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Εργαστήριο Βιοχημείας Φυτών και Φωτοβιολογίας
Υπο την επίβλεψη του Αναπλ.Καθ. κ.Κ.Κοτζαμπάση

Η συνεισφορά της φωτοσυνθετικά ενεργής ακτινοβολίας στη ρύθμιση των επαγόμενων από την UV-B ακτινοβολία αλλαγών στη δομή και λειτουργία του φωτοσυνθετικού μηχανισμού

The contribution of photosynthetically active radiation intensity to the modulation of UV-B induced changes on the structure and function of photosynthetic apparatus

Liliana Sfichi
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ΕΥΧΑΡΙΣΤΙΕΣ

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ABREVIATIONS

UV-B	ultraviolet-B radiation: 280-320 nm
PAR	photosynthetically active radiation: 400-700 nm
PSII	photosystem II
LHCII	light harvesting complex of photosystem II
Put	putrescine
Spm	spermine
Spd	spermidine
Q_A or Q_B	quinone
PQ	plastoquinone pool
Chl	chlorophyll
Fo	initial fluorescence
Fm	maximum fluorescence
Fv	variable fluorescence
ABS/RC	absorbance per reaction center (antenna size)
TRo/RC	trapping effect per reaction center
ETo/RC	electron transport per reaction center
RC/CS	density of reaction centers per cross-section
DIo/RC	rate of energy dissipation per reaction center

CONTENT

ABSTRACT

The contribution of photosynthetically active radiation (PAR; 400-700 nm) intensity to the modulation of UV-B effects on the structure and functioning of the photosynthetic apparatus has been studied in the unicellular green alga *Scenedesmus obliquus*.

Cultures of *Scenedesmus obliquus* incubated in different illumination conditions, namely darkness, low light (87 μmol m^{-2} s^{-1}) and high light (700 μmol m^{-2} s^{-1}), were irradiated for 3 h with a dose of 0,420 mW cm^{-2} UV-B radiation. The changes induced by UV-B radiation on the algal photosynthetic apparatus, were similar to those observed during photoadaptation to low light intensity, e.g. increase of antenna size, decrease of Chl *a/b* and Put/Spm ratios and decrease of active reaction center density.

PAR intensity influenced the magnitude of these responses, which were strongly expressed in cultures incubated in light conditions, versus the dark-incubated cultures. This result is consistent with the possibility that the degree of photoadaptation that cultures adopt in different illumination conditions influences their sensitivity to UV-B radiation, by means that low PAR and UV-B act synergistically producing a more intense response, whereas high PAR and UV-B act antagonistically and the effects induced by UV-B radiation are dimished. During UV-B irradiation, Put/Spm ratio decreased, but it later recovered, suggesting that this ratio constitutes an important adaptive mechanism to UV-B radiation.

The ability of cultures to recover from UV-B effects were investigated after the removal of UV-B source in cultures incubated in similar conditions as those used during UV-B irradiation, or after transfer to different conditions, such as darkness for cultures incubated in low light conditions during UV-B irradiation. Recovery was partially accomplished in cultures maintained in continuous light conditions, while in other illumination conditions, the changes caused by UV-B radiation were intensified and recovery did not occur. These results are consistent to the hypothesis that mechanisms assuring the recovering ability of UV-B irradiated cultures are light-regulated and function in both UV-B irradiation and recovery periods.

INTRODUCTION

The shortest wavelengths within the solar spectrum that reach the earth's surface are in the ultraviolet-B (UV-B:280-320 nm) region and constitutes 1,5 % of total solar irradiance prior to attenuation by the earth's atmosphere. The actual amount of UV-B radiation reaching any given location on the earth's surface varies, but under normal atmospheric conditions, does not exceed 0,5 % of the sun's total energy output (Frederick et al., 1989; Blumthaler, 1993).

On physicochemical basis, the energy contribution of UV-B radiation is, therefore, of minor importance; however, on the basis of its photobiological effects, it is highly important to the earth's biosphere (Setlow, 1974; Giese, 1976). Since plants in a natural environment are unavoidably exposed for long time periods of UV-B radiation, it is often a source of considerable stress to them (Caldwell, 1971; Jordan, 1996). This stress has become of major concern because the stratospheric ozone layer, which is the primary attenuator of solar UV-B radiation, has shown signs of erosion caused by emissions of halogenated chemicals, such as chlorofluorocarbons (Kerr and McElroy, 1993). The potential impact of these changes projecting increases in UV-B radiation on plant physiology has been investigated the object of investigation for the last two decades, and the data collected from yhese various reports roughly involve 300 species abd varieties at plants. Nearly one-third to one-half of these plants showed physiological damage and/or growth reductions in response to UV-B radiation (Teramura and Sullivan, 1991, 1994).

Although UV-B radiation can affect a number of processes at the molecular level with respect to nucleic acids, proteins, lipids, pigments and phytohormones (Teramura, 1983; Tevini and Teramura, 1989; Bornmann and Teramura, 1993), the physiological changes which limit photosynthetic capacity after plant exposure to UV-B radiation are considered of an enormous importance. Changes in physiology occuring with increased UV-B radiation include both direct structural damage to the photosynthetic apparatus, and indirect changes in growth and morphology that may reduce light perception and competitiveness (reviewed in Jansen et al., 1998). UV-B impinges on various aspects of photosynthesis but effects on photosystem II (PS II) have drawn considerable attention (Wilson and Greenberg, 1993; Rusell et al., 1995;

Krause et al., 1999, Friso et al., 1995; Nedunchezian and Kulandaivelu, 1991). PSII is a highly structurated protein-pigment complex embedded in the thylakoid membrane, which transfers electrons liberated from light – induced oxidation of water to membrane soluble PQ molecules (Anderson and Styring, 1991). The redox cofactors of PS II electron transport are bound to or contained by the D1 and D2 protein subunits, which form the reaction center of PSII (Nanba and Satoh, 1987). A cluster of four Mn ions catalyses water oxidation and electrons liberated during this process are transferred to the reaction center chlorophyll, P680, via a redox active tyrosine residue, Tyr-Z, of the D1 protein. PSII contains another redox-active tyrosine, called Tyr-D, on the D2 subunit, which can donate electrons to P680 , but not connected to the water-oxidizing complex. On the acceptor side of PSII, the electron produced by the light induced charge separation event, reduces a pheophytin molecule and then the first (Q_A) and second (Q_B) PQ electron acceptors (Andersson and Styring, 1991). Q_A is a firmly bound component of the reaction center complex, which undergoes one – electron reduction, whereas Q_B is a mobile electron carrier, which takes up two electrons sequentially from Q_A before leaving its binding site formed by the D1 protein. Most observations support the notion that UV-B preferentially inactivates the water-oxidizing complex with additional effects on the Q_A and Q_B acceptors, as well as on the Tyr-Z and Tyr-D donors (Renger et al., 1989; Vass et al., 1996; Giacometti et al., 1996). The acceptor or reducing side of the D1 and D2 proteins can be modified by UV-B radiation with a subsequent change in the number and activity of the quinone binding sites (Renger et al., 1989). Specifically, it has been suggested that UV-B radiation primarily modifies the binding sides on the PSII acceptor side with a simultaneous blocking of pheophytin, the primary electron acceptor (Renger et al., 1986). In a confirming study, high levels of UV-B radiation also inhibited formation of the semiquinone anion (Q_A^-) and decreased the overall photoreduction of plastoquinone (Melis et al., 1992). UV-B radiation also decreases chlorophyll (Chl) flurorescence with the fast components accelerated and the slow components retarded, suggesting the formation of additional quenchers of exciton energy (Renger et al., 1991). It has been indicated that plastoquinone with its three redox states (quinone, semiquinone anion and the quinol) may act as a primary UV-B photosensitive molecule since all these forms absorb to the same extent in the UV-B region (Melis et al., 1992). UV-B driven D1 protein degradation is believed to occur via the plastoquinone anion, a reactive species that is formed upon exposure to UV-B

(Jansen et al., 1993). The primary D1 cleavage site resides near the plastoquinone-binding niche.

It is not clear how much of the reduction in PS II activity is due to increased nucleic acid damage with supplemental UV-B radiation. The results obtained by Jordan et al. (1996) suggest rapid changes in the regulation of gene expression with increased UV-B radiation but much of the detail concerning this regulation still remain unknown. Supplemental UV-B radiation may decrease the activity and content of the PSII complex with a resulting decrease in electron transport, and presumably ATP synthesis, it could be corresponding decrease in the photosynthetic capacity and maximum quantum yield (Teramura et Ziska, 1996).

Previous studies have shown that carbon reduction processes were directly affected by UV-B radiation (Teramura et Ziska, 1996). Under UV-B exposure, ribulose 1,5- bisphosphate carboxylase/oxygenase (Rubisco) content may decline either as part of a general decrease in total leaf protein or by specific inhibition of transcription with a decline in the small subunits of Rubisco (Jordan et al., 1992). Decline in Rubisco activity and content may occur due to limitations in the rate of supply ATP and NADPH generated by the light photosynthetic reactions, which can determine the extent of Rubisco regeneration (Strid et al., 1990; Sullivan and Teramura, 1990). It has, also, been suggested that observed increases in dark respiration, upon UV-B exposure, may represent additional energy needed for repair, but no conclusive data are available concerning the impact of UV-B radiation on respiratory metabolism (Teramura and Ziska, 1996).

UV-B radiation may affect photosynthesis indirectly by photobleaching and photodegradation of photosynthetic pigments (Strid and Porra, 1992). High levels of UV-B radiation in combination with low levels of PAR have significantly reduced chlorophyll content in bean, barley and corn, pea and soybean (reviewed in Teramura and Ziska, 1996). However, increases as well as decreases in photosynthetic pigments have been observed with increased UV-B radiation (Correia et al., 1999; Day and Vogelmann, 1995). Strid and Porra (1992) suggested that the lowering of the chlorophyll content occurred earlier than the appearance of pigments absorbing in UV-B region, namely flavonoids. This response was proportional to the length of the UV-B treatment but the excessive radiation was deleterious because the synthesis of flavonoids proceeds faster during the recovery period than during continued UV-B treatment, which suggests that the prolonged treatment damages the biosynthetic

system induced by short exposure periods. The synthesis of flavonoids in UV-B treated plants was indicated in a number of experiments (Wellman, 1975; Tevini et al., 1981; Chappell and Hahlbrock, 1984). This response could be regard as a plant strategy to minimize the flux of harmful radiation into crucial parts of plant tissue, such as the photosynthetic apparatus in the chloroplast. It is also possible that those flavonoids containing phenolic groups, newly formed in response to exposure to UV-B radiation, can act as free radical scavengers and assist in combating the harmful biological effects of oxygen-related free radicals produced by UV-B (Bors et al., 1990).

In addition to these biochemical and molecular changes, UV-B can lead to anatomical changes in the photosynthetic apparatus, such as dilation of the thylakoid membrane and disintegration of the chloroplast envelope (Brandle et al., 1977; He et al., 1994). UV-B irradiation can also indirectly reduce the photosynthetic capacity by reducing leaf area with a subsequent decrease in light interception (Teramura and Ziska, 1996). Increases in leaf thickness or changes in epicuticular waxes or leaf pubescence have also been observed (Cen and Bornmann, 1990; Tevini and Steinmuller, 1987).

In a number of studies, UV-B radiation has been found to induce stomatal closure or to affect stomata number or density (Teramura and Ziska, 1996). Also, some morphological changes can be attributable to UV-B induced photooxidation of auxin (IAA) (Beggs et al., 1986).

It has been shown that UV-B effects vary in relation to background PAR (photosynthetically active radiation, 400-700 nm) (Bornmann, 1991). The low levels of UV-B required for DNA dimerization (Strid et al., 1994) are, in the biosphere, always accompanied by considerably higher levels of UV-A (10-20 fold) and PAR (60-600 fold). These wavelengths are suited to drive and control the photoreactivation process. In Arabidopsis, the accumulation of transcripts encoding photoreactivating enzymes is enhanced by PAR, blue or UV-A, but not by red or UV-B wavelengths (Jansen et al., 1998).

Rapid PAR-driven turnover of D1 protein (D2 is stable under PAR) has also been proposed as a part of a damage-repair cycle essential, which maintains PSII function under photoinhibitory conditions (Aro et al., 1993). By analogy, it is possible that UV-B driven D1-D2 turnover is also part of a repair cycle, preventing accumulation of UV-inactivated PSII. However, UV-B effects are often seen under

high UV-B fluences and/or low accompanying PAR (Jansen et al., 1998); low fluences of UV-B stimulate the general phenylpropanoid pathway, resulting in accumulation of flavonoid and sinapic esters (Day and Vogelmann, 1995; Li et al., 1993). These compounds play a protective role by specifically absorbing in the wavelength region from 280 to 340 nm (but not in the PAR waveband, which would diminish photosynthetic yields). Flavonoids also possess free radical scavenging activity, which might offer additional protection to cells accumulating these compounds (Rice-Evans et al., 1997).

Active oxygen species play a role in mediating UV-B damage (Strid et al., 1994; Rao et al., 1996; Mackerness et al., 1999). Scavenging of AOS (active oxygen species) or other radical species, through enzymatic or non-enzymatic systems, can alleviate the UV-B stress (Jansen et al., 1996). In turn, low fluences of UV-B induce scavenging capacity by up-regulation of genes encoding enzymatic or non-enzymatic scavengers (Day and Vogelmann, 1995; Rao et al., 1996). Specific compounds such as polyamines, waxes and alkaloids have been found to accumulate under UV-B exposure, suggesting their role in UV tolerance (Jansen et al., 1998). Polyamines accumulate in response to environmentally relevant doses of UV-B and PAR. In soybean, a correlation was found between levels of polyamines and tolerance to UV-B (Kramer et al., 1992).

A series of experimental data demonstrated the involvement of polyamines in the photosynthetic apparatus structure and functioning. Kotzabasis et al. (1993a) reported that the main polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) are associated with the light-harvesting complex (LHC) and PSII complex of spinach and that highly purified PS II core antenna and reaction center particles of PS II contain in considerable concentrations only the tetramine Spm. Del Duca et al. (1994) showed the apoproteins of the chlorophyll a/b antenna complex (LHCII, CP 24, CP26, CP29) and the large subunit of Rubisco to be substrates of a plastidic transglutaminase. This enzyme catalyzes the incorporation of polyamines into their target proteins. Besford et al. (1993) identified D1, D2, Cyt f and the large subunit of Rubisco as proteins that can be stabilized by the addition of exogenous polyamines. Andreadakis and Kotzabasis (1996) suggested that polyamines as well as the corresponding plastidal enzyme activities of ornithine decarboxylase (ODC), arginine decarboxylase (ADC) and diamine oxydase (DAO) are photoregulated and undergo considerable changes during chloroplast photodevelopment. These data strongly

support the hypothesis that polyamines play an important role in the development of structure and functioning of the photosynthetic apparatus, possibly due to their capability to "stabilize" chlorophyll protein complexes. Furthermore, data obtained by Kotzabasis et al. (1999) show that polyamines play a regulatory role during photoadaptation. Specifically, a decrease of the intracellular Put level and, viceversa, an increase of Spm, indicated by a raised Spm/Put ratio simulate a low-light photoadapted photosynthetic apparatus. It was concluded that photoadaptative responses can be modulated by inhibition or induction of polyamine biosynthesis.

Several experiments have demonstrated that polyamines are induced in response to many stressor systems (Smith, 1985; Slocum et al., 1984; Kramer et al., 1991). Membrane damage appears to be involved in the phytotoxicity of environmental stressors such as ozone (Heath, 1987) and chilling (Wang, 1990). Polyamines can bind to membrane surfaces via ionic interactions with phospholipids and act to inhibit lipid peroxidation (Kitada et al., 1979; Tadolini, 1988). Direct application of polyamines to plant tissue has been shown to inhibit the development of injury induced by ozone (Ormrod and Beckerson, 1986; Bors et al., 1989) and chilling (Kramer et al., 1991), There is also evidence that membrane damage may be involved in UV-B stress (Kramer et al., 1991; Murphy, 1983). The inhibition of photosynthesis by UV-B may involve the disruption of chloroplast membrane structure (Brandle et al., 1977). Upon UV-B exposure, the total levels of chloroplast lipids decrease and the ratio of the individual polar lipids is altered in several species (Tevini et al., 1981). Possibly, the radical-scavenging activity of polyamines and polyamines conjugates moderates UV-B stress, as it have been demonstrated for other stress situations. Direct application of exogenous polyamines to plant tissue has been shown to inhibit the development of injury induced by ozone (Bors et al., 1989; Langebartels et al., 1991) and chilling (Kramer et al., 1991). UV-B radiation induces accumulation of lipid peroxidation products in cucumber leaves concomitant with a reduction in the ratio of unsaturated/saturated fatty acids (Kramer et al., 1991). The magnitude of this effect was correlated with cultivar sensitivity to UV-B. Increased polyamine levels also resulted from UV-B exposure in cucumber (Kramer et al., 1991). Kramer et al. (1992) showed that the effects of UV-B on polyamine levels in soybean were greatly influenced by PAR levels in a dose-dependent fashion. They, also, suggested that UV-B sensitivity could involve differences in polyamine

accumulation, based on the finding that a UV-B resistant cultivar of soybean accumulated highly polyamines comparative to a sensitive cultivar.

