REVIEW ARTICLE

UV-B effects on leaves – oxidative stress and acclimation in controlled environments

Gyula Czégény, Anikó Mátai, Éva Hideg*

Department of Plant Biology, University of Pécs, Hungary

Abstract

As the steady decline in the Earth's stratospheric ozone layer and parallel increase in solar ultraviolet-B (UV-B: 280–315 nm) has come to an end, the focus of plant UV research has been shifted from regarding UV-B as threatening plant life to recognizing it as a regulatory factor. While UV-B photoreceptor mediated signaling is increasingly understood, the role of UV-B inducible reactive oxygen species is still to be explored. Earlier experiments with high UV-B irradiation doses and isolated thylakoid membranes demonstrated the potential of UV-B to trigger oxidative stress. However, under realistic UV conditions pro-oxidants cannot be reliably traced in more complex biological samples possessing an array of antioxidant defenses. In the absence of direct experimental evidence we must rely on indications and propose hypotheses on how and whether pro-oxidants, such as reactive oxygen species contribute to acclimative responses. Here we briefly review how a balance between pro-oxidants and antioxidants is affected by UV-B in whole plant experiments performed in controlled environments. A working hypothesis is proposed in which the extents of UV-induced peroxidase and superoxide dismutase activations affect the success of acclimation to UV-B.

Keywords

Abbreviations

PAR, photosynthetically active radiation; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; UV-A, 315-400 nm ultraviolet radiation; UV-B, 280-315 nm ultraviolet radiation

*corresponding author: Department of Plant Biology, Institute of Biology, Faculty of Sciences, University of Pécs, Ifjúság u. 6. H-7624 Pécs, Hungary, Tel.: +3672 503600, Fax: +3672 503634, E-mail address: ehideg@gamma.ttk.pte.hu

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1. Introduction

1.1. A changing image of UV-B

The ultraviolet-B (UV-B, 280-315 nm) light has long been recognized as detrimental for plants. Research on the mechanism on damage by UV-B was boosted by the thinning of stratospheric ozone in the late 1970s. Several reports were based on model experiments using artificial UV-B sources with unrealistically high doses or emission spectra including high energy (below 280 nm) components, and UV-B has thus been solely regarded as a stressor for a long time. The identification of distinct UV-B-inducible pathways corresponding to different doses [1,2], together with efforts from the research community to harmonize experimental protocols (as documented in a recent book [3]) contributed to a more thorough and subtle understanding of the complexity of responses to UV-B.

In addition to being a potential source of oxidative stress, solar UV-B is recognized as a key environmental signal, affecting development and metabolism [4,5]. Responses involve both UV-B-specific signaling and non-specific pathways. Photomorphogenic signaling in response to low intensity UV-B regulates the expression of genes involved in protection against UV [2], such as the synthesis of UV-absorbing phenylpropanoids [6]. The non-specific pathway involves reactive oxygen species (ROS) as well as the hormones (salicylic and jasmonic acids). It is assumed to be activated by higher UV-B [1]. Survival in UV-B is strongly dependent on maintaining low cellular ROS concentrations. The subject of this review is the effect of pro-oxidants (ROS and other free radicals) and antioxidants on successful acclimation to UV-B. We focus on acclimation as adjustment achieved during a relatively short time, for example in a laboratory experiment in response to the onset of UV-B, or prompted outdoor by a change in solar UV; rather than on adaptation as a genetically encoded feature of a species due to an evolutionary process. There is no consensus on the use of UV radiation units and this makes results from different laboratories difficult to compare, as explained in the Appendix. UV-B radiation sources used in key references and their characteristic radiation parameters are listed in Table A.1.

1.2. UV effects on leaves

UV-B induced photomorphological changes in leaves include reduced leaf size, increased leaf thickness and the synthesis of phenolic compounds [4]. These changes also affect optical properties of leaves and thus may alter the amount of quanta reaching the photosynthetic apparatus. The main influence of UV-B on photosynthesis is believed to be more direct. Protein complexes engaged in the light reactions, as well as specific enzymes of the dark reaction are functionally impaired by UV-B (see reference [7] for a recent review). The action spectrum of the UV-B effect on photosynthesis does not reveal a specific target due to the presence of numerous UV-B absorbing biomolecules [8]. Damage to photosystem II is mostly attributed to ROS produced as by-product of electron transport malfunctioning, caused by UV-B absorption in the oxygen evolving complex [9] or other quinone redox components [10]. A sequential, integrative model assuming specific redox states of the donor side and involving several redox components has also been proposed [11]. Conclusions on the effects of UV-B on photosynthetic electron transport are mostly based on experiments with isolated membrane complexes lacking protective functions. In this way, models of UV-B effects on photosynthesis might not translate to environmentally relevant damage. Although UV-induced free radical production in leaves was only detectable under extreme laboratory conditions applying short irradiation times and high fluxes [12], there are several indications that oxidative stress and the ability of plants to override it are essential parts of responses to UV [4]. Here we address experimental and interpretational challenges and also discuss future perspectives.

