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DIFFERENCES IN DIURNAL PATTERNS OF CHLOROPHYLL FLUORESCENCE IN LEAVES FROM DIFFERENT SIDES OF THE GINKGO (*GINKGO BILOBA* L.) CANOPY

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Abstract

Diurnal patterns of chlorophyll *a* fluorescence transient kinetics in leaves from the east (E) and west (W) sides of the ginkgo (*Ginkgo biloba* L.) canopy were studied on sunny day. The results showed that the performance index on a basis of absorption (PI_{abs}), maximum quantum efficiency of PSII (F_v/F_m) and potential activity of PS II (F_v/F_o) in the leaves from the E decreased more drastically than those from the W at noon, suggesting the severe photoinhibition in the leaves from the E. However, the photoinhibition was more severe in the leaves from the W in the afternoon. With the variation of photon flux density (PFD), ratio of variable fluorescence F_k to the amplitude F_J - F_o (W_k) increased firstly, then followed by a decrease in the two group leaves. However, efficiency that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (Ψ_o) and probability that an absorbed photon will move an electron into the electron transport chain (φE_o) decreased firstly, then followed by an increase in the two group leaves. These results demonstrate that both the donor and acceptor sides of PS II were damaged in the leaves in different directions. Diurnal changes in dissipation per active reaction center indicate that the excessively excited energy per active reaction center was higher in the leaves from the E at noon while that was higher in the leaves from the W in the afternoon. The results also exhibited that PI_{abs} are more sensitive to the variation of PFD and can reflect the extent of photoinhibition in ginkgo leaves in more detail, compared with F_v/F_m and F_v/F_o.

Introduction

Light is one of the most important environmental factors that regulate the development of the photosynthetic apparatus in higher plants. Plants utilize radiant energy via photosynthesis and convert it into stable chemical energy. However, under conditions where the harvested light energy exceeds the requirements to drive photochemical reactions and protective mechanisms become over-burdened, photoinhibition may take place (Powles 1984, Aro *et al.* 1993). Photoinhibition can occur in the field under natural conditions (Huang *et al.* 2006). As photosynthesis is the basis of crop yields, photoinhibition has an adverse effect on photosynthesis and the accumulation of dry biomass, which can lead to a carbon assimilation decrease of about 10% (Long *et al.* 1994).

Recently, changes in the chlorophyll *a* fluorescence OJIP transient have been used to evaluate the extent of damage to the photosynthetic apparatus under several environmental stresses. The OJIP transient has useful and practical advantages: it is non-destructive, easy, and allows rapid testing of any type of chlorophyll-containing sample in any form (Strasser *et al.* 1995, 2004). Changes in chlorophyll fluorescence emission, arising mainly from PS II, provide information on the photochemical activity of PSII and the status of the plastoquinone pool (Strauss *et al.* 2006). The photosynthetic apparatus, and particularly PS II, is sensitive to various abiotic stresses. Several researchers have reported that the measurement of OJIP transients is a sensitive and reliable method for the detection and quantification of abiotic stresses-induced changes in the

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PSII and PSI of plants (Strasser *et al.* 2004, Strauss *et al.* 2006, Jiang *et al.* 2008, Stefanov *et al.* 2011).

Ginkgo biloba L., also known as maidenhair tree, is a well-known living gymnosperm fossil with edible seeds, medicinal efficacy, and ornamental value, and is the only representative of the Ginkgoaceae family (Yang and Chen 2014). In ginkgo plants, chlorophyll fluorescence measurements have been shown to be a useful technique for studying changes in the performance of the photosynthetic apparatus under the effect of adverse environmental factors (Loreto *et al.* 2003, Gregoriou *et al.* 2007, Melgar *et al.* 2009). 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). However, there have been no studies focusing on diurnal variations of chlorophyll *a* fluorescence transient kinetics in chloroplasts of ginkgo leaves in different directions.

Materials and Methods

Ten-year-old female ginkgo plants cv. '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). They received standard horticultural practices, diseases and pest control. 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.

