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UV-B IRRADIATION IN RICE: A GENERAL GLANCE ABOUT THE CONTROL OF OXIDATIVE BURST WITHIN THE CHLOROPLAST LAMELLAE

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Summary: Ultraviolet-B (UV-B) radiation constitutes a minor part of the solar spectrum, being most of this radiation absorbed by the UV-screening stratospheric ozone layer. Yet, a global depletion of the ozone layer, largely due to the release of chlorofluorocarbons caused by human activities, is increasing solar UV-B radiation at the earth's surface. Accordingly, in spite of considerable intra- and interspecific variability in sensitivity of crop plants, UV-B radiation is increasingly being absorbed by a large number of biomolecules (*e.g.*, nucleic acids, proteins, lipids), therefore leading to their photoexcitation, what might promote changes on multiple biological processes, both with damaging or regulatory importance. Nevertheless, despite the diversity of UV targets in plants, depending of the growth conditions, the photosynthetic apparatus is amongst the main targets of UV-B, and its damage contributes significantly to the overall metabolic impairment. In this paper, a parallel is drawn between UV-B irradiation and the control of the oxidative burst exerted by Mn and ascorbate in the lamellae of *Oryza sativa* L.

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Abbreviations: A_{max} – Photosynthetic capacity; APX – Ascorbate Peroxidase; Chl – Chlorophyll; C_i = Intercellular CO_2 Concentration; Cyt – Cytochrome; CuZnSOD – Cu,Zn Superoxide Dismutase; DEPS - De-epoxidation State Involving the Components of the Xanthophyll Cycle; DHAR – Dehydroascorbate Reductase; F_o – Basal Fluorescence; F_v/F_m – Maximal Photochemical Efficiency of PSII; F_v'/F_m' - Photochemical Efficiency of PSII under photosynthetic steady-state conditions; GSH – Reduced Glutathione; GSSG – Oxidized Glutathione; LHCII – Light Harvesting Chlorophyll a/b Binding Protein of Photosystem II; MGDG - Monogalactosyldiacylglycerol; PS – Photosystem; Q_A – Quinone A; Q_B – Quinone B; q_p – Photochemical Quenching; ROS – Reactive Oxygen Species; RuBisCO – Ribulose 1,5-phosphate carboxylase/oxygenase; ϕ_e – Quantum Yield of Photosynthetic Non-Cyclic Electron Transport.

1. Introduction

Although UV-A radiation is hardly absorbed in the stratospheric, most of the UV radiation does not reach Earth's surface, since UV-C radiation is completely absorbed by the atmospheric gases and UV-B radiation is mostly absorbed by the ozone layer. Yet, as in the last years the ozone layer is being depleted by free radical catalysts (namely nitric oxide, nitrous oxide and hydroxyl, as well as atomic chlorine and bromine), ozone molecules breakdown in the stratosphere is resulting in a decrease of the effectiveness of UV radiation absorption. Therefore, more radiation reaches the Earth surface, with each 1% reduction in ozone causing an increase of 1.3-1.8% in UV-B radiation reaching the biosphere (McKenzie et al., 2003; Lidon et al., 2012a). Nowadays ozone levels over the northern hemisphere have been dropping by 4% per decade. Additionally, current stratospheric ozone levels are at the lowest point (since measurements began in 1970s) and global terrestrial UV-B radiation levels range between 0 and 12 kJ m^{-2} on a given day, with near Equator and mid-latitudes receiving higher doses (McKenzie et al., 2011).

Rice is one of the most worldwide grown staple food crops, being its production and consumption dominated by that part of Asia, from Pakistan in the west to Japan in the east (also called the ''Rice-producing Asia''), accounting for 90% of world rice production (Dawe et al., 2010). In 2013 the worldwide rice production reached *ca*. 744.9 million Ton of paddy rice (or 497.6 million Ton, milled basis) using an area of *ca*. 165 million ha (FAO, 2014). In this context, considering that as well as that, some estimates point that UV-B in the Earth surface might increase of 5–10% over temperate latitudes within the next 15 years (Tian and Yu, 2009), we aim at giving here a synoptical overview addressed to the UV-B impact on rice plant physiology, particularly in what concerns specific targets in the photosynthetic key metabolism.

