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ORIGINAL ARTICLE

Varying light regimes in naturally growing *Jatropha curcus*: pigment, proline and photosynthetic performance

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Light stress is a major abiotic stress which adversely affects productivity of the plants. Tolerance to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development. In many cases, high yield potential can contribute to yield in moderate stress environment. We studied chlorophyll (Chl) *a* fluorescence parameters and analyzed D1 core protein in one year old plants of *Jatropha curcus* under different light regimes (10–1200 µmol m^{-2} s^{-1}) in sun and shade plants. Chl *a* fluorescence provides insights into the responses of the photosynthetic system to increasing irradiance. Total Chl content was 1.43 and 0.61 mg/g^{-1} FM for shade and sun exposed plants respectively. The effective quantum yield *(ΔF/Fm')* of the sun plants was lower as compared to shade plants but the amount of the D1 core protein was higher in plants grown under high light intensity. A decrease in *ΔF/Fm'* indicates down regulation of photosynthesis or photoinhibition. D1 protein is the membrane protein complex of the PSII reaction centre. The degradation of D1 protein may regulate the functioning of the PSII repair cycle under photoinhibitory conditions. It has been shown that low-light grown or shade plants are more susceptible to photoinhibition than high light or sun plants. This higher susceptibility is accompanied by slow degradation of damaged D1 protein. High light intensity or exposure to photooxidation leads to the irreversible damage in photosynthetic performance and consequently has an overall inhibitory effect on crop productivity.

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Crop plants are exposed to various environmental factors, such as drought, salinity and extreme temperatures that affect soil productivity and crop production worldwide. In such environment it is often difficult to distinguish between the effects of the multiple stresses, but high temperature is certainly a major factor in plant productivity (Karim et al. 2003). The problem faced

by the plants under conditions of high solar radiation and temperature is energy absorption by the leaves, which can easily raise the temperature of the leaf by 5°C or more above ambient. High temperature limit for photosynthesis is generally marked by an abrupt increase in Chl fluorescence, which is easily monitored in intact leaves. Productivity of crop 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). Despite the necessity of light for autotrophic organisms, no plant is capable of using 100% of maximum solar irradiation for photosynthesis (Demming-Adams et al. 1997). When irradiance exceeds that which can be used for photochemistry, other protective mechanisms must be used to dissipate excess excitation energy or damage will occur. A larger percentage of absorbed energy is used in photosynthesis, reducing the need for alternative dissipating mechanisms and minimizing the risk of photodamage. 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 PSII photochemistry *(Fv/Fm)*, is the net result of a complex set of interacting cellular and leaf level processes (Kumar and Kasturi 2009; Ribeiro et al. 2004). *Fv/Fm* is used frequently as an expression of photoinhibition (Giaveno et al. 2007; Kitao et al. 2000). 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).

D1 protein is encoded by a small multigene family that consists of the psbA1, psbA2 and psbA3 genes. The psbA1 gene is non-functional, while the psbA2 and psbA3 genes are expressed in response to light (Tyystjärvi et al. 1998) and both encode the identical D1 protein (Guskov et al. 2009). The D1 protein despite being at the heart of the membrane protein complex of the photosystem II (PSII) reaction centre undergoes a very high turnover and is generally believed to be of importance for repair of photoinhibitory damage to PSII, since light-induced damage to the reaction centre of PSII should first hit the D1 protein. When thylakoids are exposed to excessive visible light, the PSII dimer dissociates into two monomers (Aro et al. 2005), but the most significant change takes place inside the monomeric PSII, where the reaction centre-binding D1 protein is photodamaged and degraded by specific proteases (Sudhir et al. 2005). Strong illumination of the grana may readily cause damage to the PSII complexes and endogenous cationic radicals, because the grana are rich in PSII complexes. Close association of light-harvesting protein complex of PSII (LHCII) with the PSII core complexes should also stimulate reactive oxygen species (ROS) generation in the grana. Unstacking of the thylakoid, which is also expected to lead to random distribution of the LHCII from the PSII core, may be important to avoid photodamage to

PSII. The recovery from photoinhibition requires an increased synthesis of D1 protein (Khatoon et al. 2009).

