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Photoinhibition and photosynthetic acclimation of rice (*Oryza sativa* L. cv Jyothi) plants grown under different light intensities and photoinhibited under field conditions

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Thirty-days old rice (*Oryza sativa* L. cv. Jyothi) plants grown under the greenhouse (150-200 µmol m^{-2} s^{-1}) or shade (600-800 µmol $m^{-2}s^{-1}$) were exposed to 7 days of full sunlight and compared with plants grown under direct sunlight (1200-2200 µmol $m^{-2}s^{-1}$).Transfer of greenhouse and shade plants to full sunlight for a day resulted in a decline in their photosynthetic efficiency (F_v/F_m) and an increase in non-photochemical quenching (qN). The decline in F_v/F_m was much greater in transferred greenhouse plants (33%) as compared to transferred shade-plants (20%). Sun-plants did not show much variation in the F_v/F_m ratio (4%) from their predawn measurements (control). The sun-grown plants showed a higher pool of xanthophyll pigments (violaxanthin + antheraxanthin + zeaxanthin). Transfer of greenhouse and shade-plants to full sunlight resulted in an increase in lutein, Chl *a/b* ratio, antheraxanthin (A) and zeaxanthin (Z) content. Increase in A and Z was correlated with the increase in the qN. The increase in the A and Z content was due to increase in the activity of violaxanthin de-epoxidase. Greenhouse and shade plants on exposure to sunlight showed an increase in lipid peroxidation (LPO). Prolonged exposure of greenhouse and shade plants up to 7 days resulted in recovery of the F_v/F_m, an increase in Z and A and a decline in the LPO. The study demonstrated that rice plants grown at lower light intensities initially underwent photoinhibitory damage on exposure to full sunlight, but were able to acclimate to the high irradiance by dissipating the excess light through various mechanisms such as an increase in lutein, high Chl *a/b* ratio and xanthophyll cycle, suggesting use of energy dissipation as a mechanism of protection against high irradiance, but to different extent and to some extent by different processes. The study was unique, as plants were grown and photoinhibited under natural conditions rather than the artificial light, as was the case in most of the studies so far. Results showed better adaptation of high-light grown plants and suggested role for chl a/b ratio and lutein, in addition to xanthophylls cycle in shade plants. Low-light grown plants could also completely adapt to full level of sunlight within 3 days of the treatment and xanthophylls cycle (measured as V, A and Z) and activity of de-epoxidase seemed to be important in this adaptation.

Keywords: Energy dissipation, Lipid peroxidation, Non-photochemical quenching, Photosynthetic acclimation, Photosynthetic efficiency, Rice plants, Sunlight, Xanthophyll cycle, Chlorophyll a/b ratio, Violaxanthin de-epoxidase

Photoinhibition occurs when light energy absorbed by plants exceeds the capacity of light utilization in photosynthesis[1,2]. The excess of absorbed light energy leads to production of reactive oxygen species (ROS) and damage the photosynthetic apparatus, if not dissipated safely[3]. Due to its specific physiochemical properties, photosystem II (PSII) is more susceptible to photoinhibition and/or photo-oxidative damage than photosystem I (PSI)[4]. Photoinhibition of PSII can be easily detected *in vivo* by a decrease in the 'dark-adapted' ratio of variable to maximum chlorophyll *a* fluorescence[5]. Photo-oxidative damage to cellular lipids can be used to indicate oxidative damage to biological membranes[6]. The susceptibility of plants to photoinhibition depends on the species and growth light environments. In general, the plants grown at low-light intensities are more susceptible to photoinhibition than at high-light intensities[3]. Since photoinhibition lowers plant growth and productivity, its avoidance is essential for the survival of plants in natural habitats for which plants have developed various processes[7].

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Abbreviations: A, antheraxanthin; Chl, chlorophyll; DPS, de-epoxidation state; F_v/F_m. photosynthetic efficiency of photosystem II; LHCII, light harvesting complex II; L, lutein; LPO, lipid peroxidation; MDA, malonaldehyde; PFD, photon flux density; PSI. photosystem I; PSII. photosystem II; qN, non-photochemical quenching; qP, photochemical quenching; TBA, thiobarbutaric acid; TCA, trichloroacetic acid; V, violaxanthin; VDE, violaxanthin de-epoxidase; XCP, xanthophyll cycle pigments; Z, zeaxanthin.

