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AUXIN-LIKE COMPOUNDS ACT AS PROTECTORS AGAINST UV-B IRRADIATION IN GARDEN PEA PLANTS

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Abstract

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Pretreatment with the original auxin physiological analogues 1-[2-chloroethoxycarbonylmethyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxicarbonylmethyl]naphthalene chlormethylate (TA-14) and subsequent UV-B irradiation (180 min at λ_{max} 312 nm for 6.6 kJ·m^{-2}) of pea plants (*Pisum sativum* L.) was investigated to assess if foliar application of these compounds has ability to attenuate the negative effects caused by UV-B stress. UV-B treatment increased malondialdehyde (MDA) and proline levels as well as superoxide dismutase, catalase and guaiacol peroxidase activities, but decreased hydrogen peroxide, low-molecular thiols, total phenolics and total soluble protein contents. The pre-treatment with TA compounds decreased the oxidative stress provoked by UV-B radiation detected by lower level of MDA, increased the content of thiols and UV-absorbing compounds and had favourable effect on H_2O_2 content and enzymatic activities. Exogenous application of auxin-like compounds on pea plantlets successfully counteracted UV-B induced oxidative stress *via* activation of ROS detoxifying enzymes and non-enzymatic antioxidants.

Keywords: auxin-like compounds, antioxidants, pea, plant stress, stress markers, UV-B.

INTRODUCTION

Solar UV radiation is divided into three classes: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). As sunlight passes through the atmosphere, all UV-C and most UV-B are absorbed by ozone, water vapour and oxygen. Depletion of ozone layer due to the human activities is one of the causes that increase UV-B irradiation on Earth surface. The UV-B radiation produces a number of harmful effects in plant cells such as damage to proteins, membrane phospholipids, and DNA (Zlatev et al., 2012). To defeat from ultraviolet radiation, protective physiological responses in plants might be activated, including changes in antioxidant enzyme activities, non-enzymatic antioxidants, secondary metabolites, etc. (Gill & Tuteja, 2010; Zlatev et al., 2012). Exogenous application of different plant growth-regulating substances is also able to activate some or all of these defence systems in plants subjected to different type of abiotic stresses (Todorova et al., 2008; Ding et al., 2010; Habibi, 2012; Katerova et al., 2014; Todorova et al., 2014; Esringu et al., 2016; Sergiev et al., 2016; Aksakal et al., 2017).

Auxins are major class of plant hormones that positively influence plant growth and development processes such as cell division and enlargement, root initiation, buds formation, growth of root stem apexes as well as contribute to plant phototropism, geotropism and hydrotropism. They are also involved in plant adaptive stress responses to different stresses (Kazan, 2013), including UV-B irradiation (Van-

haelewyn et al., 2016) by stimulation of different auxin related and signalling genes. It was believed that inactivation or blockage of auxin transport caused UV morphogenic responses in turmeric plants grown under full sunlight compared to plants grown under UV exclusion (Ferreira et al., 2016). Other authors reported that UV-B radiation provoked a decrease in endogenous auxin levels (Liu & Zhong, 2009; Esringu et al., 2016). Exogenous application of synthetic auxin NAA enhanced plant tolerance by increasing endogenous auxin levels (reviewed by Llanes et al., 2016). Exogenous application of original auxin physiological analogues 1-[2-chloroethoxycarbonyl -methyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxicarbonylmethyl]naphthalene chlormethylate (TA-14) improved rapeseed (*Brassica napus*) cold hardening and overwintering (Velička et al., 2005; Anisimovienе et al., 2008; Gavelienė et al., 2013). These compounds have not been assessed against other type of abiotic stresses up to now.

The aim of the study is to evaluate the ability of auxin physiological analogues to attenuate negative consequences of UV-B irradiation in pea (*Pisum sativum* L.) plants. It was suggested that exogenous application of auxin-like compounds TA-12 and TA-14 may decrease the membrane damage and enhance the plant defence systems against harmful ROS formed by UV-B irradiation of plants.

