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Research article

Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house

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ABSTRACT

The impact of UV irradiation was studied on photosynthesis, photosystem II photochemical yields and antioxidant responses using green-house grown grapevine (*Vitis vinifera* L. cv. Chardonnay) leaves. Supplemental UV irradiation (280–400 nm) was centred in the UV-B region, and corresponded to 8.95 kJ m^{-2} d^{-1} global (280–400 nm) or 8.04 kJ m^{-2} d^{-1} UV-B (280–315 nm) biologically effective dose. UV irradiation was applied daily and its effects were evaluated after 4-days. Younger (1–3 weeks-old) leaves (YL) and older (4–6 weeks-old) leaves (OL) were affected differently, UV irradiation decreased their photochemical yields to 78% and 56%, respectively. Unlike OL, YL responded by an increase in UV-B absorbing pigment, anthocyanin and total phenolics contents. UV irradiation increased total antioxidant capacities in YL but not in OL. YL were also different in their ability to increase specific hydroxyl radical and singlet oxygen neutralizing capacities in response to the supplemental UV irradiation, which is reported here for the first time. Our results suggest that the ability of maintaining photosynthesis under supplemental UV is not necessarily determined by base levels of antioxidants but rather by their inducibilities in response to the irradiation and emphasise the importance of comparing leaves of the same age in UV studies. Correlations between various antioxidant capacities, pigment contents and photosynthesis parameters were also examined. However, no single element of the defence system can be picked up as decisive factor of sensitivity to UV.

1. Introduction

Sunlight contains high energy ultraviolet (UV, 280–400 nm) radiation which affects photosynthesis in various ways. Many studies have shown that high doses of UV, specially that of UV-B (280–315 nm) are damaging to plants. Cellular components such as proteins and nucleic acids absorb this radiation, resulting in biomass reduction, impaired photosynthesis and other chloroplast functions, decreased protein synthesis, damage to DNA, reviewed [1,2]. Effects of UV-B radiation include oxidative stress [3,4], and reactive oxygen species (ROS) have been shown to participate directly in the damage induced by high UV-B doses [5–7]. On the other hand, many effects of UV-B radiation concern morphogenetic changes in plants rather than damage, especially in response to lower UV doses [8,9]. In addition, UV-B affects the secondary metabolism of plants indicating that solar UV-B is to be regarded as an environmental challenge rather than a damage-inducing source of stress [10,11]. Plants protect themselves from this potentially harmful radiation by altering metabolic functions and a number of studies confirmed the role of UV-B in regulation of gene expression [9,12–14]. Due to variations in experimental conditions, data available on the effect of UV-B radiation and the antioxidant response indicate considerable differences between plant tissues and/or plant species [15–19], but all studies agree on the critical role of effective ROS neutralizing in responses to UV-B. High, damaging ROS concentrations under high UV doses can be detected by spin trapping EPR [5,7,20] or fluorescent probes [6,21] allowing comparative studies of ROS and ROS scavenging. Currently available

Abbreviations: AA, ascorbic acid; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); Fv/Fm, maximum photochemical yield of PS II in the dark adapted state; GA, gallic acid; FRAP, ferric reducing antioxidant power; HTPA, 2-hydroxyterephthalate; OL, old leaves; PAR, photosynthetically active radiation; PPFD, photosynthetic photon flux density; PS, photosystem; ROS, reactive oxygen species; TEAC, Trolox equivalent antioxidant capacity; TPA, terephthalate, 1,4-benzenedicarboxylic acid, TPA; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Y(II)-55, effective photochemical yield of PS II at 55 μmol photons m^{-2} s^{-1} PPFD; YL, young leaves.

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ROS detecting methods are, however, not sensitive enough to identify ROS directly under milder stress conditions, leaving only the possibility of assuming that increased antioxidant activities reflect a situation requiring stronger control over the increased level of ROS. In addition to measuring ROS specific antioxidant enzymes, plant studies have also borrowed methods from medicine and food chemistry to assess total antioxidant potentials of a plant extracts [22–25]. Comparisons of these general and ROS specific antioxidant capacities are of interest. Traditionally, conclusions on the chemical nature of ROS are only drawn when specific enzymes are measured (for example superoxide dismutase or peroxidases) but ROS which are not targeted by specialised enzymes cannot be studied this way. To overcome this issue, two newly developed methods measuring specific singlet oxygen [26] and hydroxyl radical [27] scavenging capacities of plant extracts were used in the present study.

The overall aim of this work was to study the role of antioxidant responses of grapevine leaves in acclimation to supplemental, UV-B centred broad band UV irradiation. In order to compare samples with different antioxidant potentials, we chose young and old (but not senescent) leaves of the same plant, and analysed photosynthesis, photochemical efficiency, pigment and antioxidant responses to UV. Using water-based leaf extracts, changes in general (total) antioxidant capacities and in specific ROS neutralizing abilities were compared and possible correlations of these were also sought. Grapevine (*Vitis vinifera* L. cv. Chardonnay) was chosen as plant material, due to its economic importance and the sensitivity of the cultivar to UV radiation [28]. Vines are advantageous for irradiation studies, as these can be trained horizontally allowing the exposure of leaves of different ages to the same dose.