The photosynthetic acclimation is important for the survival of plants. Many plant species undergo physiological changes upon exposure to high-light environments to prevent physiological damage or injury[8]. Plants rely largely on various morphological and biochemical adaptations such as thick leaves, high concentration of photosynthetic elctron transport constituents and Calvin cycle enzymes, large pools of xanthophyll cycle pigments and a high capacity for photoprotective energy dissipation, compared to those acclimated to low-light conditions[9].

Protection of photosynthesis from high-light stress in the natural environment is provided via the thermal dissipation of excess energy in PSII, measured as non-photochemical quenching[1], a process that requires the functioning of xanthophyll cycle. The process is mediated by the enzyme violaxanthin de-epoxidase (VDE) which converts di-epoxide violaxanthin (V) to epoxide-free zeaxanthin (Z) via a mono-epoxide intermediate antheraxanthin (A)[1]. VDE, a 43 kDa nuclear-DNA encoded protein[10] has a pH optimum of 5.2[11] and requires ascorbate as a reductant[12]. It is a water soluble at neutral or alkaline pH[11]. Under high-light conditions, it is activated by a decrease in the pH of thylakoid lumen and binds the membranes, but is inactive in the dark, when the pH in thylakoid lumen is neutral or alkaline[13]. The de-epoxidised xanthophyll cycle pigments participate in dissipation of excess excitation energy in the PSII antennae[2,14]. In addition to their role in energy dissipation, xanthophylls are also prevent oxidation of thylakoid membrane lipids by ROS[15,16].

Most of the work on photoinhibition has been carried out by growing plants under artificial low-light conditions and exposing them to artificial constant high-light under laboratory conditions. In the present study, we have grown the rice (*Oryza sativa* L. c.v. Jyothi) plants in three different growth conditions i.e., greenhouse, shade and direct sunlight. Greenhouse and shade grown plants have been subsequently transferred to full sunlight for a period of 7 days to compare the extent of photoinhibitory damage (F_v/F_m and lipid peroxidation) and protective mechanisms (xanthophyll cycle) with reference to study thier adaptation. We have used natural sunlight as photoinhibitory treatment in order to consider the fluctuations in the light intensities during the course of the treatment. All the experiments have been carried out using only the third leaf of 30-days old plants.

Materials and Methods

Plant material and growth conditions

Rice (*Oryza sativa* L. cv Jyothi) plants were grown in earthen pots (diameter 20 cm) containing garden soil and vermiculite in the ratio 3:1. The plants were grown for 30 days under three different growth environments. One set of plants was grown in the greenhouse (referred to as greenhouse plants), where the photon flux density (PFD) received by the plants ranged from 150-200 μmol $m^{-2}s^{-1}$ and the relative humidity (RH) was 85-90%. The second set of plants were grown in the shade (referred to as shade plants) by placing in the shadow of a wall of a building, where the peak PFD varied between 600-800 μmol $m^{-2}s^{-1}$ and RH was 70-75%. The third set of plants were grown outdoors under fully exposed conditions of sunlight (referred to as sun plants) and the peak PFD varied between 1200-2200 μmol $m^{-2}s^{-1}$ RH being 70-75%. All the pots were well watered.

Treatment conditions

Experiments were performed at Goa University from January to April 2005 and 2006. After 30 days of growth, the plants were about 20 cm in height and had three leaves in all the three growth conditions. Leaf three (youngest) was used for all measurements. Control measurements were taken at 1030 h, prior to transfer to full sunlight in the case of greenhouse and shade plants, while in sun plants, the control leaf samples were collected at predawn (0630 h). Leaf samples were collected at 1130 h (1 h), 1330 h (3 h) and 1630 h (6 h) on the 1st day and also at 1630 h of the 3rd, 5th and 7th day after transfer to full sunlight to determine the extent of acclimation under high-light conditions. The average PFD at 1130, 1330 and 1630 h was 2050, 2156, and 1336 μmol $m^{-2}s^{-1}$, respectively.

Chlorophyll fluorescence

Chlorophyll (chl) fluorescence measurements were performed on intact leaves[2] using a pulse amplitude modulation fluorometer (PAM 101, Walz, Effelrich, Germany). Rice leaves were dark-adapted for 10 min prior to measurements at room temperature. The dark-adapted leaves were exposed to a modulated light with an intensity of 4 μmol $m^{-2}s^{-1}$ to measure initial fluorescence (F_o). This was followed by an exposure to a saturating pulse of white light of 4000 μmol $m^{-2}s^{-1}$ to provide the maximum fluorescence (F_m). After measurement of F_m, leaves were allowed to reach steady-state fluorescence (F_s), while exposed to

actinic light intensity of 330 μmol m^{-2} s^{-1}. Another burst of saturating light at Fs state was used to measure F'_m. After reaching the steady-state again, leaves were exposed to far-red radiation light to measure F'_o. Calculations were carried as described[17]. Maximal efficiency of PSII (F_v/F_m) was estimated from the variable to maximum fluorescence ratio F_v/F_m = (Fm-F_o)/F_m. Non-photochemical quenching coefficients were calculated as (qN) = 1- (F'_m-F'_o)/ (F_m-F_o).

