F_v/F_m ratios were greater than 0.8 at 19:00h. At A-RZT, F_v/F_m ratio of plants grown under RZ [CO_2] of 10,000 and 50,000 ppm were greater than 0.8 at 07:00 h. However, decreased F_v/F_m ratios (<0.8) at 07:00 h were obtained from plants grown under RZ [CO_2] of 360 and 2,000 ppm. F_v/F_m ratio was significantly lower in plants grown at RZ [CO_2] of 360 ppm compared with those grown at each of elevated RZ [CO_2] throughout the day (Fig. 2*B*). F_v/F_m ratios were similar at RZ [CO_2] of 10,000 and 50,000 ppm, but they were much higher than RZ [CO_2] of 360 and 2,000 ppm at any given time from 07:00 to 19:00 h. Under RZ [CO_2] of 360 and 2,000 ppm, F_v/F_m ratios were much lower in plants grown at A-RZT (Fig. 2) than at 20°C-RZT.

Responses of midday leaf RWC (Table 2) to different RZ [CO_2] at both RZTs were similar to those of P_{Nmax} (Fig. 1*A*). Higher midday leaf RWC was observed at all elevated RZ [CO_2] than at ambient RZ [CO_2] of 360 ppm. Although the responses of P_{Nmax}, g_{smax}, and midday leaf RWC to different RZ [CO_2] were similar, compared with those plants grown at 20°C-RZT, these variables were much lower at A-RZT. However, the change of each variable under elevated RZ [CO_2] was greater at A-RZT than at 20°C-RZT (Fig. 1, Table 2).

Shoot and root productivity: The interaction term "RZ [CO_2] × temperature" of two-way *ANOVA* for FM and DM of the shoots and roots, and the shoot/root FM and DM ratios were not significant (Table 1). Separate *ANOVA* analysis showed that FM and DM of the shoots and the roots were significantly higher in plants at all elevated RZ [CO_2] than at ambient RZ [CO_2] of 360 ppm at each RZT (Fig. 3). The shoot FM of plants, which were exposed to elevated RZ [CO_2], had 12–40% and 17–69% increases at 20°C-RZT and A-RZT, respectively, compared with those plants grown at ambient RZ [CO_2] (Fig. 3*A*). There were no significant differences in the shoot FM and DM, when the plants were exposed to elevated RZ [CO_2] of 10,000 and 50,000 ppm, but they were much higher than those plants at elevated RZ [CO_2] of 2,000 ppm (Fig. 3*A*,*B*). However, the root FM and DM of the plants grown at elevated RZ [CO_2] of 50,000 ppm were significantly greater than at 10,000 ppm (Fig. 3*C*,*D*). It was also observed that the increase of the root FM was greater than that of the shoot FM under elevated RZ [CO_2] at each RZT. For example, at 20°C-RZT, the shoot and root FM at 50,000 ppm RZ [CO_2] were about 1.4 and 1.9 fold higher than those at 360 ppm RZ [CO_2]. For the plants grown at A-RZT, the shoot and root FM was 1.9 and 2.7 fold higher than that of ambient RZ [CO_2] plants, respectively (Fig. 3*A*,*C*). Similar trends were also observed for the shoot and root DM (Fig. 3*B*,*D*). Higher increases of FM and DM of the roots at elevated RZ [CO_2] resulted in significantly lower shoot/root FM and DM ratios under all elevated RZ [CO_2] compared with those of 360 ppm RZ [CO_2] at each RZT (Fig. 3*E,F*).

NO_3^-, TRN, soluble and Rubisco protein: Shoot and root NO_3^- and TRN concentrations were determined in the same plants used for measuring the plant productivity (Fig. 3). "RZ [CO_2] × RZT" of two-way *ANOVA* for NO_3^- and TRN concentration were not significant (Table 1). Separate *ANOVA* analysis indicated that NO_3^- and TRN concentrations of the shoots and roots were significantly higher in all elevated RZ [CO_2] plants than in the plants grown at 360 ppm RZ [CO_2] at each RZT (Fig. 4). There were no significant differences in all these variables when plants were exposed to elevated [CO_2] of 10,000 and 50,000 ppm, but they were significantly greater than those plants exposed to elevated RZ [CO_2] of 2,000 ppm. At each RZ [CO_2], NO_3^- , and TRN concentrations of the shoots were higher at 20°C-RZT than at A-RZT (Fig. 4*A*,*C*). The roots, however, had higher NO_3^- and TRN at A-RZT than at 20°C-RZT (Fig. 4*B*,*D*).