MATERIALS AND METHODS

Plant material and treatments

Young pea plants (*Pisum sativum* l., cv. Ran 1) were grown as water culture in a growth chamber (12 h/12 h photoperiod; 60% to 70% relative air humidity, 160 µmol·m-2·s-1 photon flux density; 24°C ± 2°C). Thirteen-day-old seedlings were leaf sprayed with 1 mM water solutions of 1-[2-chloroethoxycarbonyl -methyl]-4-naphthalenesulfonic acid calcium salt (TA-12) or 1-[2-dimethylaminoethoxicarbonylmethyl]naphthalene chlormethylate (TA-14), and 24 h later pea plants were irradiated with UV-B (Philips TL 2X20W/12 RSSLV/25, λ_{max} 312 nm) for 180 min (6.6 kJ·m^{-2}). The distance between UV-B lamp and the top leaves of the treated plants was 0.25 m ± 0.04 m. After the end of the stress program, plants were transferred back under controlled growth conditions. The physiological responses of plants were determined immediately (0h), 24 h and 48 h of the recovery period.

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 in the 3rd leaf pair.

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 & 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 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 of extinction coefficient 155 mM^{-1} cm^{-1}. Hydrogen peroxide content was estimated spectrophotometrically (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 12 000 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 & Costa (1983); superoxide dismutase (SOD, EC 1.15.1.1), Beauchamp & Fridovich (1971).

CAT activity was monitored following the decomposition of hydrogen peroxide and was deter-

mined by measuring the diminution of absorbance at 240 nm ($\varepsilon = 36.8\ mM^{-1}\ cm^{-1}$) for 60 s.

POX activity was measured using guaiacol as a substrate and following the absorbance increase at 470 nm ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) for 60 s due to guaiacol oxidation.

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 dye binding technique (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 analysed using Duncan's multiple range test at a level of significance of 0.05.

RESULTS

MDA content increased at the first two measurements (25% and 20%, respectively after 0 and 24-h recovery) upon UV-B irradiation, but at the last measurement it reached the control levels (Fig. 1A). When TA compounds were applied alone, MDA decreased and the effect of TA-14 was more pronounced (up to 18%), but finally (after 48-h recovery) it reached control value. Combinations of TA and UV-B light significantly reduced the MDA level compared to plants treated only with UV-B. At the second measurement, MDA content reached control levels, but at the last one (after 48-h recovery) it dropped significantly.

At the first measurement, hydrogen peroxide content (Fig. 1B) did not alter considerably upon single treatments with auxin-like substances, while significant decrease (up to 65%) was detected after UV-B irradiation (either applied alone or in combination with TA compounds). Twenty-four hours later H_2O_2 levels were lower than the control in all treated plants, which continued till the end of the experimental period. The effect of UV-B exposure was most pronounced and diminution in H_2O_2 content reached 84% 24 h after recovery. Treatments with TA compounds in combination with UV-B caused reduction of H_2O_2 levels, but less expressed compared to plants irradiated only with UV-B.

Initially (0-h recovery) TA-12 and TA-14 applied alone increased slightly (17 and 14%, respectively)

