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Relationship between xanthophyll cycle and non-photochemical quenching in rice (*Oryza sativa* L.) plants in response to light stress

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Thirty days old rice plants grown under low and moderate light conditions were transferred to full sunlight to observe the extent of photoinhibitory damage and protective mechanism, and the relationship between xanthophyll cycle and non-photochemical quenching (qN) under changing light environment. Control plants (low, moderate and sun grown) exhibited similar Fv/Fm ratio, indicating similar photosynthetic efficiency prior to light stress. On exposure to the high light treatment, low light grown plants exhibited faster and higher degree of photoinhibition compared to moderate and high light grown plants. Moderate and high light grown plants showed relatively less photoinhibition and also showed higher qN, indicating better capacity of energy dissipation. Increase in qN in moderate light and sun grown plants was accompanied by conversion of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) indicating operation of Z-dependent thermal dissipation. Rice plants fed with ascorbate (AsA), a stimulator of the de-epoxidation state of V to Z, showed higher Fv/Fm ratio and qN than the plants fed with dithiothreitol (DTT) an inhibitor of xanthophyll cycle. This indicated that an increased amount of energy reached PS II reaction centre, due to absence of A and Z formation, thereby causing greater damage to photosynthesis in DTT fed rice plants. The present data confirmed the relationship between qN and Z in dissipating the excess light energy, thereby protecting plants against photodamage.

Keywords: Energy dissipation, Light stress, Non-photochemical quenching, Rice, Xanthophyll cycle

Quantity of light in natural environment can vary over several orders of magnitude and on a time scale that ranges from seconds to seasons. Light intensity on a sunny day (~2200 µmol m^{-2} s^{-1}) far exceeds the utilization capacity of most plants (~600-800 µmol m^{-2} s^{-1}). The rate of light absorption by the plants is linear to the incident light but utilization of absorbed quanta is ~10% at full sunlight[1]. This excess absorbed light energy can result in overexcitation of the photosynthetic system and cause damage to the photosynthetic reaction centre resulting in loss to productivity, thus needs to be dissipated to minimize damage to the photosynthetic apparatus. Plants protect themselves from high light stress through the thermal dissipation of excess energy in photosystem II (PS II) measured as non-photochemical quenching (qN)[2], a process requiring the presence of the xanthophyll cycle[3].

In xanthophyll cycle Z is formed by de-epoxidation of V through the intermediate A[4]. Conversion of V to Z takes place when plants are illuminated with high light, while low light or darkness stimulates the reverse reaction i.e. conversion of Z back to V. Two enzymes localized on opposite sides of the thylakoid membrane are engaged in this process: violaxanthin de-epoxidase (VDE) present on the thylakoid lumen side of the membrane, catalyses the de-epoxidation of V to Z and zeaxanthin epoxidase (ZE), carrying out the reverse reaction of epoxidation of Z to V, is localized on the stromal side of the thylakoid membrane[5]. Both A and Z protect the plants from photoinhibition by dissipating the excess light energy as heat[5]. Violaxanthin de-epoxidase is a 43 kDa nuclear-DNA encoded protein[6], with a *p*H optimum of 5.2 and requires ascorbate as a reductant[7] and galactolipid monogalactosyl diacylglycerol[8].

The present study was undertaken to study the adaptation to changing light environment in rice plants. Further experiment was done to study the impact of inhibition and stimulation, of xanthophyll cycle and its relation with energy dissipation (measured as qN) under high light conditions. We also studied the role of VDE activity in the

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Abbreviations: A-antheraxanthin; AsA-ascorbate; DEI-de-epoxidation index; DTT-dithiothreitol; Fv/Fm-quantum efficiency of PS II; LHC II-light harvesting complex II; PFD-photon flux density; PS II-photosystem II; qN-non-photochemical quenching; V-violaxanthin; VDE-violaxanthin epoxidase; XCP-xanthophyll cycle pigments; Z-zeaxanthin; ZE- zeaxanthin epoxidase.

xanthophyll cycle of rice plants exposed to high light stress using isolated PS II particles containing light harvesting complex II (LHC II). In the present study, natural sunlight was used as photoinhibitory treatment in order to consider the fluctuations in the light intensities during the course of treatment.

