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Root-zone temperature effects on photosynthesis, ^{14}C-photoassimilate partitioning and growth of temperate lettuce (*Lactuca sativa* cv. 'Panama') in the tropics

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Abstract

The effect of root growth temperature on maximal photosynthetic CO_2 assimilation (P_{max}), carbohydrate content, ^{14}C-photoassimilate partitioning, growth, and root morphology of lettuce was studied after transfer of the root system from cool root-zone temperature (C-RZT) of 20 °C to hot ambient-RZT (A-RZT) and vice versa. Four days after RZT transfer, P_{max} and leaf total soluble sugar content were highest and lowest, respectively, in C-RZT and A-RZT plants. P_{max} and total leaf soluble sugar content were much lower in plants transferred from C- to A-RZT (C→A-RZT) than in C-RZT plants. However, these two parameters were much higher in plants transferred from A- to C-RZT (A→C-RZT) than in A-RZT plants. A-RZT and C→A-RZT plants had higher root total soluble sugar content than A→C-RZT and C-RZT plants. Leaf total insoluble sugar content was similar in leaves of all plants while it was the highest in the roots of C-RZT plants. Developing leaves of C-RZT plants had higher ^{14}C-photoassimilate content than A-RZT plants. The A→C-RZT plants also had higher ^{14}C-photoassimilate content in their developing leaves than A-RZT plants. However, more ^{14}C-photoassimilates were translocated to the roots of A-RZT and C→A-RZT plants, but they were mainly used for root thickening than for its elongation. Increases in leaf area, shoot and root fresh mass were slower in C→A-RZT than in C-RZT plants. Conversely, A→C-RZT plants had higher increases in these parameters than A-RZT plants. Lower root/shoot ratio (R/S) in C-RZT than in A-RZT plants confirmed that more photoassimilates were channelled to the shoots than to the roots of C-RZT plants. Roots of C-RZT plants had greater total length with a greater number of tips and surface area, and smaller average diameter as compared to A-RZT plants. In C→A-RZT plants, there was root thickening but the increases in its length, tip number and surface area decreased. The reverse was observed for A→C-RZT plants. These results further supported the idea that newly fixed photoassimilates contributed more to root thickening than to root elongation in A-RZT and C→A-RZT plants.

Additional key words: ^{14}C-photoassimilate, carbohydrate, lettuce, photosynthetic CO_2 assimilation, root morphology, root-zone temperature

Introduction

In Singapore, certain temperate and subtropical vegetables are grown all year round by simply cooling their roots while their aerial portions are subjected to hot fluctuating temperatures. This has attracted a lot of attention because it overcomes the problem of growing temperate crops in the hot tropical regions of the world. This discovery stimulated our previous studies in the attempt to understand the physiology of certain temperate crops grown at C-RZT while their shoots were exposed to hot, fluctuating tropical greenhouse conditions (He and Lee 1998a,b, 2004, He *et al*. 2001). Our studies indicated that the aeroponics system can be a powerful tool for the study of RZT on photosynthesis, water relations, shoot development, root morphology, shoot-root interaction and mineral nutrition (He and Lee 1998a,b, Dodd *et al*. 2000, He *et al*. 2001, Tan *et al*. 2002). Several studies

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Abbreviations: A-RZT – ambient root-zone temperature; C-RZT – cool root-zone temperature ; A→C-RZT – transfer from ambient to C-RZT; C→A-RZT – transfer from C-RZT to A-RZT; DM – dry mass; FM – fresh mass; P_{max} – maximal photosynthetic CO_2 assimilation, R/S – root/shoot ratio, RZT – root zone-temperature.

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revealed that RZT had a strong influence on root growth and development (Sattelmacher *et al.* 1990, Pardales *et al*. 1991, Xu and Huang 2001). The water deficit observed in the previous study (He *et al*. 2001) may partially be due to poor root development and hence shoot productivity could be restricted by water and nutrient uptake. It is generally observed that not only root but also shoot development is highly dependent on RZT (Gosselin and Trudel 1984, Bode *et al*. 1998, Xu and Huang 2000a). While maintaining air temperature at 35 ^{o}C, reducing soil temperature from a supraoptimal level of 35 ^{o}C to an optimal level of 20 ^{o}C increased canopy photosynthesis, total carbohydrate content in roots and shoots, and root and shoot growth of creeping bentgrass (*Agrostis palustris* Huds.) (Xu and Huang 2000a,b). Gosselin and Trudel (1984) discovered that when shoots were maintained at 25 ^{o}C, greenhouse-grown *Lycopersicon esculentum* showed maximum shoot DM, leaf area and fruit mass at RZT of 23.6 ^{o}C while high RZT (34.9 ^{o}C) had an inhibitory effect on these parameters. Bode *et al*. (1998) also found that RZT greater than 35 ^{o}C resulted in sharp linear decreases in fresh mass (FM), dry mass (DM) and leaf number of *Cucumis melo* L. 'Gold Star'. They further concluded that *C. melo* grown at whole plant temperature of 25 ^{o}C attained the maximum plant FM while those grown at 40 ^{o}C-RZT with the same 25 ^{o}C shoot temperature had the least FM.