Although the present knowledge concerning the impact of UV-B radiation is increasing, several gaps exist in our present understanding of how photosynthesis is affected by UV-B radiation. Many of the detrimental UV-B effects noted under laboratory conditions are not observed in field experiments (Jansen et al., 1998). Two likely reasons underlying the discrepancy between laboratory and field studies are the unnatural amplification of damaging reactions as a result of the excessive UV-B fluence rates used in the laboratory, and a failure to take into consideration naturally occurring tolerance mechanisms (Jansen et al., 1999). PAR contribution to the modulation of plant reactions to UV-B radiation is not well understood. Several investigators have found that high PAR can alleviate to a certain extent the stress induced by UV-B irradiation (Mirecki and Teramura, 1984; Cen and Bornman, 1991; Kramer et al., 1992). In contrast, other data suggested that not light intensity but a certain UV-B/visible light ratio can provide some protection to UV-B stress (Warner and Caldwell, 1983).

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In the present study we investigated a series of alterations that UV-B radiation induces in the photosynthetic apparatus of *Scenedesmus obliquus*, in order to distinguish between reactions comprising the adaptive responses to UV-B and reactions reflecting damages in the photosynthetic apparatus. The contribution of PAR to the modulation of UV-B induced effects was assessed by comparing the behavior of cultures exposed to UV-B irradiation under different illumination conditions, namely dark, low PAR and high PAR conditions. Also, the recovering ability of the irradiated cultures in different illumination conditions was estimated after removal of the UV-B source. Chlorophyll and protein amounts, as well as the physicochemical properties of the photosynthetic apparatus were estimated in these experiments, by recording the structural and functional alterations induced by UV-B irradiation to the photosynthetic apparatus. In parallel, qualitative and quantitative measurements of the intracellular polyamine levels were carried out to elucidate whether polyamine amount fluctuations constitute a primary cellular response to UV-B irradiation, since both an adaptive and protective role has been attributed to these compounds (Smith, 1985).

MATERIAL AND METHODS

1.Organism and growth conditions

Cultures of the unicellular green alga *Scenedesmus obliquus*, (fam. *Chlorophyceae*) wild type, strain D_3 (Gaffron, 1939), were grown autotrophically in liquid culture medium (Bishop and Senger, 1971) [Table 1] in a temperature-controlled water bath (30^0C), in front of a panel of white fluorescent lamps (Osram 36W/10), which provided a photon fluence rate of 120 μmol m^{-2} s^{-1}. The cultures were continuously percolated with air enriched with 3% (v/v) carbon dioxide.

Table 1: The chemical composition of liquid medium used for *Scenedesmus obligus* cultures (Gafron, 1939).

INGREDIENTS	QUANTITY (g/l)
Macroelements	
$CaCl_2$ $2H_2O$	1,5
KNO_3	80
$MgSO_4$ $7H_2O$	24,6
NaCl	47
Na_2HPO_4 $2H_2O$	17,8
NaH_2PO_4 H_2O	40,5
Na Citrate $2H_2O$	16,5
$Fe_2(SO_4)$ H_2O	0,4
Microelements	
H_3BO_3	2,86
$MnCl_2$ $4H_2O$	1,81
$ZnSO_4$ $7H_2O$	0,22
$CuSO_4$ $5H_2O$	0,079
MoO+ (85-99,5%)	0,0177
$C_6H_5FeO_7$ $5H_2O$	18,025

2.Experimental conditions: UV-B exposure and recovery

Prior to each irradiation experiment, a volume of 600 ml suspension cultures was equally distributed in two open, cylindrical glass containers (Φ 15 cm), forming a layer of 4,5 cm height, with continuous stirring in a temperature-controlled room (26^0C).

A Vilbert-Lourmet VL6W lamp was used as UV-B light source for one of the two glass containers. The other one was used as control and was covered during UV-B irradiation with a plastic filter (thin film UV filter Edmund Scientific Company, U.S.A.), which cut all radiation below 320 nm. The UV-B intensity used for *Scenedesmus* culture irradiation was 0,420 mW cm^{-2}, as measured by a Vilber-Lourmet radiometer equipped with a 312 nm sensor.

UV-B irradiation treatment was performed for 3h in three different experimental conditions: darkness, low PAR (85 μmol m^{-2} s^{-1}) and high PAR (700 μmol m^{-2} s^{-1}), after a period of 2h adaptation of the cultures to the above-described conditions. For recovery, cultures were maintained for 4 h in the same conditions as those applied during irradiation, or were transferred in different conditions, as shown in Table 2.

Table 2: Schematic representation of illumination conditions applied during UV-B irradiation and after UV-B irradiation (recovery).

UV-B IRRADIATION conditions 3 h	**RECOVERY conditions** 4 h		
	LOW LIGHT	DARKNESS	HIGH LIGHT
LOW LIGHT	■	■	
DARKNESS	■	■	
HIGH LIGHT			■

3.Pigment extraction and estimation

After harvesting of the cells by centrifugation (1500 g, 5 min), the algal pellet was exhaustively extracted with hot methanol under safe dim green light until it was

colorless. The amount of chlorophyll (Chl) was photometrically determined according to the method of Holden (1965) and calculated as followed:

Chl a: (16,5xE665)-(8,3xE650)

Chl b: (33,8xE650)-(12,5xE665)

4. Polyamine determination and analysis

For polyamine determination, cells were harvested by centrifugation (1500 g, 10 min), the pellets resuspended in 1 N NaOH and then hydrolyzed according to the procedure of Tiburcio et al. (1985). 0.2 ml from the hydrolyzate were mixed with 36% HCl in a ratio of 1:1 (v/v) and, after transfer into ampoules, the samples were subjected to hydrolysis at 110^0C for 18h. The hydrolysis products were evaporated at 70-80^0C. The dried products were redissolved in 0.2 ml of 5%(v/v) perchloric acid. For the qualitative and quantitative estimation of polyamines, the samples were benzoylated according to the modified method of Flores and Galston (1982). For that purpose, 1 ml 2N NaOH and 10 µl benzoylchloride were added to 0.2 ml of the polyamine containing hydrolyzate and the mixture vortexed for 30 s. After 20 min incubation at room temperature, 2 ml of saturated NaCl solution were added to stop the reaction. The benzoylpolyamines were extracted three times into 2-3 ml diethylether, all ether phases collected and evaporated to dryness. The remaining benzoylpolyamines were redissolved in 0.2 ml of 63% (v/v) methanol and 20 µl aliquots of this solution was injected into the high performance liquid chromatography (HPLC) system for the analysis of the polyamines according to the method of Kotzabasis et al. (1993). The analyses were performed with a Shimadzu Liquid Chromatography apparatus (LC-10AD) equipped with a data-processing unit (DPU), a diode array detector (Shimadzu SPD-M10A) and a narrow-bore column (C_{18}, 2,1x200mm, 5µm particle size Hypersyl, Hewlett-Packard). To estimate directly the amount of each polyamine, the method of Kotzabasis et al. (1993) was again followed.

5. Fluorescence induction measurements

For the fluorescence induction measurements the portable Plant Efficiency Analyser, PEA (Hansatech Instruments) was used. Maximum yield of photochemistry (Fv/Fm), absorbance per reaction center (ABS/RC), the rate of primary photochemistry per reaction center (TR_0/RC), the rate for electron transport per reaction center (ET_0/RC), the amount of energy dissipated per reaction center (DI_0/RC) and the number of photosynthetic reaction centers per unit area (RC/CS) were measured according to the JIP method of Strasser and Strasser (1995). The method is based on the measurement of a fast fluorescence transient with a 10 μs resolution in a time span of 40 μs to 1 s. Fluorescence was measured at a 12 bit resolution and excited by six light emitting diodes providing an intensity of 600W m^{-2} of red (650 nm) light. This method allows the dynamic description of a photosynthetic sample at a given physiological state.

6. Determination of the packed cell volume (PCV)

The PCV of a cell suspension was determined by centrifugation at 1500 g for 5 min using hematocrit tubes (Senger, 1970).

8. Statistics

All experiments developed in continuous dark or light conditions were performed at least in triplicate and the results represent an average of all these values.

RESULTS AND DISCUSSION

Despite the fact that a multitude of data about UV-B induced -plant responses have been accumulated, it is difficult to evaluate the environmental relevance of UV-B effects on photosynthesis (Jansen et al., 1999). Many of the detrimental UV-B effects noted under laboratory conditions are not observed in field experiments (Fiscus and Booker, 1995; Rozema et al., 1997; Jansen et al., 1998). The response of a plant to UV-B radiation is the result of a balancing act involving damaging reactions, repair and acclimation responses (Jansen et al., 1999). Indeed, two likely reasons underlying the discrepancy between laboratory and field studies are the unnatural amplification of damaging reactions as a result of the excessive UV-B fluence rates used in the laboratory and a failure to take into consideration naturally occurring tolerance mechanisms (Fiscus and Booker, 1995; Rozema et al., 1997; Jansen et al., 1998). In addition to unrealistically high UV-B irradiance used, another criticism of many previous studies has been the low PAR under which the plants were grown and irradiated. Only a few studies have examined the effects of UV-B radiation on plants grown under relatively high PAR, which more approximate the natural conditions (Mirecki and Teramura, 1984; Bornman and Vogelman, 1991; Flores-Moya et al., 1999; Krause et al., 1999).

In this context, we considered necessary to investigate the contribution of PAR on the modulation of plant responses to UV-B irradiation and on the ability of plant cells to recover the changes induced by UV-B in the photosynthetic apparatus of *Scenedesmus* cultures. To achieve this goal, three different conditions, namely darkness, low PAR and high PAR, were used as background for UV-B irradiation and recovery.

Previously, the kinetics of PAR and UV-B radiation intensity in the solar light were established by measurements performed during day time (on 26 June). As Fig.1 depicts, UV-B radiation intensity reached unexpected levels, which were much higher as those reported by other investigators (Mirecki and Teramura, 1984; Krause et al., 1999; Bischof et al., 2000).

Different UV-B doses (0.086, 0.137, 0.175, 0.225, 0.317 and 0.420 mW cm^{-2}) similar to those found in the solar spectrum were initially tested on *Scenedesmus* cultures incubated in low light conditions (LL: 87 μmol m^{-2} s^{-1}). The photosynthetic

efficiency, described as Fv/Fm ratio was used as indicator of plant response to UV-B irradiation. As Fig. 2 shows, the photosynthetic efficiency of cultures exposed to UV-B doses higher than 0.2 mW cm^{-2} was profoundly affected.

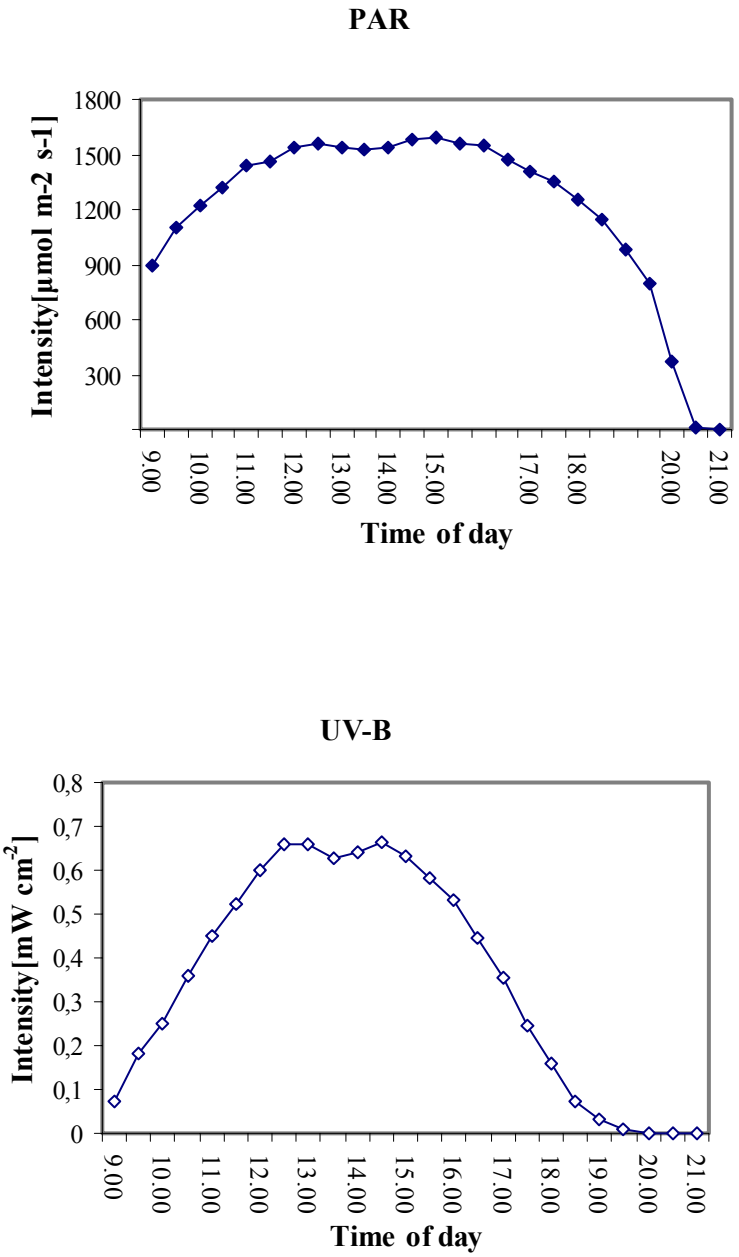


FIG. 1 : PAR and UV-B intensity kinetics in the solar light during day time (26 June, Irakleion)

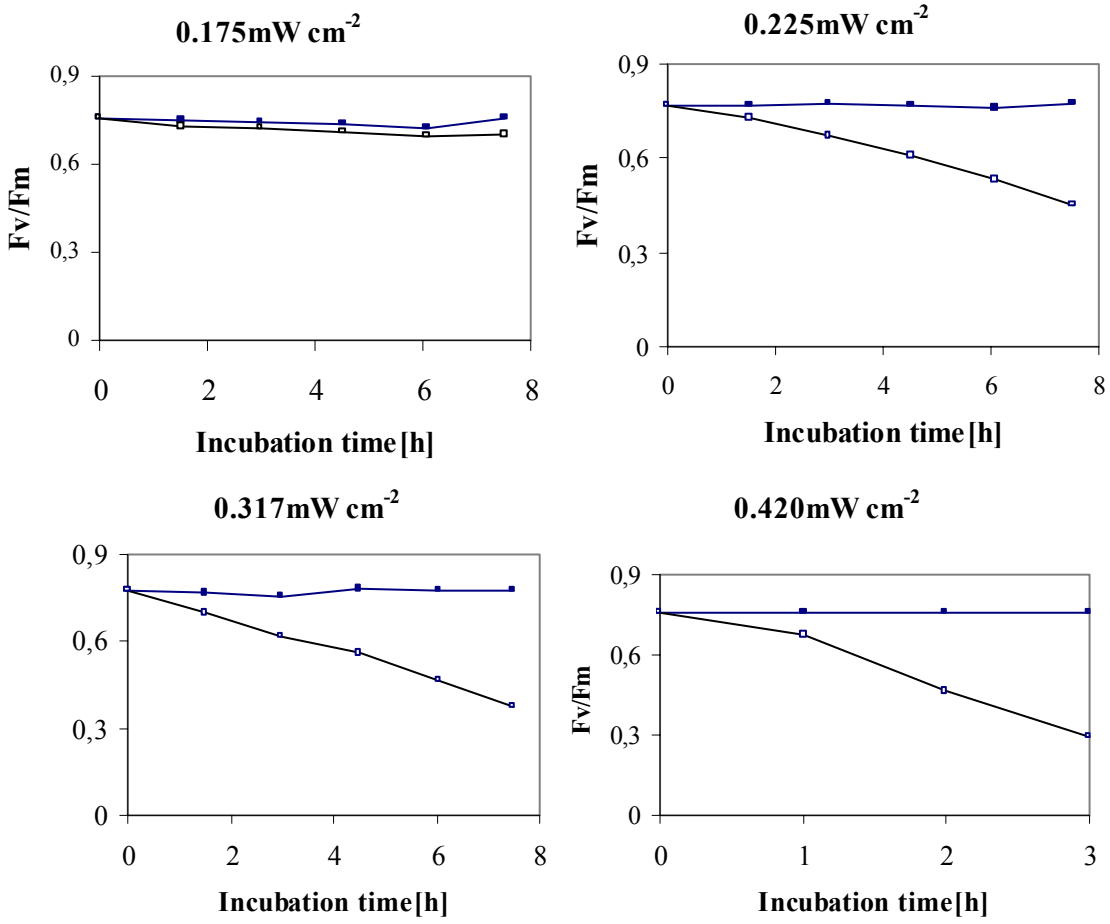


Fig.2 Changes in the photosynthetic efficiency (Fv/Fm) of *Scenedesmus* cultures exposed under low light (87 μmol m^{-2} s^{-1}) to different UV-B doses, shown above each diagram. The closed squares represent the response of control cultures. The open squares represent the response of treated cultures.

