EJEAFChe
Electronic Journal of Environmental, Agricultural and Food Chemistry

ISSN: 1579-4377

ELEVATED ULTRAVIOLET-B RADIATION INFLUENCES PHOTOSYNTHETIC PIGMENTS AND SOLUBLE CARBOHYDRATES OF SWEET ALMOND [*PRUNUS DULCIS* (MILLER) D. WEBB]

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ABSTRACT
Due to anthropogenic influences, solar UV-B irradiance at the earth's surface is increasing. To determine effects of enhanced UV-B radiation on sweet almond (*Prunus dulcis*), one-year old seedlings were submitted to four levels of UV-B radiation namely 0 (UV-BC), 0.45 (UV-B1), 1 (UV-B2), 1.50 (UV-B3), and 1.96 (UV-B3) W m^{-2} at a PAR intensity of 300 μ mol m^{-2} s^{-1} for 5 weeks. UV-B stress led to a significant decrease ($p<0.05$) in photosynthetic and non-photosynthetic pigments such as chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content, whereas a significant increase was observed for UV-B absorbing pigments (flavonoid and anthocyanin), total soluble sugars and relative electrolyte conductivity. Most of the studied parameters were only affected at high UV-B levels (> UV-B3).

KEYWORDS
ultraviolet-B radiation, Prunus dulcis, photosynthetic pigments, soluble sugars, electrolyte conductivity.

INTRODUCTION

Plants are exposed to a multitude of natural biotic and abiotic stressors. Almost all stressors affect either directly or indirectly the photosynthesis performance of leaves (Harmut and Babani, 2000). Differences in photosynthetic rates are most likely to be observed under conditions of environmental stress (Earl and Tollenaar, 1999), like e.g. drought (Rouhi *et al.* 2006) and salinity (Ranjbarfordoei *et al.* 2006). Most of the abiotic stresses are connected to anthropogenic activities which are clearly causing major changes in atmospheric chemistry and climate (Reddy *et al*., 2004). The anthropogenic destruction of earth's stratospheric protective ozone layer is of concern because the ozone column is the primary attenuator of solar UV-B radiation (280-320 nm). Reduction of the ozone layer has led to a substantial increase in UV-B radiation at the earth's surface, with the amount and intensity dependent on atmospheric and geographic factors (Madronich *et al*., 1998; Reddy *et al*., 2004). Several studies have indicated that supplemental UV-B radiation can deleteriously affect plant physiological processes (Rathore *et al*., 2003). Besides the unfavorable effects of UV-B radiation on plant physiological activities, Agrawal *et al*. (2004) reported the deleterious effects of UV-B on biochemical processes in two cultivars of wheat (*Triticum aestivum* L.). Furthermore, it has been reported that the supplementation of visible radiation with UV-B radiation causes a reduction in amounts of chlorophyll contents, soluble protein and Rubisco in leaves of C_3 plants (Laila, 2001). Numerous studies have demonstrated that the accumulation of flavonoids and anthocyanins by plants provide a defense mechanism against UV-B radiation (Bieza and Loise, 2001). UV-B radiation induces changes in plant foliar chemistry, including decreases in carbohydrates concentrations (Richard *et al*., 2000). Among different plant species, long-living trees are more susceptible to environmental changes at a much higher degree than annul crops and other herbaceous plants, because the effects may accumulate. Therefore, even small environmental changes can have a detrimental effect over the life time of trees. Historical documents show that cultivated almond (*Prunus dulcis*) originated from wild species that evolved in the mountain slopes of west-central Asia.

The species became adapted to mild, wet, and dry winters and hot summers. In west-central Asia, cultivated almond can be found in parts of Iran, Turkmenistan, Uzbekistan, Tadzhikistan, and Afghanistan (Dale and Norman, 1996; Ranjbarfordoei *et al*., 2002). In Iran, but also in other places worldwide, orchard crops such as almond, walnut, hazelnut and grapes are mainly confined to mountain regions and higher altitudes. At higher altitudes, more UV-B can reach the earth's surface, because the atmosphere tends to be cleaner and thinner than at lower elevations. In general, each km increase in altitude increases the ultraviolet flux by about 6% (Diffey, 1991). Although almond is an important orchard crop in Iran and many other countries, few experiments have addressed the influence of UV-B radiation on almond. Therefore the aim of the present study was to evaluate the effects of UV-B stress on seedlings of *P. dulcis* based on leaf pigments (photosynthetic and non-photosynthetic pigments) and soluble carbohydrates content.

