UV-B exposure, ROS and stress: inseparable companions or loosely linked associates?

Éva Hideg[1], Marcel A.K. Jansen[2] and Åke Strid[3]

[1]Institute of Biology, University of Pécs, Ifjuság u. 6. H-7624 Pécs, Hungary

[2]School of Biological, Earth and Environmental Sciences, University College Cork, North Mall, Cork, Ireland

[3]School of Science & Technology, Örebro Life Science Center, Örebro University, SE-70182 Örebro, Sweden

All authors contributed equally to this paper

Corresponding author: Jansen, M.A.K. (M.Jansen@ucc.ie).

Ultraviolet-B (UV-B) radiation has long been perceived as a stressor. However, a conceptual U-turn has taken place, and UV-B damage is now considered rare. We question whether UV-stress and UV-B-induced reactive oxygen species (ROS) are still relevant concepts, and if ROS-mediated signaling contributes to UV-B acclimation. Measurements of antioxidants and of antioxidant genes show that both low and high UV-B doses alter ROS metabolism. Yet, there is no evidence that ROS control gene expression under low UV-B. Instead, expression of antioxidant genes is linked to the UV RESISTANCE LOCUS 8 pathway. We hypothesize that low UV-B doses cause 'eustress' (good stress) and that stimuli-specific signaling pathways pre-dispose plants to a state of low alert that includes activation of antioxidant defenses.

Keywords: UV-B, stress, ROS, antioxidant, acclimation, signaling

Evaluating consequences of UV-B exposure

In the late 1980s, awareness of stratospheric ozone layer depletion triggered concerns about the potentially harmful effects of increased ultraviolet-B (UV-B) radiation. Many studies have since shown that UV-B causes damage to DNA, proteins and membranes, impedes photosynthetic activities, and impedes plant growth. Oxidative stress has been flagged as a key factor in such UV-B stress (e.g. [1]). Oxidative pressure [i.e. imbalances between the production of reactive oxygen species (ROS) and anti-oxidant scavenging capacity], has been linked to non-specific damage to DNA, proteins and lipids [2,3]. However, ROS, DNA damage and membrane degradation products also play a role in mediating UV-B protection. ROS and antioxidants orchestrate stress defense responses by adjusting gene expression, proteolysis, and thioredoxin dynamics [2,4]. Such ROS-mediated signaling is a tightly regulated process that links actual stress conditions with stress acclimation [5].

Notwithstanding the damaging potential of UV-B photons, it has become increasingly clear that under realistic UV-B exposure conditions (see glossary), UV-B does not substantially impede plant growth [6,7], and that 'the balance of current research suggests that UV-damage is probably the exception rather than the rule' [8]. Indeed, in a recent large scale study of the responses of perennial ryegrass (*Lolium perenne*) no significant effect of ambient UV-B on aboveground biomass was discernable along a latitudinal gradient (27-68°N) across Europe [9]. However, lack of stress does not mean a lack of biological impact. On the contrary, there is overwhelming evidence that UV-B is an environmental regulator, controlling gene expression, cellular and metabolic activities, and growth and development [10]. Regulatory UV-B effects can be observed under low UV-B

fluences [11] and it has been proposed that such low UV-B effects are, at least partially, mediated by the UV-B-specific UV RESISTANCE LOCUS 8 (UVR8) photoreceptor and signaling pathway [12–16].

The lack of UV-B-mediated stress observed in many studies [6] has triggered debate about the relationships between UV-B exposure, ROS and plant stress (Figure 1). In this Review, we question whether UV-B-induced ROS and UV-dependent stress are still relevant concepts, or if they are artifacts of particularly harsh UV exposure conditions. We examine the role played by generic ROS signaling under low UV-B conditions, particularly in comparison with the stimuli-specific UVR8 response pathway. Our analysis shows that low UV-B doses induce considerable alterations in antioxidant status, but that there is no direct evidence that these changes are mediated by ROS.

Is UV-B radiation a stressor?