2. ROS Production and Regulation by the Xanthophyll Cycle

A potential primary catabolism of UV-B induced physiological and biochemical injuries in the chloroplasts is related to ROS production (Tossi et al., 2009; Hideg et al., 1997, 2013; Lidon et al., 2012a), as the triplet molecular oxygen is continuously produced during the light-driven photosynthetic electron transport and therefore reduced to superoxide (Lidon and Henriques, 1993). In this context, the amount of superoxide being formed in the chloroplasts of UV-B-stressed rice

progressively rises, with the contents of hydroxyl radicals and hydrogen peroxide also increasing, due to the impairment of the photosynthetic apparatus to efficiently use the captured light energy (Lidon et al, 2012a). This pattern is coupled to the photoreduction of the ground state triplet oxygen to superoxide (in aqueous solution with a pKa of 4.9), which is followed by dismutation to hydrogen peroxide (a powerful oxidizing agent, E^o = 1.36 V, at pH 7 for the system H_2O_2/H_2O). Additionally, the uncontrolled metabolism of these precursors favors the synthesis of hydroxyl radicals (with a short lifetime and a strongly positive redox potential, +2V) in Fenton-type reactions (Lidon and Henriques, 1993; Apel and Hirt, 2004).

When sensing a sustained over-reduction of the photosynthetic components, linked to a loss of efficiency of the photochemical events the plants may trigger defensive mechanisms that helps to dissipate the excess of excitation energy, as it seems to be case in rice. Immediately after UV-B stress, the progressive oxidative burst parallels a physiological sign to the xanthophyll cycle, turning on additional removal of epoxy groups from xanthophylls (i.e., violaxanthin and antheraxanthin) through violaxanthin de-eposidase, to synthesize the related de-epoxidised xanthophyll (zeaxanthin) (Lidon and Henriques, 1993). This process implicates an initial higher accumulation of zeaxanthin, favoring energy dissipation within LHCP by non-photochemical quenching, with the amount of energy that reaches the photosynthetic reaction centres being reduced and, therefore, limiting Chl *a* photoxidation (Lidon and Henriques, 1993). Moreover, as this process requires a higher rate of consumption in the ascorbate pool (Lidon et al., 2012a), the subsequent diminishing of this chemical entity, progressively turns off the zeaxanthin recycling (Lidon et al., 2012b). This process determines, in parallel with a high DEPS, a decreasing content of all components of the xanthophyll cycle, implicating that Chls in the chloroplast lamellae becomes no longer shielded from photoxidation (Lidon et al., 2012a,b).

3. Inhibition of the Asada–Haliwell Cycle and ROS Propagation

As the production of ROS is a metabolic inevitable consequence (overexpressed under excess of photochemical energy), plants have evolved an integrated antioxidative defense mechanism that promotes the scavenging of ROS. That is the case of the integrated network of enzyme and non-enzyme (ascorbate, glutathione) molecules acting through the ascorbate-glutathione (Haliwell-Asada) cycle. Accordingly, in the first days following UV-B stress, Cu,ZnSOD activity of rice chloroplasts acts as a first line of defense against oxidative burst, dismutating superoxide to H_2O_2 (Lidon et al., 2012a). Yet, since thereafter the oxidized ascorbate is not recycled in the xanthophyll cycle, the activity of APX becomes progressively inhibited and the detoxification of hydrogen peroxide to H_2O through dehydroascorbate synthesis became restricted (Lidon et al., 2012a). In this context, ascorbate regeneration mediated by DHAR, driven by the oxidation of GSH to GSSG, becomes circumscribed to its diminished availability. Following this stage, the cytotoxic properties of ROS, linked to the evolution of inhibitory arrays of non-enzymatic and enzymatic detoxification mechanisms, prompts the

degradation of the chloroplast lamellae (Lidon et al., 2012a).

Within the rice chloroplast lamellae, acyl lipids balance is photochemically modified by UV-B absorption, as double bonds in fatty acids weaken the carbon atom bonds nearby, facilitating $H^{\cdot}$ subtraction by hydroxyl radicals (Lidon and Henriques, 1993). The unsaturated fatty acids of MGDG are mostly destroyed by ROS (Lidon and Ramalho, 2011; Lidon at al., 2012a), and therefore the disturbance of membrane assembly is greatly altered (Campos et al., 2003; Partelli et al., 2011), which affects the physicochemical properties of membrane lipid bilayers, triggering severe cellular dysfunction. This progressive inequity between the prooxidant/antioxidant systems implicates lipid peroxy radicals spread (Lidon and Henriques, 1993; Valdivieso and Mullineaux, 2009) and amplifies damage because of free radicals cascades.

