PHOTOSYNTHETICA 42 (3): 457-463, 2004

Photosynthetic utilization of radiant energy by temperate lettuce grown under natural tropical condition with manipulation of root-zone temperature

J. HE[*] and S.K. LEE

Natural Sciences Academic Group, National Institute of Education, Nanyang Technological University,
1 Nanyang Walk, Singapore 637 616

Abstract

Photosynthetic utilization of radiant energy was studied by chlorophyll (Chl) fluorescence and maximum photosynthetic O_2 evolution (P_{max}) in temperate lettuce (*Lactuca sativa* L.) grown under natural tropical fluctuating ambient temperatures but with their roots exposed to two different root-zone temperatures (RZTs): a constant 20 °C-RZT (RZT_{20}) and a fluctuating ambient RZT (RZT_a) from 23 to 40 °C. On a sunny day, irrespective of RZT, $\Delta F/F_m'$ [ratio of the variable to maximal fluorescence under irradiation (the maximal photosystem 2 quantum yield with "actinic light")] decreased and non-photochemical quenching (NPQ) increased parallel to the increase of photosynthetic photon flux density (PPFD). However, RZT_a plants showed lower $\Delta F/F_m'$ and higher NPQ than RZT_{20} plants. The electron transport rate (ETR) was much higher in RZT_{20} plants than in RZT_a plants especially during moderately sunny days. There were no significant diurnal changes in P_{max} although these values of RZT_{20} plants were much higher than those of RZT_a plants. On cloudy days, no significant diurnal changes in $\Delta F/F_m'$ and NPQ occurred, but $\Delta F/F_m'$ was higher and NPQ was lower in RZT_{20} plants than in RZT_a plants. Diurnal changes in ETR were also observed in all plants while P_{max} values throughout the whole cloudy days in both RZT_{20} and RZT_a plants were constant. Again, RZT_{20} plants had much higher values of P_{max} than RZT_a plants. During RZT transfer period, all Chl fluorescence parameters measured at midday fluctuated with PPFD. Impact of RZT on these parameters was observed 2–3 d after RZT transfer. ETR and P_{max} measured with saturating PPFD in the laboratory did not vary with the fluctuating PPFD in the greenhouse but the effects of RZT on these two parameters were observed 3–4 d after RZT transfer. Thus RZT affects photosynthetic utilization of photon energy in temperate lettuce grown under natural tropical condition.

Additional key words: chlorophyll fluorescence; electron transport rate; *Lactuca sativa*; maximum photosynthetic rate; non-photosynthetic quenching; root-zone temperature.

Introduction

Temperate vegetable crop such as lettuce has been successfully grown in the tropics by exposing roots to cool temperatures (15–25 °C) while shoots were maintained at hot ambient temperatures (He and Lee 1998a,b). Our recent study (He *et al.* 2001) showed that, irrespective of RZT, aeroponically grown lettuce exhibited substantial midday depression of CO_2 assimilation and stomatal conductance (g_s) on sunny days. RZT_a plants always showed lower CO_2 assimilation rate, g_s, and relative water content than the RZT_{20} plants. On cloudy days (PPFD<250 μmol m^{-2} s^{-1}), midday lower g_s still occurred, but a stable, irradiance-limited CO_2 assimilation was maintained. RZT transfer ($RZT_{20\rightarrow a}$) in our previous study also indicated that lettuce plants transferred from

---

Received 1 April 2004, *accepted* 31 May 2004.

[*] Corresponding author; fax: 65-68969432, e-mail: jhe@nie.edu.sg

Abbreviations: Chl, chlorophyll; ETR, electron transport rate; F_0, minimal fluorescence yield of a "dark-adapted" sample; F_m and F_v, maximal and variable fluorescence yield obtained from a dark-adapted sample upon application of a saturating light pulse; F_m', maximal variable fluorescence yield at any given time under irradiance by a saturating pulse; F_v/F_m, dark adapted ratio of variable to maximal fluorescence [the maximal photosystem 2 (PS2) quantum yield without "actinic light"]; F_t, transient fluorescence; $\Delta F/F_m'$, ratio of the variable to maximal fluorescence under irradiation (the maximal PS2 quantum yield with "actinic light"); g_s, stomatal conductance; NPQ, chlorophyll non-photochemical quenching; P_{max}, photosynthetic oxygen production at saturating irradiance; PPFD, photosynthetic photon flux density; RZT_a, ambient root-zone temperature; $RZT_{a\rightarrow 20}$, plants grown initially at RZT_a then transferred to RZT_{20}; RZT_{20}, root-zone temperature of 20±2 °C; $RZT_{20\rightarrow a}$, plants grown initially at RZT_{20} then transferred to RZT_a.

Acknowledgment: This project was supported by Academic Research Fund, Ministry of Education, Singapore.

