Species Specific Changes in Chlorophyll Fluorescence in Deciduous and Evergreen Tree Species Growing Under Short Rotation High Density Energy Plantation in North-Western Himalaya

Gopichand
1. CSIR-Institute of Himalayan Bioresource Technology Palampur (H.P) 176061
   [E-mail: gc57@rediffmail.com]

Diurnal changes in chlorophyll (chl) a fluorescence characteristics of Sun exposed and shaded leaves of some deciduous and evergreen tree species growing under short rotation high density energy plantation in north-western Himalaya, during sunny and cloudy days were recorded. Significant reduction in photochemical efficiency of photosystem (PS) 2 measured as Fv/Fm ratio during period of maximum irradiance was observed in Sun exposed leaves of some tree species. The extent of this reduction was actually a function of various plant processes. The observations were recorded in 3 years for different selected months and time. The maximum photoinhibition was recorded in between 12.00 noon to 14.00 hrs. Among the deciduous species, Salix tetrasperma showed the maximum reduction (46%), whereas Grevillea robusta showed least decline (< 31%) in the Fv/Fm ratio in 1994. In 1995, some species showed maximum reduction 60%, and other species like Trewia nudiflora, Toona ciliata, Morus alba and Melia azedarach showed least decline (15 to 19%) in the Fv/Fm ratio. In 1995, Salix tetrasperma at 14.00 hr. showed maximum reduction (34%), while at 9.00 hr, 11.00 hr and 16.00 hr it showed least decline in the Fv/Fm ratio. viz, Salix tetrasperma and Eucalyptus hybrid. Some species as Salix tetrasperma were most susceptible to high irradiances. Prolonged exposure of these plants to high irradiances resulted in lesion development on leaves characterized by reduction in pigment content and Fv/Fm ratio. Plant species with considerable midday decline in Fv/Fm ratio showed (i) slight but significant rise in initial fluorescence (Fo), (ii) pronounced decrease in Fm value, and (iii) significant reduction in area over the curve between Fo and Fm indicating the pool size of electron acceptors on the reducing side of PS2. The depression in Fv/Fm ratio though reversible could not be alleviated even after watering the plants. No reduction in Fv/Fm was observed in shade leaves of the same plants and/or when measurement were taken on cloudy days and morning (9.00 and 11.00 hr), and evening (14.00 hrs and 16.00 hr) hours. Hence high solar radiation and high atmospheric evaporative demand existing during summer/draught period could probably be the major contributory factor for this reduction.

Keyword: Salix tetrasperma, Eucalyptus, Grevillea Robusta, Toona Ciliate, Trewia Nudiflora, Morus Alba, Melia Azedarach, Populus Deltoids.

1. Introduction
It has been established that the photo-inhibition of PS II depends on the light dose (photon exposure in units mol photon m⁻², [8]), and that under a given set of conditions, combination of irradiance and duration of illumination that give the some photon exposure will result in an equal extent of photo inactivation of PS II [20, 38].

The reduction in photosynthetic capacity (photoinhibition) with a pronounced decline at midday (midday depression) has been investigated in plants under field conditions during summer [14, 22, 24, 26, 33, 39, 41]. It is manifested as reduction in the light-limited and light-saturated CO₂ assimilation, in optimal photon yield and photochemical efficiency [4, 14, 22]. Different views exist on the significance of
Photoinhibition \cite{25, 33} that limits plant productivity \cite{34}. Therefore, selection of tree species not showing and/or showing minimum midday depression may be of an advantage for biomass production.

There are a growing number of reports demonstrating the occurrence of photoinhibition under natural conditions. Natural light levels may be high enough to cause photoinhibition when plants are concomitantly exposed to environmental stress, such as high \cite{6, 17, 28} and low temperature \cite{6, 17, 28}, and insufficient supply of water \cite{10}. Recently, data have also appeared supporting the view that photoinhibition is not confined to condition of stress but occurs more frequently. A depression in the efficiency of PS II photochemistry in the midday on clear bright sunny shine days has been demonstrated in a number of plant species \cite{3, 11, 36}. In a willow canopy, this photoinhibition was over come in the evening on the same day \cite{33, 36}, Eucalyptus sp. \cite{35}, tropical forest trees \cite{5}, tropical deciduous and evergreen tree species \cite{21} and *Populus deltoides* \cite{49}.

