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RESPONSE OF EPIDERMAL BLUE-GREEN FLUORESCENCE EMISSION FROM BARLEY LEAVES TO UV RADIATION STRESS

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INTRODUCTION

Blue-green fluorescence (BGF) is emitted by higher plant pigments when excited by UV radiation. The emission spectrum of BGF varies among plant species and depends on the physiological status of the plants (Chapelle 1984). BGF has been attributed particularly to cell-wall-bound ferulic acid, although it has been suggested that flavonoids and other simple phenols can contribute to BGF (Morales 1994). The BGF signal is of interest due to its potential application in the remote sensing of plant stress. Remote sensing application techniques use ratios of BGF to chlorophyll red and far red fluorescence emission intensities to estimate plant stress (Lichtenthaler 1996). In this work we have focused on characterization of BGF response of barley leaves to different levels of photosynthetically active radiation combined with UV-A and UV-B radiation. The aim of this work is to distinguish individual fluorescent components of BGF emission. Based on different response of these components to UV-radiation we suggest fluorescence ratios appropriate for monitoring of UV plant stress.

MATERIALS AND METHODS

Plant material and acclimation conditions

Barley plants (*Hordeum vulgare* L. cv. Bonus) were grown from seeds inside growth chambers (HB1014, VB1014, Vötsch, Germany) under low or high photosynthetically active radiation (PAR; 50 or 1000 µmol m^{-2} s^{-1} – LI or HI, 16/8 h day/night cycle), 20 °C and 65% relative air humidity in the absence or presence of UV-A or UV-B radiation. The intensities of UV radiation are given in figure 1 (for 16 h per day, together with PAR illumination). After 8 days of development, plants were transferred for the next 6 days simultaneously to different light conditions (Figure 1). UV-A lamps emitting in the 350-400 nm range (Actinic BL TL-D 18W, Philips) and UV-B broadband lamps with a peak at 302 nm (TL 20W/12 RS SLV, Philips) were used. The middle segments of primary leaves of 8-day-old UVA- and UVA+ plants and of plants after 1, 3 and 6 days of UVAB and UVB exposure were used for the measurements. Sampling of leaves was done after at least 2 h-dark adaptation.

Spectral Fluorescence Procedure

We measured the fluorescence emission and excitation spectra of the adaxial surfaces of a leaves excited at 340 nm and detected at 440 and 520 nm, resp. Spectra were recorded at room temperature using a luminescence spectrofluorimeter LS50B (Perkin-Elmer, UK). We used two filters to reduce stray radiation in the spectrofluorometer. A UV-transmitting filter (UG 11, Omega Optical, Brattleboro, VT, USA; sharp cut-off above 380 nm) was placed between the excitation monochromator and the sample, while a UV-cut-off filter (GG395, Omega Optical, Brattleboro, VT, USA; sharp cut-off below 395 nm) was placed between the sample and the emission monochromator. Excitation and emission bandwidth were 5 nm. Emission spectra were corrected for the spectral sensitivity of the detection system. For the causal analysis of spectra of blue-green fluorescence in leaves, standards of 26 phenolic compounds were measured in methanol at a concentration of 0.02 mg·ml^{-1}.

The HPLC-DAD-MS qualitative and quantitative analysis of soluble phenoliccompounds was carried out on HPLC-micrOTOFQ-II system (supplier Brukers.r.o, CZ), equipped with Hypersil Gold (Thermo Scientific, USA), 50 x 2.1mm, 1.9 µm chromatographic column.

RESULTS AND DISCUSSION

Linear correlation between F435/F520 and F435/F455 fluorescence ratios indicated (expect extremely strong UVB stress, Fig 1Dd) that spectral changes of BGF emission spectra are caused by gradual decline of F435 in comparison with F455 and F520 (Fig 1Da-c). All control plants exhibit gradual decrease of F435/F520 and also F435/F455 with age. In addition, we have found that fluorescence ratio F435/F520 is much better indicator of UV stress than F455/F520, which shows no changes (except in plants exposed to extreme UVB).

In order to reveal causes of these spectral changes we performed more detailed analyses of BGF spectra of barley intact leaves and standards of phenolic compounds, together with their quantitative analysis by HPLC-DAD-MS. One of the possible reasons for the observed spectral changes could be oxidative coupling of two ester-linked ferulic acid monomers related to cell wall cross-linking (Bunzel 2010).

