"BABES-BOLYAI" UNIVERSITY FACULTY OF BIOLOGY AND GEOLOGY BIOLOGY PH. D. SCHOOL

SUMMARY OF DOCTORAL THESIS

Comparative study of physiological and molecular manifestations of salt stress tolerance in different intraspecific varieties of *Lactuca sativa L*.

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> Cluj-Napoca 2012

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Key worlds:

- hydraulic conductance
- lettuce cultivars
- osmoregulation
- salt stress tolerance
- sodium accumulation
- stomatal conductance

INTRODUCTION

A deeper insight into the molecular mechanisms of salt stress tolerance not only contributes to a better conceptual understanding of plant adaptation and acclimation to environmental changes, but also provides researchers a valuable tool to efficiently improve the bioproductive capacity of crop plants under various conditions of cultivations. Salt stress affects plants in two main ways. High concentrations of salt decrease the osmotic potential, making it harder for roots to extract water (osmotic effect), and result in toxicity symptoms (ionic effect), leading to metabolic imbalance and premature senescence of leaves (Munns, 2002; Tester and Davenport, 2003). The loss of water and the invading ions activate a concerted acclimation process that may lead to salt tolerance associated with a new steady state of growth. This acclimation includes three basic processes: restoration of turgor, regulation of ion transport across membranes, and induction of the accumulation of osmoprotectants and stress proteins. Besides these processes, several secondary responses are needed to ensure salt tolerance, e. g. the scavenging of overproduced reactive oxygen species, an increase in energy-supplying reactions, and the adjustment of the whole metabolism to the new situation. This is why multiple molecular and physiological changes may be observed in plants exposed to salt stress: drop of stomatal and root hydraulic conductance, osmotic adjustment, reduced growth rate, changes of the root to shoot ratio, nutritional disorders, change of the photosynthetically active pigments concentration (Munns and Tester, 2008; Rajendran et al., 2009).

Lettuce (*Lactuca sativa* L.) has many cultivars, which are grown on extended areas and have become a very appreciated healthy food source, rich in mineral nutrients and vitamins. Most commercially grown cultivars are developed under nonsaline conditions and are not bred to endure salt stress. Very limited information is available about salt stress reactions of the different lettuce cultivars, as well as about the physiological and biochemical parameters that may be useful for the screening of salt tolerant varieties.

The principal aim of this study is to compare salt stress tolerance of largely commercialized lettuce cultivars, and to identify more tolerant cultivars that may be suited for large-scale cultivation in areas with increasing soil salinity. For this reason, we investigated changes induced by salt stress in seed germination, in vegetative growth parameters, in root hydraulic conductivity, in the Na⁺, K⁺, Ca²⁺ content of roots, leaves and xylem sap, in the free proline and soluble sugars content of leaves, in osmotic potential of leaf tissues and xylem sap, in photosynthetically active pigments content of leaves, in photosynthetic light use efficiency, in leaf gas exchange parameters.

By evaluation of the results we expected to establish molecular and physiological markers suitable for a reliable identification of more salt-tolerant lettuce cultivars, we aimed to offer breeders a scientific tool to select among lettuce varieties when their cultivation has to be done in salt-affected areas. We also aimed to identify at least one of the mostly salt-tolerant lettuce cultivars among the frequently commercialized ones in Europe. The actuality of studying aspects of the environmental stress physiology of lettuce varieties resides mainly in the fact that there exist two modern directions in the use of lettuce in applied research activities: 1. as a test plant for bioassay of phytotoxicity of soil pollutants (Valerio *et al.*, 2007), and 2. as a source of health-promoting phytochemicals with antioxidant properties (Oh *et al.*, 2009).

MATERIALS AND METHODS

Five different cultivars of lettuce (*Lactuca sativa* L.) were used in the experiments. These were selected from among twelve cultivars examined for salt stress sensitivity, largely cultivated in Europe. The cultivars were chosen from all of the four main lettuce convarieties (butterhead, roman, looseleaf and steam lettuce).

The seeds were germinated in vermiculite substrate at 20°C and on the 7th day after germination the seedlings were placed in 15 L containers (fig. 4.7.) with a continuous circulation of full-strength Hoagland's nutrient solution. Salinization treatment was initiated on 21 days old seedlings, after two weeks of growth in Hoagland's solution. Salt stress was induced by 50 mM and 100 mM of NaCl (p. a.), and plantlets were harvested after ten days of exposure (31 days old).