In the present investigation some fast growing plant species (especially for high biomass production in a very short time duration) of different origins and character (deciduous/evergreen, tropical/temperate plants) were used to find out whether midday depression was a common occurrence in field plants during summer/draught conditions, chla fluorescence technique namely the widely used parameters. Fv/Fm the ratio of variable/maximum fluorescence-cf. The rapid assessment of changes in photosynthetic behaviour of tree species during day under field conditions, chla fluorescence technique namely the widely used parameters. Fv/Fm the ratio of variable/maximum fluorescence-cf. The rapid assessment of changes in photosynthetic behaviour of tree species during day under field conditions \cite{2, 15}. An attempt was also made to determine the relative contribution of environmental factors for midday depression under field conditions.

### 2. Materials and Methods

An experimental site of about 2 ha land located at an elevation of 1300 m on south-east facing gentle slopes in Palampur (32° 06’N, 76° E) was selected. For Experiment-I. Ten fast growing fuel-wood tree species listed below were obtained from the forest nurseries of Palampur region and used for Short Rotation High Density Energy Plantation (SRHD) system. *Grevillea robusta* Cunn (evergreen), *Jacaranda acutifolia* Humb & Bonpl. (deciduous), *Melia azedarach* Linn (deciduous), *Salix tetrasperma* Roxb. (deciduous), Eucalyptus sp. (hybrid evergreen), * Bauhinia variegata* Linn (deciduous), *Toona ciliata* Roem (deciduous), *Trewia nudiflora* Wight (deciduous), *Populus deltoides* Marsh (Clone G-3 deciduous), *Morus alba* Linn (deciduous). The Experiment I was laid out as per Split Plot design that included (i) main treatment with 10 fuel-wood tree species each (ii) Sub treatments involving 2 types of spacing i.e. 1m x 1m (10000 plant/ha) and 1m x 0.71m 14000 plants/ha and (iii) Sub-sub treatments involving 4 levels of N:P:K (2:1:1) fertilizers x 3 blocks. The Experiment II was laid out in Randomized Complete Block design with 3 replications on a 0.60 ha land adjacent to the Experiment 1. The tree saplings were planted at 1m x 1m spacing in contour rows on a gentle slope. Following treatments were included (1) main treatments = 3, sub-treatments = 9 (ii) replications = 3.

Main treatment = Irrigation (i) no irrigation (ii) 5 cm irrigation at fortnightly internal during April-June depending on soil moisture deficit (iii) 5cm irrigation at monthly internal during April-June. In both the experiments, around each plot and block two guard rows were planted at same spacing as was in adjacent plots. Fuel-wood tree species. 1. *Grevillea robusta* Cunn, 2. *Albezzia lebbeck* Lenth, 3. *Melia azedarach* Linn, 4. *Robinia pseudoacacia* Linn, 5. *Eucalyptus* spp. (hybrid), 6. *Bauhinia variegata* Linn, 7. *Toona ciliata* Roem, 8. *Populus deltoides* Marsh, (Clone G-3), 9. *Morus alba* Linn. Physiological parameters viz, photosynthesis rate, stomatal conductance/resistance (data not shown) etc. were recorded using a Portable Photosynthesis System (IRGA) Model LI-6200, LI-Cor, inc, USA, water potential was recorded with a plant water status console made in USA (data not shown) and chlorophyll fluorescence (quantum yield) parameters were measured using plant stress meter (PSM Mark II, Biomonitor, Sweden). Youngest fully expanded leaves (third or fourth leaf from the apex) of plants were darkened with leaf clips for 30 minutes (as per instrument’s
manually). Before exciting chlorophyll using an actinic light with peak at 500 nm was used. Various parameters $F_0$-constant fluorescence, $F_m$-maximum fluorescence, $F_v$-variable fluorescence and $F_v/F_m$-photochemical efficiency of Photo system PS II, $t_{1/2}$ (half the raise time from $F_0$ to $F_m$ at the P-Peak) recorded as per the manufacturer's instructions.