MATERIALS AND METHODS

Plants: One-year-old almond seedlings [*Prunus dulcis* (Mill.) D.A. Webb], of a height of 0.70 m, were purchased from an almond nursery in Saman city (situated in the province of Chaharmahal-Bakhtiary, Iran). Seedlings were brought to a glasshouse located at Shahrekord University in early March 2007 and were transferred, with the least root

disturbance, to 4000 cm^3 plastic pots filled with a mixture of local sifted soil, sand and farm yard manure in the proportion of 2:2:1 by volume. Plants were kept in the glasshouse with mean daily minimum and maximum air temperature of 22–31°C, respectively. Relative humidity of the air was around 55 %, and plants were subjected to natural PAR (600-750 μmol m^{-2} s^{-1}). In order to maintain constant soil moisture, the pots were hold at field capacity throughout the experiment.

UV-B treatments: In early July 2007, the seedlings were exposed to the UV-B treatments. UV-B radiation was artificially supplied by 36 W fluorescent lamps (UV-B, Zhejiang Yongkang Yongxin Industry Co., China) following the procedure described by Lydon *et al*., (1986). The lamps were suspended above and perpendicular to the pots and wrapped with 0.13 mm cellulose diacetate (CA) film to cut off UV-C radiation shorter than 290 nm. The CA filter on the lamps was replaced weekly to avoid photodegradation of CA properties caused by UV-B radiation. The spectral irradiance from the lamps was determined with a UV spectroradiometer (MSS2040, MSS - Electronic - Gmbh, Germany). The generalized plant action spectrum (Cadwell 1971), normalized at 300 nm, was used in accordance with the methods mentioned by Correia *et al*., (2005). Five levels of UV-B irradiation of 0, 0.45, 1.00, 1.50 and 1.96 $W.m^{-2}$ (UV-BC to UV-B4, respectively) were used for 6 h at the middle of the photoperiod (daily from 10h00 to 18h00 local time).

Pigments and metabolites: Concentration of photosynthetic pigments, flavonoids and non-structural carbohydrates were determined in fully extended leaves randomly collected from the apical section of the almond seedlings. Chlorophyll content was measured on fresh leaf tissues. Samples pulverized with liquid nitrogen. Subsequently, photosynthetic pigments were extracted by 80 % acetone and put in the freezer at -5 ºC for 24 h. Pigments were determined according to Lichtenthaler formulae (1987) using a spectrophotometer (Uvikon 930). Leaf pellets remaining after centrifugation were dried at 60 ºC in an oven and weighed. Amounts of chlorophyll *a* and *b* and total carotenoid were calculated on bas of dried mater as mg. g^{-1} dry mater.

UV-B absorbing pigments (flavonoids and anthocyanins) were extracted from fresh leaf samples using the method of Jordan *et al* (1994). Fresh leaves were ground to a powder in liquid nitrogen. The flavenoids and anthocyanins were extracted in 10 ml of acidified methanol (79:20:1, v/v, methanol: water: HCL). Absorption spectra of the extracts were spectrophotometricaly determined and flavonoid and anthocyanin concentrations were estimated from absorption intensities (A_b) at 300 and 530 nm, respectively (Nogués *et al*. 1998).

