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Diurnal changes of gas exchange, chlorophyll fluorescence, and stomatal aperture of hybrid poplar clones subjected to midday light stress

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Abstract

Diurnal changes in net photosynthetic rate (P_N), chlorophyll (Chl) fluorescence, and stomatal aperture of several hybrid poplar clones subjected to midday light stress were measured in July and August of 1996. Midday depression of P_N, photosystem 2 (PS2) efficiency, stomatal conductance (g_s), and stomatal aperture was observed in all clones, though at differing rates among them. Non-uniform stomatal closure occurred at noon and at other times, requiring a modification of intercellular CO_2 concentration (C_i). A linear relationship was found between g_s and stomatal aperture. More than half of the photons absorbed by PS2 centre dissipated thermally when subjected to light stress at noon. There was a linear relationship between the rate of PS2 photochemical electron transport (P×PFD) and P_N. There was a consensus for two fluorescence indicators ($1 - q_P/q_N$ and $(F_m' - F)/F_m'$) in assessment of susceptibility of photoinhibition in the clones. According to P_N, Chl fluorescence, and stomatal aperture, we conclude that midday depression of photosynthesis can be attributed to both stomatal and non-stomatal limitations.

Additional key words: intercellular CO_2 concentration; irradiance; net photosynthetic rate; *Populus*; stomatal aperture.

Introduction

The midday depression of photosynthesis is a common phenomenon for many plants subjected to light stress and/or concomitant high temperature stress (*e.g.*, Matos *et*

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al. 1998). Two factors either uniquely or simultaneously contribute to this process: stomatal (stomatal closure) and non-stomatal (biochemical limitation) ones. According to Farquhar and Sharkey (1982), the differentiation of stomatal and non-stomatal limitations can be accomplished through analysis of the changes in intercellular CO_2 concentration (C_i), stomatal limitation (L), and photosynthetic rates (P_N). But the validity of this method has been challenged since the first reports of stomatal heterogeneity (Laisk *et al.* 1980, Laisk 1983). The utilisation of P_N/C_i curve to describe non-stomatal limitation characteristics (Terashima 1992) is confined to cases where non-uniform stomatal closure does not occur. The correct utilisation of the method by Farquhar and Sharkey (1982) rests on the assumption that stomatal openness is uniform. The direct effect of non-uniform stomatal closure leads to overestimation of C_i (*e.g.*, Downton *et al.* 1988a,b, Terashima *et al.* 1988, Farquhar 1989, Chaves 1991, Terashima 1992). In recent years the Chl fluorescence technique has been widely used to measure plant photosynthetic function since Schreiber (1986) first introduced this commercialized technique. The Chl fluorescence technique is a quick and non-intrusive probe for the measurement of photosynthetic function (Genty *et al.* 1989), especially useful for the assessment of photosynthesis under environmental stress.

In this experiment we used several fast-growing clones of hybrid poplar, notably triploid clones possessing large leaf areas (2-3 times of leaf area of diploid clone) and susceptible to environmental stress. The aim of this work is to assess stomatal and non-stomatal limitations leading to midday depression of photosynthesis through the concurrent measurement of gas exchange, Chl fluorescence, and stomatal aperture. Especially we conducted measurements of stomatal heterogeneity using scanning electron microscopy and tried to re-evaluate the factors leading to midday depression of photosynthesis.

Materials and methods

Plants: Four clones provided by the Breeding Section of Forest Resources & Environment College at Beijing Forestry University were employed in the study. The triploid clones B342 and ZH6 and the diploid clone B11 are F_1 progeny of [(*Populus alba*×*P. glandulosa*)×(*P. tomentosa*×*P. bolleana*)], while the triploid clone B346 is F_1 progeny of [(*P. tomentosa*×*P. bolleana*)×(*P. alba*×*P. glandulosa*)]. One-year-old seedlings were cut into 15 cm long cuttings and stored with wet sand over the winter of 1995/6. In the spring of 1996 cuttings were selected for homogeneous developmental position and similar diameter, inserted in the campus nursery, and periodically watered.

Gas exchange measurements: Measurements of P_N, g_s, C_i, *etc.* were carried out under ambient conditions every two hours from 07:00-19:00 in the clear days of July and August, 1996 with a portable infrared gas analyser (*LI-6200, LI-COR*, Lincoln, Nebraska, USA). The sixth to eighth fully expanded leaf (from top) was selected and kept inside the cuvette under ambient irradiance until three stable photosynthetic measurements had been recorded.