Diurnal variations of photon flux density (PFD) were measured using a light measurement system (Model LI-188B, LI-COR Inc., USA). The value of PFD was averaged over the 3 days of measurement as conditions were similar. The sun rose around 07:00 hrs local time and the radiation increased rapidly. By 10:00 hrs, PFD was 1268 mmol m^{-2} s^{-1}, peaking at 1451 mmol m^{-2} s^{-1} at 13:00 hrs. The PFD decreased thereafter dropping below 400 mmol m^{-2} s^{-1} after 17:00 hrs (Fig. 1). The PFD showed a typical bell shaped curve over the course of the day during the period of the experiment in the field.

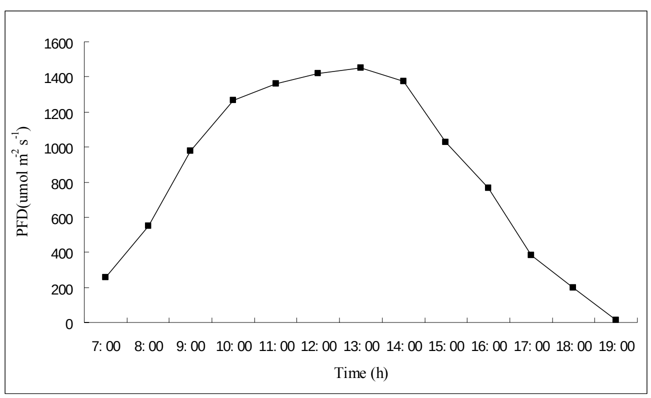

Fig 1. Diurnal variations of photon flux density.

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 3000 µmol m^{-2} s^{-1} provided by an array of three light-emitting diodes (peak 650 nm). The maximum PSII quantum yield (F_v/F_m) was determined in dark-adapted (20 min) leaves at 1 hr 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 para-meters (F_m, $F_{20\mu s}$, $F_{50\mu s}$, $F_{100\mu s}$, $F_{300\mu s}$, F_J, F_I etc.) led to the calculation and derivation of a range of new parameters according to Strasser *et al*. (1995, 2004). Chlorophyll fluorescence was analyzed on a typical sunny day. All measurements were performed on the attached fully expanded leaves with ten replicates.

Results and Discussion

Under field conditions, plants are exposed to irradiance which varies continuously over the course of the day. The performance index on a basis of absorption (PI_{abs}), maximum quantum efficiency of PSII (F_v/F_m) and potential activity of PSII (F_v/F_o) in the leaves both orienting to east and west were first decreased and then increased with the diurnal variation of light intensity (Fig. 2). However, these parameters in the leaves from the E decreased more drastically than those from the W in the forenoon and lowest peak at 11: 00 and 14: 00 to 16: 00, respectively. Apparently strong light stress induced changes in some JIP parameters such as PI_{abs}, were greater than that in

