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ORIGINAL ARTICLE

Differential responses of seven contrasting species to high light using pigment and chlorophyll *a* fluorescence

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High light intensity may induce severe photodamage to chloroplast and consequently cause decreases in the yield capacity of plants and destruction of pigments, causing an overall yellowing of the foliage. Thus, study related to light adaptation becomes necessary to understand adaptation processes in higher plants on the basis of which they are characterized as full sunlight or shade plants. Chlorophyll can be regarded as an intrinsic fluorescent probe of the photosynthetic system. The ecophysiological parameter related to plant performance and fitness i.e. *in-situ* chlorophyll fluorescence measurements were determined for different plant species in the medicinal plant garden of Banasthali University, Rajasthan. Miniaturized Pulse Amplitude Modulated Photosynthetic Yield Analyzers are primarily designed for measuring effective quantum yield ($\Delta F/Fm'$) of photosystem II under momentary ambient light in the field. Photosynthetic yield measurements and light-response curves suggested a gradation of sun-adapted to shade-adapted behaviour of these plants in following order *Withania somnifera> Catharanthus roseus> Datura stamonium> Vasica minora> Vasica adulta> Rauwolfia serpentina.* As indicated by light response curves and pigment analysis, *Datura stramonium, Withania somnifera* and *Catharanthus roseus* competed well photosynthetically and are favoured while *Rauwolfia serpentina, Vasica minora, Vasica adulta* and *Plumbago zeylanica* were observed to be less competent photosynthetically. These light response curves and resultant cardinal points study gave insight into the ecophysiological characterization of the photosynthetic capacity of the plant and provides highly interesting parameters like electron transport rate, photo-inhibition, photosynthetically active photon flux density and yield on the basis of which light adaptability was screened for seven medicinally important plants.

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The spreading acceptance of the concept of precision agriculture running (Rascher et al. 2000) generated much interest in the early stress detection of plant growth. Important here are early warning signs of plant inhibition which are directly connected to fundamental physiological processes as photosynthesis has been connected to vegetation fluorescence (Valentini et al. 1994; Smorenburg et al. 2002). Plant short-term or long-term stresses are indicated by the composition of photosynthetic pigments and their stress-induced changes, also regarded as optical signatures of leaves. In recent years, to detect the functioning and health status of plants, the screening of plant fluorescence signatures is developing as a specific tool (Lichtenthaler et al. 1999; Samson et al. 2000; Dahn et al. 1992). The application of fluorescence technology, if proven effective, has great potential for application in the field.

In general, the productivity of plants exposed to environmental stresses is dependent on the availability to develop adaptive mechanisms to avoid and tolerate stress (Willits and Peet 2001). Under normal light conditions, regular photosynthesis occurs in green plants and the absorbed sunlight is almost exclusively used for photochemical charge separation in the reaction centre, dangerous by-products, i.e. triplet states and singlet oxygen accumulate and can lead to severe photodamage (Dreuw et al. 2006). No plant has the ability to use 100% of maximum solar irradiation for photosynthesis (Demming-Adams et al. 1997). When irradiance exceeds the value which can be used for photochemistry, other protective mechanisms must be used to dissipate excess excitation energy or damage will occur. The quantum efficiency of photosynthesis of a plant is largely reduced (photoinhibition) when it is exposed to excess light level (Sudhir et al. 2005). Excess light induced photoinhibition of photosynthesis, as determined by the Chl fluorescence parameter maximal quantum yield of PS II photochemistry, is the net result of a complex set of interacting cellular and leaf level processes (Kumar and Kasturi 2009; Ribeiro et al. 2004). High light intensity may induce severe photodamage to chloroplast and consequently cause decreases in the yield capacity of plants (Hacisalihoglu and Kochian 2003) and destruction of pigment, causing an overall yellowing of the foliage (Dekov et al. 2000).

