ALTERATIONS IN INDOLEACETIC ACID, ABSCISIC ACID AND AMINOCYCLOPROPANE CARBOXYLIC ACID IN PEA PLANTS AFTER PROLONGED INFLUENCE OF LOW LEVELS ULTRAVIOLET-B AND ULTRAVIOLET-C RADIATIONS

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Summary. The response of pea (*Pisum sativum* L., *cv*. Scinado) plants to low UV-B and UV-C radiation levels was studied in the 2nd leaves in relation to IAA, ABA and ACC contents. Following regimes of UV-B and UV-C radiation were used for 7, 10 or 14 consecutive days: B1 (4.4 kJ m^{-2} d^{-1}), B2 (13.3 kJ m^{-2} d^{-1}) and C1 (0.1 kJ m^{-2} d^{-1}) and C2 (0.3 kJ m^{-2} d^{-1}). As compared to control, IAA decreased after 10 days of C2 irradiation, although B1 regime caused an increase. At the end of the experiment IAA was raised in C2-treated plants. ABA increased transiently in B1-treated plants (10d B1) but it dropped at the end of the experiment (14d UV) after both UV-B and UV-C irradiations. With one exception (7d B1), ACC accumulated in the treated plants. The increases in IAA and ABA concentrations after 10 days of B1 regime was interpreted in relation to plant adaptation. Moderate accumulation in ACC in C1- and B1-treated plants could also be connected with stimulation of ethylene signal pathway that is needed for the defence system against UV-induced damage.

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IAA reacted specifically to the difference between UV-B and UV-C treatment. It was suggested that there are different mechanisms in IAA, ABA and ACC response to prolonged influence of low UV-B and UV-C irradiations.

***Key words*:** ABA, ACC, IAA, *Pisum sativum*, UV-B, UV-C.
***Abbreviations*:** IAA - indoleacetic acid, 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, PAR - photosynthetic active radiation, PFBBr - Pentafluorobenzyl bromide, $UV\text{-}B_{BE}$ - biologically effective UV-B irradiaton.

INTRODUCTION

The depletion of the ozone layer, caused by human activity, led to an increase in UV-B (280-320 nm) radiation. The damaging effect of UV radiation increases towards shorter wavelengths: UV-A (320-400 nm) is less effective than UV-B, while UV-C (200-280 nm) are highly energetic, and quickly create high levels of injuries (Stapleton, 1992). UV-C does not reach the Earth's surface, due to its absorption in the atmosphere, with the exception of high mountain locations (Häder et al., 2007). Córdoba et al. (1997) detected a direct solar UV-C radiation, reaching the ground at Madrid (700 m a.s.l.), at levels six orders of magnitude lower than UV-B radiation. However, recently the commercial prospects are considered for treating fruits with beneficial doses (0.5-9.0 kJ m^{-2}) of UV-C that delay senescence and prevent pathogen infection during fruit post-harvest storage (Shama and Alderson, 2005).

IAA, ABA and the ethylene precursor 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 (Yang et al., 2004). 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 the stress responses and

adaptation (A-H Mackerness et al., 1999). Environmental stresses induced by ozone and UV-B stimulate ethylene production through synthesis of ACC (Ge et al., 2000; Nakajima et al., 2002; Nara and Takeuchi, 2002). It was suggested that the responses to UV-C follow pathways different from those elicited by UV-B radiation (Stapleton, 1992), but the differences regarding endogenous levels of the growth regulators have not been explored up till now. Therefore, we focused on the distinct effects of UV-B and UV-C treatments on the endogenous IAA, ABA and ACC levels.

