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Article

Changes in Carbohydrate, Phenolics and Polyamines of Pepper Plants under Elevated-UV-B Radiation

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Pepper plants (*Capsicum annuum*, cv. Manitta) were subjected to different intensities of UV-B radiation to understand alterations of primary- and secondary-metabolism such as carbohydrates, phenolic compounds and polyamines. UV-B doses with a UV-B lamp (1.2 W m^{-2}) were adjusted between 0 to 9 hr. The soluble sugars and starch contents in pepper leaves were highly influenced by UV-B treatment. The soluble sugars altered from 6.7 mg g^{-1} fw to 5.2 mg g^{-1} fw after 9 hrs of UV-B exposure. The starch contents after 3 hrs of UV-B exposure changed from 17.7 mg g^{-1} fw to 12.3 mg g^{-1} fw and then remained unchanged. The absorbance of UV-absorbing compounds reached initially maximum at all wavelengths read. On the basis of this result, we analyzed total phenolics, anthocyanin and simple free phenolic acids. Anthocyanin and free phenolic acids responded sensitively with a steady increase during UV-B treatment, although anthocyanin contents declined highly after 3 hrs of treatment. Whereas, there is no alteration of total phenolics (as gallic acid equivalent) caused by UV-B. Free polyamine levels in leaves increased rapidly and highly when UV-B was treated. The most prominent changes in polyamine induction were putrescine and spermidine (+ 70 %) after 3 hrs and spermine (+ 150 %) after 6 hrs.

Key words : Carbohydrate, Phenolic compound, Polyamine, UV-B, pepper

INTRODUCTION

Since several decades a reduction of the stratospheric ozone layer has rapidly progressed, caused by large-scale emissions of industrial air-pollutants. Consequently, the consecutive depletion of ozone layer has increased UV-B radiation reaching the earth's surface. Enhanced solar UV-B may affect the structure and function of terrestrial ecosystems either directly or indirectly. UV-B can affect photosynthesis indirectly by altering stomatal function, photosynthetic pigments, leaf anatomy and canopy morphology (Teramura & Sullivan, 1994). Therefore, an inhibition of photosynthetic processes caused by enhanced UV-B may also influence soluble carbohydrate metabolism (Yue et al., 1998). Plants are known to respond to harmful solar UV-B radiation with several defenses and repair mechanisms. PAL catalyzes the transformation of phenylalanine into trans-cinnamic acid, which is a central intermediate in the formation of complex phenolic compounds such as falvonoids, condensed tannins and lignin (Meijkamp et al., 1999). Secondary metabolites, especially phenolic acids and flavonoids as plant defense components are responsible for plant tolerance to UV-B, fulfilling the dual role as screens that reduce UV-B penetration in leaf tissues, and as antioxidants protecting from damage by reactive oxygen species (Bornman et al., 1998; Rice-Evans et al., 1997; Rozema et al., 2002). Lois and Buchanan (1994), Cullen and Neale (1994), Landry et al. (1995) and Strid et al. (1994) have all suggested that flavonoids and phenyl derivatives are involved in the protection against UV-B in higher plants. Polyamines may play a role in protecting plants from UV-B stress. Polyamines possess a number of anti-senescent activities and are induced in response to many stressors in plant systems. Bors et al. (1989) reported that polyamines were mostly involved in protective mechanisms like scavenging of active oxygen species. Recently, it has been reported that polyamines play a main role in the regulation of structure and function of the photosynthetic apparatus (Kotzabasis,

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1996; Sfichi et al., 2004). The aim of this study is to describe UV-B effects on carbohydrate, phenolics and polyamines in pepper plants. We investigated the impact of different UV-B doses on leaf component that could be a possible marker.

MATERIALS AND METHODS

Plant growth and UV-B exposure After germination in plug tray, pepper (*Capsicum annuum* L.) plants were transferred into grown at 25/20℃ (day and night), with a 12-h photoperiod under fluorescent white light (300 μmol m^{-2} s^{-1}) in a controlled environmental growth chamber. After 25 days of germination, pepper plants were subjected to UV-B irradiation with a UV-B lamp (radiation 290-/320 nm) at an irradiance of UV light (1.2 W m^{-2}) which was filtered through 0.1 mm thick cellulose acetate filters. Pepper plants were exposed to UV-B light during from 0 to 9 hrs. The biologically effective UV-B doses were determined by Caldwell (1971). All samples were collected immediately after UV-B exposure and then stored at -70℃ for further analysis.