In many plant species, negative effects imposed on the photosynthetic components are known, including through the suppression of chlorophyll synthesis (Kulandaivelu et al., 1991), the inactivation of oxygen evolution, LHCII, PSII reaction centres functionality and the thylakoid electron flux. Due to the key role of LHCII in light absorption and energy transfer to the reaction center, as well as on thylakoid stacking, any damage to these structures can result in multiple effects on the photosynthetic functioning and efficiency. Furthermore, it must be considered that after UV-B irradiation the inhibition of LHCII (Strid et al., 1990; Lidon et al., 2012a,b) is also eventually linked to a decrease in the transcription of the *cab* gene responsible for the synthesis of the chlorophyll a/b-binding proteins of LHCII, leading to the functional disconnection of LHCII from PSII (Jordan et al., 1994).

As in most plant species, including rice, following the high grana disorganization and degradation, PSII is the most sensitive component of the thylakoid membrane on exposure to UV-B radiation (Savitch et al., 2001; Lidon et al., 2012a,b). Nevertheless, strong UV-B mediated effects on PSI linear electron transport (Lidon and Ramalho, 2011) and on cyclic phosphorylation (Pang and Hays, 1991) have been also reported. In rice chloroplasts exposed to UV-B stress, D_1/D_2 polypeptides belonging to the water splitting and oxidizing sites of PSII enfolds (Lidon and Henriques, 1993). Hydroxyl radicals, synthesized during UV-B-induced decomposition of hydrogen peroxide and produced as an intermediate of water oxidation (Lidon and Henriques, 1993), seem to be responsible for this D_1 cleavage, acting on the disulphide bridges that stabilized the as the conformation of these proteins. Additionally, absorption changes in the UV-B region (Vass et al., 1999) between S_1 to S_2 and S_2 to S_3 redox transitions in the Mn cluster my also trigger proteolysis, determining general failure of Cyt b_{559} turnover, which forms part of the reaction centre core of PSII (Kamiya and Shen, 2003). In the vicinity of the water splitting centre the 49/46 kDa chlorophyll binding proteins of the inner LHCII also become destabilized, determining the inhibition of Chls and carotenes accumulation (Lidon et al., 2012a). Moreover,, the 28/26 and 22/20 kDa polypeptide that integrate the LHCII also amplify ROS-induced damage, because the efficiency of light absorption

and energy transfer to the reaction centre becomes altered, promoting a progressive functional disconnection of LHCII from PSII, decreasing the synthesis of the related Chl a/b-binding proteins (Busheva et al., 1991; Escoubas et al., 1995) and affecting Chl fluorescence emission (Lidon and Ramalho, 2011).

The main target and damage of protein structure within PSI also contributes to ROS amplification, triggering thylakoid lipids peroxidation (Lidon and Ramalho, 2011) and PS II proteolysis. This process seems to be linked to the inhibition of electron transfer components X and centres A and B associated with 18/16 kDa polypeptides, which therefore affects the Mehler reactions activities.

4. Impact on photosynthetic related parameters

Stomatal regulation is an important process limiting leaf photosynthesis. Although earlier studies have proposed that UV-B radiation does not significantly affect stomatal conductance (Keiller et al., 2003), others found reduced stomatal conductance promoted by UV-B radiation exposure, depending of growth conditions, (Pal et al., 1998; 1999; Lidon and Ramalho, 2011). Yet, despite the severe depressions of net photosynthetic and stomatal conductance rates, the C_i values were not reduced, what was accompanied by a reduction of A_{max}. Together, these results showed that the UV-B induced inhibition of photosynthesis was not related to stomatal limitation of CO_2 availability to carboxylation sites at RuBisCO level (Lidon and Ramalho, 2011), as also reported in other species exposed to UV-B stress (Keiller et al., 2003). In fact, immediately after UV-B irradiation the mesophyll imbalances may arise from damages and/or regulatory mechanisms at biochemical and biophysical level, with a wide severity extend range that is species (or even genotype) dependent. In UV-B stressed rice almost all gas exchange (both under environmental and saturating CO_2 conditions) and fluorescence of Chl *a* parameters, as well as the rates of thylakoid electron transport involving PSI and II, displayed significant negative impacts, reflecting clear performance reductions on the photosynthetic machinery, what results in lower biomass and yield in most crop plants (Kakani et al., 2003; Lidon and Ramalho, 2011; Lidon et al., 2012a,b). Depressions associated to the photochemical efficiency of PSII (F_v/F_m, $F_{v'}/F_{m'}$, q_p and ϕ_e), immediately after UV-B irradiation in rice seems to be related to the presence of regulatory mechanisms acting in the antennae, namely the photoprotective interconversion of violaxanthin to zeaxanthin or the accumulation of photochemically inactive PSII reaction centres that dissipate energy as heat (Müller et al., 2001; Lidon and Ramalho, 2011; Lidon et al., 2012a,b). These mechanisms can promote thermal dissipation but, as they are competing for excitation energy, a lower energy flow will be driven to photochemistry and a depressed PSII efficiency and thylakoid electron transport might arise, changing the Chl *a* fluorescence characteristics (Adams et al., 2002; Batista-Santos et al., 2011; Lidon and Ramalho, 2011; Lidon et al., 2012a, b).