### 3. Result

Measurements were taken throughout the day from 06.00 to 18.00 hr in Sun-exposed and shade leaves of the same plants both on Sunny and cloudy days. In each case plants were selected in such a way that at a certain time at least 60 to 70% of their leaves remained exposed to Sun. The leaves that remained exposed to Sun during morning served as shade leaves in the after noon, and vice-versa. During midday, especially between 12.00 to 14.00 hrs, plants were selected whose 60-70% of the leaves remained exposed to Sunlight. All care was taken for Sun and shade effect to select leaves at the same canopy levels. Leaves of each species were identified and logged for identified and the same leaves were used for each observation. For each species 10 plants and in each plant 5-6 leaves both on Sun and shade sides were used. Observations were recorded at least 4-5 times during summer, June 1994, (Fig.1) observations on cloudy days were also recorded (data not shown) in July and August 1994. Observations were recorded in September, October and November 1995 in Sun exposed leaves, especially in midday (Fig.2). During morning and evening observations were also recorded (data not shown). In June 1995, some hr viz., 9.00 hr, 11.00 hr, 14.00 hr and 16.00 hr were fixed for recording the observations (Fig.3). In 1996, the measurements of $F_v/F_m$ were recorded in the morning and evening hr, from the months June, July, August, September and October (Fig.4).

An experiment was also conducted to evaluate the possible role of diurnal changes in soil moisture content which can occur due to high evapo-transpiration during summer months. The weather parameters like solar radiation, air and soil temperature and relative humidity on the day of observations were also recorded.

![Fv/Fm Values](plant_species.png)

**Fig.1** $F_v/F_m$ values of some tree species recorded between 12.00 to 14.00 hrs in the month of June, 1994 after 1 year

Diurnal variation in photochemical efficiency ($F_v/F_m$) with an abrupt decline at mid-day especially during warm weather in some species, during June, September and October every year was observed. However, the agro-climatic conditions had been highly variable. The Sun days may be converted suddenly in the shade/rainy days. All the parameters including depression of $F_v/F_m$, $F_m$, $F_v$, $F_0$ and $t_{1/2}$, photosynthesis rate, stomatal conductance, and stomatal resistance were also changed drastically. However, the effect of this reduction was species specific. Some species like *S. tetrasperma*, *E. hybrid* were most susceptible to high irradiances.
They showed highly reduction in the Fv/Fm ratio upto 60% in *S. tetrasperma* and 46% *E. hybrid* in 1995 in the month of September (Fig.2). The observations were recorded in 1994, 1995 and 1996 (Fig. 1, 2, 4). In 1994 and 1995, the observations were avoided in the rainy season and to high variability of weather (data not shown). In 1994, the observations were recorded in June. The Fv/Fm ratio was less than normal, the reduction of Fv/Fm was recorded 46% in *S. tetrasperma*, followed by *G. robusta* and *M. alba* 31%. The similar results were recorded in 1995 in same month (data not shown). In 1995, all the parameters (Fv/Fm t 1/2, Fm, Fo and Fv) were recorded in September, October and November just after rainy season. In July-August, the highest variability of weather was observed. The observations were recorded at 9.00 hrs. 11.00 hrs, 14.00 hrs and 16.00 hrs (Fig.4). In 1996, Fv/Fm was recorded at midday 13.00 hrs. The Fv/Fm ratio, 0.8-0.83 was assumed as the normal value (Demmig and Bjorkman, 1987). In June, the Fv/Fm ratio was recorded less than normal from 7% to 32% (*S. tetrasperma*). In all the months at a particular time (14:00 hr) the maximum photoinhibition was recorded in *S. tetrasperma* (Fig.3).