Excitation spectra of BGF from intact leaves detected at 440 and 520 nm showed a more different shape at 77 K (Fig 2B) in comparison with room temperature spectra (Fig 2A). Emission spectra of BGF at wavelength of excitation between 340 – 500 nm showed decreased fluorescence intensity and red shift similarly as in the case of extreme UVB stress (Fig 2C). Therefore, the spectral changes of BGF observed after strong UV exposure are probably caused by a decrease in the main BGF component at 435 nm.Fluorescence analysis of 26 phenolic compound standards revealed that flavones (Apigenin, Chrysin, Fluorescence analysis of 26 phenolic compound standards revealed that flavones (Apigenin, Chrysin, Homoorientin, Isovitexin, Luteolin, Saponarin) and flavon-3-ols (Galangin, Kaempferol, Myricetin, Quercetin, Taxifolin) are non-fluorescent. Catechin showed emission maxima in the spectral range of 300 – 310 nm, hydroxybenzoic acids of 330 – 360 nm, hydroxycinnamic acids of 380 – 400 nm and stilben at 375 nm (Fig 3). In addition, because absorption and fluorescence excitation spectra of hydroxycinnamic acids did not overlap fully, several spectral forms are probably contained in each acid (Fig 3). Nevertheless, only six potentially fluorescing derivates of ferulic and sinapic acids were identified in extracts of soluble phenolic compounds of barley leaves.

CONCLUSIONS

The most pronounced spectral changes of BGF emission spectra occur after UVB exposure, although during UVA, and even high PAR irradiation the BGF spectral changes are well detectable. Moreover dynamics of the changes in BGF is accelerated with increasing intensity of UVB radiation and changes can be observed even after the removal of stress. Correlation between F435/F520 and F435/F455 fluorescence ratios clearly showed that spectral changes of BGF emission spectra are caused by gradual decline of F435 in comparison with F455 and F520. Therefore, the fluorescence ratio F435/F520(F455) is suitable for monitoring of UV plant stress. HPLC-DAD-MS analysis suggests that BGF emission could be composed of fluorescence of ferulic and sinapic acids derivates.

Detected phenolic compounds	Peak area observed in HI samples (n=6)	Peak area observed in LI samples (n=6)	EIC peak area ratios HI/LI [rel.]	Significance of differences between HI x LI
Isoorientin-7-O-[6-fer]-glc	86897	6952	12,50	YES ***
Isoorientin-7-O-[6-sinp]-glc	120571	10471	11,51	YES ***
Isoscoparin-7-O-[6-sinp]-glc	23715	4187	5,66	YES ***
Isovitexin-7-O-[6-sinp]-glc	336075	62428	5,38	YES ***
Feruloylquinic acid	200113	40552	4,93	YES **
Isovitexin-7-O-[6-fer]-glc	166319	67320	2,47	YES ***

*; **; ***: comparison of mean values using t-test – p-value 0,05, 0,01 resp. 0,001

Tab.1: *Selected phenolic compounds that potentially contribute to the BGF, detected by HPLC-DAD-MS-(MS/MS) in extracts of soluble phenolics from barley leaves grown under high and low PAR levels.*

