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BRIEF COMMUNICATION

Low doses of ultraviolet-B or ultraviolet-C radiation affect phytohormones in young pea plants

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Abstract

Pea (*Pisum sativum* L., cv. Scinado) seedlings were exposed to low doses of ultraviolet-B (UV-B; 4.4 and 13.3 kJ m^{-2} d^{-1}) or UV-C (0.1 and 0.3 kJ m^{-2} d^{-1}) radiation for 14 d. Aminocyclopropane carboxylic acid (ACC), indoleacetic acid (IAA) and abscisic acid (ABA) contents were quantified by gas chromatography coupled to mass spectrometry (GC-MS). The accumulation of ACC upon irradiation was dose-dependent. ABA content was reduced and IAA content increased upon UV-C treatment whereas the UV-B doses used did not cause significant changes in ABA and IAA contents.

Additional key words: abscisic acid, aminocyclopropane carboxylic acid, indoleacetic acid, *Pisum sativum*, stress.

Due to depletion of the ozone layer, UV-B (280 - 315 nm) radiation has an increasing negative impact on living organisms. At mid latitudes, the biologically effective radiation $UV\text{-}B_{BE}$ may reach up to around 6 kJ m^{-2} d^{-1} but in some regions higher values (about 13 kJ m^{-2} d^{-1}) were detected (Paul 2001). Except for high mountains, UV-C (200 - 280 nm) does not reach the surface of the Earth, due to its absorption in the atmosphere (Häder *et al.* 2007). Nonetheless, Córdoba *et al.* (1997) detected a direct solar UV-C radiation, reaching the ground in Madrid (700 m a.s.l.) at levels of 3×10^{-6} kJ m^{-2} d^{-1} registered by $KCl:Eu^{2+}$ dosimeters.

Abscisic acid (ABA), indole-3-acetic acid (IAA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) are important regulators of plant responses to abiotic stresses. ABA is considered as a stress hormone that modulates adaptation to various stresses including UV-B radiation (Albinsky *et al.* 1999). IAA and other auxins are involved in developmental processes like growth, apical dominance and lateral root initiation (Ljung *et al.* 2001). Ethylene is an important element in both stress response and adaptation. Environmental stresses and hormonal signals stimulate the ethylene production through synthesis of ACC (Yu and Yang 1980, Hyodo *et al.* 1985, Nara and Takeuchi 2002, Nakajima *et al.* 2002). There is scarce information regarding the influence of UV-B on IAA (Huang *et al.* 1997, Yang *et al.* 2004), ABA (Rakitina *et al.* 2001, Yang *et al.* 2004) and ACC (Nara and Takeuchi 2002) contents and until now nothing seems to be published on the effect of low UV-C doses. Therefore we concentrated upon the effects of two-week UV-B and UV-C treatments on the ACC, ABA and IAA contents in pea seedlings.

Pea (*Pisum sativum* L., cv. Scinado) seeds were germinated 3 d on moist filter paper in the dark. The seedlings were transferred to Hoagland's solution and grown in a growth chamber (12-h photoperiod, photosynthetic photon flux density of 160 μmol m^{-2} s^{-1}, temperature of 24 ± 2 °C, air humidity of 60 - 70 %). Three days later, seedlings were exposed to UV radiation

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Abbreviations: ABA - abscisic acid; ACC - aminocyclopropane carboxylic acid; B1 - 4.4 kJ m^{-2} d^{-1} UV-B; B2 - 13.3 kJ m^{-2} d^{-1} UV-B; C1 - 0.1 kJ m^{-2} d^{-1} UV-C; C2 - 0.3 kJ m^{-2} d^{-1} UV-C; IAA - indoleacetic acid; PAR - photosynthetically active radiation; PFBBr - pentafluorobenzyl bromide; UV - ultraviolet radiation; $UV\text{-}B_{BE}$ - biologically effective dose of UV-B radiation.
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in the middle of the photoperiod for 14 consecutive days. UV-B and UV-C radiations were applied in two regimes possessing different durations and doses: B1 (70 s d^{-1}, 4.4 kJ m^{-2} d^{-1}) and B2 (240 s d^{-1}, 13.3 kJ m^{-2} d^{-1}) or 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}), respectively. The distance between UV lamps and the top of plants was 0.25 ± 0.04 m. Daily UV-B_{BE} irradiations were calculated according to Caldwell (1971). B1 and B2 doses were comparable with ambient UV-B radiation and UV-C irradiations used here were lower than the dose used to achieve beneficial effects for fruits (Paul 2001, Shama and Alderson 2005). UV-B and UV-C radiations were supplied by a mercury lamp (*HPQ Philips*, Eindhoven, The Netherlands; λ_{max} 313 nm) and a germicidal lamp (*STYLO STY 115*, *GE Lighting*, Milan, Italy λ_{max} 254 nm). UV-B radiation was filtered through a 0.13 mm cellulose acetate filter to remove wavelengths below 280 nm. UV-B lamp provided 0.0631 UV-B, 0.0350 UV-A and 0.0123 kJ m^{-2} s^{-1} PAR, and UV-C lamp 0.0050 UV-C, 0.0001 UV-A and 0.0004 kJ m^{-2} s^{-1} PAR. The spectra and radiation power of the UV lamps were deter-mined with an *AvaSpec-2048* spectrometer (*Avantes*, Eerbeek, The Netherlands).

