BIOLOGIA PLANTARUM 53 (1): 167-170, 2009

BRIEF COMMUNICATION

Diurnal changes in chlorophyll fluorescence and light utilization in *Colocasia esculenta* leaves grown in marshy waterlogged area

S. ROY CHOWDHURY*, ASHWANI KUMAR and N. SAHOO

Water Technology Center for Eastern Region, Indian Council of Agricultural Research, Bhubaneswar-751023, India

Abstract

Diurnal cycle of chlorophyll fluorescence parameters was done in *Colocasia esculenta* L. (swamp taro) grown in marshy land under sun or under shade. The sun leaves maintained higher electron transport rate (ETR) and steady state to initial fluorescence ratio (F_s/F_0) than shade leaves. In spite of lower ETR, higher photochemical quenching (PQ), and effective quantum yield of photosystem 2 (Φ_{PS2}) was evident in shade plants compared to plants exposed to higher irradiance. ETR increased linearly with increase in irradiance more under low irradiance ($r^2 = 0.84$) compared to higher irradiance ($r^2 = 0.62$). The maximum quantum yield of PS 2 (F_v/F_m) did not differ much in sun and shade leaves with the exception of midday when excess of light energy absorbed by plants under sun was thermally dissipated. Hence swamp taro plants adopted different strategies to utilize radiation under different irradiances. At higher irradiance, there was faster decline in proportion of open PS 2 centers (PQ) and excess light energy was dissipated through non-photochemical quenching (NPQ). Under shade, absorbed energy was effectively utilized resulting in higher Φ_{PS2}.

Additional key words: electron transport rate, non-photochemical quenching, photochemical quenching, photosystem 2.

Lower threshold of irradiance saturation of photosynthesis is of particular significance for given type of crop where entire plant part is consumed and total biomass productivity of the crop is directly proportional to sunlight received (Ort and Baker 1988). In swamp taro (*Colocasia esculenta* L.*)* every part of the plant (corms, leaves and even petioles) are consumed as vegetable. The crop normally grows in marshy waterlogged area during monsoon. Investigations on diurnal oscillation of photosynthetic parameters help in understanding plant short term adaptive strategies to maintain its growth under changing environmental conditions (Koehn and Doudrick 1999). Our previous gas exchange studies suggested that optimum rate of photosynthesis in swamp taro leaves is attained at much lower photosynthetically active radiation (PPFD) than available during its growth period (Roy Chowdhury *et al.* 2004). This finding suggested the possibility of absorption of excess light energy by the crop that might lead to photo-damage of photosynthetic apparatus. Chlorophyll fluorescence analysis helps in characterization of light utilization under stress conditions (Flexas *et al.* 2002, Kitao *et al.* 2003). However, changes in chlorophyll fluorescence at different levels of irradiance under non-limiting soil moisture warrant further investigation. In this communication we analysed the chlorophyll fluorescence characteristics in swamp taro sun and shade leaves in a diurnal cycle to understand mode of utilization of light by the crop grown under sufficient soil moisture.

The experiment was conducted at Water Technology Centre for Eastern Region, Indian Council of Agricultural Research, in October 2003 during a sunny day after cessation of monsoon. The swamp taro (*Colocasia esculenta* L.) cv. BCST 15 was planted at a spacing of 0.6 × 0.75 m in January. The saturation of the soil with water was ensured by irrigation during drought period (May - June). The crop was grown in low lying marshy area and throughout the growth period swampy conditions were

---

Received 22 December 2007, *accepted* 15 November 2008.

Abbreviations: ETR - electron transport rate; F_0 - initial fluorescence of dark adapted leaves; F_0'- minimum fluorescence of light adapted leaves; F_m - maximum fluorescence of dark adapted leaves; F_m' - maximum fluorescence of light adapted leaves; F_s - fluorescence yield at steady state; F_v - variable fluorescence ($F_v = F_m - F_0$); NPQ - non photochemical quenching of chlorophyll fluorescence; PPFD - photosynthetically active radiation; PQ - photochemical quenching of chlorophyll fluorescence.

Acknowledgements: Authors are thankful to director of WTCER for providing research facilities. The contribution of anonymous reviewers is also sincerely acknowledged for improvement of the manuscript.

