Journal of Central European Agriculture, 2014, 15(3), p.219-235 DOI: 10.5513/JCEA01/15.3.1478

Salt-mediated changes in leaf mesophyll cells of *Lycopersicon esculentum* Mill. plants

Zmiany w komórkach mezofilu liści roślin *Lycopersicon esculentum* Mill. spowodowane zasoleniem

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Abstract

Five-week-old tomato plants (*Lycopersicon esculentum)* cv. Perkoz grown in pots containing garden soil in a growth chamber were submitted to 50 or 150 mM NaCl for 1 h, 2 and 5 days. Tomato leaf anatomy generally did not change after short time salinity, except 5-day-treatment with 150 mM NaCl, where changed cell shape (shrunk and deformed) simultaneously with increased volume of intercellular spaces (IS) were observed. Although leaf hydration (H) depleted only 1 h after 150 mM NaCl treatment both salt concentrations generated two coexisting populations of salt-affected mesophyll cells: (*i*) slightly-affected (Sl-A) which showed incipient plasmolysis or slightly changed shapes, and (*ii*) severely-affected (Sv-A) which showed severe plasmolysis; serious deformation of cell shape or disorganization including cell degeneration. In Sl-A cells salinity changed location and shape of chloroplasts which were: more rounded, with oversized starch grains (SG) (2d) or more flat (5d). Salt-mediated changes were becoming more distinguished and pronounced with length of 150 mM NaCl treatment. The amount of salt-affected cells was changing during the experiment and depended on the salt concentration. In 50 mM-treated plants salt-affected cells appeared 1 h after treatment (~40%) and raised up to 78% on 2nd day, however the population of Sl-A cells dominated. In 150 mM NaCl-treated plants the percentage of affected cells raised during the experiment from 75% to 99%. Firstly Sl-A cells dominated, but on the 5th day the majority was Sv-A. Salt-affected cells were distributed quite evenly in palisade or spongy mesophyll, except 2 d after treatment with 50 mM NaCl, when their number was higher in the palisade mesophyll. Sv-A cells in the spongy mesophyll were located mostly near the bundle while in the palisade mesophyll more irregularly. Different susceptibility of cells to salt stress might be the consequence of an unequal distribution of osmotic stress and subsequent ionic stress or physiological state of cells.

Keywords: leaf hydration, mesophyll cells, osmotic stress, plasmolysis, salt stress, tomato

Streszczenie

5-tygodniowe rośliny pomidora (*Lycopersicon esculentum*) odm. Perkoz, które rosły w doniczkach z ziemią ogrodniczą w komorze hodowlanej zostały poddane działaniu NaCl o stężeniu 50 mM lub 150 mM przez okres 1 godz., 2 i 5 dni. Krótkotrwałe zasolenie nie wywołało zmian anatomicznych w liściach pomidora, za wyjątkiem zmian kształtu komórek (obkurczenie i deformacje) i towarzyszącemu im zwiększeniu powierzchni przestrzeni międzykomórkowych, co obserwowano po 5 dniach od traktowania roślin NaCl w stężeniu 150 mM. Pomimo, iż istotny spadek uwodnienia liści odnotowano jedynie po godzinie od podania 150 mM NaCl, oba zastosowane stężenia generowały powstawanie w mezofilu dwóch grup komórek: (*i*) lekko zmienionych (LZ) - w początkowych stadiach plazmolizy lub o delikatnie zmienionych kształtach oraz (*ii*) mocno zmienionych (MZ) - z zaawansowaną plazmolizą, silnie zdeformowanych lub wykazujących drastyczne zmiany w organizacji protoplastu, aż do degeneracji. W komórkach LZ zasolenie zmieniało rozmieszczenie oraz kształt chloroplastów, które były bardziej owalne na skutek przerostu ziaren skrobi (2 dz) lub mocno spłaszczone (5dz). Obserwowane zmiany były bardziej intensywne po 150 mM NaCl i nasilały się w czasie. Także ilość zmienionych komórek była zależna od stężenia. W roślinach poddanych działaniu 50 mM NaCl komórki te pojawiały się po 1 godz. (~40%), a po 2 dniach ich ilość wzrosła do 78%, przy czym dominowały komórki LZ. W roślinach poddanych działaniu 150 mM NaCl procentowy udział komórek zmienionych wzrastał w czasie eksperymentu z 75% do 99%. Początkowo dominowały komórki LZ, ale 5-tego dnia większość stanowiły komórki MZ. Zmienione komórki występowały w miękiszu palisadowym i gąbczastym w podobnej ilości, za wyjątkiem 2 dnia traktowania 50 mM NaCl, kiedy to ich liczba była większa w miękiszu palisadowym. W miękiszu gąbczastym komórki MZ były zlokalizowane głównie w okolicy wiązek przewodzących, zaś w miękiszu palisadowym były rozmieszczone w sposób bardziej nieregularny. Wydaje się, że różna wrażliwość komórek mezofilu na stres solny była skutkiem nierównomiernego rozmieszczenia stresu osmotycznego, a potem rozprzestrzeniania się toksycznych jonów, lub wynikała z różnego stanu fizjologicznego poszczególnych komórek.

Słowa kluczowe: komórki mezofilu, plazmoliza, pomidor, stres osmotyczny, stres solny, uwodnienie liści

Introduction

Salinity is one of the common environmental factors that cause reduction of plant productivity in arid and semi-arid regions. All over the world more than 6% of the total land area (800 million ha) and nearly 20% of the irrigated land have been salt-affected (FAO, 2008). In spite of the fact that irrigated areas constitute only 15% of the total cropped land areas, they produce one-third of the foods. Additionally, due to the increasing shortage of water, salinity may become a major problem also in other climatic zones (Ivitis et al., 2013). According to European Soil Portal - Soil Data and Information System in European Union (EU) countries 1-3 million hectares are salinized (Panagos et al., 2012).

