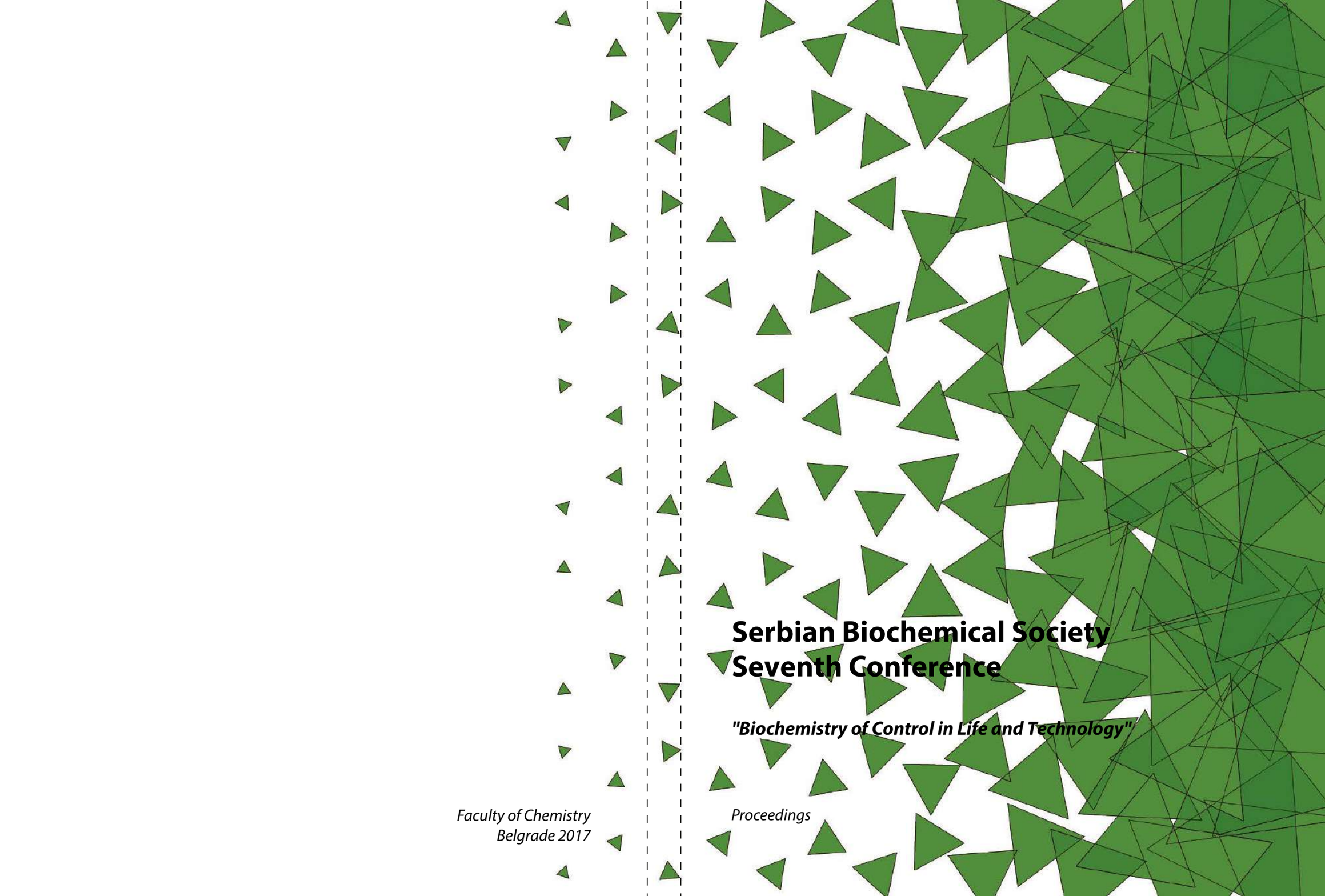

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Sun as a stressor and/or regulator of plant metabolism: responses to UV radiation and high light