RZT_{20} to RZT_a immediately decreased relative water content, resulting in stomatal limitation of CO_2 assimilation. However, non-stomatal limitation of photosynthetic O_2 evolution was not detected until 5 d after RZT transfer. These findings indicated that stomatal limitations to photosynthesis occurred prior to non-stomatal limitation of photosynthesis when lettuce plants were transferred from RZT_{20} to RZT_a. Any environmental factor resulting in decreased photosynthetic rate at a constant PPFD might lead to a greater excess of absorbed photons (Barker *et al.* 2002). This excess photon energy could cause other non-stomatal effects such as decreases in the ratios of F_v/F_m or $\Delta F/F_m'$, which have been ascribed to photoinactivation of photosystem 2 (PS2) due to high temperature and high irradiance (Björkman and Powles 1984), shoot water deficits of >30 % (Kaiser 1987), or additional stresses such as nutrient deficiency (Verhoeven *et al.* 1997). Our previous study also indicated that lettuce plants grown at RZT_a had much lower F_v/F_m than RZT_{20} plants on both sunny and cloudy days (He *et al.* 2001).

Decreases in CO_2 concentration at the chloroplast level caused by stomatal effects may reduce the photosynthetic utilization of radiant energy. When stomatal limitation of photosynthesis was more severe in RZT_a lettuce plants (He *et al.* 2001), more endogenous electron acceptors became reduced and O_2 might be the main available electron acceptor. Although oxygen can serve as electron acceptor in photorespiration (Osmond and Björkman 1972) and Mehler reaction (Schreiber and Neubauer 1990), there may be an excess of excitation energy (Baker 1991). To understand the mechanism(s) responsible for higher photosynthesis of temperate lettuce plants grown in the tropics by cooling their root only, we tested the hypothesis whether the excitation energy can be safely dissipated by photosynthetic electron transport or by events not directly concerned with photochemistry by examining in detail the interaction between RZT and irradiation on chlorophyll (Chl) fluorescence and maximum photosynthetic rates.

Materials and methods

Plants and growing methods: Butter head lettuce (*Lactuca sativa* L. cv. Palma) seeds were obtained from a commercial seed producer in Holland. Three days after germination, seedlings were transplanted onto polyurethane cubes soaked with water and placed in trays in the greenhouse. After two more days to allow seedling establishment, they were transplanted onto aeroponic troughs (Lee 1993) in a greenhouse at Nanyang Technological University, Singapore. The top of each trough was insulated by sheets of polystyrofoam on which the lettuce plants were anchored. The nutrient solution used was based on full strength *Netherlands Standard Composition*. At full strength, the conductivity of the nutrient solution was 2.2 mS. Lettuce plants were grown under 100 % of prevailing solar radiation at RZT_{20} and RZT_a (diurnal ambient temperature 23–40 °C).

Reciprocal transfer between RZTs: RZT transfer experiments were conducted 3 weeks after transplanting. Half the plants were maintained at their original RZT (either RZT_{20} or RZT_a), and the other half were transferred to the other RZT at 07:00 h. There were thus four RZT treatments: RZT_{20}, RZT_a, $RZT_{20\rightarrow a}$, and $RZT_{a\rightarrow 20}$. This experiment was performed three times at different times of the year with similar results; results are presented from only one experiment. All the leaf measurements used the fourth leaf from the base.

Chl fluorescence parameters in the greenhouse were made with a portable *PAM-2000* fluorometer (*Walz*, Effeltrich, Germany) on attached leaves following the air and leaf temperature measurements. To measure steady-state-modulated Chl fluorescence ($\Delta F/F_m' = F_m' - F_t/F_m'$), the measuring fibre optic of the *PAM-2000* fluorometer was placed as close as possible to, and yet did not shade, the leaf surface. F_t was first measured on the surface of attached leaves in the greenhouse using sunlight as the "actinic light" source. A simultaneous saturating pulse of "white light" of 6 000 μmol m^{-2} s^{-1} was applied to determine the fluorescence parameter F_m' in the irradiated state after a few minutes. At least 5 to 6 flashes were applied to leaves to obtain the F_m'. Subsequently, leaves were darkened with a black cover for 10 min. Leaves were then initially exposed to a weak measuring beam (~0.18 μmol m^{-2} s^{-1}, peak wavelength at 655 nm, 600 Hz) to give F_0, then to a 0.8 s saturation pulse (6 000 μmol m^{-2} s^{-1}) to obtain F_m. PPFD was measured close to the leaf surface simultaneously with the determination of Chl fluorescence parameters (quantum sensor connected to the *PAM-2000*). Electron transport rate (ETR) was calculated as $ETR = PPFD \times \Delta F/F_m' \times 0.50 \times 0.84$ (Rascher *et al.* 2000). $\Delta F/F_m'$ represents the effective photochemical quantum yield. The use of two photons is necessary to transport one electron (factor 0.50). Correction factor 0.84 takes into account that only a fraction of incident photons is really absorbed by photosynthesis (Rascher *et al.* 2000). NPQ was defined as: $NPQ = (F_m - F_m')/F_m'$. To measure pre-dawn F_v/F_m, *DLC-8* aluminum leaf clips (2 cm diameter, *Walz*) were used to pre-darken (10 min) the leaves prior to measurement. F_0 and F_m were recorded on attached leaves as described earlier. F_v was determined as $F_m - F_0$.