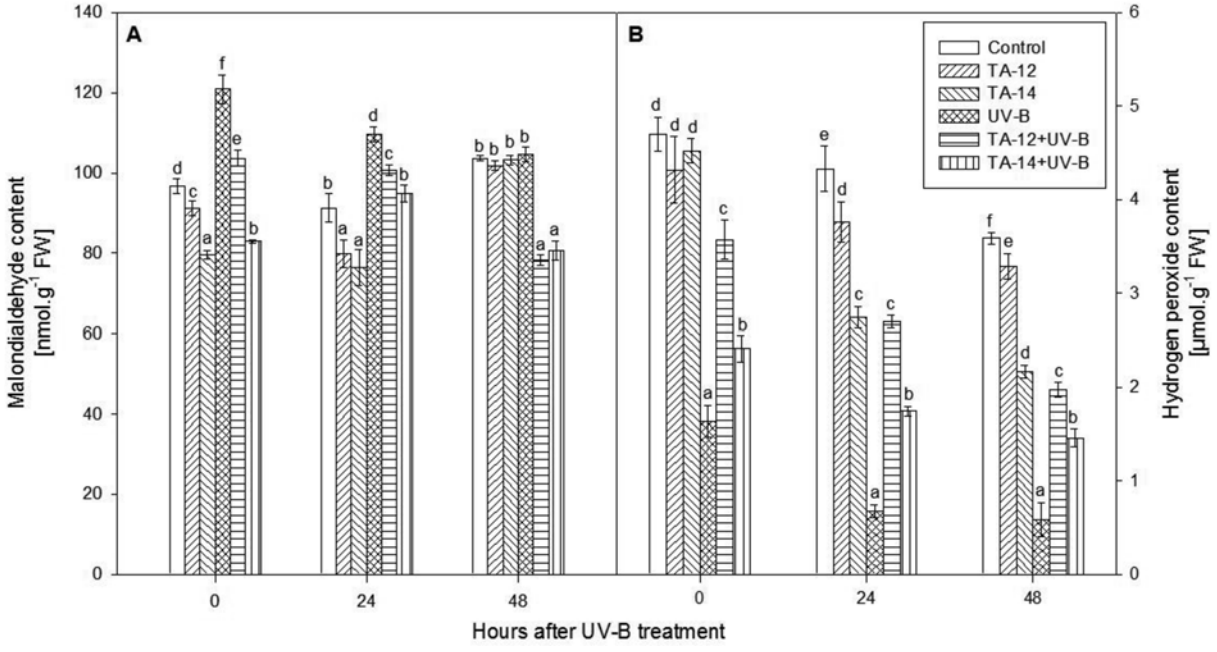

Fig. 1. Content of malondialdehyde (A) and hydrogen peroxide (B) in pea plants treated with auxin-like compounds TA-12 and TA-14 and UV-B irradiation

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

Variant	Time after UV-B 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^{c}	100	16.1 ± 0.7^{b}	100	16.6 ± 0.4^{a}	100
TA-12	19.5 ± 0.5^{d}	117	16.4 ± 0.8^{b}	102	17.0 ± 0.3^{a}	102
TA-14	18.9 ± 0.6^{d}	114	16.2 ± 0.8^{b}	101	16.6 ± 0.8^{a}	100
UV-B	12.3 ± 0.5^{a}	74	13.1 ± 0.8^{a}	81	15.4 ± 0.6^{a}	93
TA-12+UV-B	19.2 ± 1.1^{d}	116	17.1 ± 1.2^{b}	106	16.6 ± 0.5^{a}	100
TA-14+UV-B	14.4 ± 0.2^{b}	87	16.8 ± 1.0^{b}	104	16.9 ± 0.4^{a}	102

soluble protein levels, but UV-B irradiation caused a substantial reduction (26%), while combined treatments led to subtle diminution (16%, TA-12+UV-B) or increment (13%, TA-14+UV-B) compared to control (Table 1). Later (24 and 48-h recovery), the alteration in protein levels was not significant in plants treated with both TA compounds (neither applied alone nor in combination with UV-B). The reduction initially found in soluble protein content diminished with time (19 and 7%, respectively 24 and 48-h after recovery) in UV-B irradiated plants. Soluble protein content remained lower (19% and by 7%, respectively 24 and 48 h after recovery) in UV-B stressed plants than the respective controls.

Initially, SOD activity (Fig. 2A) was increased by all treatments with exception of UV-B irradiated plants, where the activity was not changed. Twenty-four hours later, SOD activity raised substantially in plants treated with UV-B (either alone or in combination with TA compounds). Finally (after 48-h recovery), SOD activity remained higher (52%) than the control in plants irradiated with UV-B, while in plants treated with TA compounds (either alone or in combination with UV-B) insignificant differences were found.

During the whole experimental period catalase activity (Fig. 2B) was not changed considerably in plants treated only with TA compounds. Irradiation with UV-B caused an increase of CAT activity by 19%, 28% and 20%, respectively 0, 24 and 48 h after recovery. Similar increase of CAT activity was detected in plants treated with TA+UV-B at the first measurement, then it tended to decline and finally reached control values.

At the first measurement point, peroxidase activity was not changed significantly after treatments (Fig. 2C). From the second measurement point, TA-14 applied alone increased POX activity (22% and 28%, respectively after 24 and 48-h recovery), while in plants treated only with TA-12 changes were not significant. UV-B irradiation applied alone considerably increased POX activity (40% and by 61%, respectively after 24 and 48-h recovery). When TA compounds were applied in combination with UV-B, POX activity increased additionally and maintained higher level than in irradiated plants until the end of the experimental period.