* Corresponding author; e-mail: somnath_rc@yahoo.com

maintained in the experimental field. In another part of the field the same cultivar was grown under natural shaded habitat. The farm yard manure was added at the time of planting and N:P:K fertilizers were applied in three doses at 2nd, 5th and 8th month after planting. Diurnal variation in chlorophyll fluorescence was measured during peak growing period (at 225th day after planting) from 07:00 till 17:00 with a pre-programmed modular chlorophyll fluorescence monitoring system (FMS 2, *Hansatech Instruments*, Norfolk, UK). The measurements were taken from leaf lamina avoiding midrib region and major veins from 3rd mature leaf of three randomly selected plants. The chlorophyll fluorescence parameters like minimum fluorescence of dark adapted leaves (F_0) and steady state fluorescence at light adapted leaves (F_s) were monitored at regular intervals. To minimise the inherent differences because of variation in chlorophyll content, structure of leaves, *etc*., the F_s values were normalized to corresponding F_0 values (Flexas *et al.* 2002). The maximum fluorescence at light adapted state (F_m’) and F_s were measured after saturating light pulse (PPFD of 8000 μmol m^{-2} s^{-1} for 1.0 s). They were used to calculate effective quantum yield of PS 2 ($\Phi_{PS2} = (F_m' - F_s)/F_m'$; Genty *et al.* 1989). The electron transport rate was derived as ETR = PPFD × 0.5 × Φ_{PS2} × 0.84 (*Hansatech* user manual). Leaf clip supplied by manufacturer was used for dark adaptation (60 min). The data of PPFD on the leaf surface was also monitored at regular intervals. The initial fluorescence in light adapted leaves was calculated as $F_0' = F_0/[(F_m - F_0/F_m) + F_0/F_m']$ (Oxborough and Baker 1997). This value was subsequently used to calculate photochemical quenching as $PQ = (F_m' - F_s)/(F_m' - F_0')$. The maximum quantum yield of PS 2 was estimated from variable (F_v) to maximum fluorescence (F_m) ratio, $F_v/F_m = (F_m - F_0)/F_m$ (Kitajima and Butler 1975). The non-photochemical quenching was calculated as $NPQ = F_m/F_m' - 1$ (Bilger and Björkman 1990, Björkman and Demmig-Adams 1994).

Leaf water potential was measured by sampling 3rd mature leaf of the three randomly selected plants at regular interval with the help of a pressure chamber (*PMS Instruments*, Corvallis, USA). The petiole of the selected leaf was cut with a sharp razor without damaging tissue and within 10 s inserted into the seal in the top of the chamber. The leaf water potential was determined following Boyer (1995). The data were statistically analyzed following Gomez and Gomez (1984).

The PPFD showed a typical bell shaped curve during the entire day in the field without shade as there was no patchy cloud over the entire period of the experiment (Table 1). However, plants which were grown under shade were under distinctly lower PPFD throughout the whole period of the day (Table 1). The leaf water potential of sun and shade leaves did not vary significantly during initial hours of the day up to 09:48 and again during later part of the day from 14:28 onwards. During the midday period, the leaves of sunlit plants, even though grown under saturated soil moisture, showed lower leaf water potential compared to shade grown plants (Table 1). The chlorophyll fluorescence technique provides a rapid, non-invasive tool for assessing photosynthetic performance. But in the field several discrepancies are likely to exist due to heterogeneity among samples (Maxwell and Johnson 2000). To minimize the differences the F_s values (Fs) were normalised to that of F_0 (Flexas *et al.* 2002). The normalized F_s/F_0 of sun leaves of swamp taro showed consistently higher values than those of plants grown under shade except for last part of the day when trend became reverse. This clearly indicates the fact that F_s/F_0 increased with increased PPFD (Table 1).

ETR increased steadily with increase in PPFD during the day in both sun and shade leaves and remained conspicuously lower in shade leaves compared to sun leaves. The midday drop in ETR in shade leaves was evident around 11:30, thereafter both sun and shade leaves showed a peak at 13:30 and then a decline (Table 1). Water deficit stress has been found to stimulate partitioning of electron flow to pathways other than CO_2 assimilation (Park *et al.* 1996, Kitao *et al.* 2003) compared to well watered plants. So in this experiment under well watered condition steady increase in ETR might favour higher CO_2 fixation especially from 08:00 to 14:00, the main period for photosynthesis in swamp taro leaves (Roy Chowdhury *et al.* 2004).

The Φ_{PS2} during initial period of the day was significantly higher in shade leaves compared to sun leaves, particularly till midday. Thereafter both leaves showed a decline and did not vary significantly from one another for rest of the day except for the very last part (Table 1). Thus even though shade plants showed lower ETR level, their photochemical efficiency was better.

