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Ecologically relevant UV-B dose combined with high PAR intensity distinctly affect plant growth and accumulation of secondary metabolites in leaves of *Centella asiatica* L. Urban

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ABSTRACT

We investigated the effects of environmentally relevant dose of ultraviolet (UV)-B and photosynthetic active radiation (PAR) on saponin accumulation in leaves on the example of *Centella asiatica* L. Urban. For this purpose, plants were exposed to one of four light regimes i.e., two PAR intensities with or without UV-B radiation. The experiment was conducted in technically complex sun simulators under almost natural irradiance and climatic conditions. As observed, UV-B radiation increased herb and leaf production as well as the content of epidermal flavonols, which was monitored by non-destructive fluorescence measurements. Specific fluorescence indices also indicate an increase in the content of anthocyanins under high PAR; this increase was likewise observed for the saponin concentrations. In contrast, UV-B radiation had no distinct effects on saponin and sapogenin concentrations. Our findings suggest that besides flavonoids, also saponins were accumulated under high PAR protecting the plant from oxidative damage. Furthermore, glycosylation of sapogenins seems to be important either for the protective function and/or for compartmentalization of the compounds. Moreover, our study revealed that younger leaves contain higher amounts of saponins, while in older leaves the sapogenins were the most abundant constituents. Concluding, our results proof that ambient dose of UV-B and high PAR intensity distinctly affect the accumulation of flavonoids and saponins, enabling the plant tissue to adapt to the light conditions.

1. Introduction

Ultraviolet (UV)-B radiation (280–315 nm) is a natural component of sunlight. Due to ozone depletion, the amount of UV-B radiation reaching the Earth's surface has increased over the last decades. Therefore, many of the early (1980s–2000s) studies focused on the impact of above-ambient UV-B levels on plants. In this context, damaging effects of UV-B radiation on DNA, as well as alterations in photosynthesis and retardations in growth and development, have frequently been reported [1–3].

Since ozone depletion has been reduced significantly, recent experiments progressively deal with low, environmentally relevant UV-B levels. These studies reveal that damaging effects on plants are predominantly induced by above-ambient UV-B levels, which trigger non-specific signalling pathways resulting in a general stress response. In contrast, low UV-B levels rather act as informational signal [4,5], stimulating the expression of genes involved in the UV-protection of plants, many of them mediated by UV-B specific photomorphogenic signalling pathways [6,7]. In this context, the up-regulation of the phenylpropanoid metabolism and the increased accumulation of flavonoids have been explored thoroughly (e.g., [8,9]). Nevertheless, plants are naturally exposed to a multitude of environmental conditions. Thus, the effects of UV-B radiation on plants and its interaction with other factors, including PAR, has to be evaluated. In this regard, it was shown that flavonoid synthesis is induced by high PAR intensity, even in the absence of UV-B radiation; though, its accumulation is additively enhanced in the presence of UV-B radiation [10–12].

Besides flavonoids, the accumulation of other secondary compounds in response to UV-B exposure, some of them probably acting as UV-protectants [13], has been recorded. Accordingly, it was proposed that the synthesis of saponins is triggered by UV-B radiation [14]. Furthermore, a few studies indicated a promoting effect of higher light intensities on saponin concentrations in plants [15–17]. However, findings are scarce and even contradictory ([18]

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and references therein). Moreover, the combination of UV-B and PAR, and their impact on the accumulation of saponins, has not yet been investigated. In the present study we aimed to examine the effects of environmentally relevant UV-B dose combined with different PAR intensities on saponin accumulation in *Centella asiatica* L. Urban (Apiaceae). *C. asiatica* accumulates both pentacyclic triterpene saponins and flavonoids in its leaves, and is thus an excellent model to study changes in the accumulation pattern of secondary metabolites in the plant tissue in response to different light regimes. Further, as reviewed in our previous work [19], the saponins of *C. asiatica*, known as centellosides, are widely used in the pharmaceutical, food and cosmetic industries. Thus, fundamental knowledge on the potential promotion of centelloside accumulation by light regimes will encourage more practical investigations aiming the target-oriented increase in saponin concentrations.

Saponins are synthesized *via* the isoprenoid pathway starting with isopentenyl pyrophosphate and dimethylallyl pyrophosphate. The cyclization of 2,3-oxidosqualene leads to the triterpenoid skeletons, such as α- and β-amyrin. The latter are modified by oxidation, hydroxylation, and other substitutions, generating the *Centella* sapogenins. Finally, the sapogenins are transformed into saponins by glycosylation processes [20,21]. On the contrary, the biosynthesis of flavonoids starts with the deamination of the amino acid phenylalanine, which is then transformed into 4-coumaroyl-CoA. 4-Coumaroyl-CoA and three molecules of malonyl-CoA are condensed to chalcone, which is isomerized to the flavanone naringenin. Naringenin is the substrate for further enzymatic reactions leading to a variety of flavonoids, including flavonols and anthocyanins [22–24].