2. Responses to UV – a balancing act

Oxidative damage has been implicated as one of the underlying agents of several abiotic stress factors counterbalanced by ROS scavenging antioxidants [13]. This frequently used model with an added UV specific dimension is illustrated in Fig.1. In a stress-free or, more realistically, low-stress state (Fig.1A) both ROS production and antioxidant activities are low. It is rather a theoretical

question whether ROS are generated in this stage at all, since the acquiring of experimental evidence is hampered by sensitivity thresholds of ROS detection methods (see 2.1.). Change in UV-B conditions, such as increased flux or the expansion of the irradiation spectrum, are for the sake of simplicity, symbolized by the appearance of UV-B in Fig.1.
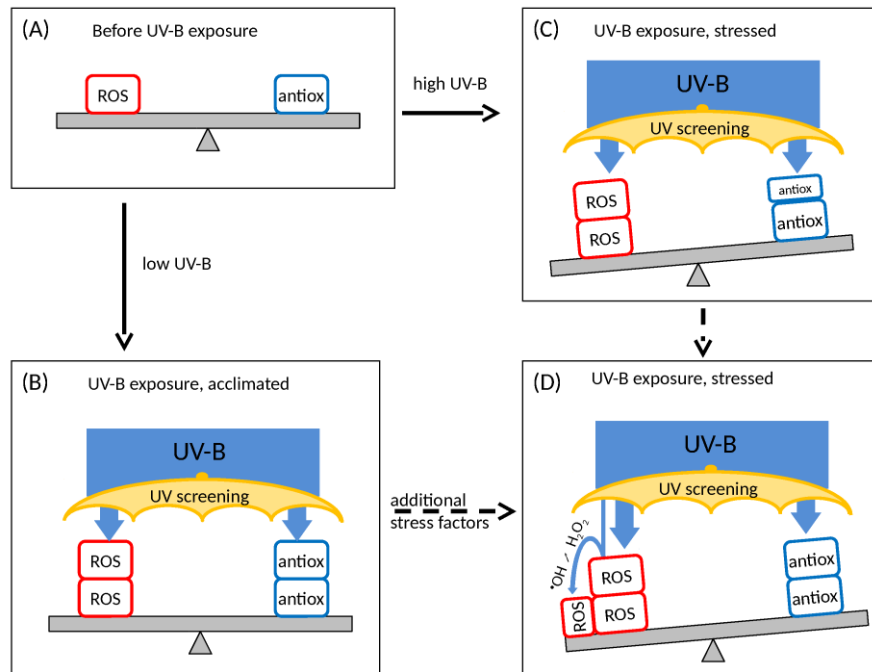

Figure 1.
UV-induced changes in the antioxidant – pro-oxidant balance in leaves
Models illustrate the balance between reactive oxygen species (ROS) production and antioxidant (antiox) activities or capacities before (A) and during (B-D) exposure to UV.

Similarly to other stressors, UV-B may increase ROS production and may also activate antioxidants or result in their production. UV-B also activates physical defenses, via the UVR8 photoreceptor mediated synthesis of UV-screening pigments, an important aspect reviewed elsewhere [2,14]. Activation of defenses determines whether the stress response is acclimation or cellular damage (Figs.1B and 1C). A special feature of UV-B radiation is that it is capable of modifying the ROS profile by photo-converting H_2O_2 to hydroxyl radicals [15] (Fig.1D) and thus increases the possibility of cellular damage (section 2.1).