Measurement of photosynthetic oxygen evolution on detached leaves: To determine P_{max}, five leaves (4th leaf from the base) were harvested at different time of the day. Leaves were then kept in a tray of distilled water in the laboratory under a PPFD of 1 350 μmol m^{-2} s^{-1} for 30 min to equilibrate. $P_{\max}$ was determined with a leaf disc O_2 electrode (*Hansatech*, King's Lynn, UK) under a PPFD of 1 350 µmol m^{-2} s^{-1} at 25 °C at saturating CO_2 (1 % CO_2 from a 1 M carbonate/bicarbonate buffer, pH 9) as described by Ball *et al.* (1987).

Ambient and leaf temperatures were measured with a *CIRAS-1* portable open system gas analyzer (*PP-System*, Hitchin, Herts, UK) just before the Chl measurements.

Total Chl was extracted in dimethyl-formamide and quantified spectrophotometrically using the procedure of Wellburn (1994).

Statistical analysis: Differences between RZT treatments were discriminated using Dunnett's procedure at $p<0.05$.

Results

Diurnal changes in PPFD, ambient and leaf temperature, and Chl fluorescence during a sunny and a cloudy day: Lettuce plants were grown in the greenhouse for 3 weeks. On a hot sunny day, PPFD showed a broad maximum (*ca.* 1 200–1 300 µmol m^{-2} s^{-1}) from 12:00 to 14:00 (Fig. 1*A*). Ambient temperature rose from 23 °C at 07:00 to a broad maximum at or above 37 °C from 12:00–14:00, and then declined to 26 °C at 19:00 (solid line in Fig. 1*B*). Midday leaf temperatures of RZT$_{20}$ plants were 2–3 °C lower than those of RZT$_a$ plants (Fig. 1*B*) ($p<0.05$). Maximum leaf temperatures of RZT$_{20}$ and RZT$_a$ plants were 39.2 and 42.5 °C, respectively, at 13:00 h. Following two consecutive bright sunny days, diurnal changes in PPFD, ambient and leaf temperatures were also measured on a cloudy day (Fig. 1*B,D*). PPFD ranged between 100–250 µmol m^{-2} s^{-1} during midday (Fig. 1*B*). The ambient (solid line in Fig. 1*D*) and midday leaf temperatures were much lower on a cloudy day than on a sunny day. However, midday leaf temperatures of RZT$_{20}$ plants were similar to those of RZT$_a$ plants on cloudy days.

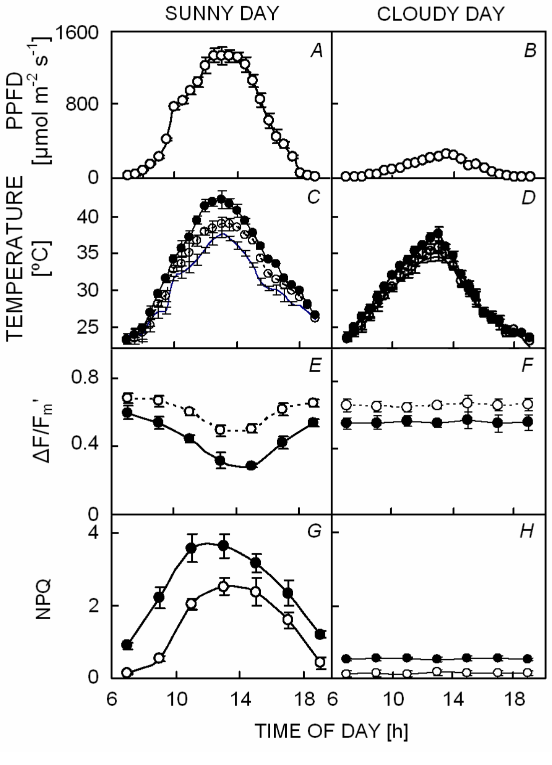

Fig. 1. Diurnal changes in photosynthetic photon flux density, PPFD (*A, B*), ambient and leaf temperatures (*C, D*), $\Delta F/F_m'$ (*E, F*), and non-photochemical quenching, NPQ (*G, H*) in lettuce plants grown under RZT$_{20}$ (○) and RZT$_a$ (●) on a sunny and a cloudy day. Means of 5 measurements at the same leaf age (the 4th leaves from the base) from 5 different plants. *Vertical bars* represent the standard error.