Total soluble sugars (sucrose, glucose and fructose) and starch were extracted from fresh leaf samples. These were ground in two x10 ml volumes of 80 % ethanol (80: 20, v/v, ethanol: water), centrifuged and supernatants adjusted to 25 ml in volumetric flasks for spectrophotometric determination of total soluble sugars. Residues were dried at 60 °C and weighed. Starch concentrations were analyzed by hydrolyzing the residues in 5 ml 3·6 % HCl at 100 °C for 3 h, centrifuging and analyzing the resultant sugars in the extracts. Soluble sugar and starch concentrations were expressed as mg g^{-1} leaf dry mass. Non-structural carbohydrate concentrations were calculated as the sum of total soluble sugars and starch (Charles *et al*. 2003).

For determination of the relative electrolyte leakage fresh leaves were placed into 50 ml capped flasks containing 17 ml distilled water. After 2 h shaking (100 rpm) at

room temperature, the conductivity of the solution (EC_i) was measured with a conductivity meter (LF315, WTW, Weilheim, Germany). After determination of EC_i, leaves were boiled in their immersion solution for 30 min, cooled at room temperature and total leaf electrolyte conductivity (EC_f) was measured. The relative electrolyte leakage was calculated as the ratio of EC_i over EC_f (Saladin *et al*, 2003).

Statistical analysis: The experimental layout was a completely randomized design. All values are means of five replicates per treatment. Statistical significance was calculated at $P < 0.05$ according to Duncan's multiple range test.

RESULTS

The changes in plant chlorophyll pigments (chl *a* and *b*) during the 5 weeks of UV-B treatments are shown in Figure 1. Exposure of almond seedlings to increasing UV-B intensity reduced the content of both pigments with a maximal loss of 29 % and 35 % at UV-B4 for chlorophyll *a* and *b*, respectively. Significant depressing effects of UV-B radiation on chl. *a*, compared to the control (UV-BC), started at UV-B2 and was maximal at UV-B4. No significant difference in chl. *a* content was observed between the control and the lowest UV-B stress level (UV-B1). Chlorophyll *b* content of the low (UV-B1) and moderate (UV-B2) stress levels was not significantly different from the control treatment. As can be seen in figure 1C, significant inhibitory effects of UV-B stress on chl. *b* were initiated at high stress levels (> UV-B3), and was most pronounced at the highest stress level (UV-B4). As a consequence of the observed effects on both chlorophyll *a* and *b* UV-B stress caused a significant decrease in total chlorophyll content (Chl *a* + *b*) when UV-B stress exceeded 1.00 W m^{-2}. The highest reduction in chlorophyll content was observed for UV-B4, and amounted 30 % compared to UV-BC (Table 1).