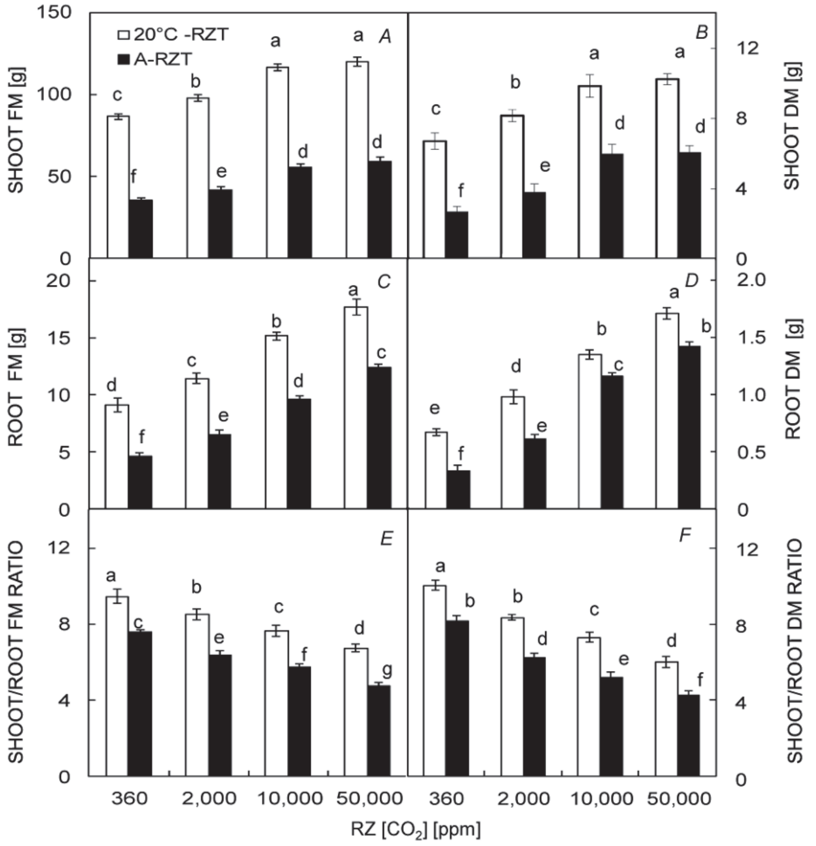

Fig. 3. Fresh mass (FM) and dry mass (DM) of shoot (*A, B*) and root (*C, D*), shoot/root ratio (*E, F*) of lettuce plants grown under different levels of elevated RZ [CO2] at 20°C-RZT (*open bars*) and A-RZT (*closed bars*) for 3 weeks. Each value is the mean of 5 measurements of 5 different leaves ($n = 5$). Vertical bars represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

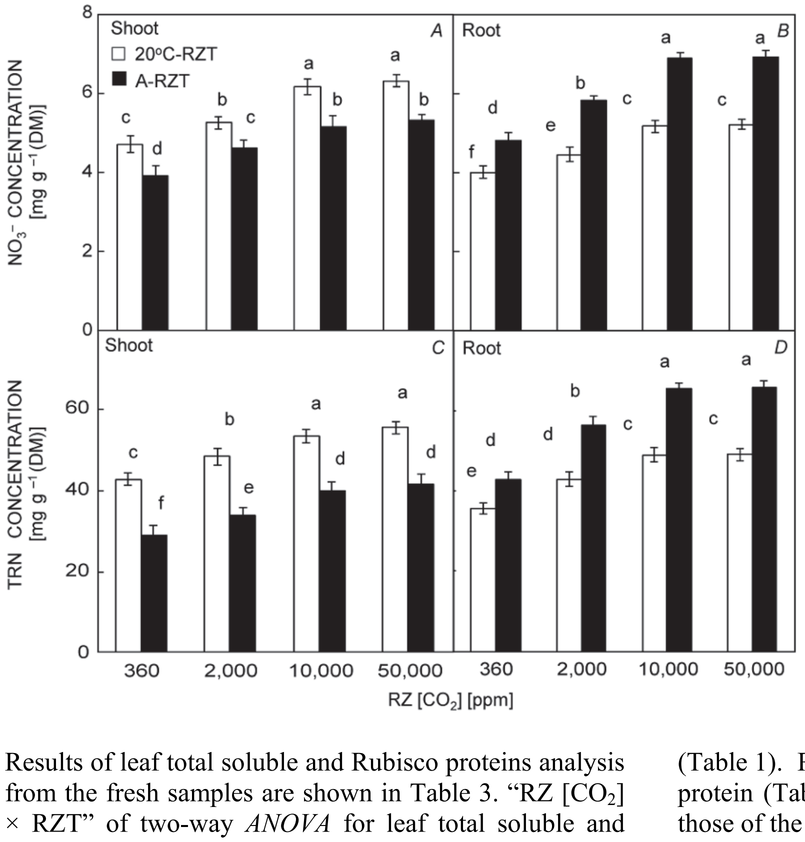

Fig. 4. NO_3^- and total reduced nitrogen (TRN) concentration of shoot (*A,B*) and root (*C,D*) of lettuce plants grown under different levels of elevated RZ [CO_2] at 20°C-RZT (*open bars*) and A-RZT (*closed bars*) for three weeks. Each value is the mean of 5 measurements of 5 different plants ($n = 5$). Vertical bars represent the standard errors. Means with *different letters* above the columns are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

Results of leaf total soluble and Rubisco proteins analysis from the fresh samples are shown in Table 3. "RZ [CO_2] × RZT" of two-way *ANOVA* for leaf total soluble and Rubisco protein concentration was not significant (Table 1). Reponses of leaf total soluble and Rubisco protein (Table 3) to RZ [CO_2] and RZT were similar to those of the shoot TRN (Fig. 4*C*).

