BIOCHEMICAL AND PHYSIOLOGICAL EVENTS FOLLOWING EXPOSURE TO UV-B RADIATION IN ICE PLANTS

S. Mapelli*[1], M. Shorina[2], I. Brambilla[1] and V. Kuznetsov[2]

[1] *National Research Council, Institute of Agricultural Biology and Biotechnology, Milan, Italy*

[2] *Russian Academy of Sciences, Timiriazev Institute of Plant Physiology, Moscow, Russia*

Summary. The common ice plant (*Mesembryanthemum crystallinum* L.) attained significance as a model organism for plant molecular biology studies on biochemical and physiological adaptation mechanisms, due to its extreme stress tolerance, drought and high salinity resistance, and its ability to shift from C3 photosynthesis to CAM in response to environmental stresses or as part of its developmental programme. At present minor attention has been paid to elevated light and UV rays effects on ice plant metabolism and its adaptation. Young ice plants have been exposed to 5 W m^{-2} UV-B light (3 to 9 kJ m^{-2} d^{-1} UV-B_{BE}) for 10 – 30 min. On the next day leaves and roots have been collected, frozen and used for determination of total contents of osmolytes, chlorophyll, polyamines and free amino acid composition. A decrease in chlorophyll and net CO_2 assimilation gave evidence that ice plant had high sensitivity to UV-B irradiation. The absence of proline in leaves accompanied with other modifications in free amino acids composition in both leaves and roots, the slight stimulation of polyamine biosynthesis as well as the lack of cadaverine in roots could be considered as indications for the damage of ice plants due to the poor metabolic adaptation to stress caused by UV-B irradiation. Our results suggest that ice plants have lower adaptive capacity to UV-B stress in comparison to drought and salinity. Data are discussed in relation to known hypotheses for *M. crystallinum* response mechanisms.

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* Corresponding author, e-mail: mapelli@ibba.cnr.it

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Abbreviations: UV-B_{BE} - biologically effective UV-B irradiaton, CAM - crassulacean acid metabolism, EST - expressed sequences tag.

INTRODUCTION

At the beginning of the evolution of life on Earth UV flux rates clearly exceeded the present values. Terrestrial plant life had become possible after development of ozone layer in the stratosphere which had absorbed all the solar UV-C and part of the UV-B radiation (Rozema et al., 1997). Instrumental monitoring of the stratospheric ozone started only in 1926 using Dobson spectrometers (Staehelin et al., 2002). A reduction of the stratospheric ozone layer has taken place over the last three decades in response to CFC emissions of anthropogenic origin. Now as a result of stratospheric ozone depletion, solar UV-B reaching the earth has been currently increasing. Since 1985 levels of ozone reduction as high as 60% have been reported for the Antarctic continent. Severe ozone depletion in Arctic since the early 1990's has been observed (Farman et al., 1985; McPeters et al., 1996). Untill present no signs of recovery have been reported, and in 2003 the second largest ozone hole ever has been announced. It is predicted that the eventual recovery of the ozone layer should be expected not earlier than 2050-2060 (WMO, 2003).

Several reports indicate that ambient levels of solar UV-B can represent an environmental stress to ecosystems. Terrestrial ecosystems include agricultural areas, agro-ecosystems, and less intensively managed lands as forests, grasslands, savannahs, deserts, tundra. In any of these environments, the normal function of ecosystems comprises many attributes that could potentially be affected by increased solar UV-B radiation, including plant biomass production, seed production, plant consumption by herbivores including insects, disease incidence of plants and animals, population fluctuations of plants and animals and changes in species composition. UV-B radiation produces several detrimental effects on plant cells such as damage to proteins, membrane lipids, and DNA (Teramura, 1983; Teramura et al., 1994; Quaite et al., 1992). UV radiation also triggers protective responses in plants, which help to reduce UV-B penetration into plant tissues, to scavenge free radicals, and to repair DNA damage (Day et al., 1992; Stapleton, 1992; Takeuchi et al., 1996; Kramer et al., 1991). Many effects of UV-B radiation concern morphogenetic changes in plants, rather than damage per se (Caldwell et al., 1995). Plant morphogenetic parameters that may change under UV-B are plant height, leaf area, leaf thickness, branching and plant phenology. The penetration of ultraviolet radiation varies among different plant species and may reflect their sensitivity. Penetration of UV-B was found to be greatest in herbaceous dicotyledons and was progressively less in woody dicotyledons, grasses, and conifers; the UV penetration also changes with leaf age (Day et al., 1992; DeLucia et al., 1992). UV-B is a triggering agent of altered gene activity, which may reflect plant function in many aspects (Jenkins et al., 1997).

