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Impact of UV-B irradiation on photosynthetic performance and chloroplast membrane components in *Oryza sativa* L.

F.C. Lidon [a], J.C. Ramalho [b,*]

[a] Dept. Ciências e Tecnologia da Biomassa, Campus da Caparica, Fac. Ciências e Tecnologia, Univ. Nova de Lisboa, 2829-516 Monte de Caparica, Portugal
[b] Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Instituto de Investigação Científica Tropical, Av. República, Quinta do Marquês, 2784-505 Oeiras, Portugal

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ABSTRACT

The impact of UV-B radiation on photosynthetic related parameters was studied in *Oryza sativa* L. cv. Safari plants, after an UV-B irradiation performed 1 h per day for 7 days (between 8 and 14 days after germination) with a ten narrow-band (λ 311 nm) that resulted in a total biological effective UV-B (UVB_{BE}) of 2.975 kJ m^{-2} day^{-1} and a total of 20.825 kJ m^{-2}. Gas exchange measurements were severely affected, showing reductions higher than 80% in net photosynthesis (P_n), stomatal conductance and photosynthetic capacity (A_{max}), 1 day after the end of the 7-days UV-B treatment. Similarly, several fluorescence parameters (F_o, F_v/F_m, F_v'/F_m', ϕ_e, q_P and q_E) and thylakoid electron transport (involving both photosystems) were also severely reduced. Concomitantly, a decline of xanthophylls, carotenes, Chl *a*, Chl (*a* + *b*) and Chl (*a*/*b*) values was accompanied by the increase of the lipoperoxidation level in chloroplast membranes, altogether reflecting a loss of protection against oxidative stress. Seven days after of the end of UV-B treatment, most fluorescence parameters recovered, but in P_n, A_{max}, thylakoid electron transport rates, Chl *a* and lipid classes, as well as the level of lipoperoxidation, the impacts were even stronger than immediately after the end of stress, denoting a clear loss of performance of photosynthetic structures. However, only a moderate impact on total lipids was observed, accompanied by some changes in the relative weight of the major chloroplast membrane lipid classes, with emphasis on the decrease of MGDG and the increase of phospholipids. That suggested an ability to *de novo* lipid synthesis allowing qualitative changes in the lipid matrix. Notably, the leaves developed after the end of UV-B irradiation showed a much lower impact, with significantly decreased values only in P_n and g_s, rises in several fluorescence parameters, thylakoid electron transport, photosynthetic pigments (xanthophylls and chls) and DEPS, while lipid classes presented values close to control. The results showed a global impact of UV-B in the photosynthetic structures and performance in irradiated leaves, but revealed also a low impairment extent in the leaves entirely developed after the end of the irradiation, reflecting a remarkable recovery of the plant after the end of stress, what could constitute an advantage under occasional UV-B exposure events in this vital worldwide staple food crop.

1. Introduction

Ultraviolet-B radiation (UV-B; 280–320 nm) constitutes a minor part of the solar spectrum and most of UV solar radiation is absorbed by the UV-screening stratospheric O_3 layer. Yet the component that reaches Earth's surface is known to elicit numerous responses at the molecular, cellular and whole-organism level in higher plants [1,2].

Although the UV-B level varies with several factors as latitude, season, time of the day, altitude, cloud cover, surface reflectance, and the thickness of the vegetation canopy [2], a global depletion of the O_3 layer, largely due to the release of chlorofluorocarbons caused by human activities, has resulted in an increase of solar UV-B radiation at the earth's surface. In the temperate latitudes, such O_3 decrease reached *ca.* 3% and 6% in the North and South hemispheres, respectively, between 2002 and 2005 (as compared to the 1970s) [3]. Despite the uncertainty of long-term predictions, it is estimated an UV-B increase of 5–10% over temperate latitudes within the next 15 years [4]. In this way, it is likely that terrestrial plants will have to deal to enhanced UV-B levels in the next decades, what lead to a significant research effort to better understand the acclimation strategies that could help photosynthetic organisms to cope with its harmful effects.