To address the question of whether UV-B radiation is a stressor, it is necessary to define stress [17]. The term 'plant stress' is commonly used by authors in a very broad sense, whereby almost every environmentally induced change in metabolic activity, growth, and developmental pattern can be referred to as stress or stress response [18]. 'Plant stress' can refer to destructive or constructive effects on plants, or for example a selecting factor driving adaptive evolution. In order to differentiate between these various aspects of stress, a general plant stress concept with unifying terminology has been developed [18-21]. This concept is based on analogy with the field of mechanics where a material can be exposed to a 'stress' (a force) which results in a 'strain' (bending). In plant sciences, the terms of 'stress factor' or 'stressor' are used to describe this imposed, external factor. Exposure of plants to a stressor can cause reversible, elastic eustress (strain or bending in

mechanics) and, once exposure exceeds a tolerance-limit, irreversible plastic distress (in mechanics: a strain resulting in rupturing) [17,20]. Eustress is an activating, stimulating stress which is a positive element in plant development, and is also referred to as 'good stress' or "constructive stress" [18-21]. When a plant experiences a mild, elastic eustress, metabolism is adjusted, and the plant acclimates to the new environment. For example, a mild water deficit, above the permanent wilting point, can induce plant hardening and increased water-use efficiency [20]. In contrast, distress is a severe stress that has a predominantly negative effect on the plant and its development, and is also referred to as "destructive stress" [18-21]. Distress occurs if the environment becomes too unfavorable for a particular plant [22]. For example, a severe water deficit below the permanent wilting point will cause severe cellular damage, and impede growth [20]. The onset of distress does, however, not always occur under the same stressor exposure conditions, as plants can increase elastic and plastic stress resistance through genetic adaptation and/or physiological acclimation. The plant stress concept generates the terminology to dissect plant stress responses, and this makes the concept particularly suitable to describe plant responses to environmental factors that cause a mixture of eu- and distress, such as for example UV-B radiation, low and high temperatures, wind and/or touch, and drought.

UV-B radiation has been amply demonstrated to induce specific changes in gene expression [23–28], increased accumulation of UV-screening pigments [29] and altered phytochemical content [30]. Many of these responses have been linked to increased UV-B tolerance, and can be induced by below ambient, chronic UV-doses which do not cause substantial damage [6,8,26]. These responses can therefore be defined as eustress. However, whereas productivity may not be directly affected by UV-radiation under

eustress conditions, regulatory changes in photosynthate allocation and morphology [31], may still cause subtle decreases in biomass accumulation [6]. In contrast, macroscopic damage, accumulation of damaged DNA and inactivation of the photosynthetic machinery are consistent with distress. The balance between eustress and distress does not simply depend on UV-dose and/or the spectral quality, but will also depend on, for example, background intensity of photosynthetically active radiation (PAR), plant acclimation state and genotype. Many early UV-B studies showed extensive distress [32,33], and this was typically associated with unrealistic experimental conditions, including high levels of UV-B and/or low levels of accompanying PAR. A review of the UV-exposure protocols used in these early studies concluded that there was little evidence to support a general impediment of photosynthesis by ambient UV-B [34]. This conclusion has been widely accepted, and is a key message of the 2011 United Nations Environment Programme assessment, which reported the minimal effects of realistic UV-B on biomass accumulation [6].

UV-B radiation as a stressor under unfavorable environmental conditions

Realistic field-based studies have shown that ambient UV-B can decrease photosynthetic activity under certain circumstances. For example, in the harsh Arctic environment, ambient levels of UV-B decrease photosynthetic performance of Arctic willow (*Salix arctica*) [35]. Several studies have demonstrated that other environmental factors can also influence the effect of UV-B on plants, which may explain the inconclusive results of many field studies. For example, water supply has been shown to influence the effect of supplemental (1.2 kJ m^{-2} d^{-1} UV above ambient) UV-B on the growth and photosynthetic electron flow of several Arctic bryophytes [36]. A study of photosynthetic soil organisms