Previous data have demonstrated that the exposure response of an organism to a stressor is a mixture of time-dependent and time-independent behavior. The early part of the exposure is described as cumulative fluence (and is, therefore, time-dependent) and the latter phase is described as a function of fluence-rate (time-independent) (Heraud and Beardall, 2000). As we observed, the decline of Fv/Fm ratio was dependent on exposure time to UV-B but the induction and the magnitude of this response were strongly correlated to UV-B dose.

Following the above-described results, 0.420 mW cm^{-2} UV-B was experimentally tested in *Scenedesmus* cultures incubated in three different illumination conditions, namely low PAR (LL: 87 μmol m^{-2} s^{-1}), high PAR (HL: 700 μmol m^{-2} s^{-1}) and darkness (D). The same procedure, consisting of 3 h irradiation followed by 4 h recovery, was applied in each of these experiments (as described in Material and Methods-Tab.2).

Data obtained from fluorescence induction measurements provided valuable information regarding the changes that occurred in the structure and function of photosynthetic apparatus of S*cenedesmus* cultures during these experiments. As Tab. 3 shows, the photosynthetic efficiency (expressed as Fv/Fm ratio) decreased under UV-B irradiation in a time-dependent manner. In addition, this response was strongly influenced by PAR intensity, being more pronounced in cultures incubated in low light conditions than in those incubated in high light or dark conditions. After the cessation of UV-B treatment, Fv/Fm ratio recovered partially, within 4 h, only in cultures incubated in continuous light conditions. In contrast, the recovery of this parameter was inhibited in dark-adapted cultures, where Fv/Fm followed a continuous decline. Similarly, Fv/Fm decreased dramatically in cultures exposed to low light conditions after UV-B irradiation in darkness or inversely (Fig.3).

The above-presented data provided an evidence that UV-B irradiation decreased the photosynthetic performance of *Scenedesmus* cultures, but the dimension of Fv/Fm changes, as well as, the capacity to recover these changes are depended on light intensity. These results are in agreement with previous experimental data, which showed that Fv/Fm ratio is down-regulated by UV-B radiation through photochemical damage of PS II (Teramura and Sullivan, 1991; Tevini and Teramura, 1989; Strid et al., 1990; He et al., 1993; Day and Vogelmann, 1995; Krause et al., 1999; Jansen et al., 1999; Rajagopal et al., 2000). In this context, it has been found that the turnover of D1 and D2 reaction center proteins is greatly enhanced by UV-B radiation with

Tab.3: Photosynthetic efficiency (Fv/Fm) of *Scenedesmus* cultures used as control (c) or exposed to 0,420mW cm^{-2} UV-B (uv), during (A) and after UV-B irradiation (B)

TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
	c	uv	c	uv	c	uv	c	uv	c	uv
0	0,753	0,753	0,759	0,76	0,756	0,756	0,753	0,753	0,77	
1	0,741	0,691	0,756	0,674	0,748	0,682	0,741	0,691	0,771	
2	0,743	0,606	0,755	0,467	0,749	0,536	0,743	0,606	0,763	
3	0,74	0,494	0,76	0,292	0,75	0,393	0,74	0,494	0,764	
4	0,735	0,352	0,751	0,362	0,743	0,357	0,741	0,159	0,769	
5	0,723	0,35	0,745	0,417	0,734	0,383	0,767	0,12	0,741	
6	0,734	0,343	0,75	0,539	0,742	0,441	0,778	0,129	0,696	
7	0,739	0,328	0,748	0,587	0,744	0,457	0,773	0,137	0,726	

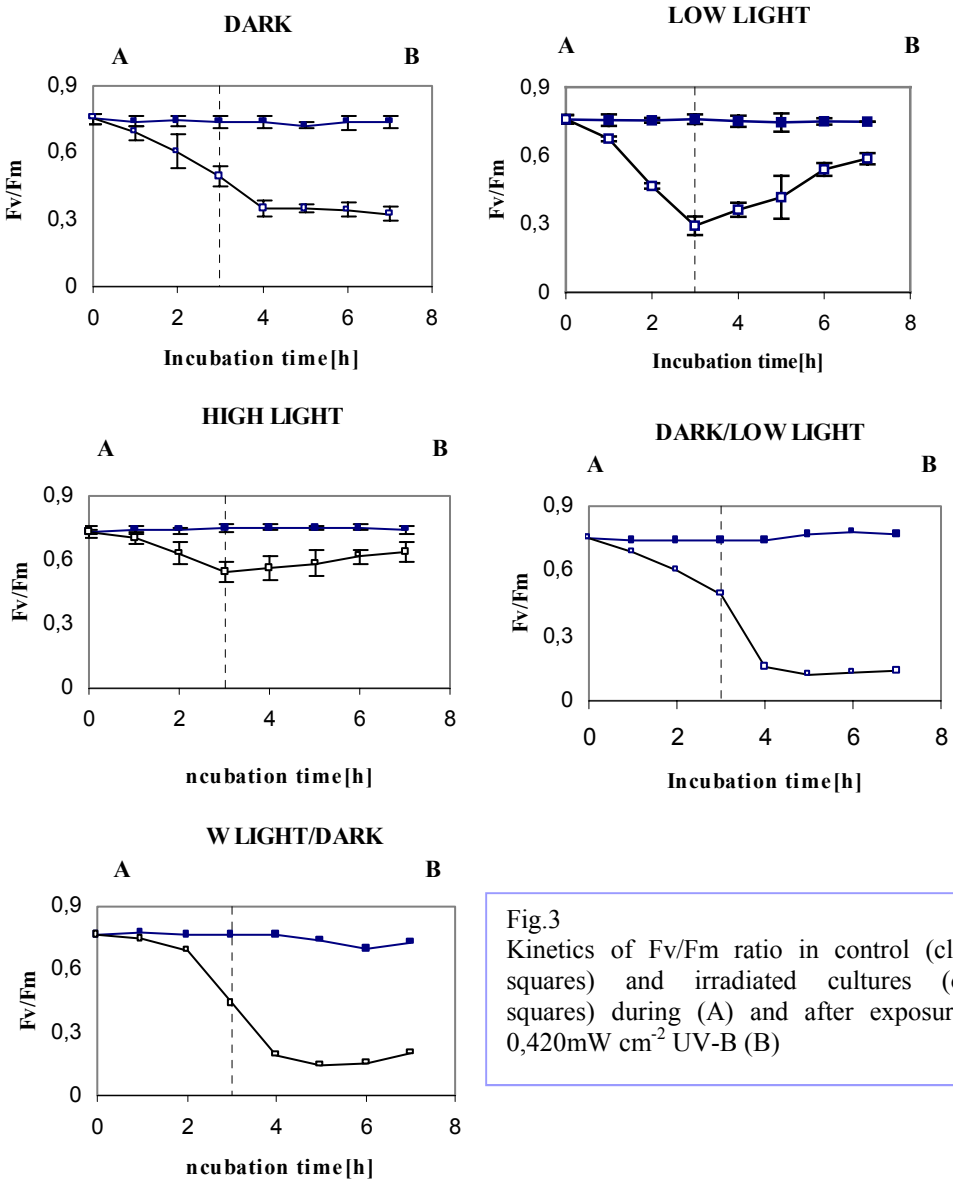
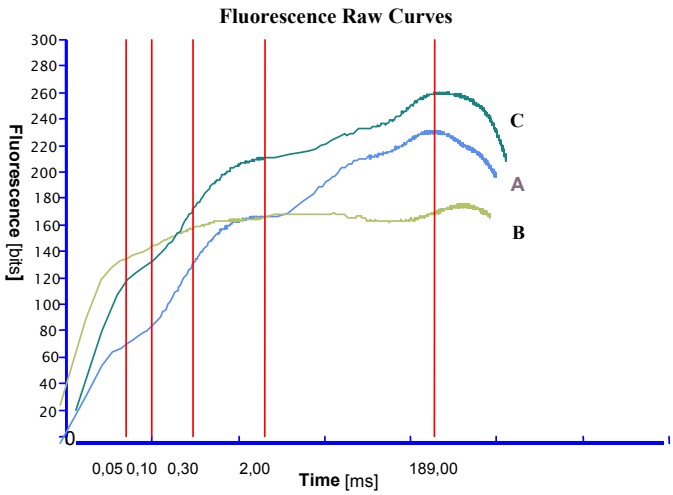


Fig.3
Kinetics of Fv/Fm ratio in control (closed squares) and irradiated cultures (open squares) during (A) and after exposure to 0,420mW cm^{-2} UV-B (B)

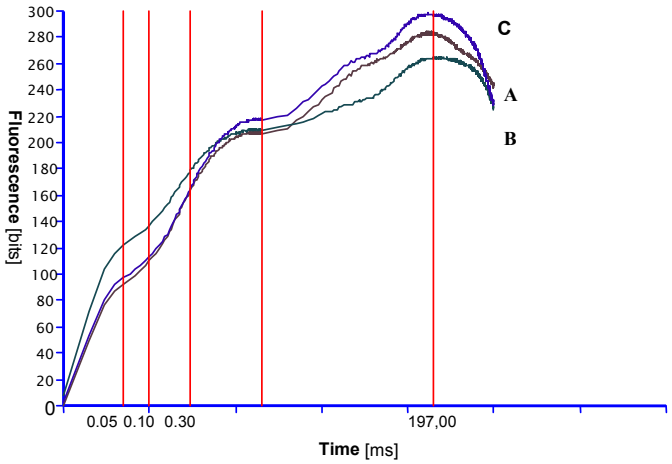
specific breakdown fragment being identified (Greenberg et al., 1989; Jansen et al., 1993; Friso et al., 1994). Protein degradation driven by UV-B radiation may be accompanied by a loss of activity of PSII reaction centers (Friso et al., 1994). As other experimental data have indicated, UV-B induces damage of PSII due to the inefficiency of the repair system that replaces damaged reaction centers with newly synthesized D1 and D2 proteins, restoring in this way the normal PSII activity (Ohad et al., 1984; Aro et al., 1993).

We previously showed that the loss of PSII activity in UV-B irradiated *Scenedesmus* cultures, as indicated by a reduced Fv/Fm ratio, was higher in light conditions than in darkness. These results are consistent with the finding that UV-B driven-D1 and D2 degradation is strongly accelerated in the presence of a background of visible light (Jansen et al., 1996). Although PAR was commonly found to diminish the impact of UV-B radiation on plants (Jansen et al., 1998), the accelerated turnover of D1 proteins under mixtures of UV-B radiation and PAR causes the reduction of photosynthetic efficiency of PSII, expressed by the decline of Fv/Fm ratio.

It is generally accepted that fluorescence induction curves, reflecting the photosynthetic activity and electron transport, have a characteristic pattern that undergoes changes when the photosynthetic systems become impaired, and can be used as an indicator of damage (Mirecki and Teramura, 1984). Therefore, we examined the polyphasic kinetics of the Chl fluorescence rise from F_0 (O level) via J-P phase to Fm (P level) of a Kautsky curve (Strasser et al., 1995) at three experimental stages applied in *Scenedesmus* cultures: prior to UV-B irradiation (A), after 3 h of UV-B irradiation (B) and after 4 h of recovery (C). As Fig.4 features, the shape of curves prior to UV-B irradiation was similar for both low light- and high light- adapted cultures, although the O-P phase was slightly increased in high light than in low light conditions (Fig.4A). The changes in fluorescence kinetics that occurred following UV-B irradiation were more dramatic in cultures incubated in low light conditions. Specifically, the O-J phase increased, whereas the J-P phase strongly decreased. In contrast, a minor increase of O-I phase accompanied by a significant reduction of I-P levels was observed in high light-adapted cultures after UV-B exposure (Fig.4B).



Low light



High light

Fig.4: Polyphasic kinetics of Chl fluorescence in cultures exposed to low light and high light prior to irradiation (A), after 3 h of irradiation with 0,420mW cm^{-2} UV-B (B) and after 4 h of recovery (C).

After 4 h of recovery in low light conditions, the O-J phase decreased but it still remained higher than prior to irradiation. Significantly, in both recovery situations, O-P phase increased over the initial level (Fig.4C). Our results show that the decline of Fv/Fm ratio in cultures exposed to UV-B irradiation resulted mainly from Fm reduction, accompanied by F_0 rise only in low light-adapted cultures. This finding is in agreement to the results of Krause et al. (1999), who found that plant exposure to direct sunlight resulted in a diminished Fm, while F_0 was insignificantly changed. On the contrary, Heraud and Beardall (2000) reported that in low light conditions, besides Fm reduction, a rise of F_0 following UV-B irradiation occured. As Surplus et al. (1998) indicated, the increase of F_0 suggests a blockage in electron flow out of PSII. In accordance with data from literature, the J-P phase is regarded as the fluorescence induction of fully functional $PSII_\alpha$ units, whereas the O-J phase represents the emission of PS II_β units that are incapable of transferring electrons from Q_A to Q_B and, possibly, are in the process of being repaired (Melis, 1991). Considering this information, our results indicate that UV-B irradiation affects highly the concentration of functional $PSII_\alpha$ units which are converted to fluorescence quenchers (Krause et al., 1990; van Wijk et al., 1993). As $PSII_\beta$ units are viewed as a reserve pool for restoring fully active $PSII_\alpha$, their increase induced by the synergistic effect of low light and UV-B radiation, may explain the increased ability of *Scenedesmus* cultures incubated in continuous low light conditions to recover from UV-B effects after the cessation of UV-B treatment.

The data provided by fluorescence induction measurements were available for the calculation of several expressions (such as ABS/RC, TR_0/RC, DI_0/RC, ET_0/RC and RC/CS) using the JIP-method (Strasser and Strasser, 1995).

The absorbance per reaction center (ABS/RC) expresses the total absorption of PSII antenna chlorophylls divided by the number of active (in the sense of Q_A reducing) reaction centers. Consequently, this flux may be regarded as a measure of light harvesting complex (antenna) size (Strasser and Strasser, 1995). Data given in Tab.4 show clearly that, comparative to control cultures, the antenna size was highly increased by UV-B irradiation. This UV-B induced effect was more strongly expressed by cultures adapted to low light conditions, comparative to those incubated in high light and dark conditions. After the cessation of UV-B treatment, the antenna size decreased, gradually, to a level closely similar to that of control. This response

was exhibited only in light-adapted cultures and was more pronounced in low light conditions than in high light conditions. Recovery did not occur in darkness, where antenna size continued to increase. Similarly, cultures transferred to darkness after UV-B irradiation in low light conditions or, inversely, cultures transferred to low light conditions after UV-B treatment in darkness showed an exacerbated increase of antenna size.

