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Research Article

BENEFICIAL EFFECTS OF AUXIN-LIKE COMPOUNDS ON PEA PLANTS IRRADIATED WITH UV-C

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Summary: The biochemical responses of garden pea plants to pretreatment with the auxin physiological analogues 1-[2-chloroethoxycarbonylmethyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxicarbonylmethyl]naphthalene chlormethylate (TA-14) and subsequent irradiation with UV-C were studied. The aim of the present investigation was to assess if foliar application of these auxin-like compounds was able to decrease the negative effects caused by UV-C irradiation. UV-C treatment increased the content of malondialdehyde, free proline, total soluble phenolics and low-molecular thiol as well as the superoxide dismutase, catalase and guaiacol peroxidase activities but decreased hydrogen peroxide (H_2O_2) and total soluble protein levels. The pretreatment with TA compounds decreased the oxidative stress provoked by UV-C radiation, recovered total soluble protein and H_2O_2 content, increased UV-absorbing compounds and low-molecular thiols earlier than in irradiated plants and had a favourable effect on enzymatic activities. Exogenous application of auxin-like compounds on pea plantlets could be interpreted in relation to their ability to counteract UV-C induced oxidative stress by increasing the content of non-enzymatic antioxidants and stabilising the antioxidant enzyme activities required for detoxification of reactive oxygen species (ROS).

Keywords: Antioxidants; auxin-like compounds; defense enzymes; pea; stress markers; UV-C stress.

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INTRODUCTION

According to the International Commission on Illumination, Ultraviolet (UV) wavelength (400 – 100 nm) is a small part of the solar radiation reaching the Earth's surface, divided into UV-A (315 – 400 nm), UV-B (280 – 315 nm) and UV-C (100 – 280 nm), and affecting negatively all living organisms. The negative effect of UV radiation increases towards the shorter wavelengths. UV-C quickly provokes high levels of injuries because of its highest energy. It causes

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overproduction of ROS and development of oxidative stress, that acts destructively on macromolecules; and affects negatively growth, development, photosynthesis, and other important processes in plants, may decrease cell viability and lead to cell death (Barnes et al., 2015). In addition, UV-C may trigger plant responses, including alteration in non-enzymatic and enzymatic antioxidant complexes (Gill and Tuteja, 2010, Gonzalez-Aguilar et al., 2010), which is controlled by phytohormone homeostasis.

Auxins control cell division and enlargement and contribute to the organogenesis of roots, leaves, buds and flowers. Under stress conditions, including UV, auxins play a role in the adaptive responses of plants (Kazan, 2013; Vanhaelewyn et al., 2016).

The priming of plants with chemicals including synthetic hormones and their physiological analogues play an important role in the regulation of plant physiological reactions under normal or stress conditions. A number of articles evidenced that different plant growth-regulating substances can modulate plant responses and increase plant tolerance to UV stress (Liu and Zhong, 2009; Hasanuzzaman et al., 2010; Katerova et al., 2012; Zhang and Li, 2012; Todorova et al., 2013; Todorova et al., 2014; Katerova et al., 2014; Esringu et al., 2016; Katerova et al., 2016, Aksakal et al., 2017). For example, exogenous application of the synthetic auxin α-naphtyl acetic acid enhanced plant tolerance by increasing endogenous auxin levels (reviewed by Llanes et al., 2016). Exogenous application of the auxin physiological analogues 1-[2-chloroethoxycarbonyl -methyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethyl-aminoethoxicarbonylmethyl]naphthalene chlormethylate (TA-14) improved cold acclimation and overwintering of oilseed rape (Velička et al., 2005; Anisimoviene et al., 2008; Gaveliene et al., 2013). To our knowledge, these compounds have not been assessed against other types of abiotic stress.

In this study for the first time we evaluated the ability of the auxin physiological analogues TA-12 and TA-14 to attenuate the negative consequences of UV-C stress in pea (*Pisum sativum* L.) plants.