MATERIALS AND METHODS

Plant material and treatment with ultraviolet radiations

Pea (*Pisum sativum* L., *cv*. Scinado) seeds were germinated 3 days on moist filter paper in dark. The seedlings were transferred into Hoagland's nutrient solution and grown in a growth chamber (12h/12h photoperiod, 160 μmol m^{-2} s^{-1} photosynthetic photon flux density, 24±2 °C, 60-70 % air humidity). When the 1st leaf appeared, seedlings were exposed to UV-B or UV-C radiation in the middle of the photoperiod for 7, 10 or 14 consecutive days. UV-B and UV-C radiations were supplied by a mercury (HPQ Phillips, Eindhoven, The Netherlands; λ_{max} 313 nm) and germicidal lamp (STYLO STY 115, GE Lighting, Italy λ_{max} 254 nm) at 0.25 ± 0.04 m distance from the top leaves. UV-B radiation was filtered trough 0.13 mm cellulose acetate to remove wavelengths below 280 nm. UV-B lamp provided 6.31 UV-B, 3.50 UV-A and 1.23 mJ cm^{-2} s^{-1} PAR, and the UV-C – 0.50 UV-C, 0.010 UV-A and 0.04 mJ cm^{-2} s^{-1} PAR. Low and moderate regimes were used for UV-B (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 UV-C (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}). $UV\text{-}B_{BE}$ was calculated using the generalized plant action spectrum (Caldwell, 1971). We used UV-B doses comparable with ambient UV-B radiation (Paul, 2001). The choice of UV-C doses was conditioned by the fact that the natural intensity for UV-C range is lower than ambient UV-B (Córdoba et al., 1997).

Endogenous ACC, ABA and IAA assay

ACC, ABA and IAA were assayed after 7, 10 or 14 consecutive UV irradiations in the 2nd leaves, 20 h after the end of a given treatment. The measurements were repeated four times and leaves were taken from four individual plants. Samples were frozen in liquid nitrogen and lyophilized. They were ground in liquid nitrogen and extracted overnight at –20 °C with 80 % (v/v) methanol. [$^2H_4$]-ACC, [$^2H_6$]-ABA and $^{13}C_6$-IAA were added as internal standards. 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 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 ml 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 the ionizing gas). Chromatograms were processed by Masslynx 3.2 software (Micromass, UK).

Statistics

Data were examined by one-way ANOVA followed by Duncan's multiple range test ($P<0.05$). Values in figures are mean ± SE ($n=4$).

RESULTS

UV-B and UV-C lights affected endogenous IAA concentrations (Fig. 1B-C). After 10 days of treatment they decreased in C2-irradiated plants, and increased after B1 regime as compared to control (Fig. 1B). Four days later (14d UV) IAA accumulated markedly upon C2 treatment, whereas no significant alterations were found in UV-B-irradiated plants. The alterations

in IAA differed in plants exposed to UV-C radiation, as compared to those after UV-B treatments (Fig. 1).

Levels of ABA increased significantly, as compared to the control, following 10 days of B1-treatment (Fig.2 B). At the end of the experiment (14d UV) ABA concentrations decreased in both UV-C and UV-B treated plants (Fig. 2C). With small exceptions, the endogenous ACC increased markedly after UV-C and UV-B lights, although the strongest alterations were found upon exposure to UV-B radiation (Fig. 3).

DISCUSSION

An opposite trend in IAA content was detected after 10 days of UV-B irradiation, as compared to UV-C regimes (Fig. 1B). By contrast to other authors who showed that IAA decreased in rice and tomato

