BIOLOGIA PLANTARUM 52 (1): 170-172, 2008

BRIEF COMMUNICATION

The effects of salicylic acid on pigment contents in ultraviolet radiation stressed pepper plants

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Abstract

Pepper (*Capsicum annuum* L.) plants were sprayed with salicylic acid (SA) and treated with ultraviolet radiation UV-A (320-390 nm), UV-B (312 nm), and UV-C (254 nm) of 6.1, 5.8, and 5.7 W m^{-2}, respectively. UV significantly reduced contents of chlorophyll (Chl) *a* and *b*, and carotenoids (Car). SA treatment moderated Chl and Car reduction in plants treated with UV-B and UV-C. The quantity of antocyanins, flavonoids, rutin, and UV-absorbing compounds in plants that were treated with UV-B, UV-C, and SA were significantly increased. Foliar spray of SA counteracted the UV effects on pepper.

Additional key words: anthocyanins, *Capsicum annuum*, carotenoids, chlorophyll, flavonoids, rutin, UV-absorbing compounds.

Protective responses stimulated by UV-radiation include increase in production of UV-absorbing compounds and secondary compounds including hydroxyl cinnamic acid derivatives, phenylpropanoids, and flavonoids which effectively absorb the UV-radiation (Hofmann *et al*. 2000, Skórska and Szwarc 2007). Anthocyanins also have a wide distribution in mature and senescent leaves. These compounds generally have two absorption maxima, one at 240 - 290 nm and the other at 500 - 550 nm. The strong UV-absorption of anthocyanins has led to the hypothesis that these compounds may protect leaves from the harmful effects of UV-radiation (Woodall and Stewart 1998).

Salicylic acid (SA) and related compounds induce significant effects on various biological aspects in plants (Raskin 1992). Exogenous application of SA increased yield and number of pods in mung bean (Singh and Kaur 1980). SA reversed the closure of stomata caused by abscisic acid (ABA) (Rai *et al*. 1986). Further, SA retarded ethylene synthesis, affected membrane depolarization, stimulated photosynthetic machinery, increased the content of chlorophyll (Chl) as well as blocked wound response in soybean (Leslie and Romani 1988, Zhao *et al*. 1995). SA is required in the signal transduction for inducing systemic acquired resistance against some pathogenic infections (Gaffney *et al*. 1993, Métraux *et al*. 1990). The aim of the present study was to investigate the effects of SA on UV-A, UV-B, and UV-C stressed plants.

In five-weeks-old pepper (*Capsicum annuum* L.) plants grown in *Vermiculite* in plant growth chamber 1.5 mM SA solutions were sprayed once on leaves in early morning, when the plants had their fourth leaf completely expanded. A constant volume was sprayed in all cases with a manual pump. Then the plants were exposed to UV-A (320-390 nm), UV-B (312 nm), and UV-C (254 nm) irradiation with a density of 6.1, 5.8, and 5.7 W m^{-2}, respectively (measured with a UV sensor model of *Leybold Didactic*, Germany). UV-A, UV-B, and UV-C lamps were purchased from the *UV-Tech* (UK). Each pot was treated with UV in their light period for 27 min per day for 14 d. The plants were divided into four groups: *1*) control, *2*) plants treated with SA (1.5 mM), *3*) plants that received UV-A, UV-B, or UV-C, *4*) plants pre-treated with SA (1.5 mM) and then exposed to UV-radiation. Four replicates were used in each experiment.

Received 19 January 2007, *accepted* 5 August 2007.
Abbreviations: Car - carotenoids, Chl - chlorophyll, SA - salicylic acid, UV - ultraviolet.
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Chlorophylls (Chl) and carotenoids (Car) were extracted in 80 % acetone and absorbances measured with a UV/visible spectrophotometer *2100-WPA* (*WPA*, UK) at 470, 648, and 663 nm and quantities of pigments were calculated as described by Lichtenthaler (1987). Anthocyanin content was measured by the method of Wagner (1979). For determination of UV- absorbing compounds leaf discs (0.65 cm diameter; three discs) were placed in 4 cm^3 of 99:1 methanol:HCl and extracted for at least 48 h at -20 °C. Absorbance was read at 305 nm (Rousseaux *et al*. 2001). To determine the absorption by flavonoids, 0.1 g of fresh leaf tissue were taken from the distal ends of an upper leaf and were extracted in 15 cm^3 glass centrifuge tubes containing 10 cm^3 ethyl alcohol: acetic acid (99:1, v:v). The samples were gently boiled for 10 min in a water bath at 80 °C and brought up to volume. Absorbance was measured at 270, 300, and 330 nm with UV-VIS spectrophotometer (Krizek *et al*. 1998). Four plants from each treatment group were chosen randomly for rutin analysis. The frozen samples (100 mg) were extracted with methanol: acetic acid: water (100:2:100 v/v) at room temperature for 1 h. The HPLC (*Agilent*, USA) analysis were performed by the injection of 20 mm^3 of extract on a *Lichrospher 100RP-18* (5 μm) column (250 × 4 mm), elution with a gradient of methanol (*A*) and 2 % acetic acid in water (*B*) (40 % *A* to 70 % *A* in 0 - 2 min, 1 cm^3 per min), and detection at 355 nm. Rutin eluted at 7.3 min and the peak area was compared with the standard.

Chl *a*, Chl *b* and Car contents (Table 1) of plants which were exposed to UV-B and UV-C decreased significantly. Photosynthetic pigment contents of plants which were exposed to UV-A did not change significantly. SA treatment moderated Chl and Car reduction ($P < 5$ %).