(cyanobacteria, lichens and mosses) under desert conditions showed that the effects of UV-B radiation were influenced by precipitation: for example, UV-B stress increased when the precipitation frequency was increased [37]. Similarly, the sensitivity of clover (*Trifolium repens*) exposed to 13.3 kJ m^{-2} d^{-1} UV-B has been shown to depend on both water availability and genotype [38]. However, not all studies show a link between water supply and UV-susceptibility. For example, UV-B (24 kJ m^{-2} d^{-1}) had no impact on photosynthesis in drought-stressed, green-house-grown olive (*Olea europea*), rosemary (*Rosmarinus officinalis*), and lavender (*Lavandula stoechas*) [39]. Nutrient supply has also been shown to influence the effect of UV-B. For example, ambient UV-B (~9 or ~15 kJ m^{-2} d^{-1}) decreased the photosynthetic activities of maize (*Zea mays*) that received low levels of nutrients, but did not affect well-fertilized plants [40]. UV-B (7.2 kJ m^{-2} day^{-1} UV above ambient) decreased the photosynthetic rates of radish (*Raphanus sativus*) grown on super-optimal nutrient levels, but not that of plants grown under optimal conditions [41]. Thus, plants that are exposed to unfavorable environmental conditions appear to be more susceptible to UV-mediated distress.

It is overly simplistic to conclude that any plant exposed to a stressor will be susceptible to UV-mediated distress. On the contrary, the literature contains numerous examples of cross-tolerances between UV-B and other environmental stressors. For example, the severity of drought stress has been shown to decrease when pea (*Pisum sativum*) [42] or tobacco (*Nicotiana tabacum*, Petit Havanna SR1) [43] were grown under supplemental UV-B (32 and ~13.2 kJ m^{-2} d^{-1}, respectively). Similarly, UV radiation diminishes drought stress in Stone pine (*Pinus pinea*) during the hot, dry Mediterranean summer [44]. In tobacco, increased drought tolerance is associated with the induction of

antioxidant defenses [43]. Furthermore, in cucumber (*Cucumis sativus*), antioxidant defenses are synergistically upregulated by a combination of drought and UV-B [45]. Thus, exposure to multiple stressors can either result in aggravated distress or in increased cross-tolerance; the factors that determine the direction of this interaction have considerable ecological and agronomical relevance.

ROS in UV-B-exposed plants

Generally, UV-B has no significant effects on photosynthesis, and just subtle effects on plant growth and development [6], implying that widespread, oxidative damage is rare under realistic UV-B levels. This does not necessarily mean that ROS formation and metabolism are unimportant. It is plausible that ROS play a role in eustress (i.e. UV-B acclimation and the readjustment of metabolism). ROS-mediated signaling is a complex process affected by individual ROS species, ROS-producing enzymes, and the oxidation–reduction states of various antioxidants [4]. The concept of a cellular redox state has been envisaged as the sum of all reducing and oxidizing redox active molecules in the cell; it is not just a control point for stress responses, but also plays a far broader regulatory role in cellular regulation [22].

In UV-B-exposed plants, increased levels of ROS may be formed as a result of disruption of metabolic activities [1,46] or owing to increased activity of membrane-localized NADPH-oxidase [47]. Visualization of production and fate of UV-induced ROS, under *in vivo* conditions, contributes to our understanding of the role of these species. However, this is technically not straightforward because of the reactivity of ROS. Target identification may appear easier, particularly in the case of high ROS concentrations. However, cascades of secondary oxidations can hide the identity of the primary ROS target

and, therefore, obscure mechanistic aspects of ROS activity [48]. Tools have been developed to visualize ROS directly or indirectly, ranging from ROS-specific reporter molecules to rather indirect indicators of ROS involvement, such as fingerprinting methods, and are overviewed below. Unfortunately, plant scientists cannot use the full range of ROS-visualizing tools that are successfully used in the medical or physical sciences. For example, inhibition of ROS production by excluding oxygen is not an option for plant physiologists. Similarly, direct identification of H_2O_2 based on its UV absorption is hampered by the abundance of UV-absorbing molecules in plants.

