Journal of Plant Physiology 222 (2018) 39–50

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

UV-B radiation modulates physiology and lipophilic metabolite profile in *Olea europaea*

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ARTICLE INFO

Keywords:
Climate change
'Galega Vulgar'
GC–MS
Photosynthesis

ABSTRACT

Ultraviolet-B (UV-B) radiation plays an important role in plant photomorphogenesis. Whilst the morpho-functional disorders induced by excessive UV irradiation are well-known, it remains unclear how this irradiation modulates the metabolome, and which metabolic shifts improve plants' tolerance to UV-B. In this study, we use an important Mediterranean crop, *Olea europaea*, to decipher the impacts of enhanced UV-B radiation on the physiological performance and lipophilic metabolite profile. Young olive plants (cv. 'Galega Vulgar') were exposed for five days to UV-B biologically effective doses of 6.5 kJ m^{-2} d^{-1} and 12.4 kJ m^{-2} d^{-1}. Cell cycle/ploidy, photosynthesis and oxidative stress, as well as GC–MS metabolites were assessed. Both UV-B treatments impaired net CO_2 assimilation rate, transpiration rate, photosynthetic pigments, and RuBisCO activity, but 12.4 kJ m^{-2} d^{-1} also decreased the photochemical quenching (qP) and the effective efficiency of PSII (Φ_{PSII}). UV-B treatments promoted mono/triperpene pathways, while only 12.4 kJ m^{-2} d^{-1} increased fatty acids and alkanes, and decreased geranylgeranyl-diphosphate. The interplay between physiology and metabolomics suggests some innate ability of these plants to tolerate moderate UV-B doses (6.5 kJ m^{-2} d^{-1}). Also their tolerance to higher doses (12.4 kJ m^{-2} d^{-1}) relies on plants' metabolic adjustments, where the accumulation of specific compounds such as long-chain alkanes, palmitic acid, oleic acid and particularly oleamide (which is described for the first time in olive leaves) play an important protective role. This is the first study demonstrating photosynthetic changes and lipophilic metabolite adjustments in olive leaves under moderate and high UV-B doses.

1. Introduction

Ultraviolet-B (UV-B) radiation is 0.5% of total solar radiation energy and comprises the high-energy solar spectrum, having considerable impact on terrestrial ecosystems (Correia et al., 2012; Verdaguer et al., 2012). Near the equator, solar UV-B radiation may reach 12 kJ m^{-2} d^{-1}, while in mid-latitudes irradiation may be higher (Forster et al., 2011). These values represent 6–14% increments over the pre-1980 levels, and according to current estimates (e.g. WMO, 2010), UV-B radiation is expected to remain elevated during the next decades. However, multiple environmental factors, such as sky cloudiness, air pollutants and aerosols can alter UV-B fluxes (Verdaguer et al., 2012). Plants have evolved molecular photoreceptors to UV-B (e.g. UV RESISTANCE LOCUS 8) which, when the plants are exposed to UV radiation, sense and transduce the signal into multiple responses, leading to adaptive changes at the cellular and systemic levels (e.g. Wargent et al., 2015; Dotto and Casati, 2017).

The Mediterranean regions are often exposed to UV-B incidences > 7 kJ m^{-2} d^{-1} (Correia et al., 2012; Martínez-Lüscher et al., 2015). Among the Mediterranean crops, *Olea europaea* is one of

Abbreviations: Chl, chlorophyll; C_i/C_a, ratio between intercellular and atmospheric CO_2 concentration; CMP, cell membrane permeability; *E*, transpiration rate; FCPV, full peak coefficient of variation; F_v/F_m, maximum efficiency of photosystem II; FW, fresh weight; F_0, minimal fluorescence yield; g_s, stomatal conductance; MDA, malondialdehyde; NPQ, non-photochemical quenching; P_N, net CO_2 assimilation rate; PSII, photosystem one; qP, photochemical quenching; ROS, reactive oxygen species; RWC, relative water content; UV-B, ultraviolet-B radiation; UV-B_1, moderate UV-B dose; UV-B_2, high UV-B dose; Φ_{PSII}, effective efficiency of photosystem II

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https://doi.org/10.1016/j.jplph.2018.01.004
Received 2 August 2017; Received in revised form 27 December 2017; Accepted 2 January 2018

the oldest and most widespread, with a great socio-economic impact worldwide. In Portugal, the cultivar 'Galega Vulgar' represents 80% of the national olive patrimony, being used in five Protected Designation of Origin regions (https://ec.europa.eu/agriculture/quality/). This ancient cultivar is under pressure for breeding programs, as both breeders and oil-producers consider the oil obtained from this cultivar of top sensorial quality and high stability (Figueiredo et al., 2014).