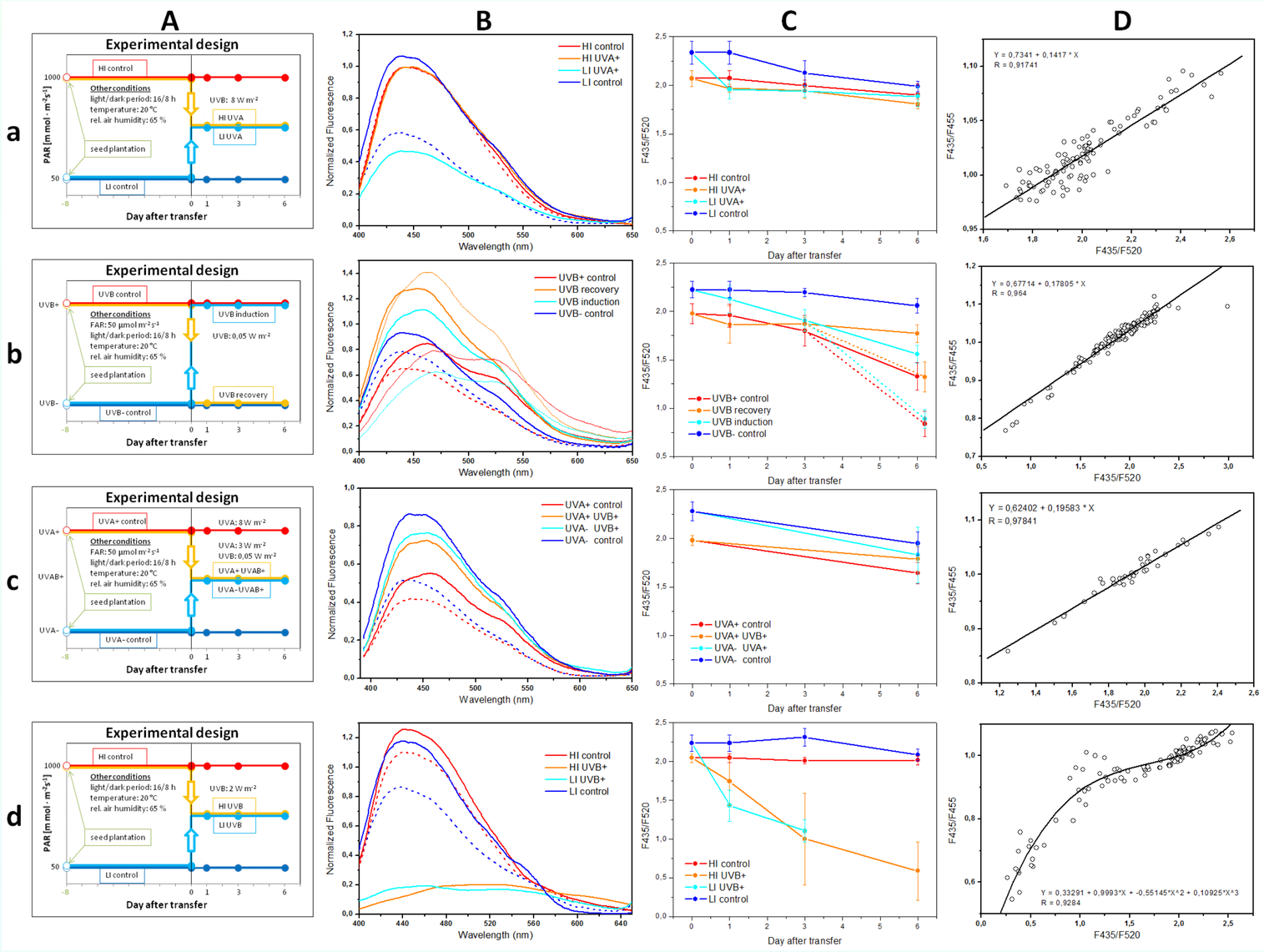

Fig.1: (A) Schemes of four different experiments with various lighting conditions. (B) Room temperature BGF fluorescence emission spectra of intact leaves from barley leaves grown under different light condition described in first column before transfer (0, dash line) and sixth day after transfer (6, solid line). Wavelength of excitation was at 340 nm, spectra were normalized at red chlorophyll maxima (for HI plants multiplied by 10 for better view). Means of 12 leaves are presented, thin solid line shows significantly different leaves (20%). (C) Dynamics of fluorescence ratio F435/F520 after the change of light conditions, n = 12. (D) The relationship between two fluorescence bands at 455 nm (F455) and 520 nm (F520).

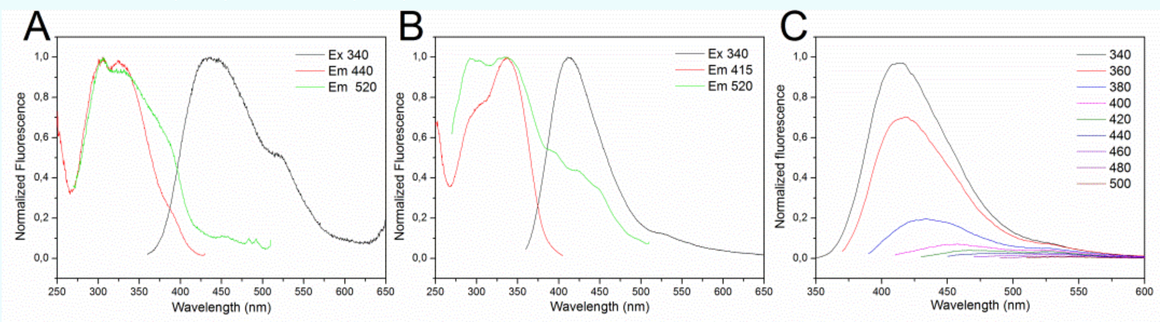

Fig.2: Room temperature (A) and 77 K (B,C) BGF fluorescence emission and excitation spectra of intact leaves from barley leaves grown under low light conditions. Wavelengths of fluorescence excitation and detection are denoted in legend.