Exposure to UV-B and UV-C significantly increased the contents of anthocyanins and UV-absorbing compounds of leaves and this effect was stimulated by SA application (Table 1).

Extracts of fresh leaves taken from plants that were treated with UV-A, UV-B, and UV-C radiation showed significant increase in UV absorbance at 270, 300, and 330 nm. SA application increased the content of flavonoids which had absorbance at 270 nm in plants which were exposed to UV-A radiation. SA application increased the content of flavonoids with absorption maximum at 300 nm in plants exposed to UV-A, UV-B, and UV-C and content of flavonoids with absorption maximum at 330 nm in plants treated with SA and then exposed to UV-B and UV-C (Table 1).

Plants exposed to UV-A, UV-B and UV-C showed significant increase in rutin content of leaves and SA application further increased rutin content in plants exposed to UV (Table 1).

The UV-induced decrease in contents of photosynthetic pigments in agreement with findings of Takeuchi *et al*. (2002) in rice. Caldwell *et al*. (1995) concluded that in sensitive plants, UV-B and UV-C significantly decreased Chl contents, primarily because UV-B destroyed the structure of chloroplast, inhibited synthesis of Chl and increased the rate of Chl degradation. Cars function as efficient quenchers of short-wave radiation (Middleton and Teramura 1993). We found that SA treatment counteracted pigment destruction. SA-treated maize plants contained more Chl and Car than control or UV-treated plants (Sinha *et al*. 1993). Also in soybean plants treatment with SA, increased pigment contents (Zhao *et al*. 1995). We found that in plants treated with SA and exposed to UV-B and UV-C, anthocyanin content increased. Similar increase and protective effects have also been suggested for anthocyanins in *Syzygium* (Woodall and Stewart 1998). Contents of flavonoids, rutin, and UV-absorbing compounds increased rapidly in response to UV-B and UV-C radiation. The flavonoids play many defensive roles in plants, and interception of UV-B by epidermal flavonoids is often proposed as an adaptive mechanism preventing UV-B from reaching the mesophyll and affecting photosynthesis (Liu *et al*. 1995). Our HPLC analysis showed an increase in rutin under UV-B, UV-C

Table 1. Changes in contents of chlorophyll (Chl) *a* and Chl *b*, carotenoids (Car) [g kg^{-1}(f.m.)], anthocyanins (Ant) [mol kg^{-1}(f.m.)], UV-absorbing compounds (UV-AC) [relative units], flavonoids (F) with absorption maxima at 270, 300 and 330 nm [%], and rutin [g kg^{-1}(f.m.)] of pepper plants in response to UV-A, UV-B, and UV-C treatments in presence or absence of salicylic acid (SA). Means ± SE, means significantly different at $P = 5$ % level are marked by different letters.

Parameter	Control	SA	UV-A	UV-B	UV-C	UV-A + SA	UV-B + SA	UV-C + SA
Chl *a*	15.67±1.11^{c}	27.58±1.06^{a}	14.93±1.25^{cd}	11.55±0.48^{de}	8.29±0.87^{e}	18.75±0.25^{bc}	20.00±2.12^{b}	17.50±2.1^{bc}
Chl *b*	10.29±0.67^{d}	32.25±1.31^{a}	9.77±1.03^{d}	6.89±0.85^{e}	4.08±0.71^{e}	31.50±0.87^{a}	20.00±1.15^{b}	13.50±0.96^{c}
Car	5.97±0.65^{c}	13.50±0.65^{a}	5.75±0.50^{c}	3.96±0.39^{d}	2.38±0.29^{e}	13.00±0.58^{a}	10.50±0.29^{b}	7.00±0.41^{c}
Ant	17.95±0.85^{e}	24.11±0.83^{c}	18.35±0.85^{e}	21.05±0.75^{d}	24.00±0.65^{c}	19.61±0.67^{de}	27.25±0.48^{b}	30.50±0.65^{a}
UV-AC	0.43±0.03^{d}	0.59±0.06^{bc}	0.50±0.05^{cd}	0.68±0.01^{b}	0.73±0.07^{b}	0.65±0.02^{b}	0.94±0.03^{a}	1.05±0.07^{a}
F_{270}	1.06±0.06^{c}	1.53±0.03^{a}	1.37±0.01^{b}	1.59±0.07^{a}	1.73±0.06^{a}	1.55±0.04^{a}	1.75±0.12^{a}	1.80±0.05^{a}
F_{300}	0.49±0.08^{f}	1.05±0.08^{e}	1.13±0.01^{e}	1.30±0.01^{c}	1.33±0.01^{b}	1.26±0.02^{bc}	1.41±0.01^{a}	1.44±0.01^{a}
F_{330}	1.28±0.01^{e}	1.31±0.02^{e}	1.54±0.02^{d}	1.72±0.02^{c}	1.74±0.03^{c}	1.56±0.03^{d}	1.85±0.03^{b}	1.93±0.03^{a}
Rutin	145.56±3.95^{g}	225.66±3.33^{f}	240.00±10.0^{f}	346.00±8.72^{d}	452.66±9.33^{b}	275.00±14.4^{e}	390.00±5.77^{c}	493.33±3.33^{a}

and SA treatments, which may protect plants against oxidative stress, or prevent the penetration of UV-radiation to the more sensitive tissue. UV radiation also increased the production of rutin in six *Hypericum* plants (Umek *et al*. 1999). The results obtained suggest that SA increases resistance of pepper plants against UV radiation and oxidative stress.

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