Plants and other organisms in nature are seldom affected by a single stress factor. Instead, they typically respond to several factors acting in concert, such as water stress, increased CO_2, mineral nutrient availability, heavy metals, air pollution, and temperature (Caldwell et al., 1998). Therefore, it is important to keep in mind that the effectiveness of UV-B radiation can be greatly increased or decreased by such factors.

Mesembryanthemum crystallinum L., the common ice plant (family: *Aizoaceae*, order: *Caryophyllales*) is native to the Namibian desert. It has been introduced into Western Australia, along the coasts of Mediterranean regions, Western United States, Mexico, Chile, and in the Caribbean, probably spread by the maritime travellers (Vivrette et al., 1977). In its native habitat, this plant germinates and becomes established during the short, cool and moist winter season, followed by drought stress coupled with increasing salinity summer (Winter at al., 1978). Salinity and water deficit affect a suite of compatible solutes in addition to the activity of ion transporters and specific water-channel proteins associated with roots, stems and leaves. However, different responses are distinguishable between plant juvenile and adult stages. Ice plants rise to relative higher fame with the discovery of stress-inducible switch from C3 photosynthesis to CAM (Winter at al., 1978). The CAM system improves water use efficiency up to five-fold compared to C-3 and C-4, and thus provides competitive advantage of CAM plants in hot and dry climate. The ice plant has also become a model system for molecular studies on biosynthesis of betalains, a class of chromogenic compounds that replace anthocyanins in most families of the *Caryophyllales* (Stafford, 1994). Furthermore, the plant gained distinction of being the only non-crop plant for which large scale EST sequencing and microarray analyses were performed. Random collection and sequencing of thousands clones provided a number of surprises. Expression profiles indicate significant differences in abundant transcripts in stressed and unstressed roots, shoots and leaves. Only about 20% of the most abundant transcripts in unstressed leaves are found also in stressed ones. Equally surprising is the number of transcripts from library of stressed ice plants that are not represented in the databases compiled for other plants (Cushman et al., 2000; http://www.tigr.org/tdb/mcgi).

In the light of the above description the question "how *M. crystallinum* responds to UV-B radiation" seems an interesting point but till now no data on this topic are available. Thus, the objective of this study was to initiate an investigation on ice plant response to UV-B irradiation during adaptation to salt stress and induction of shift to CAM. We provide evidence that the ice plant is highly sensitive to UV-B and it develops some biochemical responses divergent from that reported for salt and drought with induction of alternative biochemical modifications.

MATERIALS AND METHODS

Seeds of ice plant were germinated in perlite. After germination the two week-old plants were transferred in aerated water culture with Johnson's nutrient solution. Plants were cultivated in a climate-controlled chamber at 23-25°/15-17°C day/night temperature, 55/60% day/night relative humidity. Illumination of 350 mol m^{-2} s^{-1} was supplied by Reflax–250 sodium lamps (Russia) with a 14-h light period. Two months -old plants, programmed to shift to CAM, 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 range of 3 to 9 kJ m^{-2} d^{-1} UV-B_{BE}. Leaves and roots were collected on the next day, immediately frozen and used later for analyses of metabolic changes. Metabolites were extracted and determined according to the appropriate methods.