ACC, ABA and IAA concentrations were assayed in the 4th leaf 20 h after the last treatment (14-d UV). Following the 14-d UV exposure and preceding ACC, ABA and IAA analyses, plants were kept in normal growth conditions, including light/dark regime. The measurements were repeated four times and leaves were pooled from four individual plants. Samples were frozen in liquid nitrogen and lyophilized. Samples (about 200 mg) were ground in liquid nitrogen and extracted overnight at -20 °C with 80 % (v/v) methanol. [$^2H_4$]-ACC (*Sigma*, St. Louis, MO, USA), [$^2H_6$]-ABA (*NRC-CNRC*, Saskatchewan, Canada) and $^{13}C_6$-IAA (*Cambridge Isotope Laboratories*, Woburn, MA) were added as internal standards (50, 50 and 75 ng, respectively). The ACC-fraction was collected from *DEAE-Sephadex A25* (formiate form) with 50 % (v/v) methanol. IAA and ABA were purified by a combined solid phase extraction procedure, derivatized with PFBBr, and analyzed by gas chromatography coupled to mass spectrometry (GC-MS; Prinsen *et al.* 1991). ACC samples were purified over a strong cation-exchange resin (*Extract-Clean*, 200 mg) as described by Persson and Näsholm (2001) then eluted and derivatized with PFBBr (Smets *et al.* 2003). The derivatized forms of IAA, ABA and ACC were purified by liquid-liquid extraction (ethyl acetate-water), separated by GC (*HP 5890* series II, *Varian* column 15 m, 0.25 mm I.D., film thickness 0.25 mm; gas phase He at flow rate of 1.5 cm^3 min^{-1}, temperature gradient from 150 to 325 °C in 2 min, injection temperature 325 °C) and detected by negative ion chemical ionization GC-MS (*VG TRIO-2000* quadrupole, Manchester, UK, using methane as ionizing gas). Chromatograms were processed by *Masslynx 3.2* software (*Micromass*, Manchester, UK). The data were examined by a one-way *ANOVA* followed by Duncan's multiple range test ($\alpha < 0.05$).

The ACC content increased in a dose-dependent manner after both UV-C and UV-B treatments (Fig. 1*A*). C2 regime, which has lower daily irradiance than B1, has stronger effect on ACC accumulation than B1. It is expected because UV-C photons posses more energy than UV-B and cause higher levels of injuries (Stapleton 1992). ACC accumulation is stimulated by several stress factors such as UV-B (Nara and Takeuchi 2002), ozone (Nakajima *et al.* 2002), cadmium, copper and wounding (Yu and Yang 1980, Hyodo *et al.* 1985). Our results are in line with this, and corroborate with the general idea that ACC accumulation is indicator of plant stress (Wang *et al.* 1990).

Treatments with UV-C (C1 and C2) for 14 d led to reduction of ABA contents in the 4th leaf (to 65 and 63 %, respectively, as compared to control), whereas UV-B did not alter significantly the ABA content (Fig. 1*B*). Other authors demonstrated that UV-B increased ABA content in *Arabidopsis* and tomato plants (Rakitina *et al.* 2001, Yang *et al*, 2004). The decrease of ABA content after UV-C treatment (Fig. 1*B*) could be a direct effect of UV-C radiation on the degradation of ABA and/or an effect on its biosynthesis. ABA contains a UV-sensitive α,β-unsaturated carbonyl group, an enone structure, in its six-member ring and irradiation with UV-C (254 nm) causes isomerisation and decomposition of ABA (Todoroki *et al.* 2001). Rodrigo *et al.* (2003) report on a

