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Salt stress impact on the molecular structure and function of the photosynthetic apparatus—The protective role of polyamines

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Abstract

In the present study the green alga *Scenedesmus obliquus* was used to assess the effects of high salinity (high NaCl-concentration) on the structure and function of the photosynthetic apparatus and the possibility for alleviation by exogenous putrescine (Put). Chlorophyll fluorescence data revealed the range of the changes induced in the photosynthetic apparatus by different NaCl concentrations, which altogether pointed towards an increased excitation pressure. At the same time, changes in the levels of endogenous polyamine concentrations, both in cell and in isolated thylakoid preparations were also evidenced. Certain polyamine changes (Put reduction) were correlated with changes in the structure and function of the photosynthetic apparatus, such as the increase in the functional size of the antenna and the reduction in the density of active photosystem II reaction centers. Thus, exogenously added Put was used to compensate for this stress condition and to adjust the above mentioned changes, so that to confer some kind of tolerance to the photosynthetic apparatus against enhanced NaCl-salinity and permit cell growth even in NaCl concentrations that under natural conditions would be toxic.

Keywords: Photosynthetic apparatus; Polyamines; Salinity; Environmental stress; Chlorophyll fluorescence

1. Introduction

Salinity is a major abiotic stress in plants worldwide. Salt stress causes an initial water-deficit, due to the relatively high solute concentrations in the soil, and also ion-specific stresses resulting from changes in K^+/Na^+ ratios. Thus, it leads to increased Na^+ and Cl^- concentrations that are detrimental to plants [1,2]. Salt-stressed plants exhibit a decrease in their photosynthetic efficiency, but it is not known how this actually occurs [3]. There is increasing evidence that enhanced salinity changes photosynthetic parameters, osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content [4]. Salt also affects photosynthetic components such as enzymes, chlorophylls, and carotenoids. Changes in these parameters depend on the severity and duration of the stress [5] and also on plant species [6]. Reduced photosynthesis with increasing salinity has been attributed to: (a) stomatal closure, which leads to a reduction in intracellular CO_2 partial pressure; (b) non-stomatal factors [7,8], as the reduction in protein concentration [9]; (c) decline in photosynthetic pigments [4,10]; and (d) changes in ionic concentrations [11].

A significant inhibition of photosynthesis by high salinity seems to be associated with the photosystem II (PSII) complex. Salinity stress decreases the PSII activity [12] and inhibits the quantum yield of PSII electron transport [13]. The inhibitory target site of high salt concentration at PSII seems to vary with species [14,15]. Nevertheless, Misra et al. [16] have reported that NaCl salinity affects PSII photochemical efficiency, primary charge separation in PSII and pigment–protein complexes of thylakoid membranes. Allakhverdiev et al. [17] analysed Chl fluorescence in salt-stressed cyanobacterium

Abbreviations: Chl, chlorophyll; LHCII, light harvesting complex of PSII; PCV, packed cell volume; PS, photosystem; Put, putrescine; Spd, spermidine; Spm, spermine

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S*ynechococcus* sp., and suggested that the photochemical reaction center complex, including Q_A (the primary electron acceptor of plastoquinone), pheophytin, and the photochemical reaction center P680, was undamaged in NaCl-treated cells. Therefore, it is likely that the transport of electrons from water to P680 is blocked in these cells. NaCl interfered with the PSII-mediated transport of electrons from water to DCIP, but not from DCP to DCIP. Thus, it is likely that the oxygen-evolving machinery in PSII is damaged by the ionic effects [18].

On the other hand there have been a couple of publications reporting the importance of high endogenous polyamines and especially of putrescine to the stress tolerance of plants against high salinity [19,20]. However, there is no publication investigating the role of polyamines to the stress tolerance of the photosynthetic apparatus. Thus, the object of the present study is to investigate the effects of salinity stress on the molecular structure, function and bioenergetics of the photosynthetic apparatus and the possibility that polyamines are able to regulate this salinity effect.

2. Materials and methods

2.1. Cultures

Cultures of the unicellular freshwater green alga *Scenedesmus obliquus*, wild type strain D3 [21], were grown autotrophically in liquid culture medium [22] in a temperature-controlled water bath (30 °C) in front of a set of white fluorescent lamps (OSRAM L-40W, Munich, Germany). Cultures were grown at high light (HL: 270 μmol m^{-2} s^{-1}) intensities. The cultures were continuously percolated with air for CO_2 supply and also to avoid sedimentation. The initial cell concentration for each culture was 0.3 μl PCV ml^{-1}. Eight cultures were used for the NaCl treatment with different NaCl concentrations [0‰ (control), 2‰, 5‰, 7‰, 10‰, 15‰, 20‰, and 25‰ NaCl] in high light intensity of 270 μmol m^{-2} s^{-1}. For the Put-supplemented cultures, a stock solution (100 mM) of the polyamine putrescine (Tetramethylenediamine, Sigma) in water was first prepared. Aliquots of this solution were added to the cultures to achieve a final concentration of 1 mM.

2.2. Isolation of the thylakoid membranes

For the preparation of thylakoid membranes the algal cultures were centrifuged for 5 min at 1500×*g* and the pellets resuspended in 75 mM phosphate buffer (pH 7.4). The suspension was mixed with glass beads (Ø 0.2 mm) and broken 4 times for 1 min in a cell mill (Biospec, OK, USA). The homogenate was filtered through a sinter glass filter funnel to separate the glass beads, and centrifuged for 2 min at 500×*g* to remove unbroken cells and debris. The supernatant was additionally centrifuged for 60 min at 8000×*g*. The pellet