In order to characterize light reactions of photosynthesis, measurements of chlorophyll fluorescence have been established as a useful and informative indicator in recent years. Under ambient light-conditions, fluorometers can offer instantaneous measurements of the effective quantum yield ($\Delta F/Fm'$) of PS II. Although this parameter lead to deeper insights of ecophysiology, some additional parameters such as maximum apparent ETR (ETRmax) and saturating photosynthetically active radiation (PPFDsat) by light response curve programme of yield analyzers have attracted ecophysilogists and physiologists. These measurements are related to the leaf ontogeny and the physiological plasticity of plant and are independent of momentary ambient light conditions (Rascher et al. 2000). PS II electron transport is one of the most sensitive indicators of damage in the photosynthetic apparatus (Krause and Weis 1991). Recent chlorophyll fluorescence techniques allow evaluation of primary photochemical reactions of photosynthesis, efficiency of PS II and electron transport rate through electron carriers in particular (Rascher et al. 2000).

In the light response curve programme of most miniaturized fluorometers, the actinic light changes after short interval. Although in this way no steady state condition are applied during light curve runs, comparison of different plants measured under same environmental conditions is possible, and this has been widely used in the literature (De Mattos et al. 1997; Lüttge et al. 1998; Franco et al. 1999).

Plants with medicinal value have been extensively studied for their therapeutic uses. Traditionally, *Catharanthus roseus* has been used in folk medicine to treat diabetes and high blood pressure. As an antidiabetic remedy, it was believed to promote insulin production or to increase the body's utilization of sugars from food. *Datura stramonium* contains the alkaloids scopolamine and atropine and has been used in some cultures as a poison and hallucinogen. *Rauwolfia serpentina* or Indian Snakeroot or *Sarpagandha*, contains a number of bioactive chemicals, including ajmaline, reserpine, rescinnamine, serpentinine, and yohimbine. It had drastic psychological side effects and has been now replaced. But in herbal form rather than the form of isolated active ingredients, it is a safe and effective resource for hypertensive patients. The pharmaceutical companies have stopped producing this drug as reserpine or deserpedine or any other form. *Withania somnifera*, also known as Ashwagandha, Indian ginseng, a herb that works on a nonspecific basis to increase health and longevity. This herb is also considered as an adaptogen which is a nontoxic herb that works on a nonspecific basis to normalize physiological function, working on the neuroendocrine system. The roots and berries of the plant are used in herbal medicine. *Plumbago zeylanica,* Chitrak is used in treating intestinal troubles, dysentery, leucoderma, inflammation, piles, bronchitis, itching, diseases of the liver and consumption. The leaves of this herb work well for treating laryngitis, rheumatism, diseases of the spleen, ring worm, scabies which acts as an aphrodisiac. A tincture of the root bark is used as an anti-periodic. Root of Chitrak helps improve digestion and it stimulates the appetite. Chitrak root is also an acro-narcotic poison that can cause an abortion. *Vasica minora and Vasica adulta* are shrubs on the plains of India. Its leaf extract has been used for the treatment of bronchitis and asthma. It relieves cough and breathlessness. It is also prescribed commonly for bleeding due to peptic ulcer, piles, menorrhagia, etc. Its local use gives relief in pyorrhoea and bleeding gums.

This paper reports some results from field measurements of plant chlorophyll fluorescence. The objective was to examine the relationship between fluorescence and plant growth inhibition under stress conditions (high light), to investigate the degree of stress detectable from fluorescent measurements and to study the sensitivity and reliability of various fluorescence parameters as stress indicators and vegetation response to growing conditions and therefore characterize plants which can better perform in shade and high light intensity, thus, screening them in the basis of their saturation at a particular light intensity. The degree of stress was quantified through changes in leaf pigments since all plant stressors either directly or indirectly affect the photosynthetic apparatus and its functioning causing a decline of the leaf chlorophyll content.

In the present study, systematic analysis of enormous data obtained in the field where light-response curves runs with the Mini-PAM were performed for different plant species of medicinal importance. The main objective is to identify ways in which chlorophyll fluorescence may be used most effectively to improve plant selection process and rapidly evaluate its performance in horticulture and ethanobotanical crop improvement programme. First, different approaches of light curve recordings were tested and different plants with different modes of photosynthetic adaptations were studied in a fully light exposed medicinal plant garden.

