Adaptational changes in the lipids and fatty acid profile of the cell and thylakoid membrane of rice plants exposed to sunlight

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Abstract Adaptational changes occurring in the lipids and fatty acids of the cell and the thylakoid membrane in response to high light treatment, was studied in 30 days old rice (*Oryza sativa* L. cv. Jyothi) plants grown under low ($150\text{--}200\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) or moderate ($600\text{--}800\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) light conditions. Results were compared with rice plants grown in high ($1200\text{--}2200\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) light conditions. Exposure of rice plants and isolated chloroplast to high light, resulted in an increase in the amount of malonaldehyde, indicating oxidation of membrane lipids. Qualitative and quantitative changes in the phosphoglycerolipids and quantitative changes in neutral lipids were observed in rice plants grown under the different growth conditions. A few of the phosphoglycerolipids and neutral lipids were present exclusively in plants grown at low or moderate or high light, indicating requirement of different type of lipid composition of rice plants in response to their different growth irradiances. However, no significant quantitative changes were observed in the different saturated and unsaturated fatty acid groups of total lipids in low, moderate and high light grown rice plants, as a result of exposure to high light. No qualitative changes in the fatty acid composition due to difference in growth irradiance or high light treatment were seen. The changes observed in the phosphoglycerolipids and neutral lipid composition of cell and thylakoid membrane of low, moderate and high light grown rice plants in response to high light, are probably the result of physiological changes in the rice plants, to sustain optimum structure and function of the cell and thylakoid membrane to maintain active physiological functions to endure high light conditions.

Keywords Fatty acids · High light treatment · Lipids · Low light · Moderate light · Rice plants

Abbreviations

Chl, chlorophyll,	singlet and triplet excited states of chlorophyll
$^1\text{Chl}^*$, $^3\text{Chl}^*$	
DGDG	digalactosyl diacylglycerol
Fv/Fm	maximum quantum yield for primary photochemistry
MDA	malonaldehyde adduct formation
MGDG	monogalactosyl diacylglycerol
PAR	photosynthetically active radiation
PFD	photon flux density
PG	phosphatidylglycerol
PGL	phosphoglycerolipids
PS I	photosystem I
PS II	photosystem II
Rf	resolving front
ROS	reactive oxygen species
SQDG	sulfoquinovosyl diacylglycerol
TBA	thiobarbituric acid
TCA	trichloroacetic acid

Introduction

In the natural environment, light intensities that plants receive vary over a wide, dynamic range. Exposure of plants to higher level of photosynthetic active radiation (PAR) than required for growth can lead to a decline in the photochemical efficiency of photosystem II (PS II), a process known as photoinhibition (Aro et al. 1993; Krause 1994). In this process, two mechanisms are involved affecting either the acceptor side or the donor

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side of PS II (for a review, see Ohad et al. 2000). Acceptor side-induced photoinhibition of PS II occurs under high irradiance when it exceeds the saturation of photosynthetic electron transport (Barber and Andersson 1992). Donor side-induced photoinhibition of PS II occurs when the capacity of water oxidising complex to donate electrons to the RC P680 is inactivated by high irradiance.

Photoinhibition of photosynthesis affect various photochemical reactions, for example, in the light-harvesting antenna of photosystem II, unused light energy increases the lifetime of the excited state of chl ($^1\text{Chl}^*$), which can be converted to the triplet excited state ($^3\text{Chl}^*$). $^3\text{Chl}^*$ itself is not harmful, but it is long-lived and can transfer energy to ground-state oxygen to generate highly reactive singlet oxygen. In addition to the deleterious formation of singlet oxygen, in the Mehler reaction the direct reduction of oxygen by photosystem I (PS I), results in the formation of superoxide anion radical. These reactive oxygen species (ROS) can oxidize membrane proteins, lipids and pigments, thus resulting in membrane instability, thereby, affecting growth and survivability of the organism.

The photosynthetic membrane is composed mainly of lipids and proteins (Kirk and Tilney-Bassett 1978). The presence of lipids in the membranes facilitates energy storage and offers selective permeability of sugars, salts, substrates, etc. by maintaining appropriate fluidity. The thylakoid membranes of the chloroplast of higher plants contain three glycolipids, namely, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), and one phospholipid, phosphatidylglycerol (PG) (Joyard et al. 1998). Among the four glycerolipids, MGDG and DGDG account for about 50% and 30% respectively, of the total lipids, while SQDG and PG each account for 5–10%. In plants and eukaryotic algae, glycerolipids and fatty acids are synthesized in chloroplasts (Murata and Siegenthaler 1998), while cell membrane contain glucocerebrosides in addition to the glycerolipids, free sterols and sphingolipids, which are absent or are only minor components of photosynthetic membranes (Mudo et al. 1984). Chloroplast lipids play an important role in maintaining membrane structure and function. One of the causes for damage to chloroplasts is the release of lipids from the membranes and their oxidation, due to light and heat.