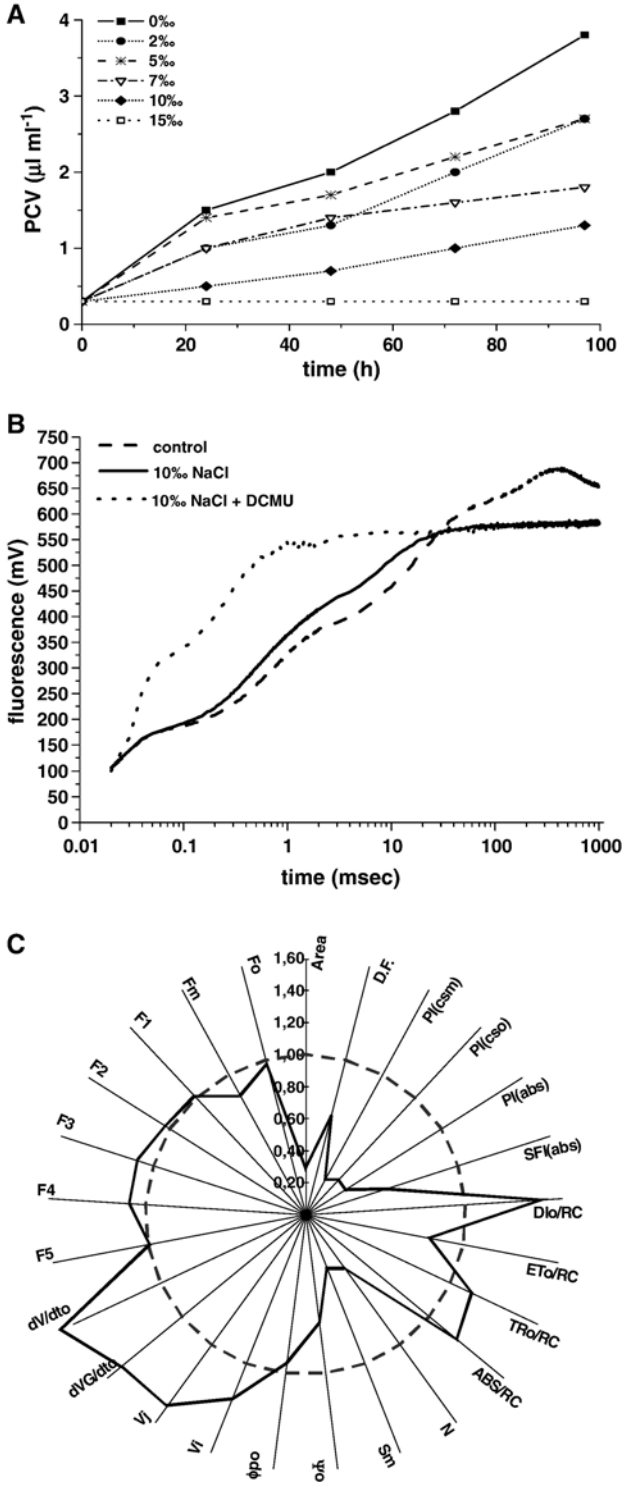

Fig. 1. (A) Culture growth measured as packed cell volume (PCV) in *Scenedesmus obliquus* autotrophic cultures. Control culture received no additional treatment, whereas the other five cultures were supplemented with NaCl upon inoculation, in different concentrations (2‰, 5‰, 7‰, 10‰, 15‰). (B) Fluorescence curves recorded from the control and the 10‰ NaCl-supplemented cultures, 96 h after onset of the experiment. The Fm value of the NaCl-treated sample was also confirmed by DCMU inhibition. (C) Radar plot with a series of parameters derived from JIP-test analyses of the fluorescence data. The plot depicts the differences provoked in the structure and function of the photosynthetic apparatus under enhanced salinity (10‰ NaCl culture; black line) in comparison to the physiological condition (control culture; grey circle). Area: the total complementary area (from time 0 to t_{Fm}) over the fluorescence induction curve is a measure of the number of quanta not emitted as fluorescence as a consequence of the photochemistry during the induction phase; D.F.: Driving Force of photosynthesis; PI: the performance indexes; SFI: the structure function index; DIo/RC: dissipation energy per active reaction center; ETo/RC: electron transport per reaction center; TRo/RC: the trapping efficiency per reaction center; ABS/RC: the effective antenna size; *N*: expresses how many times Q_A has been reduced in the time span from time 0 to t_{Fm}; Sm: a measure of the energy needed to close all reaction centers; ψ_o: the primary photochemistry; φpo: the photosynthetic efficiency; V_i: variable fluorescence at 30 ms; V_j: variable fluorescence at 2 ms; dVG/dto: expresses the excitation energy transfer between the RCs; dV/dto: expresses the rate of the RCs' closure; F1: $F_{50\ \mu s}$; F2: $F_{100\ \mu s}$; F3: $F_{300\ \mu s}$; F4: $F_{2\ ms}$; F5: $F_{30\ ms}$; Fm: Maximal fluorescence (measured when the reaction centers are closed, at P step); Fo: Initial fluorescence (measured when the reaction centers are open, at O step of the fluorescence induction curve).

consisted of two layers. The lower part of the precipitate contained mainly starch and was discarded. The upper, green layer enriched in thylakoid membranes was transferred into a small volume of 0.05 M Tricine buffer (pH 7.4) and used for the polyamine determination.

2.3. Fluorescence induction measurements

For the fluorescence induction measurements the portable Plant Efficiency Analyzer, PEA, (Hansatech Instruments, Kings's Lynn, Norfolk, UK) was used. The maximum yield of photochemistry (Fv/Fm), the effective antenna size expressed as absorbance per reaction center (ABS/RC), the amount of energy dissipated per active reaction center (DIo/RC) and the density of active photosynthetic reaction centers (RC/CS) were additionally calculated according to the JIP-test of Strasser and Strasser [23]. This method allows the dynamic description of a photosynthetic sample in a given physiological state and 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 600 W m^{-2} of red (650 nm) light. Samples were measured either in the presence or absence of the electron transfer blocker DCMU (0.1 mM), to assure the proper evaluation of Fm (maximum fluorescence).

2.4. HPLC analysis of polyamines

For polyamine analysis, cells or isolated thylakoids were suspended in 1N NaOH and then hydrolyzed according to the procedure of Tiburcio et al. [24]. A volume of 0.2 ml from the hydrolyzate was mixed with 36% HCl in a ratio of 1:1 (v/v) and incubated at 110 °C for 18 h. The hydrolysate was evaporated at 70–80 °C. The dried products were re-dissolved in 0.2 ml of 5% (v/v) perchloric acid. To identify and estimate the polyamines, the samples were derivatized by benzoylation according to the modified method of Flores and Galston [25], as described by Kotzabasis and et al. [26]. For this purpose, 1 ml of 2N NaOH and 10 μl benzoylchloride was added to 0.2 ml of the hydrolyzate and the mixture was 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 re-dissolved in 0.2 ml of 63% (v/v) methanol and 20 μl aliquots of this solution were injected into the high performance liquid chromatography (HPLC) system for the polyamine analysis, as described previously [26]. The analyses were performed with a Shimadzu Liquid Chromatography apparatus (LC-10AD) equipped with a diode array detector (Shimadzu SPD-M10A) and a narrow-bore column (C18, 2.1 × 200 mm, 5 μm particle size Hypersyl, Hewlett-Packard, USA). To estimate directly the amount of each polyamine, the method of Kotzabasis et al. [26] was followed again.

