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EFFECT OF EXPOSURE TO UV RADIATION ON GROWTH, PHOTOSYNTHESIS AND ANTIOXIDANT DEFENSE SYSTEM IN TOBACCO (*Nicotiana rustica* L. Cv. Basmas) PLANTS TREATED WITH EXOGENOUS POLYAMINES

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Summary. Tobacco (*Nicotiana rustica* L. cv. Basmas) plants were exposed to a daily dose of 10 kJ $m^{-1}d^{-1}$ of UV radiation and treated with 0.5 mM exogenous polyamines (PAs) applied to the nutrient solution for two weeks. Dry weight of leaves increased under UVAB treatment in the absence of PAs and presence of spermidine but not putrescine. In the presence of putrescine, UVA treatment caused a significant increase in Chl a content and the Chl a/b ratio. Photochemical parameters were affected neither by light treatment nor by exogenous PAs with the exception of non-photochemical quenching in UVA treated plants. In contrast to UVA, UVAB resulted in an increased net assimilation rate in the absence of PAs and presence of putrescine. Application of PAs in control plants increased net assimilation rate following elevated stomatal conductance. Both UVA and UVAB caused slight or significant reduction of the activity of antioxidant enzymes and exogenous PAs increased their activity only in control plants. In the leaves, exogenous PAs in combination with UVA resulted in a significant reduction of H_2O_2 and increased proline concentration. Our results implied that higher photosynthesic rate which was accompanied by improved antioxidant defense capacity was responsible for UVAB-induced growth stimulation. H_2O_2 content and activity of its scavenging enzymes correlated poorly with membrane damage and plant response to treatments, suggesting involvement of other components of the antioxidant defense system. Our results showed a significant role of proline in the response of plants to exogenous PAs and in protection against UV radiation treatments.

***Key words*:** Antioxidant defense system; gas exchange; leaf photochemistry; putrescine; spermidine; UV stress.

***Abbreviations*:** PAs – polyamines; Put – putrescine; ROS – reactive oxygen species; Spd – spermidine; Spm – spermine; UV radiation – ultraviolet radiation.

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INTRODUCTION

The reduction of stratospheric ozone has raised great concern about the impact of elevated solar UV-radiation flux reaching the earth's surface. The ozone reduction affects a waveband of 25 nm within the UVB radiation (280-320 nm), which is also the waveband most damaging to life. The rest of the solar radiation affecting biological photosystems, i.e. the visible photosynthetically active radiation (PAR) (400-700 nm) and UVA (320-400 nm), are unaffected by the ozone reduction (Rozema et al., 1997). The UVA component of solar radiation is less damaging than UVB according to the weighted damage action spectra of Caldwell (Caldwell, 1971), however, there is at least a 10–15-fold greater daily influence of UVA in solar radiation.

Deleterious effects of UV radiation on the growth, productivity and photosynthesis of higher plants have been extensively studied (for a review see Jenkins, 2009). The destructive action of UV irradiation results from both direct and indirect mechanisms ьха include effects on enzymes, concentrations of chlorophyll, protein and lipidя, reduction in leaf area and tissue damage. Data suggest that reactive oxygen species (ROS) are involved in the damages caused by UV radiation. Plant cells are known to have both enzymatic and non-enzymatic defense mechanisms to counteract the destructive effects of ROS. The antioxidant defense system consists of low molecular weight antioxidants such as ascorbate, glutathione, α-tocopherol and β-carotenoids as well as several antioxidant enzymes such as ascorbic acid peroxidase (APX), catalase (CAT), guaiacol peroxidase (POD), and superoxide dismutase (SOD) (Mano, 2002).

UV radiation impairs all major processes of photosynthesis including photochemical reactions and stomatal conductance. However, UVB inhibits maximum net photosynthesic rate in a variety of plants without direct correlation with chlorophyll fluorescence or PSII activity, suggesting that photodamage to PSII is not the primary reason for reduced rates of net assimilation rate (Fedina et al. 2003).

In plants polyamines are related to various kinds of environmental stresses including osmotic stress, salt stress, heavy metals and UV radiation. Endogenous titers of polyamines (PAs) increase significantly in plants exposed to various abiotic stresses (Groppa and Benavides, 2008). The most common PAs studied in plants are the diamine putrescine (Put), the triamine spermidine (Spd) and the tetramine spermine (Spm). Increased endogenous PAs concentration, especially of Put and Spm, has been reported for various plant species during UVB stress treatment (Mapelli et al., 2006, 2008; Sung et al., 2008). In tobacco plants, an increase of total PAs and Put in thylakoid membranes (Lütz et al., 2005) and callus (Zacchini and de Agazio, 2004) was reported under UV-B stress. Polyamines might play an important role in the protection mechanisms of plants during exposure to UVB radiation. Polyamines as efficient antioxidants perform a protective role by binding to negative charges of phospholipids and DNA and thereby stabilize the function of the nucleus and

the membranes (Groppa and Benavides, 2008). Moreover, PAs catabolism produces hydrogen peroxide that can enter the stress signal transduction chain promoting activation of an antioxidative defense response (Zacchini and de Agazio, 2004). Recently, it has been reported that PAs play a significant role in the regulation of the structure and function of the photosynthetic apparatus (Sfichi et al., 2004). Under UVB stress, the large subunit of Rubisco can be stabilized by PAs which is associated with the light-harvesting complex and the PSII (Kotzabasis, 1996).

