

Full Length Research Paper

Changes in photosynthetic quantum yield of developing chloroplasts in lotus (*Nelumbo nucifera* Gaertn.) leaf during vegetative, bud and flowering stages

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Photosynthetic characteristics of the rotund or orbicular leaves of a monocarpic aquatic plant lotus (*Nelumbo nucifera* Gaertn.) leaves growing under direct sun light of tropics is studied and the developmental pattern of chloroplast along the foliage during vegetative, bud and flowering stages are studied by the measurement of the quantum efficiency of PS II photochemistry or photosynthetic quantum yield (F_v/F_m) through fast Chl *a* fluorescence measurement. The quantum efficiency of PS II photochemistry (F_v/F_m) increased in the middle portions of leaf compared to that of base but decreased in the apex (margins) of lotus leaf during vegetative stage. The plant growth (development), maturation and aging (senescence) stages are accomplished with vegetative, bud and flowering stages. The efficiency of PS II quantum yield (F_v/F_m) increased from base to apex during bud stage, but that decreased gradually during flowering stage. The changes in the F_v/F_m ratio with an increase in the ratio during bud (maturation stage) and a subsequent decrease at the aging (senescence stage) clearly demonstrates that the lotus leaf development takes place by the activity of the basal meristems leading to a well defined chloroplast developmental gradient along the leaf lamina. Also, this suggests the use of Chl *a* fluorescence kinetics for the developmental studies of chloroplast in aquatic plants.

Key words: Chl *a* fluorescence, *Nelumbo nucifera*, photosynthetic electron transport, primary photochemistry, photosystem II, photosynthetic quantum yield, F_v/F_m .

INTRODUCTION

The photosynthetic metabolism of hydrophytes, a particular ecological group of flowering plants, is the least investigated area of energy metabolism. The hydrophytes include a diversity of plant species with leaves submerged in water, floating on the surface and emergent above the water. The hydrophytic plants are divided into three groups, depending on the degree of their immersion in water. According to present accepted classification (Raspopov, 1978), the aquatic vascular plants comprise helophytes (plants having only emergent leaves), neustophytes (plants with floating leaves) and hydatophytes (plants with submerged leaves).

Previous studies revealed some features of the

photosynthetic apparatus in plants differing in the extent of their contact with watery environment. According to a preliminary study by Ronzhina and Pyankov (2001), the plants with different degrees of submergence show a large variety in their mesophyll structure and a wide range of variations in the quantitative parameters of the leaf chlorenchyma. Chloroplasts are present in the epidermis of all groups of aquatic plants. It was also found that heliophilous neustophytes possess both dorsoventral and homogeneous types of mesophyll. Besides these photosynthetic peculiarities, the aquatic angiosperms show other adaptive specializations starting from seed germination to post-flowering desiccation and decomposition under water.

Lotus is an anoxia tolerant plant. These plants have green shoots with plastids in the seeds (Ushimura et al., 2001). Chl development in lotus seeds and developing

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seedlings are reported to be both light independent (Ushimura et al., 2001, Ji et al., 2001) and light dependent pathway (Ji et al., 2001). Mature dry seeds of lotus have young chloroplasts and they give rise to mature chloroplasts during germination, even in the darkness (Ushimura et al., 2001). These shoots within the seeds contain chlorophyll and chl-binding proteins CP1 and LHCP. Both PS II and PS I could be formed in dark grown lotus seedlings (Ji et al., 2001). A clear cut Shibata shift in the fluorescence emission of PS II during chloroplast development of lotus seedlings even in complete darkness, with the presence of chl-protein complexes associated with PSI, especially LHCP1 is reported (Ji et al., 2001). The two photo systems (PS II and PS I) are shown to be photo chemically active and demonstrated by the electron transport rates of 10 day dark grown lotus seedlings. This is suggested by a significant adaptive feature of the lotus seedlings for their growth and development under the hypoxic conditions in water.

Seed germination takes place under water and the developing free floating leaves emerge from water in most aquatic plants. These free floating leaves play a significant role in the carbon acquisition of the plant (Woitke et al., 2004). It is the main carbon source for the growth and development of aquatic emergent or free floating plants. The free floating leaves are adapted to high photo flux densities of direct sun light and the submerged leaves are shade tolerant. The floating leaves undergo a seasonal development and aging (Heilmeyer and Hartung, 2001).

The floating leaves apparently contribute a large proportion to the total photosynthetic electron transport during the time when both the types of leaves are fully developed (Woitke et al., 2004). The higher photosynthetic efficiency of free floating leaves are due to their peculiar adaptive features viz; the presence of stomata on the upper surface which seem to be continuously open and higher light incidence on the leaf surface facilitating the utilization of CO₂ (Maberley and Madson, 2002). The electron transport rates of free floating leaves are also reported to be 3 - 4 times higher than the submerged leaves.