Initially, both auxin-like compounds applied alone did not change significantly proline content (Fig. 3A). Later they decreased free proline content in time-dependent manner and at the end of the experimental period the reduction was 12% (for TA-12) and 26% (for TA-14). On the contrary, UV-B irradiation increased free proline levels (18%, 61% and 42%, respectively after 0, 24 and 48-h recovery). When TA-compounds were applied in combination with UV-B, they maintained proline content higher than the respective controls and significantly different than irradiated plants except TA-14+UV-B after 48-h recovery.

Initially, total phenol amount was not significantly altered by TA compounds, applied alone, while irradiation of plants with UV-B (either alone or in combination with TA compounds) caused substantial reduction (up to 41%) (Fig. 3B). Later, TA-12 applied alone raised slightly phenolic levels (13% and 17%, respectively after 24 and 48-h recovery), but when applied in combination with UV-B, the increase was considerable (41% and 52%, respectively 24 and 48 h after recovery). Similarly, TA-14 compound applied in combination with UV-B significantly increased (29% and 33%, respectively after 24 and 48 h UV-B) phenolics content, but not after single application of TA-14. At the end of the ex-

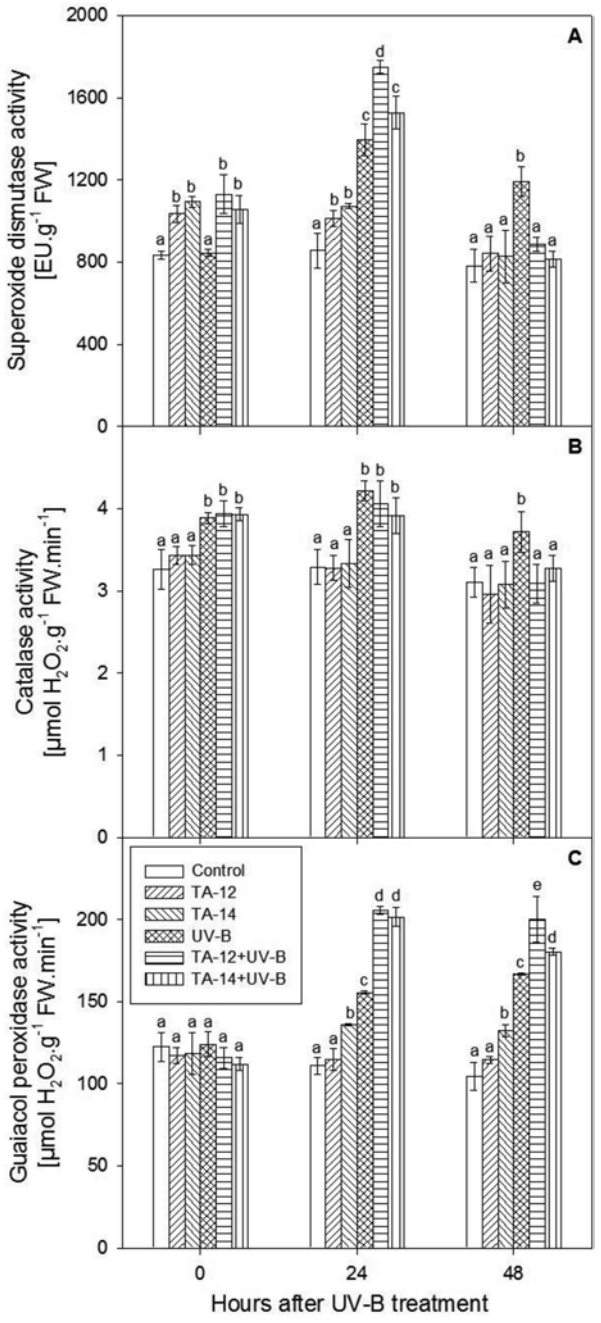

Fig. 2. Activity of superoxide dismutase (A), catalase (B) and guaiacol peroxidase (C) in pea plants treated with auxin-like compounds TA-12 and TA-14 and UV-B irradiation