Recently, the number of works has been increasing focused on the physiological effects of exogenous PAs in plants under stress conditions and the possibility to apply them as highly efficient protective substances (Velikova et al., 2000; Unal et al., 2008). The effect of exogenous PAs on growth and stress response of salt-affected plants have been extensively studied (Zhao et al., 2007; Duan et al., 2008). However, little is known whether exogenous PAs can alleviate the adverse effects of UV stress on growth and photosynthesis.

First objective of the present study was to investigate the effect of UVA and UVAB on growth and photosynthetic characteristics of tobacco plants with a focus on photochemistry and photosynthetic gas exchange of leaves. The second objective of this work was study the role of exogenous PAs on the UV radiation response of plants. A special emphasis was given to the antioxidant defense capacity in order to evaluate the possible protective role of PAs via activation of ROS scavenging system.

MATERIALS AND METHODS

Cultivation and treatments of plants

Seeds of tobacco (*Nicotiana rustica* L. cv. Basmas) plants provided by the Agricultural Research Center, Tabriz, Iran, were surface-sterilized using sodium-hypochlorite at 5% and were germinated in the dark on vermiculate saturated with 0.05 mM $CaSO_4$ solution. Seven-day-old young seedlings were pre-cultured in 50% modified Hoagland nutrient solution (Johnson et al., 1957) for three weeks. Treatments consisting of polyamines (Put and Spd) added to the nutrient solution at 0.5 mM (Zhao et al., 2007) and light (control, UVA and UA+B) started simultaneously after the transfer of 28-day-old plants to 100% nutrient solution. For UV radiation treatments, in addition to the photosynthetic active radiation (PAR, 400-700 nm) supplied by cool white fluorescent lamps throughout the day time, UVAB fluorescent lamps (30 W, Hagen, Japan) were used without a filter for UVA+B, with a transparent Plexiglass filter cutting wavelengths under 320 nm for UVA and with a yellow colored Plexiglass filter for cutting wavelengths under 400 nm for control plants with 6 h irradiance periods centered midway through the photoperiod. The photosynthetic photon flux density (PPFD) was 150 µmol m^{-2} s^{-1} for all three light treatments. The spectral outputs of the three light conditions were measured with a calibrated spectrophotometer (Shimadzu, UV-2450) and biologically effective UV doses employed were 10 kJ m^{-2} d^{-1} (Mapelli et al., 2008) calculated based on Caldwell's generalized plant damage action spectrum normalized to 300 nm (Caldwell, 1971).

Plants were grown in a growth chamber under environmentally controlled conditions at about 150 µmol $m^{-2}s^{-1}$ light intensity, 18/6 h light/dark photoperiod, 25/17°C day/night temperature and relative humidity of 60/70%.

Plant harvest and analysis

After two weeks treatment, plants were harvested. Leaves and roots were separated and washed with double-distilled water and after blotting dry, fresh weight was determined. Dry weight of samples was determined after drying at 70°C for 2 days. Before harvest, chlorophyll fluorescence and gas exchange parameters were determined.

Determination of chlorophyll fluorescence and gas exchange parameters

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark adapted and light adapted leaves. Measurements were carried out on the second youngest, fully expanded and attached leaf of four plants grown in four independent pots in four replicates. An average of four records from different parts of each individual leaf was considered for each replicate. Leaves were acclimated in the dark for 30 min using leaf clips before measurements were taken. The initial (F_0), maximum (F_m), variable ($F_v=F_m-F_0$) fluorescence as well as the maximum quantum yield of PSII (F_v/F_m) were recorded. Light adapted leaves were used for measurements of the initial (F_t) and maximum (F'_m) fluorescence. Calculations were made for $F'_v=F'_m-F_t$, excitation capture efficiency of open PSII (F'_v/F'_m), F'_0 ($F'_0=F_0/[(F_v/F_m)+(F_0/F'_m)]$), photochemical quenching ($qP=F'_m-F_t/F'_m-F'_0$) and non-photochemical quenching ($qN=1-[(F'_m-F'_0)/(F_m-F_0)]$) (Krall and Edwards, 1992).

Leaf gas exchange parameters were determined in parallel with Chl fluorescence measurements using the same leaf with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 a.m. and 13:00 p.m. at harvest. The measurements were conducted at PPFD at the leaf surface of 350 µmol $m^{-2}s^{-1}$ measured by a quantum sensor attached to the leaf chamber of the gas exchange unit. The net photosynthesic rate per unit of leaf area (A, µmol CO_2 $m^{-2}s^{-1}$), transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$) and stomatal conductance to water vapor (g_s, mol $m^{-2}s^{-1}$) were measured by the infrared gas analyzer of the portable photosynthesis system.

The concentrations of Chl a, b and total Chl was determined according to Lichtentaler and Wellburn (1985) after extraction in cold acetone and allowing the samples to stand for 24 h in the dark at 4°C.