The two sides of the balance are interconnected in a more complex way than symbolized in Fig.1. ROS may directly induce or limit antioxidant production and/or accumulation (see 2.1). Also, diverse cellular locations within the same leaf may harbor different biochemical pathways and thus result in the ROS – antioxidant balance being at different actual concentrations and activities. Interactions of UV-B and other environmental conditions, such as high intensity photosynthetically active radiation (PAR), drought or temperature stress are also capable of affecting the balance between pro-oxidant production and scavenging capacity [13]. Thus these other factors interacting with UV-B must also be considered. Responses are expected to vary between laboratory experiments and studies out of doors. Our study reviews antioxidant responses in controlled experiments that use plants grown in the absence of UV-B and then exposed to supplemental UV-B irradiation from artificial sources.

2.1. UV-B and pro-oxidants

2.1.1. Oxyradicals

Depending on experimental conditions, especially the dose and energy (wavelength) of applied UV, severe oxidative stress may promote the production of an array of ROS and other free radicals. Here we focus on ROS and do not discuss carbon-centered radicals that are secondary products of ROS mediated lipid peroxidation under long term high dose UV irradiation [16]. The key issue of understanding the role of ROS is their unambiguous identification. ROS-selective optical (colori- or fluorometric) and electron paramagnetic resonance probes offer good possibilities of detection but inherent limitations and many sources of artifacts make the use of these techniques less popular than antioxidant assays. For example, short wavelength (< 300 nm) UV irradiation of the spin trap 5,5-dimethyl-1-pyrroline N-oxide in aprotic solvents (incapable of acting as proton donors) yields nitroxide free-radicals [17] that may be misinterpreted as trapping UV-derived ROS from the biological sample. Experiments performed in water-based buffer solution are less prone to this artifact. The same spin trap was successfully applied to trap various free radicals, including hydroxyl

radicals ($\cdot$OH) in response to short (30 min) high flux UV-B (Table A.1) in thylakoid membranes prepared from spinach leaves or in detached broad bean (*Vicia faba* L.) leaves [12]. Instead of *in situ* trapping free radicals, which is hindered by the instability and potential toxicity of spin traps [18], the latter experiment relied on an attempt to have putative ROS react with 5,5-dimethyl-1-pyrroline N-oxide during a rapid preparation of a crude leaf extract immediately after the cessation of UV-B. As emphasized in the original publication [12], this technique is not reliable for identifying short-lived primary ROS products, but it is rather a demonstration of the ability of the treatment to cause oxidative stress. Similar post-irradiation spin trapping experiments with barley leaves exposed to supplemental UV-B radiation in growth chambers were interpreted accordingly, and spin adducts in leaf extracts were only used in obtaining qualitative but not quantitative conclusions: showing that above ambient (ca 2.5-fold) UV-B radiation decreased photosynthetic performance as a result of higher cellular concentrations of pro-oxidants [19]. Therefore, results based on the same technique in maize leaves in response to the UV component of tropical sunlight [20] should be interpreted with caution and cannot be the basis of identifying specific oxygen radicals.

The ability of high quasi-monochromatic UV doses (Table A.1) to promote ROS production were demonstrated using spinach leaves infiltrated with fluorometric ROS probes, and this study identified distinct ROS under different UV wavelengths: superoxide ($O_2 \cdot^-$) and singlet oxygen ($^1O_2$) dominating responses to UV-B (280-315 nm) and UV-A (315-400 nm), respectively [21]. By showing that UV-A can yield the same ROS as excess PAR this work contradicted earlier models [22] that suggested a similar damaging mechanism for UV-A and UV-B radiation. This is of special interest in regard to responses to solar UV containing all these radiations. However, the same experimental techniques failed to detect ROS in leaves exposed to lower, environmentally relevant UV doses applied in growth chambers in combination of PAR (data not shown). This is possibly due to limited sensitivities of the methods used rather than to the lack of ROS production, as suggested by several lines of indirect evidence: In addition to UV-B activation of several antioxidant pathways (detailed in section 2.2), studies using ROS scavengers and antioxidant enzyme inhibitors also indicate UV-

induced ROS production. For example, the $O_2^{\bullet-}$ reactive spin trap Tiron (1,2-dihydroxybenzene-3,5-disulphonate) reduced the effect of UV-B on the expression of *PR-1* and *PDF-1.2* genes in *Arabidopsis thaliana* leaves [23]. Using selective enzyme inhibitors, the same study confirmed the role of NADPH oxidase and peroxidases in the UV-B inducible expressions of these two genes [23].