The normalized F_s values of shade plants were consistently lower at any given point of observation even during the period of the day when shade plants had higher leaf water potential compared to sun leaves. In grape vine (Flexas *et al.* 1999, 2002) there was a decrease in normalised values of F_s with decrease in leaf water potential from -0.3 to -1.4 MPa. The decrease in F_s under lower leaf water potential was also observed in other plant species (Cornic 1994). In our study normalized F_s values consistently remained higher in sun leaves despite slightly lower leaf water potential (-0.23 MPa compared to -0.17 MPa in shade leaves). The F_m’ did not vary significantly during the day between sun and shade leaves (Table 1). During first half of the day the F_m’ values of sun and shade leaves were higher than those observed at later half of the day. But in general, sun leaves maintained comparatively higher F_m’ values than shade leaves, during major part of the observation period (Table 1).

The F_v/F_m values, which indicated maximum quantum yield of PS 2 remained near 0.80 with exception of midday period showing NPQ (Table 1). Hence it was apparent that sun leaves did not suffer any damage to reaction centres of PS 2 (photoinhibition) in spite of

Table 1. The changes in photosynthetically active radiation, PPFD, [μmol m^{-2} s^{-1})], leaf water potential [-MPa], steady state fluorescence normalised to basal dark adapted fluorescence (F_s/F_0) , electron transport rate (ETR), effective quantum yield (Φ_{PS2}), light adapted fluorescence maximum (F_m'), maximum quantum yield of open PS 2 of dark adapted leaves (F_v/F_m), photochemical (PQ) and non-photochemical (NPQ) quenchings over entire period of the day in the field where swamp taro were grown in sun (SU) or shade (SH). Each value is mean of three replications ± SE.

		07:22	08:18	09:48	10:37	11:28	12:21	13:22	14:28	15:28	16:45
PPFD	SU	274±16	929±14	1263±22	1310±121	1459±13	1473±54	1402±100	1367±28	1082±35	373±10
	SH	124±9	129±5	198±6	211±23	120±12	820±177	890±128	412±2	315±27	126±17
LWP	SU	0.08±0.002	0.10±0.005	0.13±0.027	0.15±0.003	0.19±0.005	0.23±0.003	0.24±0.009	0.21±0.01	0.20±0.02	0.18±0.01
	SH	0.10±0.005	0.12±0.005	0.14±0.008	0.14±0.004	0.15±0.004	0.18±0.005	0.19±0.005	0.20±0.003	0.18±0.003	0.16±0.009
F_s/F_0	SU	3.61±0.21	4.20±0.02	4.67±0.17	2.49±0.37	3.14±0.82	2.47±0.54	2.93±0.21	3.40±0.85	2.83±0.47	2.65±0.30
	SH	1.81±0.01	1.53±0.03	1.69±0.01	1.85±0.08	2.44±0.16	1.61±0.42	2.29±0.17	2.45±0.42	2.83±0.47	4.48±0.12
ETR	SU	71.8±1.5	229.4±6.9	226.8±6.6	224.0±50.0	304.0±38.7	269.1±50.2	303.9±39.0	190.5±43.8	81.1±39.3	108.4±7.2
	SH	39.5±2.6	40.9±1.8	95.8±0.9	61.9±5.7	34.3± 3.5	167.7±55.0	192.6±33.6	141.3±18.3	85.2± 3.8	52.2±8.8
Φ_{PS2}	SU	0.63±0.02	0.58±0.009	0.42±0.021	0.39±0.067	0.49±0.060	0.38±0.114	0.52±0.07	0.46±0.125	0.55±0.08	0.74±0.002
	SH	0.62±0.017	0.64±0.005	0.65±0.011	0.70±0.015	0.67±0.017	0.45±0.053	0.50±0.015	0.43±0.006	0.56±0.038	0.50±0.033
F_m'	SU	3792±143	4094±693	3279±60	1468±342	2503±488	1623±287	2476±564	2395±522	2576±119	3189±360
	SH	3286±96	3855±82	2426±170	2247±46	3062±337	1177±246	1919±176	1652±302	1684±333	3434±170
F_v/F_m	SU	0.86±0.000	0.85±0.000	0.84±0.000	0.68±0.040	0.71±0.070	0.73±0.020	0.68±0.160	0.82±0.009	0.81±0.011	0.79±0.037
	SH	0.82±0.007	0.78±0.002	0.79±0.003	0.79±0.009	0.80±0.008	0.81±0.020	0.64±0.070	0.63±0.600	0.80±0.009	0.72±0.085
PQ	SU	0.63±0.025	0.58±0.099	0.42±0.029	0.39±0.067	0.49±0.060	0.38±0.114	0.52±0.073	0.45±0.126	0.55±0.082	0.74±0.003
	SH	0.79±0.007	0.81±0.005	0.69±0.023	0.70±0.015	0.68±0.015	0.45±0.053	0.50±0.015	0.43±0.007	0.56±0.038	0.49±0.035
NPQ	SU		-	-	0.53±0.14	0.28±0.10	0.70±0.25	0.20±0.08	-	-	-
	SH	-	-	-	-	-	1.00±0.40	0.26±0.07	0.61±0.17	-	-