It has also been well documented that RZT not only affects root biomass but also its development and hence morphology (Kuroyanagi and Paulsen 1988, Sattelmacher *et al*. 1990, Pardales *et al*. 1991, Du and Tachibana 1994). RZT effects on root morphology could be due to alteration of photoassimilate partitioning between shoot and root. Earlier studies conducted by He and Lee (1998a, b) demonstrated that cooling the roots of *Lactuca sativa* grown in the tropics altered their growth pattern; including a higher shoot/root ratio. It has been suggested that the higher shoot/root ratio could be due to more photoassimilates being channelled into the harvestable portion of the *Lactuca sativa*. In a study with young barley, Minchin *et al*. (2002) reported that phloem loading of carbohydrate within a mature exporting leaf is shown to respond quickly to a change in temperature of the root and shoot meristem. Therefore, apart from the measurements of photosynthetic CO_2 assimilation (P_{max}) and carbohydrate content, this study also attempts to trace the ^{14}C-photoassimilate partitioning from mature source leaves of lettuce to young developing leaves and roots in response to various RZT by using $^{14}CO_2$. Growth kinetics and root morphology were also determined to investigate the relationship between photoassimilate availability and partitioning as well as growth and development of lettuce after RZT transfer. The findings may be useful for the local aeroponic vegetable growers in how to increase productivity of temperate crops grown in the tropics.

Materials and methods

Plants and cultivation: The seeds of lettuce (*Lactuca sativa* cv. 'Panama') were obtained from a commercial seed producer in Holland. After germination under dim light, seedlings with open cotyledons were transplanted into polyurethane cubes soaked in water and placed in trays. These trays were then transferred to a greenhouse where they were allowed two days of seedling establishment. The seedlings were then transplanted to the aeroponic system previously described by Lee (1993). The nutrient solution used was based on full strength Netherlands Standard Composition (Douglas 1982). The conductivity of the nutrient solution was 2.2 mS cm^{-1}. 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 C-RZT or hot A-RZT. The C-RZT was obtained by cooling the reservoir of nutrient solution to 20 ºC before it was sprayed to the plants' roots. C-RZT of 20 ºC was used since the previous studies conducted on this plant confirmed that optimal growth was obtained at 20 ºC-RZT (He and Lee 1998a,b). The RZT within the aeroponic troughs was constant throughout the experimental period except for an average variation of 2 ºC during the midday period when the temperature in the greenhouse was exceptionally high, reaching 40 ºC on some occasion.

RZT transfer was conducted three weeks after transplanting during the linear phase of growth. Some plants were maintained at C-RZT and A-RZT to act as controls. The rest ones were transferred to other RZTs so that there were four RZT treatments: C-RZT, A-RZT, C→A-RZT and A→C-RZT.

P_{max}: Four days after RZT treatments, P_{max} of newly expanded leaves (the 4th leaves from the base) of intact plants were measured with an open chamber infrared gas analysis system with a 6-cm^{2} chamber (*LI-COR Biosciences*, Lincoln, USA) in the greenhouse between 10.00 and 11.00 h. Readings were taken with an LED light source which supplied PPFD of 1 200 μmol m^{-2} s^{-1} at ambient CO_2 of 380 to 400 ppm from stable incoming air stream by using a buffer volume. Light response curves of CO_2 fixation had previously established that a PPFD of 1 200 μmol m^{-2} s^{-1} was saturating for lettuce leaf photosynthesis in this study (data not shown).

Soluble sugar: Four days after RZT transfer, 5 plants from each RZT treatment which were used for the measurements of P_{max} were harvested at 18:00 h. The whole root and all leaves of each test plant were mixed and wrapped separately in aluminium foil. The plant materials were placed in an oven set at 105 ºC and dried for 24 h. Soluble sugars were extracted using the method described by Buysse and Merckx (1994). About 0.05 g of dried leaf and root samples were extracted three times in 5 cm^3 of hot 80 % ethanol (80 ^oC). The supernatants from each extraction were combined and made to a convenient volume. An aliquot (1 cm^3) of 5 % (w/v) phenol was added to 1 cm^3 of the plant extract, followed by 5 cm^3 of concentrated H_2SO_4 and mixed thoroughly. The reaction mixture was allowed to stand for 30 min before the absorbance was recorded at 490 nm using a spectrophotometer. Total sugar content of the sample was calculated based on a calibration curve from a glucose working standard.

Insoluble sugar: Insoluble sugars were extracted from the residual plant material from the soluble sugar extraction described above. This was done by incubating the dry pellet with 5 cm^3 of 3 % HCl in a boiling water bath for 3 h. The soluble products were assayed by the same phenol-sulphuric method described above.

