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UV-B radiation modifies the acclimation processes to drought or cadmium in wheat

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ABSTRACT

Under natural conditions plants are often subjected to multiple stress factors. The main aim of the present work was to reveal how UV-B radiation affects acclimation to other abiotic stressors. Wheat seedlings grown under normal light conditions or normal light supplemented with UV-B radiation were exposed to drought or Cd stress and were screened for changes in the contents of salicylic acid and its putative precursor ortho-hydroxy-cinnamic acid, and in the activity of the key synthesis enzyme, phenylalanine ammonia lyase. Certain other protective mechanisms, such as antioxidant enzyme activities and polyamines, were also investigated. PEG treatment under UV-B radiation did not cause wilting, but resulted in more pronounced salicylic acid accumulation, which may provide protection against drought stress in wheat plants. In contrast, the high level of salicylic acid accumulation in Cd-treated plants was not further enhanced by UV-B stress, but resulted in pronounced oxidative stress and the activation of antioxidant systems and polyamine synthesis. Changes in the levels of phenolic compounds are accompanied by increased phenylalanine ammonia lyase activity in the roots, but not in the leaves. The similar pattern observed for stress-induced changes in salicylic acid and ortho-hydroxy-cinnamic acid contents suggested that salicylic acid may play a decisive role via ortho-hydroxy-cinnamic acid. The results indicated that UV-B radiation might have either a positive or negative impact under the same conditions in wheat, depending on the type of secondary abiotic stress factor. The protective or damaging effects observed may be related to changes in the levels of phenolic compounds.

1. Introduction

Plants are exposed to many environmental stresses, which are further aggravated by the effects of global climate change. In response to abiotic stress various biochemical and physiological changes are induced in plants leading to the ability of plants to cope with stress. The simultaneous exposure of plant to different abiotic stress conditions may results in the coactivation of different stress response pathways, which might have a synergistic or antagonistic effect on each other (Fraire-Vela′zquez et al., 2011; Peleg and Blumwald, 2011). Furthermore, when the plant exposed firstly to a single stress agent, is capable to increasing its resistance to a subsequent stress factor, leading to the so called cross-acclimation (Çakirlar et al., 2011).

Water deficit is a multidimensional stress affecting plants at various levels of their organization, not only manifested at the morphological level but also at the physiological level [e.g. decrease in water potential and photosynthetic rate (Ashraf and Harris, 2013)] and at the biochemical and molecular level (as formation of radical scavenging compounds, accumulation of compatible organic solutes, changes in endogenous phytohormone contents and lipid composition) (Yordanov et al., 2000; Aimar et al., 2011).

The effects of heavy metals on plant species have been well studied (Pál et al., 2006a; Yadav, 2010), as a quite complex phenomenon can evoke several parallel and consecutive changes and events. In responses to oxidative stress caused by Cd, the members of the antioxidant defence mechanisms are influenced (such as antioxidant enzymes, proline and polyamines) (Lin et al., 2007; Hegedűs et al., 2001; Ekmekci et al., 2008; Pál et al., 2006b).

Salicylic acid (SA), an endogenous plant growth regulator, participates in many physiological and metabolic reactions (Yusuf et al., 2013; Janda et al., 2012). Endogenous SA level rised in several species when they are exposed to stress conditions. For example, the endogenous SA content exhibited a concentration-dependent increase in maize plants treated with Cd (Pál et al.,

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; $\Delta F/F_m'$, actual quantum efficiency of photosystem II; GR, glutathione reductase; GST, glutathione-*S*-transferase; G-POD, guaiacol peroxidase; MDA, malondialdehyde; *o*HCA, *ortho*-hydroxy-cinnamic acid; PAL, phenylalanine ammonia lyase; PAs, polyamines; PUT, putrescine; SA, salicylic acid; SPD, spermidine; SPN, spermine.

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2005); or the endogenous SA accumulation during drought stress has also been demonstrated on several occasions (Munné-Bosch and Penuelas, 2003; Bandurska and Stroinski, 2005; Abreu and Munné-Bosch, 2008; Aimar et al., 2011). It was found that exogenous SA enhances the chilling tolerance of various plant species and this enhanced tolerance is accompanied by the increased activity of certain antioxidant enzymes (Horváth et al., 2007), furthermore endogenous SA was reported to protect rice plants from oxidative damage caused by aging as well as biotic and abiotic stress by modulating the redox balance (Yang et al., 2004). Exogenous SA also affects the polyamine metabolism in various plants (Németh et al., 2002; Szepesi et al., 2011); and it was found that the different concentrations of SA had different effects on polyamine metabolism (Wang and Zhang, 2011). It was suggested that relationship exists between endogenous SA and PAs contents and antioxidant activities (Pál et al., 2013); and even between these protective compounds and the sensitivity or resistance of plants to various biotic stresses (Liu et al., 2007; Talieva and Kondrat'eva, 2002). However, it is still not clear how endogenous SA and PAs contents influence each other.