Tab. 4: Absorbance per reaction center (ABS/RC) in control (c) and cultures treated with 0,420 mW cm^{-2} UV-B (uv) during (A) and after UV-B exposure (B)

TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW
	c	uv	c	uv	c	uv	c	uv	c
0	3,16	3,212	3,048	3,04	3,529	3,486	3,16	3,212	
1	3,423	3,96	2,998	3,867	3,413	3,74	3,423	3,96	
2	3,433	4,732	2,924	6,236	3,198	4,747	3,433	4,732	
3	3,365	6,043	3,143	10,078	3,242	6,421	3,365	6,043	
4	3,268	7,394	3,103	7,983	3,22	5,458	3,5	15,027	
5	3,55	7,958	3,076	6,803	3,209	5,267	3,295	18,365	
6	3,363	9,065	3,079	5,115	3,201	4,419	3,148	23,203	
7	3,3	8,434	3,057	4,505	3,122	4,278	3,097	20,305	

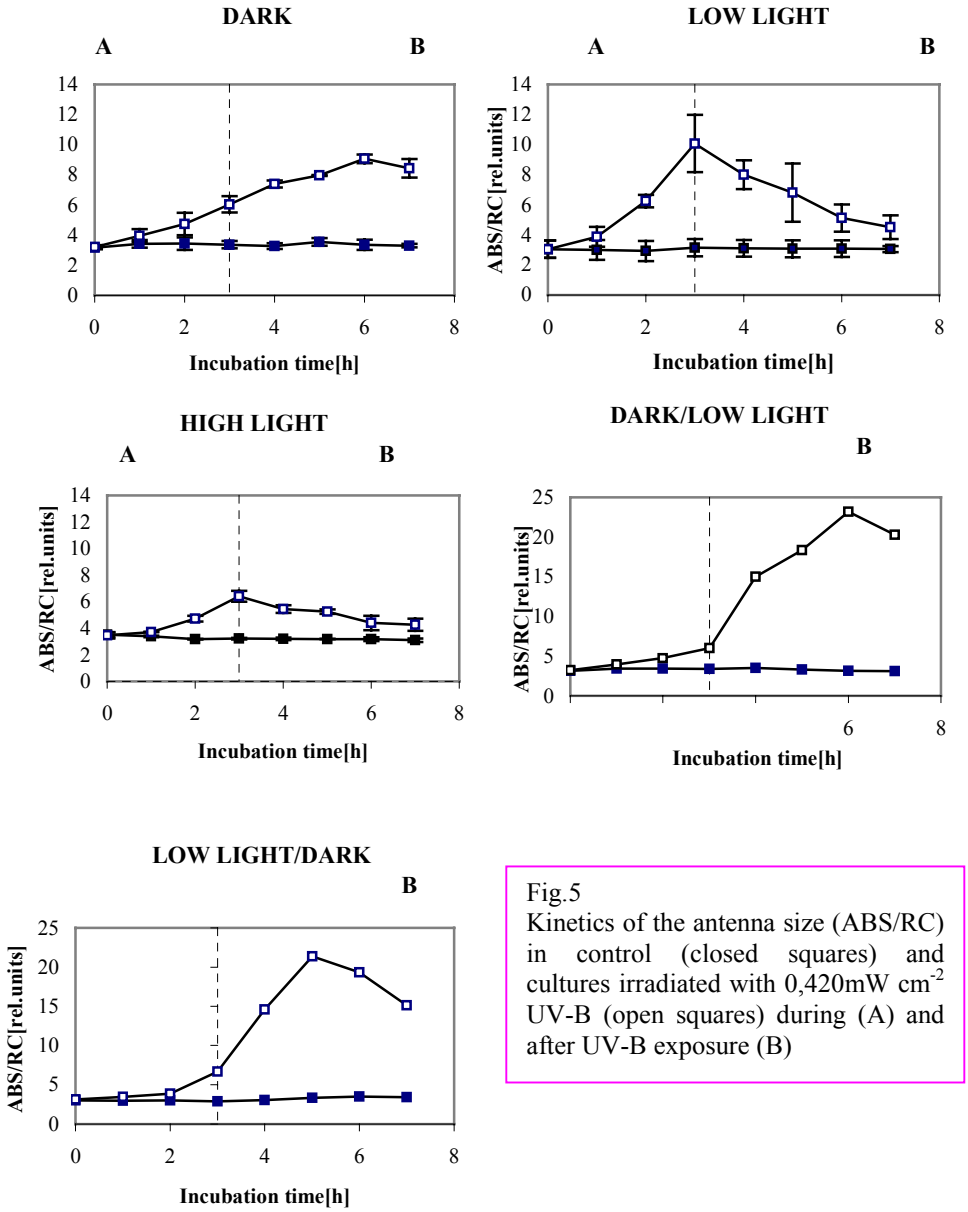


Fig.5
Kinetics of the antenna size (ABS/RC) in control (closed squares) and cultures irradiated with 0,420mW cm^{-2} UV-B (open squares) during (A) and after UV-B exposure (B)

As Fig.5 features, antenna size became highly variable in *Scenedesmus* cultures exposed to UV-B irradiation. It has been suggested that antenna size increase can result from the increase of chlorophyll molecule number per reaction center, the inactivation of some reaction centers or the modification of the rate constants for any excitation energy transfer (Strasser et al., 1978). Our results indicate that at least two of these mechanisms (e.g. the last ones) were responsible for antenna size increase.

The increase of antenna under UV-B irradiation suggests that *Scenedesmus obliquus* cells adopt this strategy in order to avoid the overexcitation and photodestruction of PSII reaction centers. There is increasing evidence that the antenna pigments, beside their role in light harvesting, also serve a photoprotective function in photosynthesis, dealing with excess absorbed light energy by dissipating it in the antenna as heat, as well as by limiting and quenching of triplet chlorophyll in the antenna (Melis, 1991). Furthermore, antenna pigments contribute to the limitation of AOS (active oxygen species) generation and damage to the PSII reaction centers (Demmig-Adams, 1990; Horton and Ruban, 1996). Thus, antenna pigments and pigment-protein complexes play essential roles in both light harvesting and photoprotection by regulating the absorption and utilization of light-energy in photosynthesis.

The kinetics of antenna size showed that *Scenedesmus* cultures possess a great capability to adapt surprisingly fast to different conditions of irradiation which demonstrates the flexibility of photosynthetic apparatus to respond to changes in environmental conditions. According to Hoffmann and Senger (1988), the light-harvesting complex (LHC) seems to play a main role in *Scenedesmus obliquus* adaptation to different irradiance conditions.

As a consequence of UV-B-induced overexcitation of PS II, the trapping effect per reaction center (TR_0/RC) was also altered. This expression stands for the initial energy flux from the antenna to the reaction center, which it is trapped there until a Q_A is reduced to Q_A^- (Strasser and Strasser, 1995). Data listed in Tab.5 show that control cultures exhibited slight differences in the trapping effect per reaction center. The highest values of this parameter were found in dark-adapted cultures and the lowest ones in cultures incubated low light conditions. Under UV-B treatment, TR_0/RC increased almost equally between cultures incubated in different illumination conditions. After the cessation of UV-B treatment, TR_0/RC recovered partially in cultures incubated in continuous light conditions. On the contrary, it increased in

cultures transferred to recover in illumination conditions different from those used as background for UV-B treatment. As Fig.6 depicts, the kinetics of primary photochemistry rate in irradiated cultures exhibit a dynamic phase consisting of TR_0/RC increase followed by a stationary phase characterized by small oscillations at a constant level of TR_0/RC. This behavior suggests that, under inhibitory irradiance conditions, the excitation rate of the open reaction centers is maintained constantly. Such a regulation, called "cruise control", is a strategy that plant cells adopt to avoid the over-excitation of the reaction centers (Gruszecki et al., 1995). Consequently, antenna size and, thus, energy surplus dissipation also increases. The data obtained from DI_0/RC measurements confirmed this hypothesis.

DI_0/RC expresses the total energy dissipation per amount of active reaction centers and it showed to be strongly related to the culture conditions used (Strasser and Strasser, 1995). From the data listed in Tab.6, one can see that, at control level, the rate of energy dissipated per reaction center increased in darkness. Moreover, cultures previously exposed to low light conditions, exhibited after their transfer in darkness, an increase of DI_0/RC values. On the contrary, the energy dissipation rate lowered in cultures incubated in low light conditions as a consequence of their degree of photoadaptation to low PAR intensity.

The rate of energy dissipation increased significantly in cultures exposed to UV-B irradiation, especially in those incubated in low light conditions. After 4 h of dark recovery, the energy dissipation rate enhanced so in cultures exposed to UV-B treatment in dark conditions, as well as in those incubated during UV-B irradiation in low light conditions. A similar response was obtained for cultures transferred in low light after dark irradiation. DI_0/RC recovered partially in cultures maintained in continuous light, especially in low light conditions(Fig.7).

According to data available thus far, the total light energy flux absorbed by a sample can be split in the energy flux conserved as free energy in chemical components (denoted as trapping flux) and the energy flux, which is dissipated as heat or fluorescence through non-photochemical quenching (NPQ) (Strasser et al., 1995). There are two classes of models for the explanation of mechanisms and regulation of NPQ. In one class, NPQ is proposed to occur in the reaction center, and this requires the conversion of a variable fraction of PSII reaction centers to a photochemically inactive state with an increased ability to thermal dissipation of energy (Kreiger et al., 1992). In the other class, the quenching is proposed to occur in the pigment bed and

this requires the appearance of specific quenching centers or processes associated with the pigment bed of PSII that increase the rate of thermal de-excitation in PSII (Genty et al., 1989; Horton and Ruban, 1996). In both models, NPQ is proposed to be, at least partially, modulated by intrathylakoid pH with a low pH producing a large NPQ. Experimental data demonstrated that, in high light-adapted plants, NPQ increased highly comparative to low light-adapted plants (Ruban et al., 1993). Indeed, our results denoted that the rate of energy dissipation in control cultures exposed to high light exceeds that found in low light conditions. Moreover, the highest energy dissipation occurred in darkness, showing that, to a certain extent, photosynthesis is inhibited. All these data denote the increased capacity of *Scenedesmus* cultures to adapt to different irradiance conditions. Considering the behavior exhibited by control cultures, one can expect that, under UV-B exposure, similar differences will be registered. As it was previously showed a different situation was found in UV-B treated *Scenedesmus* cultures, with low light cultures showing the highest energy dissipation rate (i.e. highest NPQ) (Fig.7). The increase of energy dissipation rate results, probably, from the inactivation of some reaction centers, which are transformed into dissipative sinks for the excitation energy following UV-B treatment (Tevini et al., 1991). Moreover, data resulted from RC/CS determination confirm this hypothesis.

Tab.5
Energy trapped in the reaction center (TR0/RC) in control and irradiated cultures, during (A) and after exposure to 0,420mW cm-2 UV-B (B)

TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT	
	c	uv	c	uv	c	uv	c	uv	c	u
0	2,224	2,235	2,065	2,075	2,144	2,155	2,224	2,235	2,191	
1	2,356	2,515	2,017	2,272	2,186	2,394	2,356	2,515	2,143	
2	2,367	2,622	2,177	2,746	2,272	2,684	2,367	2,622	2,169	
3	2,29	2,846	2,193	2,753	2,241	2,8	2,29	2,846	2,076	
4	2,313	2,877	2,083	2,754	2,198	2,815	2,42	2,9	2,212	
5	2,398	2,726	2,151	2,624	2,275	2,675	2,345	2,922	2,301	
6	2,291	2,915	2,109	2,524	2,2	2,719	2,292	3	2,261	
7	2,262	2,764	2,155	2,46	2,208	2,612	2,237	2,8	2,294	

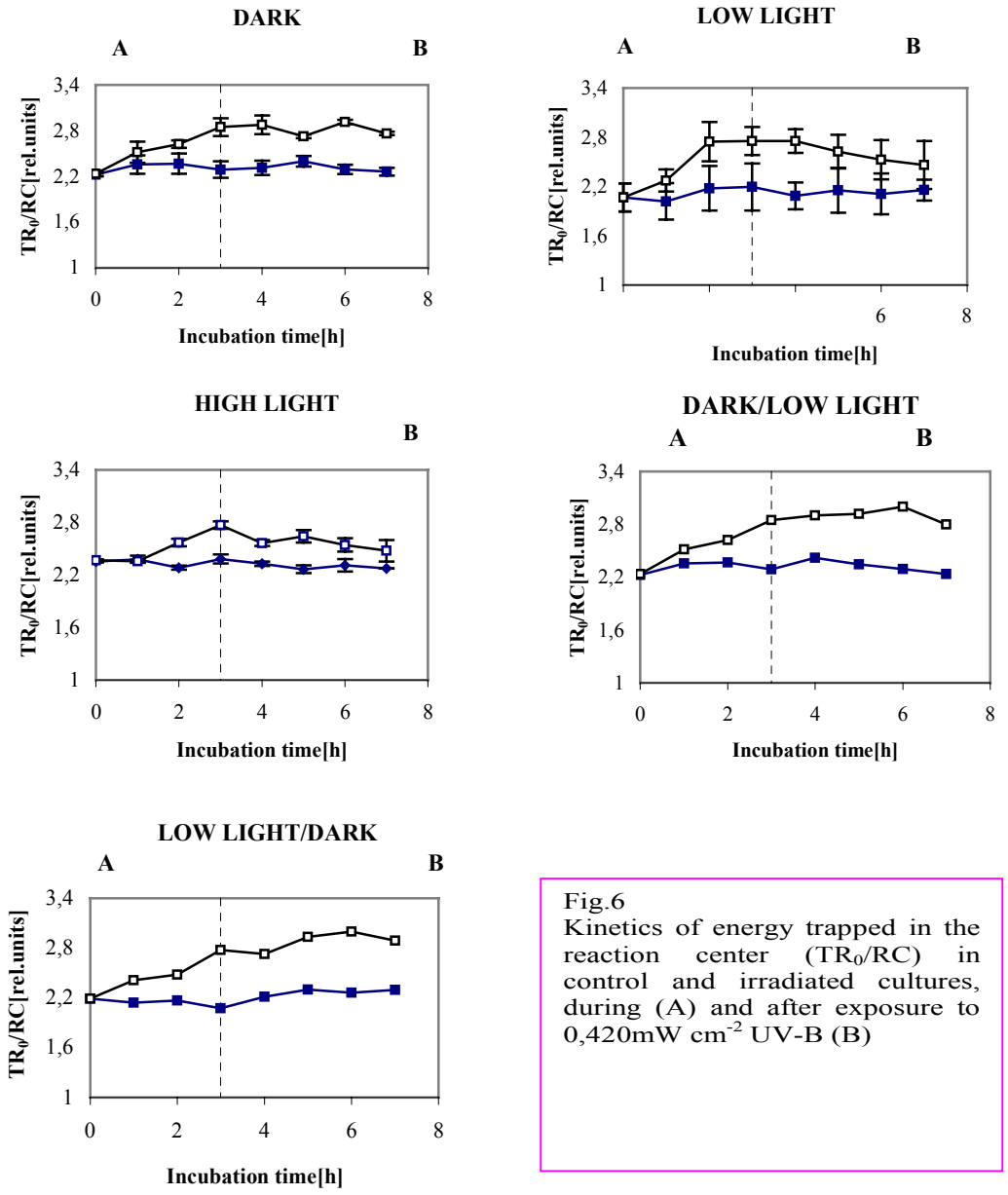


Fig.6
Kinetics of energy trapped in the reaction center (TR_0/RC) in control and irradiated cultures, during (A) and after exposure to 0,420mW cm^{-2} UV-B (B)

Tab.6: Energy dissipation per reaction center (DI_0/RC) in control (c) and irradiated (uv) cultures, during (A) and after exposure to 0,420 mW cm^{-2} UV-B radiation(B)

TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
	c	uv	c	uv	c	uv	c	uv	c	uv
0	0,985	0,976	0,884	0,871	1,155	1,155	0,831	0,86	0,985	0,976
1	1,068	1,445	0,881	1,445	1,059	1,33	0,82	1,061	1,068	1,445
2	1,083	2,109	0,847	3,49	0,961	1,857	0,864	1,387	1,083	2,109
3	1,049	3,156	0,921	5,325	0,949	2,568	0,798	3,889	1,049	3,156
4	1,129	4,048	0,919	4,729	0,94	2,667	0,855	11,85	1,08	12,628
5	1,105	4,749	0,924	2,678	0,943	2,416	1,029	18,461	0,95	16,143
6	0,967	6,221	0,92	2,941	0,957	2,03	1,223	16,33	0,856	20,203
7	1,008	6,08	0,901	2,545	0,954	1,763	1,117	12,212	0,86	17,505

