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PROLONGED INFLUENCE OF SHORT PULSES ULTRAVIOLET-C RADIATION ON YOUNG PEA PLANT DOES NOT ALTER IMPORTANT ANTIOXIDANT DEFENSE ENZYME ACTIVITIES IN YOUNG LEAVES

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Summary. The behavior of the antioxidant defense enzyme activity in the youngest leaves (3rd, 4th or 5th) after exposure of intact pea (*Pisum sativum* L., cv. Scinado) plants for 10, 14 or 21 consecutive days, to low doses of ultraviolet-C (UV-C) radiation was studied. Two UV-C regimes were used: C1 (20 s d^{-1}, 0.1 kJ m^{-2} d^{-1}) and C2 (60 s d^{-1}, 0.3 kJ m^{-2} d^{-1}).
Only C1 regime led to a slight increase in hydrogen peroxide (H_2O_2) content after 10 and 14 days of irradiation (20% and 27%, respectively as compared to control). The C2 regime did not induce a noticeable increase in H_2O_2 as compared to C1, suggesting that H_2O_2 could be accepted as a signal for activation of defense responses rather than a stress marker. The activity of superoxide dismutase decreased at the end of the stress programme in C2-irradiated plants. An increase was found in the activities of glutathione-S-transferase (39%, 10 days C2) and glutathione-reductase (23%, 10 days C2; 12%, 14 days C1; 11%, 14 days C2). In conclusion, the activation of the antioxidant defense system seemed negligible when pea plants were exposed to prolonged and short pulses of UV-C radiation, although the possibility of isoenzyme changes could not be excluded and needs additional experiments.

Key words: glutathione-reductase, glutathione-S-transferase, hydrogen peroxide, superoxide dismutase, UV-C radiation.

Abbreviations: CDNB – 1-chloro-2,4-dinitrobenzene; DTNB – 5, 5'-dithiobis-(2-nitrobenzoic acid); GR – glutathione-reductase; GST – glutathione-S-transferase; H_2O_2 – hydrogen peroxide; PPFD – photosynthetic photon flux density; ROS – reactive oxygen species; SOD – superoxide dismutase; UV – ultraviolet radiation.

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INTRODUCTION

Ultraviolet radiation is divided into three classes: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). Due to its highest energy UV-C quickly creates high levels of damage, more than UV-B and UV-A. At present, UV-C does not reach the Earth's surface, except high mountain locations, due to its absorption in the atmosphere (Häder et al., 2007). Direct solar UV-C radiation (0.3 μJ cm^{-2}) is registered at ground level at Madrid on clear-sky days, being six orders of magnitude lower than UV-B (Córdoba et al., 1997).

High levels of UV-C radiation can generate oxidative stress in plants through overproduction of ROS (H_2O_2, singlet oxygen, superoxide and hydroxyl radicals) (Zachini and de Agazio, 2004; Procházková and Wilhelmová, 2007). Nonenzymatic compounds consisting of lipid soluble membrane associated antioxidants (α-tocopherol, β-carotene), and water soluble reductants (glutathione, ascorbate, phenolics), and enzymatic antioxidants (SOD, catalase, peroxidases, GSTs, GR) are induced in response to oxidative stress (Jaleel et al., 2009). Antioxidant response under low UV-C doses was studied in rose cells (Murphy and Huerta, 1990), fruits (Barka, 2001), and older pea leaves, where Katerova et al. (2008) found that enzymatic antioxidants failed to activate under prolonged exposure to UV-C. In this study the focus is on the effect of prolonged and short pulses of UV-C irradiation on the youngest pea leaf nodes.

MATERIALS AND METHODS

Plant material and UV-C irradiation

Pea (*Pisum sativum* L., cv. Scinado) seeds were germinated for 3 days on moistened filter paper in the dark. The seedlings were transferred into Hoagland's nutrient solution and grown in a growth chamber (12h/12h photoperiod, PPFD 160 μmol m^{-2} s^{-1}, 24±2ºC, 60-70% air humidity). When the 1st leaf appeared, seedlings were exposed to short pulses of UV-C (C1 – 20 s d^{-1}, 0.1 kJ m^{-2} d^{-1}; C2 – 60 s d^{-1}, 0.3 kJ m^{-2} d^{-1}) irradiation in the middle of photoperiod for 10, 14 or 21 consecutive days. Germicidal lamp (STYLO STY 115, GE Lighting, Italy λ_{max} 254 nm) was used at 0.25±0.04 m distance from the top leaves. UV-C lamp provides 0.50 UV-C, 0.010 UV-A and 0.04 mJ cm^{-2} s^{-1} PAR.