Total osmolytes were extracted in water of high purity and were measured with a cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Free proline was extracted, derivatised with acid ninhydrin and the absorbance of sample was read according to Bates et al. (1973). Free amino acids composition was determined by HPLC as described by Mapelli and co-workers (2001). Sucrose and reducing sugars were assayed according to Prado and co-workers (1998). Total chlorophylls were extracted with 80% acetone and estimated according to Arnon (1949). Polyamines analysis (as benzoyl derivatives measured with HPLC) was carried out as described by Shevyakova and co-workers (2001). Carbon dioxide and water exchange rates in expanded leaves were measured using a LCA3 portable photosynthesis system (Analytical Development Co., Hoddeston, UK). The data presented are average of at least two independent experiments. Samples were analysed three times. Five measurements have been performed for gas exchange analyses consecutively on two leaves for each plant. Mean values and the respective standard errors were calculated and presented on the figures.

RESULTS

UV-B light affected osmolytes content (Fig. 1). Evident decrease in this parameter was observed in roots as a result of lower UV-B doses while after receiving a higher dose of irradiation osmolytes content registered conciderable recovery reaching values comparable to the control. Observed changes indicate clearly the modifying effect of UV-B irradiation on the solute content. However, the opposite trend between root and leaf solute content could be due to the effect of water and solutes sink from root to leaves especially after application 9 kJ m^{-2} d^{-1} dose. This is supported by the observation that if leaves maintained about 5% of dry mass for all the treatments, the dry matter in root decreased with about 7% to less then 5% in control and high UV dose-treated plants. A decrease of chlorophyll content and chlorophyll a/b

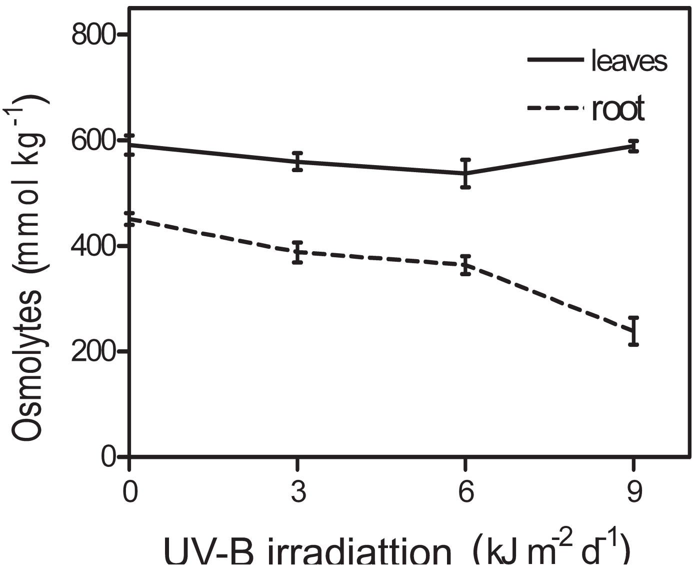

Figure 1. The effect of different UV-B doses on total osmolytes present in the 1st - 2nd leaves and roots of UV-B irradiated ice plants.

ratio (Fig. 2) proved that leaves were damaged by UV-B. The same effect has been reported for many other plant species and especially the change of chlorophyll a/b ratio in plants submitted to stress has been concidered as an indication for lack of acclimation response. Since photosynthesis is dependent on chlorophyll content,

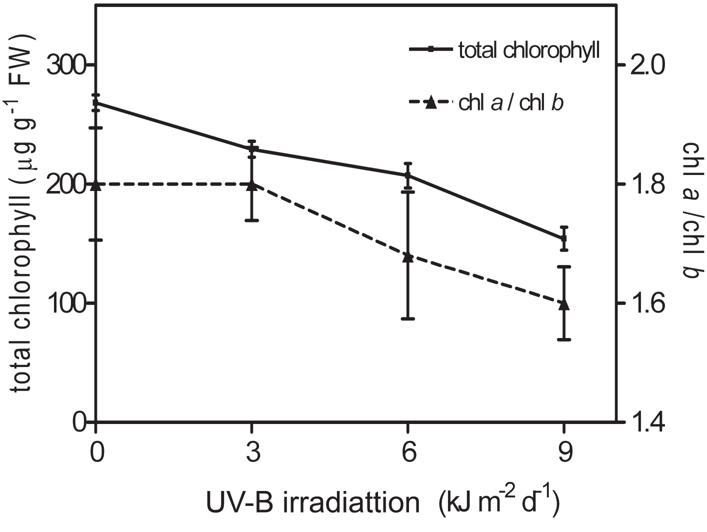

Figure 2. Changes in chlorophyll content and chl a/ chl b ratio in the 1st - 2nd leaves of ice plants after exposure to different doses of UV-B irradiation.