MATERIALS AND METHODS

Plant material and treatments

Young pea plants (*Pisum sativum* L., cv. Ran-1) were grown as a water culture under controlled growth conditions (12/12 h photoperiod, 150 μmol m^{-2} s^{-1} photon flux density, 24/22°C day/night temperatures; 80% relative humidity). Thirteen days old pea seedlings were sprayed until leaves become completely wet with 1 mM solutions of auxin physiological analogues 1-[2-chloroethoxycarbonylmethyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylamino-ethoxicarbonylmethyl]naphthalene chlor-methylate (TA-14). Twenty four hours later plants were irradiated with UV-C for 30 min (9 kJ m^{-2}). Germicidal lamp (STYLO STY 115, GE Lighting, Italy, λmax 254 nm) was used to supply the UV-C radiation, which provided 0.50 mJ cm^{-2} s^{-1} UV-C, 0.010 mJ cm^{-2} s^{-1} UV-A and 0.04 mJ cm^{-2} s^{-1} photosynthetically active radiation. The spectra and radiation power of the UV-C lamp were determined with AvaSpec-2048 spectrometer (Avantes,

The Netherlands). The distance between the UV-C lamp and the top leaves of treated plants was 0.25 ± 0.04 m.

After the end of the stress treatment, the plants were transferred back under controlled growth conditions. Physiological responses of plants were determined on the 3rd leaf pair on 0, 24 h and 48 h after the end of UV-C irradiation.

Biochemical analyses

Selected parameters such as content of free proline, malondialdehyde, total phenols, thiol-containing compounds, hydrogen peroxide, total soluble protein and activities of catalase, guaiacol peroxidase, and superoxide dismutase were measured.

Fresh leaf material (approximately 300 mg) was homogenized with 0.1% (w/v) trichloroacetic acid for determination of free proline, soluble phenols, free thiol-containing compounds, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). Free proline was extracted, derivatized with acid ninhydrin, and absorbance was read at 520 nm according to Bates et al. (1973). Total phenolics content was determined with Folin-Ciocalteu reagent supplemented with sodium carbonate and absorbance was read at 725 nm according to the method of Swain and Goldstein (1964). Gallic acid was used as a reference standard. Content of free thiol-containing compounds was determined with Ellman's reagent, absorbance was read at 412 nm, and quantity was calculated by using the molar extinction coefficient 13.6 $mM^{-1}cm^{-1}$ (Ellman, 1959). Malondialdehyde content was estimated as a parameter reflecting biomembrane integrity deterioration. It was determined as thiobarbituric acid-reagent product according to Kramer et al. (1991) by using the extinction coefficient 155 mM^{-1} cm^{-1}. Hydrogen peroxide content was estimated spectrophotometrically according to Alexieva et al. (2001). The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2.

For the assay of antioxidant enzymes, fresh plant material (approximately 200 mg) was homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (w/v). The homogenates were centrifuged at 15 000 x *g* for 15 min. The enzyme activities were determined according to the following methods: catalase (CAT, EC 1.11.1.6), Aebi (1984); guaiacol peroxidase (POX, EC 1.11.1.7), Dias and Kosta (1983); superoxide dismutase (SOD, EC 1.15.1.1), Beauchamp and Fridovich (1971).

CAT activity was monitored following decomposition of H_2O_2 and was determined by measuring the decrease of absorbance at 240 nm (ε = 36.8 mM^{-1} cm^{-1}) for 60 s.

POX activity was measured using guaiacol as an electron donor and following the absorbance increase at 470 nm (ε = 26.6 mM^{-1} cm^{-1}) for 60 s.

Total SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitrobluetrazolium (NBT). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT, which was monitored at 560 nm.

Soluble protein was determined by the dye binding technique according to Bradford (1976) using bovine serum albumin as a protein standard.