MATERIALS AND METHODS

Study site and Plants: Chlorophyll fluorescence measurements of different plant species of medicinal value were performed in the Medicinal Plant Garden on fully light-exposed plants close to the Department of Bioscience and Biotechnology of Banasthali University, Rajasthan (India) on the sunny day in the month of August.

For a survey to gain insights in ecophysiology of the vegetation in the medicinal plant garden, seven different plant species were selected. The ecophysiological parameters related to the plant performance and fitness i.e. in-situ chlorophyll fluorescence measurements were determined for 7 different plant species in the garden namely, *Catharanthus roseus, Datura stramonium, Rauwolfia serpentina, Withania somnifera*, *Plumbago zeylanica, Vasica adulta and Vasica minora.*

Measurements of chlorophyll a fluorescence parameters: Chl *a* fluorescence of PSII was measured on attached leaves with a Mini-PAM fluorometer (Walz, Effeltrich, Germany) using the protocol described by Kumari et al. (2005). Fluorescence parameters of Chl *a* of PS II recorded were minimal fluorescence of light adapted leaves, F, as well as maximal fluorescence of light adapted leaves under a saturating light pulse of a duration of 800 ms, Fm', which gave effective quantum efficiency or yield of PS II as $\Delta F/F_m' = (F_m'-F)/ F_m'$ (Genty *et al.* 1989). For the plants used in this study no specific reflection factors were known numerically and no specific anatomical structures such as hairs or reflecting cuticle were present. Thus the empirical reflection of 0.84 was chosen. Apparent photosynthetic electron transport rate (ETR) was then obtained as $ETR = 0.5 \times 0.84 \times (\Delta F/F_m') \times PPFD$, where the factors 0.5 and 0.84 account for an assumed equal excitation of both PS I and PS II and a light reflection of 16%, respectively (Schreiber *et al.,* 1986; 1995a, b).

Mini-PAM offers light response curves programme which forms instant light-response curves of ΔF/Fm' where actinic light increases in 8 steps for 4 minutes thus generating 9 measurements at a short interval of about 30 s (Rascher *et al.* 2000). An internal halogen lamp of the instrument acts as light source, using the fibre optics and the leaf clip holder. All light curves were taken in the morning between 8 and 10 a.m. to minimize build up of acute photoinhibition before the measurements.

Measurements of photosynthetic pigments:

On the same day as the fluorescence measurements were made, leaf chlorophyll *a*, chlorophyll *b* and carotenoid concentrations were determined. Fresh leaves of the experimental plants at the study site were collected and immediately processed for the determination of photosynthetic pigments. 100 mg leaves were homogenized in 5 ml of chilled 80% acetone by grinding in a pre-cooled mortar and pestle until the powdered material became completely non-green. The homogenate was centrifuged for 5 min at 3000 rpm at 4 °C in a cooling centrifuge. The pellet was discarded and the supernatant was re-adjusted to 5 ml using acetone. To 1.6 ml of the supernatant, 0.4 ml double distilled water was added. The absorbance of the resulting supernatant was recorded at 480, 645 and 663 nm. Different pigments were estimated using the following formula by Arnon (1949) as given below:

Total chlorophyll (*a* and *b*) (mg/l) = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll *a* (mg/l) = 12.7 (A_{663}) – 2.69 (A_{645})

Chlorophyll *b* (mg/l) = 22.9 (A_{645}) – 4.68 (A_{663})

Carotenoids (mg/l) = A_{480} x 200

The above formula expressed the pigment content in g/g FW of sample.

Statistical Analysis

Physiological and biochemical data were presented as mean ± standard deviation (SD) of three biological replicates.