Table 3. Leaf soluble and Rubisco protein concentrations of lettuce plants grown under different levels of elevated RZ [CO_2] at 20°C-RZT and A-RZT for three weeks. Each value is the mean of 5 measurements of 5 different plants (n = 5). Means with *different letters* are statistically different (p<0.001) as determined by *Tukey*'s multiple comparison test.

RZT	RZ [CO_2] [ppm]	Leaf soluble protein [g m^{-2}]	Leaf Rubisco protein [g m^{-2}]
20°C-RZT	360	5.75 ± 0.15^c	3.85 ± 0.15^c
	2,000	6.82 ± 0.18^b	4.52 ± 0.18^b
	10,000	8.02 ± 0.12^a	5.34 ± 0.16^a
	50,000	8.23 ± 0.14^a	5.73 ± 0.13^a
A-RZT	360	4.32 ± 0.17^e	2.32 ± 0.15^e
	2,000	5.13 ± 0.10^d	3.11 ± 0.15^d
	10,000	6.89 ± 0.14^b	4.59 ± 0.15^b
	50,000	6.94 ± 0.15^b	4.64 ± 0.15^b

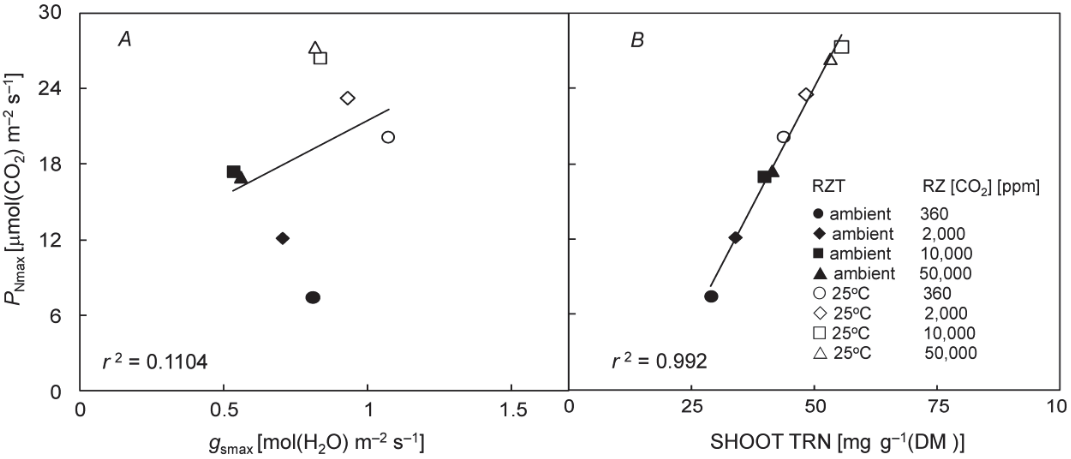

Fig. 5. Correlations between light-saturated net photosynthetic rate (P_{Nmax}) and light-saturated stomatal conductance (g_{smax}), (*A*) (data derived from Fig. 1) and P_{Nmax} and shoot total reduced nitrogen (TRN) concentration (*B*) (data derived from Figs. 1*A*, 4*C*) of lettuce plants grown under different levels of elevated RZ [CO_2] at 20ºC- and A-RZT for three weeks.