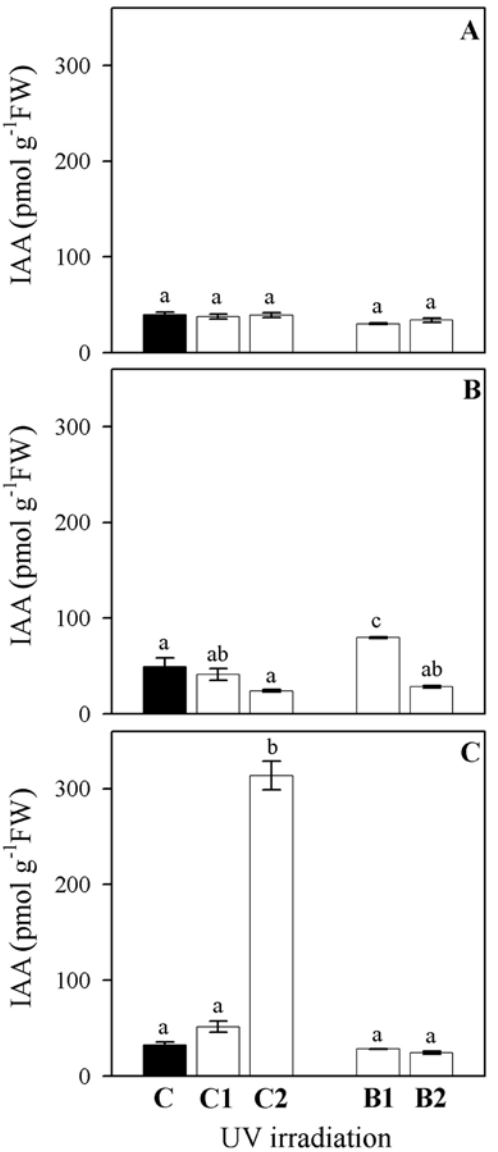
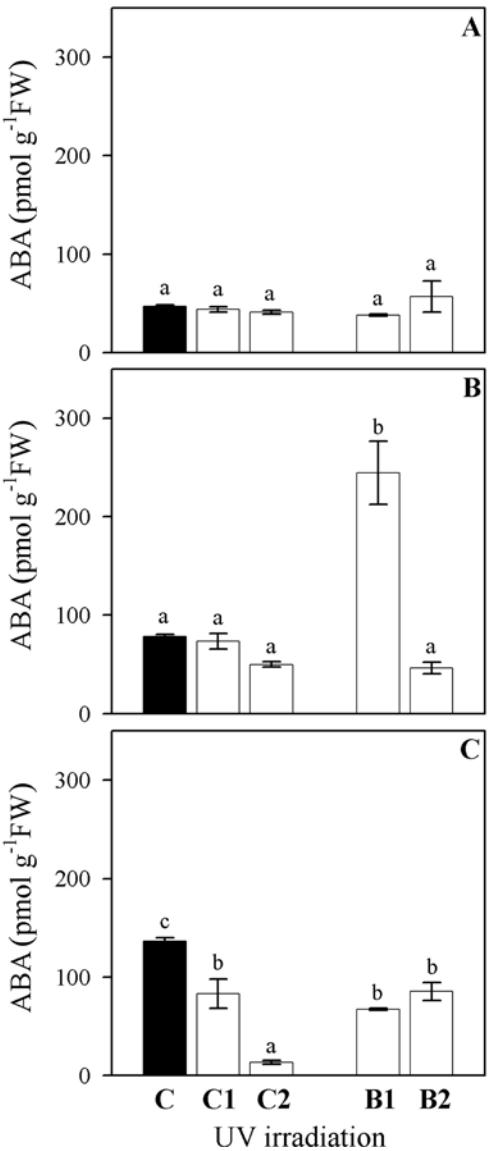


Fig. 1. Endogenous IAA concentration in leaves of pea plants exposed to low doses of UV-C (C1: 0.1 kJ m^{-2} d^{-1}, C2: 0.3 kJ m^{-2} d^{-1}) and UV-B_{BE} (B1: 4.4 kJ m^{-2} d^{-1}, B2: 13.3 kJ m^{-2} d^{-1}) radiation for 7 (A), 10 (B) and 14 (C) consecutive days. Control is labelled C. Different letters indicate statistically significant difference (P<0.05, Duncan's multiple range test). Means ± SE (n=4).