Membrane lipid plays both structural and regulatory roles in organism's adaptation and survival when subjected to stress conditions. The lipid composition and/or metabolism of plants and algae have been shown to be altered by a number of environmental influences. These include temperature (Wang et al. 2000), the salts available in soils (Wu et al. 2005), light (Bhandari and Sharma 2006) and xenobiotics such as pesticides (Renault et al. 1997).

In this study, we have tried to find out how membrane lipids and fatty acids will be affected in rice plants grown in different light conditions and subjected to high light stress, both under *in vivo* and *in vitro* conditions and relate it to the oxidation of membrane lipids and extent of recovery.

Materials and methods

Plant material and growth conditions Rice (*Oryza sativa* L. cv Jyothi) plants were grown in earthen pots having a diameter of 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 at low light intensity in the greenhouse (referred to as low light grown plants), where the photon flux density (PFD) received by the plants ranged from 150–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the relative humidity (RH) was 85–90%. The second set of plants were grown at moderate light intensity in the shade (referred to as moderate light grown plants) by placing the plants in the shadow of a wall of a building, where the peak PFD varied between 600–800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 70–75% RH, while the third set of plants were grown outdoors under fully exposed conditions of sunlight (referred to as high light grown plants) and the peak PFD varied between 1200–2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with 70–75% RH. The light intensity was measured using a photometer (Licor LI-189). All the pots were well watered.

High light treatment in vivo Experiments were performed in the campus of Goa University from January to April 2006 and 2007. After 30 days of growth the plants were about 20 cm in height and had 3 leaves in all the three light conditions. Control measurements were taken at 1030 h, prior to transfer to full sunlight in the case of low and moderate light grown plants, while in high light grown plants the control leaf samples were collected at predawn (0630 h). Leaf samples were collected at 1630 h of the 1st, 3rd, 5th and 7th day after transfer to full sunlight, to determine the quantitative and qualitative changes in the lipid composition and fatty acid profile on exposure to high light stress.

Isolation of chloroplasts Chloroplast isolation was carried out with 30 days old rice plants grown in the different light conditions according to Sharma and Singhal (1992). Chl estimation of the isolated chloroplasts was done according to Arnon (1949).

High light treatment in vitro Isolated chloroplasts, having a concentration of 1 mg of chl/ml of chloroplasts, were treated for 3 and 6 h with a light intensity of 1200 $\mu\text{mol m}^{-2} \text{s}^{-2}$, using a halogen cold light source. 6 ml of the chloroplast

suspension was treated in a double-layered glass cuvette through which temperature-controlled water was circulated to maintain a constant temperature of 30°C during the treatment. The chloroplast suspension was kept stirred at low speed during the treatment using a small magnetic flea in order to avoid self-shading. The stirring speed was low enough so as not to cause any mechanical damage.

Chlorophyll fluorescence Chlorophyll fluorescence measurements were performed on intact leaves or isolated chloroplasts, which were dark adapted for 10 min prior to measurements at room temperature, using a pulse amplitude modulation fluorometer (PAM 101, Walz, Effelrich, Germany) as described by Sharma et al. (1998), to measure the extent of photoinhibition. The dark adapted leaves were exposed to a modulated light with an intensity of $4 \mu\text{mol m}^{-2} \text{s}^{-1}$ to measure initial fluorescence (F_0). This was followed by an exposure to a saturating pulse of white light of $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$ to measure 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 $330 \mu\text{mol m}^{-2} \text{s}^{-1}$. Another burst of saturating light at F_s state was used to measure F'_m . After reaching the steady state again, leaves were exposed to far-red light to measure F'_0 . Calculations were carried out according to Schreiber et al. (1986). The Maximum quantum yield for primary photochemistry (F_v/F_m) was estimated from the variable to maximum fluorescence ratio, $F_v/F_m = (F_m - F_0)/F_m$.

Peroxidation of cell and thylakoid membrane lipids 0.5 g of leaf tissue or 3 ml of chloroplast suspension was ground in 0.5% trichloroacetic acid (TCA). The homogenate was made up to 5 ml and centrifuged at 4000 g for 15 min. The supernatant was collected and used for measuring the peroxidation of membrane lipids. 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 8.0). A glass marble was placed on the top of the test tube to prevent evaporation and was incubated 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 was taken on spectrophotometer at 532 nm and 600 nm (UV-2450 UV-visible spectrophotometer, Shimadzu). The absorbance at 600 nm was subtracted from absorbance at 530 nm in order to correct for the non-specific turbidity. The amount of MDA-TBA adduct formed was quantified according to Halliwell and Gutteridge (1989) and Sharma et al. (1998) by using extinction coefficient of MDA $155 \mu\text{mol cm}^{-1} \text{m}^{-1}$.