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In their natural environment, plants are constantly exposed to dynamic changes of solar radiation, which mainly consists of infrared (IR, >700 nm), photosynthetically active radiation (PAR, 400-700 nm) and minor portion of ultraviolet (UV) radiation (UV-B, 290-315 nm and UV-A, 315-400 nm). Sunlight is not only the primary source of energy in photosynthesis, it is also an important signal which regulates plant growth and development. During the period from the 1970s to 1990s, investigations on UV-B effects on organisms were in the centre of attention due to alarming depletion of stratospheric ozone layer and increased UV-B radiation reaching the Earth's surface. UV-B radiation has been perceived only as a stressor. A decade later, new data obtained using realistic UV-B doses and realistic UV-B:UV-A:PAR ratio, clearly show that UV-B is very important environmental cue and regulator of plant metabolism, rather than a stressor. In the recent years, great progress has been made in understanding the mechanisms of light signals' perception. However, the complications arise from the overlapping of the acclimative responses to UV-B radiation and high PAR intensity, imposing cross-tolerance to different components of solar radiation. Moreover, information on other constituents involved in the UV-B response, such as reactive oxygen species in relation to their tissue- and subcellular-localization is scarce. Our latest findings using leaf variegation as a model with metabolically contrasting tissues show specific responses to UV-B radiation and high light in relation to antioxidative metabolism, photosynthesis, carbohydrate metabolism, and distribution of phenolics.

High light intensity

Light is the most important environmental factor for plants, as it provides the source of energy for plant life. Therefore, plants have developed complex mechanisms to perceive light quality, quantity, duration and direction, and to adjust their metabolism and development accordingly. Processes such as seed germination, seedling photomorphogenesis, chloroplast development and movement, phototropism, shade avoidance, circadian rhythms and flower induction are light-regulated.

Plants perceive light signals through several protein photoreceptors: five phytochromes (PHY A-E), which are sensitive to red and far-red light (600-750 nm), and two cryptochromes (CRY1 and CRY2), two phototropins (PHOT1 and PHOT2) and zeitlupe proteins (ZTLs) for blue, and UV-A radiation (315-500 nm), while UV-B radiation is sensed by UV Resistant Locus 8 (UVR8) [1,2]. During the recent years, great efforts have

been made to characterize the organization of light-regulated transcriptional networks in the model plant *Arabidopsis thaliana*; particularly related to photomorphogenic responses.

Every day, plants are exposed to varying photosynthetically active radiation (PAR) intensities in the field [3,4]. Under the low light intensity, photosynthetic efficiency is maximal, while its capacity is limited. With the increase in the light intensity, the rate of the light-dependent reaction, and therefore photosynthesis in general, increases proportionately; however, photochemical efficiency drops, until photosynthetic rate achieves the plateau. Above this point PAR intensity overcomes metabolic requirements and the capacity of dissipation mechanisms, finally resulting in increased rate of reactive oxygen species (ROS) generation, followed by the inhibition of photosynthesis and CO_2 assimilation [5]. This condition is called photooxidative stress. It is difficult to define which PAR intensity is a stressor for a particular plant species since this depends on the developmental stage, previous light adaptation, and on other environmental conditions, including drought, high salinity, nutrient deprivation, or temperature stress [6]. Even optimal PAR intensity might be a stressor in combination with conditions that limit CO_2 availability or decrease the rate of the Calvin-Benson cycle [7]. Under such conditions, down-regulation of photosystem II (PS II) is a major mechanism of photoprotection, as well as efficient dissipation of the excess of photon energy by a decrease of pH and zeaxanthin accumulation [8]. Molecular oxygen has an important role in energy dissipation under photoinhibitory conditions, as it serves as an electron acceptor in two photosynthetic processes, photorespiration and in the Mehler reaction coupled to the water-water cycle at photosystem I (PS I), where it is reduced to superoxide anion radical [9,10].