Statistics

All experiments were repeated three times with three replicates each. The data reported are mean values ±SE. The significance of differences was statistically analyzed using Duncan's multiple range test at a level of significance of 0.05.

RESULTS

The alterations found in MDA content in plants treated only with UV-C were best expressed at the beginning of the experiment and diminished with time (Fig. 1A). Immediately after UV-C irradiation (0 h after UV-C treatment) MDA accumulated (30%), then (24 h after UV-C) it dropped (10%) and at the last measurement (48 h after UV-C) almost reached the control levels. On the contrary, when TA compounds were applied alone MDA content decreased and the effect of TA-14 was more pronounced – up to 18%. Pretreatment with TA reduced MDA in plants irradiated with UV-C as compared to those treated with UV-C only. MDA content was near the control values 0 and 24 h after UV-C treatment but at the last measurement point it was significantly lower than the control (17%).

At the first measurement H_2O_2 content did not alter significantly (Fig. 1B). Twenty-four hours later H_2O_2 decreased after the treatments. This trend continued till the end of the experimental period and was expressed mainly in UV-C treated plants leading to 76% and 82% reduction of H_2O_2 content, 24 and 48 h after UV-C irradiation, respectively. Treatments with TA compounds (either applied alone or in combination with UV-C) caused a reduction in H_2O_2 levels compared to control, but it was less expressed as compared to plants treated with UV-C only.

SOD activity (Fig. 2A) was increased following all treatments and the induction of SOD activity was more pronounced in UV-C treated plants (62% above the

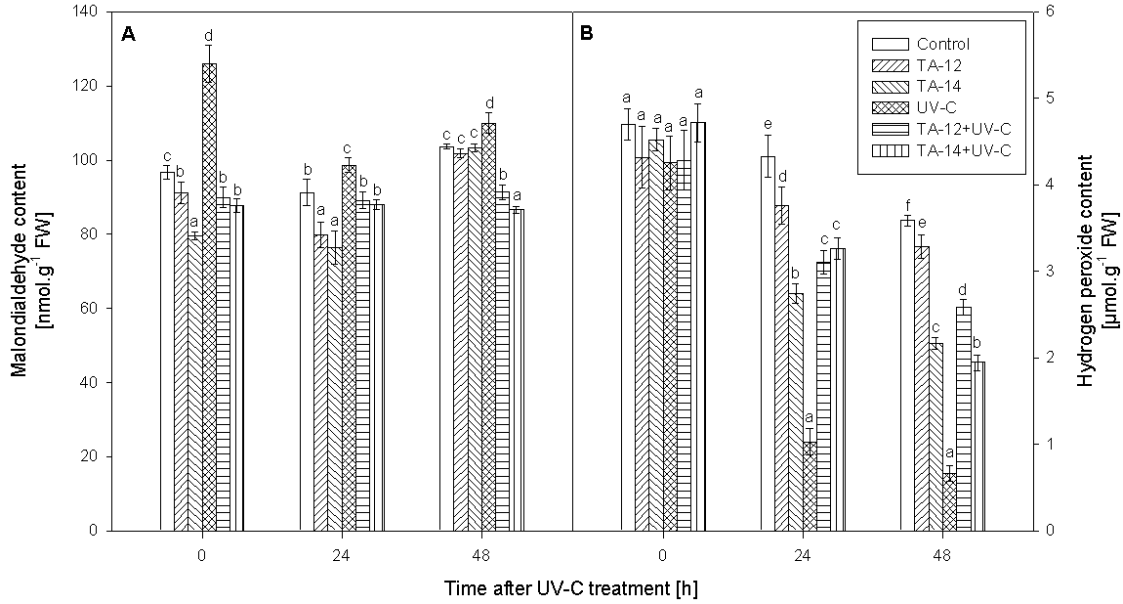

Figure 1. Content of malondialdehyde (A) and hydrogen peroxide (B) in pea plants treated with the auxin-like compounds TA-12 and TA-14 and irradiated with UV-C.

control). Later (24 h after UV-C) SOD activity raised additionally (up to 124%) in plants treated with UV-C. At the last measurement point SOD activity remained higher (61%) than the control only in UV-C treated plants, while treatments with TA compounds, applied either alone or in combination with UV-C, led to insignificant differences.