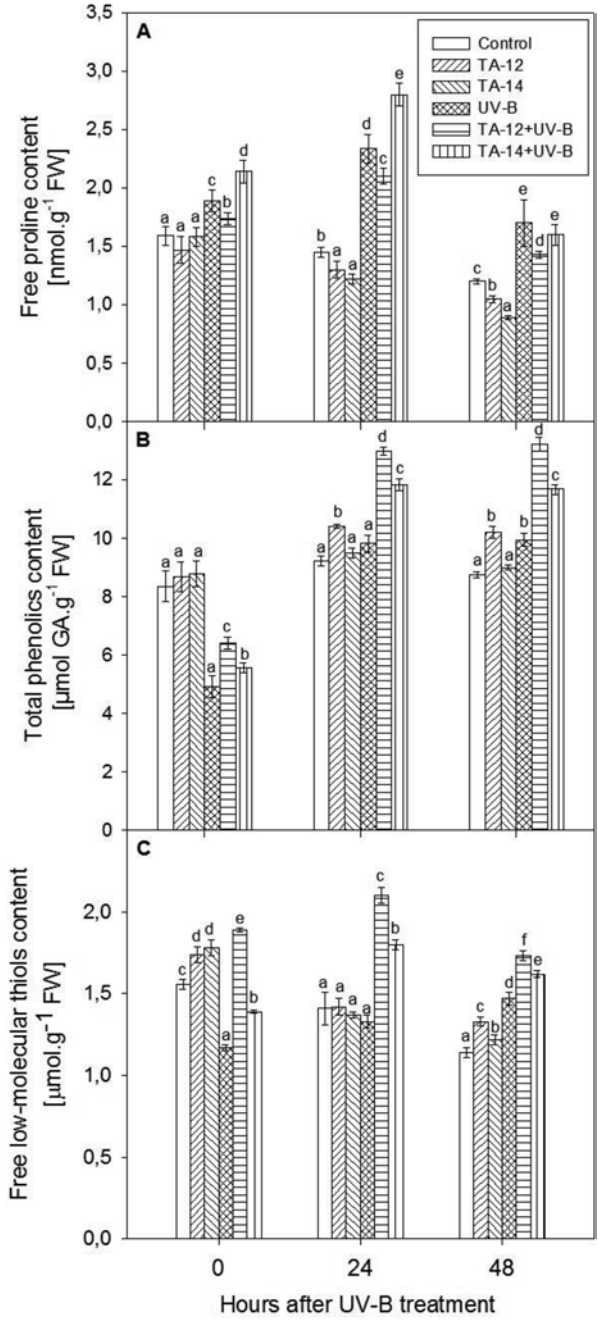

Fig. 3. Content of free proline (A), total phenolics (B) and low-molecular thiols (C) in pea plants treated with auxin-like compounds TA-12 and TA-14 and UV-B irradiation

perimental period, phenolics were slightly enhanced (14%) in UV-B treated plants.

Initially, UV-B decreased the amount of low-molecular thiol-containing compounds, then (24 h later) it started to increase, and finally the level become higher (29%) than the respective control (Fig. 3C). Both TA compounds (either applied alone or in combination with UV-B) also raised free thiols content. The exception of this trend in plants treated only with TA was detected at the second measurement point, when the quantities of thiol-containing compounds were comparable with those of the control. Appli-

cation of auxin-like compounds in irradiated plants kept the quantity of thiol-containing compounds higher than respective amount measured in plants treated with UV-B.

DISCUSSION

Exposure of plants to various types of abiotic stresses, including UV irradiation, leads to increase in ROS production and disturbance of common plant physiological processes. An increased quantity of MDA, resulting from peroxidation and fragmentation of unsaturated fatty acids (Kramer et al., 1991) is usually associated with the negative effects of ROS on cell biomembranes. The considerable accumulation of MDA quantity found after UV-B exposure indicates the occurrence of oxidative stress damages in the cell biomembranes of pea seedlings. TA compounds applied alone did not provoke such MDA accumulation, showing low cellular damage for the respective plants. Further, the MDA amount was lower in seedlings subjected to combined treatment than to UV-B only, revealing that both TA compounds could assist plants to overcome the negative effect of irradiation. Therefore, it could be suggested that ROS accumulation due to UV-B exposure is partly alleviated by preliminary application of auxin-like compounds.

