FREE AND BOUND POLYAMINES CHANGES IN DIFFERENT PLANTS AS A CONSEQUENCE OF UV-B LIGHT IRRADIATION

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Summary. All abiotic and biotic stresses induce or involve oxidative stress to some degree, and the plant ability to control oxidant levels is highly correlated with stress tolerance. Polyamines (PAs) have antioxidant properties and their induction in response to stress is well known. *Mesembryanthemum crystallinum* L., *Thellungiella halophila* Mey., *Plantago major* L. and *Geum urbanum* L. plants were grown for six weeks in water culture with modified Winter nutrient medium, 14 h at 350 μmol/m^2 s^1, 23 °C – 16 °C. Six-week-old plants were exposed to the range of 3 to 9 kJ m^{-2} d^{-1} UV-B irradiation. Starting from the following day, leaves and roots were used for analyses of PA. Free, soluble bound and insoluble conjugated fractions were extracted and determined. After UV-B irradiation the plants with different adaptation strategy showed different dynamics of PAs content. *M. crystallinum*, *T. halophila*, *P. major* are known to have a more effective antioxidant system than *G. urbanum*. The data provided evidence that the UV-B stress caused PA divergent responses and fractionations in halophyte (*M. crystallinum* and *T. halophila*) and glycophyte (*P. major* and *G. urbamum*)

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plants. The changes in the levels and fractions of putrescine (Put), spermidine (Spd) and spermine (Spm), in roots and leaves, correlated with higher tolerance of *M. crystallinum*, and *T. halophila*. The effect of UV-B irradiation depended on the exposition intensity, and the strong effect on PAs was evident after 9 kJ m^{-2} d^{-1} application. The lighter was the UV-B irradiation, the higher and faster was the capacity in the higher tolerant plant to go back to the PAs level in the control plants.

Key words: UV-B radiation, polyamines.
Abbreviations: $UV\text{-}B_{BE}$ = biologically effective UV-B irradiaton; PCA = perchloric acid; ROS = reactive oxygen species.

INTRODUCTION

Putrescine, spermidine and spermine are the main polyamines found in all living cells. They are organic polycations displaying a high biological activity. PAs are present in all compartments of the plant cell and participate in diverse fundamental processes in the cell. The total PA concentration and the ratio between individual PAs vary markedly in dependence of plant species and developmental stage. The free PAs level depends not only on their synthesis, but also on their transport, degradation and conjugation. Putrescine degradation is catalysed by diamine oxidase, whereas Spd and Spm are oxidised by polyamine oxidase (Flores and Filner, 1985). PAs can be bound to low- or high-weight molecules (phenolic acids, proteins, nucleic acids, membrane structures) (Martin-Tanguy, 2001). In the last years, PAs have been extensively studied due to their participation in the reaction of plants against several environmental stresses (Bouchereau et al., 1999; Kuznetsov et al., 2006; Groppa and Benavides, 2008). Plant physiologists studying stress give more attention to free PA. The relevance of conjugated PAs, on the other hand, has been under observation by several researchers. PA produce amide bonds with carboxylic groups. Such conjugates are important for the control of the intracellular PA concentrations and for their interaction with components of the cell wall. Some researchers indicate

that PA conjugates could regulate the intracellular PA pool, serve for PA transport, or even as substrates for aminooxidases and peroxidases. An important property of PA conjugates is their antioxidant activity. Bors et al. (1989) showed that free PAs were less efficient radical scavengers than their conjugates. Some reports have demonstrated high levels of endogenous bound PAs to proteins in dividing young tissues. Protein-bound PAs have been also found to be involved in photosynthetic functions (Margosiak et al., 1990). Furthermore, the discovery of PAs linked to cell wall polysaccharides or membranous fractions can indicate a function in formation of anchoring the cell wall polysaccharide net to the plasma membrane or in coupling membrane proteins to cytoplasmic structural proteins (Serafini-Fracassini et al., 1986).