Even short-term salinity might have serious negative effect on plants (Hernandez and Almansa, 2002), because both Na^+ and Cl^- ions are highly water soluble, easily taken up and transported to shoots (Silva et al., 2008; Snoussi et al., 2005). Salt decreases osmotic potential of a soil solution and causes the “physiological drought” therefore

the osmotic effect of salinity called “osmotic phase” is observed in cells as the first symptom (Munns, 2002). The loss of protoplasmic water leads to the concentration of ions. Salt-mediated water imbalance in plants which appears during the first hours can cause shrinkage of vacuoles and protoplasts thus leading to plasmolysis. Plasmolysed cells can regain their volume when plants adjust to a new water status which usually takes a few days: however, de-plasmolysis in cells in which a protoplast separates from a cell wall is irreversible (Bennici and Tani, 2009). In a leaf at osmotic shock, the phenomenon of dehydration in which a plasma membrane and cell wall remain in contact known as “cytorrhysis” exists (Carpita et al., 1979). The protoplasts shrink and because in such a state the liquids have very high viscosity, the protoplast often pulls cell walls which changes the cell shape and may even result in cell wall collapse. It is irreversible and leads to cell death.

Continued transport of salt to the leaves results in salt-specific effects. Cl^- and Na^+ at high tissue concentrations effectively diminish or inhibit metabolic functions (Debouba et al., 2006; James et al., 2006; Tarchoune et al., 2012). At the beginning accumulation of ions takes place in the vacuoles (James et al., 2006), subsequently they are building-up in cell walls or in the cytoplasm which results in cell death because of dehydration or poisoning, respectively (Munns, 2002).

It has been reported that salinity-induced injury of leaf depends on the age of an organ (De Lacerda et al., 2006; Mitsuya et al., 2003), but the differences among cells of the same tissue of the same plant have been poorly studied. Therefore, the aim of this study was to investigate the effect of two NaCl concentrations on tomato leaf parenchyma cells, with regard to cell disturbance degree and localization of salt-affected cells in palisade and spongy mesophyll which provide better knowledge of mechanism of salt injury. The possible explanations of different cell reactions during the short-salinity stress period have been discussed.

Material and Methods

Plant material and treatment

Tomato plants (*Lycopersicon esculentum* Mill.) cv. Perkoz were grown in pots containing garden soil in a growth chamber under 350 $\mu mol*m^{-2}*s^{-1}$ light intensity, with 16/8 h day/night cycles, at 23°C. Five-week-old tomato plants were subjected to 50 mM or 150 mM solutions of NaCl (moderate or severe stress, respectively) imposed in a single dose (30 ml per pot). The control plants were watered with tap water. First re-watering of plants took place 24 hours after salt application and then they were watered every day. The salt-induced changes in mesophyll cells of the 3rd (from the bottom), young but fully expanded leaf, as well as its hydration (H) were investigated at three points of time after salt application which represent: osmotic shock phase (1 h), osmotic adjusting phase (2 d) and osmotic adjustment/salt-specific effect phase (5 d).

Leaf hydration (H)

The leaf H was calculated using the equation:

$H = (FW - DW) * DW^{-1}$ (Munné-Bosch and Alegre, 2002) where FW - fresh weight of leaf; DW - dry weight of leaf after drying at 80˚C for 2 days. The results are expressed in grams of H_2O per gram of DW.

Light microscopy (LM)

Ten small samples of the 3rd leaves of the control and salt-treated plants were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.8, at 0-4 °C for 4 h. After washing in the buffer and post-fixation with 1% osmium tetraoxide they were dehydrated in the graded ethanol series and embedded in Epon-Spurr resin (Glińska and Gapińska, 2013).

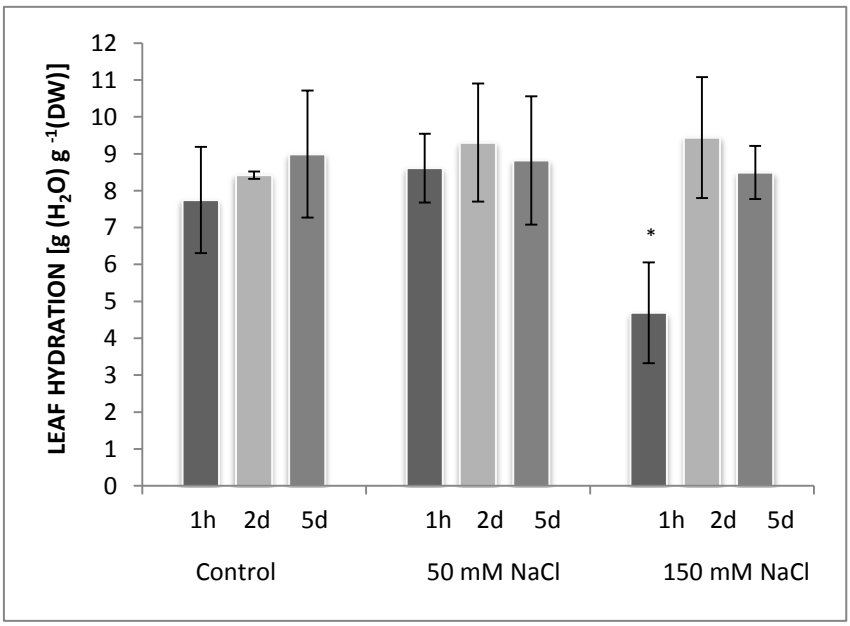
Semi-thin sections (1 μm) were cut with a glass knife on ultramicrotome (*Ultracut, Reichert-Jung*, Germany), stained with 1% toluidine blue and analyzed with light microscope (*Eclipse 50i, Nicon*, Japan) equipped with camera (*Power Shot digital 640, Canon*, Japan). The organization of 400-700 mesophyll cells from 5 cross-sections was examined for each treatment. The magnification was 400 x plus digital zoom.