Fresh weight (FW) of roots and shoots (stem with leaves) was recorded separately, for five plants from each cultivar and salinity combination. For dry weight (DW) determination, the plant material was kept at 65°C for 5 days. The actual and relative water content of the leaves was also determined. The concentrations of inorganic cations in shoots and in the xylem sap were determined by inductively coupled plasma spectrometry, leaf gas exchange parameters were measured using a portable photosynthesis system (model LCA-4), parameters of the root hydraulic conductance of lettuce plants exposed to salt stress treatments were measured by natural exudation of detached roots. The concentration of free proline, soluble sugars, photosynthetic pigments and the products of membrane lipid peroxidation were determined spectrophotometrically.

We measured and computed different parameters of the induced chlorophyll *a* fluorescence with a pulse amplitude modulated chlorophyll fluorometer (PAM-FMS2, Hansatech). Determination of leaf tissue and xylem sap osmotic potential was realized with an automatic freezing-point depression osmometer.

Investigation of seed germination process in response to salt stress was performed with 12 cultivars of lettuce. Control plants were supplied with distilled water, while the experimental variants were watered with identical amounts of distilled water supplemented with 50 mM, 100 mM and 150 mM NaCl (p. a.).

Each determination was made with 5 replicates, significant differences between treatment means were determined using the post-ANOVA Tukey test.

RESULTS AND DISCUSSION

Effect of salinity on seed germination of different lettuce cultivars

Germination of seeds is the first critical step in the ontogeny of spermatophytes that is very sensitive to adverse environmental conditions. Even without any salt stress, the time scale and the frequency of germination vary among lettuce cultivars: in some varieties germination of most seeds begins during the first day after hydration, in others there is a gradual germination preceded by a lag phase of several days.



Fig. 1. Germination percentage of the Parella Green cultivar of lettuce



Fig. 2. Germination percentage of the Paris Island lettuce cultivar



Fig. 3. Germination percentage of the Valdor cultivar



Fig. 4. Germination percentage of Salad Red Bowl cultivar



Fig. 5. Germination percentage of the Asparagina lettuce cultivar

Seed germination of different lettuce cultivars exhibits a differential sensitivity to salinity stress (Fig. 1-5.), enabling a selection of more tolerant varieties at this critical early developmental stage. Mainly similar results were published for bean (Jeannette *et al.*, 2002), cabbage (Ashraf and McNeilly, 1990), safflower cultivars (Demir and Aril, 2003). From the examined lettuce varieties, Salad Red Bowl and Paris Island showed the less sensitivity of germination to increased salinity. On the other hand, Asparagina exhibited the most pronounced inhibition of germination under salt stress conditions, which is in agreement with the fresh biomass measurements. We can conclude that in more tolerant lettuce cultivars high salinity only delays seed germination without significantly affecting the total germination percentage, while in sensitive cultivars emergence of embryos is inhibited by salt stress.

The impact of salt stress on biomass production

Salt stress inhibits plant growth by perturbing the osmotic and ionic balance of young cells. Influence on biomass accumulation is directly related with influence on productivity of crop plants. This is especially valid for lettuce, where the vegetative shoot is used in alimentation. The high amount of NaCl in the nutrient solution reduced significantly the growth rate of vegetative shoot (stem and leaves) fresh weight in all five lettuce cultivars (Fig. 6.).



Fig. 6. Effect of 50 mM and 100 mM NaCl on the shoot fresh weight (FW) of five lettuce cultivars. Data are means of five plants \pm SE. Different letters indicate significant differences according to the Tukey test (P < 0.05).



Fig. 7. Effect of 50 mM and 100 mM NaCl on the root fresh weight (FW) of five lettuce cultivars. Data are means of five plants \pm SE. Different letters indicate significant differences according to the Tukey test (P < 0.05).

The cultivar Aspargina suffered the most pronounced shoot growth reduction at both salinity levels. This indicates that from among the five cultivars that were investigated, this is the most sensitive one to salt stress in terms of biomass production. In contrast, 50 mM NaCl had no significant effect on shoot growth of the Paris Island cultivar, which may be considered a more tolerant one.

Root growth was less affected by salinity than leaf growth (Fig. 7.), and root elongation rate recovered remarkably well after several days of exposure to NaCl. This can be related to the osmotic effect of salt stress, the impaired water supply stimulating root growth towards new water sources.

