Short Communication

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DIURNAL CHANGES IN GAS EXCHANGE AND CHLOROPHYLL FLUORESCENCE IN GINKGO LEAVES UNDER FIELD CONDITIONS

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ABSTRACT

Gas exchange and chlorophyll *a* fluorescence were investigated to survey the diurnal changes in photosynthesis of ginkgo (*Ginkgo biloba* L.) leaves. The net CO_2 assimilation rate showed a bi-peak diurnal pattern and midday depression of CO_2 assimilation was observed at 13:00 h. Depression in CO_2 assimilation at midday was not limited by stomatal factors, but rather non-stomatal factors because the loss in net CO_2 assimilation rate was not followed by significant reductions in stomatal conductance and intracellular CO_2 concentration. Midday depression in CO_2 assimilation was associated with reversible inactivation of photosystem II reaction centers and increases of antenna heat dissipation in response to the strong midday sunlight. Performance index (PI) on absorption basis (PI_{abs}) is more sensitive than the maximum photochemical efficiency of photosystem (PS) II (variable to maximum fluorescence ratio, F_v/F_m) to the changes in light and temperature. It is concluded that the dissipation of excessive excitation energy in the PS II may be the major protective mechanism to prevent the chloroplast from photodamage.

Key words: *Ginkgo biloba*, chlorophyll fluorescence, gas exchange.

INTRODUCTION

Ginkgo biloba L., which is widely cultivated as a medical and fruit plant in China, is the most ancient living gymnosperm, and is the only representative of the Ginkgo aceae family. Such unique characteristics of ginkgo have attracted worldwide interest in plant science research. Our previous research showed that photo-protection was significantly strengthened at the early stages of leaf expansion in ginkgo under natural environmental conditions and photosynthetic decline in ginkgo leaves during natural senescence (Yang *et al.*, 2012; 2013).

Photosynthesis is the source of dry matter production in higher plants. Photosynthetic efficiency is usually determined by measuring CO_2 assimilation by leaves in the light, but can also be determined by measuring the extent of chlorophyll fluorescence in leaves that have been kept in the dark (Yang *et al.*, 2010). Two ratios are often used in this analysis: the maximum quantum efficiency (i.e., the trapping flux / absorption flux) and the potential photosynthetic capacity of photosystem II (PS II), indicated by F_v/F_m and F_v/F_o, respectively (Sarijeva *et al.*, 2007). The ratio F_v/F_m has been defined as the "vitality index" and used to describe the behaviour of many plants under different environmental conditions. Photosynthesis cannot proceed in the absence of photons, but no leaf can utilize all of those absorbed by antenna systems during exposure to full sunlight. Excessive photons are dangerous to plants because they can inhibit photosynthesis and may lead to photo-oxidative destruction of the photosynthetic apparatus (photoinhibition) (Foyer and Noctor1999). The midday depression of photosynthesis is a common phenomenon for many plants (Huang *et al*., 2006). Although there are several studies about the midday depression in CO_2 assimilation in field crops (Wang *et al*., 2005), as far as we know, little information related to photoinhibition and photoprotection of ginkgo (*Ginkgo biloba* L.) is available yet.

In the present study, the diurnal changes in gas exchange and chlorophyll fluorescence were examined in leaves of ginkgo in order to describe their midday photoinhibition and photoprotection under field conditions.

MATERIAS AND METHODS

Plant material: Ten-year-old male ginkgo (*Ginkgo biloba* L.) plants cultivar 'Dafozhi' were grown in the experimental fields at the Forest Management Centre of Jiangdu, Jiangsu Province, P.R. China (32°26′N, 119°38′E). Jiangdu is located in the monsoon climate area of the north subtropical zone with four distinctive seasons; its annual average temperature is 14.9 °C, and the annual precipitation is about 1,000 mm.

Leaf gas exchange: The net photosynthesis rate (P_n), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) were measured in the field on sun exposed leaves with a portable photosynthetic system (*CIRAS-2*, *PP Systems*, Hitchin, UK) at 2h intervals from 7:00-19:00 local time. To avoid potential photoinhibition from high light stress, the measurements were conducted under saturating photosynthetic photon flux densities (PPFD) 1000 µmol m^{-2} s^{-1} from a light-emitting diode (red and white) light source. The leaves in the cuvette were exposed to ambient CO_2 concentration 350 µmol mol^{-1}, relative humidity, and temperature about 25 °C.