Assay of antioxidant enzymes and related metabolites

Determination of the activity of antioxidant enzymes and concentration of related metabolites was done according to the optimized protocols described elsewhere (Hajiboland and Hasani, 2007). Fresh samples of the second youngest, fully expanded leaves and roots were ground in the presence of liquid nitrogen and measurements were done spectrophotometrically (Specord 200, Analytical Jena, Germany).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was

measured by determining ascorbic acid oxidation. One unit of APX oxidizes ascorbic acid at the rate of 1 µmol min^{-1} at 25°C. Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in the absorbance of H_2O_2 at 240 nm. A unit activity was taken as the amount of enzyme, which decomposes 1 µmol of H_2O_2 min^{-1}. Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test. The enzyme unit was calculated as enzyme protein required for the formation of 1 µmol tetraguaiacol min^{-1}. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation test. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction as measured at 560 nm, compared with control samples without an enzyme aliquot. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid (Sigma) at 532 nm. MDA levels were calculated from a 1,1,3,3-tetraethoxypropane (Sigma) standard curve. The concentration of H_2O_2 was determined using potassium titanium-oxalate at 508 nm. Proline was extracted with 3% sulfosalicylic acid. After centrifugation the supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then the absorbance was read at 520 nm. Proline (Sigma) was used for production of a standard curve. Soluble proteins were determined using a commercial Bradford reagent (Sigma) and BSA (Merck) as a standard (Hajiboland and Hasani, 2007).

The experiments were performed in a complete randomized block design in 4 replications. Statistical analyses were carried out using sigma stat (3.02). Differences between the means were detected using a one-way analysis of variance, in conjunction with the Tukey's test ($P<0.05$).

RESULTS

Dry weight (DW) of leaves was not affected by UVA treatments, while UVAB radiation caused a significant increase of leaf DW. Root DW was reduced by UVA, but not by UVAB treatment. In general, exogenous PAs did not influence plants growth. However, a significant reduction of shoot DW by Put and an increase of root DW by Spd were observed for UVAB and UVA treated plants, respectively (Fig. 1).

Leaf Chl a, Chl b, total Chl and Chl a/b ratio were not affected by light treatments in the absence of exogenous PAs. In the presence of Put, UVA treatment caused a significant increase in Chl a, total Chl and the Chl a/b ratio. In Spd treated leaves, a significant effect of UVA was observed only for Chl a/b ratio (12% increase over control) (Table 1).

Photochemical efficiency of PSII (F_v/F_m), excitation capture efficiency of open PSII (F'_v/F'_m) and photochemical quenching (q_p) were affected neither by light treatment nor by exogenous PAs. Non-photochemical quenching (qN), however, was increased under UVA and UVAB treatments significantly or as a trend in the absence of PAs (Table 2).

UVA treatment caused a reduction of stomatal conductance (g_s), net assimilation rate (A) and transpiration (E) in the presence and absence of PAs. In contrast, UVAB increased g_s, A and E. However, this effect was observed only in

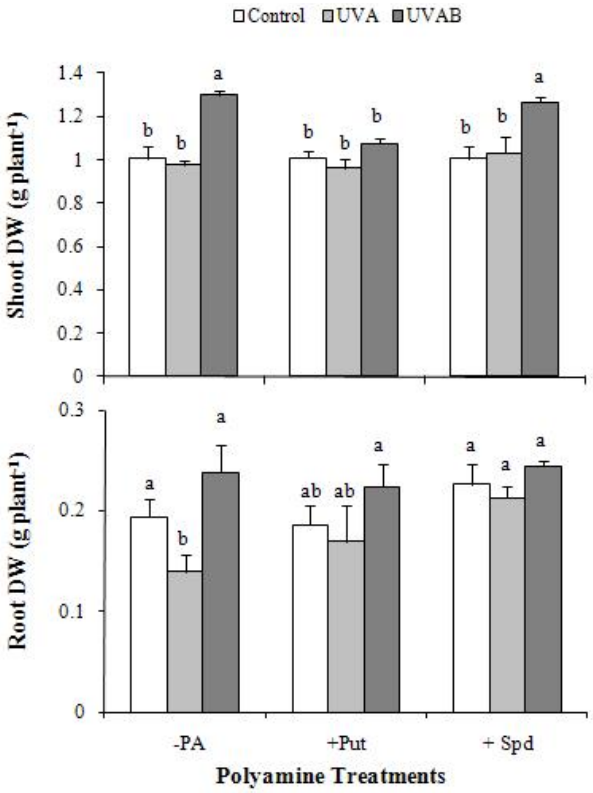

Fig. 1. Dry weight (g plant^{-1}) of leaves and roots of tobacco (*Nicotiana rustica* L. cv. Basmas) plants grown in nutrient solution without polyamine addition (−PA), with 0.5 mM putrescine (+Put) or spermidine (+Spd) under three light conditions. The means refer to 4 repetitions ± SD. Bars indicated by the same letter are not significantly different ($P<0.05$).

Table 1. Concentration of chlorophyll a, b, total (mg g^{-1} FW) and the Chl a/b ratio in leaves of tobacco (*Nicotiana rustica* L. cv. Basmas) plants grown in nutrient solution without polyamine addition (−PA), with 0.5 mM putrescine (+Put) or spermidine (+Spd) under three light conditions. The means refer to 4 repetitions ± SD. Data of each parameter followed by the same letter are not significantly different ($P<0.05$).