The developing leaves are submerged and also the free floating leaves lose the buoyancy after the flower or fruit maturation and sink gradually under water making the leaf to submerge. The characteristic peculiarity of free floating leaves, although studied, but the photosynthetic characteristic of the primary photo-chemistry of PS II is meager. In the present study, the free floating lotus leaf is used to elucidate the changes in the quantum efficiency of PS II photochemistry or photosynthetic quantum yield (F_v/F_m) through fast Chl *a* fluorescence measurement during (i) the development or vegetative, (ii) maturation or bud stage and (iii) aging or senescent stage or flowering stage to find out the process of chloroplast development in lotus leaves. Although there have been report about the development of dicot leaves by the activity of apical meristem and that of the ascicular leaves of monocot

plants by the basal meristems (Sinha, 1999), it is still not clear how the rotund leaves of aquatic angiosperms develop. In the present study we utilize the biophysical elucidation of the chloroplast developmental gradient along the foliage (Dilnawaz et al., 2001) to explain the pattern of leaf development in lotus leaves. It has been explained earlier that the function of the age and the developmental stage of the cells are reflected by the developmental status of the chloroplasts within these cells.

The older cells having mature chloroplasts and younger cells have developing chloroplasts, which can be characterized by biophysical, biochemical or molecular techniques (Dilnawaz et al., 2001). We use a fast Chl *a* fluorescence technique to characterize the developmental gradient along the orbicular or rotund leaves of lotus during vegetative, bud and flowering stages.

MATERIALS AND METHODS

Site description and plant material

Lotus (*Nelumbo nucifera* Gaertn.) plants growing in ponds were selected for the present experiment at the vegetative (no floral parts developing) stage, bud (flowers not open) stage and flowering (flowers in full bloom) stage. The sunlight intensity on the leaf surface was 1250 to 1425 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ during the experimental period starting from 6.30 to 7.30 h. However, the plants received sunlight intensities between 1175 to 1950 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ during 6.30 to 17.30 h with an average light period of 12.30 h for March to May. The air temperature was maximum 43°C and minimum 18°C during the experimental period. The water temperature in the pond was minimum 15°C in night to maximum 28°C in the mid-day. Only fully developed leaves were taken for the study. Photosynthetic fluorescence measurements were done inside the pond, without taking out the plants out of water, in order to avoid any problem of rapid dehydration or desiccation during dark adaptation. The leaves were clipped randomly at equal intervals starting from the base (center) to the apex (margin) of the orbicular foliage as shown in Figure 1.

Photosynthetic measurements

Photosynthetic measurements of lotus leaves were done by fast fluorescence kinetics at room temperature with a plant efficiency analyser (Handy-PEA, Hansatech Ltd., UK) according to Strasser et al. (1995). The leaf samples were dark adapted for 15 min inside the pond using the leaf clip before the fluorescence measurements. Dark-adapted leaves were illuminated homogeneously over an area of 4 mm diameter with an array of high intensity light-emitting diodes (λ_{max} 650 nm, 600 W m^{-2}) and fluorescence signals were detected using a PIN photodiode after passing through a long-pass filter (50% transmission at 720 nm). Fluorescence transients were recorded from 10 μsec to 1 sec with the data acquisition rate of 10 μsec for first 2 msec and then at the rate of 1 msec with a resolution of 12 bits. The fluorescence signal at 50 μsec after the onset of illumination was considered as F_0 (Strasser et al. 1995). A typical fluorescence curve of the lotus leaves is shown in Figure 2.

Theoretical considerations for the OJIP transients

The fast fluorescence kinetics of all oxygenic photosynthetic materials shows a transient fluorescence rise known as O, J, I and

NYMPHEA AND LOTUS

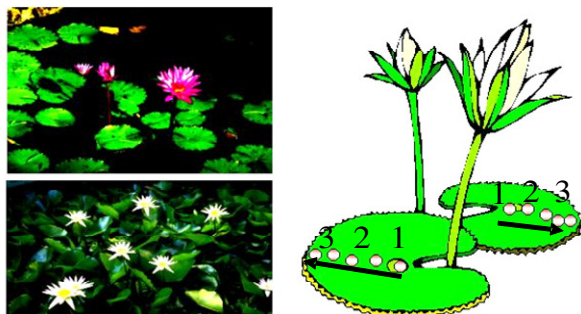


Figure 1. Picture of the lotus and water lily (nymphaea) plants with flowering. The schematic representation of the leaf sections (discs) of lotus taken for the present study. The basal section (#1) is taken at the adjoining portions where the leaf is subtended by the petiole. Then the sections proceeded at equal intervals from 1 - 3 (apex or margins) of the orbicular leaf.