Table 1. Changes in carbon dioxide assimilation and water transpiration in *M. crystallinum* after application of 3 kJ m^{-2} d^{-1} UV-B.

	Net CO_2 assimilation (μmol m^{-2} s^{-1})		**Water transpiration (mmol m^{-2} s^{-1})**	
	control	UV-B treated	control	UV-B treated
day after treatment	5.58±0.12	5.85±0.23	4.60±0.18	4.53±0.22
1 day	6.08±0.23	5.090.48	4.02±0.24	5.33±0.18
2 days	5.85±0.25	3.99±0.22	4.82±0.22	6.33±0.49
3 days	5.80±0.27	2.49±0.22	4.09±0.19	4.43±0.51
4 days	5.20±0.14	1.710.30	4.15±0.21	3.92±0.44

UV-B treatments were found to affect also net carbon dioxide assimilation in ice plant (Table 1). Decrease in this parameter was detected for the period of several days and the intensity of the process was related to the UV-B dose applied. The net CO_2 assimilation was accompanied with a water transpiration stream maintained at similar levels between the control and the UV-B treated individuals, indicating that intense water transport from roots to shoots was required.

Free amino acids and polyamines were analysed as compatible solutes mobilized as a consequence of UV-B irradiation. The total free amino acids content is shown on Figure 3 and their percentage distribution is presented in Table 2. Amino

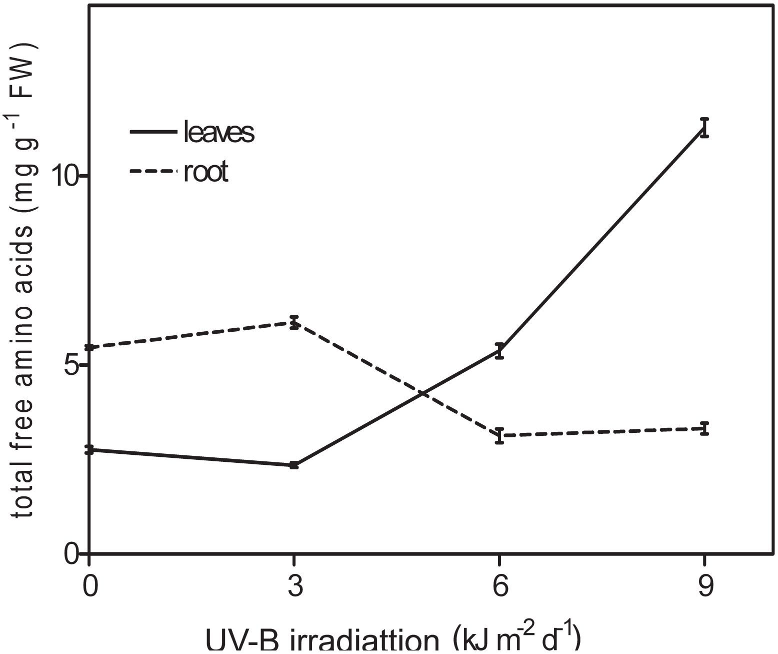

Figure 3. Total free amino acids content in the 1st - 2nd leaves and roots of ice plants after exposure to different doses of UV-B light.

Table 2. Changes in the amino acid composition of the 1st-2nd leaves and roots of ice plants irradiated with different doses of UV-B (corresponding values given in % of the total amino acids concentration is presented in Figure 3).