Extraction of photosynthetic pigments

The leaf sample (0.25 g) was homogenizedin 100% acetone to a final volume of 5 ml extract using a mortar and pestle[2] and the homogenate was then incubated overnight at 4°C in the dark. Extract was centrifuged at 4000 *g* for 10 min and the supernatant was stored at -70°C freezer for subsequent HPLC analysis of pigments. The sample was filtered through 0.2 μm-nylon filter prior to loading on HPLC.

Assay of VDE activity

The 2.4 ml of 50 mM sodium acetate-HCl buffer (pH 4.9) was added to 0.1 ml of water, 0.1 ml of suspension of PSII particles and 0.3 ml of VDE extract[18]. The reaction was started by the addition of 0.08 ml of 0.8 M sodium ascorbate and the mixture was incubated at 30°C for 3 h. The reaction was stopped by the addition of 0.09 ml of 0.2 M dithiothreitol to quench VDE activity. The particles were collected by centrifugation at 8000 *g* for 60 min and extracted with 1 ml acetone: methanol (7:2, v/v) by vortexing, followed by centrifugation at 8000 *g* for 30 min[18]. To the supernatant, 0.08 ml of dichloromethane was added. Dichloromethane was mixed with 1/100 vol. of 1 M Tris-HCl buffer (pH 8.0) to prevent the acidification that causes the rearrangement of 5,6-epoxide of violaxanthin to the furanoid-5, 8-epoxide. The colorless insoluble materials were precipitated by centrifugation at 8000 *g* for 30 min and supernatant was used for analysis of xanthophylls cycle pigments (XCP) by HPLC (Waters)[2].

The PSII particles from the control and one day sun-exposed leaves were prepared as described previously[19]. The 20 g of leaves were macerated with 10 ml of 50 mM Na-K phosphate buffer (pH 6.4), 0.3 M sucrose and 100 mM NaCl (1:1:1, v/v) to which 30 ml of Triton-X 100 (2%, w/v) was added. The mixture was filtered through eight layers of muslin cloth and the filtrate was centrifuged at 10,000 *g* for 60 min at 4ºC. The pellet was suspended in 1 ml of 40 mM Na-K phosphate buffer (pH 6.4) and centrifuged at 1000 *g* for 1 min and the supernatant was then centrifuged at 10,000 *g* for 60 min at 4ºC. The resulting pellet was washed twice with 6 ml of 25 mM MES-NaOH buffer (pH 6.5), 0.3 M sucrose and 10 mM NaCl (1:1:1 v/v) and suspended in 1 ml of the same medium. Extraction of VDE was carried out as described elsewhere[18]. The chloroplast suspension (5 ml) containing thylakoid membranes were washed twice with 10 ml of 50 mM Tris-HCl buffer (pH 7.5) and 1 mM $MgCl_2$ (1:1, v/v) by centrifugation at 8000 *g* for 30 min at 4ºC. The washed thylakoid membranes were suspended in 5 ml of the same buffer and sonicated for 20-times for 12 s at 10 s intervals. The suspension was centrifuged at 10,000 *g* for 60 min at 4°C and the supernatant was designated as the VDE extract.

Identification of xanthophyll cycle pigments (XCP)

Identification and separation of pigments was carried out using a reverse-phase column (Waters Spherisorb ODS 2 5 μm, 4.6 mm * 250 mm) and a detection programme (Waters 2996 Phase diode array detector)[2]. 20 µl of the pigment extract was injected into the HPLC and the gradient for separation was 0-100% ethyl acetate in acetonitrile/water (9:1) over 32 min, with a flow rate of 1.2 ml/min. The pigments were detected at 450 nm and quantitated on a fresh weight basis from peak area value using β-carotene as external standard. Zeaxanthin was well seperated from lutein. The de-epoxidation state (DPS) of xanthophyll cycle pigments was calculated as: DPS = (A + Z)/(V + A +Z)[20], where Z is zeaxanthin, A is antheraxanthin and V is violaxanthin.