2.5. Polarographic measurements

Maximal net photosynthetic rate was determined polarographically at 30 °C with a Clark type electrode system (Hansatech Instruments, Kings's Lynn, Norfolk, UK). The actinic light (470 W m^{-2}) was generated with two lamps (ENX360W/82V) and its intensity measured with a sensitive photoradiometer (International Light, Newburyport, MA, 01950). The infrared part of the applied irradiation was filtered off by inserting a 2% $CuSO_4$-containing cuvette (4 cm path length) into the light beam. The cell suspension was primarily adjusted to 10 μl PCV ml^{-1}.

2.6. Chlorophyll extraction and estimation

Chlorophyll content was extracted and photometrically estimated according to the method of Holden [27].

2.7. Estimation of protein content

The total protein content was determined according to the method of Bradford [28].

2.8. Statistical analysis

Data in Fig. 1A represent the averages calculated from two measurements. All the other presented data represent the average, calculated from 3 to 5 samples. The standard deviations were also calculated. For statistical analyses the SPSS 14.0 was used. Two-independent-samples *t*-test (for the data in Fig. 3A and B) and ANOVA (for the data in Fig. 6) were performed with 95% confidence level. Individual differences were analyzed by Tukey HSD test.

3. Results

3.1. Salt stress impact on the photosynthetic apparatus and the role of polyamines

To study the effect of high NaCl-concentration on the photosynthetic apparatus we used the unicellular, green alga, *S. obliquus*. Six collateral cultures with different concentrations of NaCl (0, 2, 5, 7, 10, 15‰) were used and the growth rate of the alga, expressed in PCV (packed cell volume), was measured in a time period of 100 h (Fig. 1A). Results demonstrated that there was growth in all the cultures except that of 15‰ NaCl. The growth rate of the cultures was inversely proportional to the NaCl concentration.

To record the possible changes in the molecular structure and function of the photosynthetic apparatus, the Chl a fluorescence (Kautsky curve) in the cultures without and with NaCl was measured. In order to verify the correct evaluation of Fm in the fluorescence curves of the NaCl cultures, DCMU was also added to the samples during fluorescence induction measurements. For comparison, data from the control culture and the 10‰ (170 mM) NaCl supplemented culture (with and without DCMU) are depicted in Fig. 1B. Increased NaCl concentration results in decrease of Fm and diminution of the I step of the fluorescence curve. Further processing and analysis of the data with JIP-test, provide a series of factors indicative of the molecular structure and function of the photosynthetic apparatus. Overall, this kind of analyses displayed great differences induced by the enhanced NaCl concentration (Fig. 1C). The Driving Force (D.F.) of photosynthesis, the performance indexes (PI) and the structure function index (SFI) were all greatly lowered. Also, the energy needed to close all reaction centers (Area, Sm) and the turnover number (*N*) of the reaction centers were lowered. Instead, the dissipation energy per active reaction center (DIo/RC) and the effective antenna size (ABS/RC) were increased. An indication for the increase in the antenna size (LHCII size) is given also by the Chl a/b ratio: in the control culture the value of this ratio was 3.210 ± 0.038, whereas in the NaCl-treated culture the ratio was significantly reduced to 3.061 ± 0.014. The trapping efficiency per reaction center (TRo/RC) showed also an increase and the electron transport per reaction center (ETo/RC) was reduced. Overall, the kinetics of the fluorescence was greatly changed (dV/dto, dVG/dto, V_i, V_j, Fm), although little was the impact on the photosynthetic efficiency (φpo). Among these changes, the parameters dV/dto (proportional to the rate of the accumulation of the fraction of closed reaction centers due to the PQ-redox state and the antenna-effect) and V_j (expressing the relative variable fluorescence at the J-phase of the transient) stand out.

On the other hand, the primary photochemistry yield (ψ_o: the probability that an exciton absorbed is used for electron transport beyond Q_A) was reduced (Fig. 1C).

JIP-analyses from cultures grown in various salt concentrations (0 to 15‰ NaCl) also showed that the increase of NaCl concentration results in a gradual increase of the effective antenna size (ABS/RC), decreasing simultaneously the density of the active photosynthetic reaction centers (RC/CSo) and the electron transport per reaction center (ETo/RC). The consequence of these changes is the decrease of the primary photochemistry yield (ψ_o) and the increase of the non-photochemical quenching of energy (DIo/RC), both resulting in decrease of the performance index (PI), the structure function index (SFI) and the photosynthetic efficiency or maximum photochemistry yield (Fv/Fm). The overall photosynthetic driving force (DF) also dropped to very low values (Fig. 2).

Previous results have suggested that thylakoid associated polyamines and especially the ratio putrescine to spermine (Put/Spm) is one of the main regulatory factors for the structure and function of the photosynthetic apparatus [29]. Under natural conditions mainly the changes in the Put content adjust the Put/Spm ratio [30,31]. Polyamine analysis from whole cells did not exhibit significant differences (Fig. 3A), but analysis from isolated thylakoids showed clearly a decrease in polyamine content under the 10‰ NaCl treatment, and especially decrease in Put (Fig. 3B) which resulted also in decrease of the ratio Put/Spm (Fig. 3C).

3.2. The diamine Putrescine can confer protection against enhanced salinity

Since the decrease of Put content regulates the differentiation of the photosynthetic apparatus we proceeded by testing whether the exogenously supplied Put could adjust the sensitivity of the photosynthetic apparatus to NaCl. For this purpose the molecular structure and function of the photosynthetic apparatus from four cultures (0‰ NaCl; 10‰ NaCl; 10‰ NaCl+1 mM Put; 1 mM Put) was assessed. JIP measurements