Carbohydrates Soluble sugars were extracted by heating leaf discs in 80 % of ethanol, suggested by Roe method (1955). Soluble sugars were analyzed by the reaction of 1.0 ml of the alcoholic extract with 2.0 ml fresh 0.2% anthrone in sulfuric acid (w/v) and the absorbance was read at 630nm. After the extraction of the soluble fractions, the solid fraction was used for starch analysis. Starch was extracted with 9.3 N perchloric acid first and 4.6 N perchloric acid again. The extracts were combined, and starch concentration was determined after reaction with the anthrone reagent. Glucose was used as standard for soluble sugars and starch.

Assay of UV-absorbing compounds and phenolic acids Determination of the absorbance of methanolic extract was examined according to Mirecki method (1984) with slight modification. Fresh leaf samples (0.2 g) were subjected to an extraction solution (MeOH : HCl : water = 79 : 1 : 20) and centrifuged. The absorbance at 270, 280, 300 and 320 nm was measured for analysis. The samples, equivalent to 1g of fresh leaves, were homogenized in 5 ml of 50 % MeOH in water including 1 N HCl with modification of Kader's method (1996). An aliquot of extracts was partitioned with 4 ml of ethyl ether ($\times$ 3), dried under N_2 gas and the resultant contained free phenolic acids (FPA). The fraction was reconstituted in MeOH and used for HPLC analysis (Waters Assoc., Milford, MA, USA). The mobile phase consisted of 50 % acetonitrile including 0.5 % acetic acid (solvent A) and 2 % acetic acids in water (v/v) (solvent B). The gradient program of mobile phase was as follows: 90 % A to 70 % A in 15 min, 70 % A to 50 % A in 15 min and 50 % to 10 % A in 20 min. The peaks were monitored simultaneously at 320 nm.

Total anthocyanin pigment content Concentration of monomeric anthocyanins was determined by the pH differential method as described by Wrolstad (1976). In brief, frozen leaf samples (1 g) were homogenized with 10 ml of 4 % acetic acid in ACN, the extracts were shaken at 200 rpm for 1 hr at 30℃, filtered through 0.45 μm Nylon membrane filter, and an aliquot (0.2 ml) was mixed with 1.0 ml of corresponding buffers (A, 0.025 M KCl (pH 1.0); B, 0.4M Na-acetate (pH 4.5)). The mixture was read at 510 and 700 nm, respectively. Anthocyanin pigment was calculated as mg cyanidin-3-glucoside per liter using the extinction coefficient of 29,600.

Polyamines Leaf discs frozen in liquid nitrogen were homogenized with 5 % cold PCA. The homogenates were kept for 1 h at room temperature and then centrifuged for 25 min at 9,000 rpm. The benzoyl polyamines (Redmond & Tseng, 1979) were separated on a 5 μm - 25 cm x 4.6 mm (RP-C18) column, using elution gradient from 60 to 45 % acetonitrile in the mobile phase. Free polyamines were monitored with an UV detector (254 nm). The peaks were identified with reference to the retention times of polyamine standards prepared as described above. Quantitative determination was based on external standards.

Data analysis This experimental design was a randomized complete design with five replicates. The data obtained from this study were subjected to analysis of variance (ANOVA). The multiple comparisons between treatments were made using LSD ($p< 0.05$). The statistical software used was SAS (version 8. 12).

RESULTS AND DISCUSSION

The contents for soluble sugars and starch are presented in Fig. 1. It was observed that biosynthesis of soluble

sugars and starch was strongly influenced by UV-B treatment. Soluble sugars in pepper leaves reduced to about 20 % in proportion with increase in UV-B dose. The amount of soluble sugars was 6.7 mg g^{-1} fw at the initiation of UV-B treatment, however decreased in 5.2 mg g^{-1} fw after 9 hrs of UV-B exposure. Generally the amount of starch was relatively high compared with soluble sugars. Like soluble sugars, starch responded sensitively by enhanced UV-B level. Starch content before UV-B treatment was 17.7 mg g^{-1} fw, however decreased in 12.3 mg g^{-1} fw after 3 hrs of UV-B treatment and then remained unchanged until the termination of UV-B treatment. Carbohydrates were rapidly and strongly influenced as supplemental UV-B light was irradiated. The supplemental UV-B radiation induced a decrease of soluble carbohydrates in leaves of *Triticum aestivum* (Yue et al., 1998) and *Oryza sativ*a (Sung et al., 2005), and decreased sucrose and starch contents in leaves of the moss *Polyrichum commune* (Barsig et al., 1998). However, some studies reported that UV-B induced an accumulation of starch in cucumber (Britz and Adamse's, 1994) and maize (Santos et al, 1993).