Extraction of total lipids Total lipids were extracted according to the method described by Turnham and Northcote (1984) and Bhandari and Sharma (2006).

Separation of total lipids by thin layer chromatography Separation of total lipids into polar and neutral lipids was carried out by thin layer chromatography on silica gel H according to Liljenberg and Von Arnold (1987). The different bands of lipids were identified by their R_f values against known standards.

Quantitative estimation of glycolipids and phospholipids Amount of glycolipids present in total lipids was determined by the phenol-sulfuric acid method according to Kushwaha and Kates (1981). Phospholipids were estimated by determining the amount of phosphorus in lipid fraction according to Bartlett method (Christie 1982).

Analysis of fatty acids Before the fatty acid composition was determined by gas chromatography, volatile methyl ester derivatives of the fatty acid components were prepared according to the method described by Christie (1982) and Bhandari and Sharma (2006). Methyl esters of fatty acids (1 μl) were run on a Shimadzu gas chromatograph (GC-2014) equipped with hydrogen flame ionization detector and chromatopack data processor. The column (6 mm $\times$ 2 mm i.d, stainless steel) was packed with DEGS 10% on 80–100 mesh chromosorb W-HP (Chemlabs, Bangalore). Column temperature was 180°C and injector temperature was 220°C and nitrogen was used as carrier gas (flow rate 30 ml/min). Sample (1 μl) was injected to the injector port of gas chromatograph using 10 μl fixed-needle syringe. Fatty acid methyl esters peaks were identified by comparing their retention times with methyl esters of pure fatty acid standards and were quantified by using the peak areas of individual fatty acids calculated according to Christie (1982).

Statistical analysis The experimental data were tested for significance by using a Students *t*-test for 2 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 ($P < 0.05$) is noted in figure legends wherever appropriate.

Results

Changes in lipid peroxidation of cell membrane as a result of high light treatment under in vivo conditions Figure 1a shows the relative increase in MDA content when plants were transferred from low light to high light. Transfer of low light grown plants to direct sunlight for 3 days resulted

Fig. 1 a MDA content ($\mu\text{mol}/0.1 \text{ g F.W.}$) in low (LL), moderate (ML) and high (HL) light grown 30 days old rice plants before and after exposure to direct sunlight. In low and moderate grown plants control refers to plants prior to transfer to direct sunlight and in high light grown plants control refers to measurements made at predawn (0630 h). **b** MDA content ($\mu\text{mol}/0.1 \text{ g F.W.}$) in chloroplasts isolated from low (LL), moderate (ML) and high (HL) light grown 30 days old rice plants and thereafter treated with artificial light ($1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Control refers to chloroplasts not treated with high light. ($n=3, \pm \text{SD}$). **a**—significantly different from control; **b**—significantly different from 1630 h; **c**—significantly different from 3 D ($p<0.05$)

in an increase in the MDA content by 92% than that of its control plants (i.e. prior to transfer to direct sunlight). Prolonged exposure to sunlight till 7 days led to a small decline in the amount of lipid peroxidation as compared to that observed at the end of the third day, but peroxidation level was still 63% higher than its control plants.

Moderate light grown plants also exhibited a steady increase (up to 59%) in the MDA content on transferring the plants to direct sunlight for 6 h. Prolonged exposure to sunlight up to 7 days did not show further increase in the level of peroxidation but the level of lipid peroxidation remained comparatively higher (15%) than its control plants (Fig. 1a). High light grown plants showed higher level of MDA content even in control plants (i.e. predawn), and the level of peroxidation linearly increased during the course of the day (Fig. 1a).

Comparison showed that, low light grown plants prior to transfer to direct sunlight (control plants) had lower MDA content than control moderate and control high light (i.e. predawn) grown plants (Fig. 1a).

Changes in lipid peroxidation of thylakoid membrane as a result of high light treatment under in vitro conditions Figure 1b shows the effect of artificial light ($1200 \mu\text{mol m}^{-2} \text{ s}^{-2}$) at 30°C , on the peroxidation of thylakoid membrane lipids of chloroplasts isolated from plants grown under the different growth conditions. Level of lipid peroxidation (MDA content) was highest in chloroplasts isolated from low light and lowest in chloroplasts isolated from high light grown plants, however, percent increase in the MDA content in chloroplasts isolated from plants grown under all three light conditions was more or less similar.

Qualitative changes in phosphoglycolipids of cell membrane as a result of high light treatment under in vivo conditions In low light grown plants prior to transfer to direct sunlight (control plants) eight different phosphoglycolipids (PGL) spots were seen (Fig. 2a). The tentative identification of these lipids has been listed in Table 1. Exposure of low light grown plants to sunlight for a period of 7 days resulted in qualitative changes in PGL. After one to 3 days of sun exposure, two new lipids were observed in addition to the lipids observed in control plants. However, after 7 days of sun exposure, low light grown plants exhibited more or less a similar lipid profile as control plants. In moderate light grown plants only three different PGL spots were observed, which remained unchanged after transfer to direct sunlight over a period of 7 days (Fig. 2b). High light grown plants exhibited six different PGL spots (Fig. 2c).