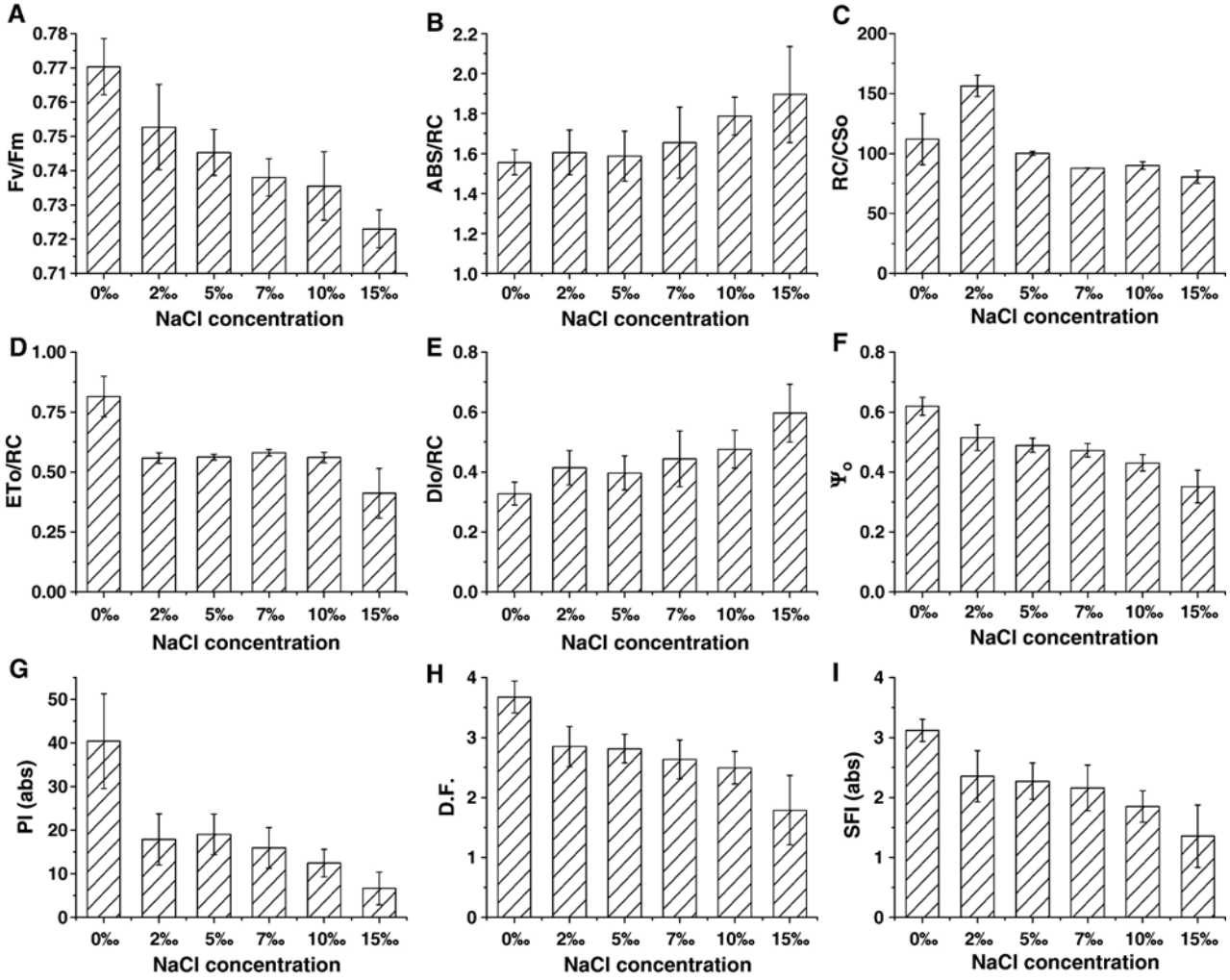

Fig. 2. Changes in the parameters calculated by the JIP-test analysis of chlorophyll fluorescence data, among cultures supplemented with various NaCl concentrations (0‰, 2‰, 5‰, 7‰, 10‰, 15‰) for 96 h. (A) Photosynthetic efficiency (Fv/Fm); (B) functional size of the antenna per active reaction center (ABS/RC); (C) density of active reaction centers (RC/CSo); (D) electron transport rate per active reaction center (ETo/RC); (E) non-photochemical energy dissipated per active reaction center (DIo/RC); (F) primary photochemistry yield (ψ_o); (G) Performance Index [PI(abs)]; (H) Driving Force of photosynthesis (D.F.); (I) Structure–Function Index [SFI(abs)].

clearly showed that addition of exogenous Put in cultures with 10‰ NaCl (10‰NaCl+1 mM Put treatment) shifted the fluorescence curve and therefore all previously mentioned fluorescence parameters (Fv/Fm, ABS/RC, RC/CS, ψ_o, ETo/RC, DIo/RC, PI, SFI, DF) towards the respective values of control culture (Figs. 4 and 5). Polarographic measurements for the maximal net photosynthetic rate from the four above mentioned cultures confirmed this tendency (Fig. 6). The NaCl-treated culture, compared to control, showed a strong decrease of the maximal photosynthetic rate, whereas the NaCl-treated culture additionally supplemented with Put (10‰NaCl+1 mM Put) presented a maximal net photosynthetic rate closer to the control one (Fig. 6).

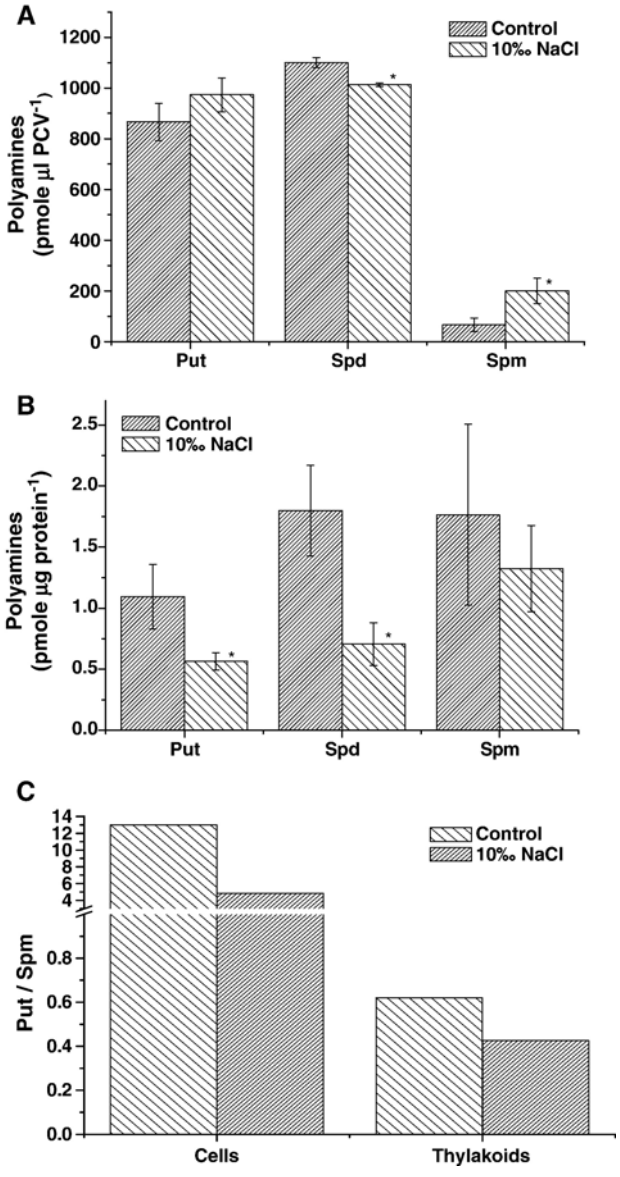

Fig. 3. Polyamine (Put, Spd, Spm) concentrations in cell (A) and thylakoid (B) preparations from control and 10‰ NaCl-supplemented cultures, after 96 h of treatment. Any significant difference (two-independent-samples *t*-test analysis, $p < 0.05$) of each polyamine concentration of the NaCl-treated culture from its respective control is indicated by an asterisk (*). (C) Put/Spm ratio in cells and thylakoid preparations from the same cultures.