$^{14}CO_2$ feeding and ^{14}C-photoassimilate partitioning: $^{14}CO_2$ was fed to the 4th leaf from the base at 10.00 h (1.91 GBq $mmol^{-1}$, *Amersham International*, Buckinghamshire, England) in a small glass vial (total volume: 1 cm^3, diameter: 1 cm). The glass vial was attached to the abaxial surface of the leaf lamina using an adhesive (masking tape) which lined the circumference of the open end of each vial, forming a gas-tight chamber. The $^{14}CO_2$ from a 10 μl droplet of $Na_2{}^{14}CO_3$ (0.19 MBq or 5 μCi) was liberated using excess 0.1 M H_2SO_4 injected into the vial through a hole on its side. The hole was then immediately sealed with vaseline. The possibility of $^{14}CO_2$ leakage was ruled out by determining the amount of $^{14}CO_2$ in unfed plants placed near the test plants. No ^{14}C was detected in these unfed plants. Experimental plants were supplied with $^{14}CO_2$ at 10.00 h and the feeding was halted after 1 h. The plants were harvested at different time intervals of 11.00, 12.00, 13.00, 14.00 h to follow translocation of ^{14}C-photoassimilates. During the harvest, the plants were separated into a test leaf (the youngest mature leaf), upper leaves (developing leaves), lower leaves (fully developed old leaves) and roots, wrapped with an aluminium foil and immediately immersed in liquid nitrogen. The plant materials were dried in an oven set at 105°C before their DM was recorded. 0.03 g of each plant part was oxidised using a *Packard Sample Oxidiser* (*Model 307*). *Carbo-sorb E* (*Packard*) and scintillation cocktail (*Permafluor E+, Packard*) were added simultaneously automatically by the oxidiser to trap the $^{14}CO_2$ released and for radioactivity counting, respectively. The amount of ^{14}C-photoassimilates in each sample was measured using a scintillation counter (*Beckman LS6000L*).

Leaf number and area: The leaves of each test plant were detached from its stem to determine its total leaf number. These leaves were subsequently used to obtain their total leaf area by means of the *Area Measurement System* (*Delta T-Devices Ltd*, Cambridge, England).

Shoot and root FM and DM: Each test plant was separated into its shoot and root after removing the whole plant from the aeroponic troughs. Shoot and root FM were recorded before they were wrapped in the aluminium foil. The plant materials were placed in an oven set at 105 ºC, dried for 24 h and then their DM were determined.

Root morphology The root region of each test plant was detached from the shoot and then placed in a tray of water. The water served to spread out the roots and keep them moist. The roots were first scanned with *WIN MAC RHIZO* scanner before total length, root tips, surface area and average root diameter were determined by the programme *WIN MAC RHIZO V 3.9*.

Statistical analysis: Data from each experimental treatment were assessed by analysis of variance (*ANOVA*) and compared with the control using Dunnett's procedure at $p<0.05$.

Results

P_{max} and carbohydrates: Four days after RZT transfer, P_{max} was highest and lowest, respectively, in C-RZT and hot A-RZT plants. P_{max} was much lower in C→A-RZT plants than in C-RZT plants ($p<0.05$). However, it was much higher in A→C-RZT than in A-RZT plants ($p<0.05$, Fig. 1). From the same leaves of the same measurements, responses of stomatal conductance (g_s) to different RZTs showed the similar trends as those of P_{max} (data not shown). Leaf total soluble sugar contents (Fig. 2*A*) among the RZT treatments were very similar to those of P_{max}. A-RZT plants had higher root total soluble sugar content than that of C-RZT plants ($p<0.05$). Root total soluble sugar contents were higher in A-RZT and C→A-RZT than in A→C-RZT and C-RZT plants, respectively (Fig. 2*B*, $p<0.05$). Leaf total insoluble sugar content was similar in all plants (Fig. 2*C*). C-RZT and A-RZT plants, respectively, had the highest and lowest root total insoluble sugar content among the different treatments (Fig. 2*D*). The C→A-RZT plants had much lower root total insoluble sugar content than C-RZT plants ($p<0.05$). For A→C-RZT plants, it was lower that than that of A-RZT plants (Fig. 2*D*, $p<0.05$).

$^{14}CO_2$ assimilation and ^{14}C-photoassimilate partitioning: Lettuce plants grown at C-RZT and A-RZT for 3 weeks were supplied with $^{14}CO_2$ at 10.00 h and the $^{14}CO_2$ feeding was halted after 1 h. In order to find out the rate of transport and the percentages of ^{14}C-photoassimilates from test (source) leaves to roots, the plants were harvested 1, 2, 3 and 4 h after feeding. The total amount of ^{14}C-photoassimilates (^{14}C counting) per plant was 3-times higher in C-RZT plants than in A-RZT plants 1 h after $^{14}CO_2$ feeding (data not shown), which was similar to the difference in P_{max} between C-RZT and A-RZT plants (Fig. 1). Fig. 3*A* shows the percentage of ^{14}C-photoassimilate found in the test leaf of lettuce at various time intervals after $^{14}CO_2$ feeding. At any given time, the transport of ^{14}C-photoassimilate was faster for C-RZT than for A-RZT plants. For instance, 3 h after feeding, the percentages of ^{14}C-photoassimilates left in the test leaves of A-RZT and C-RZT plants were 77 and 63 %, respectively. Fig. 3*B* shows that the percentage of root ^{14}C-photoassimilates was higher in A-RZT plants than in C-RZT plants at any given time after $^{14}CO_2$ feeding. The percentage of ^{14}C-photoassimilates in the roots of A-RZT plants increased from 5 to 14 % from 1 to 3 h after $^{14}CO_2$ feeding. However, there was no significant change in the percentage of ^{14}C-photoassimilates in the roots of C-RZT plants from 1 to 4 h after $^{14}CO_2$ feeding. Clearly, there were more ^{14}C-photoassimilates distributed to the roots of A-RZT than C-RZT plants. For all plants, there were no significant differences in ^{14}C-photoassimilates between 3 and 4 h after $^{14}CO_2$ feeding, indicating that the transport of ^{14}C-photoassimilates was very slow during midday (from 13.00 to 14.00 h), when stomata partially or completely closed (He *et al*. 2001).