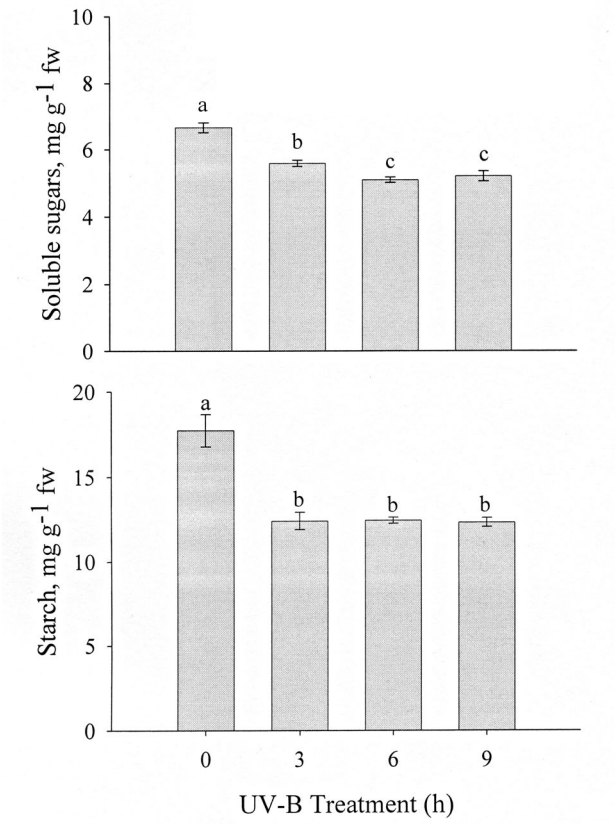

Fig 1. Changes in soluble sugars and starch in response to UV-B radiation in pepper plants. Letters above the bars are used to designate significant differences ($p < 0.05$, $n = 5$). Error bars represent standard deviation.

Following exposure to UV-B radiation, UV absorbing compounds including primarily phenolic acids and flavonoids at 270, 280, 300 and 320 nm were extracted from pepper leaves in an acidified solution (Fig. 2). These compounds reached maximum within 3 hrs and then declined. Their fluctuation was similar and a higher level was found at 270 and 320 nm. The level of UV absorbing compounds was enhanced up to 20-30 % at all wavelengths measured during experiment period. Plants treated with UV-B showed changes in phenolic compounds contents (Fig. 3, Table 1). The accumulation of phenolic compounds in cell may be related to defense against biotic as well as abiotic factors. Total phenolics as gallic acid equivalents remained relatively unchanged for up to 9 hrs of UV-B treatment whereas anthocyanin, which is colored flavonoids, resulted in a large increase (about 5.5 fold) within 3 hrs of UV-B treatment. As regarding that flavonoids have the maximal absorbance at 320 nm, it is reasonable that anthocyanin and UV-absorbing compounds in response to UV-B treatment showed the same pattern. Cinnamic acid- and benzoic acid-derivatives in UV-B treated pepper leaves resulted in a large increase except coumaric acid and benzoic acid (Table 1). The UV-B treatment for 9 hrs increased 8.0 (cinnamic acid), 2.0 (caffeic acid), 2.5 (ferulic acid) and 4.0 (sinapic acid) fold as compared to its respective control, however, coumaric contents remained relatively unchanged throughout UV-B radiation. Benzoic acid also was insensitive to UV-B treatment whereas syringic acid derived from benzoic acid showed an increase in about 3 fold higher than the control (UV-B 0 hr). An exposure to UV-B radiation may increase the concentration of UV-B-