Catalase activity (Fig. 2B) was not changed considerably in plants treated with auxin-like compounds (neither alone nor in combination with UV-C) during the whole experimental period. Irradiation with UV-C increased CAT activity (by 63%, 67% and 71%, respectively on 0, 24 and 48 h after treatment).

Peroxidase activity was not significantly altered after UV-C irradiation at the first measurement point, but later (24 and 48 h after UV-C) it increased substantially (by 113% and 92%) as compared to the respective controls (Fig. 2C). Initially, treatment with TA compounds applied alone led to a slight decrease (up to 19%) of POX but in combination with UV-C irradiation did not alter POX activity. From the second measurement point POX activity increased in a time-dependent manner (by 22% and 28%, respectively on 24 and 48 h after treatment) in plants treated only with TA-14. TA-12 did not cause considerable changes in POX activity. In combination with UV-C stress both TA compounds reduced POX activity as compared to plants irradiated only with UV-C.

There were no significant alterations in proline levels (Fig. 3A) 0 h after UV-C treatment. Later on (24 h after UV-C) both TA compounds applied alone decreased proline content, which was more pronounced in TA-14 treated plants. At the end of the experimental period proline concentration was reduced (by 12% and 26%, respectively after TA-12 and TA-14 application). On the contrary to auxin-like compounds, UV-C light increased free proline content (by 12% and 33%, respectively on 24 and 48 h after

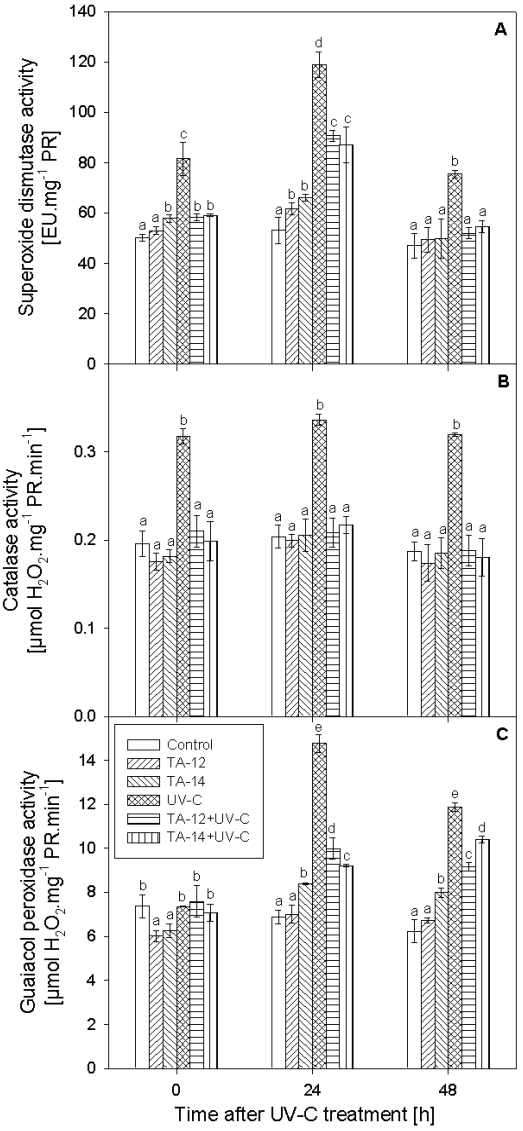

Figure 2. Activity of superoxide dismutase (A), catalase (B) and guaiacol peroxidase (C) in pea plants treated with the auxin-like compounds TA-12 and TA-14 and irradiated with UV-C.

