Photosynthesis performance in sweet almond [*Prunus dulcis* (Mill) D. Webb] exposed to supplemental UV-B radiation

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Abstract

Due to anthropogenic influences, solar UV-B irradiance at the earth's surface is increasing. To determine the effects of enhanced UV-B radiation on photosynthetic characteristics of *Prunus dulcis*, two-year-old seedlings of the species were submitted to four levels of UV-B stress, namely 0 (UV-B$_c$), 4.42 (UV-B$_1$), 7.32 (UV-B$_2$) and 9.36 (UV-B$_3$) kJ m^{-2} d^{-1}. Effects of UV-B stress on a range of chlorophyll (Chl) fluorescence parameters (FPs), Chl contents and photosynthetic gas-exchange parameters were investigated. UV-B stress promoted an increase in minimal fluorescence of dark-adapted state (F$_0$) and F$_0$/F$_m$, and a decrease in variable fluorescence (F$_v$, F$_v$/F$_m$, F$_v$/F$_0$ and F$_0$/F$_m$) due to its adverse effects on photosystem II activity. No significant change was observed for maximal fluorescence of dark-adapted state (F$_m$). Enhanced UV-B radiation caused a significant inhibition of net photosynthetic rate (P_N) at UV-B$_2$ and UV-B$_3$ levels and this was accompanied by a reduction in stomatal conductance (g_s) and transpiration rate (E). The contents of Chl *a*, *b*, and total Chl content (*a+b*) were also significantly reduced at increased UV-B stress. In general, adverse UV-B effects became significant at the highest tested radiation dose 9.36 kJ m^{-2} d^{-1}. The most sensitive indicators for UV-B stress were F$_v$/F$_0$, Chl *a* content and P_N. Significant $P<0.05$ alteration in these parameters was found indicating the drastic effect of UV-B radiation on *P. dulcis*.

Additional key words: chlorophyll content, fluorescence quenching, photosystem.

Introduction

Plants are exposed to a multitude of natural biotic and abiotic stressors. Almost all stressors affect either directly or indirectly the photosynthetic performance of leaves (Lichtenthaler 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*. 2007) 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, Balakrishnan *et al*. 2005). Several studies have indicated that supplemental UV-B radiation can deleteriously affect plant physiological processes (Rathore *et al*. 2003). However, Musil *et al*. (2003) reported that photosynthesis of *Podalyria calyptrate* was unaffected by enhanced UV-B. Supplementation of PAR radiation with UV-B radiation causes a reduction in Chl content (Mészáros *et al*. 2005), photosynthetic CO$_2$ assimilation, and photosynthetic efficiency (Xiaoqin *et al*. 2008). Furthermore, it has been reported that photosystem II is often snsitive to UV-B radiation and has often been assumed to be the most sensitive photosynthetic target for UV-B (Melise *et al*.1992). 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 less dense than at lower elevations. In general, each km increase in altitude increases the ultraviolet flux by about 6% (Diffey 1991).

Plant species vary greatly in their response to UV-B. Long-lived trees such as almond must be the most impacted by the permanent exposure and accumulation of the effects (Láposi *et al.* 2005). Although almond is an important orchard crop in Iran and many other countries, few experiments have addressed the influence of UV-B radiation on this tree. Therefore, the present study aims to clarify the effects of UV-B stress on seedlings of *P. dulcis* based on leaf photosynthesis as measured by means of Chl fluorescence, gas exchange and Chl content.

Materials and methods

Plants: Two-year-old almond seedlings [*Prunus dulcis* (Mill) D.A. Webb], of a height of 1.2 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 late March 2008 and were transferred, with the least root disturbance, to 6,000 cm^3 plastic pots filled with a mixture of local sifted soil, sand and farm yard manure in the proportion of 2:2:1 (v/v/v). Plants were kept in the glasshouse with mean daily minimum and maximum air temperature of 25–38°C, respectively. Relative humidity of the air was around 50%, and plants were subjected to the natural photoperiod till the end of July. In order to maintain constant soil moisture, the plants were uniformly watered throughout the experiment twice a day using a circulating system consisting of a water pump, gutter, and water reservoir. Each time the pumps were switched on for 30 min (early in the morning and late in the afternoon). A total of 20 almond plants, *i.e.*, five plants per treatment, were used in this experiment, each plant serving as an experimental unit.