Polyamine treatment	Light treatment	Chlorophyll concentration [mg g^{-1} FW]			
		Chl a	Chl b	Total Chl	Chl a/b
−PA	Control	1.1±0.1^{b}	0.6±0.1^{a}	1.7±0.2^{b}	1.8±0.1^{b}
	UVA	1.1±0.1^{b}	0.6±0.0^{a}	1.7±0.1^{b}	1.8±0.1^{b}
	UVAB	1.2±0.1^{b}	0.6±0.1^{a}	1.8±0.3^{b}	2.0±0.1^{b}
+Put	Control	1.2±0.1^{b}	0.6±0.1^{a}	1.8±0.2^{b}	2.0±0.1^{b}
	UVA	1.6±0.1^{a}	0.7±0.1^{a}	2.3±0.1^{a}	2.3±0.1^{a}
	UVAB	1.0±0.1^{b}	0.6±0.1^{a}	1.6±0.3^{b}	1.7±0.2^{b}
+Spd	Control	1.1±0.2^{b}	0.6±0.1^{a}	1.7±0.2^{b}	1.8±0.1^{b}
	UVA	1.2±0.1^{b}	0.5±0.1^{a}	1.7±0.2^{b}	2.4±0.1^{a}
	UVAB	1.1±0.1^{b}	0.6±0.1^{a}	1.7±0.1^{b}	1.8±0.1^{b}

Table 2. Chlorophyll fluorescence parameters including F_v/F_m (photochemical efficiency of PSII), F'_v/F'_m (excitation capture efficiency of open PSII), q_P (photochemical quenching) and q_N (non-photochemical quenching) in leaves of tobacco (*Nicotiana rustica* L. cv. Basmas) plants grown in nutrient solution without polyamine addition (−PA), with 0.5 mM putrescine (+Put) or spermidine (+Spd) under three light conditions. The means refer to 4 repetitions ± SD. Data of each parameter followed by the same letter are not significantly different (P<0.05).

Polyamine treatment	Light treatment	F_v/F_m	F'_v/F'_m	q_P	q_N
−PA	Control	0.85±0.01^a	0.60±0.03^a	0.94±0.01^a	0.28±0.08^b
	UVA	0.85±0.01^a	0.62±0.02^a	0.92±0.04^a	0.53±0.06^a
	UVAB	0.85±0.00^a	0.59±0.01^a	0.93±0.00^a	0.38±0.09^{ab}
+Put	Control	0.85±0.00^a	0.60±0.00^a	0.93±0.01^a	0.31±0.01^b
	UVA	0.85±0.01^a	0.60±0.02^a	0.93±0.01^a	0.31±0.00^b
	UVAB	0.83±0.04^a	0.58±0.03^a	0.93±0.00^a	0.25±0.07^b
+Spd	Control	0.86±0.00^a	0.62±0.02^a	0.91±0.01^a	0.33±0.04^b
	UVA	0.86±0.02^a	0.60±0.02^a	0.89±0.01^a	0.34±0.08^b
	UVAB	0.85±0.00^a	0.61±0.02^a	0.89±0.01^a	0.34±0.07^b

the absence of exogenous PAs and in Put treated plants. In the plants treated with exogenous Spd, even a reduction similar with the effect of UVA was observed due to UVAB treatment. In plants grown under control light conditions, both Put and Spd treatments caused increased net CO_2 assimilation rate following elevated stomatal conductance (Table 3).

Both UVA and UVAB treatments

Table 3. Gas exchange parameters including net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) in leaves of tobacco (*Nicotiana rustica* L. cv. Basmas) plants grown in nutrient solution without polyamine addition (−PA), with 0.5 mM putres ine (+Put) or spermidine (+Spd) under three light conditions. The means refer to 4 repetitions ± SD. Data of each parameter followed by the same letter are not significantly different (P<0.05).

Polyamine treatment	Light treatment	A [μmol $m^{-2}s^{-1}$]	E [mmol $m^{-2}s^{-1}$]	g_s [mol $m^{-2}s^{-1}$]
−PA	Control	6.45±0.14^c	2.83±0.04^{ab}	0.65±0.03^{bc}
	UVA	4.20±0.17^d	1.68±0.26^c	0.19±0.04^d
	UVAB	7.58±0.11^a	3.41±0.38^a	0.86±0.13^{ab}
+Put	Control	7.10±0.11^b	3.01±0.17^{ab}	0.82±0.18^{ab}
	UVA	4.45±0.16^d	2.00±0.60^c	0.27±0.14^d
	UVAB	7.81±0.07^a	3.06±0.29^{ab}	0.56±0.12^c
+Spd	Control	7.38±0.18^{ab}	3.21±0.20^a	1.03±0.15^a
	UVA	6.36±0.31^c	3.19±0.17^a	0.66±0.09^{bc}
	UVAB	6.08±0.21^c	2.94±0.34^{ab}	0.51±0.09^c

caused a reduction in the activity of antioxidant enzymes slightly or significantly in the presence or absence of PAs. The exception was SOD activity in the absence of PAs that was not affected by UV treatments. In the leaves of control plants, exogenous Put caused increased activity of the studied antioxidant enzymes. These changes were observed for APX, POD, CAT as a trend while for SOD the increase was significant. In contrast, application of Spd caused enhanced activity of CAT and SOD, but decreased slightly that of APX and POD (Table 4).