Materials and Methods

Plant material and growth conditions—The study was carried out using rice (*Oryza sativa* L. c.v. Jyothi) plants grown in earthen pots having a diameter of 20 cm, containing common garden soil and vermiculite in a ratio 3:1. After one week germination, each pot was thinned, to contain ~30 plants in each set of experiment. The plants were grown for thirty days under three different growth environments. One set of plants was grown in a naturally lit greenhouse where the photon flux density (PFD) received by the plants ranged from 150-200 µmol m^{-2} s^{-1} (low light) and the relative humidity (RH) was 85-90%. The second set of plants was grown in the shade by placing the pots in the shade of a building, where the peak PFD varied between 600-800 µmol $m^{-2}s^{-1}$ (moderate light) and 70-75% RH. The third set of plants was grown outdoors in the university campus, under full sunlight in a non-shaded area, where the plants received a maximum PFD of 2200 µmol $m^{-2}s^{-1}$ (high light) with 70-75% RH.

Photoinhibition of plants—After thirty days, the plants were of 20 cm in height and had three leaves irrespective of growth conditions. Third leaf from base was used for all measurements. The potted plants were transferred from growth condition to direct sunlight (high light) at 1030 to 1630 h (6 h of treatment) and leaf samples were collected at 1130, 1330 and 1630 h. Control measurements, in case of low and moderate light grown plants, were taken prior to transfer to direct sunlight (control plants), while in case of plants grown in high light the control leaf samples were collected at predawn (0630 h). Treated leaf samples were collected at 1130, 1330 and 1630 h. The average PFD at various time interval is given in Table 1.

Photoinhibition of plants fed with ascorbate (AsA) and dithiothreitol (DTT)—Rice leaves were cut from their petioles and placed in a conical flask containing AsA (5 m*M* *p*H 6.8), DTT (30 m*M* *p*H 6.4), or water (control). Solution was kept stirred at low speed continuously throughout the duration of feeding AsA or DTT, using magnetic stirrer. Prior to light treatment, rice leaves were treated with AsA/DTT for 1 h at 25°C and 200 µmol m^{-2} s^{-1} PFD and subsequently exposed to direct sunlight for 6 h (1030 to 1630 h). Control plants were fed with distilled water.

Chlorophyll fluorescence—Chlorophyll fluorescence was measured with dark adapted leaf for 10 min prior to measurement at room temperature, using a pulse amplitude modulation fluorometer (PAM 101, Walz, Effelrich, Germany)[9]. Calculations were carried out according to Schreiber *et al.*[10]. Maximal quantum efficiency of PS II (Fv/Fm) was estimated from the variable to maximum fluorescence ratio, Fv/Fm = (Fm-Fo)/Fm and non-photochemical quenching coefficients were calculated as (qN) = 1- (F′m-F′o)/(Fm-Fo).

Extraction and analysis of photosynthetic pigments—Extraction and estimation of pigments was carried out using HPLC (Waters)[11]. Identification and separation of pigments were done using a reverse phase column (Waters Spherisorb ODS 2.5 µm, 4.6 × 250 mm) using a phase diode array detector (Waters 2996). Pigment extract (20 µl) was injected into HPLC. The gradient for separation was 0–100% ethyl acetate in acetonitrile/water (9:1) over 34 min with flow rate of 1.2 ml/min. The pigments were detected at 450 nm and were quantitated on a fresh weight basis from peak area value using β-carotene as external standard.