Proline formation requires high light intensity and high temperature enhances its accumulation (Shiraishi 1996). Proline content increase is due to *de novo* synthesis, as observed by Fedina et al. (2002). Some plants accumulated several times more proline in light than in darkness (Hadidi et al. 2007; Hayashi et al. 2000). Many studies have found a positive correlation between the accumulation of proline and osmotolerance in plants (Ferdausi et al. 2009; Kamran et al. 2009). The role of proline in osmoprotection in plants remains controversial. It has been established that proline not only acts as a mediator of osmotic adjustment (Ferdausi et al. 2009), but also as a stabilizer of subcellular structures (Samuel et al. 2000), a scavenger of free radicals (Chen and Dickman 2005) and a major constituent of cell wall structural proteins in plants in morphogenesis (Chen et al. 2006). According to Saradhi et al. (1995) UV radiation induced proline accumulation has an important role in protecting plants against UV radiation promoted peroxidative processes.

Jatropha curcus L. belonging to the family Euphorbiaceae is a multipurpose shrub or small tree. It can be cultivated in all tropical and sub-tropical regions (Openshaw 2000). The present study will help in revealing information on the role of the adaptive responses in combating stress produced under different light conditions. Also, the study will open new insights into the tolerance of *J. curcus* plant under light stress. The analysis of fluorescence emission spectra may accurately indicate the onset of stress in plants. Even leaf level fluorescence measurements might be useful for physiological control of agricultural crops and for detecting plant stress in terms of Chl inhibition. The objective of the present study was evaluation of photosynthetic performance, analysis of the PSII core protein D1 and proline accumulation in different light intensity of *J. curcus* plants.

MATERIALS AND METHODS

Growth conditions: For seedling production in *J. curcus* certified seeds were obtained from the local market and treated with $HgCl_2$ (0.1%) before sowing. The seeds were germinated and grown in soil in plastic pots (22 cm) in the botanical garden of the Banasthali University.

Light treatment: For light treatment soil cultures of experimental plants were grown under different light intensity (10-1200 µmol m^{-2} s^{-1}) in sun and shade conditions. Measurements were observed on one year old plants of *J. curcus*.

Measurements of chlorophyll a fluorescence parameters: Chl *a* fluorescence of PSII was measured on attached leaves with a Mini-PAM fluoremeter (Walz, Effeltrich, Germany) using the protocol described by Kumari et al. (2005). The instant yield measurements of photosynthesis usually began at 8:00 A.M., 10:00 A.M., 12.00 P.M., 2.30 P.M. and 5.00 P.M. and are referred to as morning, forenoon, midday, early afternoon and late afternoon respectively. In the light curve programme, light intensity is increased in 8 steps each after 30 seconds. All light curves were recorded in the morning between 8 and 10 A.M. to minimize the buildup of acute photoinhibition before the measurements.

Pigment content determination: The content of Chl *a*, *b* and Cartenoid (Car) in leaf samples was determined in 80% acetone following the method of

Arnon (1949) using a double beam UV-VIS spectrophotometer (UV5704SS).

Isolation of Thylakoids: Thylakoid membranes were isolated as described by Chakraborty and Tripathy (1992). The sample preparations were protected from light and kept ice-cold during the isolation procedure. Isolated thylakoids were stored at -80 °C.

SDS-PAGE and Western Blotting: Thylakoid membrane proteins were separated by SDS-PAGE according to Laemmli (1970) in stacking and resolving gels 12.5% (containing 2 M urea) and 5% respectively. For Western blotting, electrophoresed proteins were immediately electrotransferred onto nitrocellulose membrane. (D1 primary antibody was kindly provided by Prof. E. M. Aro, University of Turku, Finland). Image was analyzed using the software package Kodak Digital Science 1D Image Analysis with the profile 1 gel densitometer application.