Peroxidation of cell membrane lipids

Lipid peroxidation (LPO) was determined by the production of TBA-MDA adduct formation, which signifies the formation of polyunsaturated fatty acid peroxides in biological systems. The 0.5 g of leaf tissue was ground in 0.5% trichloacetic acid (TCA) and the homogenate was made up to 5 ml and centrifuged at 6000 *g* for 15 min. The supernatant was collected and used for measuring the peroxidation of membrane lipids. The 1 ml of supernatant was added to the test tube containing 2.5 ml of freshly prepared (0.5%) TBA in (20%) TCA and 2.5 ml of incubation buffer (50 mM Tris HCl and 175 mM NaCl, pH 8) and allowed to incubate for 30 min at 90°C in a water bath. After incubation, it was allowed to cool and

centrifuged for 2 min at 1000 *g* to settle the debris and non-specific precipitation. The optical density (OD) was taken on spectrophotometer at 532 nm and 600 nm (UV-2450 UV-Visible spectrophotometer, Schimadzu). The absorbance at 600 nm was subtracted from that at 532 nm to correct for the non-specific turbidity. The amount of MDA-TBA adduct formed was quantitated[6,15] by using extinction coefficient of MDA 155 μmol $cm^{-1}m^{-1}$.

Statistical analysis

The experimental data were tested for significance by using a Students *t*-test for two samples, assuming either equal variances or unequal variances. All statistical tests were performed with analysis tools from Microsoft® office excel 2003. The probability of error (P<0.05) was noted, wherever appropriate.

Results

Effect of sunlight on Chl fluorescence

In the leaves of control greenhouse and shade plants (i.e. prior to transfer to full sunlight) and control leaves of sun plants (predawn), the maximum photochemical efficiency of PSII in the dark-adapted state, measured as F_v/F_m ratio was more or less same, but varied after exposure to sun (Fig. 1A). The F_v/F_m ratio in greenhouse plants decreased significantly by 46% after 3 h of transfer to sunlight (at 1330 h), however, in the case of shade and sun-plants, the decrease in F_v/F_m for the same duration of treatment (at 1330 h) was only 31% and 3%, respectively (Fig. 1A). Exposure of greenhouse and shade plants to 6 h sunlight till 1630 h resulted in a slight recovery (24 and 16%, respectively) of the F_v/F_m ratio, as compared to their respective F_v/F_m ratios measured at 1330 h on the 1st day (Fig. 1A). Three days of exposure to full sunlight resulted in a further recovery of the F_v/F_m ratio of greenhouse (39%) and shade plants (23%). Sun exposure for 5 days resulted in a recovery of 84% and 51% in greenhouse and shade plants respectively and by the end of 7th day, the F_v/F_m ratio was completely recovered and was more or less same as their respective control values (Fig. 1A).

The qN values of control greenhouse and shade leaves (prior to transfer) exceeded that of control sun leaves (predawn) (Fig. 1B). Induction of qN was stronger in greenhouse and shade plants on transfer to sunlight than in sun plants. Shade and greenhouse plants exhibited highest qN on the 1st and 3rd day, respectively after transfer to direct sunlight (Fig. 1B). Prolonged exposure till 7 days resulted in lowering of qN in both greenhouse and shade-plants than their respective values measured at the end of 1st day. In sun-plants, F_v/F_m and qN did not vary much throughout the 7 days period. Photochemical quenching (qP) also increased in greenhouse and shade-plants transferred to direct sunlight for 7 days (data not shown).

Effect of sunlight on photosynthetic pigments and xanthophyll cycle activity in leaves

Analysis of pigments showed high levels of violaxanthin (V) and low levels of antheraxanthin (A) and absence of zeaxanthin (Z) in control greenhouse, shade and sun plants, while transfer to sunlight resulted in a decline in V and accumulation of A and Z in the plants of all the three different growth conditions (Table 1). However, the sum increase in A and Z was not proportional to the decrease observed in V. Control sun plants (predawn) showed a higher pool of XCP (V + A + Z) (11.58 mg g^{-1} fresh weight; FW) than control greenhouse (8.09 mg g^{-1} FW) and shade (3.66 mg g^{-1} FW) plants.