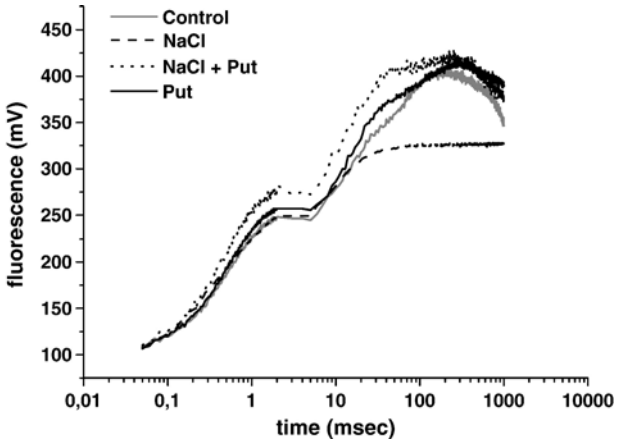

Fig. 4. Fluorescence curves measured from four *Scenedesmus obliquus* autotrophic cultures: control culture and cultures supplemented either with 10‰ NaCl or with 1 mM Put or with 10‰ NaCl+1 mM Put. Measurements were performed 96 h after onset of the treatments.

Since exogenously added Put was proved to adjust the sensitivity / tolerance of the photosynthetic apparatus and therefore of the whole cell to salinity, it seems also reasonable that addition of Put can shift the range of cell tolerance towards even higher NaCl concentrations. In order to investigate this, the growth rate from two series of cultures (with and without additional Put) grown in various NaCl concentrations (0–25‰) was measured (Fig. 7). The results showed clearly that the additional Put may reduce somewhat the growth rate of the culture at low NaCl concentrations (0–7‰), however at higher concentrations (10–25‰ NaCl) it seems to offer an advantage to the cultures, permitting their better growth compared to corresponding cultures without Put. Cultures without additional Put could not grow in NaCl concentrations higher than 10‰ and could not even survive in NaCl concentrations higher than 15‰ (Fig. 7). Conclusively, Put addition reduces the effect of high NaCl salinity and allows better growth of the culture. The cultures treated with high NaCl concentrations (15–25‰) which were additionally supplemented with Put exhibited only a relatively low stress of the photosystem II photosynthetic efficiency (Fv/Fm: 0.5).

4. Discussion

In the present contribution we studied the effects of salinity stress (NaCl) on the molecular structure and function of the photosynthetic apparatus and the protective role of polyamines. It is shown that the changes that NaCl causes to the photosynthetic apparatus can be alleviated by altering the intracellular polyamine levels, especially by increasing the thylakoid associated Put/Spm ratio.

Enhanced salinity causes increase in the size of the effective antenna per reaction center (ABS/RC; Figs. 1C and 2B). The higher the NaCl concentration, the stronger is the increase in the photosynthetic effective antenna size. At the same time the density of the reaction centers (RC/CSo; Figs. 1C and 2C) [12,32,33], the primary photochemistry (ψ_o; Figs. 1C and 2F) as well as the electron transport per active reaction center (ETo/