On a sunny day, the lowest values of $\Delta F/F_m'$ were observed between 13:00 and 15:00 when solar irradiation was highest, indicating dynamic photoinhibition (Fig. 1*E*). $\Delta F/F_m'$ gradually increased in the late afternoon with decreasing PPFD. At all times, $\Delta F/F_m'$ values were much lower in RZT$_a$ plants than in RZT$_{20}$ plants. No significant diurnal changes in $\Delta F/F_m'$ occurred on a cloudy day, irrespective of RZT. However, the $\Delta F/F_m'$ values were significantly lower ($p<0.05$) in RZT$_a$ plants than in RZT$_{20}$ plants (Fig. 1*F*). On a sunny day, NPQ increased with increasing PPFD to high values of 2.51 and 3.62 (Fig. 1*G*) at 13:00 in RZT$_{20}$ and RZT$_a$ plants, respectively, when the PPFD and the leaf temperature were highest. Significant diurnal changes in NPQ were not seen on a cloudy day (Fig. 1*H*), although the NPQ values were lower in RZT$_{20}$ plants than in RZT$_a$ plants. At each RZT, midday NPQ levels were much lower on a cloudy day (Fig. 1*H*) than on a sunny day.

ETR values were much higher in RZT$_{20}$ plants than in RZT$_a$ plants ($p<0.05$) from 11:00 to 15:00 h (Fig. 2*A*) on a sunny day. However, there were no significant diurnal changes in $P_{\max}$ (Fig. 2*C*) although they were much lower in RZT$_a$ plants than in RZT$_{20}$ plants. On a cloudy day, only at 13:00, the RZT$_{20}$ plants had a slightly higher ETR than RZT$_a$ plants ($p<0.05$). There were no significant differences in ETR between the two RZTs for the rest of time (Fig. 2*B*). Values of $P_{\max}$ throughout the whole cloudy day in all plants were constant but they were much higher in RZT$_{20}$ plants than in RZT$_a$ plants ($p<0.05$) (Fig. 2*D*).

Reciprocal transfer between RZTs: Midday $\Delta F/F_m'$ (Fig. 3*B*) varied with PPFD (Fig. 3*A*) in all plants. But midday $\Delta F/F_m'$ values were much higher in RZT$_{20}$ than in RZT$_a$ measured at the same time of the same day ($p<0.05$). From 3 d after transfer, in RZT$_{20\rightarrow a}$ plants the midday $\Delta F/F_m'$ was much lower than in RZT$_{20}$ plants

($p<0.05$) and decreased to similar low values of RZT$_a$ from day 5 after the RZT transfer. In RZT$_{a\rightarrow20}$ plants, midday ΔF/F$_m$' was higher from 3 d after transfer than in plants remaining at RZT$_a$ ($p<0.05$) and increased to the similar high value of RZT$_{20}$ plants after 5 d of RZT transfer. ETR in all plants decreased with the decrease of PPFD (Fig. 3*C*) from day 1 to day 4 after RZT transfer and then increased to higher values on day 5 when PPFD was much higher. ETR of different plants remained constant from day 5 to day 10 after transfer when PPFD over that period ranged between 925–1 325 µmol m^{-2} s^{-1} (Fig. 3*A*). Midday NPQ was much higher in RZT$_a$ plants than in RZT$_{20}$ plants (Fig. 3*D*) although the values fluctuated with PPFD (Fig. 3*A*). NPQ of RZT$_{20\rightarrow a}$ plants was higher than that of RZT$_{20}$ plants from the first day of RZT transfer and remained so throughout the 10 d after RZT transfer. They were similar to those of RZT$_a$ plants on day 4 after RZT transfer. From day 2 of RZT transfer, NPQ was much lower in RZT$_{a\rightarrow20}$ plants than in RZT$_a$ plants ($p<0.05$) and they decreased to almost the same values of RZT$_{20}$ plants from day 5 of RZT transfer.

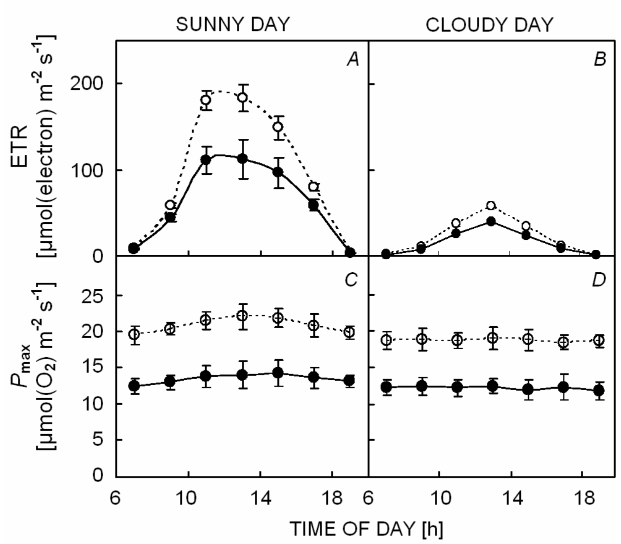

Fig. 2. Diurnal changes in electron transport rate, ETR (*A, B*) and maximal photosynthetic rate, P_{max} (*C, D*) in lettuce plants grown under RZT$_{20}$ (○) and RZT$_a$ (●) on a sunny and a cloudy day. Means of 5 measurements at the same leaf age (the 4th leaf from the base) from 5 different plants. *Vertical bars* represent the standard errors.