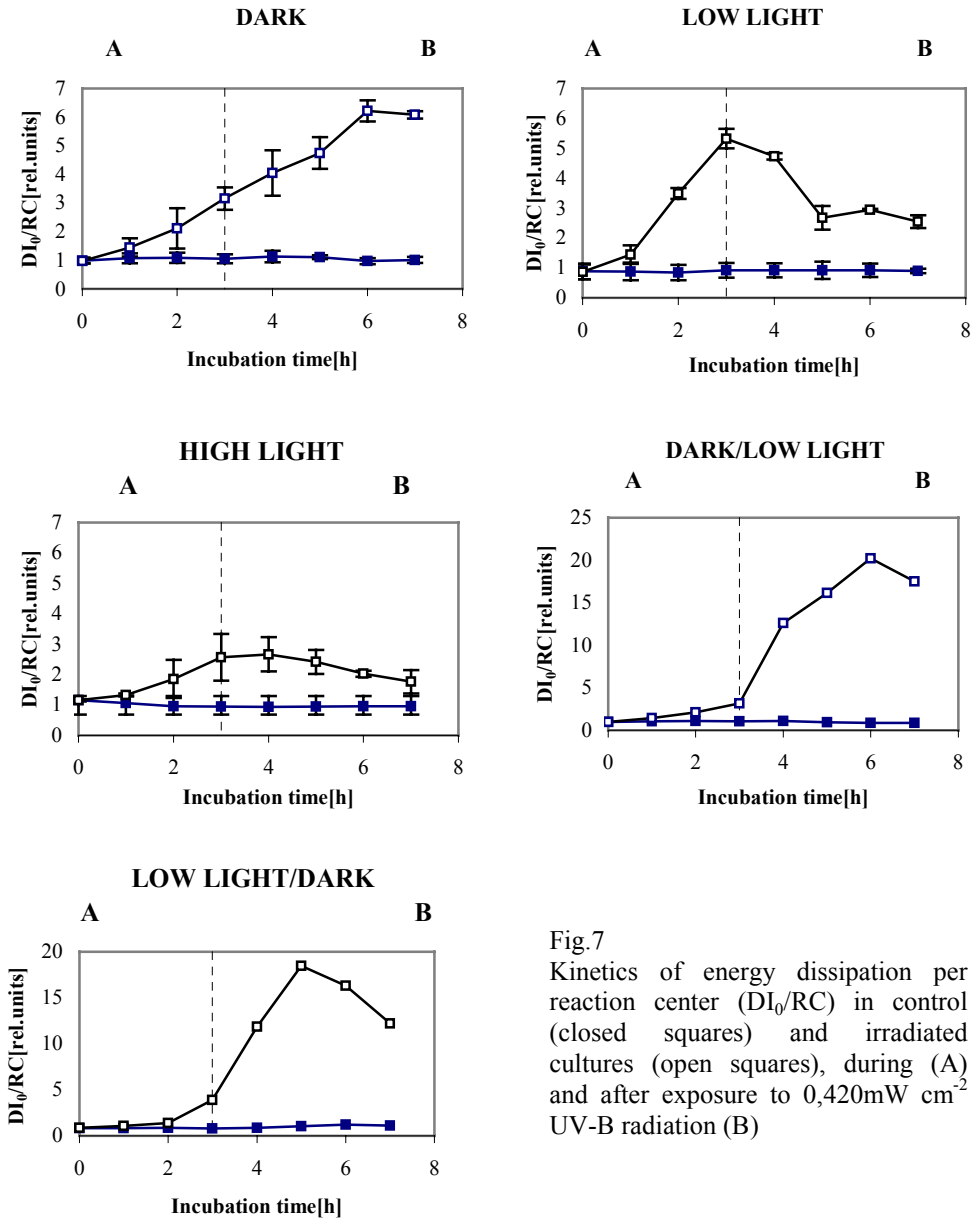


Fig.7
Kinetics of energy dissipation per reaction center (DI_0/RC) in control (closed squares) and irradiated cultures (open squares), during (A) and after exposure to 0,420mW cm^{-2} UV-B radiation (B)

The density of reaction center per cross section (RC/CS) was relative equally between controls. Tab. 7 shows that, under UV-B exposure, RC/CS was similarly decreased in dark- and high light cultures, whereas the reduction of reaction center density in low light cultures was more significant The recovery was fully accomplished in low light conditions and, only partially, in high light cultures. In darkness, recovery did not occur, the density of reaction centers following a slightly continuous decrease. A similar behavior exhibited UV-B irradiated cultures transferred to recover in different illumination conditions as those aplied during treatment.

As Fig.8 depicts, the density of reaction centers recovered only in *Scenedesmus* cultures exposed in continuous light conditions. According to Strasser et al. (1995), the increase in the number of PSII reaction centers may be more a reflection of the necessity of the electron transport capacity of PSII, as a whole, and D1 turnover to match the increased capacity of electron transport chain. To increase the number of PSII reaction centers and at the same time to maintain a balance of excitation energy between the two photosystems requires a decrease in the LHCs associated with PSII core, as it happened in light recovery of UV-B treated *Scenedesmus* cultures.

It should be noted that because the D1 protein turnover occurs in both low and high light conditions (Greenberg et al., 1989), it is not necessarily a measure of UV-induced stress. In other studies performed with higher levels of UV-B, lesions were mapped to several locations in the PS II reaction center. They corresponded to damage to the pigments, electron transport, quinones and the oxygen-evolving complex in addition to the D1 protein, and resulted in formation of inactive PSII (sometimes referred to as β-centers) (Jones and Kok, 1966; Iwanzik et al., 1983; Tevini and Pfister, 1985; Greenberg et al., 1989; Renger et al., 1989; Nedunchezhian and Kulandaivelu, 1991).

Within the framework of these UV-B irradiation experiments, one of the investigated parameters is the rate of electron transport. As Tab.8 depicts, the rate of electron transport (ET_0/RC) was higher in control cultures exposed to light comparative to those incubated in darkness. Upon UV-B exposure, ET_0/RC values decreased in all cultures, especially in those exposed to low light conditions. After a recovery period, the rate of electron transport in cultures exposed to light reached the control level. On the contrary, after 1 h of dark recovery, ET_0/RC values fell down to

67,5% compared to control and remained at this level until the end of experiment. A similar but not so exacerbated response was exhibited by irradiated cultures transferred in darkness. The kinetics of ET_0/RC reflects clearly that UV-B irradiation was responsible for the inhibition of electron transport flux, which recovered only in light conditions (Fig.9). Under UV-B irradiation the photochemical quenching of the chlorophyll fluorescence declines and the Q_A pool becomes more reduced. This requires that the limitation of electron transport lie on the acceptor side of PSII. The differences between UV-B treated samples, excluding the measure that Q_A is reduced, could be due to damage of PSII, which restrict highly the electron transport (McCormac et al., 1994).

Tab.7: Density of reaction centers per cross section (RC/CS) in control cultutres (c) and cultures irradiated with 0,420mW cm^{-2} UV-B (uv), during (A) and after UV-B exposure (B)

	TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
		c	uv	c	uv	c	uv	c	uv	c	uv
A	0	20,85	21,465	22,865	22	19,423	19,429	20,85	21,465	22,865	22
	1	22,588	19,202	22,231	21,02	21,41	19,572	22,588	19,202	22,231	21,02
	2	22,992	17,158	23,252	17,293	20,095	17,34	22,992	17,158	23,252	17,293
	3	23,008	13,688	22,514	11,198	22,532	13,111	23,008	13,688	22,514	11,198
B	4	22,596	11,777	23,514	15,242	22,532	16,972	22,2	6,655	20	8,644
	5	25,302	10,746	21,884	14,082	22,68	15,415	20,5	5,935	19,6	5,001
	6	24,34	10,53	23,956	18,241	21,508	16,259	22	4,353	18,9	5,07
	7	22,672	10,35	24,063	24,302	23,644	17,621	22,063	4,925	20,3	6,159

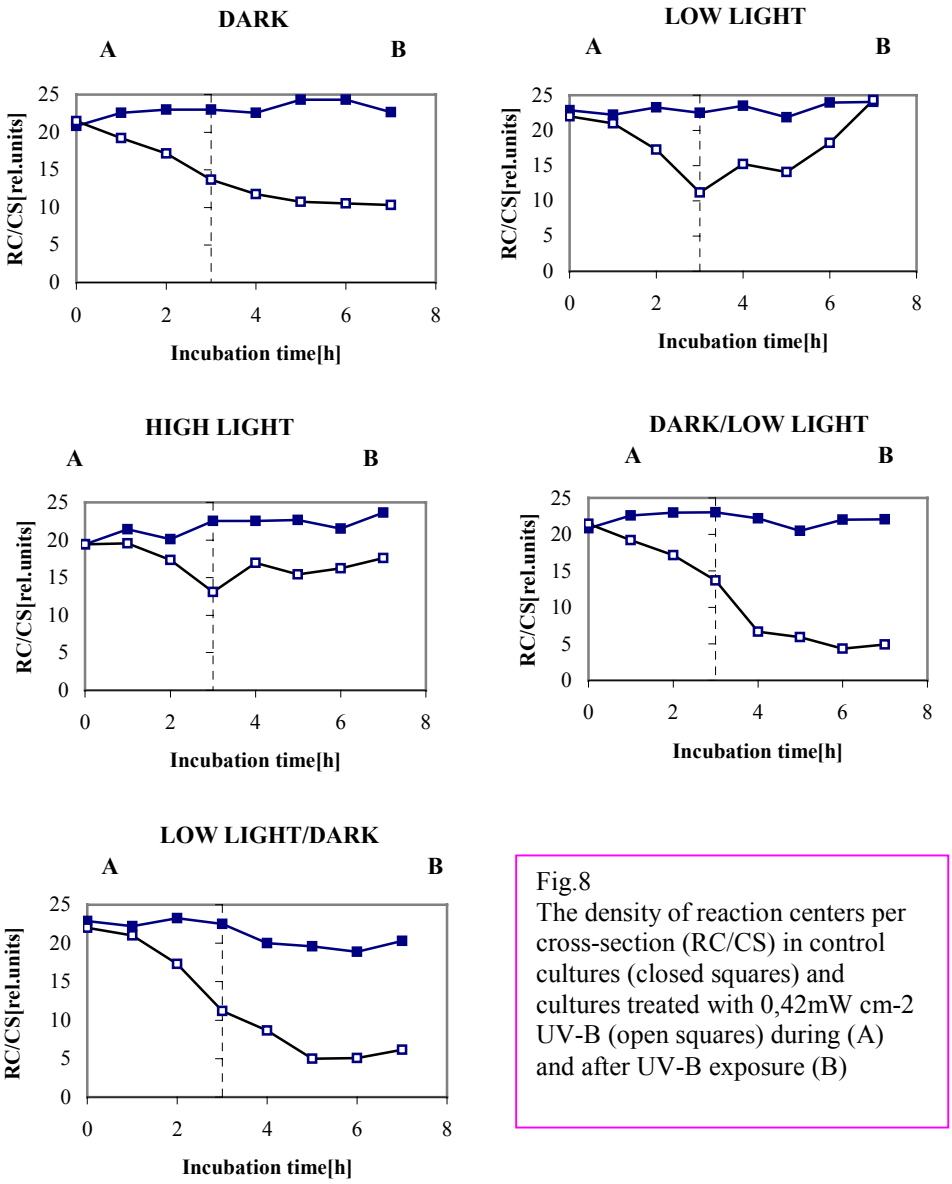


Fig.8
The density of reaction centers per cross-section (RC/CS) in control cultures (closed squares) and cultures treated with 0,42mW cm-2 UV-B (open squares) during (A) and after UV-B exposure (B)

Overall, the behavior exhibited by *Scenedesmus* cultures to UV-B irradiation suggests that significant alterations in the structure and functioning of photosynthetic apparatus have been caused. In order to maintain a constant excitation rate of PSII reaction centers (Fig.6) during UV-B inhibitory conditions, the antenna size increases contributing to the dissipation of light energy surplus. Following the kinetics of antenna size and energy dissipation rate, a linear relationship between these parameters could be established. (Fig.5, Fig.7). This demonstrates that the modification of LHC induced by UV-B, is an adaptive mechanism that cells adopt to protect themselves by UV-B radiation. The antenna size also increases due to transffer of light energy surplus from inactivated reaction centers to antenna (Strasser, 1978). The UV-B induced-inactivation of PS II reaction centers occurs independently on illumination conditions in which cultures were incubated and continues after the removal of UV-B source in dark-adapted cultures and in those transferred in different conditions of illumination after UV-B irradiation. These results suggest that mechanisms assuring the ability of cells to recover are light-dependent, and function so during UV-B irradiation as after the cessation of UV-B treatment. This hypothesis is sustained by the results obtained in cultures transferred in low light conditions after UV-B irradiation in darkness. Here, the density of inactivated reaction centres decreased rapidly after 1 h of recovery, and this suggests the inefficiency of mechanisms which assure the repair of damaged reaction centers. (Fig.8). Due to the deleterious effects of UV-B radiation on PSII, the electron transport flow is inhibited and the photosynthetic efficiency of PS II suffers a similar decline (Fig.9, Fig.3).

It is possible that the inactivation of reaction centers, as occurred in the above-described situations resulted from AOS accumulation. Experimental data suggested that the oxidative damage may induce conformational changes in the reaction centers, which could serve as a triggering or sensing signal for the primary proteolytic cleavage (Ohad et al., 1985). UV-B radiation has been shown to stimulate the generation of AOS (Murphy and Huerta, 1990; Arnotts and Murphy, 1991; Rao et al., 1996; Dai et al., 1997). The origin of these AOS is unclear but it has been proposed that UV-B exposure may lead to AOS generation, by increasing NADPH oxidase activity (Rao et al, 1996). It is proposed that AOS mediate a series of signal transduction pathways each controlling the expression of different specific genes, i.e. up-regulation of pathogenesis-related genes and down-regulation of photosynthetic

genes (Surplus et al., 1998). Consequently, it has been concluded that the antioxidant capacity of a plant tissue dictates the relative sensitivity of photosynthetic genes to UV-B induced down-regulation (Green et al., 1995; Surplus et al., 1998; Mackerness et al., 1998, 1999).