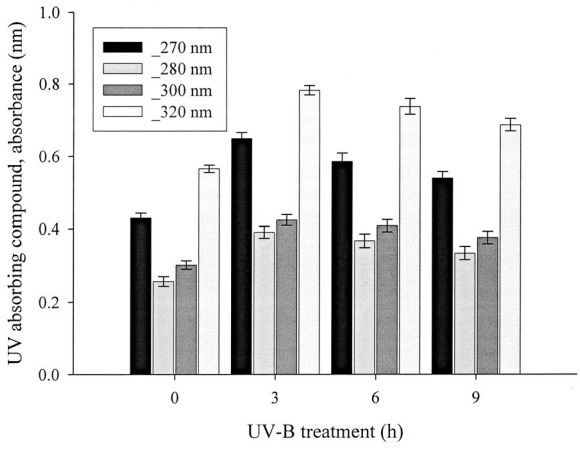

Fig. 2. Effect of supplementary UV-B radiation (1.2 W m^{-2}) on absorbance changes in UV-absorbing compounds in pepper plants ($p < 0.05$, $n = 5$). Error bars represent standard deviation.

Table 1. Compositional changes in free phenolic acids in leaves of pepper plants by elevated-UV-B radiation.

UV-B treatment	Phenolic acids, ug g^{-1} fw						
	Hydroxybenzoic	Cinnamic	Coumaric	Caffeic	Ferulic	Sinapic	Syringic
0 hr	126.5a	1.1b	4.8a	1.8b	18.7c	6.7c	2.4b
3 hr	101.0a	8.9a	5.6a	2.1b	19.0c	27.1a	2.6b
6 hr	115.6a	8.0a	5.2a	3.6a	36.7b	21.2b	7.1a
9 hr	126.3a	8.0a	5.2a	3.8a	46.3a	24.3ab	7.4a

The same letters were not significantly different within a column at LSD (P<0.05, n=5).

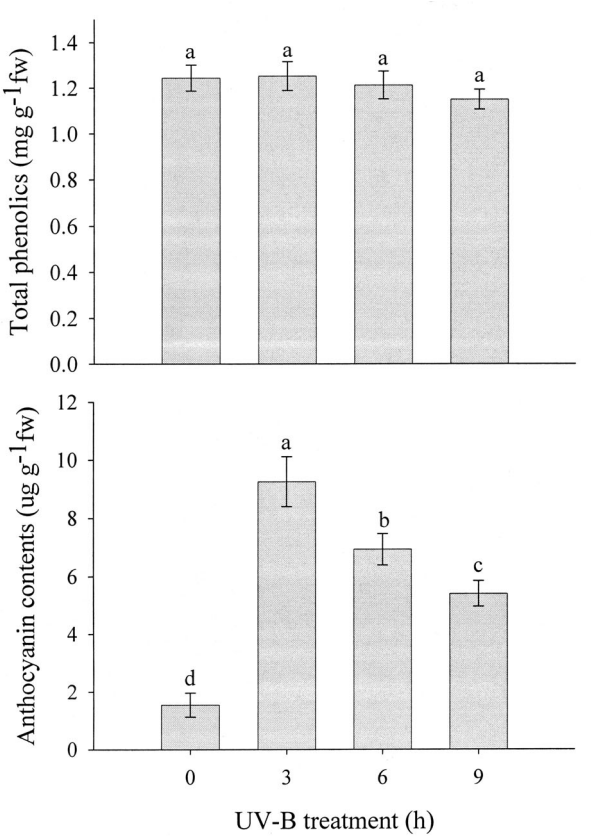

Fig. 3. Changes in total phenolic (as gallic acid equivalent) and anthocyanin during supplementary UV-B radiation (1.2 W m^{-2}). Letters above the bars are used to designate significant differences (p < 0.05, n = 5). Error bars represent standard deviation.

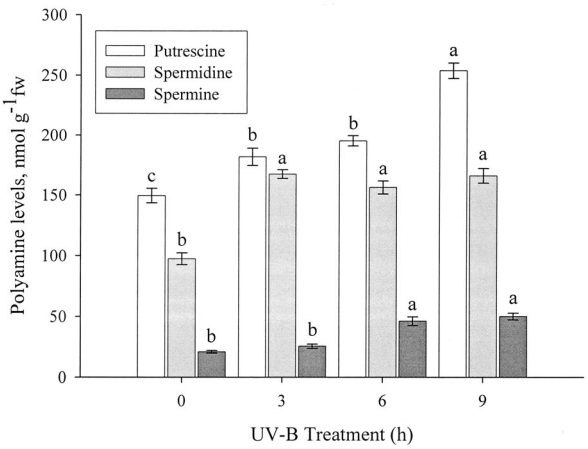

Fig. 4. Changes in free polyamine levels in the leaves of pepper plants treated with supplementary UV-B radiation (1.2 W m^{-2}). Letters above the bars are used to designate significant differences (p< 0.05, n = 5). Error bars represent standard deviation.