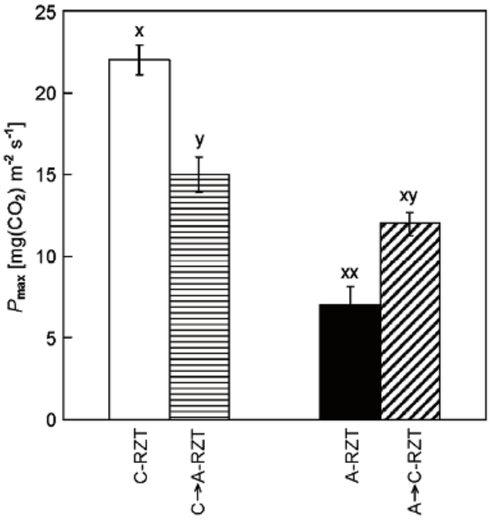

Fig. 1. Maximal photosynthetic CO_2 assimilation (P_{max}) of lettuce plants on day 4 after RZT transfer. Each reading is the mean of 5 measurements from 5 different plants and means with different letters above the columns are statistically different. *Vertical bars* represent the standard errors.

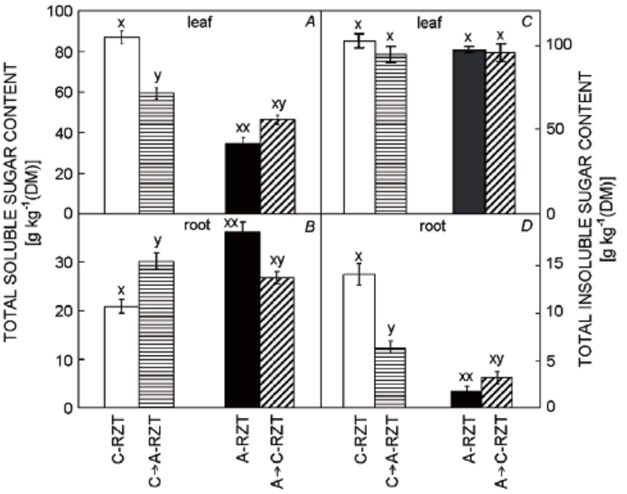

Fig. 2. Leaf and root total soluble sugar (*A,B*) and insoluble sugar (*C,D*) of lettuce plants on day 4 after RZT transfer. Each reading is the mean of 5 measurements from 5 different plants and means with different letters above the columns are statistically different. *Vertical bars* represent the standard errors.

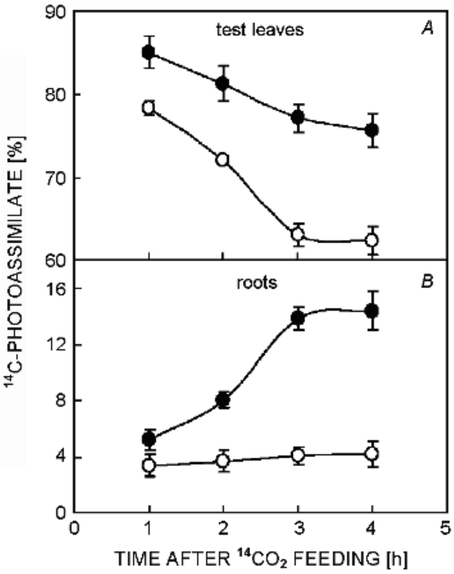

Fig. 3. Percentage of ^{14}C-photoassimilates found in test leaf (*A*) and roots (*B*) of lettuce grown at C-RZT (O) and A-RZT (●) after ^{14}C feeding for various time intervals. Each reading is the mean of four measurements from 4 different plants. *Vertical bars* represent the standard errors.