Having in mind the effects of ROS generation in chloroplasts on overall cellular metabolism, we successfully established a model system with two metabolically contrasting tissues which are spatially closely related; variegated leaves which have both photosynthetically active (green sectors) and non-photosynthetically active (white sectors) cells. Under optimal light conditions, in the leaves of variegated *Pelargonium zonale*, differential subcellular distribution of low molecular antioxidants (ascorbate and glutathione) and spatial differences in distribution of enzymatic antioxidants, soluble sugars, and phenolics in the two tissue types have been revealed [11,12]. Under conditions which accelerate Mehler reaction in chloroplasts (*e.g.* high light, HL, exposure), H_2O_2 significantly accumulated only in illuminated green sectors, and it has been restricted to the extracellular space of vascular and adjacent photosynthetically active cells and directly dependent on the activity of NADPH oxidase (Figure 1) [13]. Similar HL-induced H_2O_2 accumulation in the (peri)vascular area has been reported in *A. thaliana* and *Mesembryanthemum crystallinum* [14,15], which together with our results emphasize the tissue-specific response to HL and involvement of bundle sheet cells in rapid intercellular signal propagation during acclimatization to high light [16].

In the mesophyll tissue of *P. zonale* plants under HL, efficient scavenging of H_2O_2 in photosynthetic tissue has been attributed to catalase activity, while in the non-photosynthetic tissue peroxidases were involved in the antioxidative defence [13]. Although enzymes of ascorbate-glutathione cycle and total glutathione pool have been constitutively

higher in non-photosynthetic tissue, under progressive pro-oxidative conditions the reduction of oxidized glutathione has been more efficient in photosynthetic tissue, which is related to the photosynthetically-derived reducing equivalents.

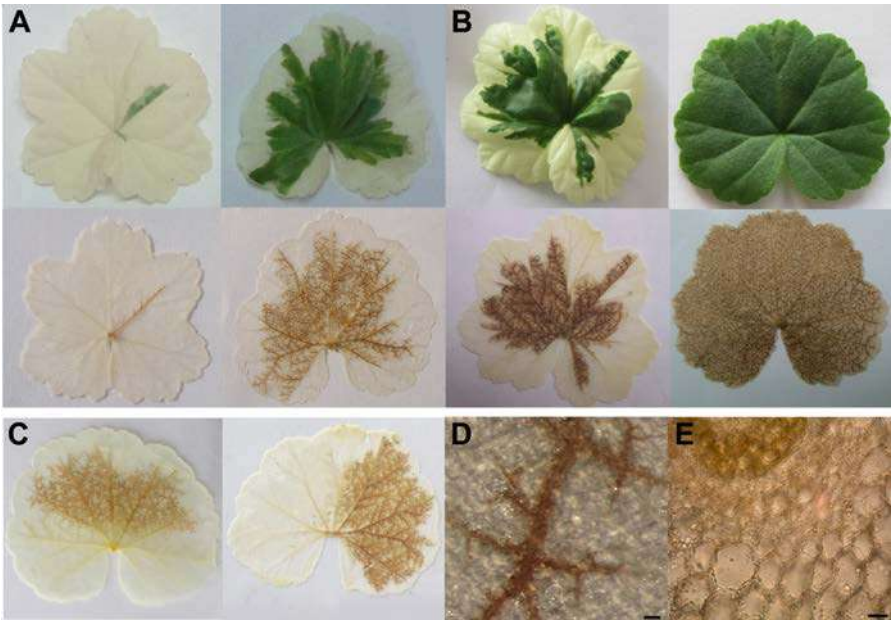

Figure 1. Accumulation of H_2O_2 in *P. zonale* leaves detected by DAB 'up-take' method: A) after 1 h-exposure to high light (HL, >1100 μmol $m^{-2}s^{-1}$ PAR); B) after 1 h-exposure to HL+100 μM paraquat. C) Light-dependent H_2O_2 accumulation in green sectors has been confirmed by partial shielding of the bottom and lateral leaf halves during 1 h exposure to HL; D) green leaf sector 5× magnified (bar represents 1 mm); E) cross section of green leaf (peri)vascular tissue (100×, bar represents 50 μm). For detailed results see [13].