Statistical analysis

For the light microscopy analysis a minimum of three samples were examined for every salt treatment from each of two series of experiments. The leaf H result represents the mean of 3 independent experiments. Sample variability is given as the standard deviation (SD) of the means. The significance of differences was determined by the Student`s t-Test. Differences at $P<0.05$ were considered significant.

Results

Leaf hydration (H)



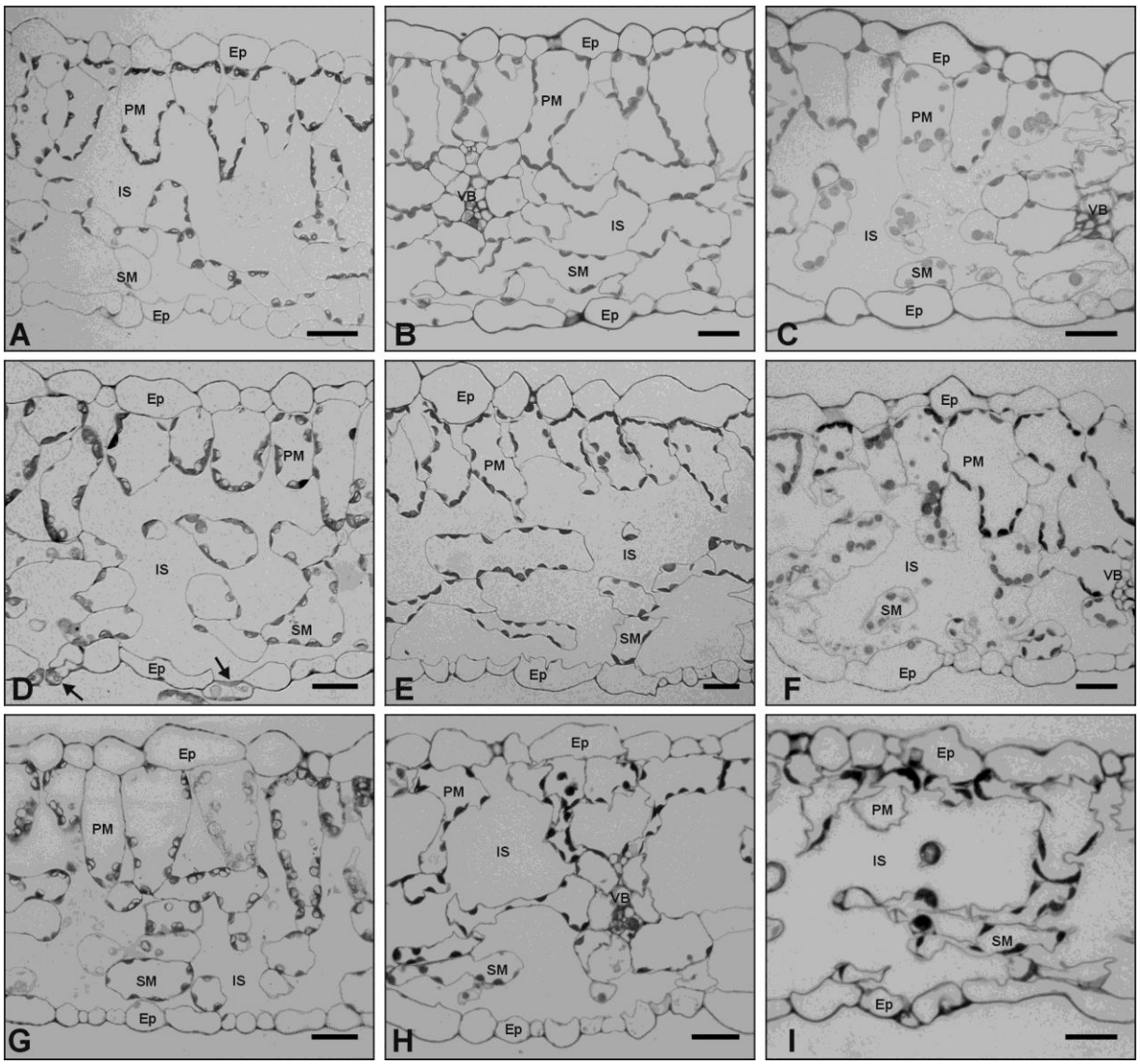
Means ± SD, n=4. Statistically significant differences between the control and salt treatment *- $P<0.05$.

Figure 1. Influence of 50 mM and 150 mM NaCl on tomato (*L. esculentum)* leaf hydration (H) 1 h, 2 and 5 days after salt application.

The intensity of the osmotic stress caused by the moderate (50 mM NaCl) or severe (150 mM NaCl) salt stress was estimated in the tomato plant leaf and was expressed as the leaf hydration (H). The results showed that in the plants grown

under the moderate salt stress, statistically significant changes in leaf H were not observed (Fig. 1). In the plants under the severe stress leaf H was depleted (by 40%) in comparison to the control plants after 1 hour, but subsequently (2 d, 5 d) it returned to the control level (112% and 94% of the control, respectively).

Leaf anatomy



Scale bars=20 μm. Ep - epidermis; PM - palisade mesophyll; SM - spongy mesophyll; VB - vascular bundle.

Figure. 2. Semi-thin cross-sections of control *L. esculentum* leaves and after treatment with NaCl: *A*) control - 2 d; *B*) 50 mM NaCl - 1 h; *C-D*) 50 mM NaCl - 2 d; *E*) 50 mM NaCl - 5 d; *F*) 150 mM NaCl - 1 h; *G*) 150 mM NaCl - 2 d; *H-I*) 150 mM NaCl - 5 d.

