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Photosynthetic response of clusterbean chloroplasts to UV-B radiation: Energy imbalance and loss in redox homeostasis between Q_A and Q_B of photosystem II

Padmanava Joshi[a,*], Sasmita Gartia[a,b], Manoj Kumar Pradhan[a], Basanti Biswal[b]

[a] Laboratory of Biophysics and Biochemistry, Anchal College, Padampur, Rajborasambar, Bargarh, 768036 Orissa, India
[b] Laboratory of Biophysics and Biochemistry, School of Life Sciences, Sambalpur University, Jyotivihar, Sambalpur, 768019 Orissa, India

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ABSTRACT

The effects of ultraviolet-B (UV-B: 280–320 nm) radiation on the photosynthetic pigments, primary photochemical reactions of thylakoids and the rate of carbon assimilation (P_n) in the cotyledons of clusterbean (*Cyamopsis tetragonoloba*) seedlings have been examined. The radiation induces an imbalance between the energy absorbed through the photophysical process of photosystem (PS) II and the energy consumed for carbon assimilation. Decline in the primary photochemistry of PS II induced by UV-B in the background of relatively stable P_n, has been implicated in the creation of the energy imbalance. The radiation induced damage of PS II hinders the flow of electron from Q_A to Q_B resulting in a loss in the redox homeostasis between the Q_A to Q_B leading to an accumulation of Q_A^-. The accumulation of Q_A^- generates an excitation pressure that diminishes the PS II-mediated O_2 evolution, maximal photochemical potential (F_v/F_m) and PS II quantum yield ($\Phi_{PS\,II}$). While UV-B radiation inactivates the carotenoid-mediated protective mechanisms, the accumulation of flavonoids seems to have a small role in protecting the photosynthetic apparatus from UV-B onslaught. The failure of protective mechanisms makes PS II further vulnerable to the radiation and facilitates the accumulation of malondialdehyde (MDA) indicating the involvement of reactive oxygen species (ROS) metabolism in UV-B-induced damage of photosynthetic apparatus of clusterbean cotyledons.

1. Introduction

The light energy absorbed by photosynthetic pigments associated with the photosystem (PS) II of chloroplasts is used to split H_2O molecule to generate electrons which are transferred through electron transport system of thylakoid for the assimilation of carbon, nitrogen and sulfur. A balance between the energy absorbed by the PS II for liberation of electrons (source) and their utilization by the terminal electron acceptors of assimilating processes (sink), often referred to as photostasis, is maintained in a photosynthetic organelle for optimization of photosynthesis [1–3]. This balance is perturbed by abiotic stress factors due to modification in the energy absorbing process of PS II, alteration in the redox state of electron carrier(s) between PS II and PS I and/or a decline in the electron sink capacity. The effects of abiotic stresses on the photostasis of photosynthesis and the acclimation mechanisms of green leaves to restore it have been studied [3]. The loss in the photostasis as induced by the stress like heavy metals [4], drought [5], nutrient limitation [6] and low temperature [7] is attributed to the loss in photosynthetic electron sink capacity. On the other hand, the impact of UV-B (UV-B: 280–320 nm) radiation, another abiotic stress, on the photostasis of photosynthesis and acclimation responses of green plants to restore it are not clearly understood, although the effects of UV-B radiation on photosynthesis have extensively been studied during the last three decades [8,9].