Tab.8
Electron transport (ET0/RC) in control (closed squares) and UV-B treated cultures (open squares) during (A) and after exposure to 0,420mW cm-2 UV-B radiation (B)

	TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
		c	uv	c	uv	c	uv	c	uv	c	uv
A	0	0,909	0,931	0,912	0,912	0,984	0,979	0,909	0,931	0,912	0,912
	1	0,856	0,909	0,937	0,942	1,039	0,928	0,856	0,909	0,935	0,942
	2	0,874	0,848	0,935	0,877	1,015	0,901	0,874	0,848	0,935	0,877
	3	0,855	0,75	0,977	0,597	1,013	0,868	0,855	0,75	0,977	0,597
B	4	0,879	0,332	0,968	0,906	1,005	0,938	1,01	0,505	0,958	0,558
	5	0,91	0,346	0,972	0,88	1,036	0,952	1,012	0,689	0,936	0,603
	6	0,914	0,341	0,996	0,931	1,066	0,977	1,001	0,89	0,982	0,633
	7	0,794	0,262	1,003	0,904	1,013	0,969	1,006	0,95	0,914	0,655

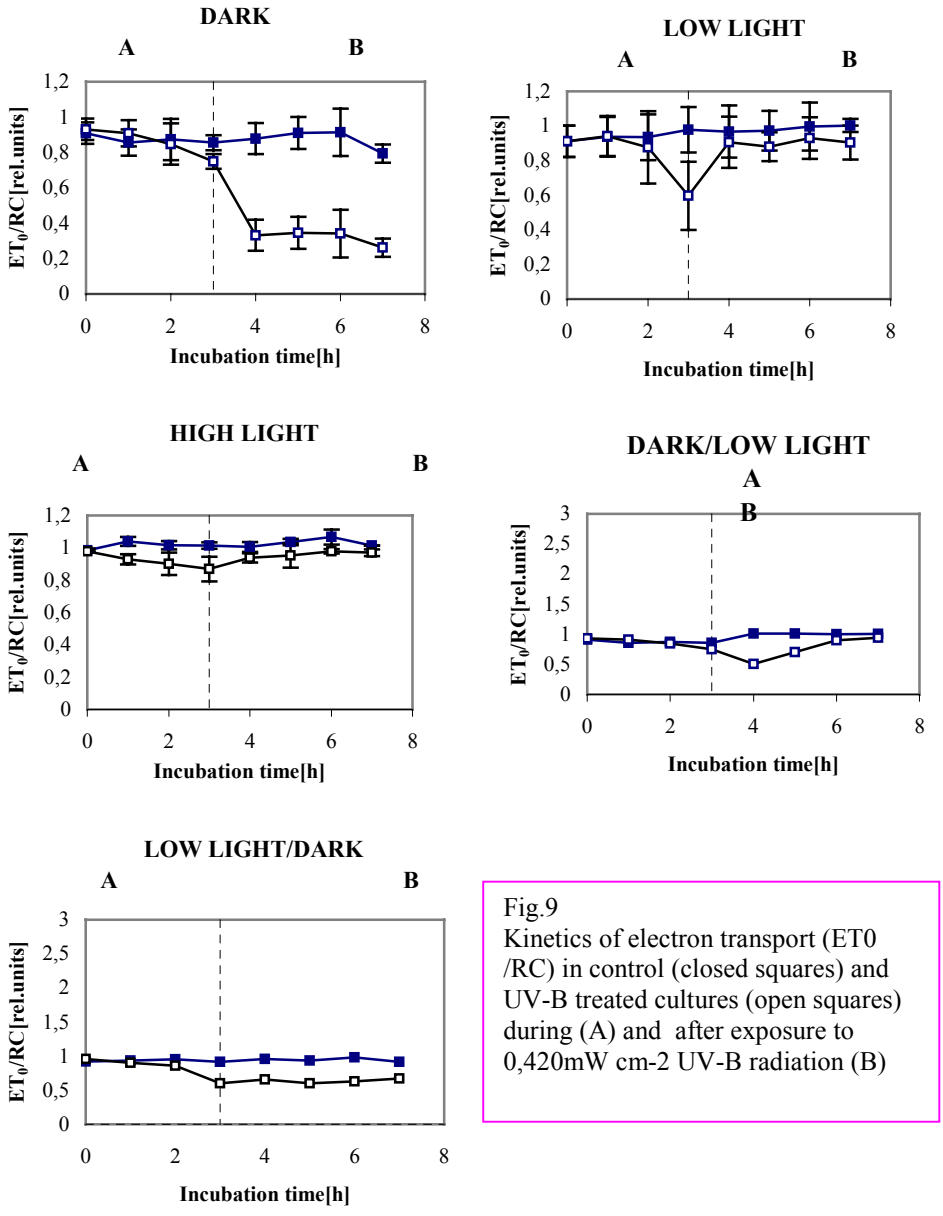


Fig.9
Kinetics of electron transport (ET0 /RC) in control (closed squares) and UV-B treated cultures (open squares) during (A) and after exposure to 0,420mW cm-2 UV-B radiation (B)

Work on the different partial reactions on the photosynthetic electron transport chain, so far, has given some contradictory results regarding the sensitive UV-B targets. The different results can be explained by different UV irradiation regimes, which were sometimes very unnatural. Several different target sites have been proposed (Bornman, 1991). These include the reaction center of PS II, the light harvesting complex (LHC), and the acceptor/donor side of PS II. Recently, it has been found that UV-B promotes dephosphorylation of thylakoid phosphoproteins and for reaction centers proteins D1 and D2 this is paralleled by degradation (Barbato et al., 1999). As it has been demonstrated, the phosphorylation of LHC II polypeptides is thought to regulate energy distribution between PS I and PS II (Allen et al., 1981); instead, phosphorylation of D1 protein from the reaction center is suggested to play a role in the regulation of its light-induced turnover (Elich et al., 1992; Kettunen et al., 1991) possibly by preventing the degradation of the phosphorylated damaged form of the protein (Rintamäki et al., 1996). Since the signal for phosphorylation is associated with the reduction level of PQ pool (Allen et al., 1981; Vener et al., 1997), it was hypothesized that UV-B light interferes with this regulation, as it induces degradation of the PQ moiety (Melis et al., 1992; Barbato et al., 1995). Several data suggested that the effect of UV-B light in the absence of visible light is similar to a dark adaptation with the deactivation of kinase activity (reviewed in Barbato et al., 1999). An experiment performed by Barbato et al. (1999) demonstrated that dark-induced dephosphorylation occurs more slowly than that observed under UV-B. Moreover, while in the dark LHC II is the complex firstly dephosphorylated (Bennet, 1980), CP 43 is firstly dephosphorylated in UV-B. All these data show that phosphorylated D1 protein is not protected against damage induced by UV-B light, but that, in light conditions, is not further degraded by protease activity (Barbato et al., 1999). These results underline the importance of light in the recovery of changes induced by UV-B treatment. This is in agreement with our results showing that, after UV-B irradiation, *Scenedesmus* cultures recovered only in light conditions.

It was observed that under UV-B irradiation *Scenedesmus* cultures respond in a time-dependent manner, suggesting an unbalancing between damaging and repair mechanisms, with the rate of damage exceeding the rate of repair. Experimental data have demonstrated that a short period of irradiation induces loses of chlorophyll proteins CP 47 and CP 43 from the PS II core antennae, but a prolonged exposure

induced the appearance of a 94 kDa protein which could have originated from the UV-B induced cross-linking of the thylakoid proteins (Rajagopal et al., 2000).

The intensity of response that *Scenedesmus* cultures exhibit to UV-B irradiation, as well as, their capacity to recover the changes induced in the functioning of photosynthetic apparatus are strictly correlated to the irradiance conditions applied during experiments. As it was shown, the changes induced by UV-B treatment in the photosynthetic activity of *Scenedesmus* cultures were more pronounced in low light conditions suggesting that high light may alleviate to a certain extent the UV-B stress. It is well established that PAR intensity influences a series of responses to UV-B stress. On this context, Mirecki and Teramura (1984) found that soybean leaves that are concomitantly irradiated with UV-B and high intensities of visible light were resistant to UV-B damage, whereas leaves irradiated concomitantly with UV-B and low intensities of visible light were sensitive. Another kind of evidence that emerges is the fact that in previously study (Cen and Bornman, 1991) with *Phaseolus vulgaris* plants exposed to different light regimes together with standard UV-B irradiation, it was shown that light intensity is a factor affecting the range of UV-B effects to the plants. Plants grown under high light conditions were most resistant to UV-B radiation, whereas low light conditions enhanced responses to UV radiation. Medium light grown plants, in general, showed a intermediate response. Other researchers too, reported that supplementary UV-B light under high PAR, shows deleterious effects with field and glasshouse plants being less sensitive to enhanced UV-B radiation, when compared to plants raised under lower irradiance (Strid et al., 1990). In contrast, Warner and Caldwell (1983), also working with soybean leaves, showed an increased inhibitory effect of UV-B light on photosynthesis when high but non-photoinhibitory visible light was present during the UV-B treatment. These results indicate the complexity of photoinhibition in the presence of visible and UV light and suggest the involvement of protective or repair mechanisms that can be modulated by either of the two light qualities.

All these data suggest that light intensity influences the sensitivity of plants to UV-B irradiation. Indeed, a stronger response to UV-B irradiation was obtained in *Scenedesmus* cultures incubated in low light conditions than in high light ones. Since the effects induced by UV-B in *Scenedesmus* cultures were similar to those observed in low light adaptation (increase of antenna size, reduction of reaction center density, reduced PQ pool), it might be affirmed that the pronounced response exhibited by low

light cultures results from the synergistic effect of UV-B and visible light. On the contrary, the adaptation to high light involves changes reverse correlated to those produced in low light photoadaptation. In this situation, high light and UV-B act antagonistically diminishing the culture responses. Thus, the degree of *Scenedesmus* cultures photoadaptation to irradiance conditions is highly correlated to their sensitivity to UV-B radiation.

Other investigators have also observed the positive role of high PAR intensity in the amelioration of UV-B-induced damage in different species. Mirecki and Teramura (1989) considered that it could be attributed to the activation of photorepair mechanisms Photorepair is thought to involve the activation of the DNA repair enzyme photolyase (Pang and Hays, 1991). However, higher levels of visible light may also contribute to protection by providing additional substrate through increases in photosynthesis for the repair or replacement of damaged organelles or tissues (Adamse and Britz, 1992).

Jordan et al. (1992) noted that increased PAR could reduce the UV-B induced down-regulation of photosynthetic genes. Mackerness et al. (1996) demonstrated that under UV-B exposure high PAR reduced the sensitivity of RNA transcripts of *rbcS*, *rbcL*, *cab*, *psbA* and hypothesized that the high PAR intensity enhanced photolyase activity and reduced the down-regulation of photosynthetic genes induced by UV-B, concluding that these changes are the result of UV-B induced gene regulation rather than being a consequence of DNA damage. High PAR is possible to increase the accumulation of flavonoids, which reduces the penetration of UV-B in the cell and PS II damage (Tevini et al., 1991; Day and Vogelmann, 1995).

The influence of PAR intensity in the recovery process is also clearly expressed. Under low light conditions, recovery in *Scenedesmus* cultures occurs more efficient than under high light conditions. Also, cultures incubated in darkness or transferred to different incubation conditions after UV-B treatment did not recover. These data demonstrate that mechanisms assuring efficient repair of damage induced by UV-B radiation are regulated by light intensity. Recovery in darkness did not occur, probably due to the dephosphorylation of reaction center proteins leading to their degradation (Aro et al., 1990). The absence of recovery in *Scenedesmus* cultures exposed to low light conditions after the application of UV-B treatment in dark conditions may be explained by the inability of these cultures to repair the damage

induced by UV-B radiation resulting in the over-accumulation of breakdown products further subjected to oxidative attack.

DNA damage and repair in plants have been examined in *Arabidopsis thaliana*, alfalfa and maize exposed to UV radiation (reviewed in Britt, 1996) and two types of DNA repair have been documented. The first type of repair is called photoreactivation and it generally occurs within few hours. Specific photolyase enzyme requires UV-A or blue light to energize direct reversal of either pyrimidine dimers created in DNA by UV-B exposure (Yasui et al., 1994; Sancar, 1996; Todo et al., 1996). The second type of repair called dark or excision repair, involves removal of damaged bases followed by synthesis of a repair patch. The kinetics of this type is considerably slower than those of photoreactivation (Degani et al., 1980). Similar to our results, Stapleton et al. (1997) observed at maize that recovery was fully accomplished in light conditions being absent in darkness.

Recovery in low light conditions after exposure to sunlight including UV-B light has been shown to occur very slowly, probably due to low capacity to restore PS II activity via protein degradation and resynthesis (Aro et al., 1993). Studies that applied artificial UV-B (Friso et al., 1994; Jansen et al., 1998) suggest that both the D1 and D2 proteins of the PS II reaction center might need replacing for recovery.

The absence of recovery in cultures incubated in low light conditions after UV-B irradiation in darkness may be caused by the light stress responsible by a strong reduction of PS II efficiency. Similar results were reported by Krause et al. (1999) who, in addition, suggested that PS II efficiency decrease may be accompanied by a reduction in photosynthetic CO_2 assimilation, as indicated by gas exchange measurements. Direct effects of UV-B light on photosynthetic carbon metabolism have not been investigated here. Therefore, one cannot exclude the possibility that the exposure of *Scenedesmus* cultures to UV-B radiation caused a primary inhibition of Calvin cycle activity. Such effect has been shown by other investigators who have observed that enhanced UV-B irradiation caused a substantial reduction in the rate of CO_2 assimilation and the amount of Rubisco with no major decline in PSII photochemistry (Nogués and Baker, 1995; Allen et al., 1997, 1998). On the other hand, it is possible that xantophyll pigments may be involved in the damage and repair processes that occurred in *Scenedesmus* cultures during and after UV-B irradiation. It has been suggested that the xantophyll cycle itself, which plays an important role in protection against photoinhibition by visible light, may become a

target of photoinhibitory UV-B radiation (Demmig-Adams, 1990). Previous data have shown that plant exposure to sunlight with a high UV-B portion may result in an increased susceptibility to photoinhibition by visible light and, thus, amplify the separate, potentially deleterious actions of UV-B and visible light (Pfündel et al., 1992). All these data showing that the xantophyll cycle was affected by UV-B radiation also suggest the possibility that this mechanism is involved in the recovery of changes induced by UV-B irradiation. It is possible that such mechanism is activated in low light cultures exposed to UV-B radiation and to contribute in recovery. The recovery of changes induced by UV-B irradiation in *Scenedesmus* cultures shows that the repair system is able to regenerate and maintain the normal population of functional PS II complexes.

The determination of the chlorophylls (Chls) and the calculation of the Chl *a/b* ratios, suggested that the cultures used as control generally show a state of adaptation that correlates with the light intensity that was applied during culturing. As data given in Tab.9 show, low light-exposed cultures possessed higher content of Chls comparative to high light-adapted cultures. On the contrary, cultures exposed in darkness contained less Chls as those incubated in light conditions. During culturing, the Chl content of samples used as control or exposed to UV-B irradiation increased. Slight differences in Chl biosynthesis attributed to UV-B radiation were mostly observed in low light and high light cultures. Thus, UV -B irradiated cultures in low light conditions exhibited a higher Chl level than the respective control, whereas UV-B-irradiated cultures exposed to high light conditions showed a lower amount of Chl comparative to corresponding control. Some minor oscillations in Chl amount occurring at the control level were also observed in dark irradiated cultures. No significant change in Chl level of control and irradiated cultures was recorded during recovery period (Fig. 10).

The Chl content in *Scenedesmus* cultures was not significanly altered by UV-B radiation. Data from literature concerning the evolution of Chl amount under enhanced UV-B irradiation are contradictory. However, increases as well decreases in photosynthetic pigments have been observed with increased UV-B radiation (Day and Vogelmann, 1995; Correia et al., 1999). Mirecki and Teramura (1984) have demonstrated that light intensity conditions in which UV-B irradiation is performed exerts a high influence on the Chl pattern. They found that low light plants exposed to UV-B in low light conditions possessed a higher Chl content as the respective control.

Tab.9; Estimation of chlorophyll content (a, b, total) and chlorophyll a/b ratio in control cultures and cultures exposed to 0,420mW cm^{-2} UV-B radiation under different experimental conditions.