The tomato leaf consisted of slightly thicker upper epidermis (Ep) coated with thin layer of cuticle, mesophyll consisting of 1 layer of palisade mesophyll (PM) and usually 3 to 4 layers of spongy mesophyll (SM) in which vascular bundles (VB) were

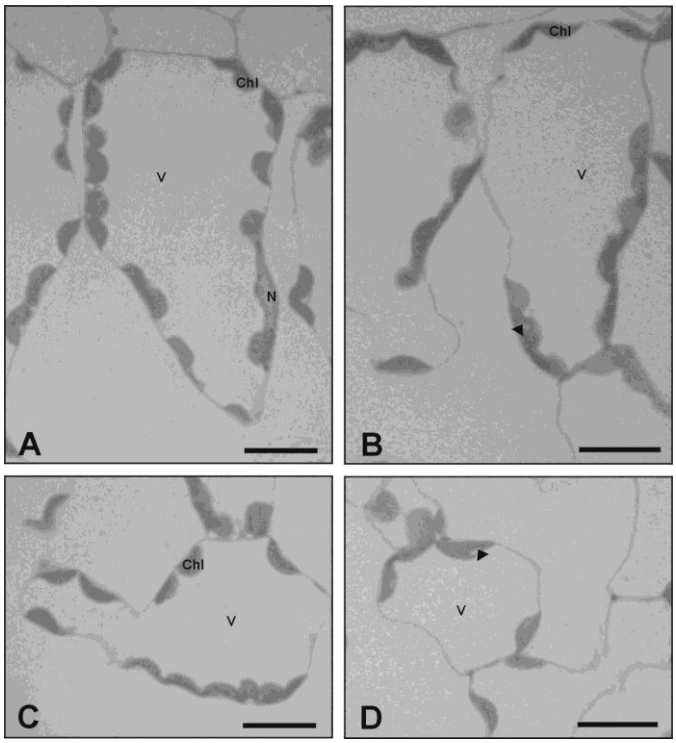
located, as well as lower Ep (Fig. 2). The stomata were distributed on both sides of the leaf blades (Fig. 2B,D). In the control plants the cells in both types of mesophyll, but especially in SM, were not very tightly packed, therefore middle-size intercellular spaces (IS) were formed (Fig. 2A).

Tomato leaf anatomy generally did not change after short time salinity (Fig. 2*B-D, F-G*), except 5-day-treatment with 50 (Fig. 2*E)* or (150 mM NaCl (Fig. 2*H-I*). The salinity brought changes in the cell shape (shrunk and deformed), volume of IS (increased), number of chloroplasts and their location and shape (see text below).

Changes of leaf mesophyll cells

Qualitative changes of mesophyll cells

Both palisade and spongy mesophyll cells of the control plants examined on semi-thin sections by light microscopy generally did not exhibit abnormalities (Fig. 3). PM cells had typical, oblong shape, slightly wider at the end adjacent to the upper Ep (Fig. 3*A-B*). Chloroplasts (Chl) in PM cells were mostly visible near the upper Ep. SM cells were oval or longish with slight weavings, characteristic of mesophyll (Fig. 3*C-D*). A big, transparent central vacuole (V) surrounded by cytoplasm with numerous, elliptical chloroplasts with small starch grains (SG) and an oval or elliptical well organized nucleus (N) could be noticed in mesophyll cells (Fig. 3*A*).



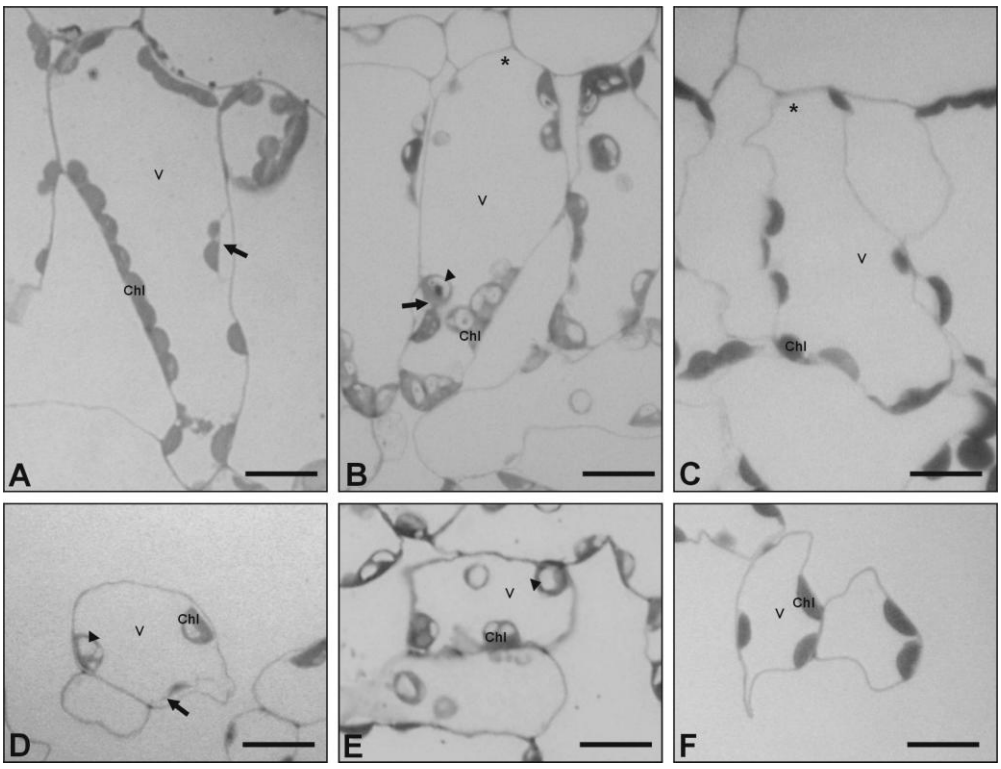
Scale bars=10 μm. Chl - chloroplast; N - nucleus; V- vacuole; black triangle - starch grain.

Figure 3. Semi-thin cross-sections of *L. esculentum* control plant leaves (stained with toluidine blue). *A*, *B*) Typical palisade mesophyll cells and *C*, *D*) spongy mesophyll cells (1 h and 5 d, respectively).