Chl fluorescence measurements were made using a pulse-modulated fluorometer *PAM 101+103* (*Walz*, Effeltrich, Germany). Just after measurements of gas exchange the leaves were excised and put into a wet black cloth and Chl fluorescence measurements were then carried out after 20 min of dark-adapted treatment.

The determined Chl fluorescence parameters were: F: fluorescence intensity at any time; F_0: minimal fluorescence (dark); F_m: maximal fluorescence (dark); F_0': minimal fluorescence (light); F_m': maximal fluorescence (light); $F_v = F_m - F_0$: variable fluorescence (dark); $F_v' = F_m' - F_0'$: variable fluorescence (light); F_v/F_m: maximal photochemical efficiency of PS2 (dark adapted leaves) (all the above parameters according to Van Kooten and Snel 1990); F_v'/F_m': photochemical efficiency of PS2 in the light, which is the efficiency if all reaction centres remained open (Bilger and Björkman 1990); $(F_m' - F)/F_m'$: actual photochemical efficiency of PS2 in the light, which is the efficiency under the actual degree of reaction centre closure (Bilger and Björkman 1990).

There are two kinds of fluorescence quenching: photochemical quenching and non-photochemical quenching. Photochemical quenching is represented by photochemical quenching coefficient $q_P = (F_m' - F)/(F_m' - F_0)$; non-photochemical quenching is expressed by two formulae, $NPQ = F_m/F_m' - 1$ (Bilger and Björkman 1990) or $q_N = 1 - (F_m' - F_0')/(F_m - F_0) = 1 - F_v'/F_v$ (Schreiber *et al.* 1989). Estimated rate of photochemistry (Genty *et al.* 1989) or apparent electron transport rate of PS2 centre is expressed by $(F_m' - F)/F_m' \times PFD$ (Winter and Lesch 1992), where PFD is photon flux density. Estimated rates of thermal dissipation (Genty *et al.* 1989) or apparent thermal dissipation rate of PS2 centre is expressed by $(1 - F_v'/F_m') \times PFD$ (Winter and Lesch 1992).

Stomatal aperture measurements by scanning electron microscopy: A 3 000 cm^3 liquid nitrogen pot and several 5 cm^3 medical bottles were brought to the plot. Leaf samples selected were similar in position and development to those used for measurement of gas exchange. After the measurements of gas exchange, the leaf samples were immediately cut into 2-3 pieces of about 0.25 cm^2 squares which were placed in the 5 cm^3 bottles. The latter were then quickly removed to the liquid nitrogen pot and stored immersed in liquid nitrogen. The leaf samples were later transferred under vacuum to the sample stage and sputter-coated with gold in an argon atmosphere (20 mA, 13 Pa, 1.5-2.0 min). The stomata were viewed and photographed at standard instrument magnifications of ×500, ×600, and ×1700 by a scanning electron microscope (*S-520* type, *Hitachi*, Japan). The width and length of stomatal apertures were then measured using the instrument ruler in three to five randomly selected areas of the leaf surface.

Results

Diurnal course of P_N and g_s: P_N in both months showed a bimodal diurnal pattern (Fig. 1*A*,*B*), indicating a significant depression of photosynthesis around noon. But the two curves were different, however: in July the morning peak was higher than the afternoon peak while in August P_N in the morning was close to that observed in the afternoon. In both months P_N of clones B342 and B11 was always higher than that of clones B346 and ZH6. In July the clones B346 and ZH6 and in August the clones B342 and B346 were subjected to light stress around noon, leading to serious photosynthetic depression.

Relative to diurnal course of P_N, the midday depression of g_s for most clones was much lower (Fig. 1*C*,*D*). Although there was similar trend of changes for diurnal courses of P_N and g_s, considerable risk may be associated with the conclusion that stomatal closure is the leading factor in the midday depression of P_N. This would especially be the case where non-uniform stomatal closure occurs.

