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Responses of epidermal phenolic compounds to light acclimation: In vivo qualitative and quantitative assessment using chlorophyll fluorescence excitation spectra in leaves of three woody species

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Abstract

Chlorophyll fluorescence (ChlF) excitation spectra were measured to assess the UV-sunscreen compounds accumulated in fully expanded leaves of three woody species belonging to different chemotaxons, (i.e. *Morus nigra* L., *Prunus mahaleb* L. and *Lagerstroemia indica* L.), grown in different light microclimates. The logarithm of the ratio of ChlF excitation spectra (logFER) between two leaves acclimated to different light microclimates was used to assess the difference in epidermal absorbance (EAbs). EAbs increased with increasing solar irradiance intercepted for the three species. This epidermal localisation of UV-absorbers was confirmed by the removal of the epidermis. It was possible to simulate EAbs as a linear combination of major phenolic compounds (Phen) identified in leaf methanol extracts by HPLC-DAD. Under UV-free radiation conditions, shaded leaves of *M. nigra* accumulated chlorogenic acid. Hydroxybenzoic acid (HBA) derivatives and hydroxycinnamic acid (HCA) derivatives greatly increased with increasing PAR irradiance under the low UV-B conditions found in the greenhouse. These traits were also observed for the HCA of the two other species. Flavonoid (FLAV) accumulation started under low UV-A irradiance, and became maximal in the adaxial epidermis of sun-exposed leaves outdoors. A decrease in the amount of HCA was observed concomitantly to the intense accumulation of FLAV for both leaf sides of the three species. Judging from the logFER, under low UV-B conditions, larger amounts of HCA are present in the epidermis in comparison to FLAV for the three species. Upon transition from the greenhouse to full sunlight outdoors, there was a decrease in leaf-soluble HCA that paralleled FLAV accumulation in reaction to increasing solar UV-B radiation in the three species. In *M. nigra*, that contains large amounts of HCA, the logFER analysis showed that this decrease occurred in the adaxial epidermis, whereas the abaxial epidermis, which is protected from direct UV-B radiation, continued to accumulate large amounts of HCA.

Keywords: Biospectroscopy; Chlorophyll fluorescence excitation spectra; Leaf polyphenols; Shading effect; Light acclimation; UV-absorbers

1. Introduction

Numerous horticultural, dicotyledonous species have exuberant vegetative growth under greenhouse conditions. They have thinner but larger leaves. They are mechanically less rigid and are more fragile than plants grown under field

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conditions. This enhanced primary plant growth under greenhouse conditions might be due to the lack of UV-B radiation needed for the induction of flavonols in the leaf epidermis [1]. Jansen et al. [2] showed that UV-B-induced flavonols in the leaf epidermis act as competitive inhibitors of efflux carriers of indol-acetic acid. Furthermore, plants growing under greenhouse conditions are known to be more sensitive to biotic and abiotic stress. Here again, the lack of flavonols is involved. The bactericidal and fungicidal properties of these phenolic compounds (Phen), or their oxidised products, contribute to increased leaf resistance against pathogens [3]. The proportion of different phenolic compounds, flavonoids (FLAV), hydroxycinnamic acids (HBA) and tannins, in leaves is known to be under the control of other environmental factors, including pathogen attack [4], and low temperatures [5]. Flavonol accumulation in leaves has also been found to increase significantly in response to nitrogen starvation [6]. It has been seen that nitrogen fertilisation can be employed to manipulate the flavonol content of vegetative tissues in tomato [6]. So, a better understanding of the environmental control of flavonol accumulation will be useful to improve horticultural practice and to reduce phytochemical input for greenhouse production.