Direct ROS measurements

Owing to its physical characteristics, singlet oxygen ($^1O_2$) is the only ROS that can be detected without the use of a reporter. The monomolar infrared (1270 nm) photoemission of $^1O_2$ has been used to demonstrate the presence of this ROS in illuminated, isolated reaction centers of photosystem II [49]. So far, singlet oxygen has not been detected in intact leaves by this method. Singlet oxygen as well as other ROS can be visualized using colorimetric, electron paramagnetic resonance (EPR) or fluorescent ROS reporter molecules. Externally supplied reporter molecules compete with natural ROS targets and undergo a discernible physical change, such as a change in color, fluorescence or EPR absorption upon oxidation [50]. The presence of $^1O_2$ and superoxide radicals has been demonstrated in spinach (*Spinacia oleracea*) leaves using selective fluorescent probes, but only in response to high, damaging UV doses [51]. Similarly, ROS have been detected in broad bean (*Vicia faba*) leaves [46] and isolated rice (*Oryza sativa*) thylakoids [1] treated with high intensity UV-B by using EPR spin trapping reporters. Thus, there is direct evidence for increased ROS production under conditions typically associated with distress.

Antioxidants and oxidized targets

Oxidized, endogenous target molecules can also be used as ROS reporter molecules. For example, accumulation malondialdehyde (MDA) [43,52] or of DNA thymine dimers [53], products of ROS-mediated oxidation of polyunsaturated membrane lipids and of DNA, respectively, imply the presence of ROS. MDA has been reported in the leaves of rice cultivars treated with UV-B (13 kJ m^{-2} day^{-1}) [54]. Absence of MDA in plants exposed to low UV-B doses may imply lack of oxidative stress. However, this is not necessarily the case given that MDA may undergo secondary reactions and/or catabolism [55].

Because of the balance between pro-oxidants and antioxidants, changes in the oxidation–reduction state of antioxidants provide a further tool for deducing changes in ROS concentrations. A short period of exposure to 0.46 kJ m^{-2} UV causes a fourfold increase in the level of oxidized dehydroascorbate radical in broad bean (*Vicia faba*) leaves, reflecting UV-induced oxidative pressure [56]. However, changes in the redox state of the ascorbate–dehydroascorbate redox pair cannot simply be equated to oxidative pressure because of concomitant re-reduction reactions by glutathione and, ultimately, NADP(H). In pea, acute exposure to 1.4 W m^{-2} UV-B has been shown to result in the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) decreasing to just 6-10% of control values [57], again indicating UV-induced oxidative pressure. Furthermore, it is not just the Halliwell–Asada antioxidant system that needs to be considered, any molecule with radical scavenging capacity can provide information about ROS [58]. Plants contain large numbers of non-enzymatic antioxidants, including phenolics, carotenoids, cytochromes, tocopherols and tocotrienols, polyamines and proteins that carry redox active S-groups, creating a dynamic network of redox interactions [22]. Using the oxidation–reduction state of

extracted antioxidants to evaluate ROS involvement in UV-B responses is an indirect tool, but this is still an attractive choice owing to the sensitivity of the method.

When plants are exposed to low, chronic UV-B conditions, another effect of UV-B exposure becomes clear: pool sizes of antioxidants such as ascorbate, GSH, xanthophylls and α-tocopherol are increased (compare with [21]), indicating greater anti-oxidative defenses. For example, exposure of spinach to low, chronic UV-B (2 weeks exposure to 1 kJ m^{-2} day^{-1}) resulted in a 2.7-fold increase in ascorbate levels [59], whereas α-tocopherol levels increased about eightfold in spinach and lettuce (*Lactuca sativa*) that were exposed to UV-B for one week [60]. Exposure to 1.4 W m^{-2} UV-B resulted in a 4.5-fold increase in total GSH levels in pea [57]. It has been argued that the functional role of the well-documented UV-B-mediated accumulation of phenylpropanoids and flavonoids is primarily to increase ROS scavenging activity [29,61]. Flavonoid accumulation occurs under both low and high UV-B conditions. In particular, the UV-induced increase in the quercetin:kaempferol-ratio [62] represents an increase in ROS scavenging activity, rather than an increase in UV absorbance. Thus, there is considerable evidence for changes in antioxidant metabolism under conditions of both distress and eustress.