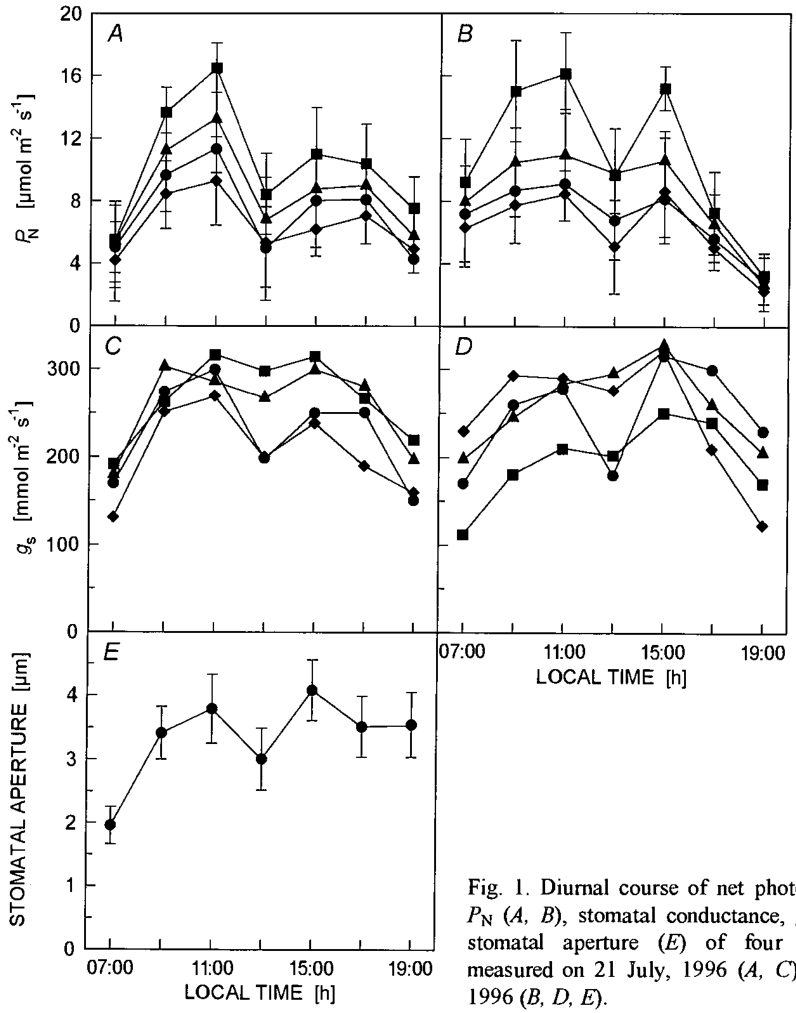

Fig. 1. Diurnal course of net photosynthetic rate, P_N (*A*, *B*), stomatal conductance, g_s (*C*, *D*), and stomatal aperture (*E*) of four poplar clones measured on 21 July, 1996 (*A*, *C*) or 14 August 1996 (*B*, *D*, *E*).

Diurnal course of stomatal aperture measured by scanning electron microscopy: Patterns of variation in stomatal aperture through the course of a single day (14 August, 1996), and across the surface of individual leaves were recorded under ambient conditions with the example of triploid clone ZH6 (Fig. 1*E*). Two scanning electron micrographs (magnification of ×500), each with about 15 stomata, from each sampling time were used to calculate the mean and standard deviation of stomatal apertures. Plotting the mean of stomatal apertures against time in the two clones (Fig. 1*E*) indicates that the stomata were closed in the morning but gradually opened with increase of ambient irradiance and temperature. Average stomatal aperture reached its maximum between 10:00 and 11:00 h, and from 15:00 h to sunset the stomata gradually closed. The stomata were nearly closed at noon (around 13:00 h) when subjected to strong irradiance.

A similar diurnal pattern was found for both stomatal aperture and g_s, which was confirmed by their linear regression (Fig. 2). The relationship coefficients reached a 5 % significance level.

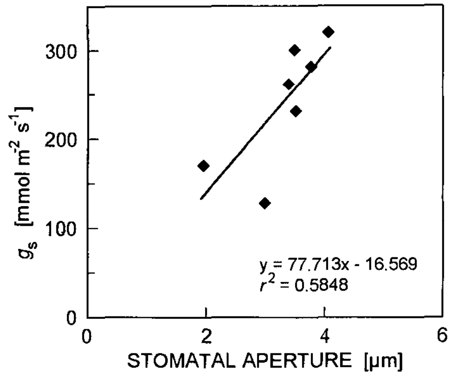

Fig. 2. Linear relationship between stomatal aperture and stomatal conductance (g_s) of poplar clone ZH6 measured on 14 August 1996.

