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UV-B-induced compounds as affected by proline and NaCl in *Hordeum vulgare* L. cv. Alfa

Ivanka Fedina[a,*], Maya Velitchkova[b], Katya Georgieva[a], Irena Grigorova[a]

[a] *Academic Metodi Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Academic Georgi Bonchev Street, Building 21, Sofia 1113, Bulgaria*
[b] *Institute of Biophysics, Bulgarian Academy of Sciences, Georgi Bonchev Street, Building 21, Sofia 1113, Bulgaria*

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Abstract

From the leaves of barley seedlings (*Hordeum vulgare* L. cv. Alfa) UV-B induced compounds, with maximum absorbance at 438 nm (A_{438}) were extracted. The relationship between the level of UV-B induced compounds and UV-B tolerance of barley seedlings was investigated. The level of these compounds depended on the time of UV-B irradiation. They increased 4 h after UV-B treatment, reached maximum after 24 h and then declined. Contrary, the syntheses of UV-absorbing compounds extracted in acidified methanol continued for a long period after UV exposure and after 120 h the values of A_{300} are higher. The content of UV-induced compounds enhanced in the plants treated with proline before UV-B irradiation and decreased as a result of NaCl pretreatment in a concentration depending manner. A physiological response to UV-B irradiation was evaluated by measuring the oxygen evolution rate, chlorophyll fluorescence and chlorophyll/carotenoids ratio. No correlation was found between the level of A_{438} and UV-B tolerance of barley seedlings. It is possible these compounds to play a subtle role in plant UV-B protection than simple UV-B screening or to serve as stress markers.
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Keywords: Chlorophyll fluorescence; Flavonoids; *Hordeum vulgare*; Oxygen evolution; UV-B; UV-B absorbing compounds

1. Introduction

Enhanced UV-B can affect lipids, nucleic acids, and proteins in leaves of higher plants, and specifically targets the photosynthesis. Photosynthetic damage is associated with stomatal behaviour, photosynthetic enzymes and pigments (Teramura and Sullivan, 1994; Tevini, 1994), electron transport chain (Tevini et al., 1991), as well as disruption of the chloroplast membrane (Bornman, 1989). To cope with UV-B radiation

Abbreviations: AOS, active oxygen species; Chl, chlorophyll; F_0, chlorophyll fluorescence of dark-adapted state; F_m, maximal fluorescence of PSII in dark-adapted state; F_v, variable chlorophyll fluorescence; qN, non-photochemical fluorescence quenching; qP, photochemical quenching; PPFD, photosynthetic photon flux density; TCA, trichloracetic acid

* Corresponding author. Tel.: +359 2 9792620; fax: +359 2 73 99 52.
E-mail address: fedina@obzor.bio21.bas.bg (I. Fedina).

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damage, plants evolved a variety of mechanisms. Protective responses are also stimulated by UV-B radiation, including increased production of UV-B-absorbing compounds. UV-absorbing compounds are mainly phenylpropanoids such as cinnamoyl esters, flavones, flavonols, and anthocyanins esterrified with cinnamic acids. These compounds are likely to afford protection to the plants by absorbing in the UV-B region of the spectrum and thereby diminishing the penetration of UV radiation. In addition, they may offer additional protection by having antioxidant activity (Brown et al., 1998). In plants, the protective role of flavonoid polyphenolics in the expression of tolerance to UV-B radiation has been shown repeatedly (Li et al., 1993; Lavola et al., 2003). It has been reported in many studies that flavonoids and related phenolic compounds specifically increased when plants are exposed to enhanced UV-B radiation (Beggs and Wellmann, 1994; Wagner et al., 2003) and were linearly-dependent on UV-B fluence (Cen and Bornman, 1993). Kolb et al. (2001) observed that PSII is protected against UV-B damage by epidermal screening, related to increased leaf phenolics (A_{314} and A_{360}), however, UV-B inhibition of CO_2 assimilation rates was not diminished. According to Middleton and Teramura (1993), although both UV-B absorbing compounds and carotenoids increased in response to UV-B irradiation, only carotenoids and not the UV-B absorbing compound (A_{300}) could be related to protection of photosynthesis. We observed that UV-B exposure induced synthesis of yellow colored compounds, extracted in 0.1% trichloracetic acid, with maximum absorbance at 438 and suggested that it should improve epidermal UV screening and should protect the leaf from UV-B damage (Fedina et al., 2003a,b). Although, some laboratory experiments have demonstrated that the concentration of phenolic compounds increases with increasing irradiance (Cen and Bornman, 1990; Day, 1993), the experimental data regarding the protective role of these compounds against UV-B radiation are still few and speculative.

Recently, we showed that NaCl pre-exposure decreased H_2O_2 generation and lipid peroxidation and alleviated the inhibitory effect of UV-B on PSII activity. It was suggested that the proline accumulated under salt stress conditions might be one of the reasons for the observed tolerance of barley seedlings to UV-B radiation (Fedina et al., 2003a,b).

In this work the relationship between the level of UV-B induced compounds and UV-B tolerance of barley seedlings as affected by pretreatment with NaCl and proline was investigated. Chlorophyll fluorescence and oxygen evolution (as a tool for securing, rapid, non-destructive detection of stress effects in intact leaves) was measured for evaluating the seedlings response to irradiation.

2. Materials and methods

2.1. Plant growth and treatment

Seeds of *Hordeum vulgare* L. cv. Alfa germinated for 2 days at 25 °C. Seedlings were grown as a water culture. The seedlings were grown under 12 h photoperiod, relative humidity 60% and day/night temperatures 25/20 °C. Three days old plants were supplied with 10^{-6}, 10^{-5} and 5×10^{-5} M proline or 100, 150 and 200 mM NaCl and after 4 days, the whole plants were irradiated with UV-B light. The irradiated seedlings were returned back to growth chamber under above described light regime. As a source of UV-B irradiation a mercury lamp was used with a characteristic emission in the range 280–320 nm (type HPQ 125W; N.V. Phillips Gloeilampenfabriken, Einhoven), 64.4 μmol m^{-2} s^{-1} (0.5×10^{-4} W cm^{-2}), where the UV-B irradiation is about 80% of the total emission. The distance between the lamp and plants was 25 cm. To cut off the radiation below 280 nm a cellulose acetate filter (0.13 mm) was used and plants received 49 kJ m^{-2} d^{-1} biologically effective UV-B radiation. Chlorophyll fluorescence, oxygen evolution, UV-B induced and UV-B absorbing compounds were measured at 4, 24, 48 and 120 h after UV-B irradiation, as described below.

The data presented are means of three different experiments, each including at least three replications. Experimental data were analyzed with the Student's *t*-test.

2.2. Chlorophyll fluorescence

Chlorophyll fluorescence induction of leaf disks was measured with a pulse amplitude modulation fluorometer (PAM 101–103, H. Walz, Germany) as described by Schreiber et al. (1986). The initial fluorescence yield in weak modulated light (0.075 μmol m^{-2} s^{-1}

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