Fig. 4*A,B* shows the values of ETR and P_{max} measured with saturating PPFD in the laboratory. Both exhibited very similar trends. ETR and P_{max} of plants grown at one RZT were constant over the 10 d after RZT transfer, with ETR and P_{max} of RZT$_a$ plants much lower than that of RZT$_{20}$ plants. In RZT$_{20\rightarrow a}$ plants, decreases in both ETR and P_{max} were observed 4 d after the transfer. Ten days after RZT transfer, ETR and P_{max} had declined by 38 and 30 %, respectively. In RZT$_{a\rightarrow20}$ plants, 90 and 92 % increases in ETR and P_{max} were recorded from 3 d after the transfer in comparison to RZT$_{20}$ plants. A clear linear relationship was established when the values of ETR measured under saturating PPFD were plotted against the P_{max} measured in the same leaves under the same PPFD (Fig. 5).

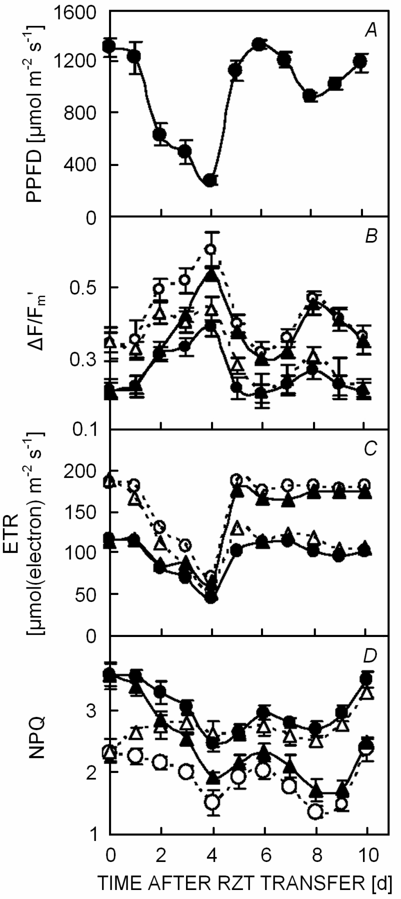

Fig. 3. Changes in photosynthetic photon flux density, PPFD (*A*), ΔF/F$_m$' (*B*), electron transport rate, ETR (*C*), and non-photochemical quenching, NPQ (*D*) at midday of lettuce plants grown and maintained at RZT$_{20}$ (○) and RZT$_a$ (●) and those grown at RZT$_{20}$ but transferred to RZT$_a$ (Δ) or grown at RZT$_a$ and transferred to RZT$_{20}$ (▲). Means of 5 measurements at the same leaf age (the 4th leaf from the base) from 5 different plants. *Vertical bars* represent the standard errors.

Average pre-dawn F$_v$/F$_m$ values were 0.81 and 0.73 for RZT$_{20}$ and RZT$_a$ plants, respectively (Fig. 6*A*). Chl content of RZT$_a$ plants was 20 % lower than that of RZT$_{20}$ plants (Fig. 6*B*). There were no significant changes in both pre-dawn F$_v$/F$_m$ and Chl content during the first 5 d in RZT$_{20\rightarrow a}$ plants. 10 d after transfer, these parameters had decreased by 8–12 % ($p<0.05$). Pre-dawn F$_v$/F$_m$ and Chl content increased from days 3 and 5, respectively, after transition in RZT$_{a\rightarrow20}$ plants and recovered to 99 and 95 % (of those maintained at RZT$_{20}$) after 10 d.

Discussion

Photoinhibition generally occurs at irradiances greater than half of full sunlight, in conjunction with other stresses that inhibit carbon metabolism, such as water deficit or high temperature (Long *et al.* 1994). There is also increasing evidence that natural sunlight alone is strong enough to cause photoinhibition (Raven 1994, He *et al.* 1996). Our previous paper reports that temperate crops such as lettuce exhibit poor growth, low photosynthetic rate, and lower potential quantum yield of PS2 (F_v/F_m) in the tropical greenhouse (He and Lee 1998a, He *et al.* 2001). However, by only cooling their roots while shoots were maintained at hot ambient temperatures, lettuce plants had higher P_{max} and productivity under full sunlight as compared to the plants grown under reduced sunlight (He and Lee 1998b). In the present study, photosynthetic utilization of radiant energy in temperate lettuce grown in the tropics was investigated *in situ* by monitoring the effective quantum yield of PS2 ($\Delta F/F_m$'). Trend of diurnal changes in $\Delta F/F_m$' in the present study was very similar to that of F_v/F_m reported previously (He *et al.* 2001). The present results indicate that cooling the roots of a temperate lettuce growing in a hot tropical greenhouse alleviates photoinhibition of PS2 during moderately sunny days. This was reflected in higher $\Delta F/F_m$' and ETR and lower NPQ in RZT_{20} plants than in RZT_a plants.The midday decline in the maximal photochemical efficiency of PS2 corresponding to the maximum PPFD and leaf temperature is termed 'dynamic photoinhibition' and has been observed widely in nature, even in plants grown under optimal conditions (Ögren and Evans 1992, He *et al.* 1996, 2001).