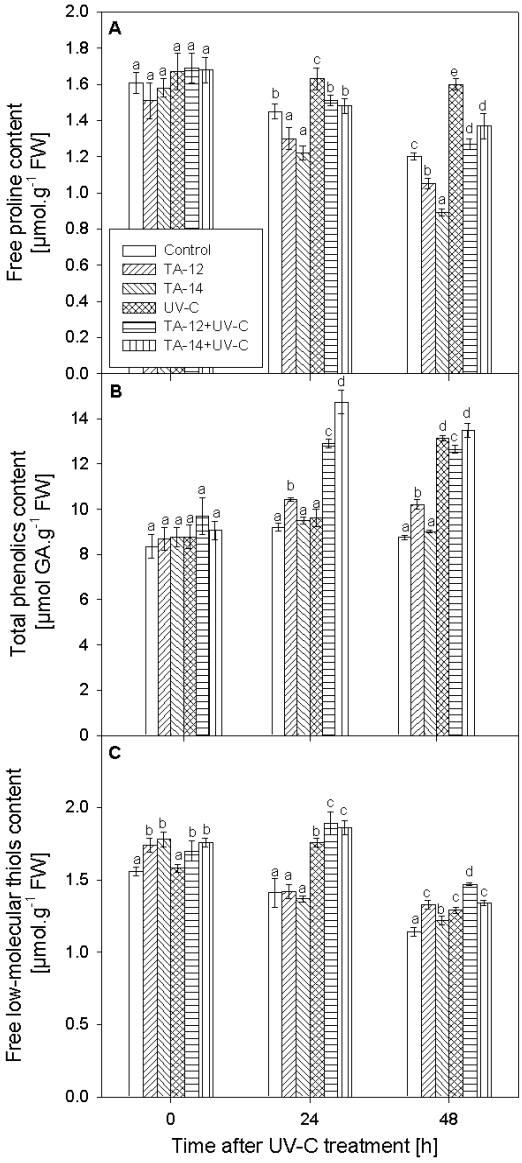

Figure 3. Content of free proline (A), total phenolics (B) and low-molecular thiols (C) in pea plants treated with the auxin-like compounds TA-12 and TA-14 and irradiated with UV-C.

treatment). Both TA-compounds, applied in combination with UV-C treatment, maintained proline content below the levels measured in irradiated plants.

Initially, total phenol amounts were not altered significantly (Fig. 3B). At the end of the experimental period phenol compounds increased considerably (by 50%) in UV-C treated plants. TA-12 applied alone slightly enhanced phenolics level (by 13% and 17%, respectively on 24 and 48 h after UV-C), but when it was combined with UV-C irradiation the rise was substantial (by 40% and 45%, respectively on 24 and 48 h after UV-C). TA-14 compound applied alone did not alter phenolics content but in combination with UV-C the increase was substantial (on 80% and 54%, on 24 and 48 h after UV treatment) as compared to the respective controls.

UV-C light did not change the amount of low-molecular thiol-containing compounds immediately after irradiation, but later on (24 and 48 h after UV treatment) an increase was detected (by 21 and 13% compared with the control) (Fig. 3C). Both TA compounds applied either alone or in combination with UV-C also raised free thiol concentrations. An exception of this trend was detected at the second measurement point (24 h) in both variants treated with TA only and the quantities of thiol-containing compounds were comparable with those of the control.

Immediately after UV-C treatment both physiological auxin analogues applied alone increased soluble protein levels (by 17% and 14%, respectively for TA-12 and TA-14), while UV-C irradiation decreased protein content by 17% (Table 1). These alterations diminished with time but in irradiated plants soluble protein content remained lower (by 18% and 7%, respectively on 24 and 48 h after UV-C) than the respective controls. Both TA compounds applied in combination with UV-C did not alter significantly protein levels during the whole experimental period.