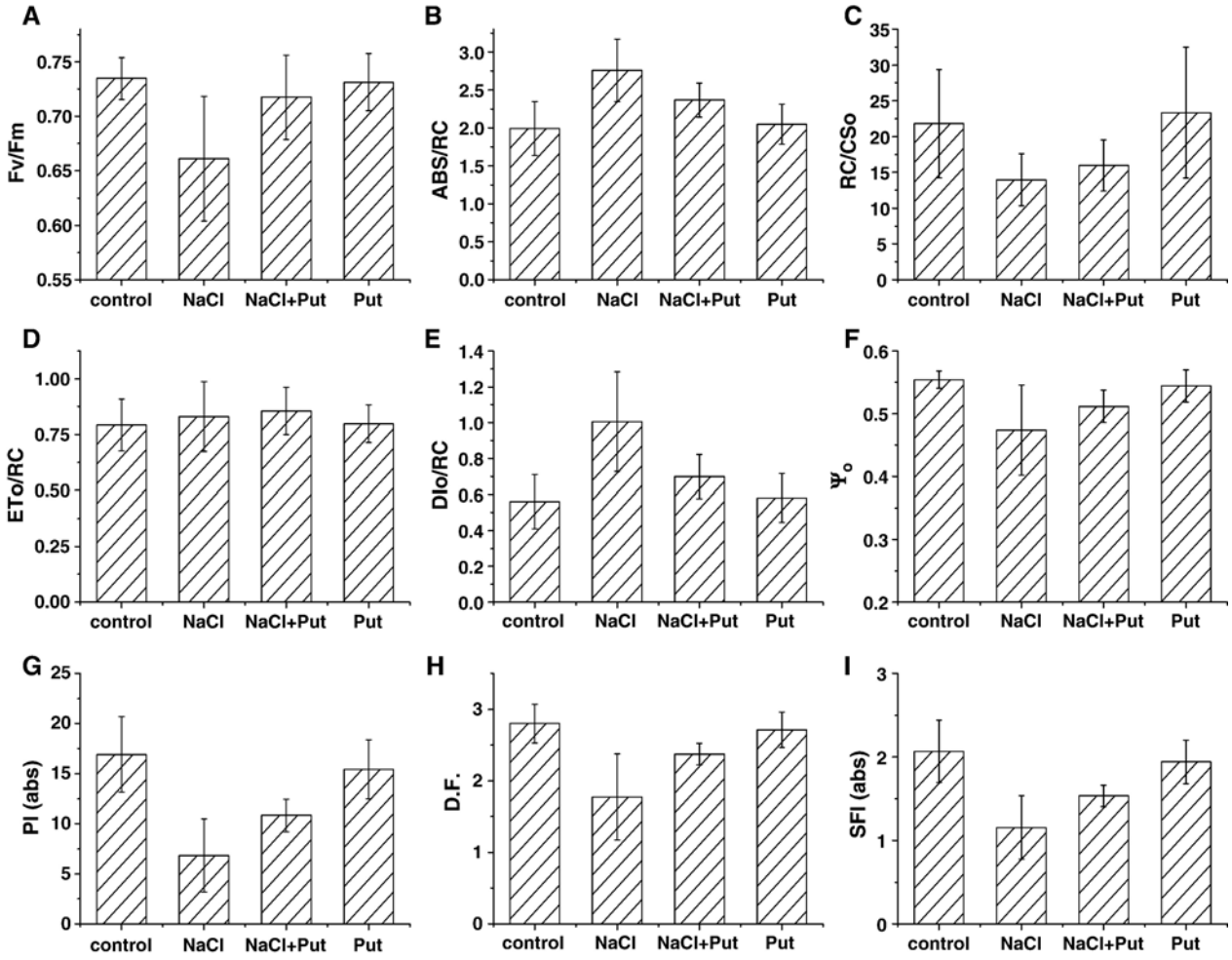

Fig. 5. Changes in the parameters calculated by the JIP-test analysis of chlorophyll fluorescence data, among four cultures: Control; 10‰ NaCl; 10‰ NaCl+1 mM Put; 1 mM Put 96 h after onset of treatment. (A) Photosynthetic efficiency (Fv/Fm); (B) functional size of the antenna per active reaction center (ABS/RC); (C) density of active reaction centers (RC/CSo); (D) electron transport rate per active reaction center (ETo/RC); (E) non-photochemical energy dissipated per active reaction center (DIo/RC); (F) Primary photochemistry yield (ψ_o); (G) Performance Index [PI(abs)]; (H) Driving Force of photosynthesis (D.F.); (I) Structure–Function Index [SFI(abs)].

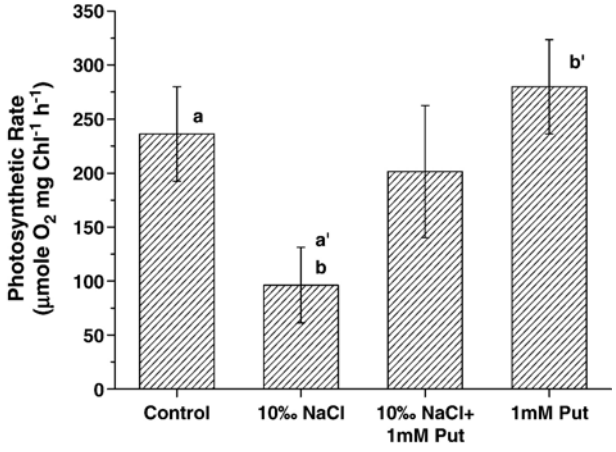

Fig. 6. Net photosynthetic rate measured as oxygen evolution from *Scenedesmus obliquus* autotrophic cultures (control culture and same cultures supplemented with either 10‰ NaCl or 1 mM Put or both 10‰ NaCl+1 mM Put), 96 h after onset of treatment. Significant differences (ANOVA, $p < 0.05$) between bars are indicated by same letters (e.g. a and a′).

RC; Figs. 1C and 2D) [12,17,18,34] decrease. All the inactivated reaction centers receive the initial amount of light energy that is transferred to the active reaction centers which cannot exploit it efficiently. The ineffective energy exploitation [35] leads to an extreme increase of the dissipation energy (DIo/RC; Figs. 1C and 2E) [12] and therefore to a decrease of the photosynthetic efficiency (Fv/Fm; Figs. 1C and 2A) [12,17,18,36]. Altogether, these changes are harmonized with the changes induced in the photosynthetic apparatus when it is adapted to lower light conditions [29]. Apart from the NaCl-induced antenna changes it cannot be excluded that the treatment leads to a more reduced PQ-pool in dark-adapted samples. The effect of the treatment on the parameters dV/dto and V_j (Fig. 1C) is similar to the changes observed in [37], where anaerobiosis was responsible for a more reduced PQ-pool. These results led us think that it is possible to control the tolerance/sensitivity of the photosynthetic apparatus and of the whole organism to enhanced salinity, by changing the molecular structure and function of the photosynthetic apparatus and especially by changing the functional antenna size. Previous

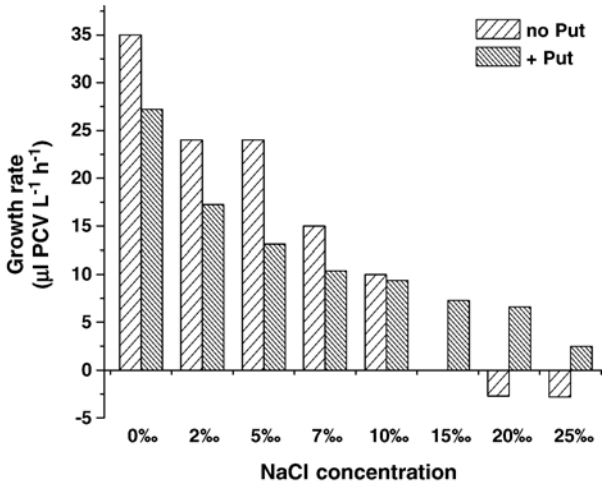

Fig. 7. Growth rate in two series of cultures supplemented with various NaCl concentrations (0‰, 2‰, 5‰, 7‰, 10‰, 15‰, 20‰, 25‰,) with or without additional Put (1 mM) supplementation. Negative growth rate values emerge in non-viable cultures.

work [29] has shown that polyamines have a significant role in the modulation of the antenna size. According to this report, exogenously supplied Put in salt stressed cultures was expected to increase the Put/Spm ratio in thylakoids and lead to a decrease of the functional antenna size. Indeed, Put addition was effective in decreasing the functional antenna size (ABS/RC; Fig. 5B). While NaCl causes increase of the functional antenna size per reaction center, the increase of the Put/Spm ratio, induced by the exogenously supplied Put, causes the opposite result thus compensating for the NaCl effect and therefore conferring protection to the photosynthetic apparatus against high NaCl-salinity. Furthermore, the decrease of the antenna size due to the exogenous Put, leads to an enhanced reaction center density (RC/CSm; Figs. 4 and 5C), possibly because of a lower rate of inactivation of the donor side of PSII combined with the activity of the PSII repair cycle. Thus, it also led to the increase of the electron transport per active reaction center (ETo/RC; Fig. 5D) and of the primary photochemistry (ψ_o; Fig. 5F). Overall, these effects resulted in an improved "management" of the excitation energy, a fact that is supported by the decrement of non-photochemical dissipation energy (DIo/RC; Fig. 5E), the increment in the quantum yield of PSII primary photochemistry (Fv/Fm, Fig. 5A) and the maximal photosynthetic activity (Fig. 6).