absorbing compounds in the epidermis. Phenolic acids, ferulic- and synipic-acid, involved in lignin biosysthesis exhibited higher amounts. In this study we observed changes in anthocyanin and simple phenolic acids (cinnamic acid- and benzoic acid-deveratives) in response to supplementary UV-B radiation (Figs. 2-3, Table 1) which also indicate that phenolic acids and anthocyanin are synthesized by UV-B treatment and accumulate in the epidermal layer to protect the more sensitive sites against UV-B impact. Hydroxycinnamic acids are important structural components which serve to cross-link polymers in plant cell walls, and ferulic- and coumaric- acids especially exhibited the highest concentration (Kroon & Williamson, 1999). Hydrocinnamic acid derivatives are constituents of lignin, our results also appeared to promote the synthesis of them. The analyses of polyamine contents in the leaves of control and high UV-B treated plants revealed a different pattern for endogenous polyamine regulation (Fig. 4). UV-B treated plants showed remarkable changes in polyamine levels already after 3 hrs of exposure to UV-B radiation. As UV-B treatment continued (i. e., 3 hrs treatment) plants have already started to increase polyamine levels, with spermidine showing higher alteration than spermine and putrescine. This UV-B mediated induction of polyamines was less obvious after 3 hrs except for spermine, which showing an increase after 6 hrs of UV-B treatment. Polyamine levels increased about 21 % (putrescine), 73 % (spermidine) and 23 % (spermine) after 3 hrs of UV-B treatment compared with the control (UV-B 0 hr), and about 70 % (putrescine and spermidine) and 150 % (spermine) after 9 hrs. Similar results have also been reported by some authors (Kramer et al., 1992; Sung et al., 2005). They reported that intracellular polyamines, especially putrescine and spermidine, increased during UV-B stress treatment. UV-

B, forming free radicals, evoke oxidative stress in plants, which form polyamines as radical scavengers (Navakoudis et al. 2003), and the large subunit of Rubisco under UV-B stress can be stabilized by polyamines which associated with the light harvesting complex and the photosystem II (Besford et al. 1993). In our study, free polyamines were up-regulated under elevated-UV-B. Even from the onset of the UV-B treatment, their levels increased rapidly.

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자외선 조사에 의한 고추 유묘의 탄수화물 합성과 항산화물질 변화

성좌경[1] · 이수연[1] · 박재홍[3] · 이상민[1] · 이용환[1] · 최두회[1] · 김태완[4] · 송범헌[3,*]

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고추 유묘기에 탄수화물 합성, 페놀화합물 및 폴리아민에 대한 자외선의 영향을 알아보기 위하여 수행하였다. 고추 유묘는 1.2 W m^{-2} 강도의 자외선 하에서 0부터 9시간까지 조사되었다. 수용성 당과 전분은 자외선 처리에 의해 크게 영향을 받았다. 수용성 당은 처리 전 (6.7 mg g^{-1} fw)에서 자외선 9시간 처리 후에 5.2 mg g^{-1} fw으로 약 22% 감소하였다. 전분은 자외선 처리 후 3시간 만에 30%까지 급격히 감소한 후 처리기간에 상관없이 변화가 없었다. 자외선 흡수물질에 대한 흡광도 분석을 한 결과, 처리 3시간 이후에 최고 흡광도를 나타내었으며 이를 토대로 하여 페놀화합물과 안토시아닌을 조사하였다. 흡광도 분석결과와는 다르게 안토시아닌 함량은 자외선 처리가 증가함에 따라 감소하였으며, 총 페놀화합물 함량은 자외선에 영향을 받지 않았다. 그러나 주요 단순 페놀산 함량은 크게 영향을 받았는데, cinnamic acid, ferulic acid, sinapic acid 및 syringic acid는 자외선 처리 후 2~8배 증가하였다. 식물체내 주요 항산화물질의 하나인 폴리아민도 자외선에 의해 70~15% 증가하였다. 위의 결과로 볼 때, 자외선은 식물체의 탄수화물의 합성을 억제하며, 식물체는 스트레스에 저항하기 위하여 방어물질인 페놀화합물 및 폴리아민의 합성을 촉진하였다.