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ORIGINAL RESEARCH

Biophysical Analysis Demonstrates Relationship Between Photosynthesis in Leaflets of *Azadirachta indica*

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ABSTRACT

Dynamics of photosynthesis in leaflets of *Azadirachta indica* was evaluated through the analysis of polyphasic chlorophyll fluorescence OJIP kinetics. In the present study, a gradual decrease in ABS/CS (absorbance per cross section), TR/CS (trapped energy per cross section) and ET/CS (electron transfer per cross section) was noted from bottom to apical leaflets. On the basis of results, it is hypothesized that fully developed leaflets act as 'source' and young leaflets as 'sink'. The study indicates that age and pattern of leaflet's arrangement influence photosynthesis in compound leaves of *A. indica*.

KEY WORDS: *Compound leaf, Leaflets, Photosynthesis, Chlorophyll fluorescence, JIP Test*

INTRODUCTION

The functional importance of leaf shape variation and evolution of compound leaves has been the subject of debate among botanists for many decades. Even within a single genus, variations in leaf shape and number of leaflets in compound leaves suggest that there is no one ecological strategy provokes leaf shape (Fig. 1). Current molecular evidence reveals that compound leaves are not dissections of simple leaves, but iterations of simple leaf lamina pieces (Efroni *et al.* 2010). Class I *KNOTTED-like* genes have been found to play an essential role in the development of compound leaves (Bharathan *et al.*, 2002). It has been suggested that compound leaves provide a number of physiological and mechanical direct advantages *i.e.* lower drag to intense winds, or more proficient heat dissipation abilities (Vogel 1989, Niinemets 1998). Variation in leaf structure and numbers of leaflets in compound leaves among the angiosperms has evolved during evolution as a

strategic morphological adaptation to use maximum resources for growth and development of plant species.

Measurements of chlorophyll fluorescence provide a non-invasive technique to monitor photosynthetic processes in plants, algae and cyanobacteria (Govindjee, 1995). The chlorophyll *a* fluorescence represents a very small fraction of the energy that is dissipated from the photosynthetic mechanism. It is known that the kinetics of fluorescence transients is polyphasic when plotted on a logarithmic time scale. Such plots clearly indicate the intermediate J and I steps between the initial step O and the maximum final level P (Strasser *et al.*, 1995). Recently, changes in the chlorophyll *a* fluorescence OJIP transient have been used to evaluate the performance of photosynthesis in plants. The JIP-test, proposed by Strasser and Strasser (1995), is used to translate the original measurements of fluorescence transient into various phenomenological and biophysical

expressions that quantify the function of PSII (Tóth *et al.*, 2007). It is based on the theory of energy flow in thylakoid membranes and enables an understanding of the relationships between the biophysical side of photosynthesis and various fluorescence parameters (Strasser and Strasser, 1995).

Leaf photosynthesis is affected by many plant factors such as leaf maturity level, leaf arrangement, sink effects, and mutual shading (Constable and Rawson, 1980; Lieth and Pasion, 1990). The present attempts were made to analyze photosynthesis in leaflets of *A. indica*. To the best of our knowledge, this is the first study which shows physiological relationship between leaflets of compound leaves. These results also describe the effect of position and age on leaflet's photosynthesis of field-grown *A. indica*.

RESULTS AND DISCUSSION

Leaflet's position and their maturity level play an important role in determining plant photosynthesis. In the present investigation, a gradual decrease in photosynthesis was noted from basal to apical leaflets *i.e.* bottom leaflets having highest level of photosynthesis while apical leaflets having

lowest. Upon illumination of dark-adapted compound leaves of *A. indica*, the chlorophyll *a* fluorescence emission exhibited a polyphasic rise from F_0 to F_m , via two intermediate steps (F_J and F_I) paralleling the closure of the reaction centers (Strasser *et al.*, 1995). Polyphasic chlorophyll fluorescence OJIP analysis showed a perfect physiological relationship between the leaflets of *A. indica*. Chlorophyll *a* fluorescence increased continuously from initial (F_0) to maximal (F_m) intensity in all leaflets.