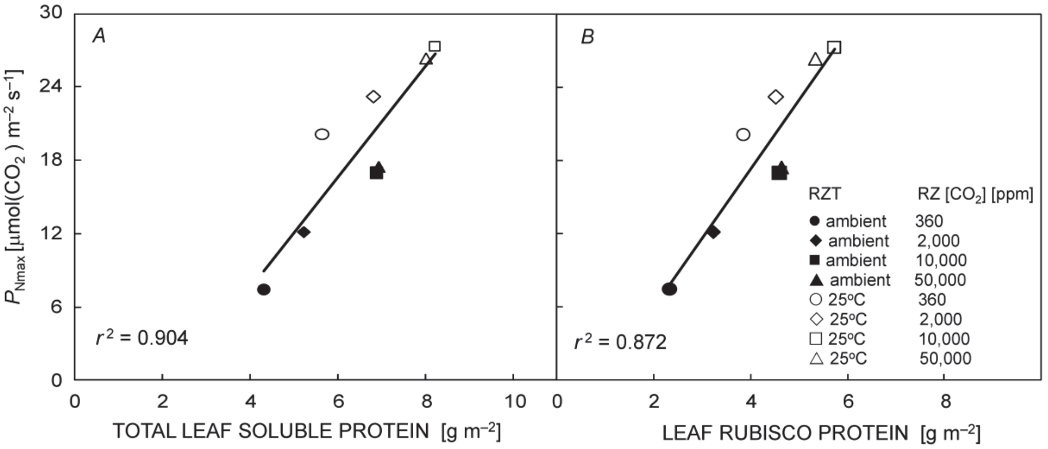

Fig. 6. Correlations between P_{Nmax} and total leaf soluble (*A*) and Rubisco concentration (B) (data derived from Fig. 1*A*, Table 3) of lettuce plants grown under different levels of elevated RZ [CO_2] at 20ºC- and A-RZT for three weeks.

Correlations between P_{Nmax} and g_{smax}, shoot TRN, leaf soluble and Rubisco proteins: Across all treatments, there was no correlation between P_{Nmax} and g_{smax} (Fig. 5*A*, p=0.14) but these variables were negatively correlated within each RZT. However, close linear correlations between P_{Nmax} and shoot TRN concentrations (Fig. 5*B*), total leaf soluble (Fig. 6*A*) and Rubisco protein concentrations (Fig. 6*B*) were established in the plants grown under different RZ [CO_2] and RZTs (p<0.001).

Discussion

The present study showed that P_{Nmax} increased with increasing RZ [CO_2] (Fig. 1*A*) with a RZ [CO_2] of 10,000 ppm sufficient for maximising productivity of aeroponically grown lettuce plants at both RZTs (Fig. 3). However, g_{smax} was significantly lower at higher RZ [CO_2] than at ambient RZ [CO_2] (Fig. 1*B*) and therefore, the enhancement of P_{Nmax} under elevated RZ [CO_2] was not due to the increase in g_{smax}. Obviously, there was more internal CO_2 available to the plants grown under elevated RZ [CO_2] as dissolved CO_2 in the xylem sap could be carried upward in the stem when plants were transpiring (Teskey and McGuire 2005) and fixed in green tissues (McGuire *et al.* 2009). Under A-RZT, reduced g_{smax} is the main cause of reduced P_{Nmax}, because it reduces CO_2 availability for carboxylating reaction (He *et al.* 2001). In the present study, although it was unable to provide the amount of elevated RZ [CO_2] needed to obtain the effects on $P_{Nmax,}$ higher P_{Nmax} observed in plants treated with higher elevated RZ CO_2 supported our hypothesis that elevated RZ [CO_2] enhanced photosynthesis. Our previous (He *et al.* 2001) and present (Table 2) studies showed that temperate lettuce plants grown under tropical A-RZT had midday leaf RWC below 80–70%, had lower g_{smax}, and they showed symptoms of wilting during midday. However, stomata were still partially open and all plants were still transpiring. Although it was not directly measured, the amount of CO_2 dissolved in the nutrition solution might still be higher in the transpiration stream (despite the lower g_{smax} and lower transpiration rate), when RZ was supplied with elevated RZ [CO_2] compared with ambient RZ [CO_2].

In this aeroponic study, A-RZT plants had similar responses to elevated RZ [CO_2] as the 20°C-RZT plants. They had higher P_{Nmax} (Fig. 1*A*) with much lower g_{smax} (Fig. 1*B*) under the same elevated RZ [CO_2]. The magnitudes of increases in P_{Nmax} under elevated RZ [CO_2] were even greater at A-RZT than at 20°C-RZT. As mentioned earlier, higher P_{Nmax} obtained for the plants grown at elevated RZ [CO_2] might partially result from using dissolved CO_2 *via* transpiration stream from roots. According to Cramer and Richards (1999), hydroponically grown tomato (*L. esculentum*) seedlings benefited significantly from elevated, dissolved CO_2 when exposed to high-temperature stress at high-light intensities due to partial closure of the stomata and lower water-use efficiency. In this study, the lettuce plants grown under elevated RZ [CO_2] maintained higher midday RWC (Table 2) accompanied by lower g_{smax} (Fig. 1*B*).

Cooling the roots to 20°C-RZT allowed higher P_{Nmax} due to a reduction of dynamic photoinhibition and a prevention of chronic photoinhibition compared with the A-RZT plants (He *et al.* 2001). In the present study, none of the plants grown at 20ºC-RZT showed chronic photoinhibition as they all had F_v/F_m higher than 0.8 at 07:00 h (Fig. 2*A*). The plants grown at 20°C-RZT under higher RZ [CO_2] of 10,000 and 50,000 ppm had constantly high values of F_v/F_m ratio (> 0.8) throughout the day, suggesting that dynamic photoinhibition did not occur in these plants (Fig. 2*A*). However, the plants, which were grown at 20°C-RZT under RZ [CO_2] of 360 and 2,000 ppm, exhibited dynamic photoinhibition. Both chronic and dynamic photoinhibition were observed in A-RZT plants under lower RZ [CO_2], but the effects were much lesser under elevated RZ [CO_2] of 2,000 ppm than under RZ [CO_2] of 360 ppm (Fig. 2*B*). We also found that decreases in midday F_v/F_m ratio were much lower under elevated RZ [CO_2] than under ambient RZ [CO_2] in lettuce grown under fluctuating air temperature from 20–36°C, under a maximal PPFD of 2,100 μmol m^{-2} s^{-1} in New Zealand (He *et al.* 2007).