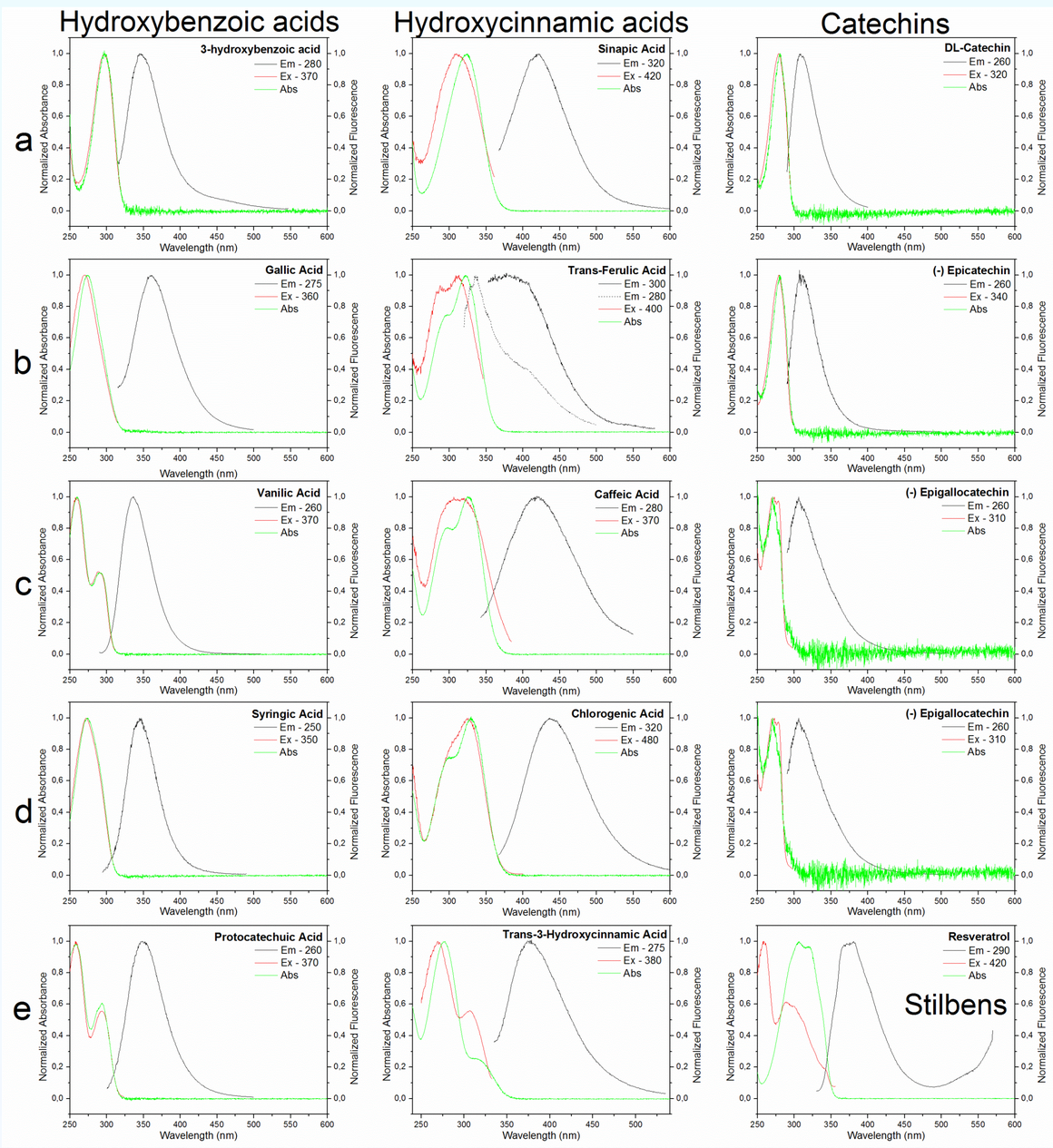

Fig.3: Examples of normalized absorption (green line) and fluorescence excitation (red line) and emission (black line) spectra of phenolic compounds standard in methanol. Wavelength of fluorescence excitation and detection are denoted in legend. Main absorbance maxima of catechins are below 250 nm up to 40 times higher than absorbance around 300 nm.

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Acknowledgements

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