Estimation of violaxanthin de-epoxidase (VDE) activity

Preparation PS II particles—PS II particles having LHC II, from control and treated (6 h sun exposed) leaves were prepared according to Kuwabara *et al.*[12] with some modifications. Leaves (20 g) were macerated in a buffer containing 10 ml of 50 m*M* Na-K phosphate buffer (*p*H 6.4), 0.3 *M* sucrose and 100

Table 1—Photon flux density (PFD, µmol m^{-2} s^{-1}) of clear day during study period

Time of day (h)	Photon flux density (µmol m^{-2} s^{-1})
0600 (pre dawn)	150
0730	615
0830	946
0930	1401
1030	1823
1130	2050
1230	2184
1330	2156
1430	1997
1530	1707
1630	1326
1730	1137
1830	706

m*M* NaCl (1:1:1,v/v), to which 30 ml of Triton-X 100 (2%, w/v) was added. The mixture was filtered through 8 layers of muslin cloth and the filtrate was centrifuged at 8000 rpm for 60 min at -4ºC. The pellet was suspended in 5 ml of 40 m*M* Na-K phosphate buffer (*p*H 6.4) and centrifuged at 1000 rpm for 1 min. The supernatant was then centrifuged at 8000 rpm for 60 min at -4ºC. The resulting pellet was washed twice with 6 ml of buffer containing 25 m*M* MES-NaOH (*p*H 6.5), 0.3 *M* sucrose and 10 m*M* NaCl (1:1:1 v/v), and was suspended in 1 ml of the same medium.

Assay of VDE activity—Assay of VDE activity was carried out as described by De las Rivas *et al.*[13] in a reaction medium containing 2.4 ml of 50 m*M* sodium acetate-HCl buffer (*p*H 4.9) and 0.1 ml of the suspension of PS II particles. The reaction was initiated by addition of 0.08 ml of 0.8 *M* sodium ascorbate. The mixture was incubated at 30°C for 3 h. The reaction was stopped by addition of 0.09 ml of 0.2 *M* dithiothreitol to quench VDE activity. The particles were collected by centrifugation at 8000 rpm for 60 min and analyzed for the contents of xanthophyll cycle pigments by HPLC. The de-epoxidation state of PS II particles were described in terms of the de-epoxidation index (DEI), i.e., (A+2Z)/(V+A+Z)[14].

Extraction and determination of xanthophyll cycle pigments (XCP)—After reaction the XCP from the PS II particles were extracted with 1 ml of acetone:methanol (7:2, v/v) by vortexing, followed by centrifugation at 8000 rpm for 30 min[14]. To the supernatant 0.08 ml of dichloromethane was added. DTT had been mixed with 1/100 vol of 1 *M* Tris-HCl buffer (*p*H 8.0) to prevent the acidification that causes the rearrangement of 5,6-epoxide of V to the furanoid-5,8-epoxide. The colorless insoluble materials were precipitated by centrifugation at 8000 rpm for 30 min. The supernatant was used for analysis of XCP by HPLC (Waters)[11].

Statistical analysis—The experimental data were tested for significance by using a Student's *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.

Results

Effect of sunlight on chlorophyll fluorescence

Transfer of low light grown plants to direct sunlight for 1 h (up to 1130 h) resulted in an initial decline of 51% in the Fv/Fm ratio, of quantum efficiency of PS II, as compared to control, which recovered linearly with increase in the duration of the sunlight treatment (Table 2). Moderate light grown

Table 2—Effect of sunlight on the photosynthetic efficiency (Fv/Fm), non-photochemical quenching (qN) and pigment content (mg/g FW) in low, moderate and high light grown plants. In low and moderate light grown plants control refers to plants prior to transfer to direct sunlight while in high light grown plants control refers to measurements taken at predawn (0600 h).

[Values are mean ± SD of 3 replications]