FLAV synthesis was found to be proportional to the UV-B dose received, even in cell culture [7]. In addition, numerous experiments confirmed increasing leaf Phen content with increasing intercepted UV-B doses [8–10]. The more exposed adaxial side of bifacial leaves accumulates larger amounts of FLAV than the abaxial side [11]. In a wide variety of dicotyledonous species, quercetin and kaempferol derivatives are known to be mainly glycosylated and located in epidermal vacuoles where they absorb UV light (*Vitis vinifera* cv; [12] *Arabidopsis thaliana* Heyn, *Beta vulgaris* L., *Nicotiana tabacum* L., *Pisum sativum* L., *Phaseolus vulgaris* L., *Spinacia oleracea* L. [13]). In addition to this epidermal UV-screening by FLAV, the relative contribution of hydroxycinnamic acids (HCA) was a matter of recent debate [14]. Caldwell et al. originally considered FLAV to be the only, or the main, screening pigment [15]. Accordingly, Markstädler et al. [16] found that HCA contribute minimally to UV-B screening in leaves of *Vicia faba* L. Yet, HCA accumulation partly replaces FLAV in *Arabidopsis* mutants that are defective in the first step specific to FLAV biosynthesis, when it is exposed to UV-B [17]. Sheahan [14] and Kolb et al. [12] further suggested that FLAV played only a minor role in *Arabidopsis* UV-screening and that HCA derivatives (sinapate esters) are the main contributors to epidermal screening. Burchard et al. [18] highlighted that HCA are the dominant UV-B protective compounds in the early stages of primary rye leaves development (*Secale cereale* L.), followed by FLAV in later stages and in reaction to UV exposure. Depending on the species, HCA are either largely unaffected by light microclimate [18] or increase with irradiance [12]. In *Phillyrea latifolia* L., full-sun exposed leaves accumulate FLAV in the epidermis, subepidermal layers and trichomes, whereas less-exposed leaves accumulate HCA in these tissues [19]. Some HBA, such as gallic acid, decrease whereas FLAV content increases under higher UV-B radiation levels [20]. A light-induced decrease in the HCA/FLAV ratio was observed by other authors [21]. If the epidermal FLAV metabolism is markedly stimulated by light, epidermal HCA esters and mesophyll FLAV are less responsive, and seem to be under endogenous control of leaf development and differentiation [22]. However, some plant species specifically accumulate HCA in high-light irradiance conditions, and some other species in situation of oxidative stress (i.e. chlorogenic acid in *Mahonia repens* (Lindl.) G. Don [23]; echinacoside in *Ligustrum vulgare* L. [21,24]; gallotannins [25]).

Knowledge of the distribution of different Phen in plant tissue could help in an improved assessment of their physiological role. Peeling off the epidermis has often been used for that purpose (e.g. [11]). Still, this technique is not only destructive but also inapplicable to many woody species. More recently, a spectral non-destructive method was introduced, based on the screening of chlorophyll fluorescence (ChlF) that reveals the components present in the epidermis [13]. By comparing whole leaf ChlF excitation spectra, acquired from 230 to 650 nm on two different leaves (or leaf sides), the type and amount of Phen that differ in their epidermis can be obtained [13]. This technique allows us to compare UV-sunscreening efficiency of leaves acclimated to different microclimates and to investigate the changes in the FLAV/HCA ratio.

The aims of the experiments presented here were: (i) to characterise and to quantify *in vivo* the leaf epidermal Phen of three weedy species during acclimation to different light microclimates; (ii) to discriminate between HCA and FLAV induction during light acclimation; (iii) to compare three species from different chemotaxons known to have different constitutive phenolic compounds; (iv) to assess the local response of FLAV and HCA to UV-radiation by comparing the changes on the two sides of the leaf.

2. Method and materials

2.1. Plant material and experimental design

Three deciduous woody species that accumulate different groups of Phen in their leaves were selected from the pioneering work of Bate-Smith [26]. The leaves of *Morus nigra* L. (*Moraceae*) are thought to accumulate mainly HCA, such as chlorogenic acid, but are is poor in flavonols and anthocyanins. The leaves of *Prunus mahaleb* L. (*Rosaceae*, *Prunoideae*) mainly accumulate quercetin and kaempferol derivatives, two flavonols. The leaves of *Lagerstroemia indica* L. 'Red Imperator' (*Lythraceae*) are described to be poor in both FLAV and HCA. The leaves of these three species are simple and their colour uniform.

The plants were grown in Angers, France (lat. 47°30′N, long. 0.35°W, alt. 56 m) in a glass greenhouse in which shading was installed, and outdoors in a plant nursery near