Qualitative changes in phosphoglycolipids of thylakoid membrane as a result of high light treatment under in vitro conditions Chloroplasts isolated from low light grown plants (control) had eight different PGL spots (Fig. 3a), while chloroplasts isolated from moderate light grown plants (control) had eleven different PGL spots (Fig. 3b). Chloroplasts isolated from high light grown plants had nine different PGL spots (Fig. 3c). Six hours of artificial high light treatment did not result in any qualitative changes in PGL of chloroplasts isolated from the three different light conditions.

Qualitative changes in neutral lipids of cell membrane as a result of high light treatment under in vivo conditions Low light grown plants prior to transfer to direct sunlight (control plants) showed the presence of five different neutral lipid spots (Fig. 4a). No qualitative changes were observed in neutral lipids even after 7 days of sun exposure

to low light grown plants. Moderate light grown plants prior to transfer to direct sunlight had nine different neutral lipid spots predominant being 1,3 diacyl glycerol and monoglyceride (Fig. 4b; Table 1). Moderate light grown plants also did not exhibit any qualitative changes in the neutral lipids on exposure to direct sunlight for as long as 7 days. High light grown plants had eight different neutral lipids (Fig. 4c).

Qualitative changes in neutral lipids of thylakoid membrane as a result of high light treatment under in vitro conditions Chloroplasts isolated from low light grown plants showed the presence of ten different neutral lipid spots (Fig. 5a). Chloroplasts isolated from moderate light grown plants, however, had nine neutral lipids (Fig. 5b). Chloroplasts isolated from high light grown plants showed the presence of 11 neutral lipids (Fig. 5c). No qualitative changes were observed in the neutral lipids in chloroplasts isolated from plants grown in the three different light conditions.

Quantitative estimation of glycolipids of cell membrane under in vivo conditions Glycolipid content in low light grown plants increased as a result of exposure to sunlight (Fig. 6a). It was seen that exposure to sunlight for a day increased the glycolipid content by 22%, which was further increased to 108% at the end of 3 days of sunlight treatment, as compared to control plants. However, longer exposure to sunlight for 7 days resulted in decline of 31% as compared to the plants exposed to 3 day of sunlight.

Glycolipid content of moderate light grown plants showed an increase of 40% as a result of one day of sun treatment, which remained constant during the sun exposure for 3 days (Fig. 6a). Seven days of sun exposure resulted in an increase of only 9% in glycolipid content as compared to its control. Though moderate light grown

Fig. 2 TLC profile of phosphoglycolipids in the cell membrane of low **a**, moderate **b** and high light **c** grown 30 days old rice plants exposed to direct sunlight for a period of 7 days. In low and moderate light grown plants control refers to plants prior to transfer to direct sunlight and in high light grown plants control refers to predawn (0630 h). In low and moderate light grown plants data has been shown till 7 days while in high light grown plants data at the end of a day has been shown. Volumetrically a constant amount of lipid sample was loaded in all the three cases

Table 1 Identification of phosphoglycolipids and neutral lipids based on their R_f values

Sr. No.	Phosphoglycolipids		Neutral lipids	
	R _f × 100	Possible lipid component	R _f × 100	Possible lipid component
1	6	Phosphatidyl inositol	6	?
2	12	Sulpholipid	8	1,3 diacyl glycerol
3	25	Digalactosyldiglyceride	10	Monoglyceride
4	32	Digalactosyldiglyceride	15	Long chain fatty acid
5	37	?	17	Monoglyceride
6	41	Phosphatidylglycerol	18	Fatty acids
7	46	Phosphatidylglycerol	22	Monoglyceride
8	48	Phosphatidyl inositol	24	Co-enzyme Q
9	52	Phosphatidylethanolamine	28	?
10	53	Phosphatidyl serine	31	Diglyceride
11	60	Phosphatidyl N-methylethanolamine	32	Triglycerols
12	64	Phosphatidylethanolamine	39	Diglyceride
13	67	Phosphatidyl N,N-dimethylethanolamine	43	?
14	72	Monogalactosyl diglyceride	46	?
15	74	?	49	?
16	76	Monogalactosyl diglyceride	55	?
17	80	?	58	Alkyl diacyl glycerols
18	85	Phosphatidylethanolamine	61	Fatty acid methyl esters
19	87	?	81	?
20	90	Monogalactosyl diglyceride	87	Fatty acid
21	94	Monogalactosyl diglyceride	90	Trialkyl glycerol ethers
22	96	?	94	Esters
23			96	?

plants had a higher level of content of glycolipids (0.55 mg/g) as compared to low light grown plants (0.53 mg/g), transfer to direct sunlight resulted in greater accumulation of glycolipid content in low light grown plants.