Table 1. Total soluble protein content in pea plants treated with the auxin-like compounds TA-12 and TA-14 and irradiated with UV-C.

Variant	Time after UV-C irradiation					
	0 h		24 h		48 h	
	mg/g FW	% to control	mg/g FW	% to control	mg/g FW	% to control
Control	16.6±0.7^{b}	100	16.1±0.7^{b}	100	16.6±0.4^{a}	100
TA-12	19.5±0.5^{c}	117	16.4±0.8^{b}	102	17.0±0.3^{a}	102
TA-14	18.9±0.6^{c}	114	16.2±0.8^{b}	101	16.6±0.8^{a}	100
UV-C	13.8±0.1^{a}	83	13.2±0.8^{a}	82	15.4±0.6^{a}	93
TA-12+UV-C	16.7±0.1^{b}	101	16.9±0.4^{b}	105	17.4±0.9^{a}	105
TA-14+UV-C	16.4±0.3^{b}	98	16.2±0.6^{b}	104	16.4±0.7^{a}	99

DISCUSSION

All types of abiotic stress, including UV irradiation, lead to overall disturbance of plant metabolism that is caused by enhanced ROS production. The negative effects of ROS on biomembrane integrity is usually associated with increased quantity of MDA which results from peroxidation and fragmentation of unsaturated fatty acids (Kramer et al., 1991). The significant accumulation of MDA found after UV-C exposure indicates the presence of oxidative stress damages in membranes of pea plants. The fact that the MDA amount was lower in plants subjected to combined treatment than that in UV-C treated plants showed that both TA compounds could assist plants to overcome the negative effect of UV-C light on biomembrane integrity and ROS accumulation is partly alleviated.

The formation and scavenging of ROS are in a delicate balance in plants grown under normal growth conditions. This balance is impaired in plants subjected to stress factors (Gill and Tuteja, 2010). Furthermore, plant tolerance to stress factors has been associated with their antioxidant capacity. Thus increased levels of antioxidants may prevent stress induced damages. Some of the most important antioxidant enzymes triggered in response to ROS generation are SOD, CAT, and POX. SOD is a metal-containing enzyme catalyzing the dismutation of superoxide to H_2O_2. CAT and POX detoxify H_2O_2 (Gill and Tuteja, 2010). CAT is a tetrameric heme-containing enzyme that directly scavenges H_2O_2 (Gill and Tuteja, 2010). Peroxidases use various substrates (i.e. phenolics) to scavenge H_2O_2 and represent key enzymes in UV stress reactions and tolerance (Jansen, 2002). In addition, under UV irradiation POX participates in such physiological functions in plants as lignin biosynthesis and cell wall linkage (Marjamaa et al., 2009, Reglinski et al., 2013).

H_2O_2 constantly decreased in UV-C-treated plants during the experimental period although the SOD activity was enhanced (Fig. 1B, Fig. 2A). Along with the role of H_2O_2 as a ROS it is believed that a relatively small increase of the level of H_2O_2 takes part in stress

responses controlling major physiological processes like signaling (including hormonal transduction) and primary plant metabolism. Since H_2O_2 is a non-radical ROS which has longer half-life than the other ROS it can act as a long-distance signaling molecule (reviewed by Ślezak et al., 2007). Therefore, the substantial reduction of H_2O_2 observed under UV-C irradiation might disturb signalling transduction and physiological stress responses in pea plants. Preliminary application of TA compounds prior to UV-C irradiation kept the H_2O_2 concentration above those in UV-C treated plants. It could be suggested that pretreatment with TA compounds restored partially H_2O_2 level in UV-C irradiated plants.