In roots, a similar trend for enzymes activity was observed for the effect of PAs and light treatment for APX and CAT. In contrast, the activities of POD and SOD rather increased by UVAB treatment in the presence of Put. In Spd treated plants, a significant effect of UVAB was observed only on POD activity but not

Table 4. Specific activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in leaves and roots of tobacco (*Nicotiana rustica* L. cv. Basmas) plants grown in nutrient solution without polyamine addition (−PA), with 0.5 mM putrescine (+Put) or spermidine (+Spd) under three light conditions. The means refer to 4 repetitions ± SD. Data of each parameter within each organ followed by the same letter are not significantly different ($P<0.05$).

Polyamine treatment	Light treatment	APX [μmol mg^{-1} protein min^{-1}]	CAT [μmol mg^{-1} protein min^{-1}]	POD [μmol mg^{-1} protein min^{-1}]	SOD [U mg^{-1} protein]
		Leaves			
−PA	Control	14.3±0.86^{ab}	3134±326^{ab}	5.5±1.02^{a}	3.9±0.18^{c}
	UVA	9.3±1.44^{c}	2080±433^{bc}	3.3±1.32^{abc}	3.6±0.11^{c}
	UVAB	9.3±1.19^{c}	2086±690^{bc}	2.4±0.34^{cd}	3.9±0.16^{c}
+Put	Control	18.1±1.86^{a}	5686±1407^{a}	6.1±0.54^{a}	7.3±0.17^{a}
	UVA	7.1±2.14^{c}	2119±167^{c}	3.5±0.35^{abc}	3.4±0.11^{c}
	UVAB	7.8±0.82^{c}	1365±494^{c}	1.9±0.11^{d}	2.9±0.14^{d}
+Spd	Control	10.9±1.36^{b}	3884±1915^{ab}	4.4±0.61^{ab}	4.9±0.15^{b}
	UVA	6.7±1.51^{c}	1419±245^{c}	2.8±0.37^{cd}	2.8±0.09^{d}
	UVAB	13.0±2.77^{b}	1663±730^{c}	2.3±0.36^{cd}	2.8±0.09^{d}
		Roots			
−PA	Control	19.7±2.29^{ab}	1046±298^{b}	41.6±1.98^{ab}	8.9±0.20^{a}
	UVA	15.0±1.81^{bc}	323±30^{c}	49.9±4.74^{a}	8.3±0.27^{b}
	UVAB	16.5±3.20^{b}	171±10^{c}	46.7±4.35^{a}	7.1±0.42^{c}
+Put	Control	23.2±0.97^{a}	2284±389^{a}	33.9±2.07^{c}	3.7±0.06^{e}
	UVA	14.4±0.36^{c}	442±81^{c}	45.7±3.64^{ab}	9.2±0.23^{a}
	UVAB	16.8±1.71^{bc}	124±10^{c}	44.2±1.91^{ab}	7.4±0.04^{c}
+Spd	Control	20.1±2.04^{ab}	1308±42^{b}	39.2±2.49^{bc}	6.0±0.17^{d}
	UVA	15.4±0.38^{bc}	392±58^{c}	25.1±1.18^{d}	4.2±0.07^{e}
	UVAB	13.3±1.51^{c}	188±17^{c}	51.9±1.74^{a}	5.8±0.19^{d}

SOD activity. In Put treated plants, UVA caused a significant increase on root SOD activity (Table 4).

In leaves, UVA treatment caused an increase in H_2O_2 content in the absence of exogenous PAs, however, a significant reduction of H_2O_2 concentration was observed in the presence of Put and Spd due to UVA treatment (Table 5). In contrast to UVA, UVAB increased H_2O_2 concentration in the presence or absence of PAs. MDA content decreased due to UVAB in the presence of Put but not Spd. Proline concentration was increased due to UVA and UVAB in the absence of PAs and presence of Put but not Spd. Exogenous Put but not Spd caused an increase of proline concentration in UV treated plants, while both PAs were effective in the absence of UV radiation. PAs and UVA and UVAB increased protein concentration in the leaves while PAs treatment reduced protein content in the absence of light treatment. In the leaves

Table 5. Concentration of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline and total soluble protein in leaves and roots of tobacco (*Nicotiana rustica* L. cv. Basmas) plants grown in nutrient solution without polyamine addition (−PA), with 0.5 mM putrescine (+Put) or spermidine (+Spd) under three light conditions. The means refer to 4 repetitions ± SD. Data of each parameter within each organ followed by the same letter are not significantly different ($P<0.05$).