Therefore, the question is what is the primary signal which triggered the unexpectedly strong response to HL in non-photosynthetically active tissue? This signal could be transferred from photosynthetic cells, but not through the involvement of extracellular H_2O_2 originating from NADPH oxidase activity (Figure 1). On the other hand, it is possible that non-photosynthetic tissue responded to HL independently, provoked by some intrinsically generated factor.

Besides investigation of the tissue- and cell-specific tolerance to HL, extensive progress has recently been made towards characterizing rapid changes in nuclear gene expression triggered by HL [17]. These transcriptional changes are regulated in a photosynthesis-dependent manner and the signals generated in the chloroplasts are transported to the nucleus [18]. Moreover, chloroplastic signals can be transferred to neighbouring cells and

distal parts of the plant. Indeed, signals from reduced chloroplasts (optimal conditions) activate transport via plasmodesmata, which allows symplastic communication and exchange of molecules, while signals from oxidized plastids (paraquat-treated) inhibit this intercellular transport [19]. Light-provoked chloroplast retrograde signalling, mediated by the redox state of plastoquinone pool, regulates nuclear alternative splicing, which together with an unknown signalling molecule induce the same change further downstream, in the roots [20]. The intercessory signalling molecule is still not identified, although some evidence suggests it might be a certain redox-sensitive protein kinase.

Anthocyanins, red and blue pigments in flowers and fruits, have a role in attracting pollinators and in seed dispersal. With their strong absorption in the 260-280 nm and 500-550 nm ranges, anthocyanins absorb the green range of PAR, protecting photosynthetic apparatus in the leaves from light excess (excitation pressure) and from potential photooxidative stress [21-23]. Moreover, anthocyanidins are efficient antioxidants, and they can serve as an electron donor for class III peroxidases in H_2O_2 scavenging, followed by re-reduction by ascorbate in vacuoles [24].

Accumulation of the anthocyanins is one of the most recognizable HL-inducible symptoms, and that is why they have been included in the most of the studies exploring the mechanisms of plant responses to HL, particularly those involved in light-mediated redox chloroplast signalling.

What is the relationship between photosynthesis-dependant redox changes and anthocyanins accumulation under HL? Studies with mutants deficient in H_2O_2 scavenging enzymes (chloroplastic ascorbate peroxidases, APX, and catalase) showed that high concentration of H_2O_2 derived from chloroplasts and peroxisomes may be important for HL-dependent induction of anthocyanins [25,26]. In addition, Maruta and co-workers showed that chloroplastic H_2O_2 generated under photooxidative stress induced by paraquat, enhanced the expression of anthocyanin biosynthesis and genes associated with its regulation even under lower PAR intensities [27]. Besides H_2O_2, other signals derived from photosynthesizing chloroplasts responsible for anthocyanin accumulation may be overreduction of the components of the photosynthetic electron transport chain (PETC), such as plastoquinone [28]. In favour of these findings, Arabidopsis *trol* mutant, with altered electron partitioning between energy-conserving and (energy)-dissipating pathways, has abolished production of anthocyanins comparing to wild type genotype [29].

Very recently, by using HyPer2, a genetically encoded fluorescent H_2O_2 sensor, it has been shown that H_2O_2 accumulated in HL exposed chloroplasts, directly transfers from chloroplasts to nuclei, avoiding the cytosol, and enabling the photosynthetic control over the gene expression [30]. Moreover, the propensity of the main chloroplastic scavenger of H_2O_2: chloroplastic APX to inactivation by H_2O_2 can be regarded as a signalling function [31]. The chloroplastic APXs (thylakoid bound-APX even more than the stromal APX) are very sensitive to inactivation in the presence of H_2O_2 (>2 nM) under low ascorbate content (<20 μM) [32].