Stomatal heterogeneity and modification of C_i: Generally, stomatal heterogeneity refers to the tempo-spatial non-uniformity within a single leaf. Especially 'patchy stomatal distribution (patchy closed stomata appear in the areas of mostly open stomata)' is the important case that has received great attention within the decade. To correctly analyze the stomatal/non-stomatal factors leading to photosynthetic depression, it should be investigated firstly whether stomatal heterogeneity occurs within leaf blade. If the mean of stomatal aperture is large enough with the least standard deviation, the stomatal openness is thought to be uniform. Otherwise, stomatal heterogeneity exists. Basing on this principle and using scanning electron microscopy, Zhang and Gao (1998) established a model to assess the diurnal uniformity of stomatal openness, by which we can modify C_i. According to the values of diurnal stomatal aperture, heterogeneity of stomatal openness was found at noon and other time. So the C_i had to be modified. The C_i-modification is provided in the following formula:

$$Ci' = \left(\frac{\bar{x}_i}{\bar{x}_{\max}} \cdot \frac{\bar{x}_i}{\bar{x}_i + \sigma_i}\right) Ci + \left(1 - \frac{\bar{x}_i}{\bar{x}_{\max}} \cdot \frac{\bar{x}_i}{\bar{x}_i + \sigma_i}\right) \Gamma,$$

$\bar{x}_{\max}$ represents the mean of diurnal maximum stomatal apertures, $\bar{x}_i$ represents the mean of the stomatal apertures at transient time in a day, and σ_i is the standard deviation of stomatal apertures at transient time in a day, Γ is the CO_2 compensation concentration. When the stomata are closed, C_i is supposed to be its Γ. C_i' represents modified C_i.

After modification of C_i, the stomatal/non-stomatal factors leading to midday depression of photosynthesis should be re-evaluated. According to Farquhar and Sharkey (1982), if P_N and C_i change in opposite directions, the decline of photosynthesis arises from non-stomatal limitation; if P_N and C_i change in the same direction (*e.g.*, both are decreasing), the judging criteria rest on the changing directions of the stomatal limitation: the increase of stomatal limitation definitely leads to the conclusion that stomatal closure is the main cause of reduced P_N, whereas the decrease of stomatal limitation does not lead to the above conclusion.

The importance of the C_i-modification is confirmed with values for clone ZH6 subjected to midday depression of photosynthesis (Fig. 3). Stomatal limitation (L) increased during the time 11:00-13:00 h, meanwhile its C_i also was sharply increased (Fig. 3*A*). Photosynthetic depression (from 11:00 to 13:00 h) should result from a non-stomatal factor. With consideration of stomatal heterogeneity, we modified the

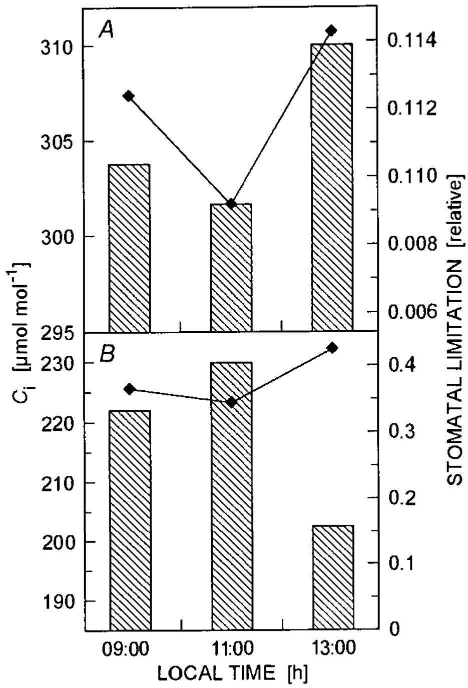

Fig. 3. Diurnal course of intercellular CO_2 concentration, C_i (*A*) or C_i' (*B*) (*columns*) and stomatal limitation (L in *A*, L' in *B*, *lines*) of poplar clone ZH6 before (*A*) and after (*B*) modification of C_i on 14 August 1996.

C_i and L [after modification, C_i' and L' are used to represent the original ones, Γ = 90 μmol(CO_2) mol^{-1}], finding that the C_i' substantially decreased at 13:00 h, being lower than the value at 11:00 h. In this case, stomatal factor (closure) is thought to attribute to the midday depression of photosynthesis. Through modification of C_i, the stomatal factor related to midday depression of photosynthesis in some cases is restored.

Diurnal course of photochemical efficiency of PS2 centre: The typically diurnal pattern of maximal PS2 efficiency in mid-August showed an inverse "bell" shape (Fig. 4*A*). It represented potential maximal photochemical conversion efficiency of PS2 centre. The PS2 efficiency was maximal at dawn and sunset, and reached its minimum around noon. The PS2 photochemical conversion efficiency of diploid clone B11 was higher than that of the other three triploid clones, the lowest was for clone ZH6.