Proline Measurement: For proline determination, 0.1 g of fresh leaf tissue was taken from the plants and proline content was measured according to Bates et al. (1973).

Statistics: The statistical analyses were done using the *Indostat* software. The one-way ANOVA pertaining to all observations in drought stress resulted in 5% level of difference during different days of the treatments. Results are presented as means ± SD.

RESULTS AND DISCUSSION

Effect of light stress on photosynthetic performance

J. curcus plants were grown under controlled environmental conditions in the botanical garden, Banasthali University. The plants were located in sun (high light) and shade (low light) conditions. Instant yield measurements and light curves were made on one year old plants. The instant measurements in the experimental plants were made at different times of the day. The results of various photosynthetic parameters as measured by Mini-PAM on *J. curcus* are given in the Fig. 1. Fv/Fm of *J. curcus* in sun and shade plants was found 0.798 ± 0.035 and 0.827 ± 0.009 respectively. Actual PSII efficiency was also affected by the increasing solar intensity throughout the day. In *J. curcus* plant, $\Delta F/Fm'$ was reduced during the middle part of the day followed by a slight recovery in afternoon. $\Delta F/Fm'$ value was highest in shade plants 41% as compared with the sun plants. The photosynthetic photon flux density *(PPFD)* values were 20 folds higher in sun plants exposed to strong intensity of light as compared to shade plants. Plants of high light intensity showed higher electron transport rate *(ETR)* (53.5 µmol m^{-2} s^{-1}) than the plants in shade (6.6 µmol m^{-2} s^{-1}, respectively). In *J. curcus* plants 92% increase in non photochemical quenching *(NPQ)* was observed in the low intensity instead shade plants.

Light curves charactetize intrinsic photosynthetic capacity of the leaves. Light curves were recorded on the same day of the experiment. These results are presented in Table 1. The cardinal points studied revealed that saturating *PPFD* ($PPFD_{sat}$) *was* 812 and 533 µmol m^{-2} s^{-1} in sun and shade plants respectively. The ETR_{max} reached a value of 79.3 ± 20.2 µmol m^{-2} s^{-1} in sun plants as compared with shade plants (38.6 ± 8.4 µmol m^{-2} s^{-1}). $\Delta F/Fm'_{sat}$ was lower 31% in sun exposed plants when compared with shade plants.

