Effects of Elevated Root-Zone CO_2 and Root-Zone Temperature on Productivity and Photosynthesis of Aeroponically Grown Lettuce Plants

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Abstract: Elevated root-zone (RZ) [CO_2] resulted in significantly higher maximal photosynthetic CO_2 assimilation rate (A) but lower stomatal conductance (g_s) in aeroponically grown temperate lettuce in the tropics. Higher midday leaf relative water content (RWC) was observed at elevated RZ [CO_2]. Grown at 20 °C-RZ temperature (RZT), all plants accumulated more biomass than at ambient (A)-RZT. The increase of biomass was greater in roots than in shoots supported by lower shoot/root ratio under elevated RZ [CO_2]. The percentage increase in biomass under elevated RZ [CO_2] was greater at A-RZT although the total biomass was higher at 20 °C-RZT. NO_3^- and total reduced N concentrations of shoot and root, total leaf soluble and Rubisco protein were significantly higher in all elevated RZ [CO_2] plants than in ambine RZ [CO_2] (360 ppm) at both RZTs. Roots, however, under each RZ [CO_2] at A-RZT had significantly higher NO_3^- and total reduced N concentration than at 20 °C-RZT. At each RZ [CO_2], total leaf soluble and Rubisco protein concentration was significant greater at 20 °C-RZT than at A-RZT.

Keywords: Elevated root-zone [CO_2]; Root-zone temperature; Photosynthesis; Total reduced N; Rubisco

Introduction

In Singapore, the temperate vegetable crops have been successfully grown in the tropics by cooling the RZ only with aeroponic systems. When grown among soil particles, plant roots normally are colonized by microorganisms and evolve more CO_2 than sterile roots. It is not unusual for CO_2 in the rhizosphere to be up to more than 10-fold higher than the atmospheric CO_2 concentration (De Jong and Schappert, 1972; Norstadt and Porter, 1984). Based on these findings, we often questioned whether the low CO_2 level in the RZ of aeroponically grown plants could regulate plant growth. Our previous study showed that elevated RZ [CO_2] reduced the negative impacts of high air temperature on growth, photosynthesis and N metabolism of lettuce plants (He *et al*., 2007, 2010). In the present study, our experimental design using aeroponically grown lettuce plants, enables us to investigate the effects of elevated RZ CO_2 and RZT not only on A and g_s but also the plant growth especially the root growth associated with the uptake and accumulation of NO_3^-. The total product of N metabolism (*i.e.*, the total reduced N), total soluble and Rubisco protein were also determined. This research may have practical significance to aeroponic vegetable production by growing plants under elevated RZ [CO_2] to enhance productivity. By studying the responses of plant growth and photosynthesis to elevated RZ [CO_2] and RZT, this not only contributes to the scientific literature but also helps the growers to improve the production of aeroponically grown vegetable crops.

Materials and Methods

Plant materials: The plant material used was Crisphead-type lettuce plants (*Lactuca sativa* L. cv. 'Wintergreen', South Pacific Seeds Ltd, New Zealand). After germination, the seedlings were transplanted to the aeroponic system. The nutrient solution used was based on full strength Netherlands Standard Composition. At full strength, the

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conductivity of the nutrient solution measured 2.2 mS. The aerial parts of plants were subjected to the fluctuations of ambient temperature ranging from 23 to 38 ºC under 100% prevailing solar radiation. The maximum photosynthetic photon flux density (PPFD) on the plant canopy on sunny days was about 1,200 μmol m^{-2} s^{-1}. Relative humidity in the greenhouse was between 65% and 95%. Roots were grown in either 20 ºC-RZT or hot A-RZT. Three weeks after transplanting, four different RZ [CO_2] (ambient, 360 ppm and elevated concentrations of 2,000, 5,000, 10,000 ppm) were imposed on plants at each of the two RZTs.

Measurements of A and g_s: Two weeks after different elevated RZ [CO_2] treatments, *A* and g_s of the newly expanded leaves (the 6th leaves from the base) were measured between 0900 h to 1100 h with an open infrared gas analysis system with a 6 cm^2 chamber (LI-COR) in the greenhouse using intact plants. Readings were taken with an LED light source which supplied 1,200 μmol m^{-2} s^{-1} of PPFD. Average ambient [CO_2] and relative humidity in the chamber were 360 ±5 μmol mol^{-1} and 70% respectively. Leaf chamber temperature was set according to prevailing ambient conditions (35 °C).

Measurement of RWC: [Fresh weight (FW) – Dry weight (DW)]/(Turgid weight – DW) × 100%.