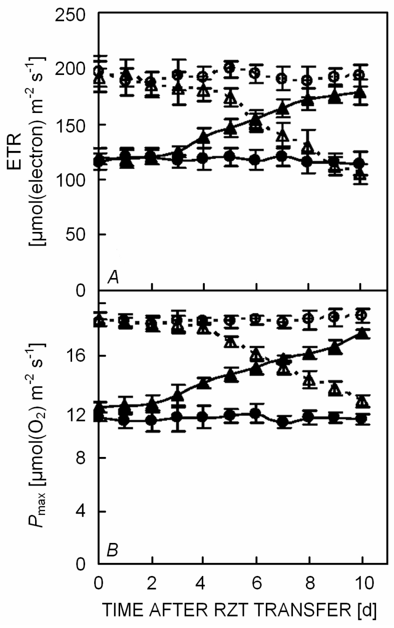

Fig. 4. Changes in electron transport rate, ETR (*A*) and maximal photosynthetic rate, P_{max} (*B*) measured in laboratory with saturating photosynthetic photon flux density, PPFD at midday of lettuce plants grown and maintained at RZT_{20} (○) and RZT_a (●) and those grown at RZT_{20} but transferred to RZT_a (Δ) or grown at RZT_a and transferred to RZT_{20} (▲). Means of 5 measurements at the same leaf age (the 4th leaf from the base) from 5 different plants. *Vertical bars* represent the standard errors.

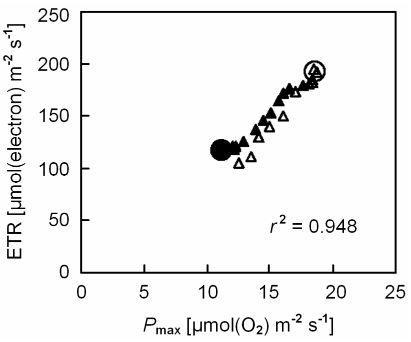

Fig. 5. Electron transport rate, ETR (values from Fig. 4*A*) plotted against maximal photosynthetic rate, P_{max} (values from Fig. 4*B*) of lettuce plants grown and maintained at RZT_{20} (○) or RZT_a (●) and those grown at RZT_{20} but transferred to RZT_a (Δ) or grown at RZT_a and transferred to RZT_{20} (▲). Data for plants maintained at one RZT are averaged from 10 d of measurements. Error bars were omitted for clarity.

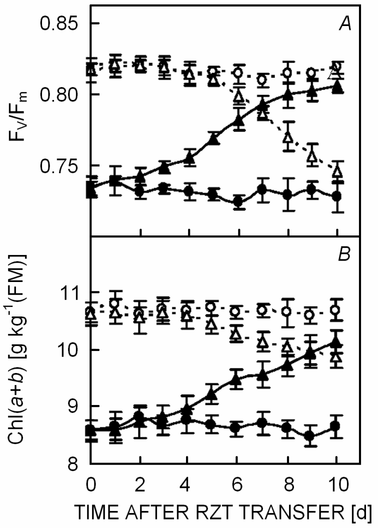

Fig. 6. Changes in predawn F_v/F_m ratio and chlorophyll content of lettuce plants grown and maintained at RZT_{20} (○) and RZT_a (●) and those grown at RZT_{20} but transferred to RZT_a (Δ) or those grown at RZT_a and transferred to RZT_{20} (▲). Means of 5 measurements at the same leaf age (the 4th leaves from the base) from 5 different plants. *Vertical bars* represent the standard errors.

Our present study with temperate lettuce grown at the tropics shows at different RZTs different responses of lettuce to fluctuating PPFD and leaf temperature. We found that cooling the roots of temperate lettuce could increase photosynthetic utilization of radiant energy and thus alleviate the photoinactivation of PS2 during moderately sunny days (Fig. 1). This finding was further confirmed in the RZT transfer experiments. Although the midday $\Delta F/F_m$ varied with prevailing PPFD when plants were transferred from RZT_{20} to RZT_a, all these parameters significantly decreased from day 5 after transfer regardless of variation in PPFD (Fig. 3). In $RZT_{a\rightarrow20}$ plants, however, $\Delta F/F_m$ was higher from 3 d after RZT transfer compared with those that remained at RZT_a ($p<0.01$). In the present study, 'dynamic photoinhibition' was absent on cloudy days irrespective of RZT (Fig. 1*F,H*). Severe water stress in RZT_a plants could potentially lead to increased susceptibility to photoinhibition even at low irradiance (on a cloudy day) if excess excitation energy cannot be dissipated safely (He *et al.* 2001). However, plants may prevent this through the down-regulation of quantum yield of PS2. This could be explained by the lower $\Delta F/F_m$ in RZT_a plants than in RZT_{20} plants. Chronic photoinhibition defined as a sustained low pre-dawn F_v/F_m (Osmond 1994) had occurred in RZT_a plants, and was only alleviated 2–3 d following transfer to RZT_{20} (Fig. 6*A*). $RZT_{20\rightarrow a}$ plants also showed chronic photoinhibition 6 d after the RZT transfer, coincident with changes in P_{max} (Fig. 4). Such chronic photoinhibition was correlated with a 20 % reduction in Chl content compared to Chl content in RZT_{20} plants (Fig. 6*B*). This Chl loss seems to be a photoprotective strategy to reduce photon absorption (Verhoeven *et al.* 1997).