Biochemical analyses

Plant material was collected from the youngest leaves, 20 h after the end of a given treatment – 10 (3rd leaf), 14 (4th leaf) and 21 days UV-C (5th leaf). Enzymes were assayed using the crude leaf extract after homogenization at 4ºC in 100 mM phosphate buffer (pH 7.6), containing 5mM EDTA and 1 mM DTT, plus 1% (w/v) soluble polyvinylpyrrolidone. The activities of antioxidant enzymes were determined: SOD (EC 1.15.1.1) - Beauchamp and Fridovich, 1971; GST (EC 2.5.1.18) - Gronwald et al. (1987); GR (EC 1.6.4.2) - Smith and Vilrbeller (1988). Soluble protein was determined by the method

of Bradford (1976). Hydrogen peroxide was measured spectrophotometrically (Alexieva et al., 2001). Each experiment was repeated two times in three replicates. Values are means ± SE.

RESULTS

In C1-treated plants hydrogen peroxide level increased slightly after 10 and 14 days (3rd and 4th leaves) and after 21 days (5th leaves) it reached the control (Fig. 1A). In C2-treated plants the endogenous H_2O_2 level was lower than in C1-treated plants and it was negatively affected after 21 days (5th leaves). Neither treatment with UV-C irradiation (C1 and C2) provoked a significant increase in SOD activity (Fig. 1B). After 21 days of C2 regime a slight decrease (17%) was observed in 5th leaves. GST activity increased (39%) in 3rd leaves after 10 days of C2 regime but the other alterations were not significant (Fig. 2A). A slight increase in GR activity was found after 10 days (23%, C1, 3rd leaves) and 14 days (about 12%, C1 and C2, 4th leaves)

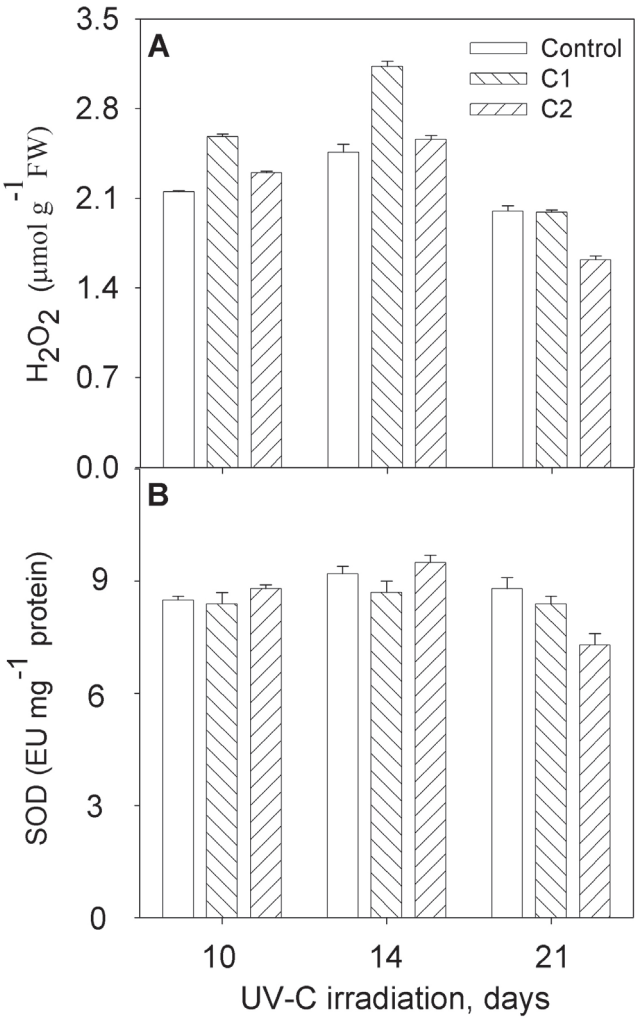

Fig. 1. Hydrogen peroxide levels (A) and SOD enzyme activity (B) in the youngest pea leaves after 10, 14 and 21 days of exposure (3rd, 4th and 5th leaf, respectively) to low doses of UV-C (Control – 0, C1 - 0.1 kJ $m^{-2}d^{-1}$, C2 - 0.3 kJ $m^{-2}d^{-1}$). Means ± SE, $n = 6$.