In all the plant species the decline in photochemical efficiency Fv/Fm was recorded only on their Sun exposed leaves, when the same leaves were shaded under natural conditions either by same plant or clouds, they did not show reduction. The maximum decline in Fv/Fm ratio was recorded between 12.00 to 14.00 hrs which corresponded with high solar radiation with high air temperature and low atmospheric humidity.

4. Discussion
The variations in photochemical efficiency of photosystem 2 (Fv/Fm) with a sudden decline at midday during lien period were recorded in some fuel-wood tree species. The enhancement of photoinhibition upon initial water stress was mainly related to rise in leaf temperature causing increased susceptibility to photoinhibition [35]. They had reported that the inhibition of Fv/Fm was largely the result of a decrease in Fm as studied from recovery studies. With photoinhibition at high temperature the rise of Fo did not appear until recovery was under way [35]. In relation to photoinhibition in the test species under the study, the depression in the quantum yield Fv/Fm was a light-induced depression as measured after 30 minutes of darkness. It was observed that the photoinhibition was recorded in horizontal as well as vertical leaves. However, horizontal leaves were exposed to full day length, while the vertical leaves received about one-third day light. Similar results were reported [35], which varied from species to species, but in some...
species it was almost unchanged. The recovery also varied from species to species. This rate was independent of the actual level of photoinhibition at least within the range of 10-60% photoinhibition. The results showed in (Fig.3) that S. tetrasperma was the possible exception with up to 60% inhibition. The recovery from photoinhibition was also similar between the species.

In terms of growth parameters, there was no significant difference between irrigated and non-irrigated plots. These observations were confirmed by water potential measurements up to 3 years. During dry spell, especially in May, June, September and October months, in hot clear days in horizontal leaves the stomata were closed, which increased the leaf temperature. Thus the photoinhibition was accelerated and the subsequent recovery of Fv/Fm got delayed. Similar observations were reported [18, 29]. Vertical leaves intercept less light thus reduce the extent of photoinhibition.

![Graph showing Fv/Fm values of some tree species recorded in the month of June 1995 at different hours](image)

Among the deciduous species, S. Tetrasperma was the most susceptible to high irradiances. It showed not only early reduction (11.00 hrs) in the Fv/Fm ratio, but also exhibited slow recovery in the ratio at the end of the day at 16.00 hrs (Fig.3). The leaves of S. tetrasperma exposed to Sun for a longer period during lien period showed lesion development, which caused a significant decrease in Fv/Fm ratio. On the other hand, plants grown in shade or shaded by other plants by same species or other species showed neither the lesion development nor reduction in Fv/Fm. [21] had reported maximum reduction in Fv/Fm in some tropical species like Boehmeria regulosa, Celtis australis and Dalbergia sissoo. Our investigations agreed with findings [21]. In all the tested fuel wood tree species, the significant decline in Fv/Fm was recorded only on their Sun exposed leaves, when the same leaves were shaded under natural conditions they did not show any reduction in Fv/Fm values. After continuous observations, we have fixed time to find out the maximum reduction in Fv/Fm values at a certain time. At 14.00 hrs the maximum reduction in Fv/Fm ratio was 19-60%. Some deciduous species as Trewia nudiflora, Toona Ciliata, J. acutifolia, Morus Alba and Melia azedarch showed about 19% reduction, while Eucalyptus hybrid and S. tetrasperma showed 46% and 60% reduction, respectively. The difference between Sun exposed and shade leaves was also recorded. On the other hand, no reduction in Fv/Fm was observed in cloudy days. The temperature difference between Sun exposed and shaded leaves was also recorded. The difference in fluorescence characteristics could be attributed to the leaf age. Similar observations were recorded [19, 40]. Thus, our findings fully agree with these
observations. He further reported that watering of plants at hourly intervals did not bring any significant change in chl fluorescence characteristic of Sun exposed leaves. It has been concluded with our observations that the photodamage is directly proportional to absorbed quantum flux density (Q) i.e. damage to PS II is not confined to high light, but may occur irrespective of flux density of quanta. The same type of results was obtained \cite{7, 38, 46}. Sustained curbing of PS II efficiency is also a balance between damage and repair, which takes place simultaneously. Our results indicate that decline in PS II and overall efficiency is a result of photodamage and our findings fully agree with the findings\cite{30, 34, 44}. It was evident that photoinhibition observed in the field in response to diurnal variability in absorbed quantum flux density (Q) is not related to damage, but results from a safe down regulation of PS II efficiency through increases in non-radiative energy dissipation. The same type of study has been done \cite{13, 16, 23, 27, 47}. Thus our findings fully agreed with these findings. The foliar pool of chlorophyll intermediates to test the hypothesis that turnover rates of foliar chlorophyll (chl) increases in high light to maintain the balance between destruction and synthesis \cite{31}. They further suggested that the leaves possess the capacity to adjust chl turn over rate in response to light availability, maintaining a constant foliar chl concentration despite its continuous destruction. These observations have confirmed our findings, i.e. The reduction in FV/FM values over time and repair of the same in the evening hours.