The formation and scavenging of ROS are normal physiological processes in plants grown under ordinary growth conditions. However, in plants subjected to different stress factors, ROS formation usually prevails their scavenging ability (Gill & Tuteja, 2010). In that case if the antioxidant capacity is increased, the stress damages might be prevented. SOD, CAT, and peroxidases (POX) are some of the most important antioxidant enzymes triggered in response to ROS generation. A metal-containing enzyme SOD catalyses the dismutation of superoxide anion to H_2O_2. Further, H_2O_2 can be scavenged directly by CAT (a tetrameric heme-containing enzyme) and indirectly by POX, which use various substrates like phenolics (Gill & Tuteja, 2010). In addition, POX are key enzymes in UV stress reactions and tolerance (Jansen, 2002) since it has been reported that under UV radiation POX contribute to lignin biosynthesis and cell wall linkage (Marjamaa et al., 2009; Reglinski et al., 2013).

The H_2O_2 was found to be constantly low in UV-B-treated plants, although SOD activity increased gradually during the experimental period. Actually, beside its role as a ROS, H_2O_2 is believed to have important role in stress response *via* participation in different physiological processes like hormonal signalling and primary plant metabolism in cells. H_2O_2 can act as a long-distance signalling molecule, because it is a non-radical ROS, which has longer half-life than the other ROS (reviewed by Ślesak et al., 2007). Therefore, the severe reduction of H_2O_2 observed under UV-B irradiation caused distress to pea plants, which might disturb plant metabolism. We found that pre-treatment with TA compounds kept the H_2O_2 levels higher than those in plants treated only with UV-B. It seems that pre-treatment with TA compounds influence positively H_2O_2 content in irradiated plants.

The superoxide anion is the dominant ROS, while singlet oxygen is minor in UV-B–treated leaves as it has been demonstrated by Hideg et al. (2002). Therefore, the substantially increased SOD activity in irradiated (either alone or in combination with TA treatment) pea seedlings probably indicated that this enzyme system was switched on to detoxify the superoxide anion induced by UV-B irradiation. In our study the activities of CAT and POX also increased significantly. An amplified POX and CAT activities have been reported in different crop and medicinal plants upon UV-B stress (Alexieva et al., 2001; Kumari et al., 2010; Hagh et al., 2012; Todorova et al., 2014; Esringu et al., 2016). The increased CAT and POX activities could be an adaptive reaction to prevent oxidative damages *via* reduction of the H_2O_2 levels produced by SOD and providing protection against the oxidative stress provoked by UV-B. The exogenous application of both TA compounds had different effects on the activity of H_2O_2 detoxifying enzymes measured in TA+UV-B-treated seedlings. TA pre-treatments caused time-dependent decrease in the activity of CAT, while POX increased in TA+UV-B-treated seedlings compared to plants irradiated only with UV-B. This indicates that POX was one of the major enzymes that assisted adaptation reactions of pea plants to oxidative stress induced by UV-B stress. The adaptation was probably activated by auxin-like compounds through stimulation of lignification processes by POX, which is

in accordance with earlier reports of other authors (Marjamaa et al., 2009; Reglinski et al., 2013). A compensation mechanism activated by TA pre-treatment before UV-B stress in order to attenuate ROS (and H_2O_2 in particular) through induction of CAT activity, immediately after UV-B stress could not be excluded.

The non-enzymatic antioxidant defence in plants includes different components. Plant phenolics, low-molecular thiols and proline are essential elements of this defence as they possess antioxidative properties (Gill & Tuteja, 2010). An overproduction of different types of compatible solutes, mainly proline is one of the most important responses of plants to different abiotic stresses, including UV radiation (Ashraf & Foolad, 2007). Proline usually increases in the cytosol, where it has multifunctional role in improving plant tolerance to stress factors (Szabados & Savoure, 2010). Abundance of proline amount was detected not only in crops (Alexieva et al., 2001), but also in other plant species (Kumari et al., 2010; Kumar et al., 2016) subjected to UV-B irradiation. Here, we found that proline was raised by UV-B irradiation (either applied alone or in combination with TA compounds). Proline overproduction could have a positive effect on reduction of UV-B-induced damage in pea plants. As proposed earlier, the positive effect might be caused due to the removal of excess H^+ in cytosol (Alexieva et al., 2001).

