Effects of high light exposure on leaf gas exchange parameters and xanthophyll cycle pigments in rice seedlings

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Abstract

The rice seedlings of Aathira and Swarnaprabha varieties were exposed to 8 h of high light (2000 $\mu molm^{-2}s^{-1}$) and the effects on leaf gas exchange parameters and interconversions of xanthophyll cycle pigments were analyzed. High light exposure resulted in decrease of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) both in Aathira and Swarnaprabha leaves. However, the rate of reduction of P_n, g_s and E was more prominent in Swarnaprabha than Aathira seedlings upon treatment with high light stress. The xanthophyll pigment profile analyzed by HPLC showed significant difference between Aathira and Swarnaprabha leaves after exposure to 8 h of high light irradiation. HL exposure in rice leaves led to the reduction of violaxanthin and concomitant accumulation of antheraxanthin and zeaxanthin in both Aathira and Swarnaprabha seedlings. However, conversion of violaxanthin to zeaxanthin was maximum in Aathira than Swarnaprabha leaves upon exposure to high light irradiation. Moreover, the de-epoxidation state of xanthophyll cycle pigments was enhanced while the epoxidation state of xanthophyll cycle pigments was decreased upon exposure to 8 h of high light irradiation in rice leaves. The proportion of the xanthophyll cycle pool to total chlorophyll was tremendously increased in Aathira than Swarnaprabha upon high light exposure, revealing the superior nature of Aathira variety in terms of tolerance potential towards high light exposure.

Key words: gas exchange, high light, xanthophyll cycle

Introduction

Plants require light for photosynthetic process, but absorption of excess light can lead to photooxidative damage to the photosynthetic apparatus and decrease the photosynthetic efficiency. Thus absorbed solar energy may become excessive for plants when it exceeds the capacity to utilize the same for photosynthesis. When light energy exceeds cell's needs it results in the production of reactive oxygen species which damage cell components including photosynthetic apparatus causing light induced photoinhibition (Nishiyama *et al.*, 2006; Gururani *et al.*, 2015).

Gas exchange measurements are a non-destructive, non-invasive technique to monitor accurate photosynthetic carbon assimilation rate in plants. Measurements are rapid and can be used to examine the altered photosynthetic rates under different environmental stress conditions (Massacci *et al.*, 2008; Wang *et al.*, 2016). The main gas exchange parameters such as the photosynthetic rate (P_n), transpiration rate (E) and the stomatal behavior, generally fluctuates at varying degrees when plants are subjected to high light (HL) irradiation and these variations are species specific (Sanusi *et al.*, 2011; Lu *et al.*, 2017).

Xanthophyll cycle is one of the most efficient mechanisms protecting plants and other photosynthesizing organisms under over excitation conditions resulted by various environmental factors (Kromdijk *et al.*, 2016; Junker *et al.*, 2017). It is well documented that plants under increasing HL stress employ a greater level of xanthophyll cycle interconversion, whereby conversion of particular group of carotenoids, violaxanthin, antheraxanthin and zeaxanthin occurs by enzyme catalyzed de-epoxidations. Under moderate stress conditions, violaxanthin function as the most abundant antenna pigment by transferring energy to Chl *a*. With increasing stress dosage or intensity, violaxanthin is biochemically converted to pigment zeaxanthin *via* the intermediate antheraxanthin by the activity of violaxanthin de-epoxidase and excess excitation energy can be harmlessly dissipated as heat through the formation of zeaxanthin or antheraxanthin in the antenna pigment complexes of PSII (Chen and Gallie, 2012). As a consequence, quenching of excess energy helps to keep PSII reaction centers open and maintain the electron transport and thus protects the reaction centers from photoinhibition (Goss and Jakob, 2010). In this study, we made an attempt to analyze the effects of HL exposure on leaf gas exchange parameters and interconversions of xanthophyll cycle pigments in rice seedlings.

Materials and methods

Two varieties of rice seeds (Aathira and Swarnaprabha) were germinated and grown in plastic bottles containing absorbent cotton soaked with half strength Hoagland solution. After 10 d of growth, rice seedlings were exposed to high light (2000 $\mu mol m^{-2} s^{-1}$), provided by 1000 W PAR64 metal halide lamps (Philips, Netherlands). Air was circulated around the seedlings and thus the temperature was maintained at $24 \pm 2^{o}C$. Photosynthetically active radiation in terms of light intensity at the surface of the leaves at one hour interval was measured by a solar radiation monitor (EMCON, India). Leaf gas exchange parameters and xanthophyll cycle pigments were analyzed after rice seedlings were exposed to 8 h of high light stress.