Like the maximal PS2 efficiency (F_v/F_m), both the open and actual PS2 efficiencies (F_v'/F_m') and (F_m' - F)/F_m', respectively, were maximal at dawn and sunset, and at their lowest at noon (Fig. 4*B,C*). In daytime, values for the clone B11 were always higher than that for the other three clones in either (F_v'/F_m') or (F_m' - F)/F_m', while the B342 and B346 clones were close in both parameters. In clone ZH6 there was diurnal difference between both indicators. ZH6 reached a high level of F_v'/F_m' in the morning and its lowest level two hours later than the other three clones, meanwhile clone ZH6 always kept the lowest level of (F_m' - F)/F_m' in daytime though still showing the same trend as the other three clones.

Fig. 4*D* shows the diurnal course of photochemical quenching with the example of two clones in the August representing the allocation of energy of photosynthetically active radiation (PAR) absorbed by PS2 antenna pigments to photosynthetic electron transport. Photochemical quenching can represent "opening" degree of the PS2 centre. Clone ZH6 showed the most serious closure of PS2 centre around noon and the PS2 centre started to close two hours earlier than the other three clones.

In opposition to photochemical quenching, non-photochemical quenching represents the allocation of PAR energy absorbed by PS2 antenna pigments to thermal dissipation. The diurnal pattern of non-chemical quenching showed a dome shape (Fig. 4*E,F*). Clone B346 had higher level of NPQ than the other clones, which showed that PS2 of clone B346 had a high efficiency of dissipating excessive photons, on the other hand clones ZH6 and B11 had very low level of NPQ in daytime.

We can assess the susceptibility of photoinhibition using some Chl fluorescence parameters. (F_m' - F)/F_m' is commonly taken as an effective indicator for assessing the susceptibility of photoinhibition, while Osmond *et al.* (1995) took 1 - q_P/q_N as an indicator. Table 1 compares the susceptibility of photoinhibition by using the two indicators.

According to the meaning of the parameters, the higher the values of 1 - q_P/q_N, the more susceptible to photoinhibition the clones should be. In opposite to 1 - q_P/q_N, higher value of (F_m' - F)/F_m' usually has less susceptibility to photoinhibition. In general, the two indicators assessing susceptibility of photoinhibition were in consensus.

Table 1. Susceptibility to photoinhibition of four poplar clones (B346, B342, B11, ZH6) determined by two indicators.

	B346	B342	B11	ZH6
1 - q_P/q_N	0.1284	0.0282	0.0136	0.1567
$(F_m' - F)/F_m'$	0.3241	0.3208	0.3555	0.2756