Determination of NO_3^-: It was determined using a Flow Injection Analyser (Model QuikChem 8000, Lachat Instruments Inc, Milwaukee, WI, U.S.A.) as described by He *et al*. (2010)

Determination of total soluble and Rubisco protein: Leaf discs were harvested in the middle of the photoperiod and immediately stored in liquid nitrogen. Total soluble and Rubisco protein were determined as describe by Jordan *et al*. (1992).

Statistical analysis: A two-way ANOVA was first used to test for the effect of RZ [CO_2] and RZT on all parameters. A separate ANOVA was then used to discriminate means across all treatments. All statistical analyses were carried out using MINITAB software (MINITAB, Inc., Release 15, 2007).

Results and Discussion

A, g_s and RWC

The interaction term "RZ [CO_2] x RZT" of two-way ANOVA for *A, gs* and midday leaf RWC was respectively, not significant (Table 1). Separate ANOVA analysis showed that elevated RZ [CO_2] resulted in significantly higher maximal *A* but lower g_s in all plants at both RZTs (Figs. 1A and 1B). Higher midday leaf RWC was also observed at elevated RZ [CO_2] (Fig. 1C). The degree of change in each parameter under elevated RZ [CO_2] was greater at A-RZT than at 20 °C-RZT.

Table 1 Two way analysis of variance of physiological variables, with P Values presented for each main effect and their interaction.

	RZ[CO_2]	Temperature	Interaction
A (Fig. 1A)	< 0.001	< 0.001	0.45
g_s (Fig. 1B)	< 0.001	< 0.001	0.89
RWC (Fig. 1C)	< 0.001	< 0.001	0.19
Shoot DW (Fig. 2A)	< 0.001	< 0.001	0.73
Root DW (Fig. 2B)	< 0.001	< 0.001	0.39
Shoot/root Ratio DW (Fig. 2C)	< 0.001	< 0.001	0.82
Shoot NO_3^- (Fig. 3A)	< 0.001	< 0.001	0.33
Shoot total N (Fig. 3B)	< 0.001	< 0.001	0.61
Root NO_3^- (Fig. 3C)	< 0.001	< 0.001	0.95
Root total N (Fig. 3D)	< 0.001	< 0.001	0.16
Total soluble protein (Fig. 3E)	< 0.001	< 0.001	0.21
Rubisco Protein (Fig. 3F)	< 0.001	< 0.001	0.54

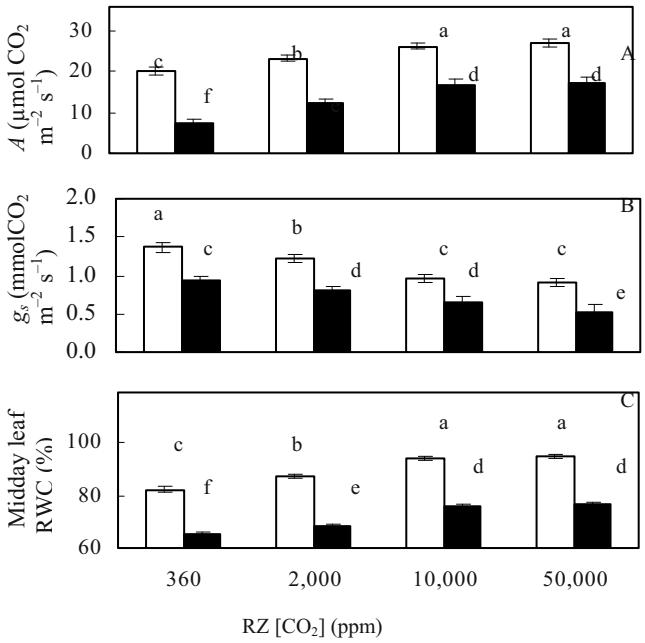

Fig. 1 *A* (A), g_s (B) and midday leaf RWC (C) of lettuce plants grown under different levels of elevated RZ [CO_2] at 20 °C-RZT (open bars) and A-RZT (closed bars) for 2 weeks. Each value is the mean of 5 measurements of 5 different leaves. Vertical bars represent the standard errors. Means with different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

Productivity of shoot and root

"RZ [CO_2] x RZT" of two-way ANOVA for DW of shoot and root, and shoot/root ratios was respectively, not significant (Table 1). Separate ANOVA analysis showed that DW of shoot and root were significantly higher in plants at all elevated RZ [CO_2] than at ambient RZ [CO_2] (360 ppm) at both RZTs. The increase of biomass was greater in roots than in shoots supported by lower shoot/root ratio under elevated RZ [CO_2]. The percentage of increase in biomass under elevated RZ [CO_2] was greater at A-RZT than at 20 ^{o}C-RZT although the total biomass was higher at 20 ^{o}C-RZT.