TIME[h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
	c	uv	c	uv	c	uv	c	uv	c	uv
Chl a (µg/µl PCV)										
0	3,493	3,493	6,489	6,479	4,261	4,261	3,493	3,493	6,489	6,489
1	3,456	3,77	7,005	6,91	4,869	4,356	3,456	3,77	7,005	6,91
2	3,784	3,434	6,966	7,372	5,037	4,759	3,784	3,434	6,966	7,31
3	3,8	3,719	7,035	7,398	4,949	4,741	3,8	3,719	7,035	7,34
4	3,77	3,24	7,301	7,419	5,258	4,388	3,492	3,321	6,766	6,205
5	3,72	3,481	7,382	7,32	5,251	4,739	3,68	3,481	6,71	6,214
6	3,85	3,601	7,717	7,362	5,039	5,033	3,808	3,461	6,903	6,55
7	3,965	3,655	7,81	7,51	5,4952	5,2622	3,758	3,527	6,747	6,721
Chl b (µg/µl PCV)										
0	1,17	1,17	2,562	2,562	1,522	1,522	1,17	1,17	2,562	2,562
1	1,15	1,248	2,503	2,715	1,524	1,53	1,15	1,248	2,503	2,715
2	1,277	1,157	2,53	2,757	1,597	1,513	1,277	1,157	2,53	2,757
3	1,269	1,302	2,647	2,88	1,596	1,559	1,269	1,302	2,647	2,88
4	1,406	1,105	2,627	2,726	1,774	1,63	1,183	1,231	2,638	2,727
5	2,025	1,923	2,627	2,674	1,664	1,601	1,257	1,286	2,48	2,727
6	2,075	2,128	2,893	2,82	1,757	1,798	1,282	1,266	2,76	2,94
7	2,27	2,32	2,869	2,748	1,847	1,833	1,303	1,296	2,72	3,001
Total chls (µg/µl PCV)										
0	4,663	4,663	9,051	9,05	5,783	5,783	4,663	4,663	9,051	9,051
1	4,606	5,018	9,509	9,625	6,393	5,886	4,606	5,018	9,508	9,625
2	5,061	4,591	9,496	10,129	6,634	6,272	5,061	4,591	9,496	10,067
3	5,069	5,021	9,683	10,278	6,544	6,299	5,069	5,021	9,682	10,22
4	5,176	4,345	9,928	10,145	7,033	6,018	4,675	4,553	9,404	8,931
5	5,745	5,405	10,009	9,994	6,915	6,341	4,936	4,767	9,19	8,941
6	5,926	5,728	10,611	10,182	6,795	6,831	5,09	4,727	9,663	9,49
7	6,235	5,975	10,678	10,258	7,342	7,095	5,061	4,823	9,467	9,722
Chl a/b										
0	2,985	2,985	2,533	2,529	2,799	2,799	2,985	2,985	2,533	2,533
1	3,006	3,02	2,799	2,545	3,195	2,847	3,006	3,02	2,799	2,545
2	2,963	2,967	2,753	2,674	3,154	3,145	2,963	2,967	2,753	2,651
3	2,994	2,857	2,658	2,569	3,101	3,041	2,994	2,857	2,658	2,549
4	2,681	2,932	2,779	2,722	2,963	2,692	2,953	2,697	2,565	2,275
5	1,836	1,81	2,81	2,737	3,156	2,96	2,929	2,707	2,706	2,279
6	1,855	1,692	2,667	2,611	2,868	2,8	2,97	2,735	2,501	2,228
7	1,747	1,575	2,722	2,733	2,976	2,871	2,884	2,722	2,48	2,24

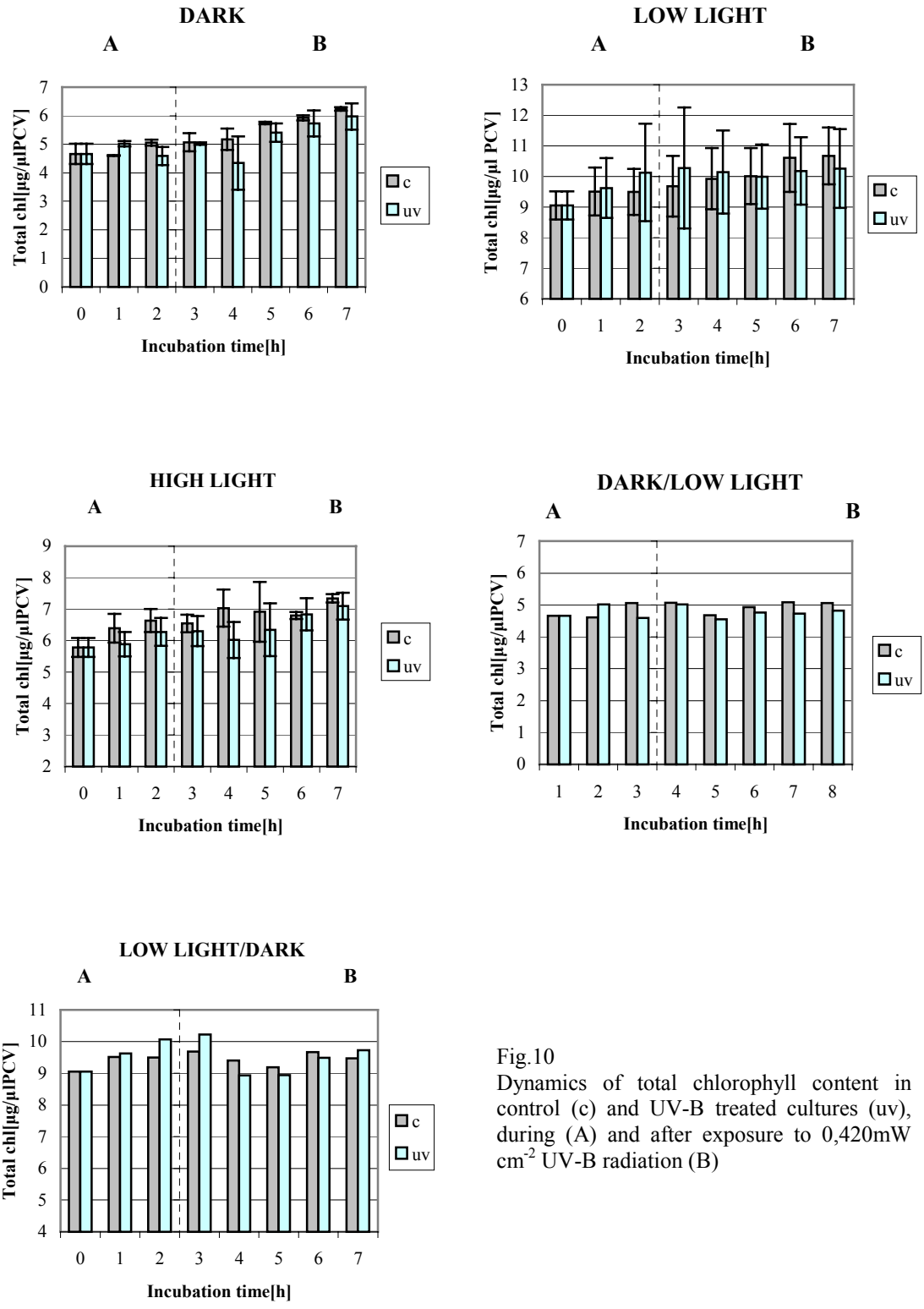


Fig.10
Dynamics of total chlorophyll content in control (c) and UV-B treated cultures (uv), during (A) and after exposure to 0,420mW cm^{-2} UV-B radiation (B)

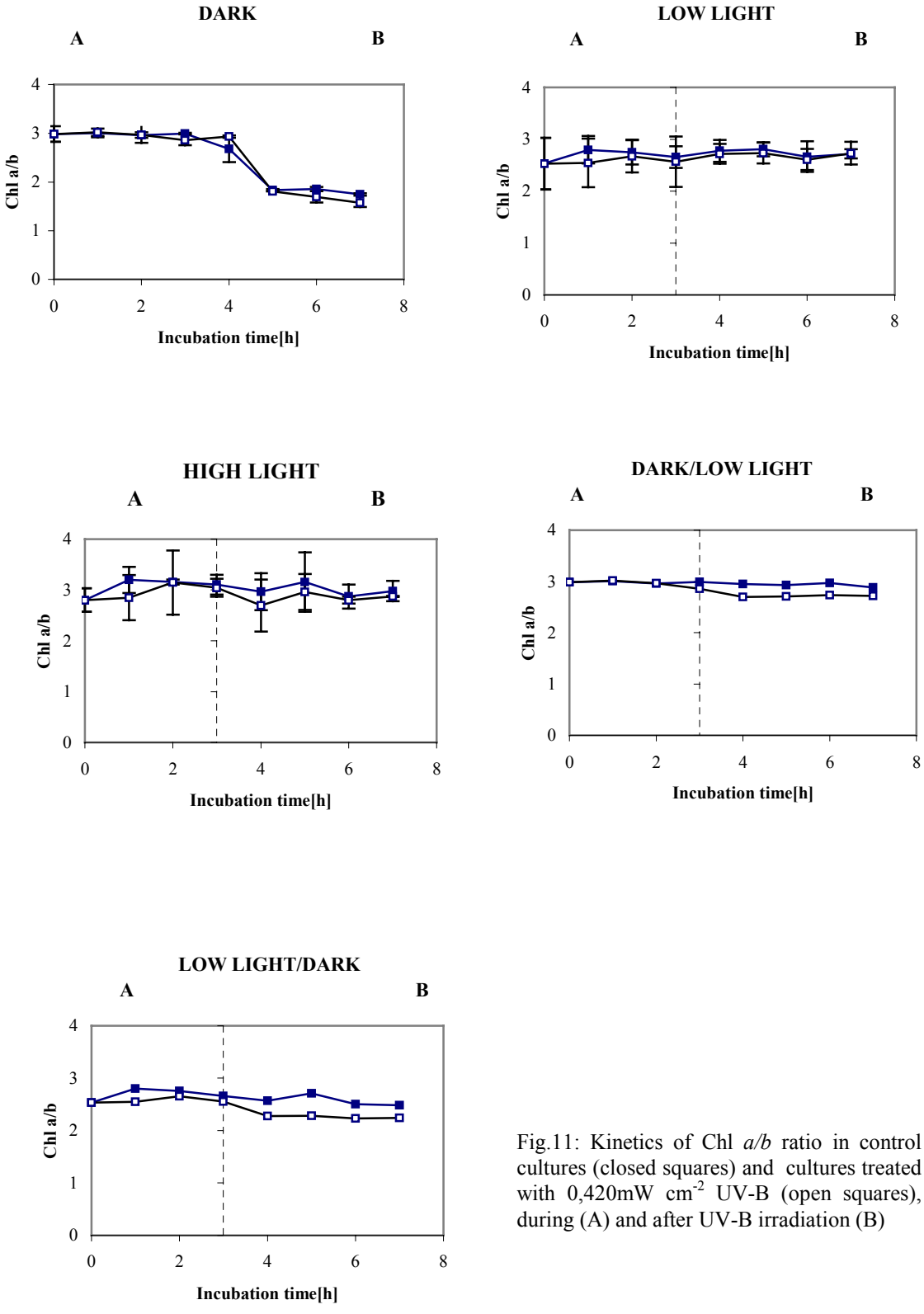


Fig.11: Kinetics of Chl *a/b* ratio in control cultures (closed squares) and cultures treated with 0,420mW cm^{-2} UV-B (open squares), during (A) and after UV-B irradiation (B)

Also, Chl b concentration was significantly reduced in low light plants irradiated in high light conditions. Our results are also consistent with these data, which indicate that low light stimulates Chl biosynthesis in *Scenedesmus* cultures exposed to UV-B irradiation. This may be corelated to the increase of antenna size that is highly expressed in low light conditions (Strasser et al., 1995). On the contrary, reduction in Chl content induced by UV-B irradiation was found in darh and high light- adapted *Scenedesmus* cultures. Similar results were also reported in studies on higher plants, but was also described for the dinoflagellate and for a variety of algae (Bischof et al., 2000). In contrast to our data, the reduction of Chl level in these studies was associated with the experimental conditions of UV-B treatment; respectively, high levels of UV-B radiation in combination with low levels of PAR have significantly reduced chlorophyll content in several species (Teramura, 1983). Data accumulated until now show that the lowering of chlorophyll levels, as it was observed in dark and high light adapted *Scenedesmus* cultures under UV-B irradiation, is believed to result from the degradation of chl a and b by chlorophyllase activity. Previous results revealed the presence of Chlides a and b during Chl breakdown caused by exposure to enhanced UV-B radiation (Rodriguez et al., 1987; Strid and Porra, 1992). This intermediates accumulated at a low concentration, which supposed their subsequent degradation. In addition to photodegradation of existing chlorophyll (Brandle et al., 1977; He et al., 1994), the reduction of Chl level, probably involve lower rates of Chl synthesis, as UV-B can reduce the levels of RNA transcripts for proteins involved in chlorophyll production (Strid et al., 1990; Jordan et al., 1992, 1994, 1996; He et al., 1993). Data obtained by us suggest that there are no specific effects of enhanced UV-B radiation on enzymes involved in the chlorophyll biosynthetic pathway but instead a down-regulation of Chl biosynthesis was found in darkness and in a less extent in high light *Scenedesmus* cultures. The results obtained in low light cultures are consistent with the finding that the increase of Chl level in conditions in which photosynthesis decreased implies that UV-B radiation is not a limiting factor for Chl biosynthesis (Mirecki and Teramura, 1984).

The values calculated for Chl *a/b* ratios were lower in low light cultures than in high light or dark-adapted cultures (Tab.9), showing clearly the adaptation of cultures to PAR intensity. Chl a/b ratio is a factor indicating the LHC II antenna size. Since Chl b is exclusively bound to LHC II and I antenna, an increase in this ratio indicates a lower Chl b concentration and, consequently, a lower antenna size (Anderson et al., 1988). As we expected, in cultures exposed to UV-B irradiation the Chl *a/b* ratio was lowered; this confirms our finding, previously mentioned, that antenna size increased during irradiation. At recovery, Chl *a/b* ratio decreased in dark conditions, whereas it increased to control level in cultures exposed to low or high light conditions. Chl *a/b*

also increased in cultures transferred after UV-B irradiation in different illumination conditions than those used during UV-B exposure, but it remained below the control level (Fig. 11).

The oscillations of Chl *a/b* under UV-B treatment was also observed in studies performed by other investigators. Vu et al. (1981) reported that chlorophyll a/b ratios decreased with increasing UV-B irradiance in soybean but increased in pea at high UV irradiance (Vu et al., 1983). Tevini et al. (1981) concluded that UV-B irradiance inhibited the biosynthesis of chlorophyll b more than a, since a/b ratios increased in several species. In contrast, Teramura et al. (1980) reported that no important change occurs in Chl *a/b* during UV-B irradiation. The fluctuations of Chl *a/b* ratios in irradiated *Scenedesmus* cultures, although not very significant quantitatively are correlated to the oscillations in antenna size. The changes that occurred in Chl content, as well as in Chl *a/b* ratio indicate that cultures show a highly adaptive behavior to the conditions applied during experiments. The investigation of polyamine pattern provided supplementary data about this behavior.

The participation of polyamines in the assembly of the photosynthetic apparatus (Kotzabasis et al., 1993a) and their involvement in the photosynthetic activity (Kotzabasis and Senger, 1994) and chloroplast photodevelopment (Andreadakis and Kotzabasis, 1996) prompted us to examine the intracellular changes that occur during UV-B exposure and recovery periods. Data resulted from the quantitative determination of the Put and Spm content have been used for the calculation of Put/Spm ratios, which may be considered an indicator of degree of adaptation that cultures adopt in the experimental conditions used (Kotzabasis et al., 1999).

As data listed in Tab. 10 show, control cultures incubated in light conditions possessed an elevated amount of Put, which increased gradually during culturing. Under UV-B irradiation, the Put level initially decreased (after 1h in dark-adapted cultures, and 2h in cultures incubated in low and high light conditions), but it recovered rapidly, so that in the third hour of treatment both control and irradiated cultures exhibited a similar Put level. After the cessation of UV-B treatment, Put initially increased in cultures incubated in continuous light conditions, while it decreased in cultures transferred after treatment in different conditions of illumination. In darkness, the amount of Put in UV-B treated cultures exhibited similar kinetics to the control. From the second hour of recovery, Put level in irradiated cultures reached the control level in all the experimental situations investigated. Until the end of experiments, Put content in both control and irradiated cultures showed similar kinetics. As Fig. 12 features, Put level increased in cultures exposed to UV-B radiation in light conditions. This result is in agreement with other data showing that under photoinhibitory conditions, such as those produced by high light or UV-B light, Put level increased (Dondini et al., 1994). In *Arabidopsis thaliana*, Campos et al. (1991)

found that UV-B radiation caused an increase in the concentration of conjugated polyamines, which was correlated with enhanced levels of PAL transcripts. This result is also consistent with findings reported by other investigators (Kramer et al., 1991; Krizek et al., 1993)

Previous experimental data have shown that polyamine pattern undergoes changes in response to different environmental conditions. Kotzabasis et al. (1999) suggested that a decreased Put/Spm ratio simulates a low light photoadapted photosynthetic apparatus and, thus, this ratio could be used to appreciate the behavior of plant cells during photoadaptation.

As data listed in Tab.10 indicate, dark and low light adapted cultures exhibited a low Put/Spm ratio comparative to high light cultures, suggesting that *Scenedesmus c*ultures are photoadapted to the irradiance conditions used. Under UV-B exposure, Put/Spm ratio followed a similar pattern of changes as that of Put, with an increase after 1 h of irradiation followed by recovery to control level. In dark adapted cultures Put/Spm ratio was permanently kept below the respective control level, during both UV-B treatment and recovery periods. On the contrary, in cultures incubated in low and high light conditions, this ratio overcame the control values in the third hour of irradiation and remained unchanged until the end of recovery period. Put/Spm ratio, also, decreased in cultures transferred in darkness after UV-B irradiation in low light conditions and inversely (Fig.13).