The F_o and F_m values decreased what agrees with the strong Chl *a* reduction, as those parameters proportional to the latter. However, since the F_v/F_m also decreased, associated to the strong impacts on Chl *a*,

xanthophylls and lipoperoxides contents, not readily reversible impairments (both at biophysical and biochemical levels) will be involved. These results reflect a high photosynthetic sensitivity in the irradiated leaves, showing that photosystems are UV-B sensitive targets, particularly PSII (Lidon and Ramalho, 2011; Lidon et al, 2012a, b), as also found for a large number of *Arabidopsis* accessions (Jansen et al., 2010). Indeed, PSII sensitivity has been reported as a main factor for UV inhibition of photosynthesis, which seems to linked to oxy radicals production on the acceptor side at the level of Q_A and Q_B electron acceptors (Melis et al., 1992) and on the donor side at the level of primary donor, the redox-active tyrosine and the Mn cluster (Keiller et al., 2003; Pfündel, 2003; Sicora et al., 2003).

Still, it is noteworthy that that the new rice leaves of UV-B exposed plants, developed after the end of the irradiation, presented a notable recovery with an almost absence of impact in many of the studied parameters. Still, it was found some imbalances between energy capture and its use through photochemistry what justified the presence of thermal dissipation mechanisms at a higher level than control plants (without UV-B exposure). Furthermore, it was concluded that such remarkable plant recovery after the end of UV-B stress constitutes an advantage under occasional UV-B exposure events under field conditions (Lidon and Ramalho, 2011).

5. Regulatory Mechanism Driven by Mn on ROS Control

Independently of UV-B irradiation prevails a positive correlation between the exogenous Mn concentrations of the growth medium and the build-up of this metal's accumulation in the leaves, which points an alteration on metal regulatory mechanisms (Lidon and Teixeira, 2000a,b; Lidon, 2001; 2002; Doncheva et al., 2005; Najeeb et al., 2009; Yao et al., 2012), characterized by a rapid and steady movement to that organ. Within the leaves, changes in the cell regulation also implicate the chloroplasts lamellae, a feature reported without UV-B application (Hayakawa et al., 1985; Lidon and Teixeira, 2000a; Yao et al., 2012), and that bring about the rise of electrostatic interactions and therefore upper thylakoid stacking (Lidon and Teixeira, 2000b).

To attain the regulatory mechanisms driven by Mn kinetics on ROS control, the synthesis of SOD has been reported in different species (Del Rio et al., 1978; 1985; Jackson et al., 1978; Hayakawa et al., 1985; Foy et al., 1988; Lidon and Teixeira, 2000a). Paralleling with these reports, after UV-B irradiation, Mn accumulation in the chloroplasts lamellae also elicits the synthesis *de novo* of an intrinsic Mn-binding protein (with a Mn(II) centre, with a small axial distortion (E/D = 0.027)) that acts as a superoxide scavenger. In fact, it was considered that the enzymatic catalysis displayed by this Mn metalloprotein that mimics superoxide dismutase, can protect against UV-B injury, upholding the chloroplast architecture and functioning at a photochemical level (Lidon and Ramalho, 2014), therefore reinforcing the crucial role of ROS control on the rice response to UV-B stress.

6. Conclusion

In rice chloroplasts, UV-B sensitivity is expressed by rapid tissues necrosis in the leaves directly irradiated, a process that follows the rate of sequential

reduction of triplet molecular oxygen produced during the photosynthetic light reactions. If UV-B irradiation becomes lethal, this process implicates unbalanced ascorbate peroxidation, which becomes an increasing limitation to the functioning of the enzymatic antioxidant systems. ROS propagation triggers increasing MGDG peroxidation of chloroplast membranes and photosystems proteolysis, degrading thylakoid structure and functioning. Moreover, Mn tolerance at a cellular level combined with UV-B irradiation, determines Mn binding into proteins of the lamellae of chloroplasts of *Oryza sativa*, having catalytic properties to superoxide dismutation that can protect against UV-B injury, contributing to the upholding the chloroplast architecture and functioning at a photochemical level. Finally, it should be underlined remarkable rice plant recovery once UV-B stress ends, as the new leaves present a complete recovery in many of the functional parameters analyzed, constituting an advantage under occasional UV-B exposure under field conditions.

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