RESULTS

Chlorophyll *a* fluorescence parameters:

All seven varieties were assessed for their photosynthetic performance, electron transport rate at saturating light intensity. Cardinal points offered easy analysis of PPFDsat, ETRmax, ΔF/Fm'sat and it was observed that PPFDsat values of *W. somnifera*, *D. stramonium* and *C. roseus* were very high which suggests that the saturation of these plants required higher values of PPFD i.e. high light intensities. While the PPFD values of remaining plants were low in comparison to above mentioned plants as indicated in the fig. 1 -3. It was observed that among the two species of *V. adulta* and *V. minor*, *V. adulta* is more tolerant to sunlight shown by its higher PPFDsat value as well as the ETRmax value was 115.8 µmol m^{-2} s^{-1} while that for *V. minora* is around 74.6 µmol m^{-2} s^{-1}. The phosynthetic performance as shown by ΔF/Fm' values were also higher for *V. adulta* and 50% more photosynthetic capacity was observed.

Table 1. Pigments content for seven contrasting plant species of medicinal importance grown under natural light conditions. The values are expressed as mean ± SD for triplicates (n=3).

Plant species	**Car (µg /g FW)**	**Chl *b* (µg /g FW)**	**Chl *a* (µg /g FW)**	**Total Chl (µg /g FW)**	**Chl *a:b***	**Car:Chl**
Catharanthus roseus	11.20 ± 0.01	0.33 ± 0.01	1.06 ± 0.03	1.39 ± 0.02	3.24 ± 0.18	8.05 ± 0.11
Withania somnifera	8.78 ± 0.06	0.31 ± 0.01	0.57 ± 0.01	0.88 ± 0.01	1.82 ± 0.07	9.94 ± 0.16
Datura stramonium	6.62 ± 0.02	0.15 ± 0.02	0.71 ± 0.00	0.86 ± 0.01	4.73 ± 0.57	7.73 ± 0.12
Vasica adulta	5.57 ± 0.02	0.07 ± 0.01	0.73 ± 0.00	0.80 ± 0.01	10.19 ± 1.39	6.93 ± 0.09
Vasica minora	4.94 ± 0.04	0.14 ± 0.01	0.46 ± 0.00	0.61 ± 0.01	3.27 ± 0.15	8.14 ± 0.15
Rauwolfia serpentina	4.43 ± 0.03	0.13 ± 0.01	0.40 ± 0.00	0.53 ± 0.01	2.98 ± 0.25	8.30 ± 0.26
Plumbago zeylenica	4.05 ± 0.07	0.12 ± 0.01	0.39 ± 0.01	0.50 ± 0.01	3.31 ± 0.35	8.03 ± 0.07