Decreased photosynthetic electron consumption is one of important direct consequences of reduction in CO_2 supply, causing a downregulation of photosynthetic efficiency and increased requirement for additional electron and O_2 consumption, such as photorespiration and the Mehler reaction (Cornic and Fresneau 2002). When stomatal limitation of photosynthesis was more severe in A-RZT plants, more endogenous electron acceptors became reduced and O_2 might be the main available electron acceptor and this could result in photodamage of photosynthetic apparatus. It was reported that transport of carbon from the roots to shoot *via* xylem could alleviate not only photoinhibition but also photorespiration (Cramer and Richards 1999). According to Cornic and Fresneau (2002), up to a leaf RWC of about 70%, it is likely that stomata closure plays the main role in the decline of leaf photosynthesis. The CO_2 concentration in the chloroplasts declines as stomata close in drying leaves. As a consequence, ribulose-1,5-bisphosphate oxygenation increases in C_3 plants and it becomes the main sink for photosynthetic electrons. In the present study, higher midday leaf RWC (> 70%, Table 2) of lettuce plants grown under elevated RZ [CO_2] could help them to alleviate both photorespiration and photoinhibition.

Reduction of photoinhibition and photorespiration may be important in determining the growth and biomass accumulation, especially, when stomata are partially closed under high temperatures during midday. Lettuce plants that accumulated more biomass at elevated RZ [CO_2] than at ambient RZ [CO_2] at both RZTs (Fig. 3) supported this assumption. In the present study, we found greater increases in the root (Fig. 3*C*,*D*) than in the shoot FM and DM (Fig. 3*A*,*B*) at elevated RZ [CO_2] than at ambient RZ [CO_2]. Thus, the lower shoot/root FM and DM ratios (Fig. 3*E*,*F*) were obtained from plants grown at elevated RZ [CO_2] compared with those grown at ambient RZ [CO_2]. The percentage of increase in biomass under elevated RZ [CO_2] was greater at A-RZT than at 20ºC-RZT although the total biomass was higher at 20ºC-RZT. It has been reported that the temperature of a sink affects its metabolic rate and hence its capacity to utilize carbohydrates (Schurr *et al.* 2000, He *et al.* 2009). Our previous results showed that roots of 20ºC-RZT lettuce plants did not have only greater biomass but also longer total length with a greater number of tips and surface area, and smaller average diameter of roots as compared with A-RZT plants (He *et al.* 2009). Plants typically have more extensive and active root systems at elevated atmospheric CO_2 concentrations than at ambient CO_2 (Norby 1994), which allows them to explore larger volumes of soil in search of nutrients (Norby *et al.* 1992). In this study, elevated RZ [CO_2] also stimulated the root development in lettuce plants grown at both RZTs (data not shown). The larger root system with more fine roots resulted in greater uptake of nutrient such as NO_3^- under elevated RZ [CO_2]. On the other hand, acclimation of photosynthesis to growth at elevated atmospheric CO_2 was reported to depend on the source/sink ratio within the plant and 'sink capacity' (Makino and Mae 1999). Larger root systems increased their capacity for utilizing photoassimilates (Ainsworth and Rogers 2007, He *et al.* 2009, 2010).

It was reported that acclimation of photosynthesis is connected with the N-status of the plant (Evans 1989). The components of chloroplasts represent a large proportion of total leaf N (Evans 1989). Leaf N content per unit area often declines under elevated CO_2 (Ellsworth *et al.* 2004), which could be due to larger leaf biomass requiring higher N investment into Rubisco. Decreased N concentration is usually interpreted as the evidence that NO_3^- uptake and assimilation have not kept pace with photosynthesis and growth under elevated [CO_2] (Pettersson and McDonald 1994). In the present study, higher NO_3^- uptake and higher concentration of TRN in lettuce plants were observed in the plants grown under elevated RZ [CO_2] compared with those grown under ambient RZ [CO_2] at both RZTs (Fig. 4). Increased uptake of NO_3^- by roots under enriched RZ [CO_2] has been reported in hydroponically grown tomato plants (van der Merwe and Cramer, 2000). Plants grown under elevated RZ [CO_2] had higher P_{Nmax} and higher productivity at both RZTs; it could be also partially due to dissolved CO_2 incorporation, which allowed the improved incorporation of N into amino acids in the roots as a consequence of greater supplies of anaplerotic carbon for protein synthesis (Cramer and Lewis 1993; Viktor and Cramer 2003). NO_3^- incorporation into organic, N containing compounds involves the reduction of NO_3^- to NO_2^- *via* the cytosolic enzyme nitrate reductase (NR), which is an NO_3^--inducible enzyme and is under a complex regulation (Cookson *et al.* 2005). It was reported that TRN content decreased at high RZT (Du and Tachibana 1994, He *et al.* 2001). Decrease in NR activity could be also linked to the decline in the rate of photosynthesis due to stomata closure (Kaiser and Brendel-Benisch 1991). In the study with wheat (*Triticum durum* L.) plants, Fresneau *et al.* (2007) concluded that a drought-induced decrease of the leaf internal CO_2 concentration partially triggered the decrease in NR activity. Effects of RZ [CO_2] on NR activity deserve further studies.