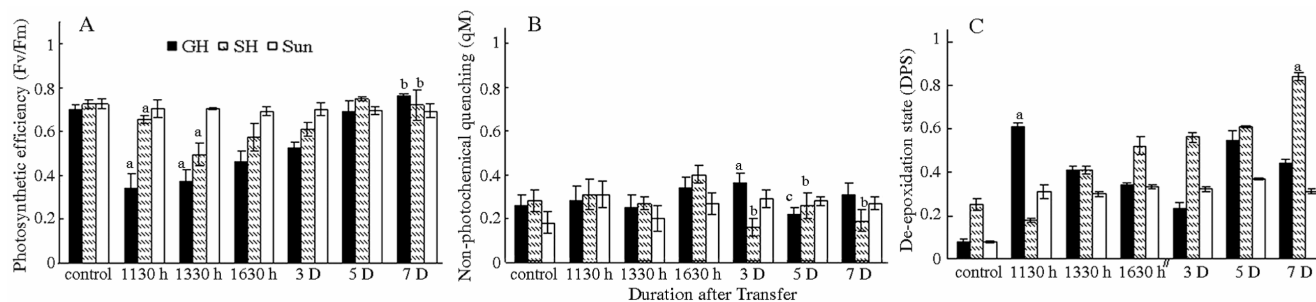

Fig. 1—Effect of sunlight on (A) photochemical efficiency (F_v/F_m), (B) non-photochemical quenching (qN) and (C) de-epoxidation state (DPS) in 30-days old rice plants grown in greenhouse (150-200 μmol m^{-2} s^{-1}), shade (600-800 μmol m^{-2} s^{-1}) and direct sunlight (1200-2200 μmol m^{-2} s^{-1}) [In greenhouse and shade plants control readings were taken before the plants were transferred to direct sunlight and in sun plants, control readings were taken at predawn (0630 h). (n = 3, ± SD). a - significantly different from control (p<0.05); b - significantly different from 1630 h (p < 0.05); c - significantly different from 3 D (p<0.05)]

Table 1—Effect of sunlight on pigment content (mg g^{-1}FW) of 30-days old rice plants grown in the greenhouse (150-200 µmol $m^{-2}s^{-1}$), shade (600-800 µmol $m^{-2}s^{-1}$) and direct sunlight (1200-2200 µmol $m^{-2}s^{-1}$)
In greenhouse and shade plants, control readings were taken before the plants were transferred to direct sunlight and in sun plants were taken at predawn (0630 h),(n =3, ± SD)

	Violaxanthin (V)	Antheraxanthin (A)	Zeaxanthin (Z)	V + A + Z	lutein (L)	Chl *a/b*
Glasshouse plants						
Control	7.44 ± 2	0.65 ± 0.04	0	8.09 ± 3	20 ± 7	0.18 ± 0.05
1130 h	0.45 ± 0.05	0.71 ± 0.01	0	1.16 ± 1	18 ± 5	0.12 ± 0.01
1330 h	3 ± 1	1.67 ± 1	0.42 ± 0.03	5.09 ± 2	15 ± 3	0.2 ± 0.02
1630 h	4.5 ± 2	2 ± 1	0.37 ± 0.01	6.87 ± 2	16 ± 4	0.18 ± 0.06
3 Day	7.7 ± 3	1.06 ± 0.05	0.15 ± 0.02	8.91 ± 4	12 ± 2	0.19 ± 0.01
5 Day	3.4 ± 1	2.34 ± 1	1.97 ± 0.08	7.71 ± 3	16 ± 3	0.26 ± 0.02
7 Day	3.11 ± 1	1.91 ± 0.08	0.58 ± 0.02	5.6 ± 2	22 ± 7	0.16 ± 0.03
Shade plants						
Control	2.74 ± 1	0.92 ± 0.06	0	3.66 ± 1	15 ± 3	0.2 ± 0.02
1130 h	5.33 ± 2	1.2 ± 0.07	0	6.53 ± 3	34 ± 8	0.16 ± 0.01
1330 h	2.4 ± 1	1.4 ± 0.04	0.33 ± 0.05	4.13 ± 2	20 ± 7	0.2 ± 0.04
1630 h	2.72 ± 1	1.65 ± 0.03	1.3 ± 0.06	5.67 ± 3	32 ± 6	0.19 ± 0.02
3 Day	3.6 ± 2	3.6 ± 1	1.17 ± 0.09	8.37 ± 4	24 ± 8	0.21 ± 0.05
5 Day	2.16 ± 1	2.6 ± 1	0.87 ± 0.04	5.63 ± 2	22 ± 5	0.16 ± 0.01
7 Day	0.5 ± 0.05	1.2 ± 0.08	1.56 ± 0.09	3.26 ± 1	12 ± 3	0.44 ± 0.07
Sun plants						
Control	10.6 ± 4	0.98 ± 0.04	0	11.58 ± 6	32 ± 6	0.75 ± 0.04
1130 h	6.21 ± 3	2.61 ± 0.09	0.2 ± 0.01	9.02 ± 5	26 ± 5	0.70 ± 0.04
1330 h	6.27 ± 2	1.91 ± 0.07	0.6 ± 0.03	8.78 ± 3	19 ± 4	0.78 ± 0.06
1630 h	4.97 ± 3	1.5 ± 0.04	1 ± 0.05	7.47 ± 2	14 ± 4	0.87 ± 0.06
3 Day	4.50 ± 2	1.3 ± 0.03	0.9 ± 0.04	6.7 ± 2	12 ± 2	0.88 ± 0.07
5 Day	4.65 ± 2	1.6 ± 0.02	1.2 ± 0.09	7.45 ± 3	14 ± 6	0.89 ± 0.08
7 Day	4.72 ± 3	1.2 ± 0.05	1 ± 0.05	6.92 ± 2	13 ± 4	0.86 ± 0.05