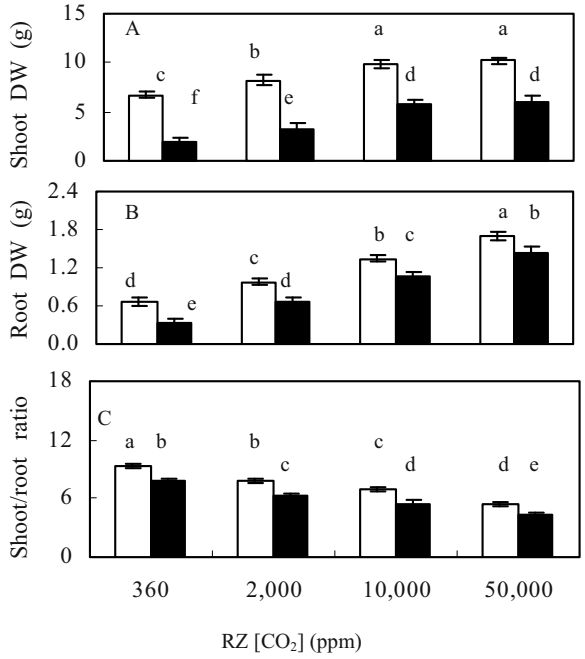

Fig. 2 DW of shoot (A) and root (B), shoot/root ratio (C) of lettuce plants grown under different levels of elevated RZ [CO2] at 20 °C-RZT (open bars) and A-RZT (closed bars) for 3 weeks. Each value is the mean of 5 measurements of 5 different leaves. Vertical bars represent the standard errors. Means with different letters above the bars are statistically different (p < 0.001) as determined by Tukey's multiple comparison test.

The above results showed that A increased with increasing RZ [CO_2] (Fig. 1A) with a RZ [CO_2] of 10,000 ppm sufficient for maximising productivity (Fig. 2). However, g_s was significantly lower at higher RZ [CO_2] than at ambient RZ [CO_2] (Fig. 1B) and therefore, the enhancement of A under elevated RZ [CO_2] was not due to an increase in g_s. Obviously, there was more internal CO_2 available to plants grown under elevated RZ [CO_2] (He *et al*., 2007, 2010) 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).