Two intermediate peaks: F_J (chlorophyll fluorescence at 2 ms.) and F_I (chlorophyll fluorescence at 30 ms) were observed between F_0 and F_m forming a typical O-J-I-P curve (Fig. 2 a, b). All leaflets showed same value of initial fluorescence (F_0). Similar value of F_0 shows that light harvesting complexes in each leaflet are associated with reaction centers. On the other hand, a significant variation in F_m was observed between all leaflets. Higher F_m was observed in fully developed old leaflets. The decreased level of F_m in young leaflets indicates the presence of inactivated oxygen evolving complex (Yamashita and Butler, 1968; Schreiber and Neubauer, 1987).



Figure 1: Leaves of *A. indica* showing variations in number of leaflets.

A wide variation in specific and phenomenological fluxes of leaflets was observed in present investigations (Fig. 2 c). ABS/CS was found higher in mature leaflets in comparison to younger ones. It may be due to the higher concentration of light harvesting pigments in mature leaflets. Young leaflets showed low concentration of chlorophyll pigments as denoted in pipeline models (Fig. 3). Less TR/CS in comparison to mature leaflets was noted in developing leaflets. Reduced TR/CS denotes the presence of undeveloped light harvesting complexes in immature leaflets. A reduced rate of electron transportation per cross section

(ET/CS) was noticed in young leaflets as compared to mature leaflets. Reduction in ET/CS is correlated with decreased value of ABS/CS and TR/CS. Similar results were also observed in leaflets arranged at left side of rachis. Constable and Rawson (1980) reported that leaf's position and age affect net photosynthetic rate in plants. Leaf age and structure influence the number of mesophyll cells and subsequently influence photosynthesis (Araus *et al.*, 1997). Young expanding leaves are characterized by low efficiency of electron transport and CO_2 fixation process (Greer and Halligan, 2001).

Table1: Formulae and glossary of terms used by the JIP-test for the analysis of Chlorophyll a fluorescence transient OJIP emitted by dark-adapted photosynthetic samples

Data extracted from the recorded fluorescence transient O-J-I-P

| | |
|--------------------------|---|
| $F_0 \equiv F_{50\mu s}$ | minimal fluorescence, when all PS II reaction centers are open (at t=0) |
| $F_m = F_P$ | maximal fluorescence, when all PS II reaction centers are closed |
| $F_{100\mu s}$ | fluorescence at 100 μs |
| $F_{300\mu s}$ | fluorescence at 300 μs |
| $F_J \equiv F_{2ms}$ | fluorescence at the J-step (2 ms) of O-J-I-P |
| $F_I \equiv F_{30ms}$ | fluorescence at the I-step (30 ms) of O-J-I-P |
| $F_V \equiv F_m - F_0$ | maximal variable fluorescence |

Specific energy fluxes (per Q_A -reducing PSII reaction center - RC)

| | |
|---|---|
| $ABS/RC = M_0 \cdot (1/V_J) \cdot (1/\phi_{P_0})$ | absorption flux per reaction center |
| $TR/RC = M \cdot (1/V_J)$ | trapped energy flux per reaction center |
| $ET/RC = M \cdot (1/V_J) \cdot \psi_0$ | electron transport flux per reaction center |
| $DI/RC = (ABS/RC) - (TR/RC)$ | dissipated energy flux per reaction center |

Phenomenological energy fluxes (per excited cross section – CS)

| | |
|--|---|
| ABS/CS | absorption flux per cross section |
| $TR/CS = \phi_{P_0} \cdot (ABS/CS)$ | trapped energy flux per cross section |
| $ET/CS = \phi_{P_0} \cdot \psi_0 \cdot (ABS/CS)$ | electron transport flux per cross section |
| $DI/CS = (ABS/CS) - (TR/CS)$ | dissipated energy flux per cross section |

Density of reaction centers

| | |
|---|--|
| $RC/CS = \phi_{P_0} \cdot (V_J/M_0) \cdot ABS/CS$ | density of reaction centers (Q_A -reducing PSII reaction centers) |
|---|--|