Current progress in studying light response in plants shows that the role of visible light goes far beyond photosynthesis. It is clear that visible light can be perceived via several

photoreceptors, which specifically mediate regulation of important processes in the plant cell. However, there are non-specific mediators in the photosynthesizing chloroplasts, generated under HL (plastoquinone redox state, oxidized metabolites, H_2O_2), which are able to transfer the signal to the nucleus and regulate the expression of genes responsible for acclimative and protective responses.

UV-B radiation

Ultraviolet radiation makes about 7% of the total solar radiation, consisting of UV-B (290-315 nm) and UV-A (315-400 nm) radiation [33]. First investigations of the influence of UV-B radiation on plant metabolism and growth indicated its harmful effects, including cyclobutane pyrimidine dimer formation in a DNA strand, enhanced ROS generation, oxidative stress and damage of cellular membrane and proteins, including D1 and D2 subunits of PS II, and Rubisco [34,35]. However, these detrimental effects have been attributed to unrealistic UV-B: UV-A: PAR ratios, high UV-B doses applied, unrealistic UV-B spectrum, plant history, as well as to combined effects of other environmental stressors (high light, drought, high temperature, nutrient deprivation) [36,37]. Today, it is accepted that ecologically relevant, low doses of UV-B radiation act as an important environmental cue and regulator of plant growth and development [2,38,39].

In plant cells, UV-B radiation activates at least two independent signalling pathways that regulate the expression of different sets of genes, depending on its fluence rate [40,41]. One is UV-B stress response, and the second one is crucial for UV-B acclimation response, and it is triggered by UVR8, specific UV-B receptor [42].

In the absence of UV-B radiation, UVR8 protein exists as a homodimer, maintained by cation-π interactions between positively charged (arginine and lysine residues) and aromatic amino acids (tryptophan and tyrosine residues), and charge-stabilized hydrogen bonds at the dimer interface (Figure 2) [43,44]. Exposure to UV-B radiation induces excitation of Trp285 and Trp233 indole rings, resulting in the disruption of cation-π interactions and UVR8 monomerization [45]. UV-B-mediated UVR8 monomers accumulate in the nucleus and interact with the protein COP1 (Constitutively Photomorphogenic 1). During the night, COP1, an E3 ubiquitin ligase, is responsible for targeting photomorphogenesis-promoting transcription factors (such as Elongated Hypocotyl 5, HY5 and HY5 Homolog, HYH) for proteolytic degradation. Under UV-B radiation, UVR8 monomers inhibit the association of COP1 with ubiquitin-proteasome apparatus and, thus, enable gene transcription mediated by HY5 and HYH (Figure 2) [46]. The accumulation of HY5 and/or HYH transcriptional factors in the nucleus is required for the expression of protective genes involved in the UV tolerance, including those for biosynthesis and metabolism of flavonoids and antioxidative defence, especially the glutathione metabolism (glutathione reductase, glutathione peroxidase, glutathione-S-transferase, peroxiredoxins and glutaredoxin) [47,48].

Blue light and UV-A radiation inhibit COP1 via cryptochromes, disabling it to alter transcriptional factors like HY5 (Figure 2) [49]. For effective repression of photomorphogenesis induced by visible light, it is required that COP1 and accessory

proteins SPA (Suppressor of Phy A) form complexes with other components of the ubiquitin-proteasome system, while in UV-B response COP1 does not require SPA [50]. Contrasting to its negative role in the regulation of the visible light response, COP1 is an important positive regulator of responses to low UV-B doses (induction of UV-B responsive genes is impaired in mutants deficient in COP1), coordinating the HY5-dependent and the HYH-dependent pathways in signalling transduction. Therefore, acclimative responses to UV-B radiation and high PAR intensity may overlap, imposing cross-tolerance [51,52]. For example, high PAR-induced flavon-3-ols and hydroxycinnamates in epidermis may attenuate UV-B radiation [53]. Besides phenolics, blue light and UV-B radiation up-regulate photolyases, enzymes involved in the repair of cyclobutane pyrimidine dimers of DNA [54,55]. However, the exact mechanism by which COP1 integrates these different stimuli remains unknown. Two possibilities are proposed: either some unknown molecules/processes are involved in the UV-B response, or other functional domains of COP1, not necessarily the ones with E3 ligase activity, may interact with certain proteins [38].