At the beginning of the evolution of life on earth UV flux rates clearly exceeded the present values. Terrestrial plant life was made possible by development of an ozone layer in the stratosphere which absorbs all of the solar UV-C and part of the UV-B radiation (Rozema et al., 1997). A reduction of the stratospheric ozone layer has taken place over the last three decades in response of anthropogenic activities as a result of which solar UV-B reaching the earth is currently increasing. Several reports indicate that ambient levels of solar UV-B can represent an environmental stress to ecosystems. UV-B radiation produces several detrimental effects on plant cells, such as damage to proteins, membrane lipids, DNA (Teramura, 1983; Teramura et al., 1994; Quaite et al., 1992) and an increase in ROS (Brosche and Strid, 2003). UV radiation also triggers protective responses in plants, including changes in antioxidant enzyme activities as well as PAs content. In *Phaseolus vulgaris* free PAs showed a marked decrease in response to UV-B radiation (Smith et al., 2001). In tobacco cultivars Lutz et al. (2005) described an increase of total PAs (especially putrescine in thylakoid membranes) as one of the primary protective mechanisms of the photosynthetic apparatus against UV-B. Ultraviolet radiation penetration varies among different plant species and may be reflected in the sensitivity of these species (Day et al., 1992; DeLucia et al., 1992). Therefore, it is important to keep in mind that the effectiveness of UV-B radiation could be greatly affected by relationship of plant species.

The aim of this study was to compare PAs in plants with different

adaptive strategy to environmental conditions. Among all terrestrial plants halophytes comprise only 2 % while the remaining 98% of all species are glycophytes being sensitive or relatively tolerant to salinity. The content and distribution of PAs (free, conjugated to low molecular compounds, or bound to macromolecules) in *Mesembryanthemum crystallinum* L., *Thellungiella halophila* Mey. (halophytes) as well as in *Plantago major* L., *Geum urbanum* L. (glycophytes) as affected by UV-B irradiation have been investigated.

MATERIALS AND METHODS

Seeds of *M. crystallinum* L., *T. halophila* Mey., *P. major* L. and *G. urbanum* L. were germinated in perlite. Two-week-old seedlings were transferred to an aerated water culture with a modified Winter nutrient medium. Plants were cultivated in a climate-controlled chamber at 23-25/ 15-17 ^{o}C day/night temperatures, 55/ 60 % day/night relative humidity, illumination of 350 µmol m^{-2} s^{-1} supplied by Reflax–250 sodium lamps (Russia) with a 14-h light period. Six-week-old plants were exposed to 10–30 min of 5 W/m^{-2} UV-B (Sankyo G15T8E UV-B lamp 280-340 nm peak 306 nm) equivalent to the range of 3 to 9 kJ m^{-2} d^{-1} UV-B_{BE}. The following days leaves and roots were collected, immediately frozen and later used for polyamine analyses. Frozen dry plant material (50 mg) was homogenised with 1.5 ml of 5 % PCA and extracts were centrifuged for 10 min at 15000 *g*. Aliquots of the supernatant were hydrolysed (6 N HCl at 100 °C for 18 h) in order to release PAs from their conjugates. The pellets were resuspended in the original volume of 5 % PCA. Aliquots of the suspension were hydrolysed (6 N HCl at 100 °C for 18 h) in order to release pellet bound PAs. The hydrolysates were dried under N_2 and resuspended in the original volume of PCA. Aliquots of PCA supernatant (free PAs), of the same hydrolysed supernatant (soluble bound PAs) and from hydrolysed pellet (insoluble bound PAs) were dansylated as described elsewhere (Torrigiani et al., 1987) and separated by HPLC essentially as described in Bouchereau et al. (1999).

In each experiment roots and leaves from three plants, for species and treatments, were collected and processed individually. At least 2 independent

experiments and three PAs analyses per sample were carried out.