Rubisco has a relatively poor affinity for CO_2 and competing reaction with O_2 and it is widely thought to limit the irradiance-saturated rate of photosynthesis (Andrews and Lorimer 1987). The low efficiency of Rubisco is partly offset by the vast amount of Rubisco protein in the leaf (Woodrow and Berry 1988). Stitt and Schulze (1994) concluded that Rubisco as a 'reserve ' protein could be advantageous in providing greater efficiency of photosynthesis in response to fluctuating conditions within a day. In our previous study (He *et al.* 2001), stomata closure in response to high A-RZT was observed immediately, when lettuce plants were transferred from 20ºC-RZT to A-RZT. However, decreases in P_{Nmax} in A-RZT plants cannot be simply recovered after increasing g_{smax} by transferring the lettuce plants back to 20ºC-RZT as Rubisco activity and Rubisco protein were also affected by A-RZT due to the N deficiency of these plants (He and Lee 2001). In the present study, a larger root system within the aeroponic system under elevated RZ [CO_2] at 20ºC-RZT increased not only the shoot NO_3^- and concentration of TRN, but also the leaf soluble and Rubisco protein (Table 3). In the analysis of the cause of the decrease in N across the elevated [CO_2] experiments, Long *et al.* (2004) suggest that almost all of the decline in leaf N could be accounted for by a decrease in Rubisco that comprises 27% of total leaf N (Makino *et al.* 1997). It has been reported that reduced amount of Rubisco results in lower photosynthetic CO_2 assimilation (Makino *et al.* 1997, Makino and Mae 1999). Although the benefits of higher Rubisco content are partially offset by lower Rubisco activation in high-N leaves (Ray *et al.* 2003), P_{Nmax} is generally higher in high-N leaves than in low-N leaves (Makino *et al.* 2003). Increases in shoot N, leaf soluble and Rubisco protein concentrations result in higher photosynthetic CO_2 assimilation (Li *et al.* 2009). In the present study, there were positive linear relationships between P_{Nmax} and the shoot TRN concentration (Fig. 5*B*), leaf soluble protein (Fig. 6*A*) and Rubisco concentration (Fig. 6*B*). These findings further suggested that the increased P_{Nmax} under elevated RZ [CO_2] at both high A-RZT and 20ºC-RZT could be partially due to higher shoot TRN concentration, higher soluble and Rubisco proteins, but not due to g_{smax} (Fig. 5*A*) (He *et al.* 2007, 2010).

Conclusions: We observed the benefits of elevated RZ [CO_2] at both RZTs within a short term of treatments by increased P_{Nmax}, decreased g_{smax} with less reduced midday leaf RWC and F_v/F_m ratio and elevated Rubisco content resulting from higher levels of TRN and soluble protein. A larger root system, which in turns not only enhanced

NO_3^- uptake but also increased the capacity for utilizing photoassimilates, indicated the acclimation of photosynthesis to elevated RZ [CO_2] though altering the source/sink ratio with increased 'sink capacity'. In conclusion, the increased P_{Nmax} and productivity under elevated RZ [CO_2] at both RZTs was due to alleviation of water loss, chronic and dynamic of photoinhibition, and higher turnover of Calvin cycle enzymes with higher concentration of Rubisco proteins.

References

Ainsworth, E.A., Rogers, A.: The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. – Plant Cell Environ. **30**: 258-270, 2007.

Andrews, J.T., Lorimer, G.M.: Rubisco: structure, mechanism and prospects for improvement. – In: Hatch, M.D., Boardman, N.K., (ed.): The Biochemistry of Plants. Vol. 10. Pp. 132-219. Academic Press, New York 1987.

Chaves, M.M., Oliveira, M.M.: Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. – J. Exp. Bot. **55**: 2365-2384, 2004.

Cookson, S.J., Williams, L.E., Miller, A.J.: Light-dark changes in cytosolic nitrate pools depend on nitrate reductase activity in *Arabidopsis* leaf cells. – Plant Physiol. **138**: 1097-1105, 2005.

Cornic, G., Fresneau, C.: Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought. – Ann. Bot. **89**: 887-894, 2002.