Polyamine treatment	Light treatment	H_2O_2 [μmol g^{-1} FW]	MDA [pmol g^{-1} FW]	Proline [nmol g^{-1} FW]	Protein [mg g^{-1} FW]
			Leaves		
−PA	Control	318±9 ^{cd}	276±5 ^{a}	1.9±0.07 ^{g}	62±2.5 ^{e}
	UVA	362±4 ^{c}	207±4 ^{c}	3.4±0.09 ^{c}	83±2.0 ^{c}
	UVAB	630±58 ^{b}	183±4 ^{d}	2.4±0.09 ^{f}	70±1.8 ^{d}
+Put	Control	410±13 ^{c}	173±3 ^{e}	3.4±0.07 ^{c}	49±1.8 ^{g}
	UVA	250±5 ^{d}	225±3 ^{b}	10.2±0.19 ^{a}	92±0.8 ^{b}
	UVAB	802±8 ^{a}	88±1 ^{g}	4.4±0.06 ^{b}	113±2.7 ^{a}
+Spd	Control	389±7 ^{c}	90±2 ^{g}	3.0±0.09 ^{d}	54±0.7 ^{f}
	UVA	270±5 ^{d}	88±2 ^{g}	2.7±0.12 ^{e}	92±2.2 ^{b}
	UVAB	580±28 ^{b}	115±4 ^{f}	2.1±0.09 ^{g}	83±1.8 ^{c}
			Roots		
−PA	Control	478±8 ^{a}	27±3 ^{e}	1.5±0.09 ^{c}	43±0.7 ^{b}
	UVA	255±5 ^{f}	41±3 ^{d}	0.9±0.06 ^{d}	44±2.1 ^{b}
	UVAB	402±5 ^{b}	78±5 ^{b}	0.5±0.07 ^{e}	51±2.8 ^{a}
+Put	Control	384±4 ^{c}	28±2 ^{e}	2.3±0.06 ^{a}	42±1.8 ^{b}
	UVA	330±11 ^{d}	42±3 ^{d}	1.9±0.07 ^{b}	43±1.1 ^{b}
	UVAB	221±4 ^{gh}	57±4 ^{c}	0.2±0.03 ^{f}	50±0.8 ^{a}
+Spd	Control	283±8 ^{e}	15±1 ^{f}	0.5±.05 ^{e}	42±1.0 ^{b}
	UVA	207±10 ^{h}	39±2 ^{d}	0.2±0.04 ^{f}	50±1.2 ^{a}
	UVAB	237±5 ^{g}	135±4 ^{a}	0.8±0.11 ^{d}	43±1.8 ^{b}

of control plants, exogenous PAs caused a significant reduction of MDA and increased proline concentration. Under these conditions, leaf H_2O_2 concentration was not affected by PAs and protein concentration decreased (Table 5).

In roots, UVA and UVAB caused a significant reduction of proline content in the presence or absence of PAs. Protein concentration of roots was not affected by Pas while it was increased under UVAB treatment in the absence and presence of Put and due to UVA in the presence of Spd. H_2O_2 content decreased by both PAs application and UV treatments. MDA content decreased under Spd but not Put treatment in control plants. Both UVA and UVAB treatments increased MDA in roots significantly in the presence and absence of exogenous PAs. Proline concentration increased in the presence of exogenous Put but it was lowered after Spd application in the control plants (Table 5).

DISCUSSION

In the present work, UV radiation applied at a dose of 10 kJ m^{-2} d^{-1} was not obviously effective enough for a considerable inhibition of plant growth, and even growth stimulation was observed under UVAB treatment. Detrimental effects of UV photons are the result of their destructive interactions with many cellular molecules such as proteins, nucleic acids bases or membrane lipids (Rozema et al., 1997). The lack of UV radiation effect on the reduction of plant weight observed in this work implied that UV radiation did not cause any serious damage to cellular components affecting plant performance considerably. This was supported by the data of Chl and protein concentration that were not reduced by UV treatments in the absence of PAs. *However*, this *did not* necessarily *mean* that UV radiation did not influence biochemical parameters such as enzymes activities. Although a reduction in biomass accumulation is often a reliable indication of plant sensitivity to UV-B radiation because it represents the cumulative effect of damaged or inhibited physiological functions, measurements of other physiological parameters have also proved to be useful indicators of UV-B tolerance or sensitivity (Smith et al., 2000).

A significant stimulating effect of UVAB radiation on the shoot growth was detected in this work. Increased dry matter production under UVB treatment at a dose of 15.8 kJ m^{-2} d^{-1} was reported for some vegetable crops such as *Lactuca sativa* and *Solanum melongena* (Smith et al., 2000). The stimulatory effect of UVAB on dry matter production observed in the present work was associated with higher stomatal conductance and net assimilation rate that were retained also in Put treated plants. In addition, proline content was higher and protein content and integration of membranes was greater in UVAB treated plants compared to the control. Although H_2O_2 was found to accumulate, MDA concentration was considerably lower under UVAB radiation treatments thus demonstrating rather an improved membrane protection under these conditions.

Photosynthesis is dependent on the light harvesting properties of chlorophyll and UV-induced reduction in Chl may be expected to result in lower biomass production and hence it can be a useful

indicator of UV sensitivity (Smith et al., 2000). UV radiation applied at a dose similar or even lower than the dose used in this study influenced negatively leaf Chl content in ice plants (Mapelli et al., 2006) but caused only a slight reduction of Chl in potato, associated with the reduction of the fractional volume of plastids and increased thylakoids (Santos et al., 2004). In the present study, UV radiation did not influence negatively leaf Chl concentration, in contrast, UVA in combination with exogenous PAs caused increased Chl a concentration or Chl a/b ratio or both. This response was mainly associated with a prominent increase of proline concentration and/or a reduction of H_2O_2 and MDA contents that were even less than control plants without UV radiation treatment. It could be suggested that exogenous PAs improved background protecting ability of photosynthetic membranes of UVA treated leaves via reduced H_2O_2 production and a rise of proline content. Polyamines are associated with the light harvesting complex and PSII and protect these complexes against photodamages (Kotzabasis, 1996).