Chlorophyll fluorescence: *In vivo* chlorophyll fluorescence was measured using a *Handy-PEA* chlorophyll fluorometer (*Hansatech Instruments*, King's Lynn, Norfolk, UK). The transient red radiation was about 3 000 µmol m^{-2} s^{-1} provided by an array of three light-emitting diodes (peak 650 nm). The maximum PS II quantum yield (F_v/F_m) was determined in dark-adapted (20 min) leaves at 2h intervals from 7:00-19:00 local time according to Strasser *et al.* (1995). OJIP transient was analyzed according to the JIP-test. The JIP-test analysis was performed using the professional *PEA Plus* and *Biolyzer HP3* software developed by R. Maldonado Rodriguez and freely available at www.unige.ch/sciences/biologie/bioen/bioindex.html. From OJIP transient, the extracted parameters (F_m, $F_{20µs}$, $F_{50µs}$, $F_{100µs}$, $F_{300µs}$, F_J, F_I *etc.*) led to the calculation and derivation of a range of new parameters according to Strasser *et al.* (1995, 2004). Gas exchange and chlorophyll fluorescence were analyzed on a typical sunny day. All measurements were performed on the attached fully expanded leaves with five replicates.

RESULTS AND DISCUSSION

Diurnal changes in environmental variables on a typical sunny day are presented in Fig. 1. Air temperature was lowest (23.6 ºC) at 7:00 h and then gradually increased, reaching a maximum of 34.5 ºC at 13:00 h (Fig. 1A). Photosynthetic photon flux density (PPFD) also reached its maximum (1459µmol m^{-2} s^{-1}) at 13:00 h (Fig. 1B).

Leaf net CO_2 assimilation rate (P_n) and Gs showed a bimodal diurnal pattern (Fig. 2). P_n was low in the early morning, increasing with time and reaching a maximum (ca. 7.8µmol m^{-2} s^{-1}) at 11:00 h. Thereafter, P_n decreased independent of the increase in incident PPFD (Fig. 2A). The second peak for P_n appeared at 15:00 h, although its value (6.5µmol m^{-2} s^{-1}) was significantly lower than the first peak. Meanwhile, the first peak for G_s was 158 mmol m^{-2} s^{-1} at 11:00 h and the second peak for G_s was 114 mmol m^{-2} s^{-1} at 15:00 h (Fig. 2B). C_i increased from 202µmol mol^{-1} at 7:00 h to its maximum value (254µmol mol^{-1}) at noon when midday depression of photosynthesis was observed. Afterwards, it gradually decreased (Fig. 2C).

Although PPFD was highest at 13:00, a drop of CO_2 assimilation was observed (Figs. 1 and 2). Apparently, light absorbed by the plants exceeded the photo-utilization capacity in chloroplast. Since the decrease in P_n at midday was accompanied by an increase in C_i, the midday depression in P_n could be attributed to the decreased photosynthetic activity of mesophyll cells, rather than the closure of stomata.

The maximum PSII efficiency (F_v/F_m) was 0.793 in the early morning. Afterwards it decreased with the increase of PPFD (Fig. 3). Although F_v/F_m fell to 0.697 at midday, it recovered to a value close to 0.792 in the late afternoon. Changes in PI_{abs} followed the patterns of F_v/F_m and F_v/F_o, but the amplitude of PI_{abs} was much greater than that of F_v/F_m, indicating PI_{abs} is more sensitive than F_v/F_m to the changes in light and temperature.

F_v/F_m is widely used as an indicator of photoinhibition (Wang *et al*, 2005). In the present study, reversible change in F_v/F_m, F_v/F_o and PI_{abs} was found during the day, suggesting that photoprotection rather than photodamage occurred. The decrease in F_v/F_m, F_v/F_o and PI_{abs} is likely to be due to the reversible inactivation or down regulation of PSII, rather than the photodamage to PSII.

ABS/RC and TR_o/RC increased significantly, while RC/CS decreased significantly at noon, indicating that the reaction centers of PSII were damaged, leading to the light energy transfer efficiency of PSII decrease and excess excitation energy increase. DI_o/RC increased at noon, indicating some energy dissipation mechanisms were used to dissipate excess excitation energy at this time (Fig. 4). It is evident that the photochemistry process in the noon was low, and the absorbed light energy was dissipated mostly by antennae and reaction center.