Tab.10: Putrescine (Put) content in control (closed squares) and irradiated cultures (open squares) during (A) and after exposure to 0,420mW cm-2 UV-B (B)

	Time [h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
		c	uv	c	uv	c	uv	c	uv	c	uv
A	0	0,559	0,559	0,655	0,655	0,64	0,64	0,559	0,559	0,655	0,655
	1	0,61	0,496	0,777	0,76	0,63	0,6	0,61	0,496	0,777	0,76
	2	0,547	0,549	0,85	0,733	0,76	0,7	0,547	0,7	0,85	0,733
	3	0,478	0,488	0,833	0,846	0,8	0,83	0,478	0,488	0,833	0,846
B	4	0,456	0,41	0,852	0,996	0,87	0,99	0,582	0,34	0,593	0,646
	5	0,572	0,552	0,915	0,813	0,95	0,88	0,641	0,423	0,645	0,684
	6	0,469	0,523	0,964	0,931	0,92	1	0,766	0,517	0,668	0,611
	7	0,57	0,455	1,099	1,004	1,15	1,14	0,7	0,61	0,69	0,632

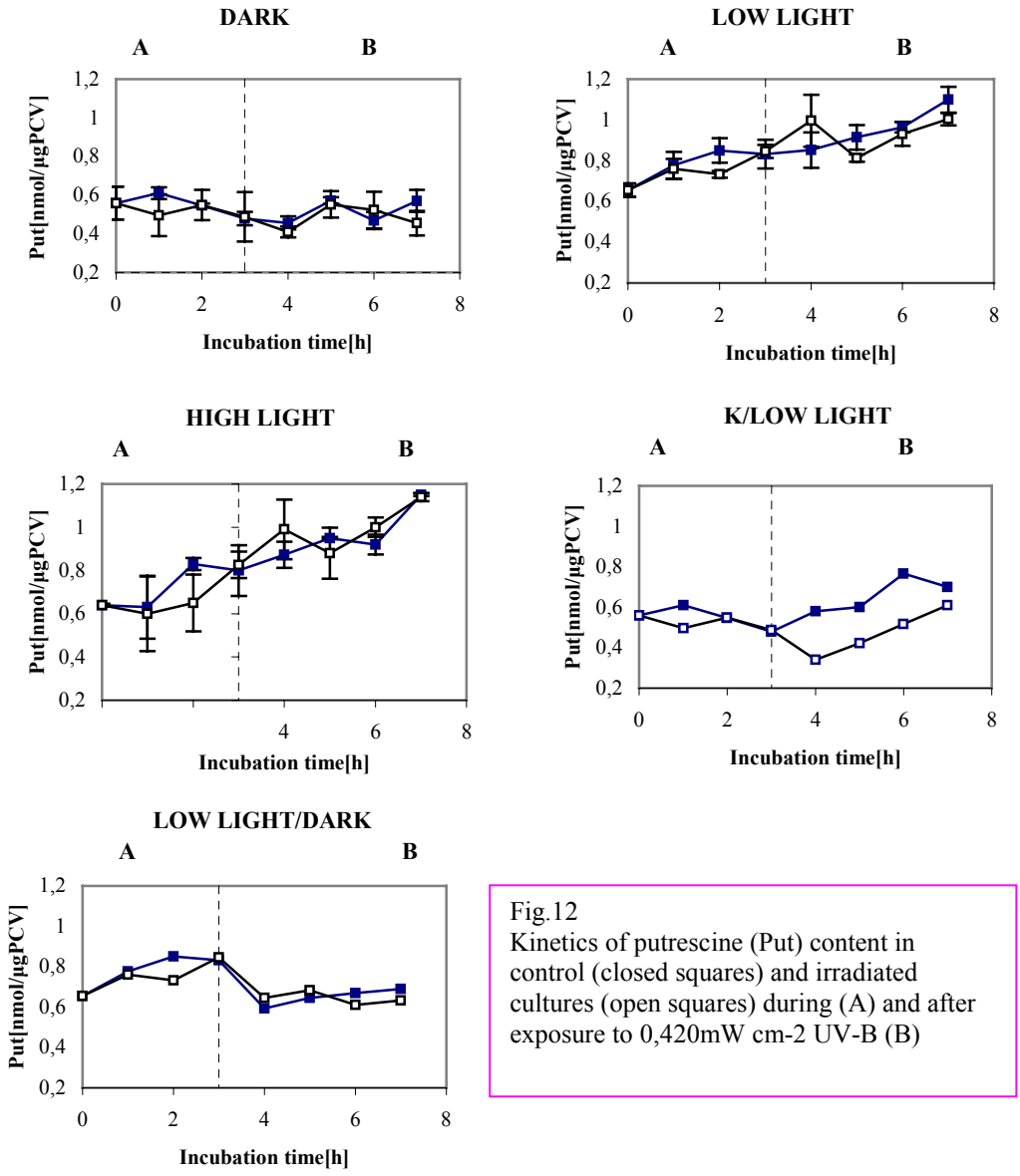


Fig.12
Kinetics of putrescine (Put) content in control (closed squares) and irradiated cultures (open squares) during (A) and after exposure to 0,420mW cm-2 UV-B (B)

Tab.11: Put/Spm ratio in control (closed squares) and irradiated cultures (open squares), during (A) and after exposure to 0,420mW cm-2 UV-B radiation (B).

	Time [h]	DARK		LOW LIGHT		HIGH LIGHT		DARK/LOW LIGHT		LOW LIGHT/DARK	
		c	uv	c	uv	c	uv	c	uv	c	uv
A	0	3,263	3,163	3,588	3,538	4,37	4,47	3,263	3,163	3,588	3,538
	1	3,688	2,753	3,711	3,38	4,35	4,19	3,688	2,753	3,711	3,38
	2	3,13	3,01	3,866	3,75	4,28	4,07	3,13	3,01	3,866	3,75
	3	3,429	3,244	3,776	3,822	4,01	4,24	3,429	3,244	3,776	3,822
B	4	4,078	3,04	3,844	3,99	3,73	3,99	3,52	2,941	3,38	3,083
	5	3,01	2,461	3,833	4,113	3,77	4,02	3,12	3,255	3,47	2,894
	6	3,54	2,972	4,007	4,015	4,03	4,18	2,56	2,906	3,596	2,544
	7	3,164	2,836	3,959	3,831	4,34	4,41	2,7	2,846	3,682	2,964

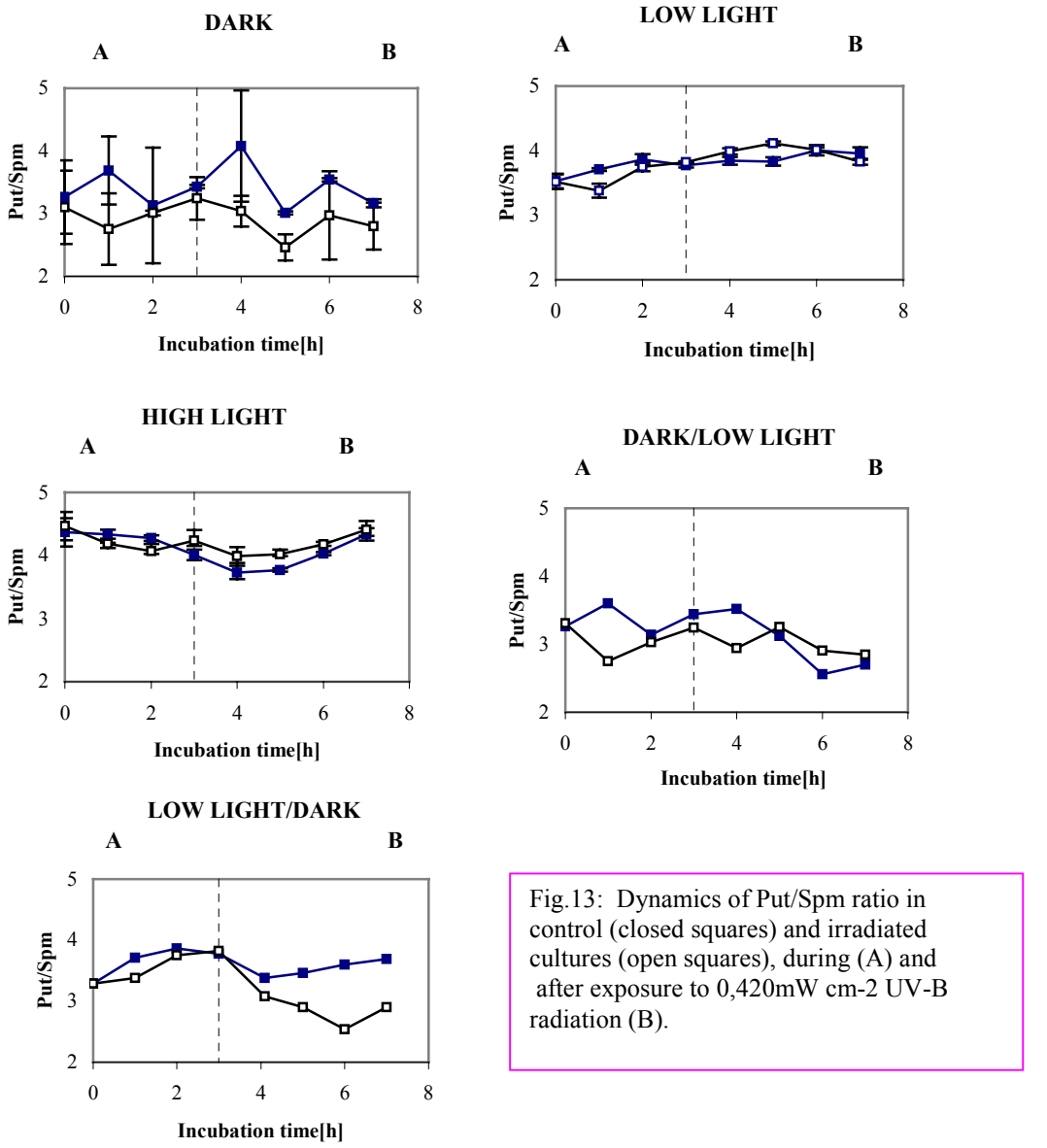


Fig.13: Dynamics of Put/Spm ratio in control (closed squares) and irradiated cultures (open squares), during (A) and after exposure to 0,420mW cm-2 UV-B radiation (B).

Combining the data obtained for Put amount and Put/Spm ratio, we observed that a decrease in Put level was accompanied by a similar decline in Put/Spm ratio. Experimental data have indicated that the inhibition of Put biosynthesis as indicated by a low Put/Spm ratio leads to changes in structure and functioning of the photosynthetic apparatus similar to those observed with low light adapted cultures, e.g. an enlargement of the LHC accompanied by a decrease of the number of reaction centers per unit areas and Chl *a/b* ratio. (Kotzabasis et al., 1999) These responses were observed in cultures exposed to UV-B irradiation and are consistent with the changes observed in antenna size and Chl *a/b* ratio. At recovery an opposite situation was found in light-incubated cultures, where Put and, consequently, Put/Spm ratio increased but in dark incubated cultures this ratio remained low. These modifications suggest the changes that occurred in the antenna size during the exposure of *Scenedesmus* cultures to UV-B irradiation.

The lowering of Put/Spm ratio under UV-B irradiation seems to play an initial protective role, since Besford et al. (1993) have demonstrated that a decrease of Put level accompanied by an increase of Spm content, retards effectively the degradation of D1 and D2 proteins of PS II. Consistent with this finding, the decrease of Put/Spm ratio indicated an adaptation of the photosynthetic apparatus to the UV-B radiation.

The data obtained from the investigation of polyamine pattern during and after UV-B treatment suggest once more that UV-B radiation induced changes similar to those found in low light adaptation. The differences between cultures are determined by light intensity, since UV-B and visible light act synergistically in low light conditions, and antagonistically in high light conditions. The finding that the effects of UV-B on polyamine levels are greatly influenced by PAR levels in a dose-dependent fashion, supports this hypothesis (Kramer et al., 1992).

The pattern of changes in Put and Spm biosynthesis, as indicated by the above-discussed data, suggest that cultures are capable to adapt their polyamine levels in a way to resist to UV-B radiation. It is most probable that indeed polyamines, and especially Put, play a major role in plant protection against radicals formed by UV-B radiation.

However, one cannot totally exclude the probability that polyamine alterations are caused in plants under UV-B treatment as a side effect only, without constituting the primary responsive effect. For this reason, complementary experiments are required, intending to clear out this controversial point. Exogenously manipulated

polyamines would greatly contribute into establishing our present hypothesis of Put being the primary plant protective component that mobilizes under UV-B exposure. This indeed could be true, since other experiments revealed that ultimately it is the endogenous polyamine concentration differences that confer plant tolerance/sensitivity against a stressor. Since a stress agent produce oxidative stress by forming free radicals, it is quite probable that the plants respond by forming the protective polyamines that can stabilize the photosynthetic apparatus and thus act as radical scavengers. Kramer et al. (1992) have suggested that UV-B sensitivity could involve differences in polyamine accumulation, finding that a UV-B resistant cultivar of soybean accumulated highly polyamine comparative to a sensitive cultivar.

The antenna size could be directly regulated by Put being bound to or loosened from thylakoids, thus also directly regulating the physiological state of the photosynthetic apparatus itself and, consequently, the mechanism of photosynthesis. This conclusion is also supported by previous publications, which in overall agree with the fact that one of the primary effects of UV-B to the photosynthetic apparatus lies between photosystem II (PSII) subcomplexes (Brandle et al., 1977; Renger et al., 1991; Caldwell and Flint, 1994; Fiscus and Booker, 1995; Teramura and Ziska, 1996; Navakoudis et al., 20001).

The present data demonstrate that *Scenedesmus obliquus* possess a great capability to adapt at different light conditions, disposing by the mechanisms which assure a certain degree of resistance to UV-B radiation and a high ability of recovery. The behavior adopted by this species under UV-B exposure is highly regulated by light intensity, which may contribute to reverse the deleterious effects of UV-B radiation.

CONCLUSIONS

The data presented in this study indicate that UV-B radiation exerts an inhibitory effect on photosynthesis. The degree of UV-B induced photoinhibition, as well as, the ability of plant cells to recover their photosynthetic activity is dependent on light intensity. Conclusively, our data can be summarized to the following results:

- UV-B radiation induces in the photosynthetic apparatus changes similar to those occuring during photoadaptation in low light conditions (increase of antenna size, reduction of reaction center density, decrease of chl a/b and Put/Spm ratios).

- The response of cultures to UV-B irradiation is influenced by light intensity, being more pronounced under low light conditions. Low light and UV-B radiation act synergistically producing a stronger response, while high light and UV-B radiation act antagonistically and the response to UV-B radiation is diminished.

- The strategy adopted by plant cells to resist to a certain extent to UV-B radiation is the dissipation of light energy surplus. To achieve this, antenna size increases, and some reaction centers are inactivated, being transformed into dissipative sinks for the excitation energy provided by UV-B radiation.

- Besides the changes that occurred in Chl a/b ratio which though are correlated with the modification that occurred in antenna size, total Chl content is not significantly affected by UV-B irradiation.

- The ability of *Scenedesmus* cultures to recover the changes induced by UV-B radiation is influenced by light intensity. Recovery occurs only in cultures incubated in continuous and stable light conditions, especially in low PAR conditions.

- The behavior adopted by *Scenedesmus* cultures under UV-B irradiation expresses the high capability of this species to adapt in different environmental conditions.

FUTURE RESEARCH

In order to obtain more valuable data concerning the strategies that plant cells adopt in critical environmental conditions, such as enhanced UV-B irradiation, several investigations will be done in the following directions:

- Isolation and characterization of light harvesting complexes from *Scenedesmus* cultures will provide data about the changes that occur in antenna following UV-B radiation and recovery.

- Characterization of polyamine pattern in intact chloroplasts, thylakoid membranes, PS II membranes and LHC will establish the measure in which polyamines are involved in the maintaining of structure and functioning of the photosynthetic apparatus in a stress situation.

- Addition of exogenous polyamines and polyamine inhibitors in *Scenedesmus* cultures prior to UV-B irradiation will offer information about the degree of plant sensitivity to UV-B radiation.

- Investigation of carotenoid pattern and determination of flavonoid accumulation will offere much information regarding the protective mechanisms that occurred in plant cells under UV-B stress.

- Determination of AOS accumulation following UV-B exposure, as well as, the investigation of antioxidant capacity in irradiated cells will establish the corelation between AOS accumulation and plant sensitivity to UV-B radiation.

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