Chlorophyll fluorescence curve of Lotus leaf

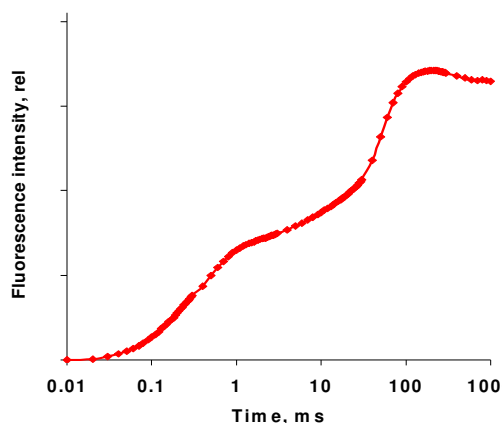


Figure 2. The polyphasic Chl *a* fluorescence induction curve of dark adapted lotus leaf.

P (Strasser and Strasser, 1995). The maximal fluorescence intensity F_P denotes the F_M values where all the reaction centres are physiologically closed. The F_o state indicates the physiological state when all the RCs are open. The flux ratios or yield ratios have been derived using the theory of energy fluxes in bio membranes (Sironval et al., 1981) and the experimental values of the OJIP-fluorescence transient values. The quantum efficiency of PS II photochemistry or photosynthetic quantum yield (F_v/F_M) through fast Chl *a* fluorescence measurement is derived according to the JIP-test (Strasser et al., 2004; Misra et al., 2001a, b).

RESULTS AND DISCUSSIONS

Photosynthetic parameters of lotus leaves are studied using fast Chl *a* fluorescence kinetics. The fast fluore-

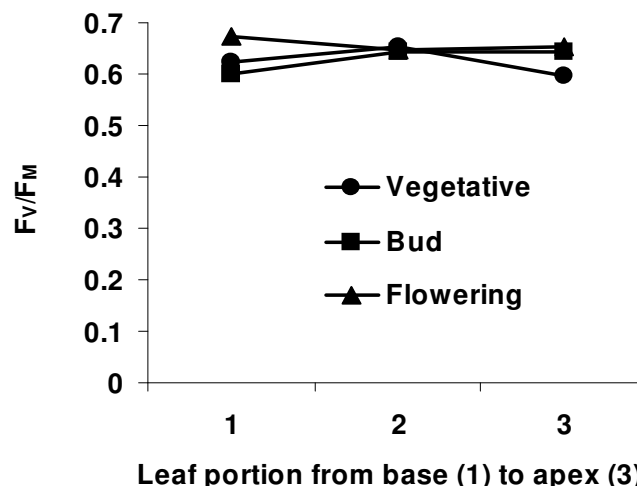


Figure 3. Changes in the quantum efficiency of PS II photochemistry or photosynthetic quantum yield (F_v/F_M) through fast Chl fluorescence measurement and calculated by OJIP curve analysis. The numbers in the X axis denote 1=base, 2=middle and 3= apex of the lotus leaf. Each data point is an average of 5 different plants.

science kinetics of all oxygenic photosynthetic materials shows a transient fluorescence rise known as O, J, I and P (Strasser and Strasser, 1995). The maximal fluorescence intensity F_P denotes the F_M values where all the reaction centres are closed. The F_o state indicates the physiological state when all the RCs are open. The fast Chl *a* fluorescence induction curve of lotus leaf is shown in Figure 2. The signals for OJIP originate at time intervals of 50 μ sec (O), 2 msec (J), 30 msec (I) and 500 msec (P).

The quantum efficiency of PS II photochemistry or photosynthetic quantum yield (F_v/F_M) through fast Chl *a* fluorescence measurement of lotus leaf showed a sequential developmental pattern during vegetative stage (Figure 3). The F_v/F_M value increased from base (segment 1 in Figure 3) to middle (segment 2 in Figure 3) but decreased subsequently in the apical segments (segment 3 in Figure 3). These changes show that the chloroplasts are developing from the basal meristematic zones of the lotus leaf, maturing at the middle portions and aging at the apical region.

This gradient is clearly demonstrated during the bud stage where the PS II quantum yield (F_v/F_M) increased gradually from base to apex. Similar changes in the photosynthetic parameters are reported in developing chloroplasts of wheat leaves, where the leaf development takes place by the activity of basal meristems (Dilnawaz et al., 2001). However, during flowering stage when the leaves start aging or senescing in monocarpic plants like wheat and aging is characterised by a gradual decrease in the photosynthetic efficiency along the chloroplast developmental gradient (Dilnawaz et al., 2001). In corollary, a sequential decrease in the PS II quantum yield

(F_v/F_m) of lotus from base to apex, clearly demonstrates the aging or senescence progression from base to apex of these leaves.