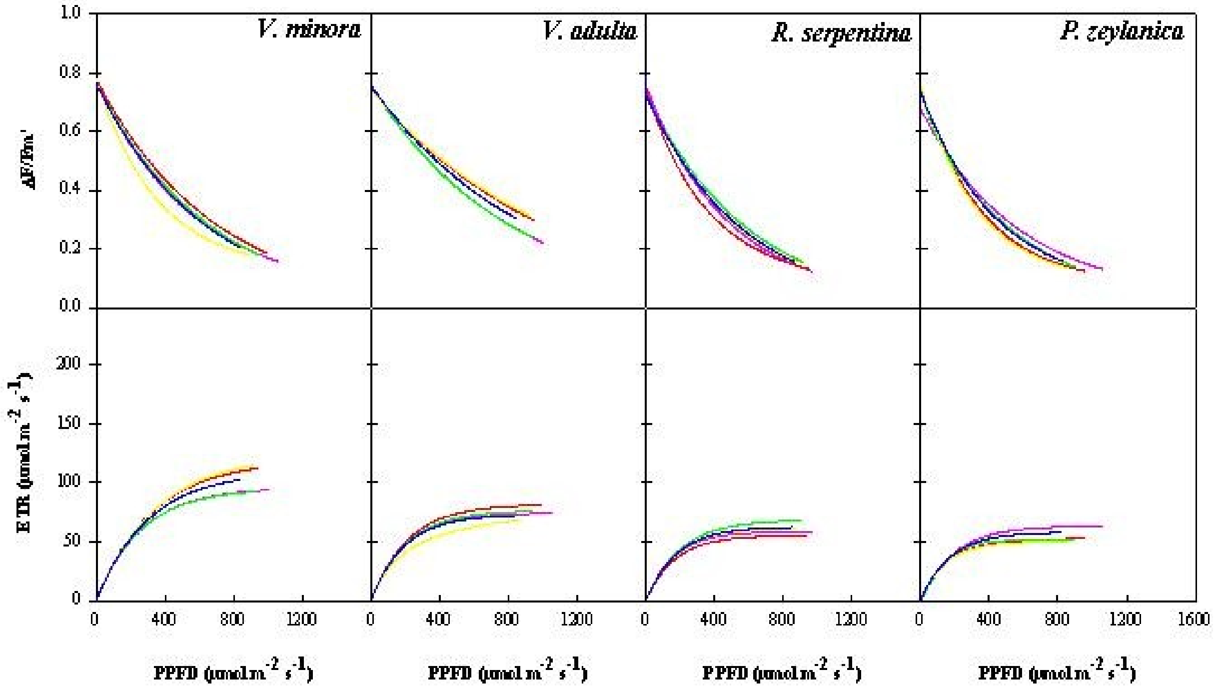

Figure 1 Regression lines for the data points of light response curves of ΔF/Fm' and ETR in relation to PPFD in sun-intolerant plant species of medicinal importance.

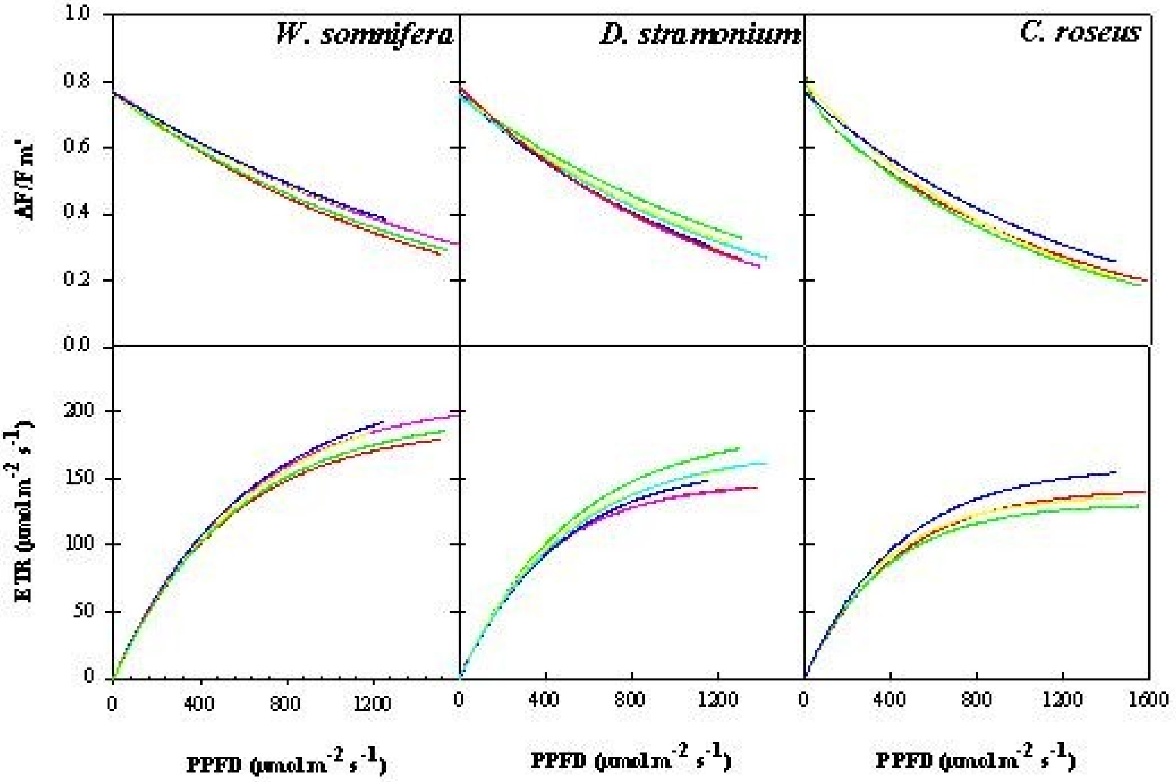

Figure 2 Regression lines for the data points of light response curves of ΔF/Fm' and ETR in relation to PPFD in sun-tolerant plant species of medicinal importance.