The activities of the enzymes detoxifying H_2O_2 in UV-C irradiated plants were also steadily increased (Fig. 2B, C). An increase in CAT and POX activity is supposed to be an adaptive reaction to prevent the damage by reducing the toxic levels of H_2O_2 produced during cell metabolism and renders protection against oxidative stress provoked by UV-C (Gonzalez-Aguilar et al., 2010). Increased POX (Erkan et al., 2008; Reglinski et al., 2013) and CAT (Mohammadi et al., 2012) activities were reported earlier in different plant species under UV-C stress. In addition, the induction of CAT and POX activity is also associated with accelerated scavenging of H_2O_2 in UV-C-treated plants (Gonzalez-Aguilar et al., 2010). In the present study, the activities of antioxidant enzymes were increased mainly by UV-C stress, while the exogenous application of TA compounds had an attenuating effect on the enzymatic activities measured in TA+UV-C-treated seedlings. TA pre-treatment normalized the level of H_2O_2 and the activities of antioxidant enzymes in TA+UV-C-treated plants as compared to those subjected only to UV-C stress. This indicates that preliminary application of auxin-like compounds could assists the adaptation reactions of pea plants to UV-C stress which was confirmed with better biochemical status.

Plant phenolics, low-molecular thiols and proline are components of the non-enzymatic antioxidant defense in plants (Gill and Tuteja, 2010). One of the most important responses of plants to different abiotic stresses, including UV radiation is an overproduction of different types of compatible solutes, mainly proline (Ashraf and Foolad, 2007). In response to stress factors, proline accumulation generally occurs in the cytosol, where it plays multiple roles in improving plant stress tolerance (Szabados and Savoure, 2010). We found that proline was increased by UV-C irradiation (Fig. 3A). However, proline content in TA+UV-C treated pea seedlings was lower than that measured in irradiated plants and almost reached control levels. Most probably exogenous application of both TA compounds provided better redox state of the plant cells and improved adaptation reactions. As a result pea plants protected from UV-C-induced damages manifested recovered proline content.

Plant low-molecular thiols pool comprises of glutathione predominantly (Foyer and Noctor, 2005). The accumulation of thiol-containing compounds (particularly glutathione) is considered a favorable response while the decrease is a negative consequence of stress. The increase of thiols in UV-C treated pea seedlings possibly assisted plants to cope with ROS damages (Fig.

3C). Similarly Erkan et al. (2008) also reported an increased amount of major non-protein thiols (i.e. glutathione) in UV-C treated strawberry fruits. In addition, Verma and Mishra (2005) noted that increased glutathione concentrations due to the exogenously applied plant growth regulator putrescine improved tolerance and adaptation of Indian mustard to salinity. The higher content of low-molecular thiols in TA+UV-C treated plants as compared to their quantities in irradiated seedlings suggested that preliminary application of auxin-like compounds improved plant adaptation reactions.

Phenolics are known as UV-screening compounds (Kolb et al., 2003). It is reported that they increase substantially in UV-treated plants and fruits (Erkan et al., 2008; Ghanati et al., 2013). However, we found that accumulation of phenolics in TA+UV-C treated plants happened earlier than in seedlings irradiated only with UV-C (Fig. 3B). It could be supposed that the earlier induction of phenolics synthesis due to TA pretreatment in plantlets subjected to subsequent UV-C irradiation resulted in their better ability to cope with UV-C stress.

The higher content of phenolics and thiol-containing compounds in seedlings exposed to combined treatment compared with UV-C stressed alone could be interpreted as an indication for better plant physiological status due to TA pretreatment.

CONCLUSION

The data in the present study suggest that exogenous application of the auxin-like compounds TA-12 and TA-14 could protect pea plants against UV-C irradiation. The protection was demonstrated by lessened membrane damages accompanied by recovered protein, hydrogen peroxide, proline content and maintained enzymatic activities as well as non-enzymatic antioxidants near to control levels. It could be concluded that the application of the auxin-like compounds tested might render beneficial and protective effects on pea plants exposed to UV-C stress through a coordinated action of non-enzymatic antioxidants and ROS detoxifying enzymes.

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