High light grown plants showed highest (0.87 mg/g) glycolipid content as compared to low (0.35 mg/g) and moderate light grown plants (0.55 mg/g), which declined

slightly (13%) at the end of one day of the exposure. However, the glycolipid content remained still higher than low and moderate light grown plants (Fig. 6a)

Quantitative estimation of phospholipids of cell membrane under in vivo conditions Phospholipid content of low light grown plants (control plants) was considerably less

Fig. 3 TLC profile of phosphoglycolipids of thylakoid membrane of chloroplasts isolated from low **a**, moderate (prior to transfer to direct sunlight) **b** and high light (predawn) **c** grown 30 days old rice plants and treated with artificial light ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 6 h. Control refers to chloroplasts not treated with high light. Volumetrically a constant amount of lipid sample was loaded in all the three cases

Fig. 4 TLC profile of neutral lipids in the cell membrane of low **a**, moderate **b** and high light **c** grown plants exposed to sunlight for a period of 7 days. In low and moderate light grown plants control refers to plants prior to transfer to direct sunlight and in high light grown

plants control refers to predawn (0630 h). In low and moderate light grown plants data has been shown till 7 days while in high light grown plants data at the end of a day has been shown. Volumetrically a constant amount of lipid sample was loaded in all the three cases

(0.4 mg/g) than seen in moderate light grown control plants (0.85 mg/g) and high light grown predawn (control) plants (0.83 mg/g; Fig. 6b). Low light grown plants on exposure to sunlight for 3 days exhibited an increase of 120% in phospholipids content as compared to its control plants. A longer exposure to sunlight (up to 7 days) did not further increase the phospholipids content. High light exposure of moderate light grown plants for 7 days resulted in a linear increase to 179% in the phospholipids content. The increase in the phospholipids content as a result of sunlight treatment was much higher (2.4 mg/g) in moderate light grown plants than the increase observed in low light grown plants (0.86 mg/g) as a result of sunlight treatment. High

light grown plants did not show any increase in the phospholipids content as compared to its predawn plants.

Plants grown in the three different light conditions exhibited lower glycolipid content than phospholipids. The treatment to high light for 7 days resulted in higher level of increase in the phospholipids than the increase observed in the glycolipid content in plants of all three different light conditions.

Quantitative estimation of glycolipids and phospholipids of thylakoid membrane under in vitro conditions Glycolipid content of chloroplasts isolated from low, moderate and high light grown plants and subsequently exposed to

Fig. 5 TLC profile of neutral lipids in the thylakoid membrane of chloroplasts isolated from low **a**, moderate (prior to transfer to direct sunlight) **b** and high light (predawn) **c** grown 30 days old rice plants and treated with artificial light ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 6 h. Control refers to chloroplasts not treated with high light. Volumetrically a constant amount of lipid sample was loaded in all the three cases

Fig. 6 Changes in glycolipids **a** and phospholipids **b** in the cell membrane of low (LL), moderate (ML) and high (HL) light grown plants before and after high light treatment. Changes in glycolipids **c** and phospholipids **d** in thylakoid membrane of chloroplasts isolated from low (LL), moderate (ML) and high (HL) light grown plants and thereafter treated with artificial light ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$). ($n=3$, $\pm$ SD). **a**—significantly different from control plants ($p<0.05$)

artificial light ($1200 \mu\text{mol m}^{-2} \text{s}^{-2}$) was more or less same (Fig. 6c). Treatment of chloroplasts isolated from low light grown plants, to artificial light resulted in 28% decline in the glycolipid content (Fig. 6c). Decrease in the glycolipids content for the same treatment for chloroplasts isolated from moderate and high light grown plants was 41% and 17% respectively as compared to their control. The phospholipid content of isolated chloroplasts from plants grown under the three different light conditions also resulted in a decline as a result of artificial light treatment (Fig. 6d).

Analysis of fatty acids of cell membrane under in vivo conditions In rice plants the fatty acid groups identified were Lauric acid C_{12} , Myristic acid C_{14} , Palmitic acid C_{16} , Stearic acid C_{18} , Oleic acid $\text{C}_{18:1}$, Linoleic acid $\text{C}_{18:2}$ and Linolenic acid $\text{C}_{18:3}$. Some slight quantitative but non-significant changes were observed in the concentration of the different saturated and unsaturated fatty acid groups of

total lipids in low, moderate and high light grown rice plants, as a result of high light treatment (Table 2). No qualitative changes were seen. (GC profile of fatty acids are given as supplementary data).