Conclusion

The quantum efficiency of PS II photochemistry or photosynthetic quantum yield (F_v/F_m) through fast Chl *a* fluorescence measurement showed a gradual change from base to apex and from vegetative to flowering stage (Figure 3). These changes suggest that there is a developmental gradient of chloroplasts in the lotus leaf. The chloroplasts in the apical sections are supposed to be relatively older as the photosynthetic efficiency decreased gradually in these sections from base to apex at flowering stage. As lotus plant is a monocarpic plant, the gradual decrease in the photosynthetic efficiency of the apical sections of the leaf with increasing plant age and flowering suggests aging phenomena of the chloroplasts as shown earlier by Dilnawaz et al. (2001) in wheat leaves. In corollary, these changes also suggest that the leaf development of lotus leaf is mostly through the basal meristematic activity of the basal meristems as reported in wheat (Dilnawaz et al., 2001). So it is concluded from this study that the rotund leaf development in lotus is through the basal meristematic activity giving rise to a distinct developmental gradient along the leaf lamina.

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REFERENCES

- Dilnawaz F, Mohapatra P, Misra M, Ramaswamy NK, Misra AN (2001). The distinctive pattern of photosystem 2 activity, photosynthetic pigment accumulation, and ribulose-1, 5-bisphosphate carboxylase/oxygenase content of chloroplasts along the axis of primary wheat leaf lamina. *Photosynthetica*, 39: 557-563.
- Heilmeyer H, Hartung W (2001). Survival strategies under extreme and complex environmental conditions: the aquatic resurrection plant *Chamaejasme intrepidus*. *Flora*, 196: 245-260.
- Ji HW, Tang CQ, Li LB, Kuang TY (2001). Photosystem development in dark grown lotus (*Nelumbo nucifera*) seedlings. *Acta Botanica Sinica*, 43: 1129-1133.
- Maberley SC, Madsen TV (2002). Fresh water angiosperm carbon concentrating mechanism: processes and patterns. *Funct. Plant. Biol.* 29: 393-405.
- Misra AN, Srivastava A, Strasser RJ (2001a). Utilization of fast chlorophyll *a* fluorescence technique in assessing the salt/ion sensitivity of mung bean and *Brassica* seedlings. *J. Plant. Physiol.*, 158: 1173-1181.
- Misra AN, Srivastava A, Strasser RJ (2001b). Fast chlorophyll *a* fluorescence kinetic analysis for the assessment of temperature and light effects: A dynamic model for stress recovery phenomena. In: *Photosynthesis*, S3-007. CSIRO Publ., Victoria, Australia.
- Raspopov IM (1978). Some Concepts of Hydrobotany. *Gidrobiol.Zh.* 14: 20-26.
- Ronzhina DA, Pyankov VI (2001). Structure of the Photosynthetic Apparatus in Leaves of Freshwater Hydrophytes: 2. Quantitative Characterization of Leaf Mesophyll and the Functional Activity of Leaves with Different Degrees of Submersion. *Russian J. Plant. Physiol.*, 48: 723-732.
- Sinha N (1999). Leaf development in angiosperms. *Annu. Rev.Plant. Physiol. Plant Mol. Biol.*, 50: 419-446.
- Sironval C, Strasser RJ, Brouers M (1981). Equivalence entre la theorie des flux et la theorie des relations entre proportions de pigments pour la description de la repartition de l'energie lumineuse absorbee par les membranes photoactives. *Bull. Acad. R. Belg.*, 67: 248-259.
- Strasser RJ, Srivastava A, Govindjee (1995). Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. *Photochem. Photobiol.*, 61: 32-42.
- Strasser BJ, Strasser RJ (1995). Measuring fast fluorescence transients to address environmental questions: The JIP test. In: Mathis, P. (Ed.) *Photosynthesis: from light to Biosphere*. Kluwer V, Acad. Publ., Dordrecht, The Netherlands, pp. 977-980.
- Strasser RJ, Tsimilli-Michael M, Srivastava A (2004). Analysis of the Fluorescence Transient. In: George C, Papageorgiou GC, Govindjee (Eds.) *Chlorophyll fluorescence: A signature of photosynthesis*. Ch. 13. *Advances in Photosynthesis and Respiration Series*. Kluwer Academic Publ., Dordrecht, Netherlands.
- Ushimaru T, Hasegawa T, Amame T, Katayama M, Tanaka S, Tsuji H (2003). Chloroplasts in seeds and dark grown seedlings of lotus. *J.Plant. Physiol.*, 160: 321-324.
- Woitke M, Hartung W, Gimmmler H, Heilmeyer H (2004). Chlorophyll fluorescence of submerged and floating leaves of the aquatic resurrection plant *Chamaejasme intrepidus*. *Funct. Plant. Biol.*, 31: 53-62.