UV-B treatments: In early August 2008, 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 (Caldwell 1971), normalized at 300 nm, was used in accordance with the methods mentioned by Correia *et al.* (2005). Four levels of UV-B irradiation of 0, 4.42, 7.32, and 9.36 kJ m^{-2} d^{-1} (UV-B$_c$ to UV-B$_3$, respectively) were used for 6 h at the middle of the photoperiod (daily from 10:00 to 16:00 h of the local time).

Gas-exchange measurements: P_N, g_s, and E were measured after 5 weeks of UV-B radiation on fully expanded leaves (fourth leaf from the apex). Measurements were made inside the glasshouse at photosynthetically active radiation (PAR, natural light) of 1,400 ± 70 µmol m^{-2} s^{-1}, air temperature of 36 ± 1.5°C and 55% RH. All measurements were carried out around midday (from 11:00 to 13:00 h of the local time) using a portable photosynthesis system (*LCA-3, ADC BioScientific Ltd.*, Hoddesdon, UK)). Atmospheric CO$_2$ concentration in the cuvette was fixed at 380 µmol mol^{-1}, and PAR intensity at 1350 ± 50 µmol m^{-2} s^{-1}. P_N, g_s and E were calculated using the equations described by von Caemmerer and Farquhar (1981). The measurements were done in the absence of UV-B to avoid instantaneous effects of the UV-B radiation.

Chl fluorescence: Chl *a* fluorescence parameters (FPS) were measured with a portable fluorometer (*PAM-2000, Walz GmbH*, Effeltrich, Germany) on the same leaves as used for gas-exchange determination. Prior to the measurements, leaves were kept in dark-adapted state (DAS) for 30 min (Genty *et al.* 1989) using light exclusion clips for dark adaptation. During DAS all reaction centers and electron carriers of the PSII are reoxidized; this situation is essential for rapid fluorescence induction kinetics and, for recording Chl FPs. The intensity of the saturation pulse was 4,000 µmol m^{-2} s^{-1} PAR with a duration of 800 ms.

The following Chl fluorescence yields (Chl FYs) were measured: minimum and maximum Chl FY in dark-adapted state (F_0) and (F_m), respectively. Based on these Chl FYs, some Chl FPs such as variable fluorescence (F_v), (the difference between F_0 and F_m), maximum quantum yield of photosynthesis (F_v/F_m), F_v/F_0 and basal quantum yield of non-photochemical processes in PSII (F_0/F_m) were calculated (Ranjbarfordoei *et al.* 2006).

Chl content: After gas-exchange and Chl fluorescence measurements, two leaves (the same leaf as used for gas exchange and Chl fluorescence, and the leaf closest to that one) were collected from each plant. Leaves were immediately wrapped in aluminum foil to avoid degradation of pigments by light. Soon afterwards, 0.5 g samples were taken from the collected leaves. These samples were then pulverized with liquid nitrogen. Subsequently, 0.25 g of each sample was extracted by 80% acetone and put in the freezer at −5ºC for 24 h.

Pigments were determined according to Lichtenthaler (1987) using a spectrophotometer (*Uvikon 930, Kontron* *Instruments*, Watford, UK). Amounts of Chl *a* and *b* were calculated according to Welburn (1994).

Results

The results in Fig. 1 show the effects of enhanced UV-B radiation on net photosynthetic rates in fully expanded leaves at different UV-B intensities. Maximum photosynthetic rate was observed at UV-B$_1$ and UV-B$_C$ (control level) and then declined significantly from UV-B$_2$. Net photosynthetic rate decreased by average 28% in UV-B treated plants compared to the control, and this effect was accompanied by decreases in g_s (41.8%) and E (20.6%) (Fig. 1).

Exposure of almond plants to the selected UV-B doses induced remarkable alterations in FYs (Table 1). Five weeks of UV-B irradiation significantly affected F$_0$ which showed an increase for UV-B$_2$ and UV-B$_3$, but no significant difference was observed between UV-B$_c$ and, UV-B$_1$. A reduction in F$_v$, and F$_v$/F$_m$, was observed with increasing UV-B intensities, but only UV-B$_2$ and UV-B$_3$ levels were significantly different from UV-B$_c$.