Analysis of other pigments indicated that control greenhouse and shade-plants (prior to transfer) had lower Chl *a/b* ratios and low levels of lutein compared to control sun plants (predawn) (Table 1). Chl a/b ratio showed many fold increase in sun-plants as compared to glasshouse and shade-plants. Transfer of glasshouse and shade-plants to full sunlight for a duration of 7 days increased the Chla a/b ratio only slightly. Exposure of shade plants to sunlight for a day also resulted in an increase in the lutein levels, in addition to A and Z, which was not observed in greenhouse and sun plants (Table 1).

Control greenhouse (prior to transfer to sun light) and sun plants (predawn) revealed similar xanthophyll cycle activity (0.08), measured as de-epoxidation state (DPS), while in comparison, control shade plants had a higher xanthophyll cycle activity (0.25), but low V + A + Z pool (Fig. 1C). Exposure of greenhouse, shade and sun plants to sunlight for a day resulted in an increase in the DPS by 325%, 108% and 312%, respectively. Increase in the xanthophyll cycle activity was correlated with the decline in V and an increase in A and Z (Table 1 and Fig. 1C). Prolonged exposure of greenhouse and shade plants to sunlight till 7 days resulted in a further increase of 29% and 61% in the xanthophyll cycle activity as compared to their respective control plants (Fig. 1A and B). While in sun plants, the xanthophyll cycle activity did not vary much during the 7 days period.

Effect of sunlight on violaxanthin de-epoxidase activity in isolated PSII particles

Results indicated highest VDE activity (refereed as DPS) under both control and treated conditions in the greenhouse plants, followed by the shade adapted plants and least activity was observed in sun plants (Fig. 2). The VDE activity increased in greenhouse, shade and sun grown plants after one day of sun exposure, as compared to prior to transfer to direct sunlight.

Effect of sunlight on peroxidation of cell membrane lipids

Prior to transfer to direct sunlight (control), greenhouse plants had lower MDA content (5.2 µmol $cm^{-1}m^{-1}$) than control shade (6.9 µmol $cm^{-1}m^{-1}$) and control sun (7.4 µmol $cm^{-1}m^{-1}$) plants (predawn) (Fig. 3). Transfer of greenhouse and shade plants to 6 h of direct sunlight resulted in an increase of 25% and 59%, respectively as compared to their respective control. Sun plants also exhibited an increase in the LPO level as the day progressed and at 1630 h on the 1st day had 45% higher levels than the control sun-plants (predawn).

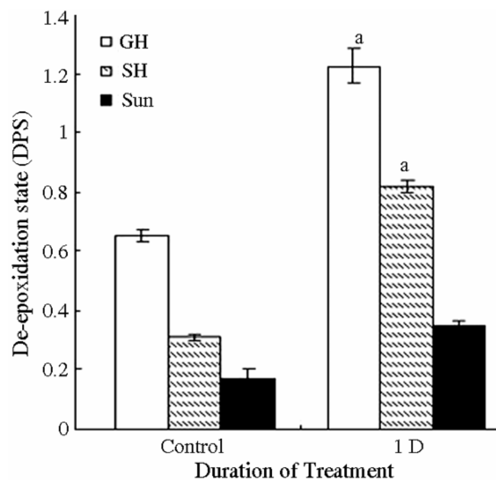

Fig. 2—Effect of sunlight on VDE activity in reactions carried out with PSII particles isolated from 30-days old control and 1 day sun-treated rice plants grown in the greenhouse (150-200 µmol m^{-2} s^{-1}), shade (600-800 µmol m^{-2} s^{-1}) and direct sunlight (1200-2200 µmol m^{-2} s^{-1}) [In greenhouse and shade plants, control refers to plants prior to transfer to direct sunlight, and in sun plants refers to predawn (0630 h). (n = 3, ± SD).). a - significantly different from control (*p*<0.05)]