Parenchyma cells were diversely influenced by salinity. Light microscopy of semi-thin sections revealed that after both 50 mM NaCl and 150 mM NaCl treatment beside unaffected cells salt-affected cells appeared, among which two coexisting categories of mesophyll cells could be distinguished: *i*) slightly-affected (Sl-A) (Fig. 4) and *ii*) severely-affected (Sv-A) (Fig. 5).

i) Slightly-affected (Sl-A) cells

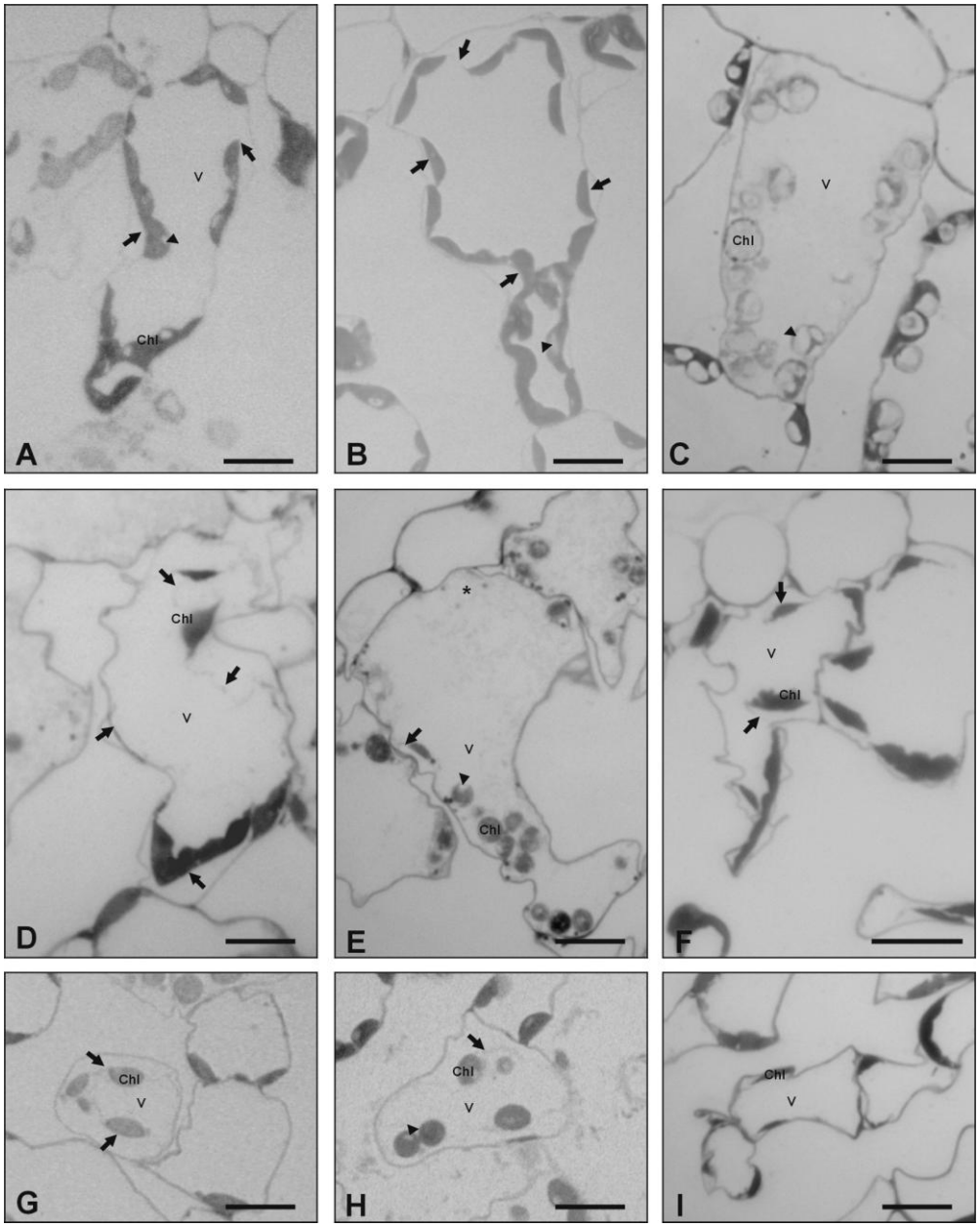
In some cells categorized as Sl-A slight invaginations of plasmalemma (arrows - incipient plasmolysis) appeared (Fig. 4*A*, *B*, *D*) while in the others, delicate reorganization of cell structure not connected with plasmolysis was seen (Fig. 4*B*, *C, E*, *F*). In the latter cells the chloroplasts were shifted. Most of them were located more centrally in the leaf (Fig. 4*B*, *C*) and had reduced contact with plasmalemma (Fig. 4*B*, *E*). Additionally, they had more rounded shape sometimes with oversized SG (Fig. 4*B*, *E* - black triangle). Sl-A cells regardless of NaCl concentration had generally unchanged (Fig. 4*A*, *B*, *D*, *E*) or only slightly changed shape (Fig. 4*C*, *F*) in comparison to the control. Cells with the first phase of plasmolysis appeared just 1 h after treatment while those with reorganized structure from the 2nd day of treatment.



Scale bars=10 μm. Arrow - incipient plasmolysis; black triangle - starch grain; asterisk – lack of chloroplast near upper epidermis; Chl - chloroplast; V- vacuole.

Figure 4. *A-C*) Slightly-affected (Sl-A) palisade mesophyll cells and *D-F*) spongy mesophyll cells in *L. esculentum* plants after treatment with NaCl: *A*) 50 mM NaCl - 1 h; *B*) 50 mM NaCl - 2 d; *C*) 50 mM NaCl - 5 d; *D*) 50 mM NaCl - 1 h; *E*) 50 mM NaCl - 2 d; *F*) 50 mM NaCl - 5 d.

ii) Severely-affected (Sv-A) cells



Scale bars=10 μm. Arrow - plasmolysis; black triangle - starch grain; asterisk - lack of chloroplast near upper epidermis; Chl - chloroplast; V- vacuole.