plants exposed for 4 weeks to ambient UV-B radiation (Huang et al., 1997; Yang et al., 2004), we found that UV-B light increased IAA concentration in B1 treated pea plants (Fig. 1B). One explanation that we could not detect a significant reduction in IAA level after B1 and B2 irradiations could be the shorter period of treatment (2 weeks) as compared with other studies. Moreover, Jansen et al. (2001) showed that UV-B tolerance was linked to IAA degradation rather than to levels of free or conjugated IAA. Thus pea could be more tolerant to UV-B radiation than tomato and rice plants because we do not detect a reduction in free IAA levels. The fact that IAA reached the control value at the end of the experiment (14 days UV-B) confirms this statement (Fig. 1C). Additionally, both IAA and ABA increased in B1-

Fig. 2. Endogenous ABA concentration in leaves of pea plants exposed to low doses of 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 for 7 (A), 10 (B) and 14 (C) consecutive days. Control is labelled C. Different letters indicate statistically significant difference (P<0.05, Duncan's multiple range test). Means ± SE (n=4).

treated plants after 10 days of irradiation (Figures 1A and 2B). Grossman and Hansen (2001) have stated that auxin-stimulated ethylene triggers an increase in ABA biosynthesis which functions as a second messenger. Although it is known that UV-B radiation caused degradation in IAA by oxidation of peroxidases (Huang et al., 1997; Jansen et al., 2001), this possibility is excluded in our study concerning UV-B treated plants but not UV-C plants (Fig. 1B). In opposite to the reduction found after 10 days of UV-C treatment, at the end of experiment (14d UV-C) IAA increased markedly after C2 regime (Fig. 1B-C). The simultaneous accumulation of IAA and ACC in C2-treated plants at the end of the experiment (14d C2) and after B1 regime (10d B1) was consistent with the fact that IAA-induced ethylene

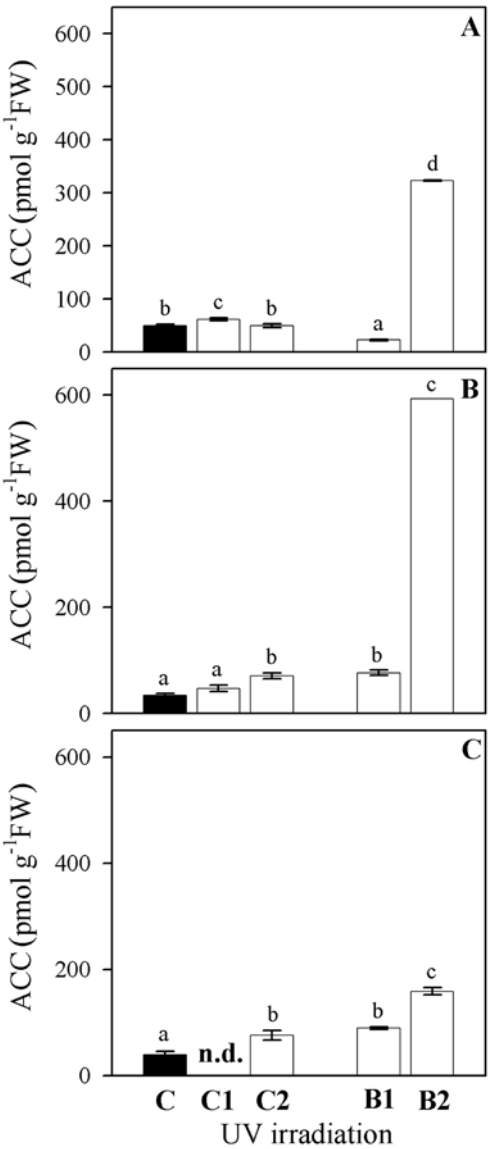


Fig. 3. Endogenous ACC concentration in leaves of pea plants exposed to low doses of UV-C (C1: 0.1 kJ m^{-2} d^{-1}, C2: 0.3 kJ m^{-2} d^{-1}) and UV-B_{BE} (B1: 4.4 kJ m^{-2} d^{-1}, B2: 13.3 kJ m^{-2} d^{-1}) radiation for 7 (A), 10 (B) and 14 (C) consecutive days. Control is labelled C. Different letters indicate statistically significant difference (P<0.05, Duncan's multiple range test). Means ± SE (n=4).

biosynthesis (Abeles, 1966) was accompanied by increased ACC content (Yu et al., 1979).