Treatment	Fv/Fm	qN	V	A	Z
Low light					
Control	0.700 ± 0.02	0.26 ± 0.05	7.44 ± 2	0.65 ± 0.2	0
1130 h	0.340 ± 0.07	0.28 ± 0.07	0.45 ± 0.1	0.71 ± 0.4	0
1330 h	0.373 ± 0.05	0.25 ± 0.06	3.00 ± 0.5	1.67 ± 0.4	0.42 ± 0.07
1630 h	0.463 ± 0.05	0.34 ± 0.05	4.50 ± 1	2.00 ± 0.5	0.37 ± 0.01
Moderate light					
Control	0.723 ± 0.02	0.28 ± 0.05	3.00 ± 0.5	1.00 ± 0.1	0
1130 h	0.653 ± 0.02	0.31 ± 0.07	6.00 ± 0.7	1.20 ± 0.3	0
1330 h	0.493 ± 0.05	0.27 ± 0.03	2.40 ± 0.4	1.40 ± 0.2	0.33 ± 0.01
1630 h	0.573 ± 0.06	0.40 ± 0.04	3.00 ± 0.3	2.00 ± 0.4	1.30 ± 0.09
High light					
Control	0.726 ± 0.02	0.18 ± 0.05	10.6 ± 1	0.98 ± 0.2	0
1130 h	0.703 ± 0.04	0.31 ± 0.06	6.21 ± 0.5	2.61 ± 0.1	0.2 ± 0.01
1330 h	0.706 ± 0.005	0.20 ± 0.06	6.27 ± 0.4	1.91 ± 0.1	0.6 ± 0.03
1630 h	0.693 ± 0.02	0.27 ± 0.05	4.97 ± 0.2	1.50 ± 0.1	1.0 ± 0.01

V–violaxanthin; A–antheraxanthin; Z–zeaxanthin

plants on photoinhibition up to 3 h showed a linear decrease (31%) in the Fv/Fm ratio, as compared to control plants (i.e. prior to transfer to direct sunlight; Table 2). However, longer (6 h) exposure to high light resulted in recovery of Fv/Fm (16%) as compared to the decline seen after 3 h of treatment. For high light grown plants, Fv/Fm showed no significant change during the course of the day as compared to control plants (i.e. predawn; Table 2).

In low light grown plants qN did not change significantly (3%) after 3 h of exposure to direct sunlight (up to 1330 h), but increased (30%) after 6 h of high light treatment, as compared to the control plants. Moderate light grown plants on exposure to 6 h sunlight showed 42% increase in the qN compared to 72% increased observed in sun grown plants exposed to light for the same duration, compared to their respective controls (Table 2).

Effect of sunlight on the xanthophyll cycle—Changes in the xanthophyll pigments were studied to find out the extent of xanthophyll cycle-dependent energy dissipation in response to high light stress. Table 2 shows xanthophyll content in low, moderate and high light grown plants in response to exposure to direct sunlight. Analysis of photosynthetic pigments showed higher level of V and lower level of A and absence of Z in controls of all the three categories, low, moderate and sun grown plants. Transfer of all the three types of plants to sunlight led to a reduction in V and accumulation of A and Z (Table 2). In low and moderate light grown plants Z was observed after 1330 h and was present till 1630 h, while in high light grown plants Z was observed within 1 h of the treatment and continued to increase linearly (Table 2). The increase in A and Z was not proportional to the decrease observed in V. The VAZ pool in control plants grown under low (8.09 mg/g) and moderate light (3.66 mg/g) was considerably lower than the predawn sun plants (11.58 mg/g; Table 2).

Effect of sunlight on chlorophyll fluorescence and xanthophyll cycle in plants fed with AsA through cut petioles

Figure 1a depicts the changes in the Fv/Fm ratio, qN and sum of A and Z of low light grown rice plants fed with AsA in response to exposure to direct sunlight. Ascorbate fed plants exposed to direct sunlight resulted in non-linear decline in Fv/Fm ratio as compared to control. Six hours of high light treatment in AsA fed plants led to 144% increase in qN than seen in the control. Sum of A and Z also showed significant increase (283%) within 1 h of the treatment as compared to control, which was further increased when exposed to sunlight till 1330 h. However, longer exposure till 1630 h (6 h of treatment) resulted in a decrease (73%) in the sum of A and Z, as compared to observed at 1330 h (Fig. 1a).

Moderate light grown plants fed with AsA showed linear decrease of 28% after 6 h exposure to sunlight in the Fv/Fm ratio (Fig. 1b). Non-photochemical quenching (qN) increased by 13% after 6 h of the sun treatment of AsA fed plants as compared to control (Fig. 1b). The sum of A and Z also increased (78%) in response to light treatment in moderate light grown plants fed with AsA (Fig. 1b).