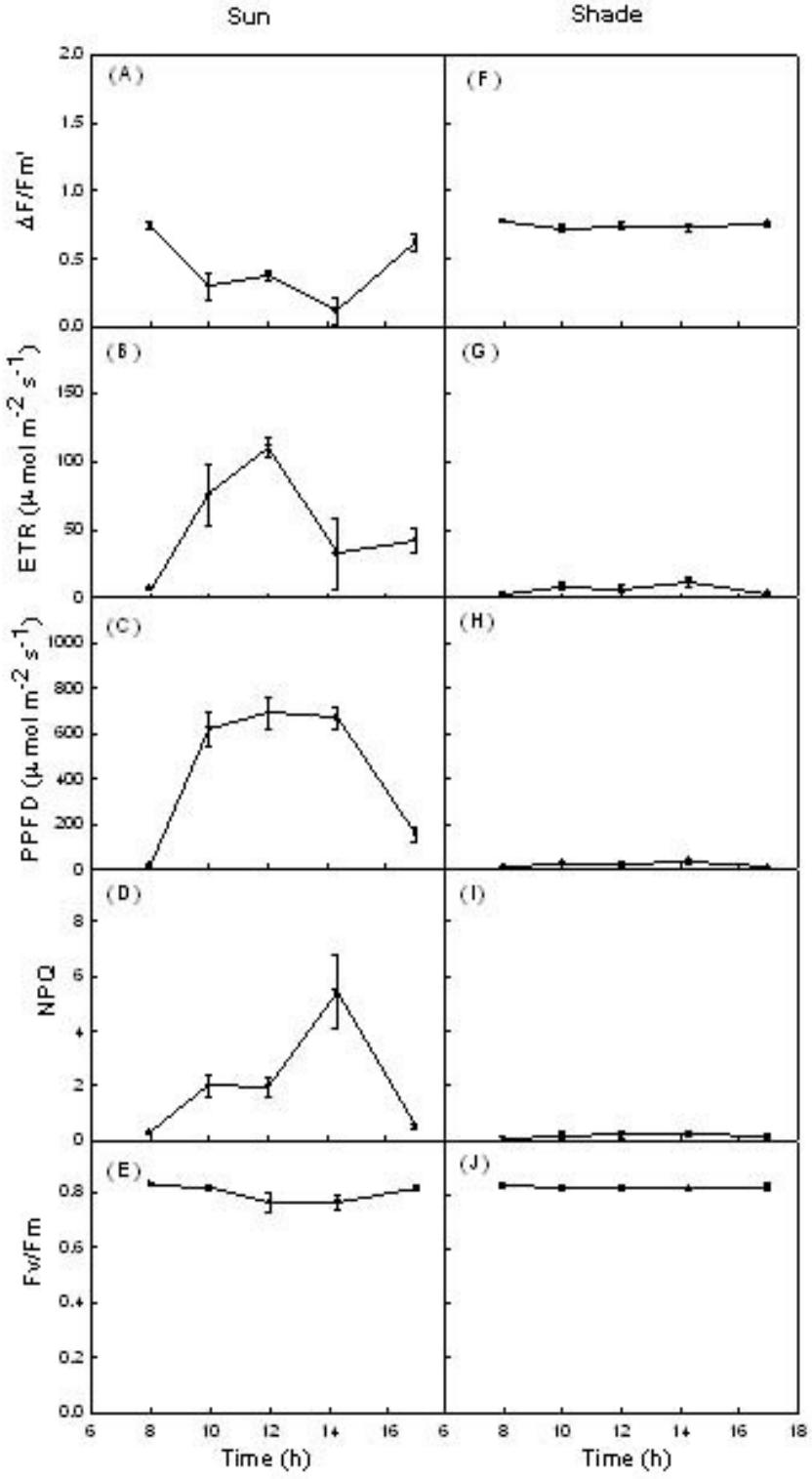

Fig. 1. Day course measurements of different parameters as (A, F) *ΔF/Fm',* (B, G) *ETR,* (C, H) *PPFD,* (D, I) *NPQ* and (E, J) *Fv/Fm* of one year old *J. curcus* plants grown under sun and shade conditions. Individual readings (n=10) are averaged and are presented with SD.

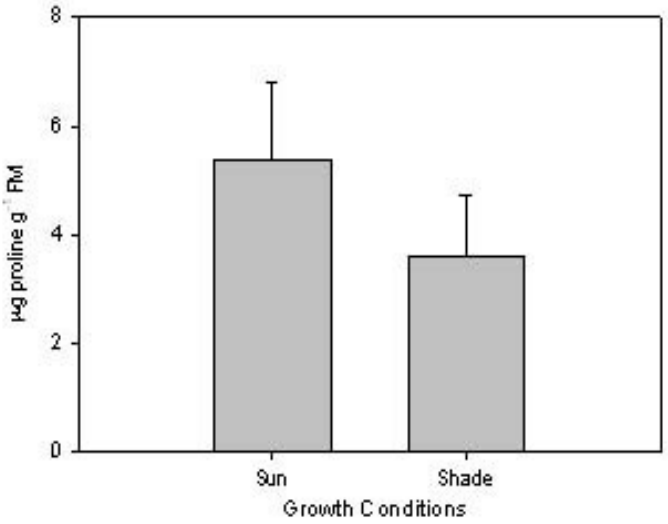

Fig. 2. Proline accumulation in one year old plants of *J. curcus* grown in sun and shade conditions.