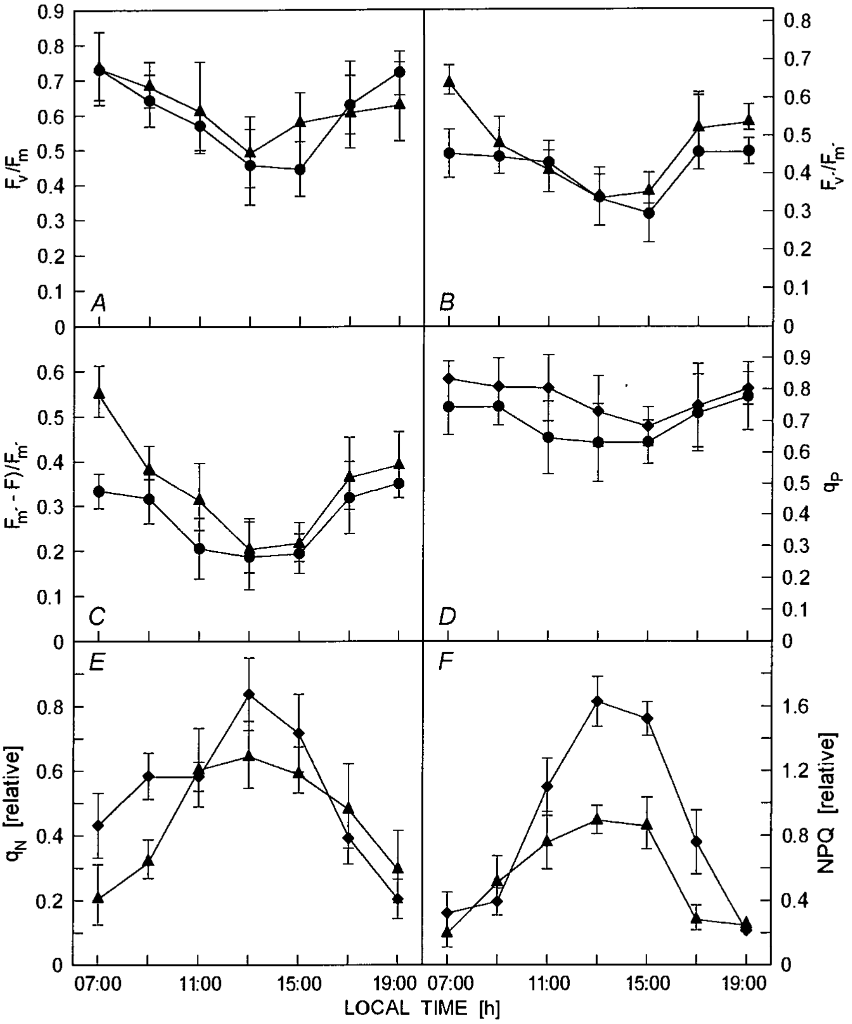

Fig. 4. Diurnal course of photosystem 2 (PS2) efficiencies [*A* - maximal F_v/F_m; *B* - at open centres F_v'/F_m'; *C* - actual $(F_m' - F)/F_m'$], photochemical quenching, q_P (*D*), and non-photochemical quenchings q_N (*E*) or NPQ (*F*) measured on 14 August 1996.

Diurnal reallocation of photons absorbed by PS2 centre: There are three channels for reallocation of photons absorbed by PS2 antenna pigments: (*1*) photosynthetic electron transport, represented by $P = F_v'/F_m' \times q_P$; (*2*) thermal dissipation, represented by $D = 1 - F_v'/F_m'$; and (*3*) detaining in the PS2 centre as excessive energy, represented by $E = F_v'/F_m' \times (1 - q_P)$.

Fig. 5 shows the diurnal course of reallocation of photons absorbed by PS2 centre to the three fractions P, D, and E in four of the clones. The fraction of photons absorbed by PS2 antenna pigment that is utilised in photosynthetic electron transport (P) reached its maximum at dawn and sunset and its minimum around noon. Meanwhile the fraction of photons absorbed by PS2 antenna pigment that is utilised in thermal dissipation (D) reached its maximum around noon, and more than 2/3 of PAR energy absorbed by PS2 centre was allocated to thermal dissipation at this time. The fraction allocated to photosynthetic electron transport in clone B11 was higher than that of clones B346 and B342. Similarly, B11 also surpassed clones B346 and B342 in the fraction of allocation to excessive energy (E). For clone ZH6, both its photosynthetic electron transport and thermal dissipation were less, and the excessive energy was almost two times as much as that of the other three clones.

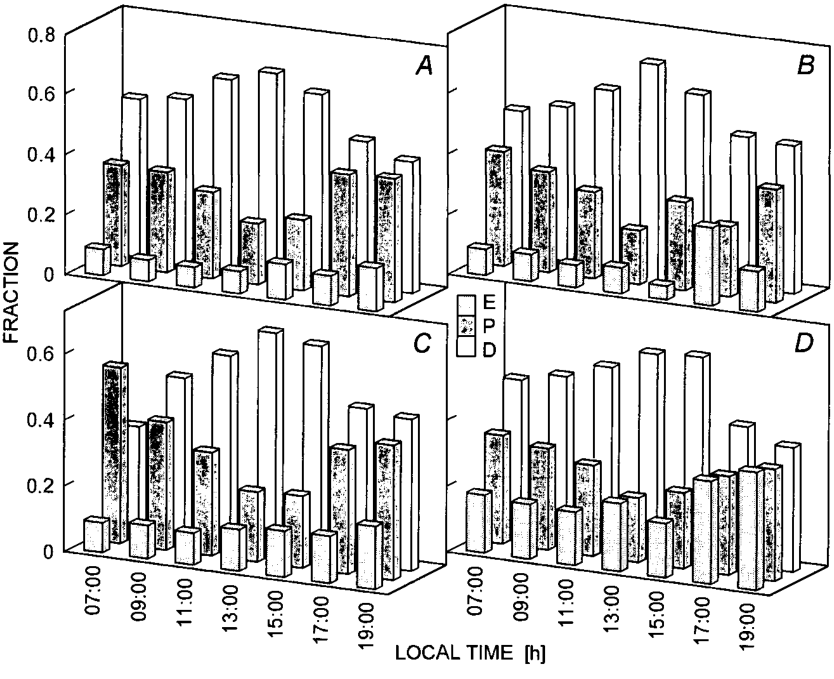

Fig. 5. Diurnal course of reallocation of PAR energy absorbed by photosystem 2 antenna pigments in poplar clones B346 (*A*), B342 (*B*), B11 (*C*), or ZH6 (*D*) on 14 August 1996. PAR energy was used for photosynthetic electron transport (P) or for thermal dissipation (D), E represents excessive energy detaining in the PS2 centre.