Our results reveal that biomass is a good and easily measurable indicator of salt sensitivity of lettuce cultivars.

Hydraulic conductance as a physiological indicator of salt stress tolerance

In terrestrial vascular plants, water transport across root tissues is a fundamental process that must be regulated efficiently under different environmental conditions, in order to allow plants to survive in a properly hydrated state. The values of root hydraulic conductance (L_0) are shown in Fig. 5.8. The most pronounced reduction was recorded in the Asparagina cultivar, for both NaCl concentrations (50

mM and 100 mM). In the case of Paris Island cultivar, both salt concentrations exerted the same degree of reduction in root hydraulic conductance.



Fig. 8. Root hydraulic conductance (L_0) of five lettuce cultivars exposed to salt stress. Each value is the mean of five samples ± SE. Different letters indicate significant differences according to the Tukey test (P < 0.05).

The reduction of the xylem sap movement from root to shoot can be beneficial for the plant, because it prevents the accumulation of toxic levels of Na⁺ and Cl⁻ ions in the leaves. This means that the plants that exhibit a more reduced hydraulic conductivity upon salt stress ensure a better protection to the leaves against ion toxicity, being more salt tolerant. On the other hand, a high shoot Na⁺ content may be beneficial by helping the plant to maintain turgor. A balance probably needs to be established between the use of Na⁺ and Cl⁻ by the plant to maintain turgor and the need to avoid their chemical toxicity (Munns and Tester, 2008). Because even under milder salinity conditions exerted by 50 mM NaCl, root hydraulic conductance decreased in a great extent, it can be considered a good physiological marker of salt stress in lettuce. Paris Island cultivar maintained a higher hydraulic conductance, which confers a more pronounced salt tolerance by enabling a better water supply of the leaves from the root.

Stomatal conductance and gas exchange of lettuce leaves under salinity

Terrestrial vascular plants regulate their gas exchange with the atmosphere through the stomatal movement. This is a crucial process both for acquisition of carbon dioxide for photosynthetic biomass production, and for the control of transpiration in the frame of water relations. The stomatal conductance (Gs) of lettuce leaves decreased when the plants were subjected to salt stress. The most significant difference from control was observed, with both salt treatments, in the case of Asparagina and Salad Bowl Red cultivars. In the Paris Island cultivar stomatal conductance decreased significantly only upon exposure to 100 mM NaCl. From all cultivars, at the 50 mM salt regime, the smallest reduction was observed in Paris Island (Fig. 9.).



Fig. 9. Stomatal conductance on leaves of five lettuce cultivars exposed to different degrees of salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

Salinity also decreased the transpiration rate, and the tendency was the same like in case of stomatal conductance (Gs). These two parameters are related to each other, as transpiration is part of the leaf gas exchange influenced by stomatal opening or closure. The most pronounced reduction in transpiration rate of leaves exposed to salinity was observed also in the Asparagina cultivar (Fig. 10.).



Fig. 10. Transpiration rate on leaves of five lettuce cultivars exposed to different degrees of salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

As a reaction to the osmotic component of salt stress, stomata tend to close in order to reduce water loss by transpiration. This impairs photosynthetic carbon uptake and a balance has to be established between water loss and carbon dioxide supply. This is achieved by regulation of stomatal movements and of gas exchange intensity. Because stomatal responses are induced by the osmotic effect of salt (Munns and Tester, 2008), it can be concluded that Paris Island has a better osmotic tolerance than the other lettuce cultivars. Ten days of treatment with 100 mM of NaCl in hydroponic cultures are probably not enough for developing the toxic effect of salt in the five lettuce cultivars used in our experiments, this is why no visible damage or premature senescence of leaves and toxicity symptoms (chlorosis, necrosis) could be detected.

Salt stress influence on shoot, root and xylem sap mineral content

An excess of NaCl can cause disorders in plant mineral nutrition with respect to nutrient availability, uptake, transport or partitioning within the plant. Different plants exhibit various mechanisms of salt exclusion, translocation, sequestration, bioaccumulation and detoxification, in order to maintain an adequate ion homeostasis needed for the normal metabolic processes in the different cell compartments. Figs. 11, 12 and 13 summarize the Na⁺, Ca^{2+} and K⁺ ion content of the shoot, root and xylem sap of five lettuce cultivars exposed to different degrees of salt stress.

All lettuce cultivars growth in saline conditions showed, in shoots as well as in roots, an increase in Na⁺ concentration. In the shoot, the highest amount of Na⁺ was found in the mostly salt tolerant lettuce cultivar (Paris Island), and the lowest Na⁺ concentration was determined in the most sensitive one (Asparagina). This reflects that from the two main strategies of salt stress tolerance, i. e. salt exclusion and salt sequestration, the latter one is used by lettuce cultivars. In the first phase of the salt stress the rapidly accumulating Na⁺ is an energetically cheap osmolite in the leaf vacuoles for the adjustment of cell turgor, and ultimately of tissue growth under the hyperosmotic stress condition imposed by salinity (Munns and Tester, 2008; Shabala et al., 2010). Figs. 12 and 13 show the reduction of potassium content in both shoots and roots as a result of salt stress, and this reduction is most probably due to the competition of Na⁺ for the same cation transporters (Alberico and Cramer, 1993; Azevedo-Neto and Tabosa, 2000). We have found no correlation between K⁺ content in shoots and roots and the salt tolerance of the examined lettuce cultivars. Under the influence of 100 mM NaCl, Ca²⁺ concentration was reduced both in shoots and roots. Ca²⁺ content dropped to the approximately same value in all cultivars, except for Valdor. No correlation could be established between calcium ion content and salt stress tolerance of the different lettuce cultivars.

In the xylem sap there was no significant difference in Na^+ concentration between salt sensitive and tolerant cultivars, however by contrasting to the shoot and root, K⁺ and Ca²⁺ content of the xylem sap increased in lettuce plants exposed to salt stress (Fig. 11.).



Fig. 11. K⁺, Ca²⁺ and Na⁺ content of xylem sap in five lettuce cultivars exposed to 50 mM and 100 mM NaCl. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05)





Fig. 12. K⁺, Ca²⁺ and Na⁺ content of shoots in five lettuce cultivars exposed to 50 mM and 100 mM NaCl. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05)



Fig. 13. K⁺, Ca²⁺ and Na⁺ content of root in five lettuce cultivars exposed to 50 mM and 100 mM NaCl. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05)

The influence of high salinity on free proline and soluble sugar concentrations in lettuce leaves

One of the most widespread osmoprotectants in vascular plants is proline. Free proline level of the leaves was increased by both salt regimes in all five lettuce cultivars. A significant difference between proline concentration of different cultivars was observed only upon exposure to 100 mM of NaCl (Fig. 14.).



Fig. 14. Free proline content of five lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

The highest proline content was determined in the Paris Island cultivar exposed to 100 mM NaCl, where free proline concentration was about twenty times higher than in control plants. In the other cultivars treated with 100 mM NaCl, this increase was smaller: threefold for Valdor, fourfold for Asparagina and Valdor, and sevenfold for the leaves of the Salad Bowl Red cultivar.

High concentrations of Na⁺ in the cytosol can be toxic for the plant cells, therefore sodium ions are sequestered in the vacuole. For balancing the osmotic potential of the concentrated vacuole sap, plants accumulate in the cytosol and in different organelles organic solutes that are compatible with metabolic activity. The higher proline accumulation of Paris Island cultivar can be considered a biochemical indicator of its better salt tolerance.

From the various organic osmolytes, sugars can contribute up to 50% of the total osmotic potential in case of some glycophytes subjected to saline conditions

(Cram, 1976). In our experiment we found no correlation between soluble sugars content of lettuce leaves and salt treatment (Fig. 15.).



Fig. 15. Soluble sugar content of five lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

Considerable variations in the accumulation of soluble sugars in response to salt stress are obvious at both interspecific and intraspecific levels and even among lines all of which are salt tolerant (Ahsraf and Fatima, 1995; Ahsraf 1990). Information regarding the role of sugars in adaptation of plants to salinity is, therefore, insufficient to conclude that they are universally associated with salt tolerance. In case of the five lettuce cultivars investigated by us, we can conclude that soluble sugars have no important role in osmotic adjustment.

Salt stress influence on leaf tissue and xylem sap osmotic potential

Many important physiological and morphological processes, such as leaf enlargement, stomatal opening, and associated leaf photosynthesis are directly affected by the reduction of leaf osmotic potential which accompanies the loss of water from leaf tissue (Jones and Turner, 1978). The results of leaf osmotic potential show that salt stress significantly increased (towards more negative values) the leaf sap osmotic potential of all cultivars. According to our measurements, Valdor and Parella Green had lower leaf osmotic potential than the other three cultivars (Fig. 16).



Fig. 16. Leaf tissue osmotic potential of five lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

Osmotic potential of xylem sap was more affected by salt stress than leaf osmotic potential. The most pronounced reduction was registered in the Asparagina cultivar, where 100 mM of NaCl treatment decreased 4 fold the xylem sap concentration. Paris Island in contrast showed just a threefold decrease, which was the smallest from all lettuce cultivars examined (Fig. 17).



Fig. 17. Xylem sap osmotic potential of five lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

We can conclude that the osmotic potential of leaf tissues varies among lettuce cultivars irrespective of salinity, while the osmotic potential of the xylem sap is more constant under control conditions, but decreases with different degrees in the cultivars that are more or less salt tolerant. In this context, measurement of xylem sap osmotic potential is more useful that determining osmotic potential of leaf tissues, if the purpose is to detect differences between cultivars with respect to salt stress sensitivity.

Effect of salinity on photosynthetically active pigments concentration

In Asparagina, Salad Bowl Red and Parella Green, a significant decrement in chlorophyll *a* content was detected in the presence of 100 mM NaCl. Interestingly, under the same salt treatment, Paris Island exhibited a moderate increment of chlorophyll *a* content. In case of Valdor, no significant difference was detected between the control and plants exposed to salt stress (Fig. 18).

Fig.18. Chlorophyll *a* content of five lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

Salt stress affected chlorophyll *b* content in a smaller extent as compared with chlorophyll *a*, except for the Parella Green cultivar, the only lettuce cultivar where a significant decrease was detected in plants exposed to salt stress. Salad Bowl Red

lettuce cultivar exposed to salt stress showed a moderate increase in chlorophyll *b* content, while chlorophyll *a* content decreased under the same condition (Fig. 19).

Fig. 19. Chlorophyll b content of five lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

The decrease of chlorophyll content may be due to an enhancement of chlorophyll degradation or to an inhibition of chlorophyll synthesis. Many reports pointed out that chlorophyll content decreased in response to increasing salt stress. (Tort and Turkyilmaz, 2004; Siler *et al.*, 2007). Our results with lettuce cultivars were generally similar, excepting that in the case of Paris Island and Valdor cultivars salt stress treatment had no significant effect on the amount of photosynthetically active pigments, which relates to a lower sensitivity of the light-harvesting antennae of these lettuce cultivars toward salt stress.

Influence of NaCl on parameters of the induced chlorophyll fluorescence in intact lettuce leaves

As induced chlorophyll fluorescence is a very sensitive, non-destructive *in situ* method for assessment of environmental stress effects on plants, the different parameters of the conventional and pulse amplification modulated chlorophyll

fluorescence are reliable physiological indicators of the site and mode of action of different stress factors that impair the primary energetic processes of photosynthesis.

Was measured the ground fluorescence (F0), the maximal fluorescence (Fm), the modulated maximal fluorescence (Fm'), the steady-state fluorescence (Fs), the vitality index or relative fluorescence decay (Rfd = (Fm-Fs)/Fs) and the two most important energetic indicators of the light phase of photosynthesis: the potential light use efficiency (Fv/Fm) and the effective quantum yield efficiency (Φ PSII) (Tab. 1.).

In the case of the five lettuce cultivars exposed to two different salt stress conditions, there were no significant changes in any of the measured induced chlorophyll fluorescence parameters. Application of 10 days of salt stress treatment with up to 100 mM of NaCl for lettuce cultivars has no damaging effect to the function of photosynthetic apparatus, this being resistant to the osmotic component of high salinity. This can be an advantageous characteristic of lettuce, because upon a few days of salt exposure the energetic efficiency of photochemical reactions is not diminished, so photosynthetic production can compensate for other functional damages. Because stress resistance, in contrast with tolerance, is an inherited feature, it is expected that salt resistance of the photosynthetic apparatus of leaves is valid for all lettuce cultivars.

 Tab. 1: Parameters of the induced chlorophyll fluorescence in intact leaves of different lettuce cultivars exposed to different salt concentrations (for abbreviations see the Material and methods section)

Cultivars	Fs	Fm'	Φ PSII	Fo	Fm	Fv/Fm	Fm/Fo	(Fm-Fs)/Fs
Asparagina								
Ø (control)	549,8	1354,8	0,59	327,4	2148,0	0,85	6,56	2,90
50mM NaCl	586,0	1488,4	0,61	295,0	1976,2	0,85	6,69	2,37
100mM NaCl	461,0	1252,6	0,63	262,6	1751,0	0,85	6,66	2,79
Valdor								
Ø	576,0	1257,8	0,54	234,0	1546,4	0,85	6,60	1,68
50mM NaCl	482,2	1190,8	0,59	258,6	1750,8	0,85	6,77	2,63
100mM NaCl	559,5	1353,2	0,59	267,2	1697,5	0,84	6,35	2,03
Salad Bowl Red								
Ø	428,8	1033,6	0,59	219,2	1360,8	0,84	6,20	2,17
50mM NaCl	397,8	927,2	0,57	193,0	1301,6	0,85	6,74	2,27
100mM NaCl	398,6	930,8	0,57	199,5	1241,1	0,83	6,22	2,11
Paris Island								
Ø	534,3	1547,0	0,66	182,0	1418,3	0,88	7,79	1,65
50mM NaCl	489,4	1435,6	0,66	172,7	1346,2	0,88	7,79	1,75
100mM NaCl	501,2	1460,8	0,66	239,2	1741,0	0,86	7,27	2,47
Parella Green								
Ø	887,2	1744,6	0,49	337,	1974,8	0,83	5,85	1,22
50mM NaCl	681,8	1571,0	0,56	286,4	1797,0	0,84	6,27	1,63
100mM NaCl	699,0	1555,0	0,55	412,8	2222,4	0,81	5,38	2,17

The level of membrane lipid peroxidation in lettuce leaves exposed to salt stress

Asparagina and Paris Island, the two mostly differing lettuce cultivars with respect to salt stress tolerance, were chosen for establishing the level of unsaturated fatty acid peroxidation in membrane lipids of leaves (Fig 20.).

Fig. 20. Lipid peroxidation in leaves of two lettuce cultivars exposed to salt stress. Bars represent means \pm SE, n = 5. Different letters indicate significant differences according to the Tukey test (P < 0.05).

Although the level of lipid peroxidation increased in both lettuce cultivars exposed to salt stress, the rate of the increment was very similar, so this parameter cannot be used as a good biochemical marker of the degree of salt stress tolerance in different lettuce cultivars. This reflects that membrane lipid peroxidation, as a result of the oxidative side-effect of salt stress, is rather a general manifestation in all lettuce cultivars, and its degree depends on the concentration of salt and on the duration of exposure, but it does not vary significantly within intraspecific lettuce varieties. Lipid peroxidation is suited for revealing the degree of salt stress, but not for distinguishing among lettuce varieties with respect to salt sensitivity.

CONCLUSIONS

From among the investigated lettuce cultivars, Paris Island exhibited the highest tolerance to salt stress exerted by 50 mM and 100 mM NaCl in hydroponic cultures, while Asparagina was the most sensitive one to high salinity. This is supported by better growth parameters, by a smaller decrease of the root hydraulic conductance and of the leaf stomatal conductance, as well as by a much higher proline content of the Paris Island cultivar exposed to salt stress. We recommend Paris Island for cultivation in areas with increasing salinity.

In all of the investigated lettuce cultivars, after ten days of exposure to 100 mM NaCl in hydroponic conditions, the osmotic effect of salt stress still dominates, instead of the previously expected ionic toxicity.

Na⁺ exclusion is not the main mechanism for salinity tolerance in lettuce cultivars. Instead, salt sequestration is used for antistress defense. This was not known previously for salt-sensitive crop plants.

Potassium and calcium ion content decreases in the shoots and roots of saltaffected lettuce plants, while the amount of these mineral nutrients increases in the xylem sap. This is also a new finding of our studies, not being reported elsewhere in the literature.

Root hydraulic conductance is a good physiological indicator of salt stress tolerance in lettuce cultivars, whereas increment of free proline content is a suitable biochemical marker of it.

Seed germination is a good physiological marker for early detection of salt tolerant lettuce cultivars, it can be useful for a preliminary screening of more sensitive and more tolerant varieties, as well as for the establishment of salt concentration range tolerated by the different lettuce cultivars. But the cultivars with a better germination rate are not necessarily those that perform a better biomass production in later developmental stages.

The soluble sugar content of leaves did not change upon exposure to salinity, reflecting that lettuce does not use simple sugars for osmoregulation.

The degree of unsaturated fatty acid peroxidation in membrane lipids is not proportional with the extent of salinity, indicating that in lettuce the oxidative sideeffect of salt stress is less prominent than in many other plants investigated so far for salinity tolerance.

Decrease in stomatal conductance correlates with sensitivity to salt stress, while photosynthetic light use efficiency is highly resistant to shorter exposure to increased salinity.

Our findings contribute to a better understanding of salt stress reactions in non-halophytes, and offer to cultivators some new tools for the selection of cultivars with different degrees of salinity tolerance.

Further investigations may reveal a possible application of salt tolerant lettuce cultivars in phytoremediation of saline soils, as well as in bioassays for salinity level. The Paris Island lettuce cultivar can be recommended for cultivation in areas affected by increased salinity, even though further field experiments should complete those undertaken in the present study.

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- Sesiunea Științifică "Actualități în biologia vegetală", ediția XI, Cluj-Napoca,
 9-10 mai 2003 (Fodorpataki L., Bartha Cs., Demeter J.Sz., Turoczy Z.: Studiul toleranței față de stresul oxidativ în culturi de celule algale, pp. 36-37)
- International Conference of Romanian Society of Biochemistry and Molecular Biology, Cluj-Napoca, 30. IX.-3. X. 2009 (Bartha Cs., Fodorpataki L., Popescu, O.: Antioxidants in two lettuce varieties exposed to salt stress)
- A 28-a Sesiune Stiintifica a SRBC, Constanta, 9-12 VI. 2010 (Keresztes Zs. Gy., Nagy E., Bartha Cs., Barna Sz., Fodorpataki L.: Modificari fiziologice si biochimice in celule algale, ca bioindicatori ai calitatii mediului acvatic)
- Second International Conference on Phycology, Limnology and Aquatic Sciences, Port Said (Egypt), 14-16 Febr. 2010, (Fodorpataki L., Keresztes Zs.Gy., Bartha Cs.: Bioindication of water pollution using molecular and physiological parameters of the green alga *Scenedesmus opoliensis* P. Richter)

- 15th International Congress of Photosynthesis, Beijing (China), 22-27. VIII.
 2010 (Bartha Cs., Fodorpataki L., Keresztes Zs. Gy., Szekely Gy., Popescu,
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- European Biotechnology Congress 2011, Istanbul (Turkey), 28. IX.-02. X. 2011 (Fodorpataki L., Keresztes Zs.Gy., **Bartha Cs.**, Marton A.L., Barna Sz.: Suitability of a green microalgal strain for wastewater bioremediation and biomass production)

PARTICIPATION IN RESEARCH PROJECTS

- "Comparative study of the ecophysiological basis of halotolerance and of acclimation to various light regimes in different crop plant species", project financed by the Sapientia Research Programmes Institute
- "Molecular phylogeny of picoalgae from Romanian salt lakes", financed by CNCSIS (Project TE 306

ACKNOWLEDGEMENTS

First and foremost I want to thank my supervisor dr. Fodorpataki László for his contributions of time, encouragement and lots of good ideas throughout my research activities and the thesis-writing period.

I also express my sincere gratitude to Dr. Micaela Carvajal Alcaraz, Dr. Carmen M. Martínez Ballesta, and for all members of the Aquaporine Group from CEBAS, for giving me the possibility to work in their laboratories in Murcia (Spain).

I am thankful to Dr. Octavian Popescu for giving me the possibility for being his Ph.D. student and working in his laboratory. Furthermore, I thank dr. Székely Gyöngyi for her support in conducting proline content determinations, as well as for the financial support offered in the frame of a research project coordinated by her.

I would like to thank to my colleagues, Keresztes Zsolt Gyula, Marosi Béla and Bücs Szilárd for their technical and moral support during the experiments. At last, but not at least, I am thankful to my wife for her continuous support and understanding during these years.

This Ph. D. programme was co-financed by the Sectorial Operational Program "Human Resources Development, Contract POSDRU 6/1.5/S/3 – Doctoral studies: through science toward society".