In conclusion, leaf net CO_2 assimilation rate in ginkgo showed a bi-peak diurnal pattern. The midday depression is mainly due to photoinhibition, but not the closure of stomata. Photoinhibition in the midday occurred as a photoprotective mechanism, which induced a reversible inactivation of PSII centers and increased thermal energy dissipation in the antennae. The dissipation of excessive excitation energy in the PS II may be the major protective mechanism to avoid the photodamage to photosynthetic apparatus.

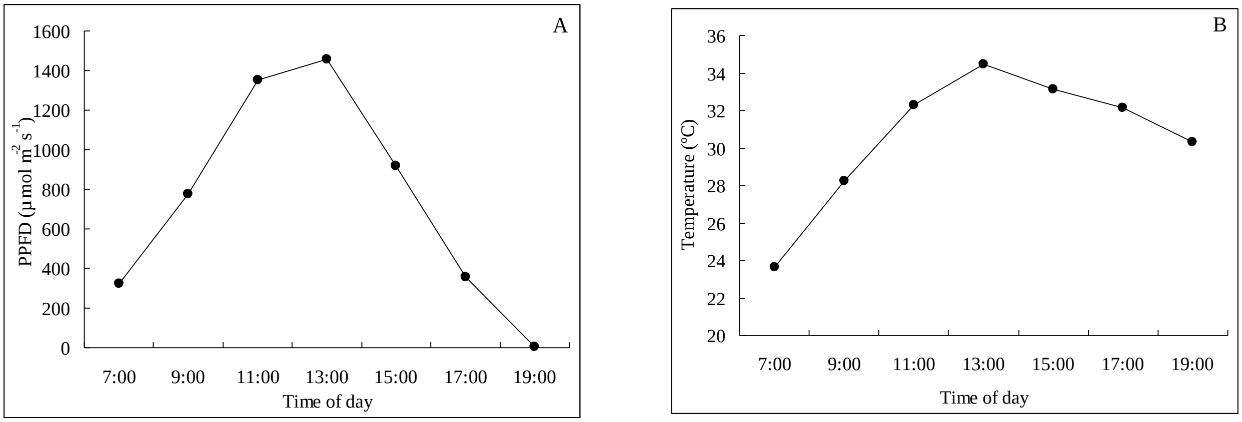

Fig. 1. Diurnal changes in photosynthetic photon flux density (PPFD) (A) and air temperature (B) on a clear day.