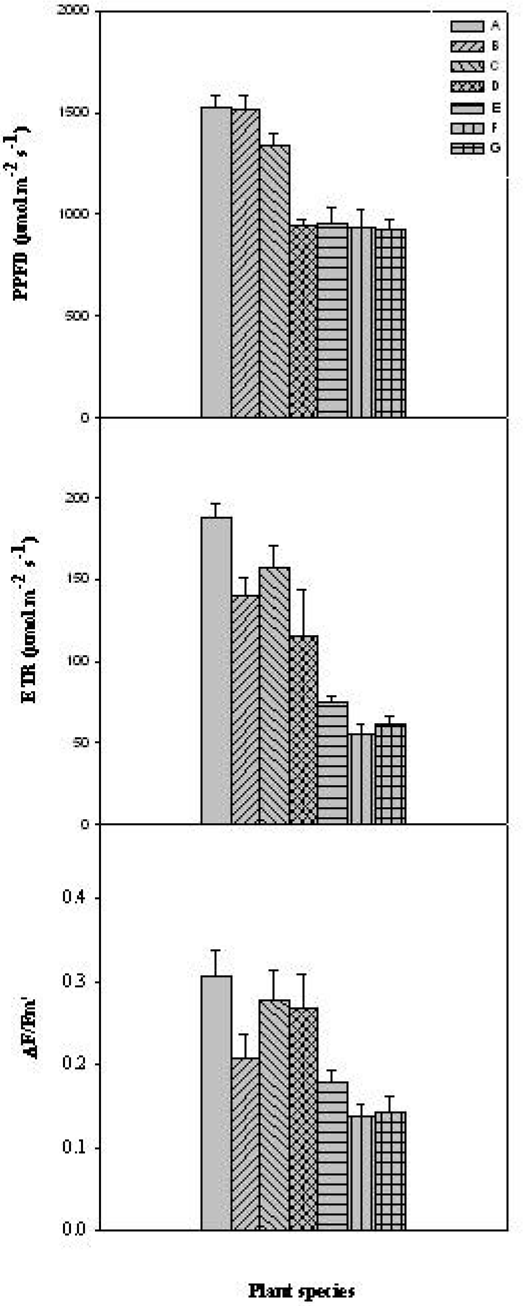

Figure 3 Changes in cardinal points of the regression lines of PPFDsat dependencies of apparent electron transport rate (ETRsat) and effective quantum yield (ΔF/Fm'sat) for seven plant species of medicinal importance. A: *W. somnifera*, B: *C. roseus*, C: *D. stramonium*, D: *V. adulta*, E: *V. minora*, F: *P. zeylanica*, G: *R. serpentina.* The values are expressed as mean ± SD (n=5).

The saturating values of PPFD in *C. roseus*, *W. somnifera* and *D. stramonium* were much higher in comparison to *P. zeylanica*, *V. adulta* and *V. minora* and *R. serpentina*. The PPFD values of *C. roseus* and *W. somnifera* reached uptil 1500-1600 µmol m^{-2} s^{-1} while that of *P. zeylanica* and *R. serpentina* were around 900 µmol m^{-2} s^{-1}. The ETRmax values were also highest for *W. somnifera* i.e. 187 µmol m^{-2} s^{-1} and lowest for *P. zeylanica* around 55.6 µmol m^{-2} s^{-1}. *C. roseus*, *W. somnifera* and *D. stramonium* showed higher ETRmax values in comparison to *P. zeylanica*, *V. adulta* and *V. minora* and *R. serpentina*. Also the quantum yield of photosynthesis was higher for these plants.

Although *C. roseus*, *W. somnifera* and *D. stramonium* have higher PPFDsat, but the ETRmax values of *D. stramonium* was 12 fold higher than *C. roseus* and that of *W. somnifera* was 34 times higher. The photosynthetic performance was highest for *W. somnifera* and *P. zeylanica* showed lowest PPFDsat, ETRmax and $\Delta F/Fm'$ among the seven plants species.