Lauric acid and Stearic acid content was highest (14.48 and 14.22 mole% respectively) in moderate light grown plants (control) and least (13.81 and 13.60 mole % respectively) in low light grown plants prior to transfer to direct sunlight (control; Table 2). Myristic acid content was highest in control high light grown plants (predawn; 14.97 mole%) and least in control low light grown plants (14.52 mole %). Palmitic acid content was highest in control low light grown plants (13.81 mole %) while it was the least in control moderate light grown plants (12.94 mole %).

Oleic acid, a single double bind unsaturated fatty acid, was highest in control moderate light grown plants (15.36 mole%) and least (14.67 mole %) in control low light grown plants (Table 2). Linoleic acid was observed to be highest in control high light grown plants (15.08 mole %)

Table 2 The fatty acid composition (mole %) in cell membrane of low, moderate and high light grown 30 days old rice plants before and after high light treatment. In low and moderate grown plants control refers to plants prior to transfer to direct sunlight and in high light

grown plants control refers to predawn (0630 h). In low and moderate grown plants data has been shown till 7 days while in high light grown plants data at the end of a day has been shown. ($n=3$, $\pm$ SD)

	Lauric Acid (C ₁₂)	Myristic Acid (C ₁₄)	Palmitic Acid (C ₁₆)	Stearic Acid (C ₁₈)	Oleic Acid (C _{18:1})	Linoleic Acid (C _{18:2})	Linolenic Acid (C _{18:3})
Low light							
C	13.81 $\pm$ 0.02	14.52 $\pm$ 0.05	13.81 $\pm$ 0.03	13.60 $\pm$ 0.01	14.67 $\pm$ 0.06	14.78 $\pm$ 0.03	14.78 $\pm$ 0.01
1 D	13.84 $\pm$ 0.02	14.48 $\pm$ 0.02	13.61 $\pm$ 0.02	13.64 $\pm$ 0.02	14.74 $\pm$ 0.02	14.69 $\pm$ 0.02	14.97 $\pm$ 0.02
3 D	13.90 $\pm$ 0.02	14.56 $\pm$ 0.02	13.68 $\pm$ 0.02	13.69 $\pm$ 0.02	14.76 $\pm$ 0.02	14.82 $\pm$ 0.02	14.58 $\pm$ 0.02
7 D	13.94 $\pm$ 0.02	14.68 $\pm$ 0.02	13.72 $\pm$ 0.02	13.71 $\pm$ 0.02	14.78 $\pm$ 0.02	14.91 $\pm$ 0.02	14.22 $\pm$ 0.02
Moderate light							
C	14.48 $\pm$ 0.02	14.92 $\pm$ 0.02	12.94 $\pm$ 0.02	14.22 $\pm$ 0.02	15.36 $\pm$ 0.02	14.98 $\pm$ 0.02	13.06 $\pm$ 0.02
1 D	13.95 $\pm$ 0.02	14.60 $\pm$ 0.02	13.94 $\pm$ 0.02	13.74 $\pm$ 0.02	14.82 $\pm$ 0.02	14.80 $\pm$ 0.02	14.12 $\pm$ 0.02
3 D	13.90 $\pm$ 0.02	14.63 $\pm$ 0.02	13.89 $\pm$ 0.02	13.73 $\pm$ 0.02	14.81 $\pm$ 0.02	14.66 $\pm$ 0.02	14.34 $\pm$ 0.02
7 D	13.96 $\pm$ 0.02	14.73 $\pm$ 0.02	13.37 $\pm$ 0.02	13.74 $\pm$ 0.02	15.01 $\pm$ 0.02	14.80 $\pm$ 0.02	14.35 $\pm$ 0.02
High light							
C	14.25 $\pm$ 0.02	14.97 $\pm$ 0.02	13.15 $\pm$ 0.02	14.11 $\pm$ 0.02	15.21 $\pm$ 0.02	15.08 $\pm$ 0.02	13.20 $\pm$ 0.02
1 D	14.19 $\pm$ 0.02	14.91 $\pm$ 0.02	13.66 $\pm$ 0.02	13.92 $\pm$ 0.02	14.91 $\pm$ 0.02	14.14 $\pm$ 0.02	14.24 $\pm$ 0.02

and least (14.78 mole %) in control low light grown plants. Linolenic acid was observed to be highest in control low light grown plants (14.78 mole %) and least in control moderate light grown plants (13.06 mole %). However, no significant difference in the distribution pattern of any seven fatty acids was observed. Table 3.

Analysis of fatty acids of thylakoid membrane under in vitro No significant qualitative and quantitative changes in the fatty acid composition were observed when isolated chloroplasts were exposed to high light; neither did we

observe any significant changes in the distribution pattern of the various fatty acids.