Relationship between gas exchange and photochemical efficiency of PS2 centre: The PS2 centre apparent photosynthetic electron transport rate is represented by $P\times PFD = (F_m' - F)/F_m'\times PFD$, and the PS2 centre apparent thermal dissipation rate is represented by $D\times PFD = (1 - F_v'/F_m')\times PFD$. The diurnal pattern of P×PFD for four clones also showed a bimodal shape (Fig. 6*A*). There were no significant differences among maximum values in the morning between the clones. The maximum values of clone B342 in the afternoon appeared two hours earlier than those of the other three clones, which meant the PS2 centre of clone B342 had higher level of apparent photosynthetic electron transport rate under strong irradiance, though this did not last for long. The P×PFD of clone ZH6 was low throughout the entire diurnal course. The diurnal pattern of D×PFD of the four clones were typically dome-like curves (Fig. 6*B*). The D×PFD maximum values of the three triploid clones were higher than that of the diploid clone B11. Genty *et al.* (1989) found that there was a significant linear relationship between the apparent quantum efficiency of CO_2 assimilation and actual PS2 efficiency $(F_m' - F)/F_m'$. We tried to use P_N and apparent photosynthetic electron transport rate (P×PFD) to establish a regression relationship (Fig. 7). The relationship coefficients of clones B346, B342, and B11 were significant at the 5 % level, while in clone ZH6 the relationship was significant at the 10 % level.

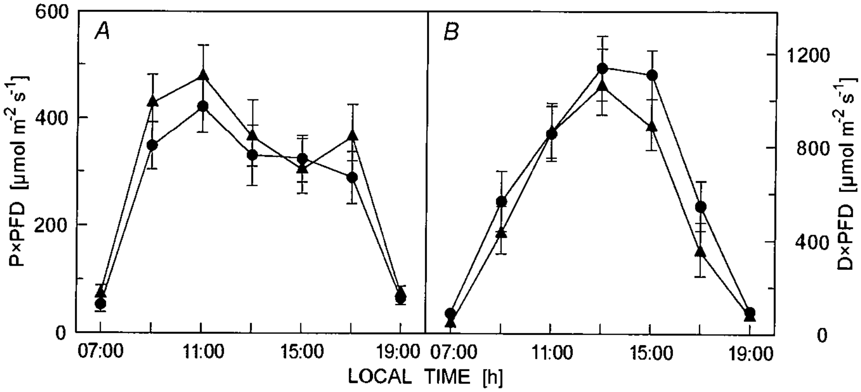

Fig. 6. Diurnal course of apparent photosystem 2 photosynthetic electron transport rate (*A*) and thermal dissipation rate (*B*) on 14 August 1996.

Discussion

Occurrence of stomatal heterogeneity makes it invalid to analyze stomatal/non-stomatal limitation of photosynthetic depression using the method of Farquhar and Sharkey (1982). The non-uniform stomatal closure can result in overestimation of C_i (*e.g.*, Downton *et al.* 1988a,b, Terashima *et al.* 1988, Farquhar 1989, Chaves 1991, Terashima 1992), supporting example can be found at the time 13:00 (Fig. 3*A*) which will lead to a wrong conclusion that midday depression of photosynthesis arises from non-stomatal factors. It is difficult to correctly identify stomatal/non-stomatal limitation for the midday depression of photosynthesis through the approach of gas exchange, because modification of C_i can lead to different results (Fig. 3).