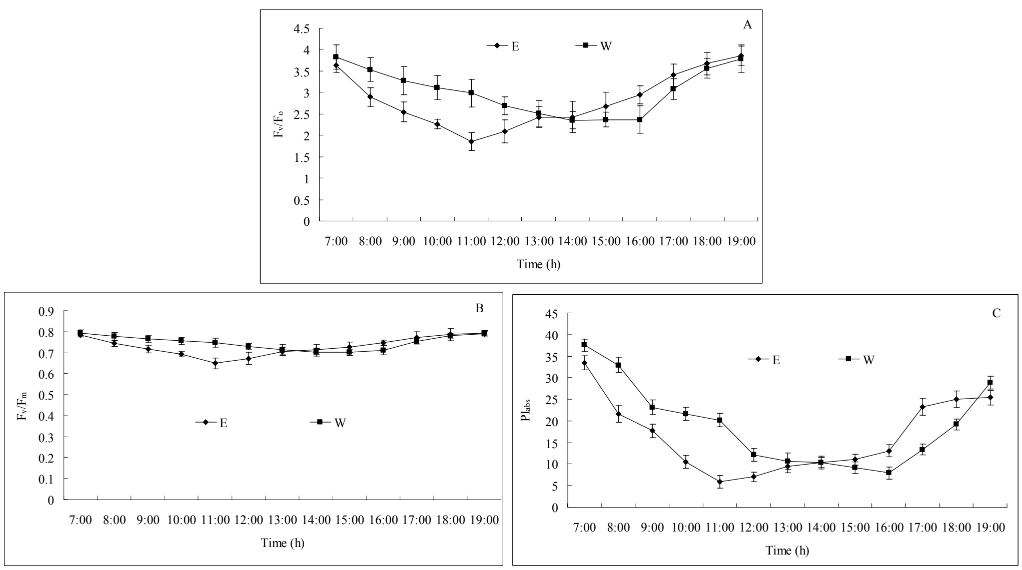

Fig. 2. Diurnal changes in the maximum quantum yield of PSII (F_v/F_m, A), the performance index (PI) on absorption basis (PI_{abs}, B) and potential activities of PSII (F_v/F_o, C) in ginkgo leaves from the E or W. Each data is the average of 10 independent measurements.

F_v/F_o and F_v/F_m, which is generally used as a stress indicator of chlorophyll *a* fluorescence. Therefore, our results indicate that, in ginkgo leaf, OJIP transients are more sensitive methods compared with using F_v/F_o and F_v/F_m for measuring physiological damage to the photosynthetic apparatus imposed by strong light stress on sunny day in the field, and can reflect the extent of photoinhibition in ginkgo leaves in more detail. Changes in the intensity and the irradiation angle of sunlight affect the utilization of actual absorbed light in ginkgo leaves.

With the variation of photon flux density (PFD), ratio of variable fluorescence F_k to the amplitude F_j - F_o (W_k) increased firstly, then followed by a decrease in these two group leaves (Fig. 3A). However, efficiency that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (Ψ_o) and probability that an absorbed photon will move an electron into the electron transport chain (φE_o) decreased firstly, then followed by an increase in these two group leaves (Fig. 3 B,C). W_k in the leaves from the E increased more rapidly than that from the W, and peak at 14:00 and 13:00, respectively. These results demonstrate that both the donor and acceptor sides of PSII were damaged in the leaves orienting to different directions. But the damage to the receptor side is more serious than that the donor side.

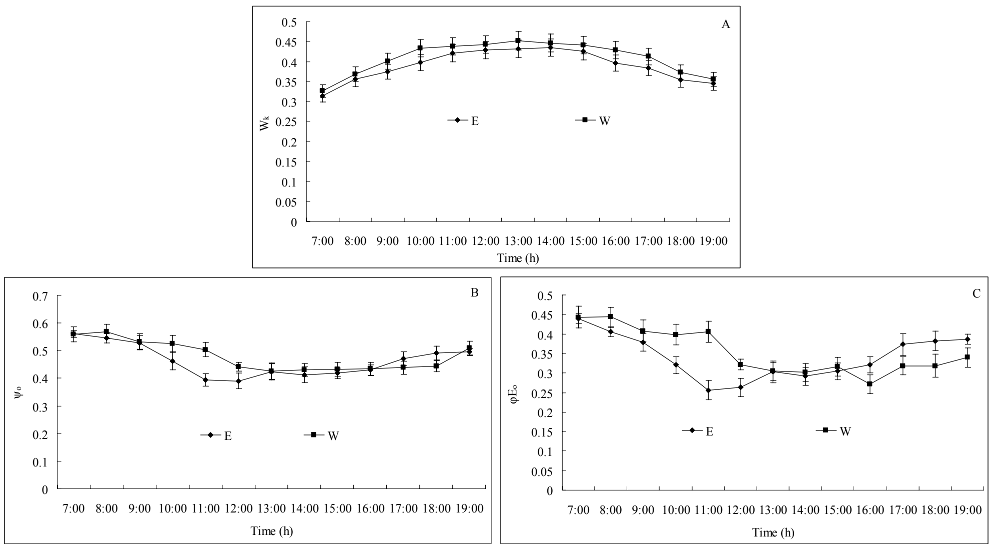

Fig. 3. Diurnal changes in ratio of variable fluorescence F_k to the amplitude F_J - Fo (W_k, A), efficiency that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_o, B) and probability that an absorbed photon will move an electron into the electron transport chain (φEo, C) in ginkgo leaves from the E or W. Each data is the average of 10 independent measurements.