In RZT transfer experiments, $^{14}CO_2$ feeding was conducted 5 days after RZT transfer. Based on the results of Fig. 3, ^{14}C-photoassimilate partitioning into different plant tissues was analysed 3 h after $^{14}CO_2$ feeding. After feeding $^{14}CO_2$ for 3 h, the percentage of ^{14}C-photoassimilates in test leaves of C-RZT plants was significantly lower than that of A-RZT plants ($p<0.05$, Fig. 4*A*), which were similar to those shown in Fig. 3*A*. However, there was no significant difference in the percentage of ^{14}C-photoassimilates in the test leaves between C-RZT and C→A-RZT plants. The A-RZT and the A→C-RZT plants also showed similar percentage of ^{14}C-photoassimilates in their test leaves (Fig. 4*A*). The results indicated that RZT transfer may not affect the transport rate of ^{14}C-photoassimilates from test leaves to other parts of lettuce within 5 days of RZT transfer. However, after 3 h of $^{14}CO_2$ feeding, lettuce plants grown at C-RZT had the highest ^{14}C-photoassimilates of 30 % found in the upper, young and developing leaves (Fig. 4*B*). of 4 % in their upper leaves. The upper developing leaves The A-RZT lettuce had the lowest ^{14}C-photoassimilates of C→A-RZT lettuce had lower percentage of ^{14}C-photoassimilates than that of C-RZT plants ($p<0.05$). Meanwhile, A→C-RZT plants had significantly higher ^{14}C-photoassimilates in the upper developing leaves compared to those of A-RZT plants ($p<0.05$). It was also noted that there was a higher percentage of ^{14}C-photoassimilates in the roots of C→A-RZT plants compared to C-RZT plants. Furthermore, for A→C-RZT transfer, the percentage of ^{14}C-photoassimilates in roots was lower than that of A-RZT plants but it was higher than that of C-RZT plants (Fig. 4*C*, $p<0.05$). These results indicated that A-RZT and C→A-RZT plants distributed more ^{14}C-photoassimilates to the roots while C-RZT and A→C-RZT plants had a higher amount of ^{14}C-photoassimilates distributed to their developing leaves.

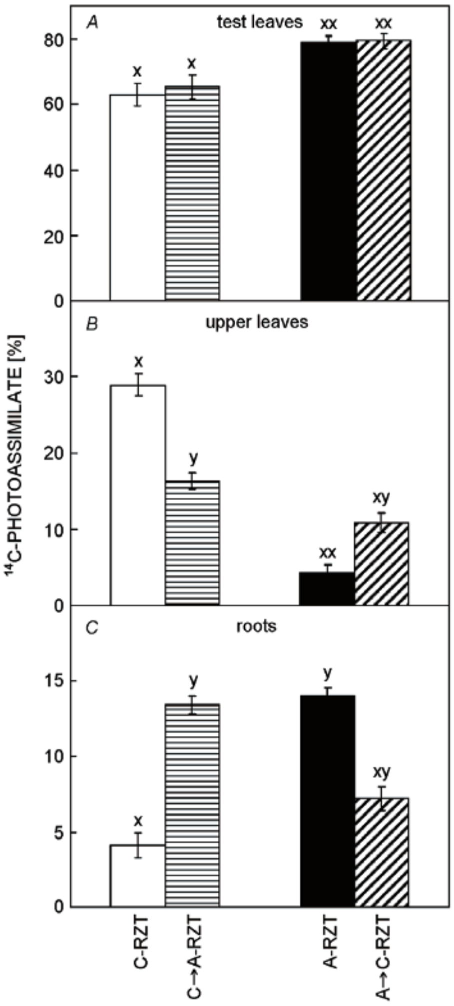

Fig. 4. Percentage of ^{14}C-photoassimilates found in test leaves (*A*), upper leaves (*B*) and roots (*C*) of lettuce grown at C-RZT, A-RZT, C→A-RZT and A→C-RZT after ^{14}C feeding for 3 h. Each reading is the mean of four measurements from 4 different plants and means with different letters above the columns are statistically different. *Vertical bars* represent the standard errors.