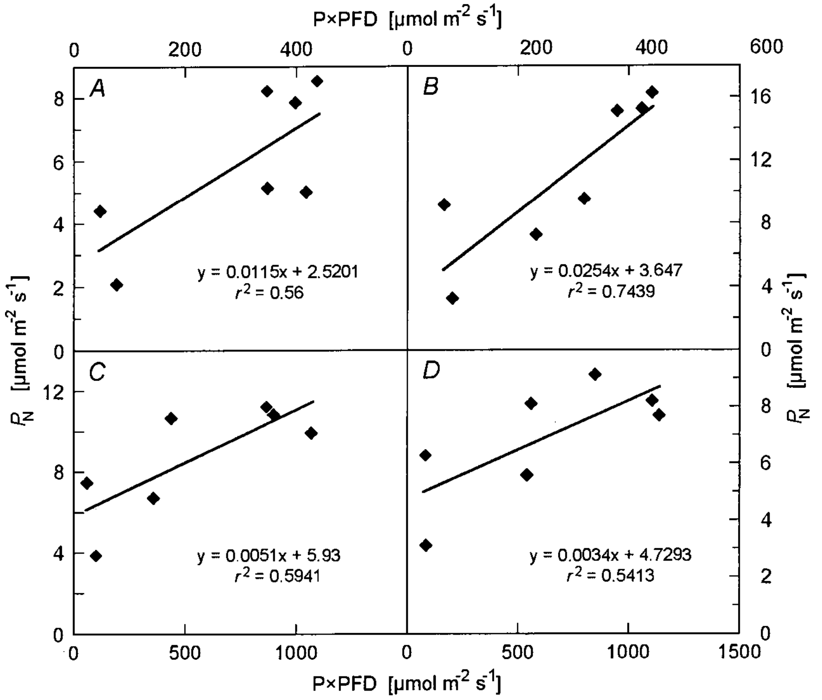

Fig. 7. Linear regression between PxPFD and net photosynthetic rate, P_N on 14 August 1996 in poplar clones B346 (*A*), B342 (*B*), B11 (*C*), and ZH6 (*D*).

Fig. 4*A*-*C* shows that all three indicators relating to PS2 efficiency [F_v/F_m, F_v'/F_m', and $(F_m' - F)/F_m'$] declined around noon, suggesting that the PS2 centres could not use excessive PAR energy and was inactivated or damaged. However, PS2 centres of most clones were not seriously photo-damaged, which was confirmed by the recoverable peak of PS2 efficiency in the afternoon (Fig. 4*A*-*C*).

To maintain high photochemical quenching, PS2 centre should maintain 'opening' status, so photochemical quenching could reflect the degree of opening of PS2 centres. The PS2 centres closed heavily around noon, which meant that strong irradiance could lead to the inactivation of PS2 and block electron transport from PS2 to PS1. When PS2 centre absorbs excessive photons, PS2 centre will dissipate *via* the thermal channel, otherwise the excessive photons will make the PS2 centre inactive or even photo-damaged. So non-photochemical quenching is a mechanism of photo-protecting the photosynthetic apparatus from damage (Ögren 1991). High efficiency of non-photochemical quenching might be an effective pathway of avoiding PS2 centre to be substantially photo-damaged. Supporting evidence can be found from the values for clone ZH6 (Fig. 4*E*,*F*). The pronounced decrease of its PS2 efficiency around noon might result from its relatively low level of NPQ.

To some extent, photochemical quenching can indicate potential photosynthetic capacity, meanwhile the resistance to environmental stresses can be mirrored by its non-photochemical quenching. Both parameters provide valuable information for helping selection of good clones (fast-growth/high resistance). Especially great emphasis should be placed on the fraction of excessive energy detaining in the PS2 centre (E). Backlog of reduced electron acceptors in PS2 could result in an increased decay of the singlet excited Chl to triplet excited Chl that might react with oxygen to form toxic singlet oxygen (Asada *et al.* 1987, Demmig-Adams *et al.* 1996b). So high fraction of excessive energy detaining in the PS2 centre has great potential to make PS2 centre photo-damaged. However, thermal energy dissipation counteracts backlog of excitation energy (Demmig-Adams *et al.* 1996a). Confirmed information can also be found from the values for clone ZH6, because its fractions of photosynthetic electron transport and thermal dissipation were in very low level, no wonder its PS2 withheld a considerable portion of excessive photons besides its allocation to photosynthetic electron transport and thermal dissipation. The excessive energy was almost twice as much as that of the other three clones. The greater detainment of excessive energy easily leads to inactivation of PS2 centre, which may be one of the main causes leading to more serious photoinhibition for clone ZH6 as compared with the other clones.

The scanning electron micrographs showed a direct evidence of noon decrease in mean stomatal aperture subjected to light stress, indicating that this stress might directly or indirectly induce stomatal closure. Incorporating the analysis for PS2 efficiency above, we suspect that both stomatal and non-stomatal factors might contribute to the midday depression of photosynthesis. In fact there is a close relationship between stomatal and non-stomatal factors. In the Chl fluorescence analysis, one of the causes leading to a decrease of photochemical quenching perhaps is the stomatal factor. When the stomata are closed, C_i decreases, because CO_2 is the final acceptor of photosynthetic electron transport chain. The decrease of CO_2 will block electron transport and decrease photochemical quenching. And *vice versa*, for example, under strong light stress the destruction of D1-protein or other photosynthetic electron carriers will result in a decrease of photosynthetic electron transport, which will in turn lead to decrease in mesophyll CO_2 assimilation efficiency. In this situation CO_2 supply is excessive rather than inadequate. As completely open stomata release too much water under strong irradiance, stomata will soon close in response to the feedback effect. In this manner the stomatal factors and non-stomatal factors interact and coexist.

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