RESULTS

The plant species studied showed different degree of resistance to UV-B irradiation. In experiments with higher UV-B intensity or with irradiation repeated for the subsequent days, we found the following order for plant resistance estimated by visual morphological parameters: *Plantago major* < *Geum urbanum* < *Thellungiella halophila* < *Mesembryanthemum crystallinum*. In *P. major* high or longer UV-B irradiation caused evident necrosis spots that developed in wilted plants. In *M. crystallinum* higher UV intensity (12-15 kJ m^{-2} d^{-1}) or several days exposition under 3-9 kJ m^{-2} d^{-1} were necessary for the appearance of visible chlorotic spots on the young leaves. However, both one day and one single treatment of UV light were enough to evidence the difference in PA levels between the plants (Fig. 1). Total PAs (free + soluble bound + pellet bound) are presented in Fig. 1. The main effect of UV-B occurred in the leaves. Put and Spd were mainly affected. The increase in UV-B intensity caused an increase in *M. crystallinum* leaves (Fig. 1 A, C). In *T. halophila* leaves only Put was evidently affected. It is interesting to note also the high level of Put in control leaves of *P. major*, 4-5 times higher in comparison to other plant species. In roots the main point of interest, comparing the plants under study, was the high level of Spm in *M. crystallinum* (Fig. 1 F); however, Spm seemed not to be influenced by UV-B irradiation. From the data presented in Fig. 1 the calculated total PAs in control plants ranged from 9.09 μmol /g DW in *G. urbanum* to 15.99 μmol /g DW in *M. crystallinum*. UV-B light caused a total PAs increase of 50.9 % in *T. halophila,* 28.3 % in *M. crystallinum* but only 13.9 % and 11.1 % in *P. major* and *G. urbanum*, respectively. Apart from the quantities described above, the PAs ratio distribution between leaves and roots (Fig. 2) seemed to give important information. *P. major* had a higher ratio in controls and UV-B caused only a modest decrease. In *M. crystallinum* and *T. halophila* the PAs leaves/roots ratio changed widely, more consistent in the former species. If the ratio was calculated based on PAs values in plants after 48 h of UV-B exposition a trend going back to the ratio value of control plants was also evident.