With the diurnal variation of light intensity, fraction of active reaction centers per excited cross-section (RC/CS) first decreased and then increased, and lowest peak at 12: 00 and 15: 00, respectively (Fig. 4).

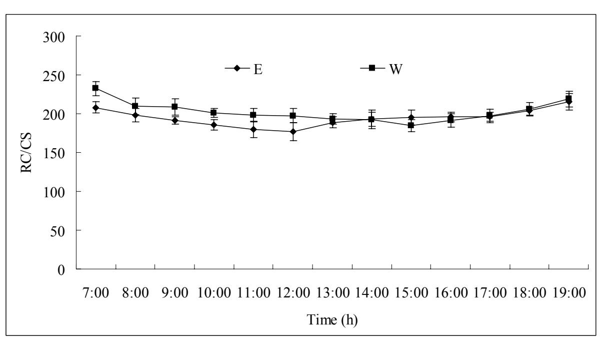

Fig. 4. Diurnal changes in fraction of active reaction centers per excited cross-section (RC/CS) in ginkgo leaves from the E or W. Each data is the average of 10 independent measurements.

The energy fluxes per active reaction centers for absorption (ABS/RC), trapping (TR_o/RC) and dissipation (DI_o/RC) in dark-adapted ginkgo leaves increased during the day and then decreased (Fig. 5). ABS/RC and TR_o/RC in the leaves from the E peak at 11: 00 while those from the W peak 14: 00 (Fig. 5 A, B). However, DI_o/RC in the leaves from the E peak at 12: 00 and that from the W peak at 16: 00 (Fig. 5C), these three parameters in these two group leaves peaked at different time, indicating that the extent of photoinhibition closely related to the quantities of absorption energy in leaves.

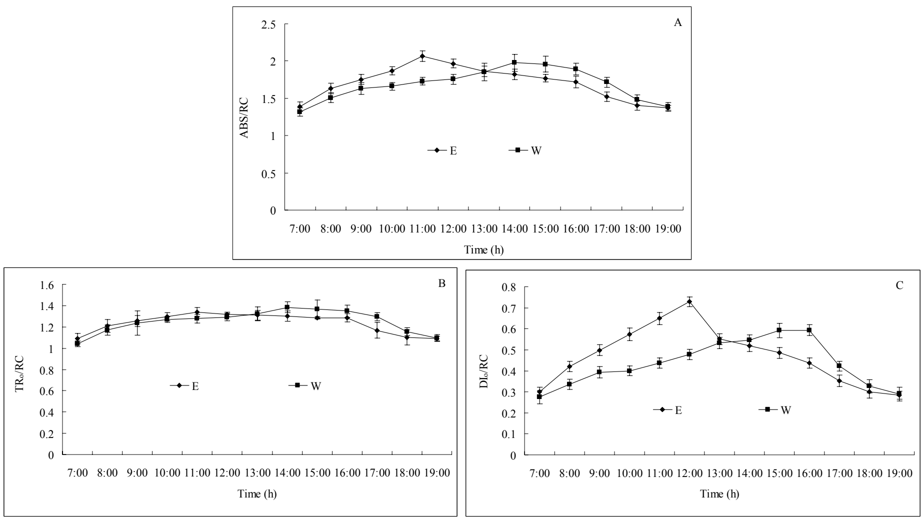

Fig. 5. Diurnal changes in energy fluxes per active reaction centers for absorption (ABS/RC A), trapping (TR_o/RC, B) and Dissipation (DI_o/RC, C) in ginkgo leaves from the E or W. Each data is the average of 10 independent measurements.

In summary, diurnal variations of chlorophyll *a* fluorescence transient kinetics in chloroplasts of ginkgo leaves in different directions were not the same on sunny day. PI_{abs} can be used as an index for high photosynthetic efficiency breeding in ginkgo. When studying diurnal variations of photoinhibition in plant leaves, much attention should be paid on the selection for growing direction of leaves.

Acknowledgements

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