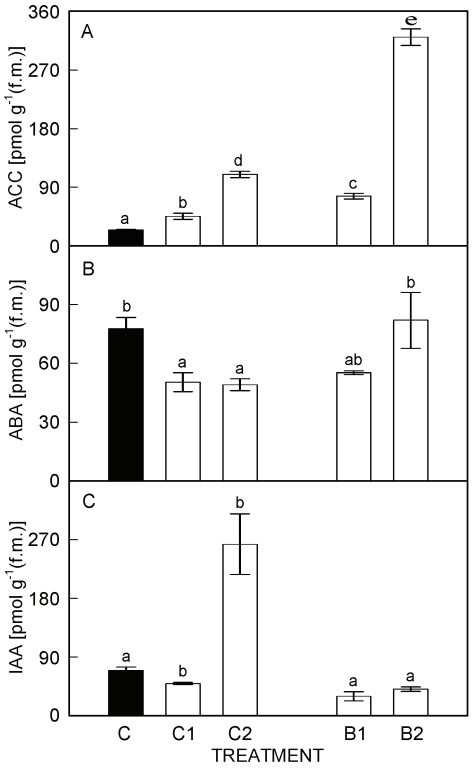

Fig. 1. Effect of pea exposure to 14 consecutive days of low doses UV-C (C1: 0.1 kJ m^{-2} d^{-1}, C2: 0.3 kJ m^{-2} d^{-1}) and UV-B_{BE} (B1: 4.4 kJ m^{-2} d^{-1}, B2: 13.3 kJ m^{-2} d^{-1}) radiation on ACC (*A*), ABA (*B*) and IAA (*C*) content in 4th leaves. Control is labelled C. Means ± SE (n = 4). Different letters indicate statistically significant difference ($\alpha < 0.05$, Duncan's multiple range test).

decreased content of ABA in citrus mutant defective in carotenoid biosynthesis and it is well known that carotenoids are precursors of ABA (Milborrow 2001). So it is possible that in UV-C treated plants biosynthetic pathway is switched from ABA to xanthophylls in order to protect plants by absorbing UV-light.

Surprisingly, we observed accumulation (377 % compared to control) of IAA upon irradiation with UV-C in C2 regime whereas the other treatments hardly affected the IAA contents (Fig. 1*C*). In these experiments, the simultaneous accumulation of IAA and ACC after 14 d of C2 treatment (Fig. 1*A*,*C*) was consistent with the fact that IAA-induced ethylene biosynthesis (Abeles 1966) was shown to be accompanied by increase in ACC content (Yu *et al.* 1979).

UV-B exposure of rice or tomato plants for 4 weeks reduces their IAA contents (Huang *et al.* 1997, Yang *et al.* 2004) because UV-B radiation could cause oxidation of IAA by peroxidases (Huang *et al.* 1997, Jansen *et al.* 2001). Changes in peroxidase activity, however, depend on plant species, it is not always increased under UV-B radiation and it could be important as defence against UV-B oxidative stress (Skórska and Szwark 2007, Agarwal 2007). We could not show any significant reduction in IAA content after UV-B treatments (Fig. 1*C*). The 14-d of treatment used in the present experiments, however, might not be sufficient to induce an effect in the endogenous auxin concentration. Jansen *et al.* (2001) show that UV-B tolerance is linked to IAA degradation rather than to the content of free or conjugated IAA. Possibly, the plant tends to keep a steady state level of free IAA through hydrolysis of IAA-conjugated and/or up-regulation of *the novo* IAA synthesis. A more active IAA metabolism or a larger pool of IAA-conjugates as compared to tomato and rice, might explain that in pea only a minor effect on the pool of free IAA is found. This possibly indicates that pea has a higher tolerance to UV-B radiation compared to tomato and rice plants. Indeed, the lack of a significant accumulation of stress hormone ABA under UV-B radiation is in agreement with this hypothesis (Fig. 1*B*). However, as the accumulation of ACC (Fig. 1*A*) indicates that pea plants are stressed under B1 and B2 regimes, it may be concluded that in pea plants lack of IAA degradation (Fig. 1*C*) is not sufficient as the only indicator for UV-B tolerance.

Prolonged (14-d) treatments of pea plants with low doses of UV-B or UV-C radiation led to increased ACC leaf contents indicating that plants are stressed. UV-C radiation reduced ABA and IAA content, while UV-B in B1 and B2 regimes that are analogous to ambient UV-B, did not change contents of these plant hormones. Further experiments, using different *Arabidopsis* mutants affected on hormone signalling, metabolism or UV response are necessary for better understanding of the exact mechanism on plant response to UV-B and UV-C stress.

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