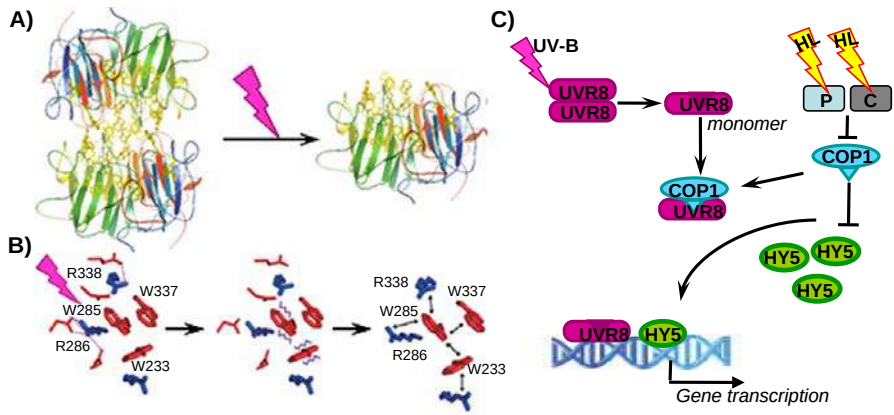

Figure 2. Schematic overview of UV-B radiation-induced activation of UVR8 receptor. A) UVR8 as a dimer in the absence of UV-B radiation. B) Important amino acids at the interface of two monomers and crucial cation-π-interactions between two Arg (R338 and R286) and three Trp (W285, W233 and W237). UV-B-induced excitation of π-electrons of indole rings W285 and W233 (zig-zag purple lines), which prevents maintaining cation-π-interactions and leads to monomerization of UVR8 dimer. C) The interaction between UVR8 monomer and COP1 - connection between visible light (HL) and UV-B perception. Detailed explanation is given in the text. P, phytochromes; K, cryptochromes. Adapted from [2,45].

Nevertheless, activation of UVR8/COP1 pathway leads to the accumulation of secondary metabolites. In particular, accumulation of flavonoids and phenylpropanoids, alkaloids and terpenoids is considered as a hallmark of UV-B response in plants. Phenolic compounds are the most abundant secondary metabolites in plants, involved in a wide range of

developmental and regulatory processes, as well as in numerous biotic and abiotic stress responses [56,57]. Therefore, UV-B radiation improves plant adaptive capacity to drought, high temperatures, pathogen and insect attack, and nutrient deficiency conditions (reviewed in Vidović et al. 2017) [39]. Plants grown in the open field, exposed to natural UV-B doses, have higher nutritional and pharmacological value than plants grown in polytunnels and glasshouses, which are non-transparent to UV radiation [58,59]. These UV-B effects have a strong impact on the agricultural, pharmaceutical and food industries.

Interaction of UV-B radiation and high PAR on primary and secondary metabolism

The importance of phenolic compounds on plant metabolism can be illustrated with the finding that over 20% carbon derived from the Kalvin-Benson cycle is introduced to the shikimate pathway [60]. Biosynthesis of phenylpropanoids and flavonoid glycosides can be regarded as an additional energy escape valve under unfavourable conditions [61]. However, the exact relationship between photosynthesis and phenolic metabolism is insufficiently investigated.