Since UV-B radiation is readily absorbed it can provoke photoexcitation of a large number of biomolecules, such as nucleic acids, proteins and lipids, resulting in changes of genetic, biochemical and physiological functions within cells, thus, implicating significant

* Corresponding author. Tel.: + 351 21 4544682; fax: + 351 21 4544689.
E-mail addresses: cochichor@mail.telepac.pt, cochichor@iict.pt (J.C. Ramalho).

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impacts on many biological processes [4], both with damaging or regulatory importance [2]. In fact, low levels of UV-B might trigger regulatory not stress responses, of photomorphogenic nature, comparable to those mediated by phytochromes, cryptochromes, and phototropins [2]. However, depending on flow rate, duration and interaction with other environmental biotic and abiotic stress factors (cold, drought and mineral levels) the UV-B radiation was reported to provoke clear impacts at several levels, including visual symptoms (*e.g.*, promoting tissue chlorosis and necrosis), leaf ultrastructure and anatomy (*e.g.*, changes in thickness of epidermal and palisade mesophyll layers), photosynthetic pigments, photosynthesis, transpiration, leaf expansion, growth, development and yield, damaging proteins and DNA and reducing genome stability [2,5–10].

Amongst the major UV-B targets are the photosynthetic structures. Accordingly, the impact in a wide number of photosynthetic components has been reported, including the suppression of Chl synthesis [11], the inactivation of oxygen evolution, LHCII, PSII reaction centres and thylakoid electron flux. Furthermore the decrease of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) content and activity, that affects V_{cmax}, accompanied with a large reduction in the expression and abundance of both large and small subunits of rubisco, would contribute to depress photosynthesis and yield [6,9,12–16].

The most common UV-B impairments/damages are related to proteins (cross-linking, aggregation, denaturation and degradation) and membrane lipids [10,17–20], namely, through photooxidation or by ROS and free radicals produced during photosensitization [18]. However, in order to cope to UV-B exposure, plants could trigger a range of protective mechanisms that includes the synthesis of UV-screening phenolic pigments (as hydroxycinnamic acids and flavonoids), the up-regulation of reactive oxygen species (ROS) scavenging molecules (as ascorbate, glutathione and α-tocopherol and enzymes from the ascorbate–glutathione cycle), better repair systems (particularly of DNA), changes in plant morphology and marked gene expression modifications [2,10,21]. A large number of rice genotypes belonging to five Asian rice ecotypes (from the Bengal region and Indonesia) and Japanese lowland and upland rice groups showed a wide range of sensitivities to UV-B radiation [22]. Knowing that the UV-B absorbing O_3 layer is naturally thinner in the equatorial tropical regions, which are the main areas of rice production, it is of paramount importance to evaluate the degree of impact and recover mechanisms implicating the photosynthetic apparatus after stressful UV-B exposure in rice. In this context, the UV-B mediated effects on photosynthetic related parameters (gas exchanges, fluorescence parameters, photosystems activity) and on the chloroplast membrane components (photosynthetic pigments and lipid classes) were analysed in exposed leaves, as well as in those developed after the end of UV-B irradiation.

Rice is one of the most widely grown crops in the world, and it is by far the most important in terms of human consumption in low- and lower-middle-income countries. Although rice is grown worldwide, rice production and consumption are dominated by that part of Asia from Pakistan in the west to Japan in the east (also called the "Rice-producing Asia") that accounts for 90% of world rice production [23]. Global harvested area around 2000 was approximately 166 million ha, which was estimated to be distributed amongst 144 million rice farm households in the world, the vast majority in developing countries [23], giving a global worldwide production of around 689 million MT in 2008 [24]. Taking into account the upmost importance in this essential worldwide staple food crop, we aim at to improve the knowledge of UV-B impact on rice plant physiology, particularly in what concerns specific targets in the photosynthetic key metabolism.