UV radiation is traditionally divided into UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm) wavelength ranges, which have increasing levels of energy and harmful effects. The ozone layer depletion and increase of the ozone hole increase the UV-B radiation, which could change the adaptive mechanisms of plants to known stressor factors. Although the effects UV-B radiation exhibit variations in the higher plant species, there are three potential targets of UV-B radiation in plant cells, namely the genetic system (Agrawal et al., 2009), the photosynthetic system (Majer and Hideg, 2012) and membrane lipids (An et al., 2000). At lower doses UV stress induces changes in morphology, gene expression and plant metabolism, mainly through the stimulation of the antioxidant system leading to acclimation (Kakani et al., 2003).

As under natural conditions plants are often subjected to multiple stress factor, the impact of a particular stress can be elevated by a simultaneous action of other stress. The main aims of the present study were to reveal (1) how UV-B treatment can affect alone and in combination with drought or Cd the synthesis of SA, a signalling molecule playing role in the acclimation processes to various types of stressors, and (2) what relationship may be exist between the changes in the SA content and the alteration, caused by UV-B radiation in the effect of Cd or drought stress on some physiological parameters and certain stress responses, which can be important in defence mechanism in wheat plants. In order to achieve our goals wheat seedlings grown under normal light condition or normal light supplemented with UV-B radiation at the same time were screened for changes in SA content, with the amount of the putative precursor *ortho*-hydroxy-cinnamic acid (*o*HCA) and the activity of the key enzyme of the phenyl-propanoid pathway, phenylalanine ammonia lyase (PAL), certain other protective compounds, antioxidant enzyme activities, contents of proline and polyamines were also investigated.

2. Materials and methods

2.1. Plant material and growth conditions

Winter wheat (*Triticum aestivum* L. Mv Emese) variety from MTA ATK, Martonvásár, Hungary was used for the experiments. Seeds were germinated for 3 days at 22 °C, then seedlings were grown on modified Hoagland solution (Pál et al., 2005) for 2 weeks at 20/18 °C with 16-h light:8-h dark periodicity in a Conviron G-48 plant growth chamber (Controlled Environments Ltd., Winnipeg, Canada) in the phytotron of the Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary at the photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1}. Half of the plants were grown at normal light (CL) and the others grown at normal light combined with UV-B radiation (UV) which was performed by 7 UV-B Narrowband TL 100 W/01 lamps from Philips (with maximal radiation at 311 nm). Dose of UV-B radiation was 38 μW cm^{-2} in the case of control plants, while 430 μW cm^{-2} in the case of UV-B-treated plants. At two-week-old stage seedlings were divided into three groups under both light condition. First part of plants was the control (C), second part of the plants was treated with 50 μM $Cd(NO_3)_2$ for 7 days (Cd) and the third part of the plants was treated with 15% polyethylene glycol (PEG-6000) for 5 days (PEG). The 1st, 2nd and 3rd leaves and roots of control, Cd-treated and PEG-treated plants were sampled at the end of the experiment. The concentrations and duration of Cd and PEG treatments were determined based on the results of phenotypic test from preliminary experiments.

2.2. Leaf rolling score

Leaf rolling is often interpreted as a strong manifestation of leaf response to water deficit. Plants showing leaf rolling at early stage of stress appears to have poor drought tolerance. Leaf rolling is scored on a scale from 1 to 5, where 1 is slightly rolled and 5 is tightly rolled (O'Toole and Cruz, 1980). Visual leaf rollings score were performed on the 5th days of PEG treatment.

2.3. Determination of chlorophyll content

The total chlorophyll content was measured on the third leaves using a SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). The SPAD-502 meter measures the transmittance of red (650 nm) and infrared (940 nm) radiation through the leaf, and calculates a relative SPAD meter value.

2.4. Chlorophyll fluorescence induction measurement

The quantum yield of photosystem II (PSII) indicated by the $\Delta F/F'_m[(F'_m - F_s)/F'_m]$ chlorophyll fluorescence induction parameter where F'_m and F_s represent the maximum and steady-state chlorophyll fluorescence levels in the light-adapted state, respectively) was measured on fully expanded leaves using a pulse amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) as described by Janda et al. (1994). In order to compare the values the measuring conditions was the same. During the fluorescence measurement the PPFD was 250 μmol m^{-2} s^{-1} as during the plant growth, and the UV-B Narrowband TL 100 W/01 lamps were switched off.

2.5. Determination of proline content

0.5 g of the samples was homogenized in a mortar with quartz sand and 4 ml distilled water. The mortar was rinsed with another dose of 4 ml distilled water. Centrifugation was carried out at 10,000 × g for 10 min. The supernatant was made up to 10 ml with distilled water and 2.5 ml of this dilution was used for proline quantification according to Bates et al. (1973). The proline content was expressed as $\mu g\,g^{-1}$ fresh plant weight (FW), was derived from a standard curve obtained by diluting 250 $\mu g\,ml^{-1}$ L-proline stock solution (Reanal, Budapest, Hungary) to concentrations of 0.5, 0.75, 1.0, 1.25 and 1.5 $\mu g\,ml^{-1}$.

2.6. Estimation of lipid peroxidation

The lipid peroxidation analysis was based on malondialdehyde (MDA) levels. 0.2 g of tissue was ground in 600 μl 0.1% (w/v) TCA, then centrifuged at 12,000 × *g* for 10 min. 300 μl of the supernatant