2.1.2. Hydrogen peroxide

Permanent metabolic production and the presence of several cellular peroxidases maintain a steady state pool of H_2O_2 [24]. H_2O_2 is a far more stable ROS than oxyradicals and thus it is more accessible to reporter molecules. The challenge of studying the ability of UV-B to promote H_2O_2 production is rather in distinguishing UV-inducible concentrations from metabolic base levels than in the detection itself. Using the cell permeable fluorescent indicator dye 2',7'-dichloro-dihydrofluorescein-diacetate a NADPH-oxidase dependent increase in guard cell H_2O_2 concentrations was observed when *Arabidopsis* leaves were exposed to 3h of near ambient UV-B irradiation (Table A.1) from a broad-band light source [25]. H_2O_2 accumulation to detectable levels was slow, requiring 3h of irradiation suggesting that the proposed pathway was also influenced by factors other than UV-B [25]. There are several reports on UV-B induced H_2O_2 production in leaves and, contrary to reports on oxyradicals (section 2.1.1.), these include experiments using closer to ambient UV-B conditions modeled by using lower UV-B doses in combination with PAR and allowing several days to acclimate to these conditions (Table A.1). A common conclusion of these studies is that simultaneous exposure to UV-B plus another stress factor, such as salt stress [26], iron deprivation [27], or an organophosphate insecticide [28] increased H_2O_2 concentrations further than these factors alone.

A unique feature of UV-B, which may explain the synergistic effect of this radiation with other stress conditions is that UV-B is capable of photosensitizing hydroxyl radical ($^{\bullet}OH$) production from H_2O_2. This was predicted using computer modeling and demonstrated in solution [15]. The relevance of this phenomenon to *in vivo* UV-B effects is indicated by the requirement of accentuated increase in H_2O_2 and $^{\bullet}OH$ detoxifying pathways in UV-B acclimated leaves [29]. This reaction may tip

the oxidant-antioxidant balance towards oxidative stress due to the strong oxidizing nature of $^\bullet$OH as illustrated in Fig.1D. Because stress conditions other than UV can increase cellular H_2O_2 levels [13], synergistic deleterious effects may be due to $^\bullet$OH photo-production from H_2O_2 [15].

Assays aimed at measuring cellular H_2O_2 concentrations may also suffer from artifacts similar to the problem with oxyradical trapping techniques. An important characteristic of the popular 3,3'-diamonibenzidine (DAB) assay [30] is the sensitivity of the chromophore to UV-B, as illustrated in Fig.2. The applied UV-B intensity was similar to fluxes used in plant acclimation experiments, but it was applied for a short time only. The same supplemental UV-B intensity had no significant effect on photosystem II photochemical quantum yield when applied for 4h daily for 4 days to tobacco leaves (unpublished data). The oxidation of DAB by H_2O_2 is catalyzed by cellular peroxidases [30], as illustrated in solution in Fig.2.
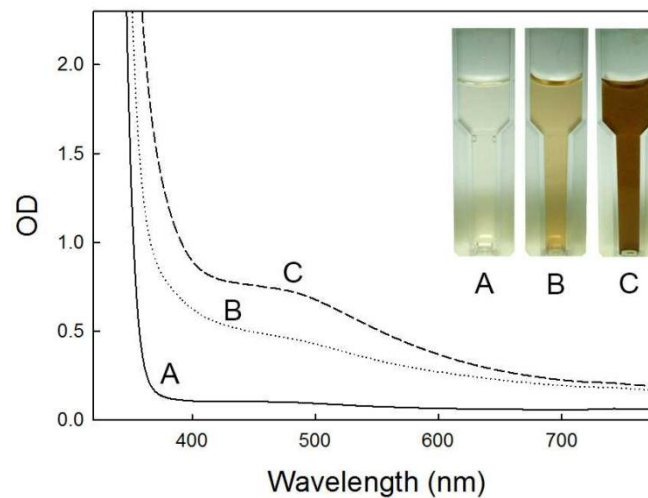

Figure 2.
In solution illustration of the sensitivity of the 3,3'-diamonibenzidine (DAB) assay to UV-B.
Absorption spectra of (A) 2.5 mM DAB before exposure to UV-B, (B) 2.5 mM DAB after 0.5 h UV irradiation from a UV-B centered broad band source (Q-Panel UVB-313EL, covered with cellulose diacetate filter) and (C) 2.5 mM DAB + 50 mM H_2O_2 + 0.01 unit horseradish peroxidase without exposure to UV-B. As experiments with DAB frequently rely on visual assessments of DAB oxidation, color changes are also illustrated in the inset as cuvette photos.