By contrast to other studies where enhanced ABA levels was reported upon UV-B irradiation in tomato and arabidopsis plants (Rakitina et al., 2001; Yang et al., 2004), a clear reduction was manifested in pea plants after UV-C treatments (Fig. 2). A transient increase in ABA detected in B1-treated plants (10d B1) might have a key role in changing gene expression leading to stress adaptation (Rock, 2000). The decrease of ABA levels could be a direct effect of UV- radiation on ABA degradation or an effect on its biosynthesis. Decreased ABA levels were observed in citrus mutant defective in carotenoid biosynthesis (Rodrigo et al., 2003) and it is known that carotenoid biosynthesis in plants is connected with that of ABA (Milborrow, 2001). So it is possible that ABA synthesis in UV-treated plants was switched to xanthophylls synthesis to protect plants by absorbing UV-light.

Irradiation with either UV-B or UV-C caused a substantial enhancement in endogenous ACC content, more pronounced when UV-B was applied. C2 regime, which had lower daily irradiance than B1, had an equal or stronger effect on ACC accumulation than B1. It is expected because UV-C photons possess more energy than UV-B and cause a higher degree of damage. ACC is stimulated by several stress factors, such as UV-B (Nara and Takeuchi, 2002) and ozone (Nakajima et al., 2002). The present results are in line with these data and corroborate with the idea that ACC concentrations are indicative for the strength of plant stress (Wang et al., 1990). Despite the fact that the effect of low UV-C radiation levels were lower than the applied UV-B intensities, we showed that both UV-lights, especially C2 and B2, led to a significant increase in ACC (Fig. 3).

Low UV irradiation levels led to stimulation of a specific response connected with plant defense (Frohnmayer and Staiger, 2003). It could be assumed that there is a difference in the specific UV-B and UV-C defense responses regarding endogenous IAA, ABA and ACC (Fig. 1-3). The C1 regime does not cause a significant alteration in IAA levels, but led to a substantial reduction in ABA and some induction in ACC. By contrast to C1 regime, B1 increased IAA level that is mentioned to have capacity in ACC induction and also caused accumulation of ABA. Possibly, accumulation in

ACC is connected with increased ethylene concentrations that are important in the ethylene signal pathway required for defence against UV-induced damage (A-H Mackerness et al., 1999). By contrast to C1 regime, transient accumulation of IAA caused by B1 could stimulate ABA synthesis required for adaptation (Rock, 2000). The possibility that ABA synthesis can be switched to xanthophylls synthesis, contributing to UV-protection by its absorption, can not be excluded after exposure of pea plants to C1 and B1 radiations. In opposite to low UV-regimes, C2 and B2 led to an obvious increase in ACC, known as a stress indicator, especially after B2. The enormous accumulation in IAA after C2 treatment (14d C2) is an additional indication for stress events (Fig. 1C)**.**

A distinct IAA response was detected after C2 and B2 regimes, also after C1 and B1 (Fig. 1). Therefore, IAA reacted specifically to the difference between UV-B and UV-C wavelengths.

Acknowledgements: This work was supported by a grant from the Interuniversity Poles of Attraction Programme (Belgian State, Prime Minister's Office-Federal Office for Scientific, Technical and Cultural Affairs; IUAP-V P5/13) and by specialization fellowship No 13DA-IZ/ JDW/BUL/2003-2004/03-2250 from the bilateral cultural program between the Ministries of Bulgarian and Flemish Community.

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