2. Materials and methods

2.1. Plant material and growth conditions

Rice (*Oryza sativa* L. cv. Safari) seeds were washed, sterilized, and germinated as in [25]. The seedlings were grown hydroponically (in 2 L pots, 10 replicates and five independent series from each treatment), at 33/26 °C (day/night), a PPFD of *ca.* 400 μmol m^{-2} s^{-1} and a 12 h photoperiod in growth chambers (EDTU700, ARALAB, Portugal). The nutrient solution developed for rice growth [26], containing (in mg L^{-1}) 40 N, 10 P, 40 K, 40 Ca, 40 Mg, 20 Al, 0.5 Mn, 0.2 B, 0.05, Mo, 0.01 Cu, and 0.01 Zn was used. Iron was added as hexahydrated $FeCl_3$ at 2 mg L^{-1}. The solutions were daily adjusted to pH 4.5, being the volume restored to its original level, and renewed every 5 days.

UV-B stress was induced in an irradiation chamber with ten narrow-band (λ 311 nm) fluorescent lamps Philips, TL 100 W/01 SLV (Fig. 1), concomitantly with an environmental PPFD of *ca.* 100 μmol m^{-2} s^{-1}. The intensity of UV-B radiation was measured with a RM-21 spectroradiometer (Gröbel UV-Electronics, Ettlingen, Germany). In the experiment for assessment of dose–response relationships, plants were irradiated 1 h per day (after 2 h of normal growth PPFD), for 7 days (between 8 and 14 days after germination), with UV-B fluxes of 22 W m^{-2}. These resulted in a total biological effective UV-B (UVB_{BE}) of 2.975 kJ m^{-2} d^{-1} and a total of 20.825 kJ m^{-2} after 7 days exposure, as weighted by Caldwell's generalized plant action spectrum [27].

The evaluations were performed during the recovery period 1, 7 and 14/15 days after the end of UV-B exposure, corresponding to 15, 21 and 28/29 days after germination, respectively. Determinations were carried out in leaves that were subjected to UV-B irradiation (analysed 1 and 7/8 days after the ending of irradiation) and in leaves entirely developed after the plant exposure to UV-B (analysis 7 and 14/15 days after the UV-B irradiation), as well as in similar leaves of control plants, not UV-B exposed.

2.2. Leaf gas exchanges

Leaf net photosynthetic rate, P_n, stomatal conductance to water vapour, g_s, and the internal CO_2 concentration, C_i, were measured under photosynthetic steady-state conditions after *ca.* 2:30 h of light exposure, using a CO_2/H_2O open system portable IRGA (CIRAS I, PP Systems, UK) with external CO_2 set to 370 μL of CO_2 L^{-1} and PPFD of *ca.* 400 μmol m^{-2} s^{-1}. Measurements were carried out in 6–8 plants per treatment between 10:30 and 12:00 h (previously observed the best day time to obtain maximal P_n and g_s values).

Measurements of O_2 evolution expressing photosynthetic capacity, A_{max}, were performed in leaf pieces (*ca.* 2.2–3.2 cm^2) under irradiance (PPFD 450 μmol m^{-2} s^{-1}) and CO_2 (*ca.* 7%) saturating conditions, at 25 °C, in a Clark-type leaf-disc O_2 electrode (LD2/2, Hansatech, Kings Lynn, UK). Saturating PPFD was provided by a Björkman lamp (Hansatech).

2.3. Chlorophyll fluorescence parameters

Chl *a* fluorescence parameters were determined under the same environmental conditions as A_{max}, using a PAM 2000 system (H. Walz, Effeltrich, Germany) coupled to the LD2/2 O_2 electrode, as in [28]. Briefly, measurements of the minimal fluorescence from the antennae, F_o, and photochemical efficiency of PSII, F_v/F_m, were performed on overnight dark-adapted leaves. F_o denotes the fluorescence emission by the excited Chl *a* molecules before excitation energy migrate to the reaction centres and was determined using a weak light (<0.5 μmol m^{-2} s^{-1}). F_v/F_m represents the maximal PSII photochemical efficiency and was obtained using a 0.8 s saturating