	Leaves				**Roots**			
	UV-B doses (kJ m^{-2} d^{-1})							
	0	3	6	9	0	3	6	9
Aspartate	4.03	3.64	2.61	2.08	4.44	7.23	15.64	14.09
Glutamate	6.10	7.44	4.19	2.10	4.48	3.72	10.14	9.07
Asparagine	2.06	2.25	2.14	4.51	0	0.80	0.16	0.4
Serine	6.11	6.76	8.21	4.95	3.95	9.52	5.84	7.18
Glutamine	24.22	18.13	28.43	34.54	18.61	18.37	18.58	18.00
Glycine	1.49	5.24	1.10	1.25	5.98	8.56	1.33	1.19
His + Thr	2.84	2.77	3.39	1.74	1.88	2.23	3.31	3.87
Arginine	19.39	15.80	15.30	15.05	1.73	1.91	4.34	4.08
GABA	2.54	0.69	0.91	1.82	1.29	0.85	1.29	1.48
Alanine	6.14	7.73	5.37	3.30	3.95	5.06	4.61	5.99
Valine	8.64	10.29	11.19	14.25	29.00	23.75	16.68	16.45
Phe + Orn	5.19	4.20	4.59	3.82	0.95	1.70	2.80	2.47
Isoleucine	0.48	0.93	.88	0.94	0.84	0.02	1.00	0.65
Lysine	2.28	1.67	2.71	1.78	1.40	1.42	0.13	1.95
Leucine	2.62	3.17	2.27	0.08	0.34	0.88	0.91	2.07
Proline	0	0	0	0	13.65	9.96	9.26	6.96
Citrulline	1.09	1.30	1.08	1.60	1.40	0.24	0.09	0.90
Tyrosine	2.49	2.30	3.33	1.19	0.35	0.89	0.80	0.42
Methionine	0.93	2.75	1.13	2.91	3.66	1.68	1.30	1.22
Tryptophane	1.38	2.94	2.18	2.08	2.09	1.21	1.79	1.56

acid concentrations were half reduced in roots and about five times increased in leaves after UV-B exposure and this effect was inhanced after radiation with higher UV doses (both concentrations and percentage composition). In this aspect leaves and roots showed some organ “peculiarity”. Increase of glutamine and valine and decrease of aspartate, glutamate, arginine and alanine were the main and most interesting changes in amino acid content in leaves. The absence of proline in leaves was surprising considering that this imino acid has been considered as a stress inducible solute. Some of the changes in free amino acids in roots were found to be opposite to those observed in leaves i.e. aspartate, glutamate, arginine and alanine increased in these organs. In control roots the presences of proline and valine was relevant (about 42%). They both decreased after UV-B irradiation and remained half the control percentage in plants exposed to 9 kJ m^{-2} d^{-1} UV-B. The percentage decrease could be concidered as more relevant considering that the quantity of the free amino acids has been also reduced by half.

UV-B treatment effects on free polyamines content is presented on Figure 4. Total polyamines generaly decreased with increasing of UV-B doses in roots. The increase in putrescine and spermidine content in leaves was related to the quantity of UV-B applied. However, general increase of total polyamines did not occurred in leaves. The absolute absence of cadaverine in roots is worth noting since this polyamine has been indicated as an relevant compound participating in adaptive mechanism responce of ice plant to other abiotic stresses.