Leaf gas exchange parameters were analyzed by using a LI-6400 portable photosynthesis system (Infra-red gas analyzer, LI-COR, Lincoln, Nebraska, USA). Leaf surfaces were cleaned and dried using tissue paper before being enclosed in the leaf chamber for gas exchange measurements. All measurements were record on fully expanded first formed rice leaves and reading was taken between 9.00 to 10.00 am. The various leaf gas exchange parameters, net photosynthetic rate, P_n ($\mu molm^{-2}s^{-1}$), stomatal conductance, g_s ($\mu molm^{-2}s^{-1}$) and transpiration rate, E ($mmolm^{-2}s^{-1}$) was measured in rice leaves after exposure to high light. P_n, g_s and E were calculated using the equations derived by von Caemmerer and Farquhar (1981).

High performance liquid chromatography (HPLC) analysis of xanthophyll cycle pigments was done by the method of Gopalakrishnan and Annamalainathan (2016). Fresh leaf samples were homogenized in 100% ice cold acetone containing 0.1% butylated hydroxytoluene (w/v) by using a pre-cold mortar and pestle. The homogenate was centrifuged for 25 min at 4°C for 8000 rpm and the supernatant was collected. Identification and separation of pigments were done on a reverse phase column, Waters Spherisorb ODS-5 µm column (250x4.6 mm) in HPLC. Samples were injected with a Rheodyne 7010 injector, with a 20 µl loop at 30°C for 60 min. The solvent system consisted of acetonitrile:methanol:water (84:14:2) and methanol:ethyl acetate (68:32). Peaks were detected at 450 nm with waters 996 photodiode array detector and integrated using Empower software.

Standards of xanthophylls (lutein, zeaxanthin and β-carotene) were used for the study. Pigments were identified by comparing their absorption spectra and retention time with standards. Level of de-epoxidation and epoxidation state of xanthophyll cycle pigments was calculated as (Z + A)/(V + A + Z) and (V+A)/(V+A+Z), respectively, where, Z - zeaxanthin, A - antheraxanthin, V - violaxanthin. The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean±standard error (SE).

Results

Treatment with 8 h of high light stress significantly decreased the P_n in both rice varieties over the control plants. However, the rate of reduction of P_n was more prominent in Swarnaprabha (52%) than Aathira seedlings (29%) upon treatment with high light stress as compared to the control leaves (Fig. 1A). Likewise, significant decrease in g_s was recorded in both Swarnaprabha (54%) and Aathira (28%) leaves when they were subjected to high light treatment as compared to the control leaves (Fig. 1B). The transpiration rate was also decreased after exposure to 8 h of high light stress and the maximum reduction was recorded in Swarnaprabha (81%) than Aathira (48 %) with respect to the control leaves (Fig. 1C).

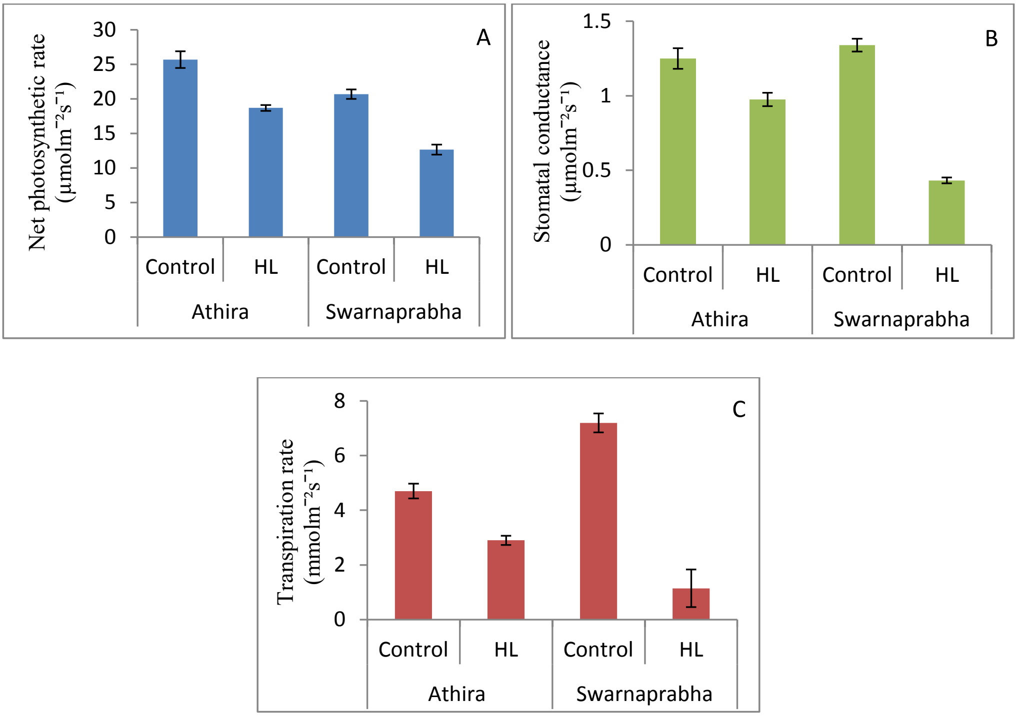

Figure 1: Net photosynthetic rate (A), stomatal conductance (B) and transpiration rate (C) of Aathira and Swarnaprabha leaves after exposure to 8 h of high light stress (2000 μmolm^{-2}s^{-1}). The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9).