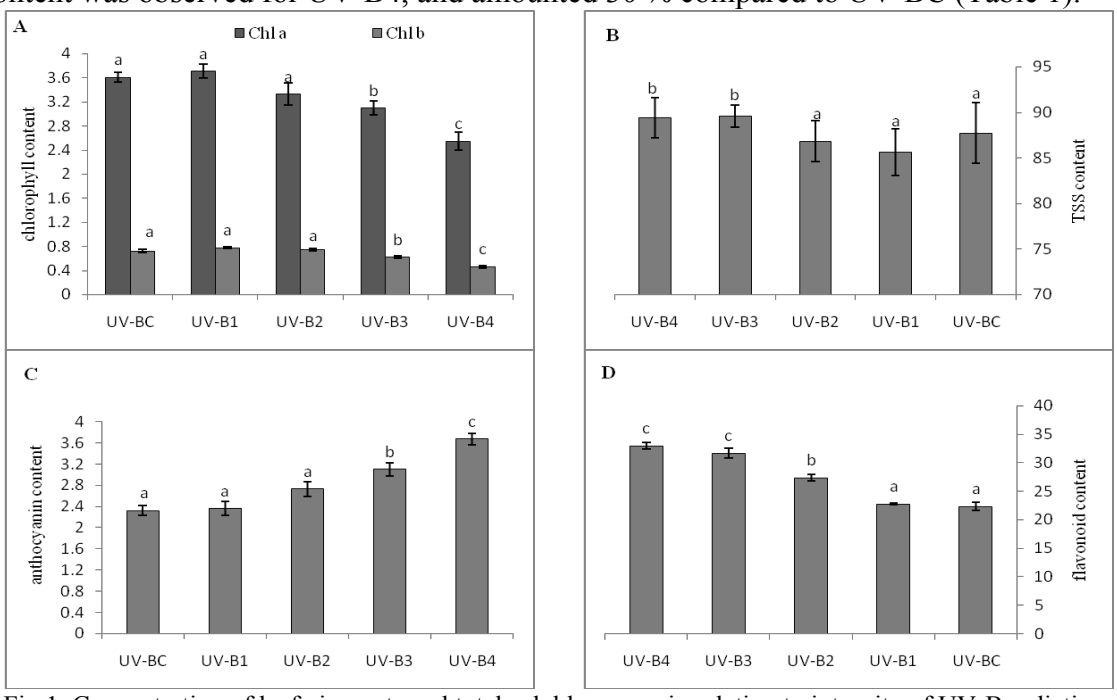

Fig.1. Concentration of leaf pigments and total soluble sugars in relation to intensity of UV-B radiation (UV-BC, UV-B1, UV-B2, UV-B3 and UV-B4). (A and B) chlorophyll (*a* and *b*) and total soluble sugars (TSS) contents [mg. g^{-1} (DM)], respectively. C and D antocyanin and flavonoid contents, expressed as absorbance per gram fresh weigh of tissue at 300 and 530 nm, respectively. Different letters express significantly different results among UV-B treatments ($P < 0.05$). Data are mean values ± S.E. ($n = 5$).

Table1. Effects of increasing UV-B radiation intensity ($W.m^{-2}$) on chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid contents (the four in mg g^{-1} dry mass), flavonoid and anthocyanin contents (expressed as absorbance per gram fresh weight of leaf tissue at 300 and 530 nm, respectively), total soluble sugars [TSS (mg g^{-1} dry weight) and electrolyte conductivity [EC (%)] in *P. dulcis* seedlings.

	UV-B radiation treatment				
Pigments and metabolites	UV-BC	UV-B1	UV-B2	UV-B3	UV-B4
Chlorophyll *a*	3.613±0.083a	3.716± 0.116a	3.331± 0.179a	3.109± 0.121b	2.553± 0.148c
Chlorophyll *b*	0.720±0.028a	0.779±0.012a	0.748±0.02a	0.623±0.014b	0.462±0.02c
Chlorophyll *a* + *b*	4.334± 0.093a	4.496± 0.11a	4.080± 0.156a	3.732± 0.115b	3.0164± 0.113c
Carotenoid	0.732±0.007a	0.738±0.012a	0.773±0.015a	0.806±0.013b	0.800±0.005b
Flavonoid	22.355±0.664a	22.765±0.209a	27.430±0.555b	31.660±0.854c	32.980±0.596c
Anthocyanin	2.322±0.09a	2.362±0.13a	2.72±0.14a	3.103±0.13b	3.670±0.114c
TSS	86.746± 3.32a	85.638± 2.51a	86.846± 2.61a	89.555± 1.22b	89.388± 2.22b
Electrolyte conductivity	14.215± 0.32a	14.13±0.12a	15.485±0.65a	17.155±0.61b	19.782±0.42c

Different letters express significantly different results between UV-B stress levels (a, b, c). Means ± SE (n = 5).

Carotenoid concentration only increased significantly by about 10 % for UV-B intensities above UV-B2, and then remained constant. Pigments that absorb UV-B radiation strongly are considered to play a major role in protecting plant from UV-B damage. Flavonoid concentration showed an increasing trend with increasing of UV-B intensity, with a significant increase of more than 41 and 47 % for the two highest UV-B stress levels (UV-B3 and UV-B4, respectively) compared to UV-BC. Enhanced UV-B intensities also had a progressive effect on the anthocyanin content. Compared to UV-BC, anthocyanin content at the most severe stress level applied (UV-B4) increased by 58 % (Figure 1A).

No change in the total soluble sugar content (TSS) was observed for UV-B intensities below UV-B3, but at higher intensities TSS increased significantly with 3 % (Fig.1D). Relative electrolyte leakage (REL) increased when UV-B intensity was above UV-B2. REL increased up to 21 and 39 % for UV-B3 and UV-B4, respectively, compared to UV-BC (Table 1).