Sun grown plants, when fed with AsA, showed no significant changes in Fv/Fm ratio at early stage but 6 h of sun exposure resulted in 27% decrease in the Fv/Fm ratio as compared to control. Non-photochemical quenching increased by 38% as a result of 6 h of exposure as compared to control (predawn plants; Fig. 1c). The sum of A and Z was initially low in AsA fed plants as compared to control. However, at 1630 h the sum of A and Z increased significantly (660%) as compared to control (Fig. 1c).

Effect of sunlight on the chlorophyll fluorescence and xanthophyll cycle in plants fed with DTT through cut petioles

Fv/Fm ratio declined in DTT fed low (78% decrease), moderate (47% decrease) and sun (53% decrease) light grown plants as compared to their respective controls. qN increased by 16% after 6 h of treatment to DTT fed low light grown plants, as compared to control, however, qN decreased by 22 and 60%, respectively in moderate and sunlight grown plants fed with DTT and exposed to 6 h of the treatment as compared to their respective control (Fig. 1d). Decrease in qN in DTT fed plants, on exposure to sunlight, was in contrast to increase observed in qN in AsA fed plants for the same treatment. At 1130 h (1 h of sun treatment) DTT fed low light grown plants exhibited similar sum of A and Z as control (0.60-0.64 mg/g; Fig. 1d). However, a decline (46%) was resulted A+Z, as compared to control after 6 h of sun exposure (Fig. 1 d).

The sum of A and Z declined linearly in DTT fed moderate light grown plants as a result of sun exposure. Six hours of sunlight treatment resulted in a decrease of 35% in the A+Z content as compared to 1130 h (Fig. 1e).

Both A and Z declined to almost zero in sun grown plants fed with DTT and exposed to sunlight (Fig. 1f).