The physiological and molecular basis for olivés drought tolerance has been extensively studied (e.g. Bacelar et al., 2007; Conde et al., 2014), but less is known about the performance of this species to high UV-B radiation (Liakoura et al., 1999; Liakopoulos et al., 2006; Koubouris et al., 2015). The few available reports indicate that *O. europaea* tolerance to enhanced UV-B radiation involves leaf cuticle thickness increase and high content of phenolic compounds (Liakoura et al., 1999; Liakopoulos et al., 2006). The epicuticular wax layer provides the first line of defence against adverse environmental conditions (Provost et al., 2013), including UV-B radiation (Ni et al., 2014). These wax structures consist of heterogeneous mixtures of lipophilic substances (e.g. terpenes, alkanes, esters, aldehydes and fatty acids), reflecting from 10 to 30% of the incident UV-B radiation (Kakani et al., 2003). Exposure to enhanced UV-B radiation resulted in changes in wax chemical compositions in other species (Gil et al., 2012). Cuticle function is complemented by inner epidermal/mesophyll layers that, by shifting metabolic pathways to accumulate secondary metabolites, protect plant cells against reactive oxygen species (ROS) (Kaling et al., 2015).

Besides, it has been described for olive and other species that excessive UV-B radiation increases ROS and impairs the photosynthetic apparatus, consequently reducing growth rates (Koubouris et al., 2015; Dotto and Casati, 2017). UV-B impairment of photosynthesis has been attributed to a destruction and/or reduction of the synthesis of photosynthetic pigments, degradation of PSII proteins, to the loss of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) small and large units' expression and to stomatal closure (Inostroza-Blancheteau et al., 2016; Escobar et al., 2017; Machado et al., 2017). UV-B cyto- and genotoxicity was demonstrated for different species (e.g. Araújo et al., 2016; Fina et al., 2017), due to direct/indirect effects on macromolecules (e.g. DNA, proteins and lipids) and consequent reduction of genome stability. Also, enhanced UV-B irradiation decreased olives' fruit yield by decreasing pollen germination and tube length (Koubouris et al., 2015).

Metabolomic approaches have contributed to identify most sensitive networks linked to these physiological adjustments in several species, and to find key stress metabolites (Rodziewicz et al., 2014). Besides the model species *Arabidopsis* (Kusano et al., 2011), few crops were screened for their metabolome changes in response to UV-B, namely *Zea mays* (Casati et al., 2011) and *Lactuca sativa* (Wargent et al., 2015). Also, the majority of the studies were focused on changes in the phenylpropanoid pathway (e.g. sinapic acid, sinapoyl malate, quinic acid), and other sunscreen protective phenolic compounds (e.g. Casati et al., 2011; Kusano et al., 2011). Metabolomic studies in *O. europaea* are also restricted to the characterization of some leaf phenolic compounds induced by UV-B radiation (Liakopoulos et al., 2006). However, for a comprehensive knowledge of the mechanisms underlying the response of plants to enhanced UV-B radiation, its impact on other metabolic pathways must been investigated (Rodziewicz et al., 2014). Several studies extended the metabolomic approaches to identify interesting lipophilic metabolites also involved in abiotic stress response and tolerance, such as epicuticular wax components (alkanes, terpenes and fatty acids), membrane fatty acids, ROS scavengers (terpenes), and metabolites related to respiration and energy usage (organic acids) (Lytovchenko et al., 2009; Mihailova et al., 2015; Wenzel et al., 2015). For instance, UV-B treatment promoted the methylerytritol phosphate pathway (MEP), increasing the levels of diterpenes as a way to cope with ROS, and stimulated mevalonic acid pathway leading to increased levels of membrane sterols and triterpenes, involved in stress adaptation (Gil et al., 2012).