Fig. 3. Western blot analysis of D1 protein under sun and shade coditions in one year old *J. curcus* plants.

Table 1. Cardinal points of the regression lines of saturating photosynthetic photon flux density ($PPFD_{sat}$) and half saturating $PPFD_{sat}$, effective quantum yield of PSII at saturating *PPFD* ($\Delta F/Fm'_{sat}$) and at half saturating *PPFD*, maximum electron transport rate (ETR_{max}) and half ETR_{max} obtained during light curves measurements of two months old plant of *J. curcus* in sun and shade conditions.

Growth Conditions	ETR_{max} (µmol m^{-2} s^{-1})	½ ETR_{max} (µmol m^{-2} s^{-1})	$PPFD_{sat}$ (µmol m^{-2} s^{-1})	½ $PPFD_{sat}$ (µmol m^{-2} s^{-1})	$\Delta F/Fm'$	½ $\Delta F/Fm'$
Sun	79.3 ± 20.2	39.6 ± 10.1	812 ± 102	143 ± 32	0.18 ±0.03	0.59 ±0.07
Shade	38.6 ± 08.4	19.3 ± 04.2	533 ± 52	079 ± 21	0.26 ± 0.06	0.63 ± 0.03

Table 2. Measurement of pigment (Chl and Car) content in one year old *J. curcus* plants under different light conditions.

Growth Conditions	Car (mg g^{-1} FM)	Chl b (mg g^{-1} FM)	Chl a (mg g^{-1} FM)	Tot. Chl (mg g^{-1} FM)	Chl a/ b	Car/Chl
Sun	4.08 ± 0.01	0.18 ± 0.00	0.43 ± 0.00	0.61 ± 0.00	2.39 ± 0.06	6.66 ± 0.06
Shade	8.29 ± 0.10	0.40 ± 0.00	1.03 ± 0.00	1.43 ± 0.00	2.55 ± 0.07	5.76 ± 0.04

The Chl fluorescence yield in the dark-adapted state was also affected by temperature. The *Fv/Fm* value reflects the potential quantum efficiency of PSII and it is used as a sensitive indicator of plant photosynthetic performance (Netto et al. 2002). High temperature stress reduced the *Fv/Fm* ratio, as well as *ΔF/Fm'* indicating that a structural and functional disorder of the photosynthetic apparatus and damage to the PSII machinery (Pereira et al. 2000; Murkowski 2001). Reductions in the *Fv/Fm* ratio and *ΔF/Fm'* under high temperature stress suggested damage to an important portion of the PSII reaction centre in *Jatropha* plants. This damage was associated with structural modification on PSII, especially in D1 protein, which in conditions of heat stress, was phosphorylated and degraded afterwards (Langjun et al. 2006). Moreover, reduction in the *Fv/Fm* ratio also suggested the occurrence of photoinhibition, also known as photodamage (Colom and Vazzana 2003). Accumulation of reduced electron acceptors may increase the generation of reactive radicals such as active oxygen species (AOS), which can induce oxidative injuries (Souza et al. 2004). These oxidative injuries could enhance Chl degradation or the inhibition of its biosynthesis (Papadakis et al. 2004), damage PSII components (Souza et al. 2004) and may inactivate chloroplast enzymes, especially those participating in CO_2 assimilation (Dekov et al. 2000).