Similarly, none of chlorophyll fluorescence parameters was affected by UV radiation negatively. An increase in qN under UVA radiation observed in the absence of exogenous PAs could be likely attributed to the increased synthesis of xanthophyll cycle pigments. UVA/blue radiation is involved in several steps in the synthesis of both carotenoids and Chl (Senger, 1987). Non-photochemical quenching reflects the capacity to dissipate excess absorbed energy as heat and is related to light-induced formation of pigments in the xanthophylls cycle (Müller et al., 2001). Increased qN was accompanied by lower stomatal conductance in UVA treated leaves that may in turn cause an excess of reducing power ($NADP^+H^+$) generated at the electron transport chain. These data suggest that UVA treated plants could dissipate excess light energy via xanthophylls cycle more efficiently.

UVA radiation influenced negatively gas exchange of leaves due to lowered stomatal conductance. Surprisingly, UVAB treatment caused rather an increase in the stomatal conductance and photosynthesic rate. UVB radiation was reported to close stomata (Poulson et al., 2006). In the present study, compounds synthesized differentially under UVA and UVAB radiations e.g. zeaxhanthin or ABA, are probably responsible for the differential behavior of leaf stomata under UVA and UVAB.

Plants treated with exogenous PAs had greater stomatal conductance, higher transpiration rate and particularly higher net assimilation rate of CO_2. The effect of exogenous PAs could be related to its effect on K^+ uptake and balance (Zhao et al., 2007) in plant cells in general and guard cells in particular. The effects of Put and Spd on the enhancement of tonoplast H^+-ATPase and H^+-PPase were demonstrated in barley roots (Liu et al., 2006). In contrast to our data, a slight or significant reduction of stomatal conductance was observed in control as well as stressed plants upon application of exogenous PAs (Iqbal and Ashraf, 2005).

Exogenous Spd but not Put mitigated the contrast effects of UVA and UVAB on gas exchange parameters. The difference between Put and Spd in their interaction

with UV treatment indicates a complex relationship between these two factors likely due to different mechanisms and/or different sites of action. Some authors suggest that the individual PAs have different roles during the plant response to stresses (Kasukabe et al., 2004).

Plant exposure to high levels of UV radiation causes an oxidative stress (Brosché and Strid, 2003) and exogenous PAs was reported to increase the activities of key enzymes involved in the antioxidative response and decrease lipid peroxidation (Yannarelli et al., 2006). In the present study, UV radiation treatment caused a reduction in the activity of antioxidant enzymes. In the UVAB treated plants this effect was expectedly accompanied by the accumulation of H_2O_2. Accumulation of H_2O_2 is an indication of lower scavenging potential of related enzymes, but could not necessarily indicate occurrence of oxidative damage. Other efficient ROS scavenging factors are likely responsible for the reduction of membrane injury under these conditions and may account for the poor correlation between H_2O_2 accumulation and membrane damage. In this case, proline may perform the role of protection particularly against more aggressive free radical species such as hydroxyl radicals which was not measured in this work.

In contrast, under UVA treatment the reduction of the activity of antioxidant enzymes was associated with lower H_2O_2 content particularly in the presence of PAs. Simultaneously, this treatment caused a reduction of membrane injury as judged by the significantly lower MDA content and increased proline concentration.

Application of PAs induced the activity of antioxidant enzymes being more pronounced for Put and mainly significant for SOD. However, exogenous PAs increased enzymes activity only in the control plants, while their effect in reduction of MDA and H_2O_2 was obvious in the UV treated plants. Probably PAs influence other components of antioxidant system e.g. antioxidant metabolites such as β-carotene, ascorbate, glutathione or proline that were not considered for assay in this study with the exception of proline.

Not only UV radiation but also PAs particularly Put caused a considerable increase of proline accumulation that correlated well with reduced MDA content in this work. Proline accumulation often occurs in plants under a variety of stress conditions, but consensus on its role in tolerance to stresses has not been achieved. A relationship between lipid peroxidation and proline accumulation was reported in plants subjected to diverse kinds of stress (Molinari et al., 2007). Proline acts as a free radical scavenger to protect plants from damage by oxidative stress (Alia and Matysik, 2001). Light dependence of proline synthesis was reported by some authors (Ábráham et al., 2003). However, the effects of PAs and UV light on increasing proline synthesis have not been studied and should be investigated further.

REFERENCES

Ábrahám E, G Rigó, G Székely, R Nagy, C Koncz, S László, 2003. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid

in *Arabidopsis*. Plant Mol Biol, 51: 363–372.

Alia MP, J Matysik, 2001. Effect of proline on the production of singlet oxygen. Amino Acids, 21: 195–200.

Brosché M, A Strid, 2003. Molecular events following perception of ultraviolet-B radiation by plants. Physiol Plant, 117: 1-10.

Caldwell MM, 1971. Solar UV irradiation and the growth and development of higher plants. In Photophysiology, Ed. AC Giese, Academic Press, New York, 131–177.

Duan J, J Li, S Guo, Y Kang, 2008. Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. J Plant Physiol, 165: 1620–1635.

Fedina IS, IT Grigorova, K Georgieva, *2003***.** Response of barley seedlings to UV-B radiation as affected by NaCl. J. Plant Physiol, 160: 205–208.

Groppa MD, MP Benavides, 2008. Polyamines and abiotic stress: recent advances. Amino Acids, 34: 35–45.