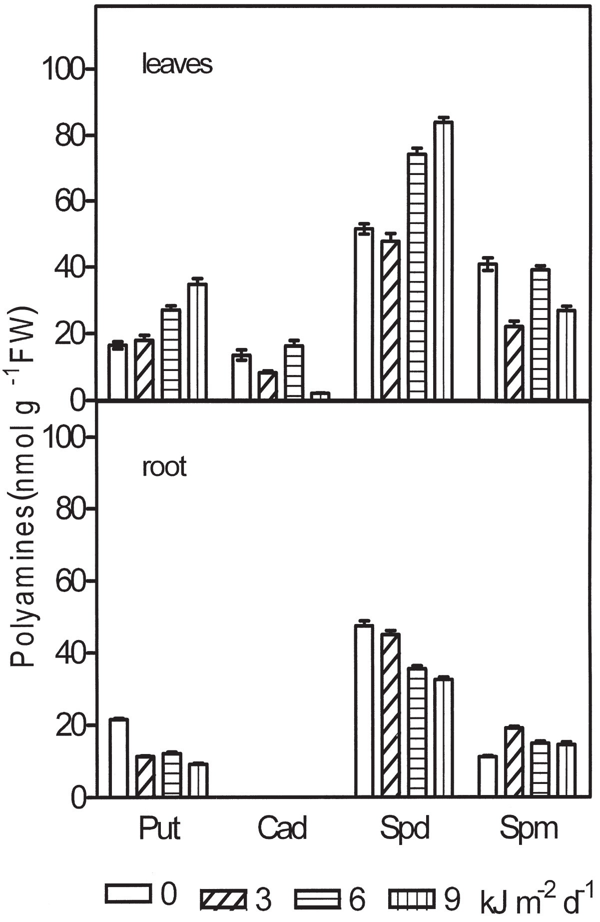

Figure 4. Variations in the polyamines content in the 1st - 2nd leaves and roots provoked by different doses of UV-B light.

DISCUSSION

In spite of the reported capacity of *M. crystallinum* plant to adapt to drought and salt stress conditions (Winter at al., 1978) the present data showed that, at least at juvenile growth stage, this plant was strongly affected by UV-B irradiation and in some aspects the effect seemed related to the UV-B doses applied. Modification of osmolytes as well as the decrease in chlorophyll content and CO_2 net assimilation are considered evidence for UV-B sensitivity. Many plants display reduced photosynthetic function and chlorosis as symptoms of UV-B stress (Renger et al., 1989; Barnes et al., 1990). On the other hand, it has been reported that plants adapted to UV-B exposure retain higher photosynthetic integrity and those with availability to maintain chlorophyll levels during UV-B exposure are less sensitive towards this kind of stress (Barnes et al., 1987; Bornman et al., 1990; Greenberg et al., 1997). The poor adaptive response to UV-B irradiation of ice plants can be related to amino acid and polyamines composition and quantities. Particularly interesting was the low quantity of proline in roots, its decrease during stress progress, and its absence in leaves. Proline is universally accepted as an imino acid induced by stress conditions and having an important role in adaptive mechanisms (Kuznetsov et al., 1999). An increase in proline content as a result of drought and salt stresses in ice plants was reported (Adams et al., 1998). The present study does not clarify the reason for the low content or absence of proline in plant tissues but some hypotheses could be suggested. The first hypothesis is related to the specific ability of the ice plants to synthesize betalains as other plants belonging to the *Caryophyllales* order do. Betalamic acid is a precursor of this class of compounds. No data for free betalamic acid in ice plant has been reported till now, but it is known that the reaction between betalamic acid and proline forms indicaxanthin (Vogt et al., 1999). It is still not clear whether this reaction is under light control.

The significant function of cadaverine, among all the other polyamines in ice plants under stress conditions, has been also documented (Shevyakova et al., 2001). Cadaverine synthesis has been proved to be under ethylene control. The role of this phytohormone in the biosynthesis of other polyamines has not been reported (Shorina et al., 2005; Kuznetsov et al., 2006). Thus, another hypothesis could be related to the absence of ethylene induction of cadaverine biosynthesis as well as the existence of a mechanism for fast catabolism or conjugation of this poliamine under the described stress conditions. These suggestions remain interesting starting points for future investigations.

Considering that plants, such as spinach and sugar beet also belong to the *Caryophyllales* order, the above hypotheses could have relevance to UV-B sensitivity of economically valued plant species. Spinach has been well used for several research aspects, but till now few data have been available about UV-B effect on this species.

Furthermore, the functional products of more than the 20 thousand sequences available in the ice plant EST database (Cushman et al., 2000; http://www.tigr.org/tdb/mcgi) are not yet established and research on sequences involved in the metabolic response of *M. crystallinum* under UV-B stress should be of interest for further investigation.

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