Two consequences are relevant to the use of DAB for *in situ* H_2O_2 detection in leaves. One is that UV-B exposed DAB gives false positive results; therefore it should be introduced into leaves only after the cessation of irradiation. This way, the assay is to be applied as a post-irradiation test, to visualize cellular H_2O_2 concentrations present immediately after the UV-B treatment, at the moment of the chromophore's delivery. The second consequence is that in addition to cellular H_2O_2 concentrations, UV-induced changes in peroxidase activities may also affect the extent of DAB color change, making quantitative H_2O_2 comparisons difficult.

2.2. UV and antioxidants

Plants generally respond to physiologically relevant doses and wavelengths of UV radiation by enhancing antioxidant enzyme activities and/or increasing cellular amounts of non enzymatic antioxidants [4]. In some cases, especially in response to higher doses reduced antioxidant activities may also be observed. Unlike ROS, antioxidant levels rarely decrease below the threshold of detection. Also, antioxidant assays are usually easier to perform than ROS assays, although the former are also not free from pitfalls. Antioxidant activities in a leaf are strongly influenced by several other factors in addition to the applied UV treatment, including growth conditions (temperature, background PAR, watering, etc.) and developmental aspects, such as age. For example, younger (1-3 weeks old) leaves of green-house grown grapevine (*Vitis vinifera* L. cv. Chardonnay) acclimated more successfully to 8.04 kJ m^{-2} d^{-1} biologically effective doses of UV-B radiation (Table A.1) than 4-6 weeks old leaves, due to an UV-induced increase in their total and ROS specific antioxidant capacities, which was not observed in older leaves [31].

Due to the interconnectedness of leaf antioxidants, it is not unusual that changes in one antioxidant affect another. It is less known that one antioxidant may also affect the outcome of an assay targeting another. Photometric assays based on the oxidation of ascorbate or the reduction of H_2O_2 to water, to measure ascorbate-peroxidase [32] or catalase [33] activities, respectively, are based on measuring changes in the UV absorption of corresponding compounds. High background

UV absorption of extracts from polyphenol rich leaves may lessen the sensitivity of these methods. An alternative is the use of polyphenol adsorbing compounds during extraction [34] or the application of non-denaturing gel electrophoresis instead of photometric assays. An advantage of the latter is the possibility of separately quantifying enzyme isoforms.

Successful acclimation to UV requires coordinated molecular level responses and some of the proposed signaling pathways are assumed to include ROS (see references [7,35] for recent reviews). Due to its general biochemical and chemical attributes, H_2O_2 fulfills the requirements of being a second messenger among the ROS [36]. The possibility of H_2O_2 conversion to the highly reactive and toxic hydroxyl radical via the Fenton reaction poses some hazard, but this can be lowered by chelation and compartmentation of potential iron and copper catalysts. An added risk is the potential UV-B photo-cleavage of H_2O_2 to $^\bullet OH$ [15], which can only be reduced by either (i) preventing UV-B from reaching H_2O_2 containing tissues or (ii) efficient H_2O_2 detoxification in tissues where UV-B photons do reach. The former strategy is facilitated by UV-induced morphology changes including the increase of epidermal UV absorption [4], and moving the site of photosynthesis away from upper regions of adaxial palisade cells towards the inside of the leaf [37]. An application of UV-inducible chlorophyll fluorescence demonstrated the penetration of 305 nm UV-B into *Arabidopsis* and soybean leaves, and that UV-B reached chlorophyll containing tissues even in sunlight acclimated leaves having strong epidermal UV screening [38]. These results show the need for the second, antioxidant line of defense. In a recent experiment with tobacco plants acclimated to supplemental UV-B, the UV-inducible relative increase in peroxidase activity was higher than in other antioxidants, for example of SOD [29]. This was complemented with a marked increase in hydroxyl radical scavenging capacities [29,31] and increases in antioxidant defenses followed a peroxidases > $^\bullet OH$ detoxification > SOD, in that order [29]. When supplemental UV-B resulted in less than 20% loss of photosynthesis, the ratio of peroxidase activation to SOD activation in UV-B acclimated tobacco leaves varied between 4 to 1 and 2 to 1, depending on the applied PAR and UV-B intensities, rather than on UV to PAR ratios. Significant increases in leaf H_2O_2 were detected when a