On the basis of our observations, it has been concluded that the enhancement of leaf photosynthetic capacity played a more important role in maintenance of open PS II centers and then increase in capacity for non-radiative energy dissipation. The same type of findings that agreed with our results \cite{31}. The leaves were unable to maintain the same degree of PS II center openness in high light, even though Fv/Fm decreased at high irradiances, allowing a greater fraction of light to be dissipated non-radiative \cite{1, 13}. They further reported that the calculation based on chlorophyll fluorescence showed that despite enhancement of non-radiative energy dissipation, leaves were frequently exposed to a certain fraction of potentially damaging excess light in the field \cite{1, 13}.

The upper canopy leaves of *Salix* lost about 6.3 to 12.6 % of potential carbon gain during the day caused by photoinhinition \cite{31}. Similar observations have also been reported \cite{28, 34}. In our observation, about 50% upper leaves were damaged by photo inhibition in case of *Salix tetrasperma* and the values of Fv/Fm reduced by 46 to 60%. Therefore, the high potential photoinhibitory loss of photosynthesis was reported at midday \cite{28, 34}. On the basis of our observations, especially in case of *Salix tetrasperma*, the photoinhibition was recorded up to 60%. It has been concluded that the reduction of Fv/Fm value more than 50% was beyond the
repair of Fv/Fm value in the same evening hours, which resulted in the damage of the plants. During the experimentation it has been proved that this species has failed to establish in this agroclimatic region. The higher values of Fv/Fm ratio could be expected for cloudy days and for shaded parts of the tree crown, which are not affected by high light [43]. We have reported similar observation in our findings.

A. During the sunny days delay was observed in the Fv/Fm ratio. The decrease was significant at mid-day (maximal values of incident PAR). The Fv/Fm ratio had a dynamic course, in the evening and in the morning the Fv/Fm ratio reached the maximum.

B. The seasonal investigations revealed that photoinhibition did not occur on particular days only when the photoinhibition processes were extremely fraught with high intensity of incident PAR together with other stresses (drought) but this phenomenon existed in forest ecosystems generally.

C. The daily course in Fv/Fm ratio was typical for Sun exposed parts of tree crown. The shaded parts had a steady level of Fv/Fm throughout the whole day. PS II quantum efficiency was stable during the whole day.

From the seasonal view-point, on cloudy days the Fv/Fm ratio from the morning measurements exhibited the lowest value in May (for both Sun exposed and shade). It could be due to climatic conditions, low temperature during nights and high intensities of PAR during the days prior to data recording observations.