slightly decreased water status of the leaves. Verhoeven *et al.* (2005) noted that predawn F_v/F_m of evergreen tree*Taxus × media* was near 0.80 and declined during the day in winter. Duan *et al.* (2005) also noted that predawn F_v/F_m of sun and shade leaves did not differ significantly in *Picea asperata,* while in midday values declined less in shade leaves. In addition, the dry climate population under showed higher midday F_v/F_m and NPQ compared to wet climate population (Duan *et al.* 2005). In tall fescue cultivars N fertilization reduced decline in stomatal conductance, net photosynthetic rate (P_N) and F_v/F_m ratio induced by heat stress (Zhao *et al.* 2007). In ozone treated tomato leaves decline in P_N was more due to closed stomata than low F_v/F_m ratio (Degl'Innocenti *et al.* 2007). In our study, rather higher Φ_{PS2} was noted in shade than in sun leaves (Table 1). This suggests that shade leaves utilised its maximum quantum yield potential more effectively than sun leaves. At the beginning of the day higher F_v/F_m of sun leaves followed by a decline between 09:48 and 10:37 was, however, not reflected in Φ_{PS2} of sun leaves. Sun leaves showed a continuous decline in Φ_{PS2} till 10:37 and then mostly showed non-significant difference with that of shade leaves. On the other hand, effective quantum yield continuously increased in shade leaves from beginning of the day to 12:30 (Table 1). The photo-damage of photosynthetic apparatus due to over excitation is effectively controlled by cyclic electron transport, photorespiration and Mehler reaction (Biehler and Fock 1996, Park *et al.* 1996, Baroli and Melis 1998). But Mehler reaction does not seem to be dominant when plants are grown under sufficient soil moisture. Our previous gas exchange studies revealed optimum photosynthetic efficiency at PPFD 1190 μmol m^{-2} s^{-1} with two peaks of P_N (09:00 - 11:00 and 15:30 - 16:00) under field condition The substomatal concentration of CO_2 remained fairly constant throughout the day suggesting that CO_2 fixation was not limited by stomatal opening (Roy Chowdhury *et al*. 2004).

The excess of energy was rather dissipated as a heat in correspondence with measurable NPQ during first half of the day in sun leaves and during second half of the day in shade leaves. The photochemical quenching, which states the ratio of open to total PS 2 centres (Schreiber *et al.* 1994, Govindjee 1995) was distinctly higher in shade leaves (0.7 - 0.8) compared to sun leaves (0.5 - 0.6) till 13:20. During initial part of the day, shade leaves maintained constant level of PQ whereas it declined in sun leaves under sharp increase in PPFD from 300 to 1300 μmol m^{-2} s^{-1} but after 13:20 the values became similar. The higher F_s/F_0 ratio observed in sun leaves could be explained by reduced PQ. This resulted in partitioning more energy to fluorescence. Similarly, shade leaves, which showed higher PQ, had greater proportion of open PS 2 centres, which allowed partitioning of absorbed energy more through photochemical reactions resulting in higher Φ_{PS2}.

Thus sun and shade grown leaves of swamp taro adopted different strategies to utilize radiation under field condition. The shaded leaves maintained higher leaf water potential, showed better photochemical efficiency, greater proportion of open PS 2 centres and maintained steady increase of ETR with increase in PPFD. The higher NPQ in sun leaves suggested lower photochemical efficiency but in turn helped in protecting the photosystems from damage due to excess energy.

References

Baroli, I., Melis, A.: Photoinhibitory damage is modulated by the rate of photosynthesis and by the photosystem II light-harvesting chlorophyll antenna size. - Planta **205**: 288-296, 1998.