Photoinactivation of PS2 measured by the decreases in $\Delta F/F_m$' can be related to competition between photochemical and non-photochemical de-excitation of the photon energy absorbed by light-harvesting complexes (Schreiber *et al.* 1998). Under normal conditions, electron transport and CO_2 fixation determine this balance at any given PPFD. Under stress, safe dissipation of excess energy may continue through electron transport processes, including photorespiration and the Mehler-ascorbate pathway as well as non-photochemical mechanism, and thus avoid over-reduction and potential damage to PS2 (Baker 1991). In the present study, NPQ which reflects energy dissipated as heat related to energization of the thylakoid membrane due to lumen acidification was also measured during the day. All lettuce showed at midday a high capacity for NPQ, the bulk of which is likely to be high energy state quenching which may protect the leaves from high PPFD-induced damage. The relative midday NPQ increase (expressed as a percentage of the initial value) was much smaller in RZT_a plants than in RZT_{20} plants, indicating a lower capacity for dissipating excess excitation energy. However, the greater NPQ values of RZT_a plants under low PPFD (Figs. 1*G,H*) indicate these plants always retained a proportion of slowly relaxing NPQ referred to as photoinhibitory quenching. More energy was also probably dissipated *via* NPQ when plants were transferred from RZT_{20} to RZT_a compared to those maintained at RZT_{20}. This indicates that excess energy was built up from the first day of RZT transfer (Fig. 3*D*). The xanthophyll cycle is involved in a process that facilitates the increased thermal dissipation of the excess of absorbed excitation energy in the light-harvesting antennae of PS2. A strong correlation between NPQ and the de-epoxidation state of the xanthophyll cycle carotenoids was found in field-grown grapevines (Flexas *et al.* 2000). The relationship between NPQ and the xanthophyll cycle in temperate lettuce grown in a tropical greenhouse merits our further study.

Despite the significant reduction in the photochemical parameter such as $\Delta F/F_m$' at mid-sunny days, P_{max} did not show any reduction (Fig. 2*C*). This contradictory phenomenon of midday maxima in both photoinhibition and photosynthesis had been observed in natural phytoplankton assemblages (Behrenfeld *et al.* 1998) and alga (Vonshak *et al.* 2001). According to these researchers, the effect of photoinhibition on photosynthesis depends upon which step of the electron transport chain is rate limiting at a given irradiance. Photosynthetic activity at sub-saturating irradiance is rate-limited by photon absorption, and as a result, energy transfer to PS2 reaction centres varies linearly with irradiance. Conversely, at photon saturation, the acceptor side of PS2 that limits the enzymatic process in the Calvin cycle limits photosynthesis. The tolerance of P_{max} to photoinhibition in these studies suggests that photoinhibition does not directly affect the rate-limiting step of photosynthesis at photon saturation. In the present study, photoinhibition was not the primary factor which caused the reduction in P_{max}. The insensitivity of P_{max} to photoinhibition during midday might result from the compensatory high values of ETR (Fig. 2*A*, Behrenfeld *et al.* 1998). Although not shown in this paper, the ETR was also measured in the laboratory at different times of day just before the measurements of P_{max} under saturating PPFD of 1 350 $\mu mol\ m^{-2}\ s^{-1}$. All ETR values obtained at different times under saturating PPFD in the laboratory were similar to those measured in the greenhouse at midday while the PPFD was highest. In the RZT transfer experiment, where ETR was measured in the greenhouse under fluctuating PPFD, there was no clear relationship between ETR (Fig. 3*C*) and P_{max} (Fig. 4*B*). However, values of ETR agree well with those of P_{max} while they were measured with saturating PPFD in the laboratory during the RZT transfer (Figs. 4 and 5). A linear relationship between ETR and leaf gross CO_2 assimilation was also found for both higher plants (Earl and Tollenaar 1998, Maxwell *et al.* 1998) and algae (Geel *et al.* 1997). However, the relationship between Chl fluorescence and photosynthetic CO_2 assimilation was not stable during fluctuations in incident PPFD (Earl and Tollenaar 1998). From the above discussion we conclude that decreases in $\Delta F/F_m$' at moderately sunny days in temperate lettuce grown in the tropics could be attributed to the regulation of the capacity of PS2 electron transport, which involves the thermal dissipation of excess excitation energy. When pre-dawn lowers F_v/F_m, loss of Chl and the decreases of

ETR and $P_{\max}$ were observed in $\mathrm{RZT_a}$ and $\mathrm{RZT_{20\to A}}$ plants and PS2 was damaged. Reduction in $P_{\max}$ could also result from the decreased capacity of enzymatic processes in the Calvin cycle, related to nitrogen deficiency and decrease in ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes as reported in our previous studies (He and Lee 2001, Tan *et al.* 2002).