In our work we hypothesized that ambient level of UV-B radiation and high PAR intensity additively promote the accumulation of saponins and their respective genins in leaves of *C. asiatica*. For this purpose *C. asiatica* plants were exposed to one of four light regimes i.e., two PAR intensities with or without UV-B radiation. The five-week experiment was conducted in technically complex sun simulators, providing an irradiance spectrum, which is very similar to the natural solar radiation [25]. Moreover, the PAR and UV-B intensities followed the natural diurnal variations of the solar irradiance, and the climatic conditions were controlled precisely. The determination of saponin and sapogenin concentrations was realized weekly. In order to elucidate whether the effects of UV-B and PAR on centelloside accumulation depend on the physiological age of the plant tissue and the duration of exposure ([26,27] and references therein), we compared the saponin and sapogenin concentrations in the leaves, which had emerged before treatment initiation and in those, which emerged during the experiment. Furthermore, we investigated the causal relationship among the accumulation of centellosides in leaves, photosynthesis as well as herb and leaf yield of *C. asiatica*. Aiming a monitoring of the specific UV-B response of the plants, we additionally recorded the accumulation of epidermal flavonols and anthocyanins *in vivo* by multiparametric fluorescence measurements. In comparison with traditional wet chemical analysis the non-destructive estimation of flavonoid amounts is less costly and enables repeated measurements of the same leaf during the whole experimental period.

2. Materials and methods

2.1. Plant material

Stock plants of *Centella asiatica* L. Urban were provided by D. Randriamampionona (Laboratoire de Biotechnologie Végétale, Université Libre de Bruxelles, Gosselies, Belgium/Institut Malgache de Recherches Appliquées, Antananarivo, Madagascar). The genome-based identification of the species was carried out by A.N. Nicolas (Institute of Systematic Botany, The New York Botanical Garden, NY, USA). Four weeks before starting the experiment, the plantlets were propagated vegetatively by cuttings obtained from the stock plant stolons. Nodules having one expanded leaf were cut off and stuck into propagation boxes filled with a mixture of peat soil, sand and perlite (3:2:1) as rooting medium. As soon as the cuttings had rooted they were transplanted into cultivation pots containing the same mixture as substrate. The plantlets were raised in a greenhouse, and 180 homogeneous plants having four to six completely expanded leaves were selected for the experiment.

2.2. Treatments and growth conditions

The five-week experiment was conducted in two sun simulators of the Helmholtz Zentrum München (Neuherberg, Germany) in June/July 2011. The sun simulators provided an irradiance spectrum, which is very similar to the natural solar radiation (Fig. S1, Supplementary data). The simulation of the spectrum from 280 to 850 nm was achieved by a combination of metal halide lamps (HQI/D, 400 W, Osram, München, Germany), quartz halogen lamps (Halostar, 300 W and 500 W, Osram, München, Germany), blue fluorescent (TLD 18, 36 W, Philips, Amsterdam, The Netherlands) and UV-B fluorescent (TL12, 40 W, Philips, Amsterdam, The Netherlands) tubes. Oversupplied infrared radiation was reduced by a layer of water. The wavelengths below 280 nm were blocked efficiently using selected borosilicate and lime glass filters. A suitable combination of these glasses allowed us the simulation of different UV-B scenarios. The natural diurnal variations of the solar irradiance were realized by switching appropriate groups of lamps on and off [25,28]. The photoperiod was 12 h with day/night temperatures of 25 °C/15 °C and relative humidity of 60%/80%. Each sun simulator was subdivided into two compartments enabling four experimental treatments with 45 plants at random placed in each compartment. After an acclimatization phase of 4 days, the PAR intensity was raised from 150 μmol m^{-2} s^{-1} to 455 μmol m^{-2} s^{-1}. Starting the experiment, the PAR intensity was maintained at 455 μmol m^{-2} s^{-1} in the first sun simulator, while it was adjusted to 835 μmol m^{-2} s^{-1} in the second one. Moreover, using UV-B absorbing acrylic glass the UV-B radiation was set at 0 W m^{-2} in one compartment and at 0.3 W m^{-2} in the other compartment of each sun simulator. Thus, the treatments will be referred to as follows: high PAR/−UV-B, high PAR/+UV-B, low PAR/−UV-B and low PAR/+UV-B. The plants were watered daily, while fertigation (EC 2.0 mS cm^{-1}, Hakaphos Blau containing 15% N, 10% P_2O_5, 15% K_2O, and 2% MgO, Compo, Münster, Germany) was realized every second day.

2.3. Multiparametric fluorescence measurements

For the *in vivo* monitoring of the UV-B specific response of the *C. asiatica* plants during the whole experimental period, the accumulation of epidermal flavonoids was recorded by non-destructive fluorescence measurements using a multiparametric portable optical sensor (Multiplex® Research, FORCE-A, Orsay, France). The accumulation of flavonols was ascertained considering the decadic logarithm of the red to UV excitation ratio of far-red chlorophyll fluorescence (FLAV index), which is proportional to the flavonol content of the leaves [29]. Moreover, the anthocyanins were evaluated by the decadic logarithm of the red to green excitation ratio of far-red chlorophyll fluorescence (ANTH_RG index), which is proportional to the anthocyanin content in the tissue [30]. The suitability of the fluorescence based FLAV and ANTH_RG indices for prediction of flavonol and anthocyanin accumulation in *C. asiatica* leaves was validated before [31]. Further, the blue and the far-red fluorescence, both excited with UV light, were used to calculate the blue-to-far red ratio (BFRR_UV index). The BFRR_UV index may