Activation of antioxidant pathways

A common strategy for studying ROS metabolism is to quantify the activity of the enzyme components of the antioxidant system as proxies for oxidative pressure [63,64]. Measured enzymes typically include Cu- or Zn-superoxide dismutases (SODs), ascorbate peroxidase, dehydroascorbate reductase, glutathione peroxidase, glutathione reductase and catalase, and their activities are mostly measured following exposure to high doses of UV-B. However, interpretation of data is complicated owing to differences in antioxidant

responses between species, between genotypes of the same species [65–67] and between leaves of different age, and/or developmental stage [52,68]. Nevertheless, there is some consensus. Elevated SOD, catalase, glutathione reductase and glutathione peroxidase activities were found in many UV-B exposure studies (compare with [69]). In winter wheat (*Triticum aestivum*), the antioxidant system was up-regulated by UV-B (4.2 or 10.3 kJ m^{-2} d^{-1}) under optimal temperatures; however, under low (10°C during daytime and 5°C at night) temperatures, UV-B decreased photosynthetic yield [70], which again emphasizes that distress is most likely to occur when plants are exposed to multiple unfavorable factors. UV-B (0.18 W m^{-2}) also induced the production of the pyridoxine biosynthesis enzyme PDX1, and increased the levels of the antioxidant pyridoxine in *Arabidopsis* (*Arabidopsis thaliana*) [71]. However, despite the publication of numerous papers on UV-B-induced antioxidant pathways, there is still considerable uncertainty regarding to what extent enzyme components of antioxidant pathways are up-regulated under eustress conditions.

UV-B-dependent expression of oxidative defense genes

The problem with the aforementioned biochemical approaches is that they are either relatively insensitive (reporter molecules), or indirect (changes in oxidation state, reduction state or the total pool size of antioxidants). Molecular approaches can potentially avoid some of these pitfalls by yielding information on expression of antioxidant pathways. Nine *Arabidopsis* DNA array studies on UV acclimation performed by five different laboratories have been published in journals or are searchable in Genevestigator (https://genevestigator.com/gv/) [23–28,72–75]. These studies used a range of daily UV-B doses (from 0.093 to 7.0 W m^{-2}), spectra, durations of UV-B exposure (from 15 minutes to 12 days) and PAR background levels (from low 25 μmol m^{-2} s^{-1} to ambient glass house

conditions that include UV-A). In a study using particularly low levels of UV-B (0.093–0.137 W m^{-2}), expression of glutathione reductase and the pyridoxine biosynthetic protein PDX1.3 were found to increase. Glutathione reductase reduced glutathione with the help of NADPH and is therefore a key component of the ascorbate–glutathione antioxidant system [23]. Glutathione peroxidase, and several glutathione transferases and glutaredoxins were shown to be upregulated following exposure to short periods of relatively high intensity UV-B [24,25]. Glutaredoxin expression was decreased in plants exposed to chronic (12 day; 0.564 kJ m^{-2} day^{-1}) UV-B, possibly reflecting a down-regulation following an initial up-regulation of expression [26]. Thus, there is considerable evidence for altered expression of glutathione-related genes across a range of UV doses and exposure times, complementing measurements of altered GSH:GSSG ratios and pool size [57], and implying that alterations in ROS metabolism are a feature of all UV-B exposure conditions.

PDX gene products are strong antioxidants that neutralize singlet oxygen, hydroxyl radicals, and superoxide [71,75,76]. The *PDX1.3* gene is up-regulated following exposure to short periods of low- [23] or high-intensity UV-B [24,25]. However, *PDX1.3* has not been found to be differentially expressed in plants exposed to chronic (12 day) UV-B, suggesting that PDX antioxidant activities are components of the fast, initial response to UV-B.

Numerous genes encoding enzymes involved in phenol metabolism such as flavonol synthase, caffeoyl-CoA *O*-methyltransferase, and 4-coumarate-CoA ligase 3 are upregulated in *Arabidopsis* following exposure to short periods of low level UV-B [23]. Short exposures to high UV-B levels induce expression of isoflavone reductase, phenylalanine ammonia lyase, cinnamoyl-CoA reductase, caffeoyl-CoA *O*-methyltransferase, leucoanthocyanidin dioxygenase [24] and flavanone 3-hydroxylase, chalcone synthase, flavonol synthase,

chalcone isomerase, dihydroflavonol reductase, cinnamoyl-CoA reductase in *Arabidopsis* [25]. Thus, the altered expression of genes involved in the biosynthesis of phenols is a shared feature of plants exposed to low and high UV-B doses. Given the well-documented accumulation of phenolic metabolites in UV-B-exposed plants, and given the important role of phenolics as antioxidants [29], it is concluded that alterations in ROS metabolism occur across all UV-B-exposure conditions.