Perusal of relevant literature reveals that UV-B radiation inflicts damage to the photosynthetic apparatus of green plants at multiple sites. The sites of damage include oxygen evolving complex (OEC) [10,11], D1/D2 reaction centre proteins [12,13] and other components on the donor and acceptor sides of PS II [14,15]. The radiation also inactivates the xanthophyll cycle associated with light harvesting complex II (LHC II) [16] and alters gene expression for synthesis of PS II reaction centre proteins [11]. Even the ambient UV-B radiation inflicts detrimental effects on photosynthesis of Arctic plants [17] and algae [18,19] above and beyond altering their ultra structure, respiration, growth and reproduction [19,20]. Inactivation of PS II induced by UV-B has been viewed to be the main reason of loss

Abbreviations: Chl, chlorophyll; Car, carotenoid; DCMU, 3-(3,4dichlorophenyl)-1,1-dimethylurea; F_m, maximum fluorescence; F_v, variable fluorescence; G_s, stomatal conductance; $K_3Fe(CN)_6$, potassium ferricyanide; MDA, malondialdehyde; PAR, photosynthetically active radiation; P_n, the rate of carbon assimilation; Q_A, primary quinone electron acceptor of PS II; Q_B, secondary quinone electron acceptor of PS II; q_p, photochemical quenching; ROS, reactive oxygen species; TL, thermoluminescence; UV-A, ultraviolet-A (320–400 nm); UV-B, ultraviolet-B (280–320 nm); $\Phi_{PS\,II}$, quantum yield of PS II.

* Corresponding author. Tel.: +91 6683223045.
E-mail address: padmanava.joshi@gmail.com (P. Joshi).

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in photosynthesis. Conversely, PS I is reported to be relatively stable [21,22]. The uneven damage of these photosystems is likely to create an energy imbalance leading to a loss in redox homeostasis in the photosynthetic apparatus a possibility that has not yet been examined. Further, most of the findings relating to UV-B effects on photosynthetic apparatus are based upon studies made with reductionist approach and to our knowledge no effort has been made to examine both primary photochemistry of thylakoids and carbon assimilation in Calvin–Benson cycle in a single plant system to establish the relative susceptibility of different major components of chloroplasts to UV-B radiation.

Simultaneous study of the primary photochemistry and carbon assimilation provides scope to analyze the UV-B-induced alteration in the efficiency of electron source and electron sink capacity which may result in energy imbalance in photosynthetic apparatus. In fact, our results, demonstrating the relative stability of the rate of carbon assimilation (P_n), with a significant loss in PS II photochemistry and a partial blockage in the electron transport chain between Q_A and Q_B suggest a change in redox homeostasis and creation of an energy imbalance in clusterbean cotyledons. We have also analyzed the effect of UV-B radiation on mechanisms of defense and adaption to alleviate the damage of the photosynthetic apparatus.

2. Materials and methods

2.1. Plant materials and UV-B exposure

Clusterbean (*Cyamopsis tetragonoloba* L.) seeds obtained from National Seeds Corporation of India, Sambalpur, were surface sterilized with 30% alcohol and then kept in running water for 3 h. These seeds were soaked in distilled water for 12 h. The well germinated seeds were grown in Petri plates on cotton soaked with distilled water as described by Joshi et al. [23]. It has been shown there that the developmental pattern of cotyledons of clusterbean seedlings exhibits juvenile phase up to d 6 and steady phase from d 7 to 10. Since the optimum photosynthesis is maintained during steady phase of leaf development, the experimental period was limited to d 10 in the present work.

A set of seedlings was exposed to UV-B radiation (Philips TL 20, Type-05, 285–320 nm with the maximum peak at 315 nm) at a fluence rate of 60 $\mu mol\ m^{-2}\ s^{-1}$ for 1 h daily from d 1 till 10. These seedlings were otherwise grown under light of equal intensity as control ones.

2.2. Estimation of photosynthetic pigments and analysis of carotenoid (Car) composition by high performance liquid chromatography (HPLC)

Pigments were extracted from cotyledons of clusterbean seedlings with 100% ice-cold acetone and the extract was used for determination of chlorophyll (Chl) and Car according to the method described by Wellburn and Lichtenthaler [24].