The *Fv/Fm* values at various periods of dark adaptation of leaves can be used to indicate the level of photoinhibition, i.e. acute or chronic. In general, low-light grown plants are more susceptible to photoinhibition than sun plants or high-light grown plants. Since photoinhibition has a potential to lower productivity and plant growth, avoidance of photoinhibition is critical for the fitness and survival of plants in natural habitats. Short-term photoinhibition is thought to be due to the buildup of an electrical gradient across the thylakoid membrane (Maxwell and Johnson 2000). We also noted that the increase in *PPFD* was accompanied by a decrease in *ΔF/Fm'* in *J. curcus* plants. High value of *ΔF/Fm'* shown in healthy plants, where infected plants presented a significant decrease in this parameters. The relation *ΔF/Fm'* provided a measure of the proportion of the light absorbed by the Chl associated with PSII what is used in photochemistry; PSII is considered an important sensitive target to environment stresses. Thus, the decline in *ΔF/Fm'* due to increasing photosynthetic active radiation *(PAR)* may reflect a closure of the reaction centres (Medina et al. 2002).

High light plants also displayed symptoms associated with photoinhibition such as reduced Chl content and necrosis of the leaf. Electron transport is inhibited under high temperature stress. It can restrict crop growth and productivity (Chang et al.

2003) and is likely to become an increasingly important factor with the changing climate conditions. Inactivation of PSII and thylakoid disorganization is considered as a key feature of high temperature stress (Daniel et al. 2000). Usually, the main contributor to *NPQ* is termed high energy state quenching and it is thought to be essential in protecting the leaf from light-induced damage (Bergantino et al. 2003). Changes in PSII activity can occur owing to changes in thylakoid membrane structure and organization. Such modifications will result in changes in all parameters of photosynthetic performance; indicate loss of PSII reaction centres or increase in *NPQ* (Maxwell and Johnson 2000). From these measurements we indicate that photosynthetic activity decline under high light conditions compared with shade plants.

Effect of light stress on pigment content

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Pigment content is higher in leaves of shade plant as compared with sun leaves (Table 2). The increase of the total Chl content in shade (1.43 mg g^{-1} FM) than in sun leaves (1.10 mg g^{-1} FM). This would show that sun leaves possess thicker cell walls, lower leaf water content and higher dry weight than shade leaves. Car is a large class of isoprenoid molecules, which are *de novo* synthesized by all photosynthetic and many non-photosynthetic organisms. Car content was higher in shade plants (22%) as compared with sun 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.

Shade plants possess much higher amounts of LHCII than sun-exposed plants and consequently, a/b ratio are higher than in sun-exposed plants (Netto et al. 2002). In all plants amount of Chl *a* was found greater than Chl b however Chl *b* was significantly increased in shade plants. Chl b is most abundant in the antennae of the light harvesting complex, whereas Chl a is concentrated around PSII. To capture as much light as possible, shade-grown plants typically have more light-harvesting complexes per unit area than do sun-grown plants that typically receive more light than needed. Therefore, it was not surprising that the Chl b content was higher in the shade-grown plants. The decrease in Chl b content in sun plants could be an indication of Chl destruction by excess irradiance.

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 photooxidation in lower light conditions. In case of *J. curcus*, where the ratio a/ b increased in the low intensity plants due to less synthesis of Chl a than to the reduction of photooxidation of Chl b in the shade. It is notable that the conditions of both Chl a and b were observed to increase under low light conditions (Wijanarko et al. 2007). We demonstrate a significant positive correlation between sensitivity to photoinhibition and Chl content of *J. curcus* leaves.

In addition to low water content and higher water saturation deficit, the sun plants have higher osmotic pressure values than the shade ones (Kornyeyev et al. 2004).

Effect of light stress on D1 core protein

In the present study, we have primarily focused on the rate of D1 protein degradation in the leaves of the *J. curcus* plant under varying light regimes. In our gels the D1 protein (32 kDa) detected after western blotting on the nitrocellulose membrane. Our data indicate that PSII membrane fragments are advantageous to study degradation of the D1 protein in the shade plants. Mean intensity and area of the band was measured in *Jatropha* plants under different light intensity by gel documentation. We found those parameters higher in sun plants, 1.1 and 1.7 folds as compared with shade plants due to the high turnover rate.