According to Foyer & Noctor (2005), glutathione is the main low-molecular non-protein thiol-containing compound, and its accumulation is considered as a favourable response, but the decrease – as a negative stress consequence. The increased amounts of non-protein thiols have been reported in different plant species after UV-B exposure (Kumari et al., 2010; Kumar et al., 2016). Similarly, in our study, a progressive increase of free thios was found in UV-B treated pea plants. This possibly indicated that non-protein thiol-containing compounds contributed in effective antioxidant defence in UV-B irradiated plants. Further, the higher content of low-molecular thiols in TA+UV-B treated plants compared to the amount in seedlings irradiated only with UV-B, could be assumed as a benignant adaptation reaction, improving redox state of the plant cells. Our data are in line with those reported earlier by Verma & Mishra (2005), who found that pre-treatment of Indian mustard seedlings with putrescine increased glutathione concentrations, enhancing the tolerance and adaptation of plants to salinity. In addition, exogenous application of putrescine, H_2S or H_2O_2 increased glutathione content that increased tolerance to UV-B by maintaining redox homeostasis in barley (Li et al., 2016). Similarly, it could be supposed that auxin-like compounds improved the redox state and assisted pea plants to cope with ROS damages induced by UV-B stress.

Plant phenolics belong to UV-screening compounds (Kolb et al., 2003) and increase substantially in different plant species subjected to UV-B radiation (Kumari et al., 2010; Ghanati et al., 2013; Kumar et al., 2016; Esringu et al., 2016). Accumulation of UV-absorbing compounds such as total phenols has been reported in pea plants upon UV-B stress (Alexieva et al., 2001). Here, it was found that UV-B irradiation caused an augmentation of phenolic compounds, but it occurred only at the end of the recovery period. However, after TA+UV-B treatment, phenolics accumulated in significant degree and was higher than that found in seedlings irradiated with UV-B. Therefore, it could be supposed that enhanced synthesis of phenolics upon TA+UV-B treatment resulted in enhanced plant ability to cope with UV-B-induced stress injuries, and it seems that TA compounds provided better protection for cells from potential oxidative damage induced by UV-B irradiation.

The higher quantity of phenolics and non-protein thiols achieved by TA pre-treatment of irradiated plants compared to those subjected only to UV-B treatment could be interpreted as an indication for better plant antioxidant capacity due to TA application.

CONCLUSION

Our data suggest that pre-treatment with auxin-like compounds TA-12 and TA-14 could protect pea plants against subsequent exposure to UV-B irradiation. This protection was established by lower level of cell membrane damages; recovered protein content to control level; retained CAT and SOD near to non-stressed activities; enhanced POX activity and non-enzymatic antioxidant quantities in TA+UV-B treated plants. It could be concluded that the exogenous application of physiological auxin analogues

might provide defensive effect on pea plants exposed to UV-B irradiation *via* activation of ROS detoxifying enzymes and non-enzymatic antioxidants.

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NUO UV-B SPINDULIUOTĖS SĖJAMĄJĮ ŽIRNĮ APSAUGANTIS AUKSINO TIPO JUNGINIŲ POVEIKIS

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Santrauka

Sėjamojo žirnio (*Pisum sativum*) lapai buvo paveikti originaliais auksinų fiziologiniais analogais 4-[2-chloroetoksikarbonilmetil]-1-naftalinsulfo rūgšties kalcio druska (TA-12) ir 1-[2-dimetilaminoetoksikarbonilmetil]naftalin chlormetilatu (TA-14), vėliau 180 min apšvitinti UV-B (λ_{max} 312 nm, 6.6 kJ·m^{-2}). Buvo įvertinta, ar minėti junginiai gali susilpninti UV-B streso sukeltas neigiamas pasekmes. UV-B apšvita padidino kontrolinių augalų malondialdehido (MDA) ir prolino kiekius, taip pat superoksido dismutazės, katalazės ir gvajakolio peroksidazės aktyvumą, tačiau sumažino vandenilio peroksido, mažo molekulinio svorio tiolio kiekį, bendrą fenolio ir bendrą tirpių baltymų kiekį. TA junginių poveikis sumažino oksidacinį stresą, kurį sukėlė UV-B spinduliuotė, buvo aptiktas mažesnis MDA kiekis, didesnis tiolių ir UV absorbuojančių junginių kiekis bei didesnis H_2O_2 kiekis ir fermentų aktyvumas. Panaudoti egzogeniškai auksino tipo junginiai sėkmingai priešinosi UV-B sukeltam oksidaciniam stresui stimuliuodami sėjamojo žirnio aktyviąsias deguonies formas (ROS) detoksikuojančius fermentus ir nefermentinius antioksidantus.