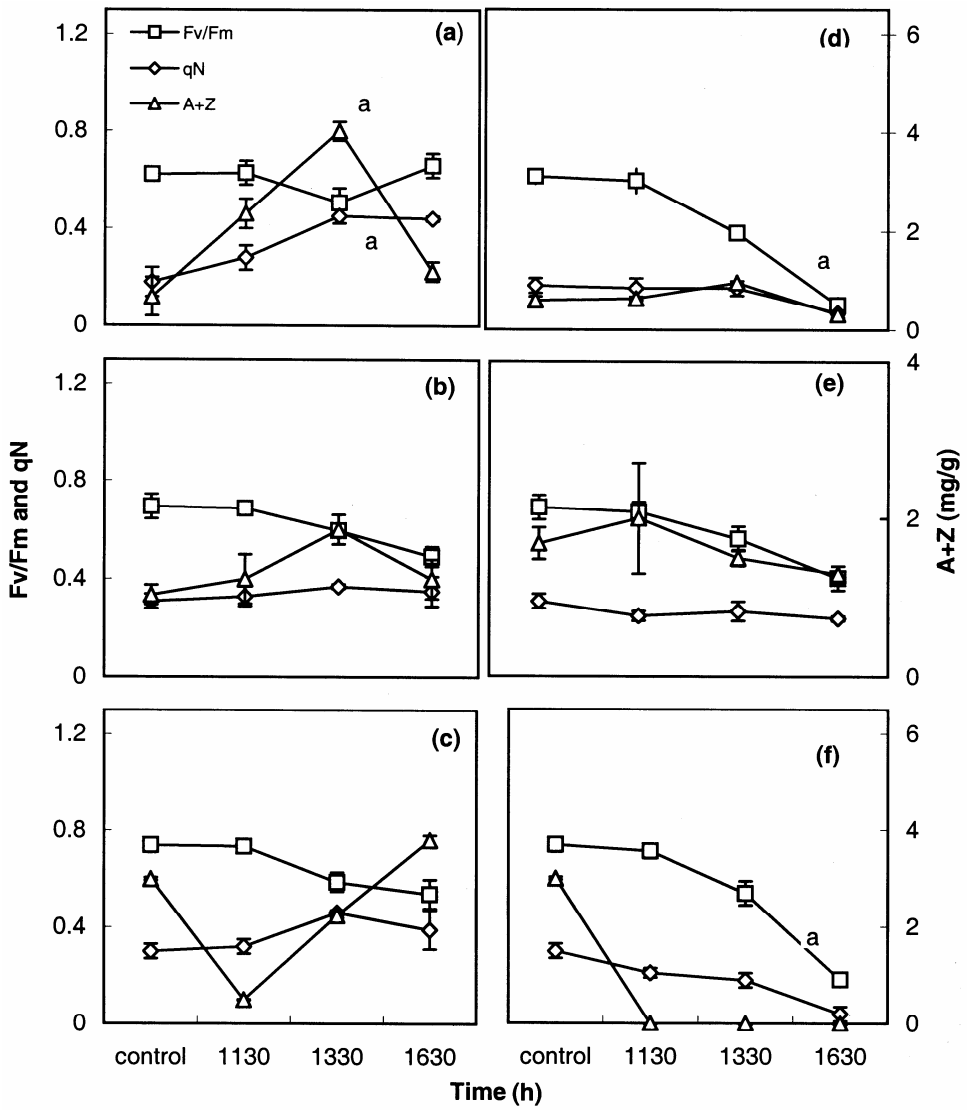

Fig. 1—Effect of direct sunlight on quatum efficiency of PS II (Fv/Fm) and non-photochemical quenching (qN) and the sum of antheraxantnin and zeaxanthin (A+Z) in 30 days old rice plants grown under low light (a and d), moderate light (b and e) and high light (c and f) fed with ascorbic acid (a,b,c) and dithiothreitol (d,e,f). Control refers to plants fed with water for 6 h under growth conditions. [[a]Significantly different from control ($P < 0.05$). Values are mean ± SD of 3 replications]

Effect of sunlight on the VDE activity in isolated PS II particles

The VDE activity increased in low, moderate and high light grown plants after 6 h of sun exposure as compared to control (Fig. 2). Results indicate highest activity of VDE, referred as de-epoxidation index (DEI), under treated conditions in the low light grown plants followed by the moderate light grown plants and least activity was observed in sun grown plants. Though actual value of DEI in sun grown plants was three times less than seen in low and moderate light grown plants, percent increase in the DEI as a result of light treatment was more or less same, as seen in low and moderate light grown plants.

Discussion

Our data indicate similar photosynthetic efficiency in control plants of all the three different growth conditions (Table 2). The higher degree of photoinhibition (decrease in the Fv/Fm ratio) observed in low light grown plants than moderate light grown plants after 1 h of high light treatment (Table 2), indicated that the former experienced higher light stress under comparable light conditions, because of its inability to handle the excess radiation, due to its acclimatization to low light, as qN was lower in low light grown plants than observed in moderate light grown plants.

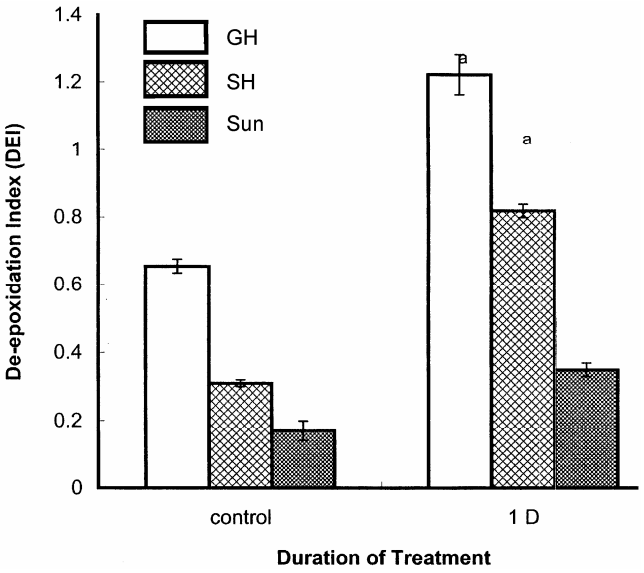

Fig. 2—Effect of sunlight on the violaxanthin de-epoxidase activity measured as DEI, in PS II particles isolated from thirty days old control and 6 h sun exposed rice plants grown at low, moderate and direct sunlight. In low and moderate grown plants control refers to plants prior to transfer to direct sunlight and in sun grown plants control refers to predawn (0600 h). [[a]Significantly different from control ($P < 0.05$). Values are mean ± SD of 3 replications]