higher (200 µmol m^{-2} s^{-1}) PAR was combined with 13.6 kJ m^{-2} d^{-1} UV-B, but there was no significant increase when a lower (50 µmol m^{-2} s^{-1}) PAR was supplemented with 3.6 kJ m^{-2} d^{-1} UV-B (unpublished data). There was no catalase activation, this enzyme was either unaffected or present at decreased activities as a result of acclimation to UV-B. Similar effects were measured in *Arabidopsis thaliana*, using the same UV source and similar UV-B doses as applied to tobacco [29] (Table A.1), including higher (approximately 5-fold) activation of peroxidases than of total SOD (1.5-fold) and no change in catalase [40]. Different conditions, similar PAR but an approximately four-times higher UV-B (Table A.1) resulted in oxidative stress in pea and wheat leaves, with marked increase not only in H_2O_2 but also in lipid peroxidation products [42]. UV-B enhanced SOD activity in this study but peroxidase enzymes were unaffected [40]. Thus the ratio of peroxidase activation to SOD activation was < 1, unlike in experiments when leaves were less stressed and peroxidases were preferentially enhanced [29,39].

UV effects in field experiments can be more complex, due to the presence of multiple factors affecting antioxidants, which may be difficult to control. Consequently, studies supplementing sunlight with UV-B from artificial sources can give diverse conclusions. For example, grapevine leaves exposed to extra UV-B had higher SOD activity than sunlight acclimated ones, but ascorbate peroxidase or peroxidase activities were basically unaffected [41]. Conversely, applying supplemental UV-B to pea (*Pisum sativum* L.) leaves grown out of doors in the tropics resulted in a larger increase in peroxidase activities than SOD, and a decrease in catalase activity [42]. In addition to examining different species, the two experiments were different in several other conditions and are only mentioned here to illustrate the diversity of outdoor responses. Less diverse results from controlled laboratory experiments suggest that acclimative antioxidant responses to UV-B are directed at H_2O_2, preferentially via peroxidases and not catalase activity [29,39], which argues for the photosynthesis derived nature of this ROS.

3. What can and what do laboratory experiments tell us about the effect of UV-B on plants?

The use of simplified model samples, such as functional, isolated leaf organelles or their membrane subunits which lack most of natural defenses has advanced the understanding of possible interactions of UV photons with biomolecules and identified several reactions as potential primary ROS sources, as reviewed recently [7]. However, results of these model experiments may not be relevant to more complex systems, such as whole leaves in sunlight. Solar UV is not the single environmental factor capable of generating ROS, therefore a possible, although arguable compromise is to use growth chambers and use only UV-B as a potential stressor. It is to be recognized and accepted that these experiments miss several important aspects of responses to ambient sunlight, for example interactions between responses to UV-B and other potential stress factors, or adaptive responses evoked by UV being present from very early stages of plant development. With the exceptions of solar simulators, experiments utilizing commercially available growth chambers or modified versions of these were unable to reproduce ambient UV to PAR dose ratios [3]. However, critical integration of growth chamber based studies into models interpreting responses to natural UV conditions may serve as building blocks of hypotheses, in analogy to incorporating conclusions of *in vitro* UV experiments with isolated thylakoid membranes into whole plant laboratory studies.

Research aimed at assessing the oxidant-antioxidant balance of UV-exposed leaves is hindered by the lack of means to measure actual cellular concentrations of ROS. Although new methods are expected to emerge, the inevitable invasive nature of working with ROS-reactive probes will always impose challenges, for example the possibility of wounding stress evoked during probe delivery, or the introduction of probes as artificial competitors into the cellular antioxidant network. Identifying more stable ROS, such as H_2O_2 is more promising (see section 2.1.2.) but at present it is not possible to perform an extensive survey of all major ROS *in vivo*, except in plants suffering from strong, near-lethal levels of oxidative stress. As a result, both qualitative and quantitative information on UV-inducible ROS in UV-B acclimated leaves is to be estimated from

antioxidant data. Because one type of ROS is in theory (and in chemistry practice) convertible into almost any other, finding the antioxidant that initially responded to UV-exposure to the largest extent cannot unambiguously identify the primary ROS products. However, acclimative changes in the antioxidant profile may highlight key ROS. Because ROS are generated in either enzymatic reactions, via energy or via electron transfer to molecular oxygen, or in a Fenton reaction chloroplast redox reactions are expected to be among the primary targets of UV-B. Accordingly, chloroplast antioxidants are more active in UV-B acclimated leaves than in controls. Actual extents of activation vary among plant species, and with age, and it is difficult to compare data from laboratories using different UV-B sources and mismatched experimental conditions. Still, some trends are clearly observable.