The xanthophyll pigment profile analyzed by HPLC showed significant difference between Aathira and Swarnaprabha leaves after exposure to 8 h of HL irradiation. Apart from the major fractions of the xanthophylls pigments, including lutein, some other carotenoids - α and β carotenes could also be detected. Neoxanthin (N) was eluted first followed by violaxanthin (V), antheraxanthin (A), lutein (L), zeaxanthin (Z), Chl *b*, Chl *a*, α and β carotenes. It was found that the content of pigments N, A and Z registered a steady increase after exposure to 8 h of HL stress in both rice varieties. In contrast, the content of V was found to decrease after HL treatment in both rice varieties as compared to the control leaves. It was showed that the Z content was not traceable in control seedlings of Aathira and Swarnaprabha leaves, while it was increased significantly after exposure to 8 h of HL treatment and reached to an extent of 7.7 μgg^{-1} FW in both Aathira and Swarnaprabha leaves. Likewise, the content of A was also enhanced to an extent of 2.3 μgg^{-1} FW in Aathira leaves after 8 h of HL exposure, but A content was not detected in untreated leaves of this variety. In Swarnaprabha leaves, A was found to increase from 1.3 μgg^{-1} FW (control) to 2.4 μgg^{-1} FW after 8 h of HL treatment (Fig. 2A).

A prominent reduction of lutein content in Aathira and Swarnaprabha was recorded after 8 h of HL (55 and 60%) with respect to their untreated leaves. The content of N was enhanced in Aathira seedlings (35%) after exposure to 8 h of HL treatment, but not in Swarnaprabha as compared to the respective control seedlings. Likewise, a significant variation in both α and β carotene content was registered after HL stress in both rice varieties. A significant increase in the accumulation of β-carotene content was registered in the leaves of Aathira (170%) and the increase in Swarnaprabha leaves was negligible (12%) upon 8 h of HL treatment over the control plants. Moreover, α-carotene was also enhanced in Aathira leaves, whereas in Swarnaprabha it was not increased after treatment with HL exposure (Fig. 2A).

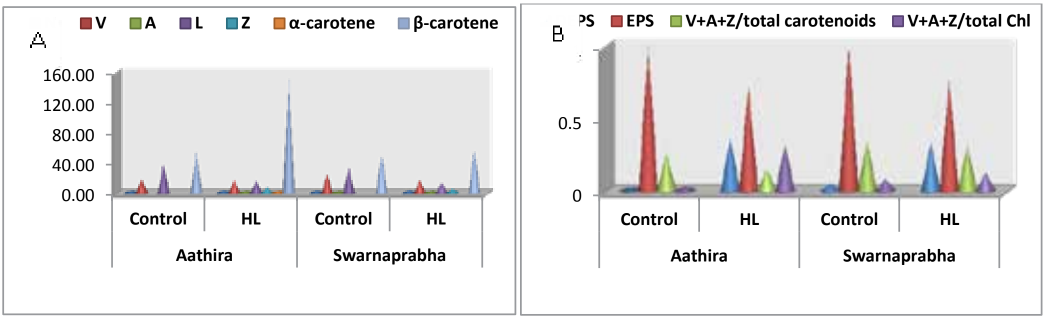

Figure 2: HPLC elution components of the photosynthetic xanthophyll pigments (A), changes in de-epoxidation (DEPS) and epoxidation (EPS) state of xanthophyll cycle pigments, the proportion of the xanthophyll cycle pool (V+A+Z) to total Chl and carotenoids (B) in Aathira and Swarnaprabha after exposure to 8 h of HL treatment. The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9).

The de-epoxidation state of xanthophyll cycle pigments (DEPS=Z+A/V+A+Z) generally exhibited higher values in HL treated Aathira and Swarnaprabha seedlings (37 and 33%) as compared with controls. In contrast, a significant decrease was found in the epoxidation state of xanthophyll cycle pigments (EPS=V+A/V+A+Z) after exposure to HL irradiation in both varieties of rice seedlings and the rate of decrease was more or less similar in both varieties (23-28%). Parallel to this, the proportion of the xanthophyll cycle pool (V+A+Z) to total Chl and carotenoids became more significant in rice seedlings upon HL exposure. The ratio of xanthophyll cycle pool to total Chl (*a*+*b*) was highly increased in Aathira (983%) and to a lesser extent in Swarnaprabha leaves (54%) after exposure to 8 h of HL stress over the control leaves. However, the ratio of xanthophyll cycle pool to total carotenoids was decreased in both Aathira and Swarnaprabha leaves after 8 h of HL exposure with respect to the control seedlings (Fig. 2B).