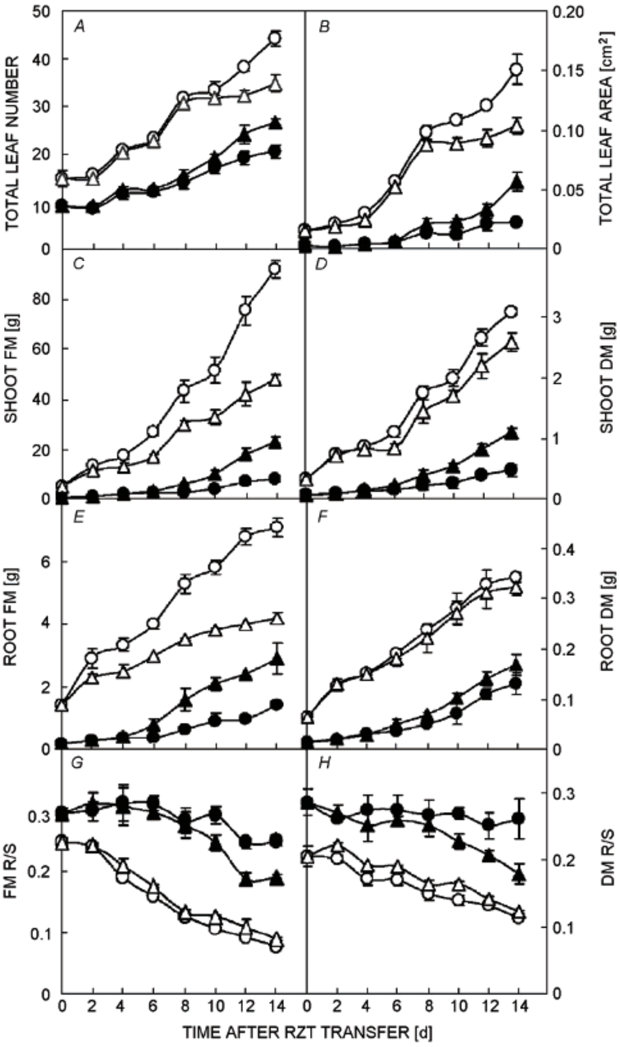

Fig. 5. Total leaf number (*A*), leaf area (*B*), shoot FM and DM (*C,D*), root FM and DM (*E,F*), and FM and DM R/S (*G,H*) of lettuce plants grown at C-RZT (O), A-RZT (●), C→A-RZT (Δ)and A→C-RZT (▲). Each point is the mean of six measurements. *Vertical bars* represent the standard errors.

Percentages of ^{14}C-photoassimilates in all the other lower leaves (fully developed old leaves) were very low (3 to 4 %) in all plants (data not shown).

Growth kinetics of leaves, shoots and roots: The increase in total leaf number for lettuce grown at C-RZT was the fastest while A-RZT plants had the lowest increase of total leaf number (Fig. 5*A*). C→A-RZT plants displayed decline in its increase in total leaf number on 12 day ($p<0.05$). After 14 days, C→A-RZT plants had 14 % fewer leaves than C-RZT plants while A→C-RZT plants had 30 % more leaves than the A-RZT plants. The C-RZT plants had the fastest increase in total leaf area while there was a little increase in total leaf area of A-RZT plants (Fig. 5*B*). In C→A-RZT, there was a significant decline in increase of total leaf area from day 10 as compared to that of C-RZT plants ($p<0.05$). For A→C-RZT plants, a significant increase in total leaf area was observed after 10 days compared to A-RZT plants. On 14 day, the total leaf area of A→C-RZT plants was 2.6 times larger than that of A-RZT plants.

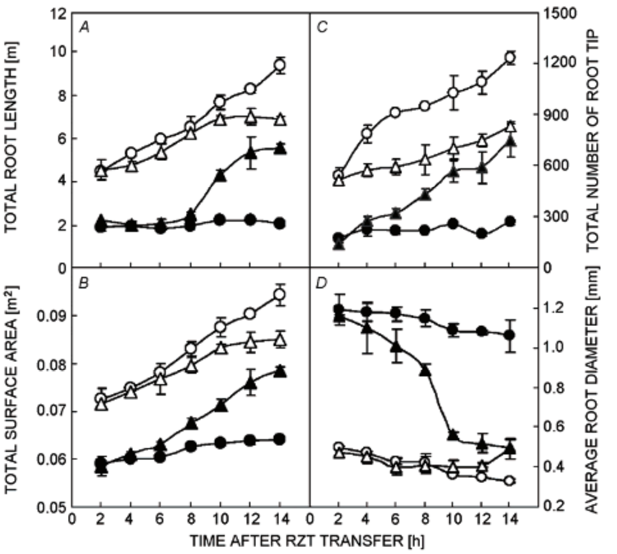

Fig. 6. Total root length (*A*), total surface area (*B*), total number of root tips (*C*) and average root diameter (*D*) of lettuce grown at C-RZT (O), A-RZT (●), C→A-RZT (Δ) and A→C-RZT (▲). Each point is the mean of four measurements. *Vertical bars* represent the standard errors.

Lettuce grown at all RZTs showed increasing shoot FM over the 2-week period (Fig. 5*C*). However, the increase was the greatest for C-RZT plants and the lowest for plants grown at A-RZT. The transfer of lettuce from C→A-RZT led to a significant decline in the increase of shoot FM starting from day 6 compared to that of C-RZT plants ($p<0.05$). On 14 day, the shoot FM of C→A-RZT plants was 48 % lower than in C-RZT plants. On the other hand, A→C-RZT promoted the increase of shoot FM. A→C-RZT lettuce had 3 times higher shoot FM than A-RZT plants 14 days after RZT transfer. The root FM of lettuce grown at different RZTs exhibited similar trends as their shoot FMs (Fig. 5*E*). The FM R/S of A-RZT lettuce was consistently higher than in C-RZT plants throughout the experimental period (Fig. 5*G*) ($p<0.05$). FM R/S of C-RZT plants decreased from day 2, indicating that there was more shoot development. The C→A-RZT and C-RZT plants displayed similar R/S despite C→A-RZT transfer. However, A→C-RZT transfer resulted in a lower R/S for A→C-RZT plants from day 10 as compared to A-RZT plants. R/S of A→C-RZT plants was 30 % lower than that of the A-RZT plants on day 14 after RZT change. Similarly to the trends of shoot FM, the lettuce plants grown at C→A-RZT showed a decline in the increase in shoot DM. Conversely, A→C-RZT caused an increase in shoot DM (Fig. 5*D*). However, transition of RZT did not significantly change the root DM of C→A-RZT and A→C-RZT plants compared to C-RZT and A-RZT plants, respectively at $p<0.05$ (Fig. 5*F*). The trend of DM R/S (Fig. 5*H*) was also similar to that of FM R/S (Fig. 5*G*).