Figure 5. *A-F*) Severely-affected palisade and *G-I*) spongy mesophyll cells in *L. esculentum* plants after treatment with NaCl: *A*) 50 mM NaCl - 1 h; *B*) 150 mM NaCl - 1 h; *C*) 150 mM NaCl - 2 d; *D-F*) 150 mM NaCl - 5 d; *G*) 150 mM NaCl - 1 h; *H*) 150 mM NaCl - 2 d; *I*) 150 mM NaCl - 5 d.

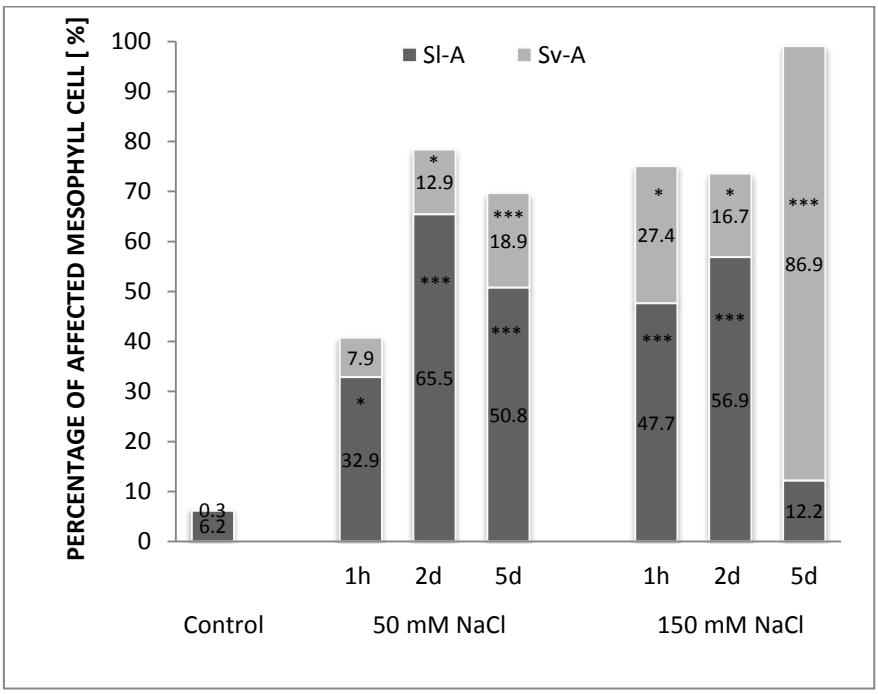
In the cells categorized as Sv-A severe plasmolysis and serious disturbances of cell shape and structure could be noticed (Fig. 5). In plasmolysed cells, vacuoles shrank, plasmalemma in many places lost contact with a cell wall and extensive periplasmic space appeared (Fig. 5*A*, *B*, *D*, *G* - arrows). Additionally, in Sv-A group

some cells were degenerated. In such cells plasmalemma and especially tonoplast were often broken down; swollen, rounded chloroplasts and other organelles seemed to be incorporated into the remnants of a vacuole; cytoplasm showed degeneration (Fig. 5*C*, *E*, *H*) and occasionally even a cell wall collapsed (Fig. 5*H*). Sometimes, especially after higher salt treatment, the shapes of cells were seriously changed (Fig. 5*D*, *E*, *F*, *I*). Because of cell wall invaginations the cell shapes were similar to a puzzle (Fig. 5*E*) and, in drastic situations, the types of mesophyll were hardly recognizable (Fig. 5*F*, *I*). Such cells usually did not show plasmolysis but cell volume was drastically diminished, chloroplasts were very flat and disrupted (Fig. 5*F*, *I*).

Quantitative changes of mesophyll cells

Both palisade and spongy mesophyll cells of the control plants were unaffected (92.7% and 94.2%, respectively), except for a few percent of slightly changed cells localized at the peripheries of the samples (Tab. 1; Fig. 6).

In the salt-treated plants the total number of cells categorized as affected as well as those falling into Sl-A or Sv-A category depended on salt concentration and was changing during the experiment (Fig. 6; Tab. 1).



Sl-A – slightly-affected cells; Sv-A - severely-affected cells. Statistically significant differences between the control and salt treatment (* - $P<0.05$; ** - $P<0.01$; *** - $P<0.001$).

Figure 6. Influence of 50 mM and 150 mM NaCl on the appearance of affected mesophyll cells in *L. esculentum* leaf.

In 50 mM NaCl-treated plants 1 h after treatment, affected cells appeared (39.8%), mostly of Sl-A category (32.9%). On the 2nd day the percentage of affected cells raised up to 78.4%. At that time the population of Sl-A cells dominated (65.5%), however the raise in Sv-A cells was statistically significant (12.9%). On the 5th day

the total number of affected cells slightly diminished (69.7%) compared to 2nd day, which was the effect of the lower number of Sl-A cells (50.6%), but not of Sv-A cells which was 18.9%.

In 150 mM NaCl-treated plants the percentage of affected mesophyll cells raised during the experiment from 75.1% 1 h after treatment to 99.1% on 5th day. Firstly (1 h, 2 d) Sl-A cells dominated (47.7%, 56.9%), but on the 5th day the situation was reverse and Sv-A cells accounted for ~87%.

Localization of affected cells

The affected cells were distributed quite evenly in the palisade or spongy mesophyll, except 2 d after treatment with 50 mM NaCl, when they were significantly more abundant in PM (22.9%) then in SM (6.8%) (Tab. 1). Sv-A cells in SM were located mostly near the vascular bundle (Fig. 2*C*), while in PM more irregularly (Fig. 2*F,G*).

Table 1. Influence of 50 mM and 150 mM NaCl on the amount [%] and distribution of slightly-affected and severely-affected cells between palisade and spongy mesophyll in *L. esculentum* leaf.