2. Materials and methods

2.1. Plant material

Grapevine (*V. vinifera* L. cv. Chardonnay) plants were grown in 20 cm diameter pots, in a mixture of garden soil and vermiculate. Plants were grown in the absence of UV, in a glass-roofed green-house under natural daylight, which provided ca 200 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at noon. During growth, individual shoots were climbed on horizontal rods compelling most of the leaves facing upwards, with adaxial surfaces at the same distance from irradiation sources, regardless of their ages. In this study, fully developed 1–3 weeks-old leaves (the first three fully developed leaves from the tip of the shoot) represented young leaves (YL) while 4–6 weeks-old non-senescent leaves (chosen from the fourth to the ninth leaves from the tip of the shoot) were regarded as old leaves (OL). For antioxidant measurements water extracts were made by first grinding 4 leaf disks (approx. 40 mg) to powder in liquid nitrogen, then in 2 mL ice cold phosphate buffer (50 mM, pH 7.0). After centrifugation (500× *g* for 5 min at 4 °C), supernatants were collected and stored at −70 °C until use. Singlet oxygen scavenging capacity measurements required more concentrated leaf extracts, and the above procedure was modified by extracting 9 leaf disks (approx. 90 mg) into 2 mL of the same buffer and centrifuging for 10 min at 3000× *g* at 4 °C. The Folin-Ciocalteu reagent was purchased from Ferak Berlin GmbH (Berlin, Germany). All other chemicals were from Sigma-Aldrich (Sigma-Aldrich Kft Budapest, Hungary).

2.2. Supplemental UV radiation

For a 4-day supplemental UV treatment plants were moved to an other location in the green-house, where visible light was lower, due to the shading of UV tubes. Here both UV irradiated plants and controls were kept under 50 μmol photons m^{-2} s^{-1} PPFD between 7 AM and 7 PM. In addition to PPFD, UV treated plants were exposed to 0.84 W m^{-2} irradiance (integrated UV-B dose, measured with a Cole-Palmer radiometer, model 97503-00, and a broad range 312 nm centred sensor) from Q-Panel UVB-313EL tubes daily, between 9 AM and 3 PM. A cellulose diacetate filter (Courtaulds Chemicals, Derby, UK) was used to exclude any effect of shorter wavelength (<280 nm) UV radiation. Spectral distribution of UV irradiance from the tube panel (Fig. 1) was measured with a Newport OSM-400UV/VIS spectrometer (Newport Corporation, Irvine CA, USA), courtesy of Dr. Andreas Albert (Helmholtz Zentrum München, Germany). The applied UV irradiance corresponded to 8.95 kJ m^{-2} d^{-1} global (280–400 nm) or 8.04 kJ m^{-2} d^{-1} UV-B (280–315 nm) biologically effective dose, calculated using the Biological Spectral Weighting Function developed by Flint and Caldwell [29]. The applied biologically effective UV-B dose corresponded to approximately 107% of ambient daily UV-B in the northern hemisphere (latitude 46°), in summer [30]. However, PPFD in our experiments was lower than in the field therefore results of this study cannot be directly extrapolated to outdoor conditions. PPFD was measured with a LI-250 radiometer (LI-COR Environmental, Lincoln, Nebraska USA).

2.3. Photosynthesis and photochemical yield measurements

Photosynthesis (gas exchange) measurements were performed on leaves attached to the vines. Photochemical yields were measured on detached leaves and were completed within 30 min after removal from the plants. Following these, 0.6 cm diameter disks were cut from the leaves which were used immediately for pigment analysis and for making leaf extracts. Photosynthesis was characterized by CO_2 uptake (CO_2 μmol m^{-2} s^{-1}) and stomatal conductance (mmol H_2O m^{-2} s^{-1}) measured on intact leaves at 300 μmol photons m^{-2} s^{-1} PPFD using a LI-6400 Portable Photosynthesis System (LI-COR Environmental, Lincoln, Nebraska USA). Photochemical yields were calculated from chlorophyll fluorescence yields measured on excised leaves using the MAXI-version of the Imaging-PAM (Heinz Walz GmbH, Effeltrich, Germany). Leaves were first kept in the dark for 20 min, which was followed by measurements of F_o, the minimal fluorescence yield of dark adapted samples and F_m, the maximal fluorescence yield obtained with the help of a saturation pulse. Maximum (potential) photochemical quantum yield of photosystem (PS) II was calculated as

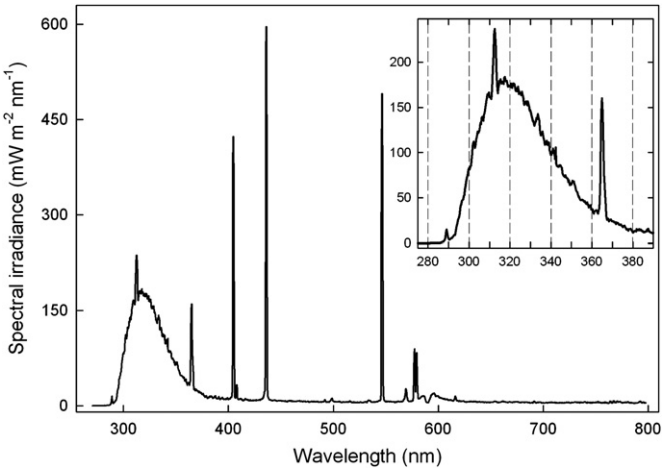

Fig. 1. Spectral distribution of irradiance from the Q-Panel UVB-313EL tube panel (covered with cellulose diacetate filter) applied in the experiments presented here. Inset: UV region of the same spectrum.