Using leaf variegation enables the examination of source-sink interactions in relation to carbon (sugars, phenolics) allocation within the same leaf, providing the same microenvironment conditions. This is also an excellent model system for studying tissue-specific responses to photosynthesis-dependent H_2O_2 generation. Since PAR, UV-A and UV-B radiation are interlaced under natural light conditions, employing variegated plants under strictly controlled and realistic conditions such as in Sun Simulators, enable relevant conclusions about specific responses to PAR and UV-B radiation. For illustration, high light in combination with UV-B radiation triggered stronger increase of APX and catalase activities and ascorbate accumulation than in the absence of UV-B radiation, only in green sectors of variegated *P. zonale* leaves (Figure 3). These findings indicate that that UV-B radiation and high PAR intensity synergistically stimulate antioxidative defence in green sectors of variegated *P. zonale* leaves [11].

Reports on UV-B effects on photosynthetic machinery and stomatal conductance are inconsistent, due to various UV-B: UV-A: PAR ratios, different periods of exposure, plant metabolic state and previous plant exposure to UV-B radiation. A large number of studies have described deleterious, minimal or negligible effects of UV-B radiation on photosynthetic activity [35,62].

The beneficial influence of ambient UV-B radiation on photosynthetic rate is rarely reported. The UVR8/COP1/HY5(HYH) signalling pathway leads to the expression of *SIG5* (which encodes sigma factor of plastidic RNA polymerase, involved in D2 protein biosynthesis) and induction of ELIP1 (Early-Light Inducible Protein 1), which may interact with D1 protein of PS I, and protect the photosynthetic apparatus from photooxidative stress [48,63]. In our study with variegated *Plectranthus coleoides* exposed to ecologically relevant UV-B radiation combined with high PAR, which simulates sunny spring conditions in mid-northern latitudes, rapid stimulation of CO_2 assimilation, was

observed already after four hours, and remained at the high level until the end of experiment [64]. In addition, after nine days of treatment, increased influx of electrons in alternative electron pathways was detected, compared with HL treatment with no UV-B radiation. Stimulation of photosynthesis might be related to an enhanced requirement for building blocks for biosynthesis of apigenin and cyanidin glycosides, particularly in the neighbouring non-photosynthetic cells (Figure 3).

Contrastingly to the results obtained with *P. coleoides*, in variegated *P. zonale* identical UV-B irradiances under the same experimental conditions, did not affect the photosynthetic rate [11]. Instead, UV-B radiation provoked carbon allocation from source (green) to sink (white) leaf tissue, decreasing trehalose concentration and, therefore, mediating regulation of starch degradation (Figure 3). Trehalose-6-phosphate is involved in carbon allocation to sink tissues (*e.g.* root), and in regulation of starch degradation [65].