Treatment	Unaffected cells [%]		Slightly-affected cells [%]		Severely-affected cells [%]	
	Palisade	Spongy	Palisade	Spongy	Palisade	Spongy
Control	92.7 ± 12.3	94.2 ± 5.6	6.9 ± 11.4	5.6 ± 5.1	0.4 ± 0.8	0.2 ± 0.5
50 mM NaCl						
1 h	55.9 ± 23.6**	60.9 ± 20.0*	31.9 ± 13.3*	33.3 ± 16.6*	12.2 ± 10.4	5.7 ± 3.9*
2 d	16.4 ± 7.7***	24.8 ± 6.5***	60.7 ± 16.4***	68.4 ± 8.8***	22.9 ± 9.0**	6.8 ± 4.7***a**
5 d	26.7 ±13.3***	32.8 ± 11.1***	55.3 ± 12.5***	47.7 ± 7.8***	18.0 ± 0.7***	19.6 ± 3.2**
150 mM NaCl						
1 h	20.4 ± 13.2***	27.4 ± 3.7***	49.0 ± 6.7***	46.9 ± 5.2 ***	30.6 ± 19.7*	25.7 ± 8.0**
2 d	20.6 ± 13.2***	30.0 ± 10.6***	58.0 ± 7.5***	56.2 ± 5.0 ***	21.4 ± 6.7*	13.8 ± 6.2**
5 d	0.0 ± 0.0***	1.5 ± 1.8***	8.4 ± 10.4	14.0 ± 9.5	91.6 ± 10.4***	84.5 ± 11.3***

Means ± SD, n=5. Statistically significant differences between the control and salt treatment (* - $P<0.05$; ** - $P<0.01$;*** - $P<0.001$) and between palisade and spongy mesophyll (a - $P<0.05$).

Discussion

Leaf hydration

Many authors indicated a decrease in water related parameters short after NaCl treatment (Boughalleb et al., 2012; Hernandez and Almansa, 2002; Kholova et al., 2010; Morales et al., 1998; Nandwal et al., 2007; Sairam et al., 2002; Stępień and Kłobus, 2006) and also after long growth in saline conditions (35 mM or 70 mM NaCl) some changes in water balance in *L. esculentum* plants were noticed (Romero-Aranda et al., 2001). We also revealed the depletion of leaf hydration (H) at high salinity in the early phase of the experiment which was correlated with cell plasmolysis observed in light microscopy. On the other hand, plasmolysis of

mesophyll cells categorized as Sv-A was noticed not only in tomato plants grown on the medium supplemented with 150 mM, but also with 50 mM NaCl, while the whole leaf H was not statistically diminished after treatment with the latter salt concentration.

Leaf anatomy

Our research revealed that short-time NaCl treatment did not significantly change leaf anatomy. However, after the longest time of treatment (5 d), enlarged intercellular spaces (IS) and deformations of cell shape were visible in PM and SM as compared to the control plants. Characteristic cell shape deformations (cytorrhysis) were caused not only by salinity (Strogonov, 1964), but also by other stresses connected with osmotic imbalance such as freezing and PEG (Rajashaker and Lafta,1996).

Probably more anatomical changes would have been visible after longer salinization period. Sam et al. (2003/4) in two *L. esculentum* cultivars also noticed larger IS after 10 d salt stress (75 and 150 mM NaCl), but only in PM. It seems that salt-induced anatomical changes differed between plant species. For example, similarly to our data, Giełwanowska et al. (2005) observed large IS and irregular mesophyll cells in *Deschampsia antarctica* plants grown in habitats exposed to high salinity and flooding. On the contrary, *Arbutus unedo* after 16 week-salt-treatment (52 and 105 mM NaCl) showed a reduction of mesophyll IS and an increase in cell size of the second layer of PM (Navarro et al., 2007). In *Brugeria parviflora* IS as well as mesophyll thickness significantly decreased (Parida et al., 2004), while in *Medicago arborea* an increased mesophyll thickness was observed (Boughalleb et al., 2009).

Changes of leaf mesophyll cells

Although at the anatomical level salt-induced changes were rather slight, the influence of salt on cells was profound. While the leaf mesophyll cells from the control plant were typical, except for a few percent of slightly changed cells localized at the ends of the samples (which was probably the effect of taking the samples from the leaf blades), both tested salt concentrations caused changes in parenchyma cells ranging from slight plasmolysis to severe, irreversible cellular damage including cell wall breakdown which might have been the effect of changing osmotic pressure. De Felipe and Sanchez Conde (1984) reported that in tomato plants increasing osmotic pressure caused breakdown of cellular and chloroplast membranes and even cell walls.

In our experiment, the cell reaction depended on the intensity of salinization. The percentage of affected cells, especially of Sv-A, raised more and faster after 150 mM NaCl then after 50 mM NaCl treatment. Moreover, after plant re-watering under severe stress, the number of affected cells increased, probably due to the fact that ions initially located only near bundles spread all over mesophyll, while in 50 mM NaCl-treated plants the number of affected cells diminished, which suggests that initially, osmotic stress was more important than the ionic one. However, only the number of Sl-A cells, whose changes were reversible, diminished. It seems that Sv-A cells whose plasmalemma completely lost contact with a cell wall, thereby making deplasmolysis impossible, later exhibited severe ultrastructural disorganizations including chloroplast disorders, in spite of subsequent plant rehydration (2 d, 5 d). Pareek et al. (1997) indicated that ultrastructural changes in leaf cells of *O. sativa* seedlings which appeared after 4 h salinity, *eg*. cytoplasmic lysis and cell wall damage, did not vanish after 16 h salinity stress recovery.