In the present study, we hypothesise that excessive UV-B radiation may compromise photosynthesis in olive plants and shift metabolic pathways, particularly lipophilic protective metabolites such as fatty acids (e.g. important for membrane stabilization), terpenes and alkanes (e.g. constituents of protective cuticles and with antioxidant roles). For that, a gas chromatography-mass spectrometry (GC–MS) – based lipophilic metabolic profiling combined with physiological studies were conducted in *O. europaea* (cv. 'Galega Vulgar') exposed to different UV-B doses in order to: a) understand olive shifts photosynthetic pathways according to the UV-B doses applied; b) assess the lipophilic-metabolome profiles in response to increased UV-B irradiation; and c) identify putative relevant or new UV-responsive lipophilic metabolites that may be related with defence strategies.

2. Material and methods

2.1. Plant material, culture conditions and UV-B treatment

Six month old potted (2L) *Olea europaea* L. plants (cultivar 'Galega Vulgar') were growth in a climate chamber (T: 25 ± 2 °C, RH 40%, 16 h d/n photoperiod and light provided by Osram cool white fluorescent lamps gave a light intensity of 600 μmol m^{-2} s^{1}). Plants with similar height (45 ± 5 cm) and age were randomly divided in 3 groups (n = 10 per group): Group 1) Control plants: plants not exposed to extra-UV-B irradiation; Group 2) UV-B_1 treatment: plants were exposed for five days to a total of 32.5 kJ m^{-2} biologically effective UV-B dose (6.5 kJ m^{-2} day^{-1}); and Group 3) UV-B_2 treatment: plants were exposed for five days to a total of 62.0 kJ m^{-2} biologically effective UV-B dose (12.4 kJ m^{-2} day^{-1}). To supply the extra UVB radiation, a system with ten UV-B linear tube Hg-lamps (Sankyo Denki G8T5E, 8W, Japan) with an energy spectrum of 280–320 nm and a major emission peak at 306–312 nm was used. The system was prepared with a borosilicate glass filter to ensure that radiation below 290 nm was blocked (UV-C ~0 kJ m^{-2}). UV-B biologically effective doses, 6.5 and 12.4 kJ m^{-2} d^{-1}, were calculated according to Correia et al. (2012). Every day, the homogeneity of the UV-B radiation was measured with a VLX calibrated radiometer (VLX 254 and 312, Vilber Lourmat, Marne-la-Vallée Cedex, France) and the spectral sensitivity and the corresponding correction factor was determined (Correia et al., 2012). The UV-B_1 treatment (moderate treatment) corresponds to a UV-B dose that can be found in several parts of the Mediterranean region (e.g. Correia et al., 2012; Martínez-Lüscher et al., 2015), while the UV-B_2 treatment is approximately the double of the UV-B_1 dose (high treatment), but still within natural values already reported (Forster et al., 2011).

2.2. Plant water status and growth

Plant water status was assessed by determining the relative water content: %RWC = 100 × (fresh weight − dry weight)/(turgid weight − dry weight) of leaf discs of eight plants per treatments (n = 8).

2.3. Cell cycle analysis and genotoxicity

To assess the impact of UV-B on cell cycle dynamics, nuclear suspensions were obtained from chopping leaves in 1 mL of Woody Plant Buffer as described by Loureiro et al. (2007). For ploidy analysis, leaves were chopped together with the same amount of an internal reference standard (*Pisum sativum* cv. 'Ctirad', cv = 9.09 pg DNA; provided by Prof. Dolezel, Institute of Experimental Botany, Olomouc, Czech Republic). After filtering with a 50 μm nylon mesh, propidium iodide (PI, Fluka) and RNAse (Sigma, St. Louis, USA) were added. Nuclei were analysed in a Beckman Coulter EPICS XL (Beckman Coulter, Hialeah, FL, USA) flow cytometer as described by Loureiro et al. (2007). The nuclear DNA content was calculated according to the following formula: