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SALT STRESS TOLERANCE OF A FRESHWATER GREEN ALGA UNDER DIFFERENT PHOTON FLUX DENSITIES

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SUMMARY. - Salinity stress and unfavorable light conditions are main limiting factors of plant productivity both in aquatic and terrestrial, natural and anthropically modified environments. This is the reason why the identification of physiological responses to the interactive effects of high salt concentration and different photon flux densities is an important requirement for the selection of tolerant and highly productive plant ecotypes under stressful environmental conditions. The aim of this study is to identify physiological parameters which enable the evaluation of the degree of salt stress tolerance and the energetic requirements of protective mechanisms based on the photochemical conversion of light energy. The axenic monoalgal cultures of *Scenedesmus opoliensis* were grown under controlled conditions in the presence of 0, 0.1 and 0.5 M NaCl at photon flux densities of 5, 50 and 100 $\mu M\ m^{-2}s^{-1}$. The dynamics of growth and biomass production, as well as the photosynthetic pigment content was determined for all the experimental variants, the significance of the results was evaluated with the one-way ANOVA and with the Tukey test. The investigated freshwater microalga can easily acclimate to high salt concentrations, under these conditions the rate of cell divisions increases, the cells develop very small light-harvesting antennae and they excrete high amounts of mucilage in which the individuals form extended aggregates.

Plant cells are generally able to live within a certain range of enhanced salt concentrations or changing salinities, since most probably all life originated in the oceans, i. e. a highly saline environment. However, during evolution, the degree of salt resistance and salt tolerance became very divergent among the present-day aquatic organisms. Algae (and cyanobacteria) have attracted considerable attention in this respect, since they are inhabitants of biotopes characterized by changing salinities and can serve as model organisms for a better undestanding of salt acclimation in the more complex physiological processes of higher plants [3, 4, 11]. Enhanced salt concentrations change the growth conditions in a manner unfavourable for most organisms. The increase in external concentrations of inorganic ions impairs the osmotic balance between the cells and their surrounding medium and forces water efflux (exosmosis) from the cells, leading to the loss of turgor pressure. In parallel, the increased exogenous ion concentration tightens the influx of these ions into the cells according to their electrochemical gradients [7, 13, 16, 20, 22, 23].

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The loss of water and the invading ions activate a concerted acclimation process that leads to salt-tolerant cells with a new steady state of growth. This acclimation process includes three basal processes: restoration of turgor, regulation of the uptake and export of ions through the cell membranes, and induction of the accumulation of osmoprotecting compatible solutes and stress proteins. The acclimation initiated by water loss due to salt stress involves water fluxes which are controlled by water channels and the concentrations of those cellular solutes that predominantly contribute to the osmotic potential [5, 14, 17]. Because the influx of sodium and chloride disturbs the cellular ion homeostasis, acclimation also involves the regulated uptake and export of potassium and sodium cations [2, 15, 16]. Elevated ion concentrations impair the function of biopolymers and induce protective measures that involve mainly the accumulation of osmoprotective compatible solutes and specific proteins. These molecules ensure the protection and renaturation of damaged functional and structural proteins, nucleic acids and membrane lipids [12, 18, 24]. Besides these main processes, several secondary responses are needed to ensure a successful salt tolerance, e. g. the scavenging of liberated free radicals, increase in energy-supplying reactions and, finally, the adjustment of the whole metabolism to the new situation [1, 21]. All these efforts are realized with an increased energy consumption and require an adequate photon flux density and photochemical conversion to cope with the stressful conditions.

Salt acclimation has generated great interest for two main reasons: 1. sodium chloride represents one of the environmental major factors of aquatic habitats, and 2. salt resistance constitutes a real biotechnological challenge in the field of renewable biomass production [8, 25, 27]. This is why we have chosen a freshwater green microalga with high bioproductive capacity to achieve a study of its salt tolerance. The aim of this study is to investigate the relationship between the salt concentration and the photosynthetically active light intensity during the development of salt stress tolerance in cell cultures of a typically freshwater species of green microalga.

Material and methods. Axenic monoalgal cultures of *Scenedesmus opoliensis* P. Richter, strain AICB 141, obtained from the culture collection of the Biological Research Institute in Cluj [6], were grown in Kuhl-Lorenzen (KL) nutrient media supplemented, according to the different experimental variants, with 0.1 M and 0.5 M sodium chloride. The control cultures were kept in the KL medium without sodium chloride. The initial pH of all the culture media was adjusted to 6.5 and the cell suspensions were illuminated continuously with fluorescent lamps at photon flux densities of 5, 50 and 100 micromoles $m^{-2}s^{-1}$ on the surface of the cultures [10]. The dynamics of cell divisions was evaluated cytometrically with a light microscope. The initial cell density of all the cultures was set to 540 cells per microliter. The photosynthetic pigment content was determined spectrophotometrically after extraction with methanol at high temperature [9, 19]. The dry algal biomass of the cultures was determined after 2 weeks of development in the presence of different concentrations of sodium chloride at the three different photon flux densities mentioned above. Every measurement was repeated 5 times. The experimental data were evaluated statistically, using ANOVA ($P < 0.05$) and the Tukey test ($P < 0.05$) [26].

Results and discussion. *Scenedesmus opoliensis* is a freshwater algal species, being present in the phytoplankton of various rivers and lakes, known for its pronounced metabolic plasticity that confers tolerance to different environmental changes in the aquatic habitats. One strain of this species was grown for years in laboratory cultures in a liquid medium with low osmotic potential and with no sodium ions. Such a culture that was in the exponential growth phase of the algal population was used to initiate the experimental setups designed to study the capacity of this alga to cope with two different concentrations of sodium chloride (0.1 M and 0.5 M, respectively) under three different photon flux densities.

In the absence of sodium chloride (control cultures) the rate of cell divisions in the algal populations is influenced by the amount of light energy available from the environment. High photon flux densities (100 µmole photons $m^{-2}s^{-1}$, that in aquatic habitats represents a higher light intensity but does not cause photoinhibition) ensure a much higher cell density in the algal cultures than low and medium light intensities (5 and 50 µmole photons $m^{-2}s^{-1}$), underlining the fact that the incident light energy is an important external factor for algal growth (Fig. 1).

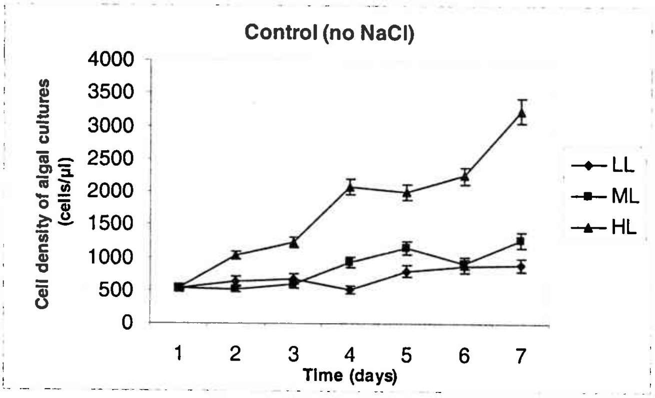


Fig. 1. *Variation of cell number in the cultures of* Scenedesmus opoliensis *grown under different photon flux densities.*
HL - high light; LL - low light; ML - medium light. The error bars indicate standard deviation (n = 5).

In the presence of a smaller amount of sodium chloride (0.1 M) there were no significant differences from the control cultures with respect to the dynamics of cell density. The light intensity remained the determinant factor for the rate of cell divisions, the high photon flux density leading to an increased algal cell density in comparison with the cultures grown under low and medium light. Multiplication of this alga under the above mentioned conditions does not show sensitivity to a relatively low concentration of sodium chloride.

In the presence of 0.5 M sodium chloride the variation of cell density of the algal cultures differs significantly from the values recorded in the control populations if the cell cultures are illuminated with high photon flux densities. In low and medium light the cell density remains on a lower level along the days, while in the intensely illuminated cultures the cell number increases more quickly than in the control, even though the maximal algal density reaches its steady state at a lower level (Fig. 2). This reflects that the salt stress caused by 0.5 M sodium chloride induces a prompt response of the algal culture reflected by the enhancement of the reproductive capacity immediately after the initiation of the stress reaction. Because this process of hardening requires extra amounts of metabolic energy, it can be sustained only by a sufficient amount of photosynthetically active light energy.

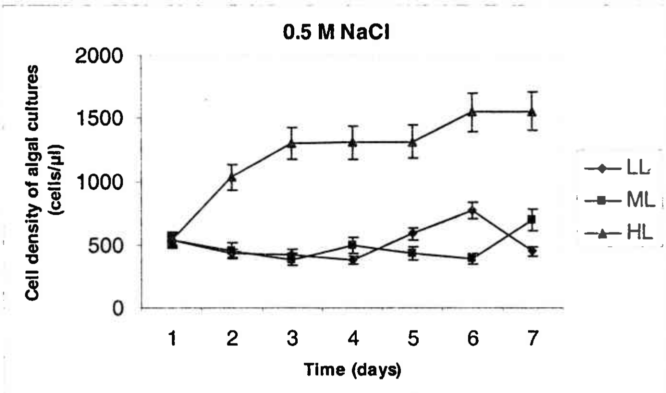
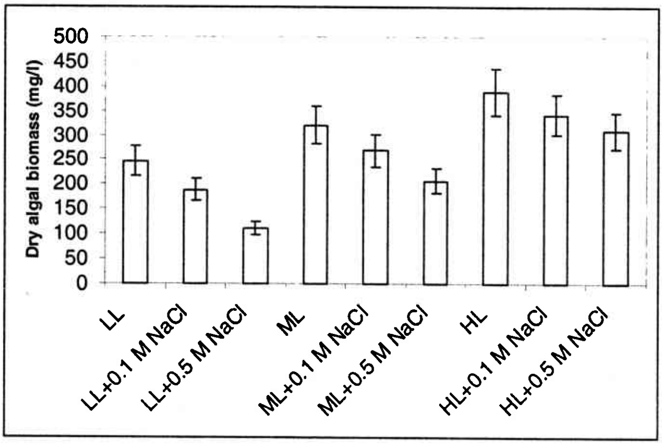


Fig. 2. *The influence of 0.5 M NaCl on the variation of cell number in the cultures of* Scenedesmus opoliensis *grown under different photon flux densities.*
HL - high light; LL - low light; ML - medium light. The error bars indicate standard deviation (n = 5).

The dry algal biomass of two weeks old cultures initiated with the same cell density was the lowest in the present of 0.5 M sodium chloride under all the three light intensities. The lower concentration of NaCl caused a less pronounced decrease of the net biomass production in comparison with the control cultures. As expected, the highest algal biomass was recorded in the presence of 100 µmole photons $m^{-2}s^{-1}$ and in the absence of sodium chloride (Fig. 3). The results are similar to those obtained with marine algae, where the control cultures were grown in seawater [17], and they reflect that the acclimation to salt stress has high energy demands and leads to a decreased biomass production. This may be the reason why the dry weight is much less affected by salt stress in the presence of a higher photon flux density, which provides more energy to sustain the physiological processes involved in the development of tolerance.

Microscopic investigations revealed that in the presence of higher concentrations of sodium chloride the algal cultures become almost colorless, the cells loose their obvious green colour, the pyrenoid of the chloroplasts is smaller and the chloroplast itself is shrunken. This indicates a partial desiccation and a loss of turgor. As a reaction to the unfavorable growth condition created by salinity, the cells excrete a high amount of mucilage in which they form extended aggregates.

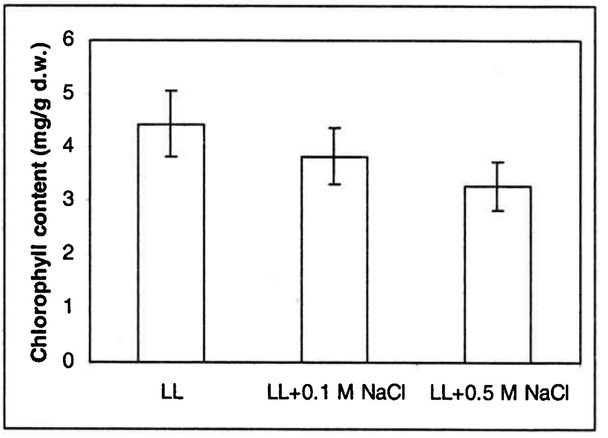


F i g. 3. *The dry weight of the 2 weeks old axenic cultures of* Scenedesmus opoliensis *grown in 3 light intensities in the presence of different amounts of sodium chloride.* HL - high light; LL - low light; ML - medium light. The error bars indicate standard deviation (n = 5). The mean values are significantly different according to the Tukey test (in all cases $P < 0.05$).