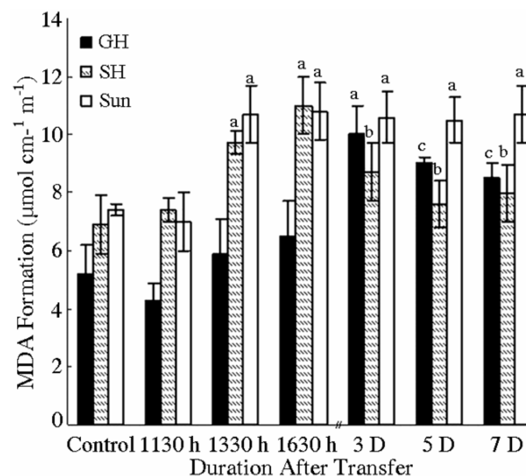

Fig. 3—Effect of sunlight on MDA-TBA adduct formation (µmol m^{-2} s^{-1}) in 30-days old rice plants grown in greenhouse (150-200 µmol m^{-2} s^{-1}), shade (600-800 µmol m^{-2} s^{-1}) and direct sunlight (1200-2200 µmol m^{-2} s^{-1}) [In greenhouse and shade plants, control readings were taken before the plants were transferred to direct sunlight and in sun plants control readings were taken at predawn (0630 h). (n = 3, ± SD). a - significantly different from control (*p*<0.05); b - significantly different from 1630 h (*p*<0.05); c - significantly different from 3 day (*p*<0.05)]

Exposure of glasshouse plants to 3 days of sunlight resulted in an increase in the MDA content to 92% than that of control plants. Seven days exposure to sunlight resulted in a slight decline (29%) in the LPO level, as compared to that observed at the end of the 3rd day, but was still 63% higher than the control greenhouse plants. In shade plants, the LPO level declined on prolonged exposure up to 7 days, but was still 15% higher than the control shade plants. The extent of LPO did not vary much in sun plants during the 7 days period.

Discussion

This study examined adjustments in photosynthetic function in rice plants transferred from greenhouse and shade (low-light) conditions to direct sunlight (high-light) and results were compared with the plants grown under direct sunlight. Acclimation of plants to different light conditions has profound influence on the structure and function of photosynthetic apparatus[27,28]. Photosynthetic efficiency of PSII was significantly reduced upon high-light exposure in greenhouse plants than in shade and sun plants (Fig. 1A). The adaptation of shade and sun plants to high-light was reflected by the low degree of photoinhibition of PSII occurring after one day of transfer to direct sunlight. Results indicated that low-light grown plants (greenhouse plants) were more sensitive to high-light stress and had less capacity for photosynthetic acclimation, following transfer to full sunlight than high-light grown plants. This was evident by an inability to attain Fv/Fm values by the glasshouse plants, similar to those grown at higher light intensities (shade and sun plants) within the same durations.

Prolonged exposure till 7 days to full sunlight resulted in photosynthetic acclimation of low-light grown plants to high-light. Since rice plants are normally grown under high-light conditions, greenhouse and shade plants exposed to full sunlight for a period of 7 days adapted to the high-light conditions by managing the excess light energy absorbed. The observed decrease in PSII efficiency was consistent with increase in non-photochemical quenching in greenhouse and shade plants, transferred to full sunlight for a day (Fig. 1B). Greenhouse and shade plants exposed to sunlight for a day tended to acclimate to the high-light conditions through better dissipation of excess energy, as observed by the increase in the qN and recovery of F_v/F_m ratio (Fig. 1A and B).

Protection of photosynthetic apparatus through qN is one of the protection mechanisms that plants have developed to avoid photodamage[9]. Study with potted trees of *Picea abies* has shown that photochemical apparatus of PSII is able to acclimate to high-light within 2 days after transfer[21]. Some cereals such as wheat and winter rye grown under high irradiance are

resistant to photoinhibition, due to an increased photosynthetic capacity, rather an increased efficiency of non-radiative dissipation[22,23]. In our study, however, rice plants grown at low-light intensities (greenhouse and shade) showed an increased efficiency of non-radiative dissipation (Fig. 1B), as well as an increased photosynthetic capacity (data not shown).