Root morphology: C-RZT lettuce showed linear increase in total root length over the 2-week period (Fig. 6*A*). The increase of total root length of C→A-RZT plants was similar to that of C-RZT plants from day 0 to 8 after RZT transfer. However, there was no further increase in total root length in C→A-RZT plants from day 10 to 14 after RZT transition. There was no significant increase in total root length of A-RZT plants over the 2-week period. A→C-RZT plants exhibited increase in total root length after eight days of RZT transfer. The total root length of A→C-RZT plants was almost threefold than that of A-RZT plants at day 14. The total root surface areas (Fig. 6*B*) at different RZTs showed similar trends as the total root length. The total root tip number of C-RZT lettuce was consistently greater than that of all the other plants (Fig. 6*C*) ($p<0.05$). Although there was an increase in total root tip number for C→A-RZT plants, the net increase of this parameter decreased significantly after C→A-RZT transfer, which was 33 % lower than that of C-RZT plants at the end of the experiment. The total root tip number of A-RZT plants did not change significantly throughout the whole experiment. For A→C-RZT plants, there was rapid increase in the total root tip number, which was significantly higher than in A-RZT plants by day 4 after RZT transfer. At day 14, total root tip number of A→C-RZT plants was 2.8 times as high as that of A-RZT plants. This demonstrated that C-RZT promoted new root formation in these plants. The A-RZT had the highest average root diameter while C-RZT plants had the least one (Fig. 6*D*), indicating finer roots in C-RZT plants and thicker roots in A-RZT ones. The average root diameter of C→A-RZT plants remained similar to that of C-RZT plants during the first 10 days of RZT transfer. However, there was a gradual thickening in C→A-RZT roots after 10 days. By day 14, the average root diameter of C→A-RZT plants was 1.5 times as thick as that of

C-RZT plants. Conversely, A→C-RZT lettuce developed new and finer roots. Thus, at day 14, the average root diameter of A→C-RZT lettuce was 53 % smaller than that of A-RZT plants.

Discussion

Like in our previous study (He *et al.* 2001), in the present study $P_{\max}$ of A-RZT and C→A-RZT plants decreased compared to that of C-RZT plants. However, when the plants were transferred from A→C-RZT, $P_{\max}$ increased significantly compared to that of A-RZT plants (Fig. 1). Higher soluble sugar content (Fig. 2*A*) was also found in the plants with a higher $P_{\max}$. Although $P_{\max}$ and soluble sugar content was 2–3 times higher in C-RZT plants than in other plants which were grown or previously grown at higher RZT, there was no significant difference in leaf total insoluble sugar among all plants (Fig. 2*C*). Lower soluble sugar production but higher insoluble sugar content in the leaves of A-RZT plants implies that these plants retain and accumulate more carbohydrates in their leaves. This was supported by the fact that the transport of newly fixed ^{14}C-photoassimilate was slower for A-RZT than for C-RZT plants (Figs. 3*A* and 4*A*). Lower root total insoluble sugar content in plants grown at higher RZTs compared to that of C-RZT plants (Fig. 2*D*) could be due to its hydrolysis, which was reflected by higher root total soluble sugar content (Fig. 2*B*). Higher root soluble sugar content could be a mechanism of osmotic adjustment used by plants grown at higher RZT to regulate their root water potential. The water deficits and stomatal limitation of photosynthesis of A-RZT lettuce observed in our previous study (He *et al.* 2001) may partially be due to poor root development which restricted the uptake of water. These data combined with our previous reports strongly suggest that high RZT not only inhibits photosynthesis and carbohydrate production but also affects the distribution and metabolism of carbohydrates (Xu and Huang 2000a,b). However, short term RZT transfer did not affect the transport rate of ^{14}C-photoassimilates as there was no significant difference in this parameter between C-RZT and C→A-RZT plants as well as A-RZT and A→C-RZT plants after 5 days of RZT transfer (Fig. 4*A*). Although short term RZT transfer did affect the rate of ^{14}C-photoassimilate transport, compared to C-RZT plants, lettuce grown or previously grown at A-RZT affected the partitioning of ^{14}C-photoassimilate among the different organs. In the present study, the results show that RZT altered the transport rates and proportions of ^{14}C-photoassimilates in different sinks (Figs. 3 and 4). The younger developing leaves of C-RZT lettuce exhibited greater sink strength (Fig. 4*B*) and thus higher growth rate of leaves (Fig. 5*A,B*). Total leaf number and leaf area data revealed that C-RZT lettuce had greater leaf expansion and initiation than A-RZT plants (Fig. 5 *A,B*). This was in accordance with other observations that RZT influenced leaf growth in *Lycopersicum esculentum* (Gosselin and Trudel, 1984), *Triticum aestivum* (Kuroyanagi and Paulsen, 1988; Al-Hamdani *et al*., 1990) and *Capsicum annuum* (Dodd *et al*., 2000). C→A-RZT caused a decline in the total leaf expansion rates while A→C-RZT increased this parameter (Fig. 5b). Other than leaf expansion and initiation, both shoot and root FM and DM were negatively affected by A-RZT (Fig. 6). This has provided further evidence that RZT has a significant impact on shoot and root development (Sattelmacher *et al*., 1990; Pardales *et al*., 1991; DeLucia *et al*., 1992; Bode *et al*., 1998). The C→A-RZT transfer generally reduced the shoot and root FM and DM. The reverse was recorded for A→C-RZT lettuce (Fig. 5*C-H*). Therefore, shoot and root productivities of these plants were considered to be responsive to RZT changes (Gosselin and Trudel, 1984; DeLucia *et al*., 1992; Bode *et al*., 1998; He and Lee, 1998a, b). The negative influence of high RZT on lettuce root development was further illustrated by root morphology studies (Fig. 6). It was clearly observed that high A-RZT had a dramatic impact on the root morphology of lettuce. The root morphological analyses revealed that high RZT inhibited root elongation, hair formation and increased root diameter. Similar results have been reported in studies of high RZT effects on different plants (Abbas Al-Ani and Hay, 1983, Pardales *et al*., 1991; Sattelmacher *et al*., 1990). Abbas Al-Ani and Hay (1983) suggested that root length and diameter appeared to be inversely related in a study using *Secale cereale* seedlings. In the present study, A-RZT lettuce had thicker and shorter roots than their C-RZT counterparts (Fig. 6). It has been reported that root thickening, or an increase in diameter, is controlled through signals emanating from shoot apices and root tips (Pierik *et al*., 1999). Root thickening may also be accompanied by associated changes in microfibril angles within expanding cell walls (Pierik *et al*., 1999). One of the chemical signals may be ethylene production in the roots caused by oxygen shortages, flooding and hot temperatures (Arshad and Frakenberger, 2002). The role of ethylene in the thick root syndrome and inhibition of root elongation has been investigated by our research team. Our results indicated that the presence of an ethylene inhibitor promoted root elongation at high RZT of 38 °C. Without the presence of the ethylene inhibitor, root elongation at high RZT was significantly inhibited (Qin *et al*. 2007).