Photosynthetic pigments

The contents of Chl *a*, Chl *b* and total Chl and the Chl *a:b* and the Car:Chl ratios were dependent on species, light conditions and species × light interactions. Car contents were higher in all sun-tolerant plant species viz. *C. roseus*, *W. somnifera* and *D. stramonium*; as compared to sun-intolerant plant species viz. *P. zeylanica*, *V. adulta* and *V. minora* and *R. serpentina*. Chl *a*, Chl *b* and total Chl was also found to be higher in the sun-tolerant plant species than other sun-intolerant plants (Table 1).

DISCUSSION

With the advent of highly interesting parameters like light-response curves and the cardinal points, there is ease in the characterization of ecophysiological studies of the photosynthetic capacity of the plant. To obtain accurate light-response curves, there is a necessity to reduce photoinhibition caused due to exposure of leaf to high light intensities or prolonged light exposure. In contrast to chronic photoinhibition, which is not reversible, acute photoinhibition is reversible after 20-30 min dark incubation and is due to energy dissipation via built up of an electrochemical gradient across the thylakoid membranes and via generation of heat during xanthophyll cycle (Thiele et al. 1998). To overcome acute inhibition all the measurements were taken early in the morning before exposure of leaf to high light thus reducing photoinhibition.

When plants are exposed to high irradiation associated with a high vapor pressure deficit, the stomata normally close to prevent water loss, which results in a decrease in the intercellular CO_2 concentration and depression of photosynthesis. In this condition, plants become more predisposed to suffer photoinhibition because of the absorption of an excess of photons over what is used in photosynthesis. The decrease of the maximum photochemical efficiency of PS II, which is maximal around 0.830 in shade plants, as observed principally in sun-intolerant plant species under high-light conditions, can reflect photodamage to PS II (Genty et al. 1989; Baker 2008). On the other hand, as observed by Kitajima (1994) and Ellis et al. (2000), the higher photosynthetic rates of the sun-tolerant plant species could be an important quencher of excess light energy.

As expected we observed the light saturation of electron transport rate for some medicinal plant species and it was found that some plant species were considered sunlight-tolerant while others fall in the category of sunlight-intolerant plant species. Sun-tolerant and light acclimatized plants have a higher photosynthetic capacity when exposed to high light than do sun-intolerant plants. The results presented here compare seven different plant species grown in Medicinal Plant Garden and indicate that among these plants *C. roseus*, *W. somnifera* and *D.*

stramonium were sun-tolerant as these plants were unaffected when grown under full solar irradiance. While the plants *P. zeylanica*, *V. adulta* and *V. minora* and *R. serpentina* were shade-plants i.e. sun-intolerant plants as their photosynthetic capacity greatly reduced under high light environments. The light curves studies enabled us to categorize the plants into sun adapted to shade adapted plants as *Withania*> *Catharanthus*> *Datura*> *Vasica adulta*>*Vasica minora*> *Rauwolfia*> *Plumbago* when their PPFDsat values were compared. Thus, on the basis of PPFDsat values *Withania*, *Datura* and *Catharanthus* were regarded as sun-adapted while remaining four were shade-adapted plants. The light curves recordings allow us to provide an assessment of the significance and evaluation under which conditions and precautions this approach can be applied effectively in the field to gain reliable information about light-response characteristics (Rascher et al. 2000).

This physiological characteristic should contribute to diminishing excess light during high irradiation exposures, maintaining a higher maximum photochemical efficiency of PS II and reducing the predisposition to photoinhibition, as observed in the sun-tolerant plant species (Kitajima 1994; Poorter 1999). Moreover, as high rates of photosynthesis are associated with high transpiration, lower leaf temperatures might also contribute to reduce photoinhibition in the sun-tolerant plants (Krause et al. 2001). Ribeiro et al. (2005) concluded that photosynthetic and transpiration rates play a major role in separating tropical trees of different successional groups under high irradiance.