Pigments extracted from cotyledons of clusterbean seedlings with 100% ice-cold acetone were centrifuged at $10{,}000 \times g$ for 10 min. The filtered supernatant was used for HPLC analysis. Chromatography was carried out on a 3.91×150 mm Nova-pak C_{18} Waters (USA) Analytical Column following the method of Rivas et al. [25]. A fixed amount of pigment extract was injected with Waters 717 plus Auto sampler. The column was equilibrated prior to injection of each sample by flushing with acetonitrile methanol (mobile phase A) for 10 min. The elution solvent A contained acetonitrile and methanol in ratio 7:1 (v/v) and solvent B (mobile phase B) contained acetonitrile, methanol, water and ethyl acetate in ratio 7:0.96:0.04:5. Mobile phases were pumped by a Waters 515 HPLC pump at a flow rate of 0.3 mL min^{-1}. The pigments were identified by comparing their retention time with those of the standard. Peaks were scanned and detected by a Waters 996 photodiode array (PDA) detector integrated with Waters millennium software.

2.3. Measurement of anthocyanin and flavonoids

Accumulation of anthocyanin in the cotyledons of clusterbean seedlings was estimated according to the method of Beggs and Wellmann [26]. A known weight of cotyledons was taken in a mixture of 5 cm^3 of ethanol:HCl (100:1) and kept in darkness for 24 h. Absorbance of the extract was measured at 546 nm. Anthocyanin content was expressed in term of absorbance at 546 nm/g fresh weight (A_{546}/g fresh wt).

Accumulation of flavonoids in the cotyledons of clusterbean seedlings was estimated following the method of Flint et al. [27]. A known weight of cotyledons was boiled vigorously for 10 min in a mixture of 5 cm^3 of ethanol and acetic acid (99:1). The final volume of the extract was adjusted to 5 cm^3 and absorption was measured at 270 nm. The pigment content was expressed in term of absorbance at 270 nm/g fresh weight (A_{270}/g fresh wt).

2.4. Measurement of malondialdehyde (MDA)

The level of thylakoid membrane lipid peroxidation of chloroplasts isolated from cotyledons of clusterbean seedlings was measured in term of MDA accumulation following the method described by Panda et al. [28].

2.5. Measurement of P_n and stomatal conductance (G_s)

The measurement of P_n and G_s in cotyledons of clusterbean seedlings was conducted with an infrared gas analyzer (CIRCAS 2, pp system, UK) fitted with an ergonomic Parkinson's universal leaf cuvette PLC6 (U). The temperature inside the leaf chamber was maintained at 25 °C. The steady state photosynthesis was measured at a saturating photon flux density of 1200 $\mu mol\ m^{-2}\ s^{-1}$ and CO_2 concentration of 375 $\mu mol\ m^{-2}\ s^{-1}$.

2.6. Measurement of pulse amplitude modulated (PAM) Chla fluorescence

Both fast and slow phases of Chla fluorescence transients were measured with a PAM fluorometer (FMS-1, Hansatech, UK) from attached cotyledons of clusterbean seedlings according to Schreiber et al. [29]. The cotyledons were dark adapted for 15 min before fluorescence measurement.

2.7. Isolation of chloroplasts and measurement of O_2 evolution

Chloroplasts were isolated from the cotyledons of clusterbean seedlings following the method of Izawa and Good [30]. Isolated chloroplasts were suspended in a medium containing 300 mM sucrose, 50 mM NaCl, 50 mM Na/K phosphate buffer (pH 6.9) with a Chl concentration of 2 mg/mL.

O_2 evolution from the isolated chloroplasts was measured with a Clark type oxygen electrode at 21 °C in rate saturating red light. The assay medium contained 30 mM Na/K phosphate buffer (pH 7.2), 30 mM NaCl and 200 mM sucrose and 0.4 mM $K_3Fe(CN)_6$. Chloroplasts containing 40 μg Chl were placed in 2 mL of reaction mixture for the measurement of PS II-mediated O_2 evolution.

2.8. Measurement of thermoluminescence (TL)

TL curves were obtained from isolated chloroplasts following the method of Desai et al. [31]. Chloroplasts containing 100 μg Chl/mL in suspension medium were frozen to liquid