According to a recent hypothesis [43], extreme $(^1O_2 + O_2^{\bullet-})/H_2O_2$ ratios in chloroplasts cause oxidative damage or initiate programmed cell death (Fig.3). Although the original hypothesis was set up to distinguish defense and death responses, here we present a modified version that may be useful for interpreting UV responses. In the absence of reliable data on ROS concentrations, the $(^1O_2 + O_2^{\bullet-})/H_2O_2$ ratio is to be translated to a ratio of antioxidant activities. Because the detoxification of $O_2^{\bullet-}$ by SOD yields H_2O_2, this is not a simple reciprocation of the ROS ratio and a set of coupled equations must be worked out. However, it is clear that in samples with low $^1O_2$ concentrations, relative amounts of $O_2^{\bullet-}$ and H_2O_2 will define the ROS ratio. $^1O_2$ production may take place in chloroplasts at all light intensities, but its concentration is kept low by non-enzymatic antioxidants and oxidative protein damage is efficiently repaired [44]. Low $^1O_2$ plastid concentrations are expected when leaves maintain most of normal photosynthetic activities under low supplemental UV-B doses. Such conditions enhance the production of flavonoids, which are good $^1O_2$ antioxidants [45] and are present in chloroplasts [46].

An application of the original model [43] to conditions when $^1O_2$ concentrations are much lower than those of $O_2^{\bullet-}$ and H_2O_2 is shown in Fig. 3. Extreme $(^1O_2 + O_2^{\bullet-})/H_2O_2$ ratios are caused by strongly imbalanced, preferential increases in peroxidases or in SOD. Successful acclimation to stress

is expected when the induction of $O_2^{\bullet-}$ and H_2O_2 detoxifications are more balanced. The following

hypothesis intends to explain how UV-B induced relative changes in SOD and peroxidase activities

affect the success of acclimation to supplementary UV-B in laboratory experiments. Antioxidant

capacities that are present before the onset of UV-B are influenced by a number of factors, such as

plant species, leaf age and growth conditions including PAR [13]. Consequently, the general model

we present here is not based on ROS scavenging capacities themselves but it compares the extent of

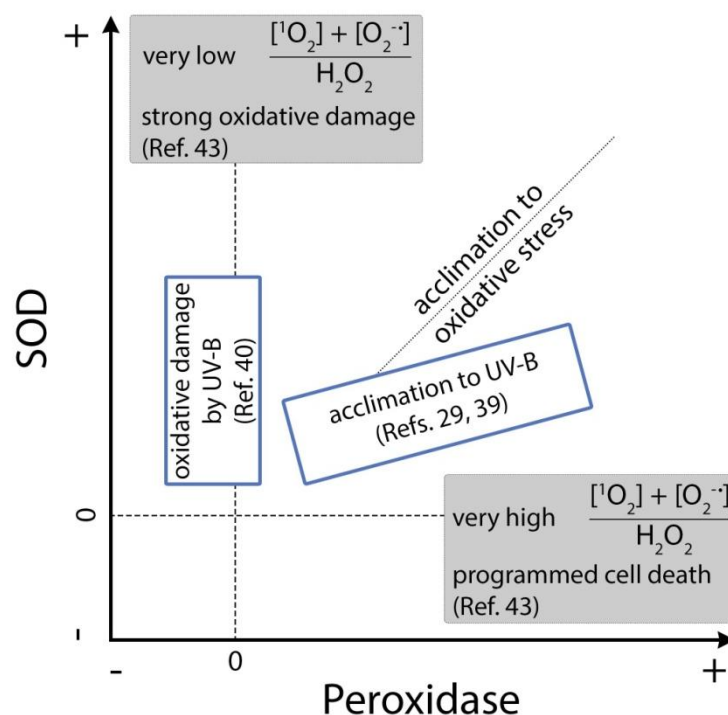UV-B induced relative changes in these capacities.