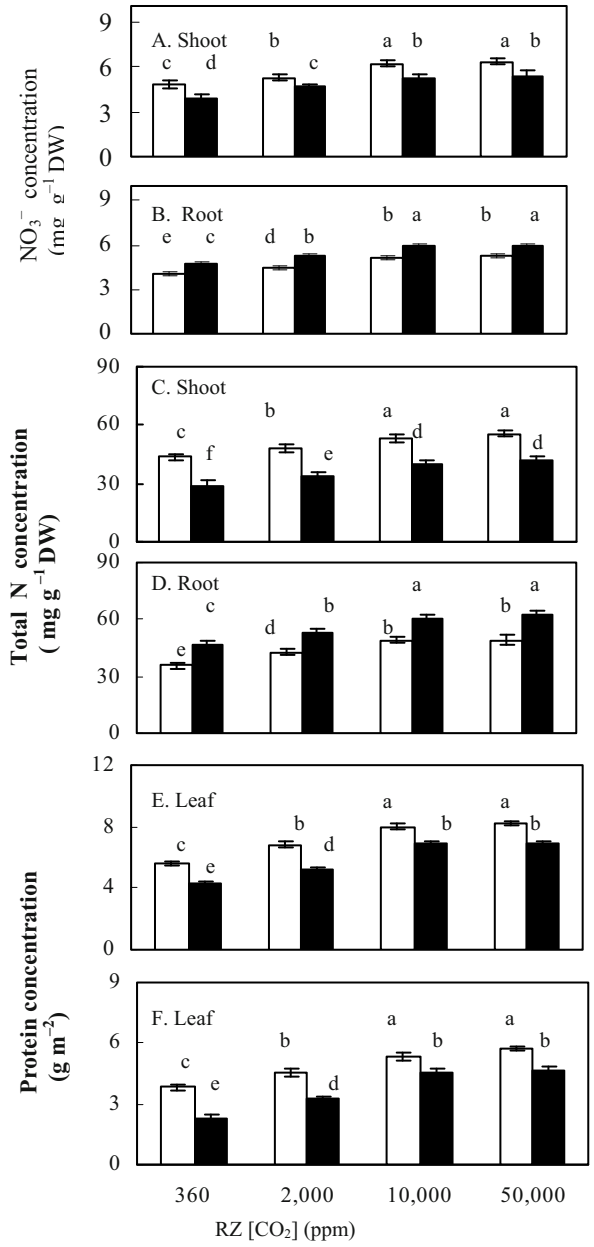

Fig. 3 NO_3^- , total reduced N concentration of shoot (A, B) and root (C, D), leaf soluble and Rubisco protein (E, F) concentrations of lettuce plants grown under different levels of elevated RZ [CO_2] at 20 °C-RZT (open bars) and A-RZT (closed bars) for three weeks. Each value is the mean of 5 measurements of 5 different plants from two different bins. 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.

NO_3^-, total reduced N, soluble and Rubisco protein concentration

"RZ [CO_2] x RZT" of two-way ANOVA for NO_3^-,

total reduced N, leaf total soluble and Rubisco protein concentration was respectively not significant (Table 1). Separate ANOVA analysis indicated that NO_3^- and total reduced N concentrations of shoot and root were significantly higher in all elevated RZ [CO_2] plants than in plants grown at RZ [CO_2] of 360 ppm at both RZTs. At each RZ [CO_2], NO_3^- and total reduced N concentration of shoot were higher at 20 °C-RZT than at A-RZT. At each RZ [CO_2] NO_3^- and total reduced N concentration of shoot were higher at 20oC-RZT than at A-RZT. Roots, however, underconcentrations were also significantly higher in all elevated RZ [CO_2] plants than in plants grown at RZ [CO_2] of 360 ppm at both RZTs.

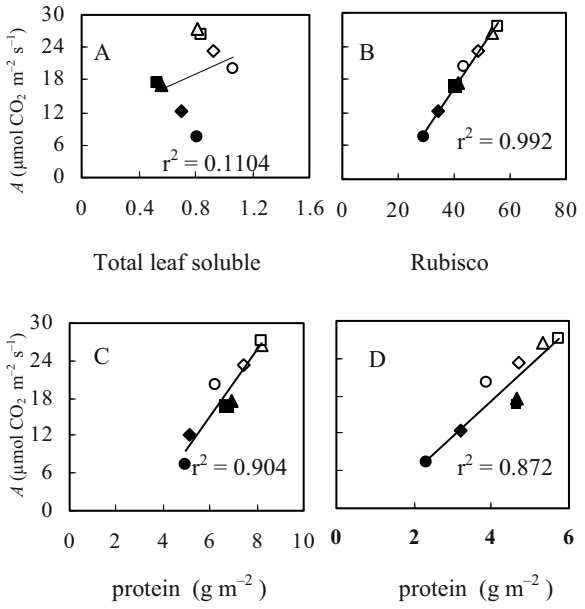

Fig. 4 Correlations between maximal *A* and maximal g_s, (A) (data derived from Fig.1) and maximal *A* and total reduced N of shoot (B), total leaf soluble (C) and Rubisco concentration (D) (data derived from Fig. 1 and Fig. 3) of lettuce plants grown under different levels of elevated RZ [CO_2] at 20$_o$C- and A-RZT for three weeks.

There was no correlation between *A* and maximal g_s (Fig. 4A, p = 0.14) but these variables were negatively correlated within each RZT. However, a close correlation between maximal *A* and total reduced N (Fig. 4B), total soluble (Fig. 4C) and Rubisco protein (Fig. 4D) concentration of shoot was established in plants grown under different RZ [CO_2], and RZTs (Figs. 4B, 4C and 4D, p < 0.001). Decreased N concentration is usually interpreted as evidence that NO_3^- uptake and assimilation have not kept pace with photosynthesis and growth in enhanced [CO_2] (Pettersson and McDonald, 1994). In this study, a larger root system under elevated RZ [CO_2] at 20 °C-RZT increased not only leaf NO_3^- but also concentration of total reduced N, leaf soluble and Rubisco protein. Plants grown under elevated RZ [CO_2] had lower g_s but higher *A* and higher productivity could also be partially due to dissolved inorganic carbon 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 (Viktor and Cramer, 2003). Increases in the leaf N and Rubisco concentration result in higher *A* (Li *et al*., 2009). The increased *A* under elevated RZ [CO_2], especially at 20 °C-RZT could partially be due to higher Rubisco protein not g_s (He *et al*., 2007, 2010).

Acknowledgements

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