Since long we have been studying the response of the photosynthetic mechanism to various environmental stresses [38–42]. However, the central mechanism by which polyamines regulate the reorganization of the photosynthetic apparatus and mainly the antenna size upon light or stress stimulation, has only recently begun to be uncovered [43]. According to these findings, the photosynthetic apparatus reacts to a stress impact by being reorganized as if in lower light conditions. Application of the diamine Put can reverse this effect. Thus, the stress impact is minimized. The results of the present study are in full agreement with this concept: significant changes are provoked as a direct response of the photosynthetic apparatus upon the environmental change (high NaCl concentration). The role of polyamines in the regulation of this response is to generate a tolerant behavior against the environmental stress condition.

The above description represents the general model of the NaCl effect and the polyamine action in the photosynthetic apparatus, as it results from the present study. There are several studies reporting results similar to ours, concerning the decrease of the electron transport in the presence of high NaCl concentration [12,17,18,34]. However, none reports the exact alterations that occur in the molecular structure and function of the photosynthetic apparatus, as well as any possibility of recovery (like the polyamine effect, presented here). In the present contribution it is clearly demonstrated that the main responses of the photosynthetic apparatus to the enhanced NaCl salinity is the increase of the functional antenna size. Due to the incident light, the antenna absorbs large amounts of energy, which however, cannot be effectively used. So, the photosynthetic apparatus is constantly under excitation pressure, which leads to energy dissipation and suppressed photosynthetic O_2 formation. Exogenously supplied Put drastically decreases the effective antenna size per active RC, both by decreasing the size of LHCII per RC and by increasing the density of the active RCs [43], so that the damaging effects are diminished. This is clearly supported by the results of the growth rate of the cultures (Fig. 7), from which it becomes obvious that the additional Put diminishes the effect of enhanced salinity and allows the culture growth under even higher salt concentrations. The fact however that Put reduces the growth rate in unstressed cell cultures is not surprising. There are quite a few publications reporting a similar dual effect of Put and also of other polyamines on the cell growth rate [44–47]. Cultures supplemented with Put grew also in 25‰ NaCl with relatively only low stress of photosystem II (Fv/Fm: 0.5), whereas cultures without additional Put could not survive in NaCl concentrations higher than 15‰ (Fig. 7).

4.1. Conclusions

Enhanced salinity is experienced as a stress condition in plants since it provokes changes in the photosynthetic apparatus, by affecting both its structure and function. The most important of these changes indicate an alteration of the molecular structure and functioning of the photosynthetic apparatus towards adaptation to lower light intensity, i.e. increased effective antenna size and decreased active reaction center population, which altogether produce a higher excitation pressure. Exogenously added Put can compensate for these changes, by inducing the reciprocal reorganization of the photosynthetic apparatus. Thus, Put confers resistance against enhanced NaCl-salinity and permits cell growth of photosynthetic organisms even in NaCl concentrations that normally would be toxic.

References

[1] T. Yamaguchi, E. Blumwald, Developing salt-tolerant crop plants: challenges and opportunities, Trends Plant. Sci. 10 (2005) 615–620.