UV-B treatment decreased the F$_v$/F$_0$ ratio with about 21%. F$_0$/F$_m$ did not change significantly with increasing UV-B intensities from UV-B$_c$ to UV-B$_1$, but a further increase led to a significant increase of the parameter from about 0.191 at UV-B$_1$ to 0.235 at UV-B$_3$. F$_m$ was not affected by the UV-B radiation treatments and remained constant (Table 1).

UV-B radiation caused a significant reduction in Chl *a* when the intensity exceeded UV-B$_2$ (Table 1). The size of reduction varied ranging from a reduction by 15% in UV-B$_2$ to a reduction by 24% in UV-B$_3$ compared to UV-B$_c$, respectively. A similar decreasing trend was also observed for Chl *b*, but the reduction of Chl *a* was somewhat greater than reduction in Chl *b*. At all treatments, UV-B radiation induced a reduction in total Chl (*a* + *b*), but a significant reduction was initiated at UV-B$_2$ and was more pronounced at UV-B$_3$. In contrast, UV-B enrichment did not result in a significant alteration in the ratio of Chl *a*/*b* (Table 1).

Fig. 1. Changes in the rates of CO_2 assimilation (P_N), stomatal conductance (g_s) and transpiration (E) in *Prunus dulcis* seedlings exposed to different intensities of UV-B radiation. *Different letters* express significant differences between treatments at $P<0.05$. Data are mean values ± SE ($n = 5$).

Discussion

Exposure of almond seedlings to increased UV-B radiation negatively and significantly affected the process of photosynthesis including CO_2 assimilation (P_N), stomatal conductance to water vapour (g_s) and transpiration rate (E). Several studies have confirmed the deleterious effects of UV-B radiation on photosynthetic processes (Keiller and Holmes 2001, Mészáros *et al*. 2001, Correia *et al*. 2005, Mészáros *et al*. 2005, Láposi *et al*. 2005, Cechin *et al*. 2007) but less is known about the way how
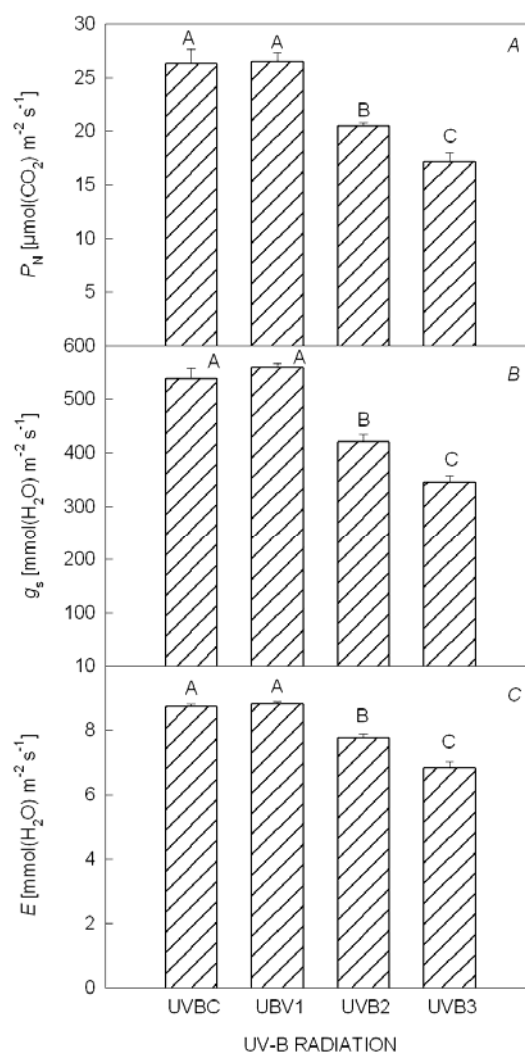
UV-B radiation affects photosynthetic activity in almond plants. The significant reduction in P_N suggests that UV-B treatment can affect photosynthesis by affecting enzyme activity, primary photochemistry, electron transport, biochemical reactions of the Calvin cycle, as well as changes in the structure of the chloroplasts (Borman 1989, Ambasht and Agrawal 1995, Balakrishnan *et al*. 2005, Cechin *at al*. 2007). In the present study, fluorescence measurements show an

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Table 1. Chlorophyll (Chl) fluorescence parameters and Chl *a* and *b* content [mg g^{-1}(FM) in *Prunus dulcis* at control and three increasing UV-B doses (UV-B$_C$: 0, UV-B$_1$: 4.42, UV-B$_2$: 7.32 and UV-B$_3$: 9.36 kJ m^{-2} d^{-1}). Different letters express significant difference between treatments ($P<0.05$).