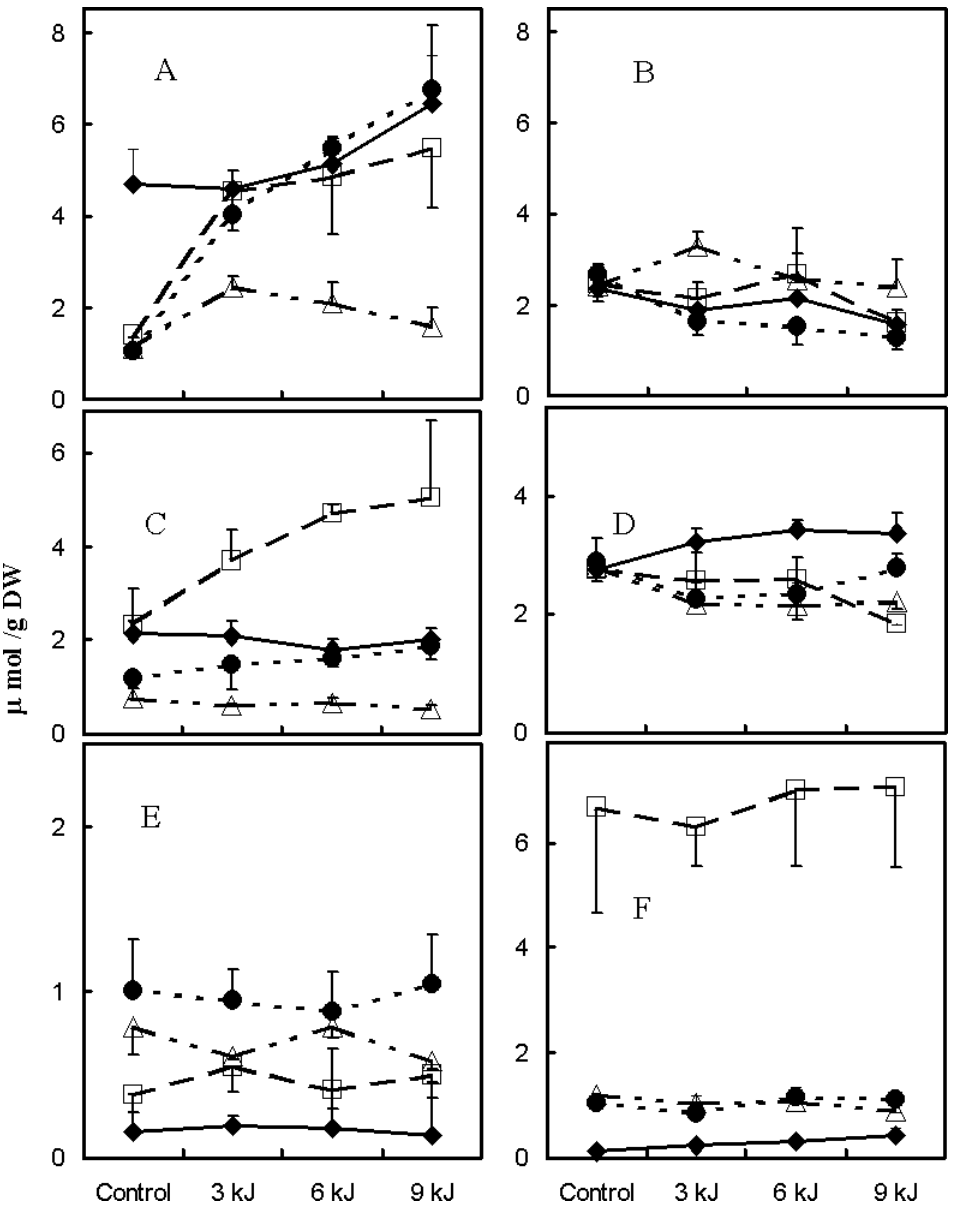

Fig.1. Changes in the content of total (free + soluble bound + pellet bound) Put (A, B), Spd (C, D) and Spm (E, F) in leaves (A, C, E) and roots (B, D, F) of *Plantago major* (◆), *Geum urbanum* (△), *Thellungiella halophila* (●), *Mesembryanthemum crystallinum* (□) after 24 h of UV-B irradiation.

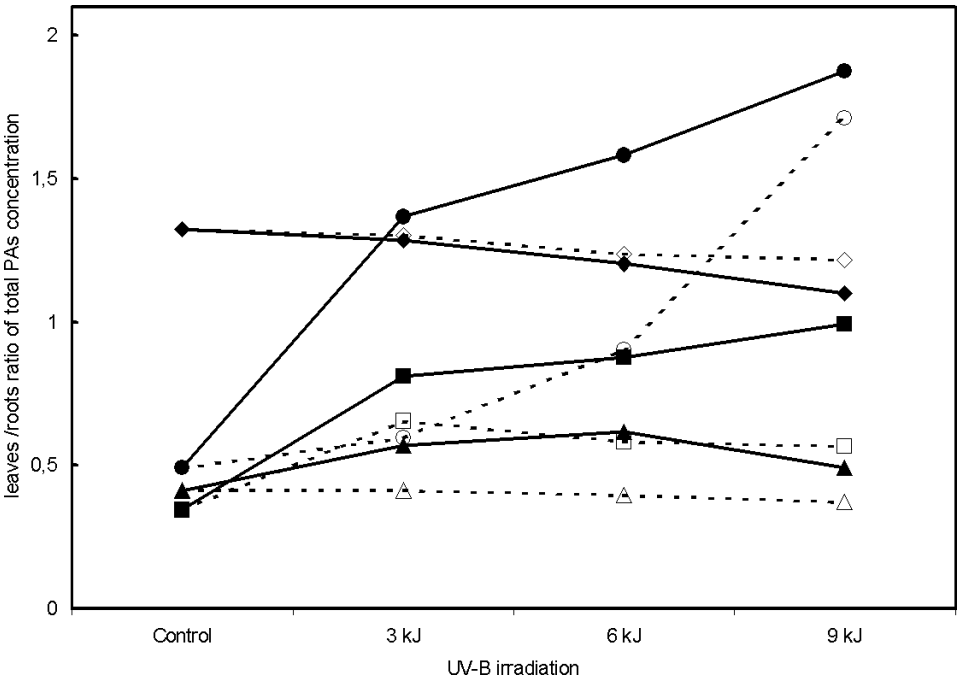

Fig. 2. Effect of UV-B irradiation on the ratio between total PAs in leaves and roots of different plant species: *Plantago major* (◆), *Geum urbanum* (△), *Thellungiella halophila* (●), *Mesembryanthemum crystallinum* (□). Continuous lines 24 h and dashed lines 48 h after UV-B irradiation.

In Table 1 the PA distribution between the free, soluble bound and pellet bound fractions and the influence of UV-B light is presented. In *P. major* leaves, PAs were relatively equally distributed in the three fractions. In *G. urbanum* the main fraction was the free PAs which in leaves and roots exceed 80 % of total PAs. In these two plant species UV-B light caused some distribution modification that, although statistically significant, did not indicate a specific trend. On the contrary, the distribution and UV-B light effect appeared to be related in *M. crystallinum* leaves where a decrease of free PA fraction and an increase in the soluble bound one were evident. The pellet bound fraction remained a marginal one. A similar trend, although with lower intensity, was observed in *T. halophila* leaves.