Discussion

HL exposure altered the fundamental processes of photosynthesis in the leaves of Aathira and Swarnaprabha as evidenced by various leaf gas exchange parameters. The reduction of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) was more prominent in Swarnaprabha than Aathira seedlings upon treatment with 8 h of HL irradiation. According to Zhang *et al.* (2016), HL irradiation induced deactivation of rubisco, which suppressed P_n and subsequently E in *Platanus orientalis, Melia azedarach* and *Solanum lycopersicum* seedlings. Thus the enhancement rate of inhibition in photosynthesis under HL exposure in Swarnaprabha, followed by Aathira could be due to the reduced carbon assimilation and inhibition of PSII photochemistry. The high reduction of stomatal conductance in Swarnaprabha and comparatively lower reduction in Aathira, results in reduction of gaseous exchange, ultimately resulting in decline of photosynthesis in Swarnaprabha leaves. Tani *et al.* (2001) concluded that the stomatal limitation to photosynthesis is responsible for the limited photosynthetic activity of *Pteridophyllum racemosum* to increased light stress conditions.

Xanthophyll cycle is one of the most efficient mechanisms protecting plants under over excitation conditions of HL stress and some components of this cycle act as quenchers of singlet chlorophyll, thus preventing the formation of reactive oxygen species. The concomitant increase of some xanthophylls and carotenes with HL exposure in Aathira and Swarnaprabha rice leaves can be attributed to the involvement of these pigments in dissipation of HL induced excess energy. HL exposure in rice leaves led to the reduction of violaxanthin and concomitant accumulation of antheraxanthin and zeaxanthin in both Aathira and Swarnaprabha seedlings. However, conversion of violaxanthin to zeaxanthin was maximum in Aathira than Swarnaprabha leaves upon exposure to HL irradiation. The results were corroborated with the findings obtained by HPLC analysis in the leaves of *Secale cereale* leaves exposed to HL illumination (1200 $\mu molm^{-2}s^{-1}$) wherein it was found that zeaxanthin content was highly enhanced after HL treatment (Janik *et al.*, 2008). Similar pattern of changes in xanthophyll pigments have been reported in *Hevea brasiliensis* after exposure to HL conditions (Gopalakrishnan and Annamalainathan, 2016).

Lutein is the most abundant xanthophyll pigment in higher plants and it has a primary role in quenching of triplet chlorophyll states (Estaban *et al.*, 2008). HL irradiated rice seedlings showed the lowest epoxidation state of xanthophyll pigments and lutein content, which suggested that the absence of lutein was compensated by increased levels of other xanthophylls derived from β-carotene (Pogson *et al.*, 1996). High NPQ status of Aathira leaves upon exposure to HL stress than Swarnaprabha could be correlated with the high xanthophyll cycle pigments accumulated in Aathira and it is fact that xanthophylls are involved in the NPQ of excitation energy in LHCII of the PSII. In addition to this, zeaxanthin presumably exert additional photoprotective effect as an antioxidant providing stabilization to thylakoid membrane lipids under extreme HL stress (Gruszecki and Strzałka, 2005).

The de-epoxidation state of xanthophyll cycle pigments was enhanced while the epoxidation state of xanthophyll cycle pigments was decreased upon exposure to 8 h of HL irradiation in rice leaves. Likewise, the proportion of the xanthophyll cycle pool to total Chl was tremendously increased in Aathira than Swarnaprabha upon HL exposure, revealing the superior nature of Aathira variety in terms of tolerance potential towards thermal dissipation of excess energy after exposure to HL and thus avoiding photooxidative stress. Photoprotection of the photosynthetic machinery to HL conditions through xanthophyll pigments was observed in *Plantago media* plants (Golovko *et al.*, 2011). Moreover, the extent of de-epoxidation of xanthophyll cycle pigments and the content of total xanthophyll cycle pigments expressed per chlorophyll was greatly enhanced in *Vinca minor* upon HL exposure (Verhoeven *et al.*, 1999). Previously it was reported that oxidative stress in plants leads to an increase in β-carotene content since the deactivation of singlet oxygen is provided by β-carotene in plants (Ramel *et al.*, 2012). Similarly, HL exposure induced accumulation of α and β-carotene content was higher in the leaves of Aathira and comparatively lower in Swarnaprabha leaves upon 8 h of HL treatment. Conclusively it was found that the higher levels of zeaxanthin and the lower level of epoxidation state of xanthophyll pigments in Aathira than Swarnaprabha leaves after HL exposure largely contributes towards photoprotection in Aathira and thus revealing the superior nature of Aathira variety in terms of tolerance potential towards HL exposure.

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