Discussion

The present study highlights the changes in the lipids of cell and thylakoid membrane of rice plants grown in the different growth conditions as a result of high light treatment. In our study, we observed a pronounced decline in the maximum quantum yield for primary photochemistry,

Table 3 The fatty acid composition (mole %) in thylakoid membrane of chloroplasts isolated from low, moderate (prior to transfer to direct sunlight) and high light (predawn) grown 30 days old rice plants and

thereafter treated with artificial light (1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 30°C). Control refers to chloroplasts not treated with high light. ($n=3$, $\pm$ SD)

	Lauric acid (C ₁₂)	Myristic acid (C ₁₄)	Palmitic acid (C ₁₆)	Stearic acid (C ₁₈)	Oleic acid (C _{18:1})	Linoleic acid (C _{18:2})	Linolenic acid (C _{18:3})
Low light							
C	13.94 $\pm$ 0.02	14.60 $\pm$ 0.02	13.51 $\pm$ 0.02	13.72 $\pm$ 0.02	14.84 $\pm$ 0.02	14.81 $\pm$ 0.02	14.55 $\pm$ 0.02
3 h	13.89 $\pm$ 0.02	14.53 $\pm$ 0.02	13.68 $\pm$ 0.02	13.68 $\pm$ 0.02	14.77 $\pm$ 0.02	14.84 $\pm$ 0.02	14.58 $\pm$ 0.02
6 h	13.58 $\pm$ 0.02	14.33 $\pm$ 0.02	13.54 $\pm$ 0.02	13.41 $\pm$ 0.02	14.48 $\pm$ 0.02	14.51 $\pm$ 0.02	15.12 $\pm$ 0.02
Moderate light							
C	13.95 $\pm$ 0.02	14.68 $\pm$ 0.02	13.93 $\pm$ 0.02	13.76 $\pm$ 0.02	14.83 $\pm$ 0.02	14.76 $\pm$ 0.02	14.06 $\pm$ 0.02
3 h	13.93 $\pm$ 0.02	14.64 $\pm$ 0.02	13.76 $\pm$ 0.02	13.71 $\pm$ 0.02	14.81 $\pm$ 0.02	14.83 $\pm$ 0.02	14.29 $\pm$ 0.02
6 h	13.83 $\pm$ 0.02	14.53 $\pm$ 0.02	13.83 $\pm$ 0.02	13.61 $\pm$ 0.02	14.70 $\pm$ 0.02	14.70 $\pm$ 0.02	14.77 $\pm$ 0.02
High light							
C	13.85 $\pm$ 0.02	14.54 $\pm$ 0.02	13.81 $\pm$ 0.02	13.65 $\pm$ 0.02	14.74 $\pm$ 0.02	14.76 $\pm$ 0.02	14.60 $\pm$ 0.02
3 h	13.81 $\pm$ 0.02	14.52 $\pm$ 0.02	13.81 $\pm$ 0.02	13.59 $\pm$ 0.02	14.69 $\pm$ 0.02	14.80 $\pm$ 0.02	14.74 $\pm$ 0.02
6 h	13.82	14.55 $\pm$ 0.02	13.78 $\pm$ 0.02	13.62 $\pm$ 0.02	14.71 $\pm$ 0.02	14.78 $\pm$ 0.02	14.71 $\pm$ 0.02

measured as Fv/Fm ratio, of low light grown plants on exposure to direct sunlight for 6 h (See inset Fig. 1a). In addition to the decrease in the Fv/Fm, an increase in MDA content was also observed as a result of high light treatment (Fig. 1a). Increases in MDA are indicative of enhanced lipid peroxyl radical formation in the cell and thylakoid membrane as a result of higher production of singlet oxygen within the light harvesting complexes during sun exposure. This indicates ROS related oxidative damage to plants under our experimental conditions.

The decrease observed in the amount of MDA after 3 days of transfer to sunlight in low and moderate light grown plants as compared to day one after transfer (Fig. 1a), in our study, could be due to the activity of both enzymatic and non-enzymatic antioxidants, as discussed by Ye et al. (2000) and Verhoeven et al. (2005). However, the increase in MDA content observed in cell membrane lipids of high light grown plants in our study (Fig. 1a), indicates limitation of protection by such antioxidative processes against oxidative damage in these plants.

In our study with isolated chloroplasts, artificial light treatment for 6 h resulted in a continuous increase in the amount of lipid peroxidation (Fig. 1b). The reason for this could be that unlike intact leaves, isolated chloroplasts did not have any functional enzymatic or non-enzymatic antioxidants, which could protect it against lipid peroxidation. Even though high light grown plants exhibited higher lipid peroxidation than low and moderate light grown plants (Fig. 1a), chloroplasts isolated from high light grown plants had lower level of lipid peroxidation compared to chloroplasts isolated from low and moderate light grown plants (Fig. 1b). The results may suggest that chloroplasts isolated from high light grown plants had greater level antioxidants, thus better of protection of thylakoid membrane lipids against ROS.