In the past, many workers had investigated Fv/Fm ratio to obtain more information about variability in photochemical efficiency of PS II. The fluorescence measurements of photosystem II unaffected by high light showed the values of Fv/Fm ratio above 0.80 [12, 33, 42]. The radiation regime in our plantation (2 ha) canopy crown was characterized by horizontal and vertical distribution of solar radiation. The plantation crown creates a heterogeneous optical system, in which certain parts (leaves) of the canopy were exposed to high levels of PAR at different times of the days and season. For a better characterization of heterogeneity of PAR for Sun exposed and shaded parts of tree crown, we had standardized certain leaves and recorded observations for sunny and cloudy days. It was evident that the values from the 60 to 70 % Sun exposed leaves were below real ones. The real values were near to that of incident PAR. So the maximal differences of PAR between Sun exposed and shaded leaves was about 30 to 60 % for sunny days, so the new shoots/leaves developed under very different radiation conditions. On cloudy days the differences of Fv/Fm were not so significant.

In our findings the Fv/Fm ratio for shaded leaves on sunny days was higher than that on cloudy days. It could be explained by higher sensitivity to increased PAR and qualitatively and quantitatively different responses to light [39]. Perhaps the shaded leaves had larger light absorbing antennae complexes than the Sun exposed leaves so the protection could be seen in the disconnection between antennae complexes and reaction centers. Under our study, the high values of PAR above 750μ mol m⁻²s⁻¹ were determined. It was the value of PAR, when the rate of photosynthesis was saturated. In the sunny days the values of radiation above saturating PAR were recorded approximately during observation between 10.00 to 16.00 hrs (depending on the month or the season), that means the photoinhibition influenced the photochemical efficiency of PSII for the majority of sunny days.

The temperature plays an important role in photoinhibition and at 30-40 °C the photochemical efficiency is adversely affected. In our studies, the temperature was recorded from 27-48 °C which lowered the photochemical efficiency of PSII when maximum irradiance on clear days was 2050 to 2200 μ mol m⁻² s⁻¹ on a horizontal plane. The horizontal leaves showed stronger photoinhibition (maximum 40 %) in comparison to vertical leaves (30% in Eucalyptus [35]. In our observations the photoinhibition in Eucalyptus
hybrid was 46%. Thus, the results fully agree with the results \cite{38}.

The *Eucalyptus* leaves showed a non-linear light dependence of photoinhibition that levelled of forward high lights as revealed by comparison of light and photoinhibition levels in vertical and horizontal leaves.

In our studies the photoinhibition fully recovered up to next morning as reported similar result in willow \cite{36}. This rate was independent of the actual level of photoinhibition at least within the range of 10-40% photoinhibition. In the stress up to 71%, the recovery from photoinhibition was also similar between the species. The temperature above 37 °C promoted photoinhibition through a slowdown of the rate of photosynthetic energy dissipation \cite{38}. They had further investigated that the rate of electron transport was decreased with increase in leaf temperature above a threshold in the range of 30-40 °C \cite{32}. At temperature below 50 s°C this effect becomes persistent due to damage of PSII \cite{39}. Our findings fully agree with these observations.

5. Acknowledgement

This study was made possible by financial support from Department of Non-conventional Energy Sources, C.G.O. Complex, Lodhi Road, New Delhi, Government of India. I am also thankful to Dr. R.D. Singh and Prof. V.S. Rama Das for their valuable help in this study.

6. References


16. Epron D, Dreyer E, Breda N. Photosynthesis of oak trees (Quercus petraca (Matt) Licbl.) during drought under field conditions : diurnal course of net CO₂
31. Niinemets Ü, Kull O. Sensitivity of photosynthetic electron transport to photoinhibition in a temperate deciduous forest canopy: Photostem II center openness, non-radiative energy dissipation and excess irradiance under field conditions 2001; 21:899–914.
42. Špunda V, Kalina J, Kuropatwa R, Mašlák M, Marek M. Responses of photosystem 2 photochemistry and pigmen: Composition in needles of Norway spruce

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samplings to increased radiation level. Photosynthetica 1993; 28(3):401–413.


46. Tyystiaarvi E, Aro EM. The rate constant of photoinhibition, measurement in lincomycin-treated leaves, is directly proportional to light intensity. Proc Natl Acad Sci USA 1996; 93:2213–2218.