[2] R. Munns, Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses, Plant. Cell Environ. 16 (1993) 15–24.
[3] H.-C. Ma, L. Fung, S.-S. Wang, A. Altman, A. Hittermann, Photosynthetic response of *Populus euphratica* to salt stress, Forest Ecol. Manag. 93 (1997) 55–61.
[4] N. Sultana, T. Ikeda, R. Itoh, Effect of NaCl salinity on photosynthesis and try matter accumulation in developing rice grains, Environ. Exp. Bot. 42 (1999) 211–220.
[5] S.K. Mishra, D. Subralimanyam, G.S. Singhal, Interactionship between salt and light stress on the primary process of photosynthesis, J. Plant Physiol. 138 (1997) 92–96.
[6] R.S. Dubey, Protein synthesis by plants under stressful conditions, in: M. Pessarakli (Ed.), Handbook of Plant and Crop Stress, Macel Dekker Inc., New York, 1994, pp. 277–299.
[7] C.P. Bethke, C.M. Drew, Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annum* during progressive exposure to NaCl salinity, Plant Physiol. 99 (1992) 219–226.
[8] Z. Nagy, G. Galiba, Drought and salt tolerance are not necessarily linked: a study on wheat varieties differing in drought resistance under consecutive water and salinity stresses, J. Plant Physiol. 145 (1995) 168–174.
[9] J.V. Sibole, E. Montero, C. Cabot, C.B. Poschenrieder, Role of sodium in the ABA-mediated long-term growth response of bean to salt stress, Physiol. Plant. 104 (1998) 299–305.
[10] K.G. Kolchevskii, N.I. Kocharyan, O. Koroleva, Effect of salinity on photosynthetic characteristics and ion accumulation in C3 and C4 plants of Ararat plain, Photosynthetica 31 (1995) 277–282.
[11] M.A. Khan, I.A. Ungar, Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions, Am. J. Bot. 84 (1997) 279–283.
[12] C. Lu, A. Vonshak, Effects of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells, Physiol. Plant. 114 (2002) 405–413.
[13] J. Xia, Y. Li, D. Zou, Effects of salinity stress on PSII in *Ulva lactuca* as probed by chlorophyll fluorescence measurements, Aquat. Bot. 80 (2004) 129–137.
[14] H. Greenway, R. Munns, Mechanisms of salt tolerance in nonhalophytes, Annu. Rev. Plant. Physiol. 31 (1980) 149–190.
[15] J.E. Backhausen, M. Klein, M. Klocke, S. Jung, R. Scheibe, Salt tolerance of potato (*Solanum tuberosum* L. var. Desirée) plants depends on light intensity and air humidity, Plant Sci. 169 (2005) 229–237.
[16] A.N. Misra, A. Srivastava, R.J. Strasser, Utilization of fast chlorophyll a fluorescence technique in assessing the salt/ion sensitivity of mung bean and Brassica seedlings, J. Plant Physiol. 158 (2001) 1173–1181.
[17] S.I. Allakhverdiev, A. Sakamoto, Y. Nishiyama, N. Murata, Inactivation of photosystems I and II in response to osmotic stress in *Synechococcus*, contribution of water channels, Plant Physiol. 122 (2000) 1201–1208.
[18] S.I. Allakhverdiev, A. Sakamoto, Y. Nishiyama, M. Inaba, N. Murata, Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiol. 123 (2000) 1047–1056.
[19] K. Urano, Y. Yoshiba, T. Nanjo, T. Ito, K. Yamaguchi-Shinozaki, K. Shinozaki, *Arabidopsis* stress-inducible gene for arginine decarboxylase *AtADC2* is required for accumulation of putrescine in salt tolerance, Biochem. Biophys. Res. Commun. 313 (2004) 369–375.
[20] Y. Kasukabe, L. He, K. Nada, S. Misawa, I. Ihara, S. Tachibana, Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*, Plant Cell Physiol. 45 (2004) 712–722.
[21] H. Gaffron, Reduction of carbon dioxide with molecular hydrogen in green algae, Nature 143 (1939) 204–205.
[22] N.I. Bishop, H. Senger, Preperations and photosynthetic properties of synchronous cultures of *Scenedesmus*, in: A. San Pietro (Ed.), Methods in Enzymology, Academic Press, New York, 1971, pp. 53–66.
[23] B.J. Strasser, R.J. Strasser, Measuring fast fluorescence transients to address environmental questions: the JIP-test, in: P. Mathis (Ed.), Photosynthesis: from Light to Biosphere, Kluwer Academic Publishers, Dordrecht, 1995, pp. 977–980.
[24] A.F. Tiburcio, R. Kaur-Sawhney, A.W. Galston, Correlation polyamines and pyrolidine alkaloids in developing tobacco callus, Plant Physiol. 78 (1985) 323–326.
[25] H.E. Flores, A.W. Galston, Analysis of polyamines in higher plants by high performance liquid chromatography, Plant Physiol. 69 (1982) 701–706.
[26] K. Kotzabasis, M.D. Christakis-Hampsas, K.A. Roubelakis-Angelakis, A narrow-bore HPLC method for the identification and quantitation of free, conjugated and bound polyamines, Anal. Biochem. 214 (1993) 484–489.
[27] M. Holden, Chlorophylls, in: T.W. Goodwin (Ed.), Chemistry and Biochemistry of Plant Pigments, Academic Press, London, 1965, pp. 461–488.
[28] M.M. Bradford, A rapid and sensitive method for the estimation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[29] K. Kotzabasis, B. Strasser, E. Navakoudis, H. Senger, D. Dörnemann, The regulatory role of polyamines in structure and functioning of the photosynthetic apparatus during photoadaptation, J. Photochem. Photobiol., B Biol. 50 (1999) 45–52.
[30] A. Andreadakis, K. Kotzabasis, Changes in the biosynthesis and catabolism of polyamines in isolated plastids during chloroplast photodevelopment, J. Photochem. Photobiol., B Biol. 33 (1996) 163–170.
[31] D. Dörnemann, E. Navakoudis, K. Kotzabasis, Changes in the polyamine content of plastidal membranes in light- and dark-grown wildtype and pigment mutants of the unicellular green alga *Scenedesmus obliquus* and their possible role in chloroplast photodevelopment, J. Photochem. Photobiol., B Biol. 36 (1996) 293–299.
[32] H. Schubert, M. Hagemann, Salt stress on 77 K fluorescence and photosynthesis in the cyanobacterium *Synechocystis* sp., PCC6083, FEMS Microbiol. Lett. 71 (1990) 169–172.
[33] H. Schubert, S. Fluda, M. Hagemann, Effects of adaptation to different salt concentrations on photosynthesis and pigmentation of the cyanobacterium *Synechocystis* sp. PCC6803, J. Plant Physiol. 142 (1993) 291–295.
[34] T. Endo, I. Schreiber, K. Asada, Suppression of quantum yield of photosystem II by hyperosmotic stress in *Chlamydomonas reinhardtii*, Plant Cell Physiol. 36 (1995) 1253–1259.
[35] K. Satoh, C.M. Smith, D.C. Fork, Effects of salinity on primary processes of photosynthesis in the red alga *Porphyra perforata*, Plant Physiol. 73 (1983) 643–647.
[36] G. Lu, J. Zhang, Role of light in the response of PSII photochemistry to salt stress in the cyanobacterium *Spirulina platensis*, J. Exp. Bot. Sl. 346 (2000) 911–917.
[37] P. Haldimann, R. Strasser, Effects of anaerobiosis as probed by the polyphasic chlorophyll *a* fluorescence rise kinetic in pea (*Pisum sativum* L.), Photosynth. Res. 62 (1999) 67–83.
[38] E. Navakoudis, C. Lütz, C. Langebartels, U. Lütz-Meindl, K. Kotzabasis, Ozone impact on the photosynthetic apparatus and the protective role of polyamines, Biochim. Biophys. Acta 1621 (2003) 160–169.
[39] L. Sfichi, N. Ioannidis, K. Kotzabasis, Thylakoid-associated polyamines adjust the UV-B sensitivity of the photosynthetic apparatus by means of light-harvesting complex II changes, Photochem. Photobiol. 80 (2004) 499–506.
[40] K. Logothetis, S. Dakanali, N. loannidis, K. Kotzabasis, The impact of high CO_2 concentration on the structure and function of the photosynthetic apparatus and the regulatory role of polyamines, J. Plant Physiol. 161 (2004) 715–724.
[41] C. Lu, N. Qui, Q. Lu, B. Wang, T. Kuang, Does salt stress lead to increased susceptibility of photosystem II to photoinhibition and changes in photosynthetic pigment composition in halophyte *Suaeda salsa* grown out doors? Plant Sci. 163 (2002) 1063–1068.
[42] C. Lütz, E. Navakoudis, H.K. Seidlitz, K. Kotzabasis, Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UV-B protection, Biochim. Biophys. Acta 1710 (2005) 24–33.
[43] E. Navakoudis, K. Vrentzou, K. Kotzabasis, A polyamine- and LHCII protease activity-based mechanism regulates the plasticity and adaptation status of the photosynthetic apparatus, Biochim. Biophys. Acta 1767 (2007) 261–271.

[44] W. Tang, R.J. Newton, Polyamines promote root elongation and growth by increasing root cell division in regenerated Virginia pine (*Pinus virginiana* Mill.) plantlets, Plant Cell Rep. 24 (2005) 581–589.

[45] K.A. Paschalidis, K.A. Roubelakis-Angelakis, Sites and regulation of polyamine catabolism in the tobacco plant. Correlations with cell division/expansion, cell cycle progression and vascular progression, Plant Physiol. 138 (2005) 2174–2184.

[46] L. Gemperlová, J. Eder, M. Cvikrová, Polyamine metabolism during the growth cycle of tobacco BY-2 cells, Plant Physiol. Biochem. 43 (2005) 375–381.

[47] K.A. Paschalidis, K.A. Roubelakis-Angelakis, Spatial and temporal distribution of polyamine levels and polyamine anabolism in different organs/tissues of the tobacco plant. Correlations with age, cell division/expansion and differentiation, Plant Physiol. 138 (2005) 142–152.