ROS and regulation of gene expression

ROS are both stress-inducing compounds and signaling molecules that control, among others, gene expression. Therefore, analyzing regulation of UV-B-dependent gene expression can shed light on the potential role of ROS in UV-acclimation. We have reviewed the expression of genes encoding proteins involved in 'traditional' antioxidative pathways, such as SOD, ascorbate and glutathione metabolic enzymes, as well as isoprenoid, phenolic, and pyridoxine biosynthetic genes, in published microarray data. Fourteen genes have been reported to be up-regulated at least twofold in different studies reported by at least two separate laboratories (Table 1). The protein products of five of these genes are involved in glutathione metabolism, seven in phenylpropanoid metabolism (cinnamates and flavonoids) and one in pyridoxine and one in isoprene biosynthesis (solanesyl diphosphate). Studies using mutants [25,27] have shown that each of these genes needed the UV-B photoreceptor UVR8 [12–14] for expression (Table 1), and that most of them were also dependent on the downstream regulatory proteins CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and ELONGATED HYPOCOTYL 5 (HY5) [27,28,72]. Thus, the genes belong to the UV-B-specific, 'low UV dose' route of gene expression [10,11] and,

therefore, support the concept that even low doses of UV-B can cause changes in antioxidant metabolism.

A pertinent question is whether ROS control the expression of the same 14 genes. To answer this, we compared gene expression under UV-B with that under oxidative stress conditions involving various types of ROS (O_3, $O_2^{\cdot-}$, H_2O_2, $^1O_2$) (Table 1) [77–101]. Stressors such as ozone [77–85,86–89], methyl viologen and high light [89,90,99] increased the expression of several genes involved in antioxidative metabolism; however, overlap with UV-B-induced genes is more or less non-existent. Similarly, expression of genes encoding several antioxidative proteins was increased in the singlet oxygen scavenging-deficient *Arabidopsis flu* mutant [94–96]. However, overlap with UV-B-induced genes was limited. Thus, plants express different enzyme systems and/or different isoenzymes when exposed to UV-B compared with general oxidative stress conditions. There are two notable exceptions to this: (i) the GRX480 glutaredoxin gene (At1g28480) was induced during most of the conditions examined; (ii) norflurazon treatment, inhibiting carotenoid biosynthesis [102] and, thus, leading to singlet oxygen formation in the chloroplast [103,104], resulted in induction of five out of the fourteen UV-B-regulated genes, which infers some overlap in action.

Expression of genes linked to eustress and antioxidative protection is not controlled by ROS, but rather through the UVR8 pathway. We therefore hypothesize that low, ecologically relevant doses of UV-B cause eustress, pre-disposing the plant to a state of 'low alert' in case conditions worsen, including activation of genes involved in generic antioxidant defense. This is in contrast to the situation under high-UV-B, distress conditions (Figure 2). For example, similarities in gene expression have been noted

between plants exposed to artificially generated ROS and plants exposed to high levels of UV-B [105]. Furthermore, the UV-B-mediated expression of several genes can be modified by treating plants with effectors of ROS metabolism, including free-radical scavengers. It was concluded that ROS mediate responses to high UV-B levels [105].

Conclusion

High levels of UV-B can cause distress in plants. Distressed plants produce elevated levels of ROS. Thus, under these conditions, UV-B exposure, ROS and stress are closely linked. Distress can also occur when plants are simultaneously exposed to ambient UV-B and unfavorable environmental conditions. By contrast, under low, chronic UV conditions, distress is a rare event, prompting the question: do ROS play a role in the cellular and organismal acclimation responses under these conditions? Both low and high levels of UV-B radiation can change antioxidant metabolism (i.e. change the size and/or oxidation–reduction state of the ascorbate, glutathione, and tocopherol pools, and induce accumulation of flavonols and related phenolics, which are strong cellular antioxidants). UV-B also affects expression of genes that impact on the cellular redox state (i.e. genes whose products are involved in glutathione, pyridoxine and phenolic metabolism). We conclude that changes in ROS and antioxidant metabolism are an intrinsic part of both eustress and distress. Nevertheless, low UV-B-induced changes in antioxidant metabolism do not appear to be linked to control of gene expression. Instead, UV-B-specific perception and signaling pathways involving UVR8, COP1 and HY5 [10] comprise the main regulatory pathway under low UV-conditions, activating antioxidant defenses before potential oxidative pressure. ROS-mediated signaling appears to be restricted to high UV-B distress conditions. This conclusion triggers two important questions for future research. Firstly,