Cramer, M.D., Lewis, O.A.M.: The influence of nitrate and nium nutrition on the growth of wheat (*Triticum aestivum*) and maize (*Zea mays*) plants. – Ann. Bot. **72**: 359-365, 1993.

Cramer, M.D., Richards, M.D.: The effect of rhizosphere dissolved inorganic carbon on gas exchange characteristics and growth rates of tomato seedlings. – J. Exp. Bot. **50**: 79-87, 1999.

De Jong, E., Schappert, H.J.V.: Calculation of soil respiration and activity from CO_2 profile in the soil. – Soil Sci. **113**: 328-333, 1972.

Douglas, J.M.: Advanced Guide to Hydroponics. – Pelham Books/Stephen Greene Press, London 1985.

Du, Y.C., Tachibana, S.: Effect of supraoptimal root temperature on the growth, root respiration and sugar content of cucumber plants. – Sci. Hort. **58**: 289-301, 1994.

Ellsworth, D.S., Reich, P.B., Naumburg, E.S. *et al.*: Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. – Global Change Biol. **10:** 2121-2138, 2004.

Evans, J.R.: Photosynthesis and nitrogen relationships in leaves of C_3 plants. – Oecologia **78**: 9-19, 1989.

Fresneau, C., Ghashghaie, J., Cornic, G.: Drought effect on nitrate reductase and sucrose-phosphate synthase activities in wheat (*Triticum durum* L.): role of leaf internal CO_2. – J. Exp. Bot. **58**: 2983-2992, 2007.

He, J.: Impact of root-zone temperature on photosynthetic efficiency of aeroponically grown temperate and subtropical vegetable crops in the tropics. – In: Buchner, T.B., Ewingen, N.H. (ed.): Theory and Applications in Energy, Biotechnology and Nanotechnology. Pp. 111-144. Nova Science Publishers, New York 2009.

He, J.: Mineral nutrition of aeroponically grown subtropical and temperate crops in the tropics with manipulation of root-zone temperature at different growth irradiances. – Plant Stress **4**: 14-30, 2010.

He, J., Austin, P.T., Nichols, M.A.: Effect of root-zone CO_2 on productivity and photosynthesis in aeroponically grown lettuce plants. – Acta Hort. **648**: 39-45, 2004.

He, J., Austin, P.T., Lee, S.K.: Effects of elevated root zone CO_2 and air temperature on photosynthetic gas exchange, nitrate uptake, and total reduced nitrogen content in aeroponically grown lettuce plants. – J. Exp. Bot. **61**: 3959-3969, 2010.

He, J., Austin, P.T., Nichols, M.A., Lee, S.K.: Elevated root-zone CO_2 protects lettuce plants from midday depression of photosynthesis. – Environ. Exp. Bot. **61:** 94-110, 2007.

He, J., Chee, C.W., Goh, C. J.: "Photoinhibition" of *Heliconia* under natural tropical conditions- Importance of leaf orientation for light interception and leaf temperature. – Plant Cell Environ. **19**: 1238-1248, 1996.

He, J., Chua, N.Y.A., Qin, L.: Interaction between iron stress and root-zone temperature on physiological aspects of aeroponically grown Chinese broccoli (*Brassica alboglabra*). – J. Plant Nutri. **31**: 1-20, 2008.

He, J., Lee, S.K.: Growth and photosynthetic characteristics of lettuce (*Lactuca sativa* L.) grown under fluctuating hot ambient temperature with the manipulation of cool root zone temperature. – J. Plant Physiol. **152**: 387-391, 1998a.

He, J., Lee, S.K.**:** Growth and photosynthetic responses of three aeroponically grown lettuce cultivars (*Lactuca sativa* L.) to different root zone temperatures and growth irradiances under tropical aerial condition. – J. Hort. Sci. Biotech. **73**: 173-180, 1998b.

He, J., Lee, S.K.: Relationship among photosynthesis, Ribulose-1,5-bisphosphate carboxylase (Rubisco) and water relations of subtropical vegetable Chinese broccoli grown in the tropics by manipulation of root-zone temperature. – Environ. Exp. Bot. **46**: 119-128, 2001.

He, J., Lee, S.K.: Photosynthetic utilization of radiant energy by temperate lettucegrown under natural tropical condition with manipulation of root-zone temperature. – Photosynthetica **42**: 457-463, 2004.

He, J., Lee, S.K., Dodd, I.C.: Limitations to photosynthesis of lettuce grown under tropical conditions: alleviation by root-zone cooling. – J. Exp. Bot**. 52**: 1323-1330, 2001.

He, J., Tan, L.P., Lee, S.K.: Root-zone temperature effects on photosynthesis, ^{14}C-photoassimilate partitioning and growth of temperate lettuce (*Lactuca sativa* cv. 'Panama') grown in the tropics. – Photosynthetica **47**: 95-103, 2009.