Plants are known to change their membrane lipid composition in response to stress (e.g. drought: Gigon et al. 2004; freezing: Welti et al. 2002; salinity: El Kahoui et al. 2004). The changes in the lipid composition and content observed in the present study may be related to adaptation and acclimation of the rice plants under the changing environmental conditions, in order to sustain optimum structure and function of the cell and thylakoid membrane to maintain active photosynthesis even under stress conditions. The slight qualitative changes observed in the phosphoglycolipids in the low light grown plants after exposure to sunlight, indicate a role of these lipids in protecting or adaptation of plants which were grown at very low light and then transferred to high sunlight (Fig. 2). No qualitative changes were observed in the neutral lipids of low and moderate light grown plants on exposure to direct sunlight (Fig. 4), indicating that the neutral lipids did not

play any significant role in the acclimation of low light grown plants to high light. Although, some of the phosphoglycolipids and neutral lipids were common to the plants grown in all the three different growth conditions, a few of the phosphoglycolipids and neutral lipids were present exclusively only in low or moderate or high light grown plants. Thus, indicating requirement of different type of lipid composition of rice plants in response to their different growth irradiances which on transfer to high light change qualitatively (more so in low light grown plants) and quantitatively on exposing to high sunlight (Fig. 2). These qualitative and quantitative changes in the lipids may be required for better functioning of the biochemical reactions under the changed conditions in order to provide acclimation. Toumi et al. (2008) observed a differential modification in the lipid composition of four grapevine cultivars with different drought tolerance when they were subjected to water stress. Their data suggests that lipid composition could also have an impact on the overall tolerance of plants to stress.

The quantitative increase in the glyco and phospholipids (Fig. 6) may be due to an increase in the enzymes involved in lipid synthesis in order to change the lipid composition of the membrane (Nishida and Murata 1996).

The thylakoid membrane has to be stabilized in a bilayer structure by increasing the concentration of the bilayer forming lipids such as DGDG and phospholipids (Mock and Kroon 2002). This probably could be the reason for the increase observed in the concentration of glycolipids and phospholipids in low and moderate light grown plants on transfer to direct sunlight (Fig. 6a and b). In higher plants it is well known that light largely stimulates “*de novo*” synthesis of lipids and fatty acids. Klyachko-Gurvich et al. (1999) observed changes in lipid or fatty acid quality during light acclimation of plants in addition to quantitative changes in chloroplast lipids. Tasaka et al. (1996) reported that this change is more distinct in combination with low temperatures. However, in our study with isolated chloroplasts, high light treatment did not result in any increase in the glyco and phospholipid content (Fig. 6c and d), since chloroplasts being an isolated system “*de novo*” synthesis of lipids was probably not possible, thus substantiating *de novo* synthesis of lipids under *in vivo* conditions.

In this study we did not observe any significant changes in the fatty acid composition, however, it was observed that a specific type of fatty acid was consistently higher in plants under specific conditions (low, moderate and high light). Similarly, a slight increase in some of the unsaturated fatty acids over saturated fatty acids was also observed. These changes may be needed for the adaptation of the plants to high light conditions to maintain the required membrane integrity and function under altered light con-

ditions. However, more studies are needed to confirm this observation.

Similar changes in the fatty acid composition under different stress conditions have been reported by various workers. Guerfel et al. (2008) observed an increase in the degree of unsaturation in leaf lipids of olive trees in response to water stress. Liao et al. (2004) reported an increase in the contents of unsaturated fatty acids in the chloroplasts of *Dioscorea zingiberensis* as a result of treatment with high irradiance and high temperature. Walsh et al. (1997) reported that the changes in the fatty acid composition due to high level of light intensity may occur as a cellular response to reduce the susceptibility of the membrane to photooxidation. Gombos et al. (1997) reported that the unsaturation of the fatty acids in membrane lipids did not affect the extent of light-induced damage but accelerated the recovery from damage. Further, it has been pointed out by Murata and Siegenthaler (1998) that the degree of fatty acid desaturation and the lipid composition in thylakoid membranes is critical in maintaining optimal photosynthesis. Similarly, in the present study the various changes observed in the quality and quantity of lipids and fatty acids indicates a vital role of these components in maintaining active physiological functions in rice plants in response to exposure to different light conditions as high light may lead to formation of ROS and may affect fatty acid composition of cell membrane affecting the membrane function.

It is concluded that high light caused depression of the maximum quantum yield for primary photochemistry (Fv/Fm). This was accompanied by peroxidation of membrane lipids as MDA increased significantly. High light exposure also caused changes in the phosphoglycolipids and neutral lipids and fatty acid composition. We propose that these changes are essential for adaptation of low light grown plants to high light regime.

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