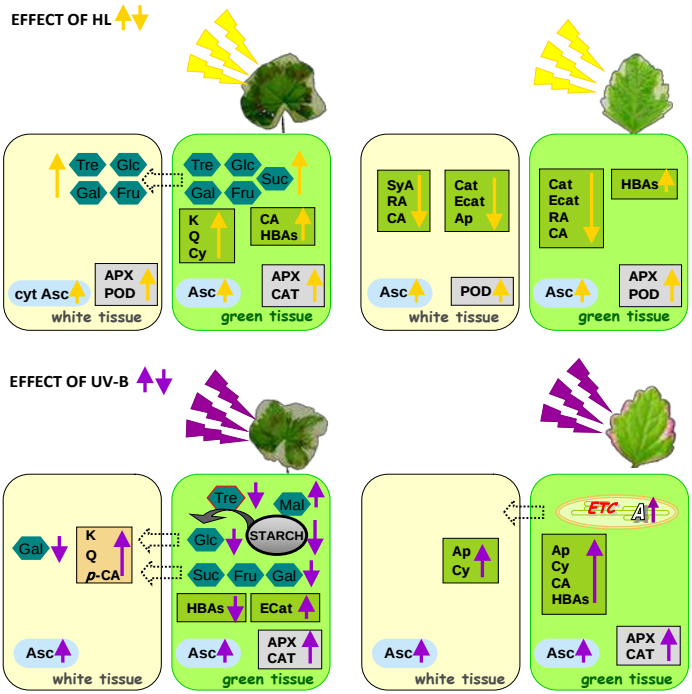

Figure 3. Schematic overview of the link between sugar, phenolic and antioxidative metabolism under high light (HL, 1350 µmol m^{-2} s^{-1} PAR) and HL combined with ecologically relevant UV-B irradiance (0.9 W m^{-2}) combined with HL in green and white leaf sectors of variegated *P. zonale* (left) and *P. coleoides* (right). The influence of UV-B radiation on photosynthesis is emphasized. Arrow directions indicate increased or reduced metabolite content/enzyme activity. Ap, apigenin: APX, ascorbate peroxidase, CA, caffeic acid; CAT, catalase, Cat, catechin; Cy, cyanidin; ECat, epicatechin; HBAs, hydroxybenzoic acids; K, kaempferol; *p*-CA, *p*-coumaric acid; POD, class III peroxidase; Q, quercetin; RA, rosmarinic acid; SyA, syringic acid. For details see [11,64].

In these two studies with variegated species, UV-B-induced accumulation of phenylpropanoids and flavonoids has been closely related to, and regulated by photosynthesis. Flavonoids and anthocyanins (carbon-rich compounds) are mostly glycosylated in the plants, with two or three sugar moieties per aglycone, and their synthesis in the cells requires triosophosphates, ATP, NADPH and malonic acid. The link between photosynthesis and anthocyanin accumulation is even more clear in the case of variegated Arabidopsis *immutans* mutant, with 200 fold higher level of anthocyanin synthase transcripts in photosynthetic than in non-photosynthetic tissue [66]. Moreover, under HL, expression of anthocyanin regulatory transcription factors has been increased more than 100 times in green leaf sectors of *immutans* plants.

At the same time, these studies showed that UV-B differentially affected photosynthesis in the two variegated species (Figure 3). Favouring the carbon assimilation and electron partitioning towards phenylpropanoid pathway enabled increased flavonoid production in both leaf tissues of *P. coleoides* under UV-B radiation (Figure 3). Alternatively, in the leaves of *P. zonale*, UV-B radiation stimulated starch degradation and sugar transport from source to sink leaf tissue, providing the building blocks for biosynthesis of *p*-coumaric acid, kaempferol and quercetin glycosides, which have been induced in white tissue (Figure 3).

Taken together, the specific influence of UV radiation and of PAR alone, on plant metabolism is difficult to resolve, due to their interconnected character and due to other simultaneous factors in the field, which should not be neglected. However, it is obvious that signals dependent on photosynthesis are crucial in the regulation of biosynthesis of phenolic compounds, which are important not only for the plant-environment interactions, but are beneficial for human health and nutrition, as well. Moreover, the species-specific, and source-sink dependence of UV-B regulation of photosynthesis and associated processes, such as induction of flavonoids has to be considered. The targets of this regulation remain to be revealed.

Conclusion and Future Perspectives

Naturally, PAR, UV-B and UV-A radiation are interlaced, and none should be taken as an isolated factor. The molecular mechanisms of functional integration of different light-mediated COP1-dependent pathways, in plants which grow under ambient conditions are still unknown. Besides photomorphogenic responses associated with COP1, high intensities of visible light can activate redox retrograde signalling pathways from chloroplasts, responsible for the acclimation responses. There is growing evidence that photosynthesis is involved in phenolics biosynthesis through redox changes in the electron transport chain, which include H_2O_2 generation at PS I. In fact, it has been suggested that carbon-rich flavonoid glycosides act as energy escape valve under photooxidative stress. Comprehensive knowledge of the signalling pathways between plastids and other cellular compartments under optimal and stress conditions will provide new solutions for developing and engineering advanced plants with improved vigour and stress tolerance.

Furthermore, the specific effects of UV-B radiation on photosynthetic electron transport, CO_2 assimilation and associated processes still have to be elucidated. Investigation of the link between primary and secondary metabolism mediated by UV-B radiation presents a challenge for future studies.

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