DISCUSSION

Several studies have indicated that increasing UV-B intensities adversely affects a number of physiological and metabolic processes in higher plants (Ambash and Agrawal, 1994). In this study, it was revealed that the response of almond plants to UV-B radiation resulted in a significant reduction of chlorophyll content [chl. *a*, chl. *b* and (chl. *a* +*b*)] while the chlorophyll *a/b* ratio significantly increased (data not shown). Chlorophyll pigments are the central part of the energy capturing system of every green plant and therefore, any significant alteration in their concentrations is likely to cause a marked effect on the plants' life (Shweta and Agrawal, 2006). Reduction in chlorophyll contents by excess UV-B radiation has been reported in sessile oak (*Quercus petraea* L.) (Mészáros *et al*., 2001) while chlorophylls of *Quercus rubra* were not affected by enhanced UV-B radiation in both greenhouse and field conditions (Bassman and Robberecht, 2006). A decreased chlorophyll *b* concentration is a more common symptom of UV-B radiation stress (Strid and Porra, 1992). This can be attributed to inhibition of biosynthesis of pigments under UV-B exposure (Charles *et al*, 2002). Mackerness *et al*.

(1999) suggested that under UV-B stress plants sacrifice their chloroplasts in order to protect the rest of the cell.

The different levels of supplementary UV-B had a clear effect on carotenoid content. Seedlings responded with increasing carotenoid concentration. These points to the photo-protection role of carotenoids in photosynthetic systems by dissipating excess excitation energy through the xanthophylls cycle (Demmig Adams and Adams, 1992).

Accumulation of UV-B absorbing pigments (flavonoid and anthocyanin) is one of the ways by which plants alleviate the harmful effects of UV-B stress. In this study, flavonoid concentrations were significantly increased in leaves of almond plants (Table 1). Increase in flavonoid content is in support of the results obtained by Shweta and Agrawal (2006) in spinach (*Spinacia oleracea* L.), by Mirna *et al*. (2004) in quinoa (*Chenopodium quinoa* Willd.) and by Rathore *et al*. (2003) in wheat (*Triticum aestivum* L.).

The marked increase of anthocyanin concentration in response to increase in UV-B radiation intensity indicates that accumulation of this pigment provides a useful tool to protect plants against the harmful effects of UV-B radiation. A strong correlation (R = 97%) was found between the amounts of anthocyanin or flavonoid accumulated by almond plants and enhancement of UV-B intensity. Several reports have also suggested increasing in flavonoid and / or anthocyanin content following UV-B exposure (Reddy *et al*., 2004; Rathore *et al*., 2003; Day, 2001; Rozema *et al*., 1997b).

Several studies on the effects of UV-B radiation on plant carbohydrates have been carried out but they have been contradictory, some indicating increases in response to UV-B (Mirna *et al*., 2004.; Marcel *et al*., 2001) and others indicating decreases (Richard *et al*, 2000; Carlos *et al*., 2005).This may be due to diversity of plant tissue or experimental conditions. In the present work, significant effects of UV-B radiation on total soluble carbohydrates (TSS) was only observed at high and severe UV-B stress levels. Such increases have been reported in UV-B irradiated leaves of pea and corn and *Phylica pubescens* Aiton. (Santos *et al*., 1993; He *et al*., 1994; Charles *et al*., 2002).

In this study, enhanced UV-B radiation resulted in a significant increment in membrane leakage (as indicated by increase in EC) at high and severe stress levels (Table 1). An increasing in EC can be attributed to oxidative stress factor such as activation of superoxid radicals (Saladin *et al*., 2003) and increase in lipid peroxidation (Laila, 2001).

In conclusion, the present study confirms that in UV-B treated almond plants there are effective mechanisms for protecting the structure of photosynthesis system. To date, little information exist on almond plant response to enhancement of UV-B radiation, further research should be focused on the subject.

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