Biehler, K., Fock, H.: Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. - Plant Physiol. **112**: 265-272, 1996.

Bilger, W., Björkman, O.: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. - Photosynth. Res. **25**: 173-185, 1990.

Björkman, O., Demmig-Adams, B.: Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. - In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp 17-47. Springer-Verlag, Berlin 1994.

Boyer, J.S.: Measuring the Water Status of Plants and Soils. - Academic Press, New York 1995.

Cornic, G.: Drought stress and high light effects on leaf photosynthesis. - In: Baker, N.R., Bowyer, J.R (ed.): Photoinhibition of Photosynthesis: from Molecular Mechanisms to the Field. Pp. 297-313. BIOS Scientific Publishers, Oxford 1994.

Degl'Innocenti, E., Guidi, L., Soldatini, G.: Effects of elevated ozone on chlorophyll *a* fluorescence in symptomatic and asymptomatic leaves of two tomato genotypes. - Biol. Plant. **51**: 313-321, 2007.

Duan, B., Lu, Y, Yin, C., Junttila, O., Li, C.: Physiological responses to drought and shade in two contrasting *Picea asperata* populations. - Physiol. Plant. **124**: 476-484, 2005.

Flexas, J., Badger, M., Chow, W.S., Medrano, H., Osmond, C.B.: Analysis of the relative increase in photosynthetic CO_2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress. - Plant Physiol. **121**: 675-684, 1999.

Flexas, J., Escalona, J.M., Evain, S., Gulias, J., Moya, I., Osmond, C.B., Medrano, H.: Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO_2 assimilation and stomatal conductance during water-stress in C_3 plants. - Physiol. Plant. **114**: 231-240, 2002.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta **990**: 87-92, 1989.

Gomez, K.A., Gomez, A.A.: Statistical Procedures for Agricultural Workers. - John Willey & Sons, New York 1984.

Govindjee: Sixty three years since Kautsky: chlorophyll *a* fluorescence. - Aust. J. Plant Physiol. **22**: 131-160, 1995.

Kitajima, M., Butler, W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. - Biochim. biophys. Acta **376**: 105-115, 1975.

Kitao, M., Lei, T.T., Koike, T., Tobita, H, Maruyama, Y.: Higher electron transport rate observed at low intercellular CO_2 concentration in long-term drought-acclimated leaves of Japanese mountain birch (*Betula ermanii*). - Physiol. Plant. **118**: 406-413, 2003.

Koehn, A.C., Doudrock, R.L.: Diurnal pattern of chlorophyll fluorescence and CO_2 fixation in orchard grown *Torreya taxifolia* (Arn.). - J. Torrey bot. Soc. **126**: 93-98, 1999.

Maxwell, K, Johnson, G.N.: Chlorophyll fluorescence: a practical guide. - J. exp. Bot. **51**: 659-668, 2000.

Ort, D.R., Baker, N.R.: Consideration of photosynthetic efficiency at low light as a major determinant of crop photosynthetic performance. - Plant Physiol. Biochem. **26**: 555-565, 1988.

Oxborough, K., Baker, N.R.: Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non photochemical components – calculation of qP and Fv'/Fm' without measuring Fo'. - Photosynth. Res. **54**: 135-142, 1997.

Park, Y.-I., Chow, W.S., Osmond, C.B., Anderson, J.M.: Electron transport to oxygen mitigates against the photoinactivation of photosystem II *in vivo*. - Photosynth. Res. **50**: 23-32, 1996.

Roy Chowdhury, S., Kannan, K., Sahoo, N. Verma, H.N.: Environmental control of diurnal variation in photosynthesis in swamp taro leaves, *Colocasia esculanta* (L.) Schott. under waterlogged conditions. - Aroideana **27**: 190-197, 2004.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. - In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology for Photosynthesis. Pp. 49-70. Springer-Verlag, Berlin 1994.

Verhoeven, A.S., Swanberg, A., Thao, M., Whiteman, J.: Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in *Taxus* × *media* growing in sun and shade environments. - Physiol. Plant. **123**: 428-434, 2005.

Zhao, W.Y., Xu, S., Li, J.L., Cui, L.J., Chen, Y.N., Wang, J.Z.: Effects of foliar application of nitrogen on the photosynthetic performance and growth of two fescue cultivars under heat stress. - Biol. Plant. **52**: 113-116, 2008.