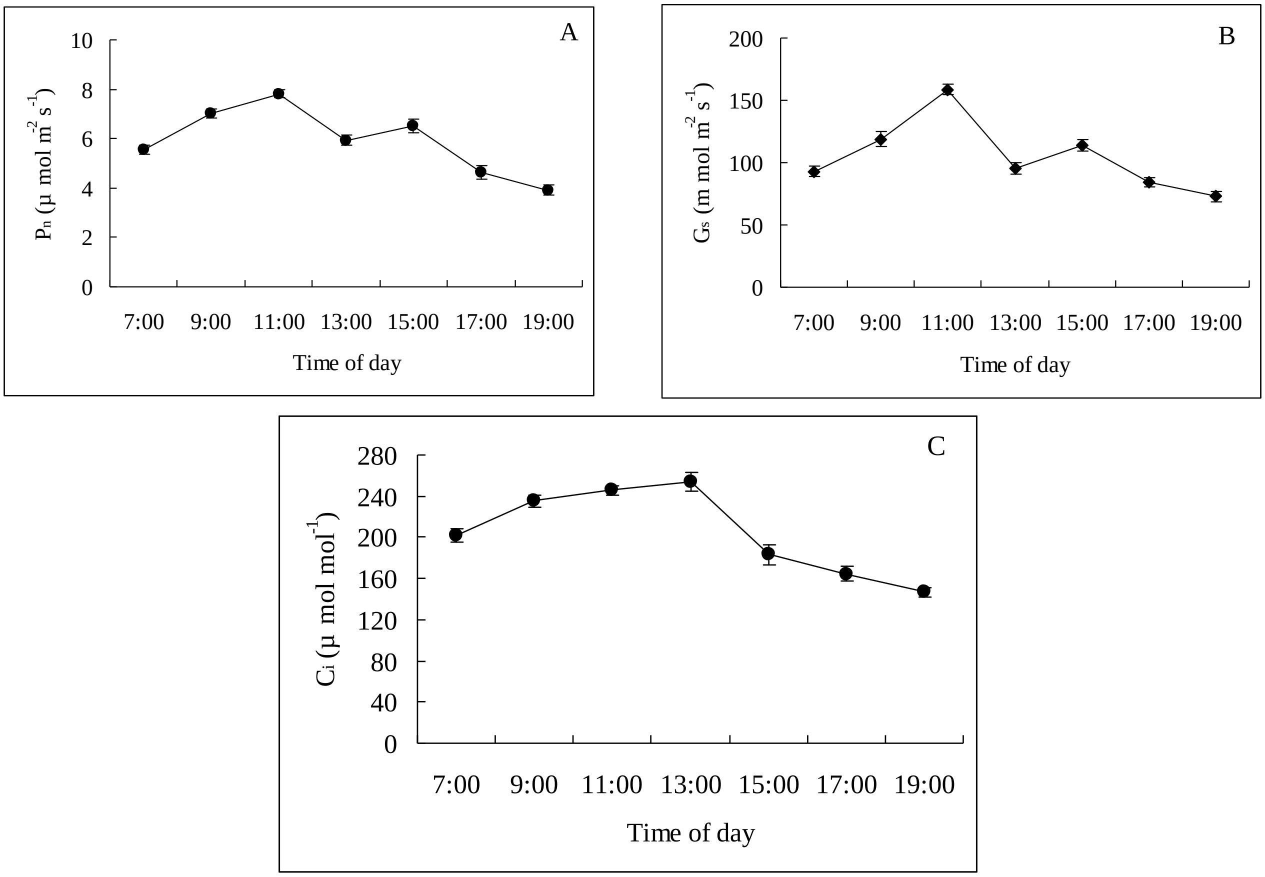

Fig. 2. Diurnal changes in net photosynthetic rates (P_n) (A), stomatal conductance (G_s) (B) and intercellular CO_2 concentration(C_i) (C) in ginkgo leaves