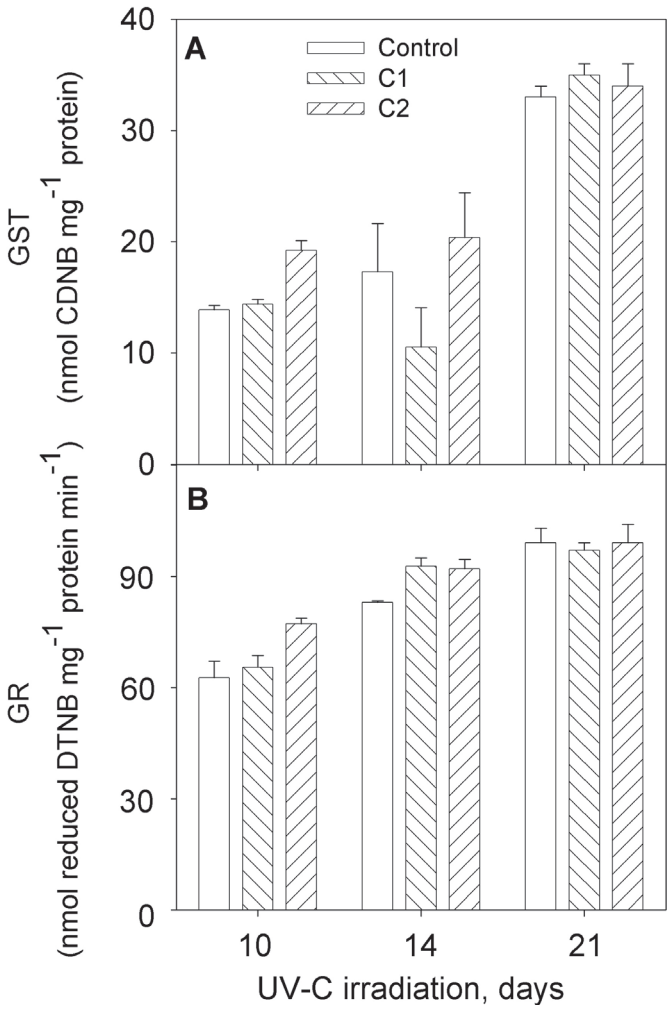

Fig. 2. GST (A) and GR (B) enzyme activities in the youngest pea leaves after 10, 14 and 21 days of exposure (3rd, 4th and 5th leaf, respectively) to low doses of UV-C (Control - 0, C1 - 0.1 kJ $m^{-2}d^{-1}$, C2 - 0.3 kJ $m^{-2}d^{-1}$). Means ± SE, n = 6.

of UV-C treatment (Fig. 2B). The control values of hydrogen peroxide and activity of SOD (Fig. 1B) remained unchanged, but the activities of GST (Fig. 2A) and GR (Fig. 2B) increased with time.

DISCUSSION

Under normal conditions both the formation and removal of ROS are in balance and besides nonenzymatic compounds, the enzymatic antioxidants are also involved. SOD is a metal-containing enzyme that catalyses dismutation of superoxide to oxygen and H_2O_2 and act as the first line of defense against ROS (Alscher et al., 2002; Jaleel et al., 2009). Then catalase and peroxidases detoxify H_2O_2 (Jaleel et al., 2009; Foyer and Noctor, 2005). GSTs are multifunctional cytosolic proteins that conjugate electrophilic substrates, including some secondary metabolites to glutathione and reduce toxic organic hydroperoxides levels (Dixon et al., 2002; Foyer and Noctor, 2005). GR is a key

enzyme in the glutathione-ascorbate cycle and can regenerate reduced glutathione from its oxidized form. Together with glutathione, it is an important component of the ROS scavenging system in plant cells. SOD, catalase, peroxidases, GR and GSTs have different isoforms, whose number and amount could vary in plants during growth, normal and stress conditions. It was suggested that probably moderate enhancement of H_2O_2 in the 2nd pea leaf after 10 or 14 days of C2 regime was not connected with considerable oxidative damage in plant tissues (Katerova et al., 2008). Indeed, the lack of a considerable increase in the youngest leaves upon C2 treatment for 10, 14 or 21 days confirmed the above suggestion (Fig. 1A). The transient enhancement of H_2O_2 generation upon C1 in the youngest (Fig. 1A) and old pea leaves (Katerova et al., 2008) could be important as a secondary messenger that induces different defense processes in plants and could play a role in lignin synthesis (Murphy and Huerta, 1990; Foyer and Noctor, 2005). The increase in GST and GR activities observed with time in the control plants could be explained with acceleration of senescence in plants that led to higher ROS generation (Procházková and Wilhelmová, 2007) and needs higher levels of enzymatic antioxidants. Similar results of the effect of UV-C radiation were reported in other studies. SOD activity was found to decrease in the 2nd pea leaf upon 7 and 10 days of C2 treatment (Katerova et al., 2008), in cultured rose cells (Murphy and Huerta, 1990) and in tomato fruits (Barka, 2001) exposed to low UV-C dose. GR increased after single treatment with a high dose UV-C in the upper tobacco callus layer, however, it was unaffected in the lower layer (Zacchini and de Agazio, 2004). GST activity increased in the 2nd pea leaves after 10 days of C1 and C2 regimes, but GR activity was slightly decreased (Katerova et al., 2008). Some GSTs could act as signaling molecules, activating phenylpropanoid metabolism following exposure to UV light (Loyall et al. 2000). The increase in GST activity after 10 days of C2 regime (Fig. 2A) could contribute to flavonoid biosynthesis regulation important for the defense against UV radiation.