Figure 3.
Our graphical interpretation of a general stress response model [43] showing how changes in superoxide radical and hydrogen peroxide scavenging (SOD and peroxidase, respectively) influence acclimation to UV-B when cellular singlet oxygen concentrations are low. Stress-induced changes in antioxidant capacities relative to controls are shown in the two axis. No change is marked by "0" and dashed lines. "−" and "+" refer to decrease and increase, respectively. Combinations of peroxidase and SOD activation that are of special interest are symbolized by boxes. According to the original model [43], extremely imbalanced peroxidase : SOD activation ratios in response to a stress condition result in cell damage (gray shaded boxes). Responses to UV-B (blue-framed boxes) are special: experimental data support our hypothesis that peroxidase : SOD activation >1 facilitate acclimation [29,39], and ratios <1 promote oxidative damage [40] as detailed in section 3.

Acclimation to UV-B is expected to be different from responses to other potential stressors

because the UV-B photo-production of $^{\bullet}OH$ from H_2O_2 [15] is to be avoided by increasing peroxidase

activities. This assumes a stronger activation of peroxidase than SOD (illustrated by the blue framed area in the lower right side of Fig.3.), because the latter also contributes to increasing H_2O_2 concentrations. On the other hand, a high peroxidase : SOD activation ratio bears the risk of creating a $({}^1O_2 + O_2{}^{\bullet-})/H_2O_2$ ratio that is high enough to induced cell death [43]. A 2-4-fold higher increase in peroxidase than in SOD has been shown to facilitate the acclimation of tobacco [29] and *Arabidopsis* [39] to supplemental UV-B (section 2.2). It is yet to be explored whether a small increase in peroxidases and no change in SOD can also achieve a similar effect. An opposite trend, a stronger increase in SOD than peroxidase or an increase in SOD only decreases the $({}^1O_2 + O_2{}^{\bullet-})/H_2O_2$ ratio. Experiments in reference [40] showed that a 1.3-1.5-fold higher SOD activity and no change in peroxidase activity corresponding to peroxidase : SOD activation ratio < 1 (as indicated by the blue framed area in the upper left of Fig.3.) failed to prevent oxidative damage under supplemental UV-B.

The upper and lower limits of the peroxidase : SOD activation ratio contributing to successful acclimation to UV-B are expected to be influenced by background PAR and strongly modified by stress factors other than UV-B. An increase in ${}^1O_2$ concentration increases $({}^1O_2 + O_2{}^{\bullet-})/H_2O_2$ and peroxidase : SOD activation ratios that allowed acclimation to UV-B under low ${}^1O_2$ may become damaging under higher ${}^1O_2$ levels. High plastid ${}^1O_2$ might occur when a combination of stress conditions prevents the regulation of photosystem II electron flow and malfunctioning redox components enhance ${}^1O_2$ production and/or protein repair is impaired [47]. Although UV-induced ${}^1O_2$ production in chloroplasts only occurs when high dose UV-A is applied alone [21], this effect might also modify leaf responses to UV-B, especially when multiple stress factors are present in addition to full spectrum sunlight. It is important to note that the translation of ROS ratio to detoxifying capacity ratios in our hypothesis also includes contributions of non enzymatic antioxidants, therefore "SOD" and "peroxidase" are rather symbols of total antioxidant capacities targeted to $O_2{}^{\bullet-}$ and H_2O_2, respectively, than names of specific enzymes.

4. Concluding remarks

The hypothesis presented in section 3. is based on laboratory experiments, performed on plants grown without UV radiation and were only exposed for supplemental UV for a relatively short (4-14 day) period. Under these conditions the photosynthetic machinery is certainly among the primary targets of UV-B. However, under field conditions, the effects of the full solar spectrum on plant growth do not always reflect decrease in photosynthesis [48]. In this way, a model centered on chloroplast oxidant-antioxidant balance might not translate to environmentally relevant conditions. Nonetheless, hypotheses based on growth chamber or greenhouse experiments have already proven useful in assessing the underlying biochemical mechanisms of UV-B damage and acclimation, as reviewed elsewhere [4,5].

As the stratospheric ozone hole is expected to recover as a result of the successful implementation of the Montreal protocol, research on extreme UV effects is no longer justified. However, because the recovery is slow and local UV conditions are expected to be modulated by various ozone modifying tropospheric conditions (for example clouds) and climate factors [49], research on plant acclimation strategies to supplemental UV-B is and will remain timely.

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