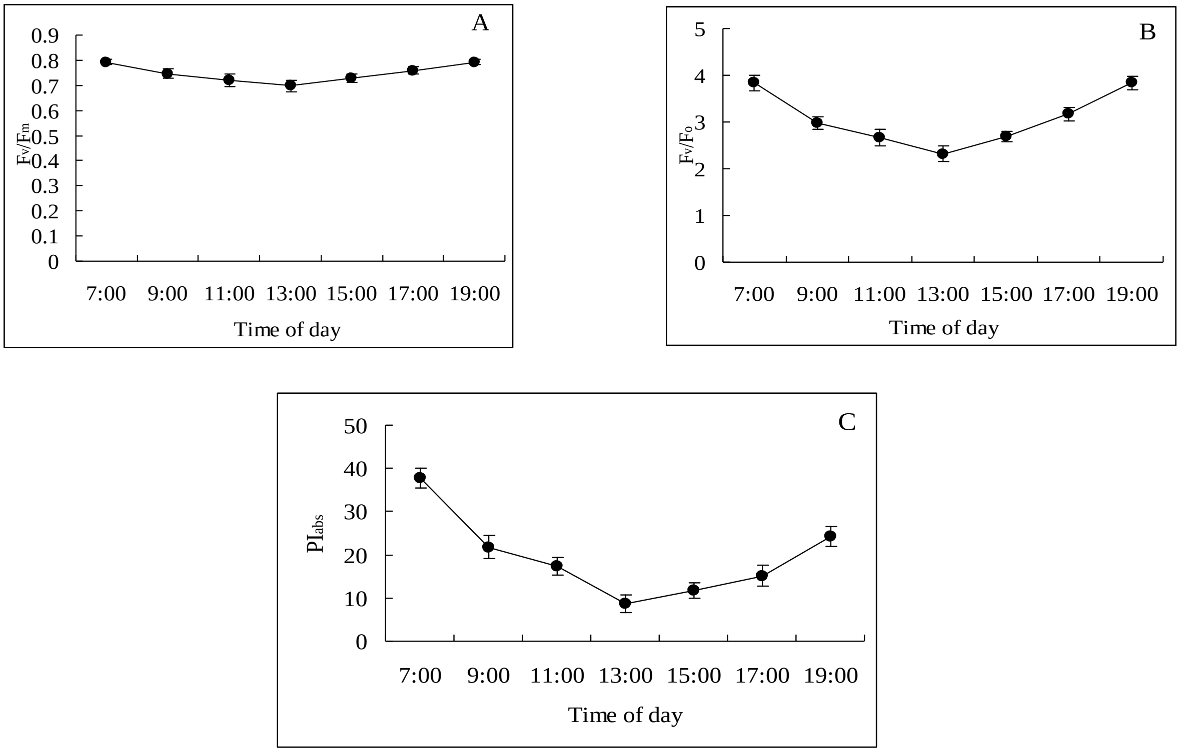

Fig. 3. Diurnal changes in the maximum quantum yield of PSII (F_v/F_m) (A), potential activities of PSII (F_v/F_o) (B) and the performance index (PI) on absorption basis (PI_{abs}) (C) in ginkgo leaves

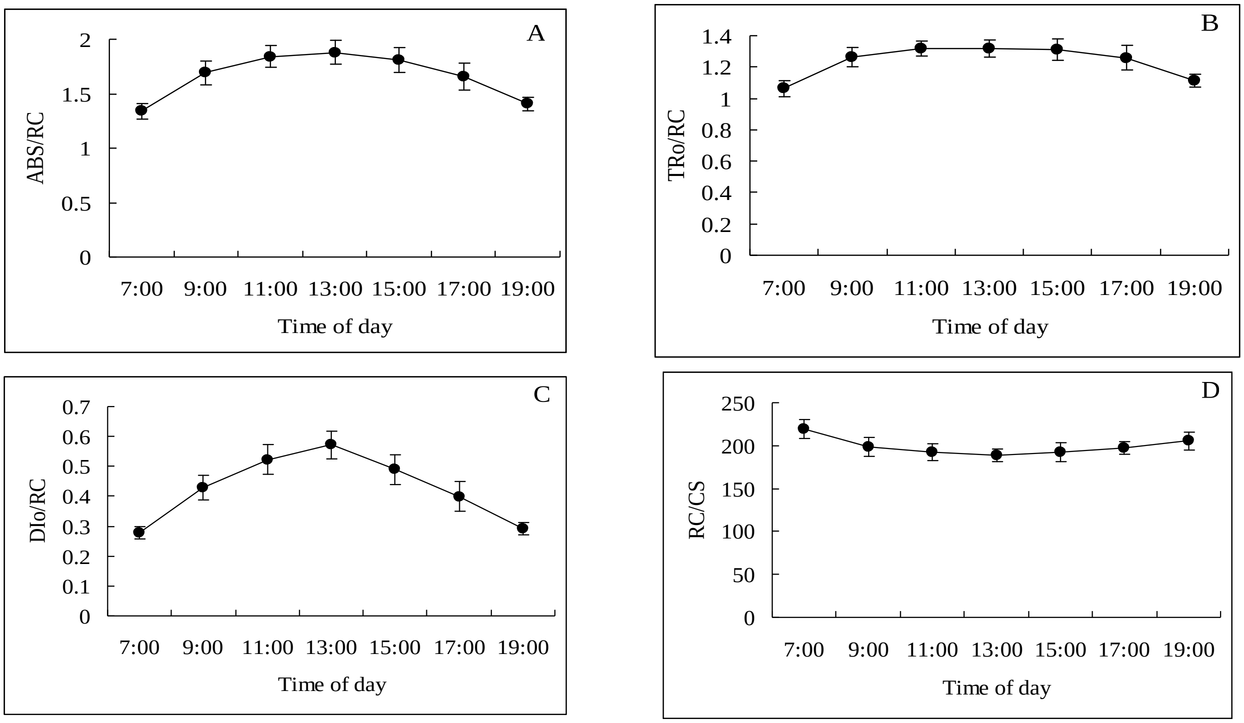

Fig. 4. Diurnal changes inenergy fluxes per excited cross-section for absorption (ABS/ CS) (A), trapping (TR_o/ CS) (B), Dissipation(DI_o/ CS) (C) and in fraction of active reactioncenters per excited cross-section (RC/ CS)(D)in ginkgo leaves

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