Parameter	UV-B treatment			
	UV-B$_C$	UV-B$_1$	UV-B$_2$	UV-B$_3$
Chl *a*	2.04 ± 0.09[a]	1.95 ± 0.01[a]	1.37 ± 0.04[b]	1.55 ± 0.03[bc]
Chl *b*	0.61 ± 0.01[a]	0.60 ± 0.02[a]	0.53 ± 0.01[b]	0.47 ± 0.02[ab]
Chl (*a+b*)	2.66 ± 0.02[a]	2.54 ± 0.05[a]	2.26 ± 0.07[b]	2.02±0.049[c]
Chl *a/b*	3.37 ± 0.16[a]	3.30 ± 0.10[a]	3.27 ± 0.02[a]	3.34 ± 0.14[a]
F_0	0.168 ± 0.003[a]	0.166 ± 0.004[a]	0.196 ± 0.005[b]	0.198 ± 0.006[b]
F_m	0.870 ± 0.006[a]	0.896 ± 0.006[a]	0.827 ± 0.004[b]	0.818 ± 0.005[abc]
F_v	0.705 ± 0.007[a]	0.702 ± 0.009[a]	0.631 ± 0.008[ab]	0.0619 ± 0.007[abc]
F_v/F_m	0.806 ± 0.004[a]	0. 807 ± 0.006[a]	0.752 ± 0.010[ab]	0.757 ± 0.007[ab]
F_0/F_m	0.193 ± 0.004[a]	0.191 ± 0.006[a]	0.237 ± 007[b]	0.242 ± 0.007[ab]
F_v/F_0	4.183 ± 0.010[a]	4.237 ± 0.174[a]	3.231 ± 0.122[b]	3.140 ± 0.125[b]

increase in F_0 which indicates the impairment of the light-harvesting complex of PSII (Krause and Weis 1991). On the other hand our data indicate that almond leaves exposed to UV-B radiation exhibited no significant changes in F_m level, which suggests the secondary electron acceptor of PSII was not probably damaged. Significant reduction of F_v indicates a reduction rate of quencher Q_A and a decrease in PSII quantum yield (Fernandez *et al.* 1997). Efficiency and stability of PSII, the major component of the photosynthetic apparatus, was monitored during the experiment by means of F_v/F_m. Alteration in F_v/F_m implies changes in photochemical conversion efficiency of PSII and, therefore, possible photoinhibition of photosynthesis. Under nonstressed conditions, F_v/F_m is almost constant (from 0.80 to 0.86) (Björkman and Demming 1987). Our most severe UV-B treatment decreased F_v/F_m to 0.7572 ± 0.016. This reduction provides clear proof for thermal dissipation processes (Scarascia Mugnozza *et al.* 1996). The principal cause of increase in F_0/F_m (induced by UV-B radiation) can be attributed to a loss of excitation energy during its transfer from the pigment bed to the RCs and to an increase of energy loss through nonphotochemical quenching processes (Roháček 2002).

UV-B exposure of *P. dulcis* plants led to a substantial reduction in the content of both Chls *a* and *b*, which reveals a possible damage in the photosynthetic capacity of chloroplasts (Kakani *et al.* 2004). Mackerness *et al.* (1999) suggested that under UV-B stress, plants sacrifice their chloroplasts in order to protect the rest of the cell. UV-B radiation might affect the photosynthetic pigments, either through inhibition of their synthesis or effects on the enzymes involved in the Chl biosynthetic pathway. In the UV-B stressed almond plants, the decrease in Chl *a*, Chl *b*, and Chl (*a+b*), suggests a possible damage of the light-harvesting complex. Similar responses were observed in *Sinapsis alba* (Jennifer *et al.* 2000) and *Helianthus annuus* L. (Cechin *et al.* 2007) plants.

Conclusion: The present investigation showed that supplemental UV-B radiation caused adverse effects on activity of photosystem II in almond seedlings leading to reductions in photosynthetic gas exchange and Chl pigments. Parameters such as F_v/F_0, Chl *a* content and P_N were useful indicators of the plant's response to UV-B.

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