Low light grown plants subjected to high light stress initially undergo photoinhibitory damage, but are able to recover partially from this damage in the course of time. Recovery was possible since the damage was temporary in nature and could be overcome by protective mechanism such as the xanthophyll cycle mediated energy dissipation. Since rice plants are genetically capable to grow under high light conditions, low and moderate light grown plants exposed to full sunlight for a longer period adapt to the high light conditions through better dissipation of excess energy as seen by the increase in the non-photochemical quenching (qN) and recovery of Fv/Fm ratio [Table 2 and xanthophylls cycle (Fig. 1)]. Longer duration of treatment (3 days and more) to full sunlight resulted in low and moderate light grown plants behaving same as sunlight grown plants (data not shown).

Lower susceptibility to photoinhibition of PS II in high light grown rice plants (moderate and high light grown plants) compared to low light grown plants observed in the present study (Table 2), could be related to faster kinetics and higher degree of V de-epoxidation and an increased pool size of xanthophyll cycle pigments. Sun grown plants are thought to have various protective system such as increased photosynthetic capacity[15], active PS II repair cycle[16], xanthophylls associated energy dissipation[17] and antioxidant activity[18], that safely dissipate excess light energy, neutralise potentially damaging products or repair damage to the photosynthetic apparatus, thus preventing or minimizing photoinhibition.

Inhibition of photosynthesis under high light has been reported earlier by several groups[17]. Pearcy and Sims[19] observed that many plant species grown at low light undergo physiological shock upon exposure to more open high light environment. They reported that sudden exposure of vegetation structurally and functionally attuned to a low irradiance to high light can induce dysfunction in photosynthesis and other physiological activities. In our study, the decrease in the quantum efficiency of photosynthesis (Fv/Fm ratio) observed in low and moderate light grown plants on sun exposure (Table 2) could be a result of physiological shock upon exposure to direct sunlight.

Increase in qN in response to high light has been reported[20,21]. Špunda *et al.*[20] have found that the photochemical apparatus of *Picea abies* was able to acclimate within two days after transfer to high light conditions by an increase in photochemical and non-photochemical quenching processes. Gray *et al.*[22] and Huner *et al.*[23] have reported that 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. However, Špundová *et al.*[24] have reported lower qN in high light grown barley as compared with low-light-grown barley.

Our data showed that depressions in PS II efficiency were accompanied by retention of Z and A in sun transferred low and moderate light grown plants (Table 2). Low and moderate light grown plants exposed to direct sunlight underwent a greater degree of conversion of V into its de-epoxidised form Z and A, similar to sun grown plants, than non-sun transferred plants (Table 2), and are indicative of an increased allocation of absorbed light towards energy dissipation during high light treatment. Increase in Z was accompanied by an increase in qN in sun exposed plants. A correlation was observed between the levels of Z+A and levels of qN in low and moderate light grown plants transferred to direct sunlight for a day (Table 2), indicating that thermal dissipation of excitation energy is dependent on the xanthophyll cycle activity. Xanthophyll cycle and its ability for energy dissipation appear to adjust continuously to

both short term and long term responses of plants to high light. Similar observation were also reported by[11,16]. A central role of Z in non-radiative dissipation of excess light energy was confirmed by Niyogi *et al*.[25] by using *npq1* mutants of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana* defective in violaxanthin de-epoxidase activity. Similarly Chang *et al*.[26] have observed that qN reduces significantly in the antisense VDE in tobacco plants. They have demonstrated that a significant level of the qN requires de-epoxidation of V. Verhoeven *et al*.[27] have reported that the transgenic tobacco with suppressed Z formation on exposure to high light for longer duration results in photoinhibition, indicating a photoprotective function for the xanthophyll cycle through energy dissipation.