Hajiboland R, BD Hasani, 2007. Responses of antioxidant defense capacity and photosynthesis of bean (*Phaseolus vulgaris* L.) plants to Cu and Mn toxicity under different light intensities. Act Biol Szeged, 51: 93–106.

Iqbal M, M Ashraf, 2005. Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. Plant Growth Regul, 46: 19–30.

Jenkins GI, 2009. Signal transduction in responses to UV-B radiation. Annu Rev Plant Biol, 60: 407–431.

Johnson CM, PR Stout, TC Broyer, AB Carlton, 1957. Comparative chlorine requirements of different plant species. Plant Soil, 8: 337–353.

Kasukabe Y, L He, K Nada, S Misawa, I Ihara, S Tachibana, 2004. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up regulates the expression of various stress- regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol, 45: 712–722.

Kotzabasis K, 1996. A role for chloroplast-associated polyamines? Bot Acta, 109: 5–7.

Krall JP, GE Edwards, 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol Plant, 86: 180–187.

Lichtenthaler HK, AR Wellburn, 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biol Soc Trans, 11: 591–592.

Liu J, B-j Yu, Y-L Liu, 2006. Effects of spermidine and spermine levels on salt tolerance associated with tonoplast H^+-ATPase and H^+-PPase activities in barley roots. Plant Growth Regul, 49: 119–126.

Lütz C, E Navakoudis, HK Seidlitz, K Kotzabasis, 2005. Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UV-B protection. Bioch Biophys Acta (BBA)-Bioenerget, 1710: 24–33.

Mano J, 2002. Early events in environmental stresses in plants

induction mechanisms of oxidative stress. In: Oxidative Stress in Plants, Eds. D Inzé, MV Montganu, Taylor and Francis, UK, pp. 217–246.

Mapelli S, M Shorina, I Brambilla, V Kuznetsov, 2006. Biochemical and physiological events following exposure to UV-B radiation in ice plants. Gen Appl Plant Physiol, Special Issue, 33–44.

Mapelli S, IM Brambilla, NL Radyukina, YuV Ivanov, AV Kartashov, R Reggiani VlV Kuznetsov, 2008. Free and bound polyamines changes in different plants as a consequence of UV-B light irradiation. Gen Appl Plant Physiol, Special Issue, 34: 55–66.

Molinari HBC, CJ Marur, E Daros, MKF Campos, JFRP Carvalho, JCB Filh, LFP Pereira, LGE Vieira, 2007. Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp.): osmotic adjustment chlorophyll fluorescence and oxidative stress. Physiol Planta, 130: 218–229.

Müller P, X-P Li, KK Niyogi, 2001. Non photochemical quenching. A response to excess light energy. Plant Physiol, 125: 1558–1566.

Poulson ME, MR Torres Boeger, R A Donahue, 2006. Response of photosynthesis to high light and drought for *Arabidopsis thaliana* grown under a UV-B enhanced light regime. Photosynth Res, 90: 79–90.

Rozema J, J Staij, L Björn, M Caldwell, 1997. UVB as an environmental factor in plant life: stress and regulation. Tree, 12: 22–28.

Santos I, F Fidalgo, JA Almeida, R Salema, 2004. Biochemical and ultrastructural changes in leaves of potato plants grown under supplementary UV-B radiation. Plant Sci, 167: 925–935.

Senger H, 1987. Blue light control of pigment biosynthesis-chlorophyll biosynthesis. In: Blue Light Responses: Phenomena and Occurrence in Plants and Microorganisms, Vol I. Ed. H Senger, CRC Press, Boca Raton, FL, pp. 75–85.

Sfichi L, N Ioannidis, K Kotzabasis, 2004. Thylakoidassociated polyamines adjust the UV-B sensitivity of the photosynthetic apparatus by means of light-harvesting complex II changes. Photochem Photobiol, 80: 499–506.

Smith JL, DJ Burritt, P Bannister, 2000. Shoot dry weight, chlorophyll and UV-B-absorbing compounds as indicators of a plants sensitivity to UV-B radiation. Ann Bot, 86: 1057–1063.

Sung J-K, S-Y Lee, S-M Lee, Y-H Lee, D-H Choi, T-W Kim, B-H, Song 2008. Changes in Carbohydrate, Phenolics and Polyamines ofPepper Plants under Elevated-UV-B Radiation. Korean J Soil Sci Fert, 4: 38–43.

Unal D, I Tuney, A Sukatar, 2008. The role of external polyamines on photosynthetic responses, lipid peroxidation, protein and Chlorophyll a content under the UV-A (352 nm) stress in *Physcia semipinnata*. J Photochem Photobiol B: Biol, 90: 64–68.

Velikova V, I Yordanov, A Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous

polyamines. Plant Sci, 151: 59–66.

Yannarelli GG, SM Gallego, ML Tomaro, 2006. Effect of UV-B radiation on the activity and isoforms of enzymes with peroxidase activity in sunflower cotyledons. Environ Exp Bot, 56: 174–181.

Zacchini M, M de Agazio, 2004. Spread of oxidative damage and antioxidative response through cell layers of tobacco callus after UV-C treatment. Plant Physiol Biochem, 42: 445–450.

Zhao F, C-P Song, J He, H Zhu, 2007. Polyamines improve K^+/Na^+ homeostasis in barley seedlings by regulating root ion channel activities. Plant Physiol, 145: 1061–1072.