Table 1. Free, soluble bound and pellet bound PAs percentage (%) distribution in leaves and roots of different plant species 24 h after exposition to different intensity of UV-B light.

	Leaves			Roots		
	Free	Sol bound	Pellet	Free	Sol bound	Pellet
			Plantago major			
Control	36.68	23.62	39.70	61.33	12.50	26.17
3 kJ 24h	27.24**	34.20**	38.56	64.96	10.46**	24.59
6 kJ 24h	26.61**	30.44**	42.95*	64.13	11.58	24.29*
9 kJ 24h	27.22**	25.70	47.08**	62.46	16.51**	21.03**
			Geum urbanum			
Control	91.07	7.43	1.50	81.91	15.72	2.37
3 kJ 24h	95.28	3.72**	1.00**	82.88	13.33**	3.79**
6 kJ 24h	85.14	13.85**	1.01**	78.04	14.83	7.13**
9 kJ 24h	89.84	8.40**	1.77**	81.73	15.62	2.64
			Thelungiella halophila			
Control	64.39	32.67	2.94	65.77	25.24	8.99
3 kJ 24h	62.42	35.75*	1.83*	63.40	27.20	9.40
6 kJ 24h	56.61*	42.16**	1.23**	66.59	27.85*	5.56**
9 kJ 24h	56.46*	42.48**	1.06**	58.53**	37.74**	3.73**
			Mesembryanthemum crystallinum			
Control	87.80	10.51	1.68	23.33	61.20	15.47
3 kJ 24 h	53.93**	43.81**	2.26**	36.51**	52.41**	11.08**
6 kJ 24h	49.67*	48.65**	1.68	32.05**	57.90	10.05**
9 kJ 24h	44.42**	54.49**	1.09**	22.77	63.60	13.63**

Data are the mean of % distribution determined for each individual plant. *, ** indicate significant difference at $P<0.05$ and $P<0.01$, respectively, between control and treatments after one-way ANOVA followed by Dunnett post t-test.

DISCUSSION

Glycophyte and halophyte plants were compared with respect to PA levels after exposure to UV-B light. The first general indication, when comparing four species, was that halophytes (*M. crystallinum* and *T. halophila*) besides specific adaptation capacity to soil salinity might be distinguished from glycophytes also for their UV-B responses. The studies on PA content

and modifications as a consequence of stresses or phenological stages of plants have been frequently analysed but a complete figure of the PA pool components and allocation in the different fractions has been highly underevaluated. In the present study the full figure of free, soluble bound and pellet bound PA fractions has been mainly considered instead of the single PA components or fractions. The importance of bound PA changes as UV-B stress protection was suggested for *Nicotiana tabacum* cultivars (Lutz et al., 2005). From the data presented here some hypotheses involving PAs can be inferred. First, UV-B light modified the leaves:roots PAs ratio (Fig. 2) in *M. crystallinum* and *T. halophila* plants where such a ratio was constitutively. In *P. major* where the ratio was constitutively high, the light treatment did not change it. Furthermore, in *P. major* the quantity of Put was also constitutively high (Fig. 1 A). Put content was clearly affected in *M. crystallinum* and *T. halophila,* plant species that can be indicated as highly tolerant to UV-B. The distribution in free and bound fractions (Table 1) showed that *P. major* control plants had a relatively high level of bound PAs. If the binding process can be necessary as a protection mechanism, such high levels in control plants can become a limiting factor for further PA binding. In *M. crystallinum* and *T. halophila* the increase in soluble bound Pas under UV-B light can be related to adaptation/resistance capacity. What kind of compounds are involved in PA binding would be important, and might be the limiting factor. The main candidate participating in PA binding are polyphenols, but amino acids or carbohydrate cannot be excluded.

If some PA species can be induced or related to plant adaptation to UV-B light requires more plant species to be investigated. At present, other plant species, wheat, triticalae, maize, false flax and sage, are under investigation and the obtained results will give further evidence to test the above hypotheses.

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