Shade leaves of *J. curcus* plants more susceptible to photoinhibition compared with high light-grown plants is a slower rate of D1 protein degradation at high light and thus, their slower repair cycle of the photodamaged PSII centres. This slower rate of D1 protein degradation is consistent with the stable long-term accumulation of photoinhibited PSII reaction centres in low-light and shade plants (Kornyeyev et al. 2004). Photoinhibition of photosynthesis is an extremely complex phenomenon, comprising both damaging and protective events, including down-regulation of PSII and reversible and irreversible photoinactivation of PSII electron transport, as well as the concomitant repair of photodamaged PSII centres via *de novo* synthesis of the D1 protein (Mahmudov et al. 2005; Alfonso et al. 2000; Sudhir et al. 2005).

Sun and high-light plants have different strategies to help combat photoinhibition, including a higher capacity for photosynthesis (Netto et al. 2002), a more active PSII repair cycle (Ferreira et al. 2007; Wijanarko et al. 2007). Shade and low light plants have very limited capacity for the PSII repair cycle (Tyystjärvi et al. 1992). D1 protein synthesis not only factor limiting the repair of photodamaged PSII centres in higher plants, but also the rate of D1 protein degradation plays a regulatory role. Photoinhibition occurs when the rate of light-induced damage to PS II centres is greater than the repair capacity (Huylenbroeck et al. 1997). However, the repair of photodamaged PSII centres constitutes a complex cycle, including degradation of photodamaged D1 protein, *de novo* synthesis and insertion of newly synthesized D1 protein into a PSII complex, possible migration of PSII complexes between appressed and nonappressed thylakoid regions and finally the activation of the PSII complex (Aro et al. 1993).

Effect of proline under light stress

The average proline contents in sun leaves are higher than in shade leaves of *J. curcus* plants. The proline range of 5.37 ± 1.45 and 3.62 ± 1.10 μg g^{-1} FM in sun and shade plants, respectively. Fig. 2 shows that variations of the proline content are wider in magnitude in sun than in shade plants. The sun leaves contain less water content as compared with shade plant. The coincidence of the proline increase with the decrease in water content and the increase in water saturation deficit and the osmotic pressure of the cell sap indicate the relationship. Shading through its effect on the water status of the plants resulted in reducing the accumulation of proline in the photosynthetic organs (Aldesuquy and Ibrahim 2001).

The low proline content in shade plants may be attributable to different factors including low

transpiration, hence the water stress is reduced and the proline content remains low. Proline synthesis in the *J. curcus* plant under stress is greatly reduced in the dark, indicating that the reducing power produced during photosynthesis might be involved in the biosynthetic pathway. Differences in proline contents in sun and shade are comparable to those in water saturation deficit (Toteva et al. 2004). Aldesuquy and Ibrahim (2001) found that there were significant negative correlations between proline concentration and shoot water content (as a percentage of shoot fresh weight) for seven genotypes of spring wheat.

CONCLUSION

J. curcus has been selected in the present study to evaluate and analyze the changes occurring in its ecophysiology. In this work, we indicate that in all measurements, the decrease of photosynthetic parameters as *Fv/Fm*, *ETR*, *ΔF/Fm'* under high light conditions. This suggests the occurrence of chronic photoinhibition due to photoinactivation of PSII centres, possibly attributable to D1 protein damage but turnover rate is very high in sun exposed plants. Sun light always fluctuates with clouds and chloroplasts tend to receive excessive light energy that can result in photooxidation. The exposure to photooxidation leads to the irreversible damage in photosystems and consequently has an overall inhibitory effect on plant productivity. Finaly we conclude that the effects of different light regimes on photosynthesis using biochemical and molecular analysis suggested acclimation and adaptive responses to plant stress conditions, resulting in improve stress tolerance and plant breeding strategies leading to better yield.

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