Jordan, B.R., He, J., Chow, W.S., Anderson, J.M.: Changes in mRNA levels and polypeptide subunits of ribulose 1,5-biophosphate carboxylase in response to supplementary ultraviolet-B radiation. – Plant Cell Environ. **15**: 91-98, 1992.

Kaiser, W.M., Brendle-Behnisch, E.: Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis. I. Modulation in vivo by CO_2 availability. – Plant Physiol. **96**: 363-367, 1991.

Lee, S.K.: Aeroponic system as a possible alternative for crop production in Singapore. – Commonwealth Agri. – Digest **3**: 1-14, 1993.

Li, Y., Gao, Y., Xu, X. *et al*: Light-saturated photosynthetic rate in high-nitrogen rice (*Oryza sativa* L.) leaves is related to chloroplastic CO_2 concentration. – J. Exp. Bot. **60**: 2351-2360, 2009.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R.: Rising atmospheric carbon dioxide: plants FACE the future. – Annu. Rev. Plant Biol. **55**: 591-628, 2004.

Makino, A., Mae, T.: Photosynthesis and plant growth at elevated levels of CO_2. – Plant Cell Physiol. **40**: 999-1006, 1999.

Makino, A., Sakuma, H., Sudo, E., Mae, T.: Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. – Plant Cell Physiol. **44**: 952-956, 2003.

Makino, A., Shimada, T., Takumi, S. *et al.*: Does decrease in ribulose-1,5-bisphosphate carboxylase by antisense rbcS lead to a higher N-use efficiency of photosynthesis under conditions of saturating CO_2 and light in rice plants? – Plant Physiol. **114**: 483-491, 1997.

McGuire, M.A., Marshall, J.D., Teskey, R.O.: Assimilation of xylem-transported 13C-labelled CO_2 in leaves and branches of sycamore (*Platanus occidentalis* L.). – J. Exp. Bot. **60**: 3809-3817, 2009.

Norby, R.J.: Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. – Plant Soil **165**: 9-20, 1994.

Norby, R.J., Gunderson, C.A., Wullschleger, S.D. *et al.*: Productivity and compensatory responses of yellow-poplar trees in elevated CO_2. – Nature **357**: 322-24, 1992.

Norstadt, F.A., Porter, L.K.: Soil gases and temperatures: a beef cattle feedlot compared to alfalfa. – Soil. Sci. Soc. Amer. J. **48**: 783-789, 1984.

Pettersson, R., McDonald, J.S.: Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. – Photosynth. Res. **39**: 389-400, 1994.

Ray, D., Sheshshayee, M.S., Mukhopadhyay, K. *et al.*: High nitrogen use efficiency in rice genotypes is associated with higher net photosynthetic rate at lower Rubisco content. Biol. – Planta **46**: 251-256, 2003.

Schurr, U., Heckenberger, U., Herdel, K. *et al.*: Leaf development in *Ricinus communis* during drought stress: dynamics of growth processes, of cellular structure and of sink-source transition. – J. Exp. Bot. **51**: 1515-1529, 2000.

Stitt, M., Schulze, D.: Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. – Plant Cell Environ. **17**: 465-487, 1994.

Tan, L.P., He, J., Lee, S.K.: Effects of root-zone temperature on the root development and nutrient uptake of *Lactuca sativa* L. cv 'Panama' grown in an aeroponic system in the tropics. – J. Plant Nutr. **25**: 297-314, 2002.

Teskey, R.O., McGuire, M.A.: Carbon dioxide transport in xylem causes errors in estimation of rates of respiration in stems and branches of trees. – Plant Cell Environ. **25**: 1571-1577, 2002.

Teskey, R.O., McGuire, M.A.: CO_2 transported in xylem sap affects CO_2 efflux from *Liquidambar styraciflua* and *Platanus occidentalis* stems, and contributes to observed wound respiration phenomena. – Trees Struct. Funct. **19**: 357-362, 2005.

Teskey, R.O., McGuire, M.A.: Measurement of stem respiration of sycamore (*Platanus occidentalis* L.) trees involves internal and external fluxes of CO_2 and possible transport of CO_2 from roots. – Plant Cell Environ. **30**: 570-579, 2007.

van der Merwe, C.A., Cramer, M.D.: The influence of dissolved inorganic carbon in the root-zone on nitrogen uptake and the interaction between carbon and nitrogen metabolism. – In: Louçao, M.A., Lips, S.H. (ed.): Nitrogen in a Sustainable Ecosystem – from the Cell to the Plant. Pp. 145-151. Backhuys Publishers, Leiden 2000.

Viktor, A., Cramer, M.D.: Variation in root-zone CO_2 concentration modifies isotopic fractionation of carnon and nitrogen in tomato seedlings. – New Phytol. **157**: 45-54, 2003.

Woodrow, I.E., Berry, J.A.: Enzymatic regulation of photosynthetic CO_2 fixation in C_3 plants. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **39**: 533-594, 1998.