Transferred greenhouse and shade plants underwent a greater degree of conversion of xanthophyll cycle i.e. V into its de-epoxidised form Z and A, as observed in the sun plants (Table 1). A correlation between Z + A and qN was observed, when greenhouse and shade plants transferred to direct sunlight for a day (Table 1 and Fig. 1B), indicating photoprotective acclimation to excess irradiance by thermal dissipation of the excess light energy absorbed. However, the relationship between qN and DPS (conversion of V to A + Z) was not found in shade grown plants exposed to sunlight for longer than 1 day. Longer than 1 day treatment resulted in higher DPS level while qN decreased, indicating that part of the qN might not be xanthophyll cycle-dependent under long-term photoinhibitory conditions.

An increase in the amounts of xanthophyll cycle carotenoids and its correlation with qN in high-light grown plants has been reported[9,24]. The enhanced accumulation of Z and A prevents photo-oxidative damage to PSII[25]. The relationship between energy dissipation and xanthophyll cycle conversion state was similar for sun plants and transferred greenhouse plants and at an early stage of photoinhibition even in shade plants. This suggested that greenhouse plants and sun plants used the same mechanisms of energy dissipation i.e., Z-dependent qN during photoinhibition. Shade plants during early stage of photoinhibition (up to 1 day) also used the same mechanism of energy dissipation, however, during longer stage of photoinhibition shade plants might use, in addition to xanthophyll cycle, other mechanism such as lutein-dependent excitation relaxation, as lutein levels increased in shade plants on transfer to full sunlight (Table 1).

Lutein might play a role in energy dissipation albeit by a different mechanism. Increased amounts of lutein in shade plants may provide protection against triplet chlorophyll and ROS under high-light stress[9,26,27]. Slight increase in Chl *a/b* ratio in shade grown plants on exposure to sun light might have also prevented overenerzization of Chl *a* molecules in the LHC and thus prevented overenerzization of the reaction center (Table 1). The Chl *a/b* ratio was lower in greenhouse plants than in shade and sun plants (Table 1), indicating a smaller light harvesting complex II (LHCII) in latter. Also, increased Chl *a/b* ratios indicated a lowered amount of peripheral Chl *a* and *b* binding LHC, resulting in a decreased absorption cross-section of PSII and consequently diminished light stress under a given photosynthetic active radiation[28]. In plants, the size of light harvesting Chl *a/b* binding complexes is not fixed, but modulates in response to changes in the light environment. Under high-light, the LHC size is reduced to avoid the potentially damaging excess excitation[29]. Similar sun/shade differences in plants grown in high irradiance compared to low irradiance have been reported[30].

The xanthophyll cycle is mediated by the VDE. Greenhouse, shade and sun plants exhibited an increase in Z and the DPS after one day of sun exposure, indicating increase in the VDE content or activity on high-light treatment. In control greenhouse and shade plants (i.e. prior to transfer to full sunlight), exogenous addition of VDE extract had a promotive effect on the VDE activity, observed as higher DPS (exogenous VDE data not shown), indicating lower levels of VDE or activity present in control (low-light grown) plants. However, in treated-plants, exogenous addition of VDE extract did not show any changes in the DPS, indicating limitation/saturation of xanthophyll cycle (data not shown).

Transfer of greenhouse and shade plants to sunlight till 1630 h resulted in an increase in MDA production (Fig. 3), indicative of increased peroxidative damage to lipids. The decrease in the amount of MDA production after 3 days of transfer to sunlight could be due to the protective role of Z or other enzymatic and non-enzymatic antioxidants. When the xanthophyll cycle is blocked by dithiothreitol (3 mM) and low temperature (3ºC), photoinhibition of electron transport is exacerbated and pronounced LPO occurred[31]. Besides promoting energy dissipation in the LHC, Z might protect plants from photooxidative stress by participating directly in quenching singlet excited state of chlorophyll, reactive singlet oxygen and/or free radicals in the thylakoid membrane, thus preventing the accumulation of lipid peroxides[16,32].

This study demonstrated that low-light grown rice plants were initially highly susceptible to high-light stress, but substantially acclimated on longer duration

(7 days) of full sunlight conditions by employing physiological mechanisms, such as non-radiative energy dissipation through the xanthophyll cycle, as seen in high-light grown plants. In addition to this, other processes might be functioning during longer duration of high light such as high level of lutein and high Chl *a/b* ratio, thus preventing overenerzization observed in this study, especially in shade grown plants. The data presented here revealed acclimative strategies with reference to pigments (xanthophyll cycle), lutein, Chl a/b ratio and xanthophyll cycle-mediated qN in greenhouse, shade and sun grown plants in their responses to natural sunlight.

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