It was interesting to note that A-RZT lettuce had higher FM and DM R/S than the C-RZT one (Fig. 5*C-H*). This further confirmed that more photoassimilates were allocated to the roots of A-RZT lettuce. It is well documented that transport of newly fixed carbon out of a source leaf must equal the capacity of the sinks to utilize it (Zamski and Schaffer, 1996; Minchin *et al*., 2002). Source-sink relationship in the short term (minutes to hours) provides most information on the mechanisms of existing metabolic and transport processes (Minchin *et al*., 2002). In a study with tomato, Walker and Ho (1977) reported that warming and cooling of sinks (fruits) resulted in changes in carbon transport out of the source. In the present study, the results show that changes of RZT altered the transport rates and proportion of ^{14}C-photoassimilate allocated to different sinks (Figs. 3 and 4). The younger developing leaves of C-RZT lettuce exhibited greater sink strength (Fig. 5*A,B*) and thus a higher growth rate of shoot and lower R/S (Fig. 5*C-H*). There have been a number of studies which concluded that plant growth was the result of interaction between source leaves and carbon partitioning among competitive sinks (Minchin *et al*., 1994; Minchin and Thorpe, 1996; Schurr *et al*., 2000). In the present study, RZT transfer resulted in changes of growth kinetics (Figs. 4 and 5*A,B*) and hence altered the strength of different sinks. For instance, when A-RZT plants were transferred to C-RZT, more ^{14}C-photoassimilates partitioned into both young developing leaves and new forming roots after 3 h $^{14}CO_2$ feeding in those 5-day RZT transferred plants (Fig. 4). For a longer period (i.e., 14 days) of RZT transfer, increases in leaves, shoots and roots of A→C-RZT were observed (Fig. 5). These results imply that the temperature of a sink may affect its metabolic rate and hence its capacity to utilize carbohydrate (Farrar, 1999). Our results also indicate that A-RZT induced greater levels of ^{14}C-photoassimilates delivered to the lettuce root (Figs. 5*C-H* and 6). However, it was surprising that the high ^{14}C-photoassimilates translocation to the roots of A-RZT lettuce, was not accompanied by a greater root development (Fig. 7). This may be attributed to the higher respiration rates in the roots which may require energy for the active uptake of water and nutrients in a poorly developed root system (Minchin *et al*., 1994; Klock *et al*., 1997). The high rate of respiration may have taken place at the expense of root development at high RZT (Du and Tachibana 1994). It is highly probable that there exists a negative feedback mechanism among root respiration, water uptake, nutrient absorption, root morphology and high RZT.

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