In conclusion, the moderate increase in H_2O_2 observed in the youngest leaves of C1-treated plants might act as a signal molecule to induce different defense systems. Exposure of pea plants to short pulses UV-C irradiation for 10, 14 and 21 consecutive days did not cause noticeable activation of the major antioxidant enzymes in the youngest leaves. The possibility of isoenzyme changes, however, could not be excluded and needs additional experiments.

REFERENCES

Alexieva V, I Sergiev, S Mapelli, E Karanov, 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ, 24: 1337-1344.

Alscher RG, N Erturk, LS Heat, 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot, 53: 1331-1341.

Barka E, 2001. Protective enzymes against reactive oxygen species during ripening of tomato (*Lycopersicon esculentum*) fruits in response to low amounts of UV-C. Aust J Plant Physiol, 28: 785-791.

Beauchamp C, I Fridovich, 1971.

Superoxide dismutase. Improved assay and an assay applicable to acrylamide gels. Anal Biochem, 44; 276-287.

Bradford M, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72: 248-254.

Córdoba C, JA Muñoz, V Cachorro, IA De Cárcer, F Cussó, FJ Jaque, 1997. The detection of solar ultraviolet-C radiation using KCl:Eu^{2+} thermoluminescence dosemeters. J Phys D App Phys, 30: 3024-3027.

Dixon D, A Lapthorn, R Edwards, 2002. Plant glutathione transferases. Genome Biol, 3: 1-10.

Foyer C, G Noctor, 2005. Oxidant and antioxidant signalling in plants; a re-evalution of the concept of oxidative stress in a physiological context. Plant Cell Environ, 28: 1056-1071.

Gronwald J, P Fuerst, C Eberlein, M Egli, 1987. Effects of herbicide antidotes on glutathione content and glutathione S-transferase activity of sorgum shoots. Pestic Biochem Physiol, 29: 66-76.

Häder D-P, HD Kumar, RC Smith, RC Worrest, 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci, 6: 267-285.

Jaleel CA, K Riadh, R Gopi, P Manivannan, J Inès, HJ Al-Juburi, ZS Hong-Bo, R Panneerselvam, 2009. Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. Acta Physiol Plant, 31: 427-436.

Katerova Z, E Shopova, L Brankova, S Ivanov, E Karanov, 2008. Alterations in antioxidant enzymes of pea plants in response to prolonged influence of short pulses ultraviolet-C radiations. Compt Rend Acad Bulg Sci, 61: 335-340.

Loyall L, K Uchida, S Braun, M Furuya, H Frohnmeyer, 2000. Glutathione and a UV light-induced glutathione S-transferase are involved in signaling to chalcone synthase in cell cultures. Plant Cell, 12: 1939-1950.

Murphy T, A Huerta, 1990. Hydrogen peroxide formation in culture rose cells in response to UV-C radiation. Phisiol Plant, 78: 247-253.

Procházková D, N Wilhelmová, 2007. The capacity of antioxidant protection during modulated ageing of bean (*Phaseolus vulgaris* L.) cotyledon 1. The antioxidant enzyme activities. Cell Biochem Funct, 25: 87-95.

Smith I, T Vierheller, C Thorne, 1988. Assays of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis (2-nitrobenzoic acid). Anal Biochem, 175: 408-413.

Zacchini M, M de Agazio, 2004. Spread of oxidative damage and antioxidative response through cell layers of tobacco callus after UV-C treatment. Plant Phisiol Biolchem, 42: 445-450.