Rice plants fed with ascorbate in our study, showed higher Fv/Fm ratios and qN values than in DTT fed rice leaves (Fig. 1). In AsA fed leaves V was de-epoxidised to Z, indicating the efficient thermal dissipation of xanthophyll cycle. On the contrary DTT inhibited de-epoxidation of the xanthophyll cycle as well as the xanthophyll cycle associated energy dissipation process in the pigment bed. Hence, a decrease in Fv/Fm ratios and qN values were observed in DTT fed rice leaves, indicating that an increased amount of energy reached the PS II reaction centre, due to the absence of Z formation, thereby causing greater damage to photosynthesis in DTT-fed rice leaves. Similar results in different study are obtained by Guo *et al*.[21] and Li *et al*.[28].

However, some authors have reported no correlation between Z and qN[29,30]. Experiments on *Arabidopsis* by Davison *et al*.[30] have indicated that doubling of amount of xanthophyll cycle carotenoids in PS II antenna had no visible effect on the maximum non-photochemical quenching. Their results have demonstrated that photoprotection mediated by the xanthophyll cycle is not solely a result of involvement of Z in non-photochemical quenching.

The xanthophyll cycle is mediated by the enzyme VDE. Increase in DEI observed in low, moderate and high light grown plants transferred to direct sunlight as compared to their respective control (i.e. plants prior to transfer to direct sunlight; Fig. 2), indicates an increase in VDE activity and in turn conversion of V to A and Z on exposure to sunlight. The enzyme triggers the de-epoxidation of V to A and Z, as seen by decrease in V and increase in A and Z on exposure to high light in the present study (Table 3). However, decrease in V was not proportional to increase in A and Z. Zeaxanthin was seen in low and moderate light grown plants on exposure to sunlight but was absent in the sun grown plants (Table 3). Absence of Z (but presence of A) in sun grown plants could be the reason for lower DEI (VDE activity) compared to VDE activity seen in low and moderate light grown plants (Fig. 2).

Table 3—Effect of sunlight on the xanthophyll cycle pigment content (µg/g fresh wt) and de-epoxidation index (DEI) in reactions carried out with PS II particles isolated from low, moderate and high light grown plants. In low and moderate light grown plants control refers to plants prior to transfer to direct sunlight while in high light grown plants control refers to measurements taken at predawn (0600 h).

[Values are mean ± SD of 3 replications]

	V	A	Z	V+A+Z	DEI
Low light					
Control	5 ± 0.2	3 ± 0.4	0	8 ± 0.5	0.37 ± 0.02
1630 h	4 ± 0.1	2 ± 0.1	4 ± 0.2	10 ± 1	1.0 ± 0.06
Moderate light					
Control	3 ± 0.3	0.25 ± 0.03	0.5 ± 0.01	3.75 ± 0.4	0.33 ± 0.01
1630 h	3 ± 0.1	1.00 ± 0.05	2 ± 0.2	6 ± 0.7	0.83 ± 0.02
High light					
Control	7 ± 0.3	1 ± 0.02	0	8 ± 1	0.12 ± 0.03
1630 h	6 ± 0.3	3 ± 0.1	0	9 ± 0.9	0.33 ± 0.02

V–violaxanthin; A–antheraxanthin; Z–zeaxanthin

Increase in VDE activity under high light conditions has also been reported previously[14,31]. In our study, the higher VDE activity observed in sun exposed low, moderate and high light grown plants could also be due to active synthesis of VDE enzyme under high light conditions. However, Eskling and Akerlund[32] have reported a decrease in the quantity and activity of VDE when spinach plants are shifted from low (100 µmol $m^{-2}s^{-1}$) to high light (950 µmol $m^{-2}s^{-1}$).

To conclude, the present study highlights the importance of the xanthophyll cycle in protecting the rice plants grown under low and moderate light conditions and subsequently transferred to high light, by dissipating the excess light energy through non-photochemical quenching. The investigation shows that the low and moderate light grown rice plants adjust physiologically to solar irradiation in a similar fashion as high light grown plants.

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