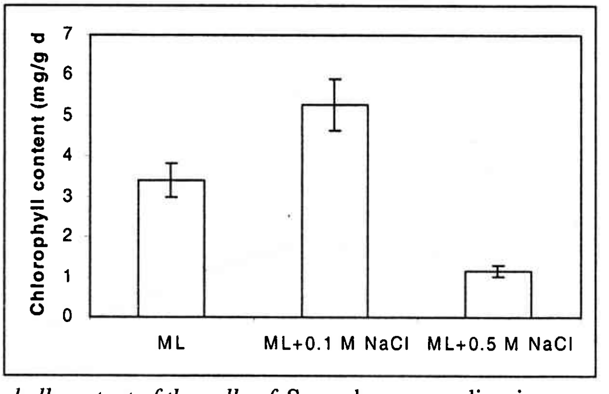
The embedded cells become protected by this mucous sheath, they survive successfully and they exhibit a relatively high rate of multiplication, even though in the presence of high photon flux densities they reduce severely the amount of photosynthetic pigments. The rate of net oxygen production of the algal culture depends strongly on the light intensity, but does not show significant differences in relation to the salt concentration if the oxygen evolution is expressed per dry biomass unit (data not shown).

In the cultures grown under low light the presence of 0.1 M and 0.5 M NaCl causes a slightly significant ($P < 0.05$) but not very pronounced decrease in the overall chlorophyll content of the algal cells. The amount of chlorophylls is kept high because the main limiting factor is the reduced energy input that induces the development of an extended light-harvesting complex in order to absorb a higher percentage of the incident low photon flux (Fig. 4).

Under the conditions of a moderate light intensity (50 μmole photons $m^{-2}s^{-1}$) the algal cells develop smaller photosynthetic pigment antennae in the thylakoid membranes of their chloroplasts and the presence of sodium chloride causes different quantitative changes depending on salt concentration: the chlorophyll content increases significantly in the presence of 0.1 M NaCl and it obviously decreases in the cultures provided with 0.5 M NaCl (Fig. 5). These results can be explained by the fact that small amount of sodium chloride create a slight stress condition compensated by the development of a more extended light-harvesting complex, while under the situation of a more severe salt stress the defense mechanisms do not allow to spend too much energy for the synthesis of many new chlorophyll molecules and binding proteins.

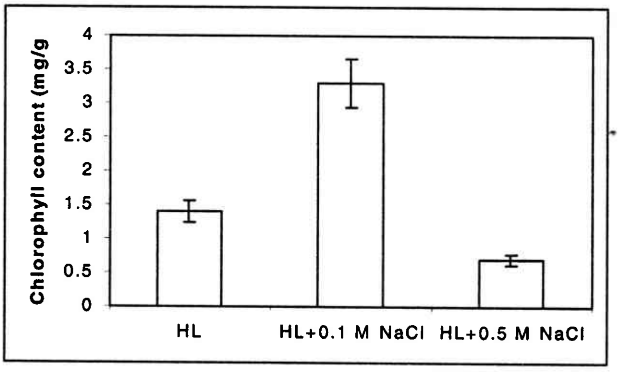


F i g. 4. *Chlorophyll content of the cells of* Scenedesmus opoliensis *grown under low photon flux density (LL) in media with different concentrations of sodium chloride.*
The error bars indicate standard deviation (n = 5). The mean values are significantly different according to the Tukey test ($P < 0.05$).



F i g. 5. *Chlorophyll content of the cells of* Scenedesmus opoliensis *grown under medium photon flux density (ML) in media with different concentrations of sodium chloride.*
The error bars indicate standard deviation (n = 5). The mean values are significantly different according to the Tukey test ($P < 0.01$).

The changes observed in medium light intensity occur even more obviously in the algal cultures grown under higher photon flux densities (increased chlorophyll content in the presence of 0.1 M NaCl and significantly decreased amounts of these pigments under the influence of 0.5 M NaCl), the reaction to mild salt stress leading to different results than the processes that develop under a more severe salt stress (Fig. 6).



F i g. 6. *Chlorophyll content of the cells of* Scenedesmus opoliensis *grown under high photon flux density (HL) in media with different concentrations of sodium chloride.* The error bars indicate standard deviation (n = 5). The mean values are significantly different according to the Tukey test ($P < 0.01$).

Conclusions. The monoalgal cultures of the freshwater species *Scenedesmus opoliensis* can acclimate to high salt concentrations, under these conditions the rate of cell divisions shows only a moderate decrease, the cells develop very small light-harvesting antennae and they excrete high amounts of mucilage in which the individuals form huge aggregates. A higher tolerance is expressed mainly if there is a relatively increased photon flux density, suggesting the role of light in supporting the energy demands of an efficient protective mechanism against physiological drought caused by the hypertonic environment and against the toxicity induced by excessive amounts of the sodium ion.

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