It is well known that levels of Chl *a* and Chl *b*, total Chl as well as the ratios of Chl *a*:*b* and Car:Chl are essential differences between sun- and shade-adapted leaves (Demming-Adams 1998; Lichtenthaler and Babani 2004; Lichtenthaler et al. 2007). We found that, on an average, sun-tolerant species grown at high sun-irradiance contained higher ratios of Chl *a*:*b* and Car:Chl (Table 1) than sun-intolerant plants. The higher Car:Chl ratios found in plants developed under high-sunlight conditions in comparison to sun-intolerant plants, resulted from both a reduction in the total chlorophyll content and an increase in carotenoid content (Demmig-Adams and Adams 1992).

According to Poorter and Rozendaal (2008), sun trees of a given species have small leaves to reduce the heat load, but light-demanding species had larger leaves than shade-tolerant ones, probably to outcompete their neighbors. Shade trees of a given species have high specific leaf area to capture more light in a light-limited environment, whereas sun-intolerant species have well-protected leaves with a lower SLA than that of light-demanding species, probably to deter herbivores and enhance leaf lifespan. Recently, Silvestrini et al. (2007) found that *H. courbaril* grown under low light (5–8% of sunlight), showed higher total chlorophyll and carotenoid contents than the pioneer *T. micrantha*, but under high light (100% of sunlight), *H. courbaril* was more susceptible to photoinhibition than the sun-tolerant species. Recently, Matos et al. (2009) determined that sun leaves of field-grown coffee trees showed well-developed photoprotection mechanisms in comparison to shade leaves, which proved sufficient for avoiding photoinhibition.

Carotenoids play an important role in the light harvesting complex and in the photoprotection of the photosystems. Several studies have shown that these compounds are very important in protecting the photosynthesis apparatus against photodamage, by interconversions among the xanthophyll molecules (Young et al. 1997; Ort 2001).

Carotenoids is a large class of isoprenoid molecules, which are synthesized *de novo* by all photosynthetic and many non-photosynthetic organisms. Car content was higher in sun-tolerant plants as compared with sun-intolerant plants. Dere et al. (1998) in their studies determined that Car pigments are the most important photosynthetic pigments and they prevented Chl and thylakoid membrane from the damage of absorbed energy by photooxidation. Sun-tolerant plants possess much higher amounts of LHC II than sun-intolerant plants and consequently, Chl *a:b* ratio are higher than in sun-tolerant plants (Netto et al. 2002). Lowest Chl ratio in sun plants is an indicator of senescence, stress and damage to the plant and the photosynthetic apparatus, which is expressed by faster break down of Chl than Car. Leaves become more yellowish-green and exhibit value for pigment as senescence progresses. Our results corroborate many studies made with sun or high light and shade or low-light leaves (Lin et al. 2009) they clearly indicate that low light-grown plants are more susceptible to photoinhibition than high light-grown plants. The Chl content increase in the low intensity plants due to reduced photo-oxidation in lower light conditions.

CONCLUSION

This experimental study was designed and performed with the goal of getting information about the dependence of fluorescence parameters to chlorophyll variations and the possibility to assess plant stress by quantifying this dependence. Convincing results were obtained proving the fluorescence signal as a valuable source of information and an efficient tool for vegetation assessment. Out of seven plant species three can be regarded as sun-tolerant while rest four as sun-intolerant plant species. Thus, chlorophyll *a* fluorescence parameters can allow assessment of sun and shade growing capacity of plants and assign optimum light conditions as required for their normal photosynthesis.

In conclusion, the light-response curves and the cardinal points of light-response curves, namely PPFDsat, ΔF/Fm'sat and therefore, maximum apparent ETR (ETRmax) can be directly calculated using Mini-PAM. These cardinal points can actually be taken as the face values, where the measurements gave the results for a PPFD range which is close to the saturation point of instant light-response curves. The careful interpretation of the parameters obtained can be an enormous advantage in ecophysiological field work. Future work is planned including study of plant fluorescence in different spectral bands and under the impact of different stress factors as well as the development of new information deriving tools based on fluorescence data.

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