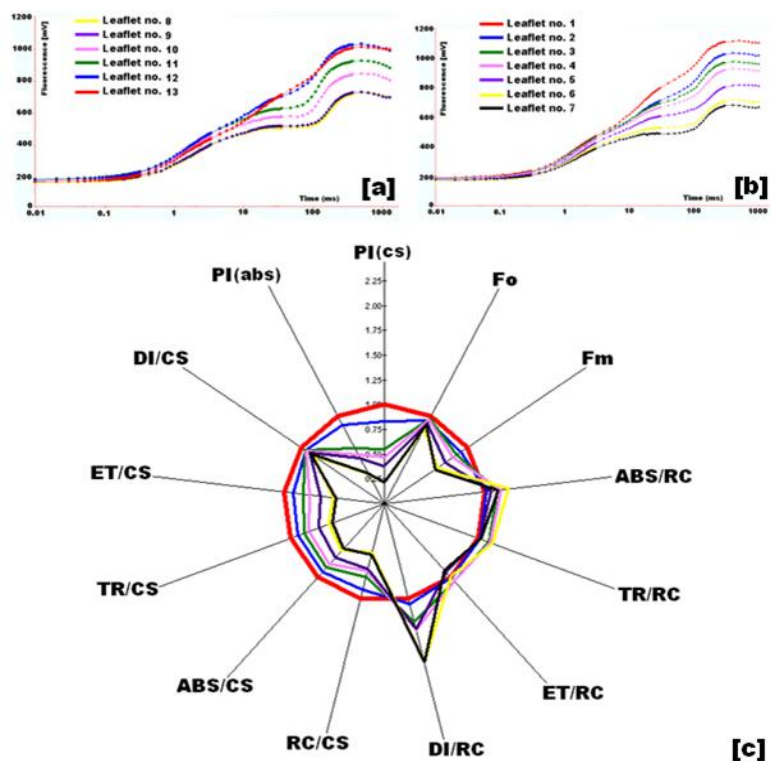


Figure 2: (a, b) Chlorophyll a fluorescence induction, (c) radar plot showing variations in various photosynthetic parameters of leaflets of *A. indica*.

In present study, low performance of photosynthesis was observed in young leaflets of *A. indica*. The density of active reactions (RC/CS) decreased gradually towards apical leaflets of right side. On the other hand, leaflets arranged at left side of rachis did not follow any uniform pattern regarding the concentration of active reaction centers. This unequal pattern of reaction centers may be due to the variation in solar irradiation and leaflet's position.

Photosynthetically, plant parts are divided into source and sink, sources being the parts where net CO₂ fixation occurs,

and sinks being the sites where carbohydrates are stored or utilized as source of energy. It is hypothesized that that mature leaflets act as source and new developing leaflets as 'sink'. Carbohydrates, synthesized into mature leaflets, are translocated to developing leaflets to act as source of energy for their development. The study clearly demonstrates that arrangement of leaflets and their age determine light absorption and thus influence photosynthesis in compound leaves of *A. indica*.