References

Baker, N.R.: A possible role for photosystem II in environmental perturbations of photosynthesis. – Physiol. Plant. **81**: 563-570, 1991.

Ball, M.C., Chow, W.S., Anderson, J.M.: Salinity-induced potassium deficiency causes loss of functional photosystem II in leaves of the grey mangrove, *Avicennia marina*, through depletion of the atrazine-binding polypeptide. – Aust. J. Plant Physiol. **14**: 351-361, 1987.

Barker, D.H., Adams, W.W., III, Demmig-Adams, B., Logan, B.A., Verhoeven, A.S., Smith, S.D.: Nocturnally retained zeaxanthin does not remain engaged in a stage primed for energy dissipation during the summer in two *Yucca* species growing in the Mojave Desert. – Plant Cell Environ. **25**: 95-103, 2002.

Behrenfeld, M.J., Prasil, O., Kolber, Z.S., Babin, M., Falkowski, P.G.: Compensatory changes in Photosystem II electron turnover rates protect photosynthesis from photoinhibition. – Photosynth. Res. **58**: 259-268, 1998.

Björkman, O., Powles, S.B.: Inhibition of photosynthetic reactions under water stress: interaction with light level. – Planta **161**: 490-504, 1984.

Earl, H.J., Tollenaar, M.: Relationship between thylakoid electron transport and photosynthetic CO_2 uptake in leaves of three maize (*Zea mays* L.) hybrids. – Photosynth. Res. **58**: 245-257, 1998.

Flexas, J., Briantais, J.-M., Cerovic, Z., Medrano, H., Medrano, H., Moya, I.: Steady-state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: a new remote sensing system. – Remote Sens. Environ. **73**: 283-297, 2000.

Geel, C., Versluis, W., Snel, J.F.H.: Estimation of oxygen evolution by marine phytoplankton from measurement of the efficiency of Photosystem II electron flow. – Photosynth. Res. **51**: 61-70, 1997.

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., Lee, S.K.: Growth and photosynthetic characteristics of lettuce (*Lactuca sativa* L.) grown under fluctuating hot ambient temperatures with the manipulation of cool rootzone 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 rootzone temperatures and growth irradiances under tropical aerial condition. – J. horticult. Sci. Biotechnol. **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., Dodd, I.M.: Limitations to photosynthesis of lettuce grown under tropical conditions: alleviation by root-zone cooling. – J. exp. Bot **52**: 1323-1330, 2001.

Kaiser, W.M.: Effects of water deficit on photosynthetic capacity. – Physiol. Plant. **71**: 142-149, 1987.

Lee, S.K.: Aeroponic system as a possible alternative for crop production in Singapore. – Common Agr. Digest **3**: 1-4, 1993.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. – Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Maxwell, K., Badger, M.R., Osmond, C.B.: A comparison of CO_2 and O_2 exchange patterns and the relationship with chlorophyll fluorescence during photosynthesis in C_3 and CAM plants. – Aust. J. Plant Physiol. **25**: 45-52, 1998.

Ögren, E., Evans, J.R.: Photoinhibition of photosynthesis *in situ* in six species of *Eucalyptus*. – Aust. J. Plant Physiol. **19**: 223-232, 1992.

Osmond, C.B.: What is photoinhibition? Some insights from comparisons of shade and sun plants. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis in the Field. Pp. 1-24. Bios Scientific Publishing, Oxford 1994.

Osmond, C.B., Björkman, O.: Simultaneous measurements of oxygen effects on net photosynthesis and glycolate metabolism in C_3 and C_4 species of *Atriplex*. – Carnegie Inst. Year Book **71**: 141-148, 1972.

Rascher, U., Liebig, M., Lüttge, U.: Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. – Plant Cell Environ. **23**: 1397-1405, 2000.

Raven, J.A.: The cost of photoinhibition to plant communities. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis – From Molecular Mechanisms to the Field. Pp. 449-464. Bios Scientific Publishing, Oxford 1994.

Schreiber, U., Bilger, W., Hormann, H., Neubauer, C.: Chlorophyll fluorescence as a diagnostic tool. Basic and some aspects of practical relevance. – In: Raghavendra, A.S. (ed.): Photosynthesis – A Comprehensive Treatise. Pp. 320-336. Cambridge University Press, Cambridge 1998.

Schreiber, U., Neubauer, C.: O_2-dependent electron flow, membrane energization and the mechanism of non-photochemical quenching of chlorophyll fluorescence. – Photosynth. Res. **25**: 279-293, 1990.

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.

Verhoeven, A.S., Demmig-Adams, B., Adams, W.W., III: Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach exposed to high light and N stress. – Plant Physiol. **113**: 817-824, 1997.

Vonshak, A., Torzillo, G., Masojidek, J., Boussiba, S.: Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the alga *Monodus subterraneus* (Eustigmatophyta). – Plant Cell Environ. **24**: 1113-1118, 2001.

Wellburn, A.R.: The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. – J. Plant Physiol. **144**: 307-313, 1994.