there is a need to elucidate the precise combination of environmental conditions, and physiological acclimation states where either eustress or distress will occur. Secondly, an important follow-up question is how plants 'balance' generic ROS-specific signaling pathways with stimuli-specific systems such as the UV-B photoreceptor-mediated responses. Understanding this balancing act should give us an insight into the fundamental issues underlying one of the most important plant characteristics, the capability to acclimate to variable environmental conditions.

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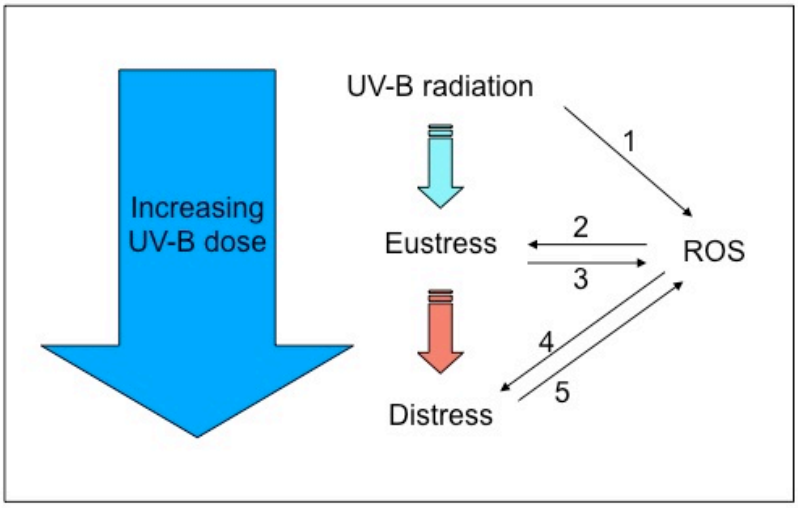
Legends

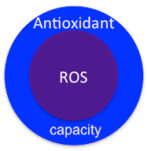
Figure 1. UV-B radiation has been well documented to induce eustress, leading to UV-acclimation. UV-B-induced distress appears to be a relatively rare phenomenon under natural light conditions. There is some evidence that UV-B exposure can directly induce ROS (1), although it is not clear to what extent this happens under realistic UV-B conditions. ROS that are formed may contribute to eustress and the UV acclimation response (2) or cause oxidative damage (4). Conversely, further ROS can be produced as part of the UV response of the plant; either as part of UV-acclimation by induction of NAPH oxidase activity (3) or as a result of metabolic disruption (distress) of, for example, photosynthetic electron transfer reactions (5).

Figure 2. ROS levels and antioxidant capacities under physiologically relevant UV-B levels (eustress) and under high UV-B conditions (distress). Under physiologically relevant UV-B levels, the ROS scavenging capacity, regulated by the UV-B-specific signaling pathway containing the UVR8 UV-B photoreceptor and the COP1 and HY5 signaling components, is sufficient to deal with the oxidative pressure inflicted by UV-B. Under high UV-B conditions, the UV-B levels are high enough to lead to a massive development of ROS, over-riding the antioxidant capacity regulated by non-specific stress pathways and contributing to both signaling and gene expression.



Ambient UV-B levels

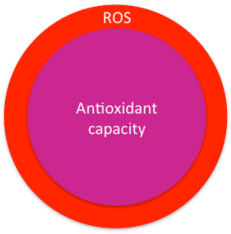
UV-B-specific gene expression regulated by UVR8
Low ROS levels
Sufficient ROS scavenging capacity



Eustress

High UV-B levels

Gene expression regulated by ROS
High ROS levels
Insufficient ROS scavenging capacity



Distress