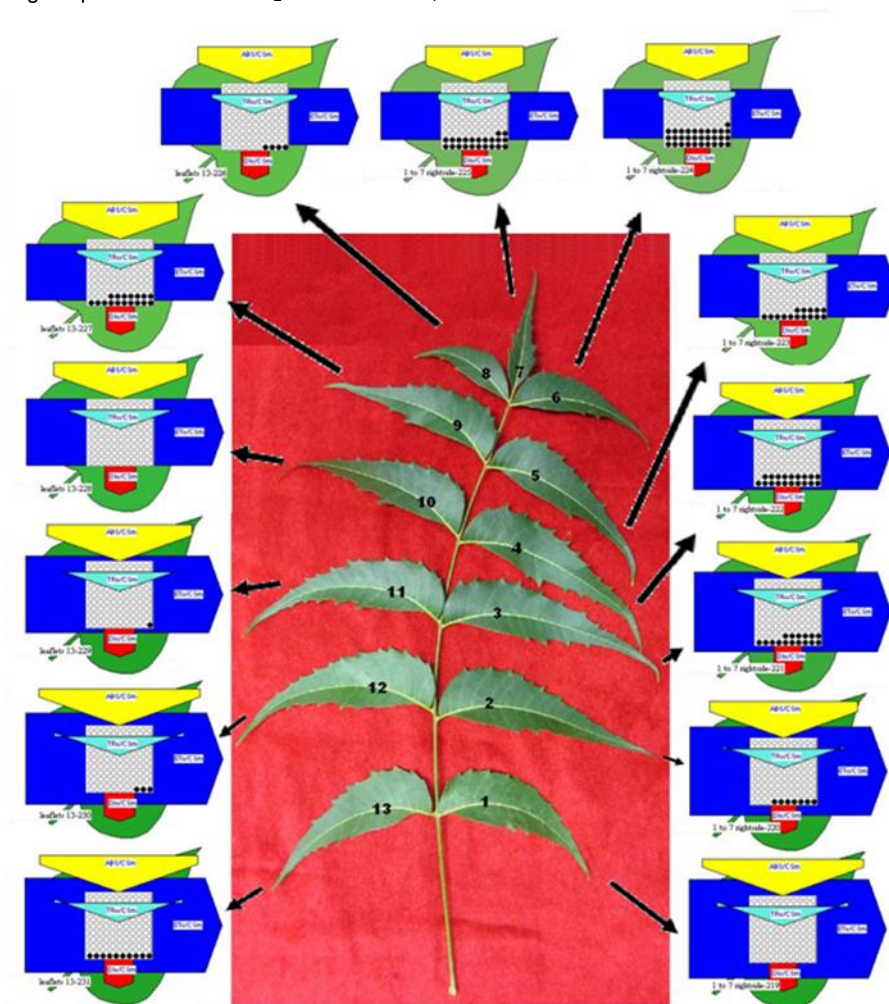


Figure 3: Models of photosynthetic process describing the rate of ABS/CS, TR/CS, ET/CS and DI/CS in leaflets of *A. indica* (block dots represent inactive PS II reaction centers).

MATERIALS AND METHODS

Plant materials and measurement of polyphasic chlorophyll fluorescence kinetics

Compound leaves were collected from healthy tree of *A. indica* growing at University College of Science Campus, Udaipur (India) and were kept in the dark for one hour. Chlorophyll *a* fluorescence

O-J-I-P transients were recorded in dark-adapted leaves with a Plant Efficiency Analyzer, PEA (Hansatech Instruments, Kings Lynn, Norfolk, U.K.). Fluorescence transients were induced over a leaf area of 4 mm diameter by a red light (peak at 650 nm) of 3000 μmolm⁻²s⁻¹ (sufficient excitation intensity to ensure closure of all PSII RCs to obtain a true fluorescence intensity of F_m) provided by a high

intensity LED array of three light emitting diodes. A total measuring time of one second was used throughout the experiments.

JIP test

The Chlorophyll a fluorescence transient O-J-I-P was analyzed according to the JIP- test (Strasser and Strasser, 1995). The fluorescence intensities determined at 50, 100 and 300 μ s ($F_{50\mu s}$, $F_{100\mu s}$ and $F_{300\mu s}$, respectively), 2 and 30 ms ($F_{2ms} = F_J$ and $F_{30ms} = F_I$) and at F_m (maximum fluorescence) were used to calculate the JIP-test parameters (Strasser and Strasser 1995). The intensity measured at 50 μ s was considered to be the initial fluorescence (F_0). The Chl a fluorescence transient was analyzed using the JIP-test using Biolyzer software (Laboratory of Bioenergetics, University of Geneva, Switzerland). The extracted and technical parameters, specific energy fluxes (per reaction center), phenomenological energy fluxes (per cross section), quantum efficiencies or flux ratios, density of reaction centers and performance indexes were calculated by using the equations of JIP- test (Table: 1).

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