We observed that the reactions of cells were not only salt-concentration-depended, which was in accordance with many earlier reports (Bennici and Tani 2009, 2012; Rahman et al., 2000; Sam et al., 2003/4; Trotta et al., 2012), but the same concentration of salinity also diversely influenced cells. Among mesophyll cells the unchanged, slightly- and severely-affected ones were visible. The data concerning such diversified reactions of cells of the same tissue in the same plant have been scanty. However, in *O. sativa* treated with 1.5% NaCl mesophyll cells degradation differed in apical and basal part of leaves (Mitsuya et al., 2002). Also in *O. sativa* after 0.3% NaCl some cells were almost destroyed, but cells without any lessions were also observed (Rahman et al., 2000). Similarly, Bennici and Tani (2012) indicated in electron microscopy that in callus of *Nicotiana tabacum* grown in the medium containing 200 mM or even 300 mM NaCl, cells with normal structure were present contiguous to dead or strongly injured cells with evident disorganization of protoplast. Also in an embryogenic culture of orchardgrass *Dactylis glomerata* subjected to 200 mM NaCl the intensity of damage varied from cell to cell (Gupta, 2007). Similar situation was also found in mesophyll after water-deficit stress in a crop plants - *Eragrostis tef* (Ginbot and Farrant, 2011). Considering the fact that the first phase of salt stress is connected with osmotic stress, such diversified response of mesophyll cells observed in the present study is not so astonishing. Dual response of cells to salinity of the same concentration suggests that the dehydration process as well as subsequent ion presence spread in mesophyll unequally. Sv-A cells in the SM were observed mostly near the vascular bundles probably because of the high concentration of Na^+ and Cl^- ions. Fricke et al. (1995) reported that Cl^- concentrations were higher in the epidermal cells of *Hordeum vulgare* above and around lateral veins than in epidermal cells located between vascular bundles. NaCl-trigered necrotic lesions in minor veins in *Pisum sativum* were also observed (Hernandez et al., 2001).

However, it seems highly probable that cell susceptible to salt stress vary. First of all, it could be connected with better osmotic adjustment (Meloni et al, 2001). Osmoprotectants accumulat in a vacuole to balance the osmotic strength inside and outside a cell might prevent cell dehydration and cell damage. Accumulation of osmoprotectants including sugars is quite common under salinity stress in plants *e.g*. *Brassica oleracea* (Elavoumoottil et al., 2003), *Lupinus albus* (Fernandez et al., 2004), *Zea mays* (Kholova et al., 2010), *Phaseolus vulgaris* (Stoeva and Kaymakanova, 2008), *Cicer arietinum* (Nandwal et al., 2007), *Tamarix ramosissima* (Carter and Nippert, 2011), *Nitraria retusa* (Boughalleb et al., 2012), *Centaurea ragusina* (Radić et al., 2013). Also in *L. esculentum,* high amounts of proline (Gapińska et al., 2007), glycinebetaine (Mäkelä et al., 2000) and soluble sugars (Khavari-Nejad and Mostofi, 1998) under salt conditions were reported. Moreover, in *Nicotiana bigelovii* callus, more negative symptoms caused by salinity were observed in the cells with chloroplasts without SG reserves than in those with SG presence (Bennici and Tani, 2009). It supports the hypothesis that sugar metabolism might be partially responsible for an unequal response of cells.

It seems that cell disturbance might be also connected with oxidative stress. Under water shortage conditions, excitation energy harnessed by chlorophyll cannot be dissipated via photosynthesis and this can lead to the formation of reactive oxygen species, which can cause considerable cellular damage. The increase in H_2O_2 content in leaf tissue after salt treatments was reported (Chaparzadeh et al., 2004; Yamane et al., 2012; Yang et al., 2009). In the present study on 2nd day after 50 mM

NaCl application, the percentage of Sv-A cells was higher in PM which is more photosynthetically active, than in SM. Moreover, in PM the cells with disturbed structure were more dispersed. Bruns and Hecht-Buchholz (1990) observed greater changes in chloroplasts of PM than of SM in NaCl treated potato. It seems that changes of chloroplast localization in Sl-A cells were also connected with minimizing oxidative stress. Redistribution of chloroplasts in cells was observed as the reaction to other stresses which caused secondary oxidative stress. In *Lemna trisulca* after strong light, lead or H_2O_2 treatments, chloroplasts accumulated at the anticlinal walls (Samardakiewicz et al., 2013). In our experiment, not only the localization but also the chloroplast structure were affected by NaCl. Big, rounded chloroplasts with oversized SG were visible in Sl-A cells after 150 mM NaCl treatment. Changes of chloroplast shapes similar to those observed by us were reported in salt-treated *L. esculentum* also by Sam et al. (2003/4). They found big, spherical chloroplasts with large SG in cv. C-28, but flat-shape chloroplasts with small SG in cv. Mariela.

In conclusion, the obtained data suggest that both 50 and 150 mM NaCl affect leaf mesophyll cells. In the leaves of plants treated with either NaCl concentration, two types of affected-cells existed: slightly- and severely-affected. Salt-affected cells were quite evenly distributed in the palisade or spongy mesophyll, except 2 d after treatment with 50 mM NaCl, when they were a more abundant in the palisade mesophyll. Sv-A cells in the palisade mesophyll were irregularly distributed probably because of additional photo-oxidative stress, while in SM were mostly concentrated near the bundle which was probably connected with more pronounced osmotic and subsequent ionic imbalance in this location. Conducted research pointed out diversified reactions of mesophyll cells of *L. esculentum* in response to salinity and their different intensity. These reactions were not only concentration- and time-dependent, but they were even more connected with location of cells in a leaf blade and their physiological state. The obtained results shed the new light to the mechanisms of agrarian plant resistance to that common environmental stress.

References

Bennici, A., Tani, C., (2009) Ultrastructural effects of salinity in *Nicotiana bigelovi var. bigelovi* callus cells and *Allium cepa* roots. Caryologia: International Journal of Cytology, Cytosystematics and Cytogentics, 62(2), 124-133.

Bennici, A., Tani, C., (2012) Ultrastructural characteristic of callus cells of *Nicotiana tabacum* L. var. BELW3 grown in presence of NaCl. Caryologia: International Journal of Cytology, Cytosystematics and Cytogentics, 65(1), 72-81. DOI: 10.1080/00087114.2012.678091

Boughalleb, F., Denden, M., Tiba, B.B., (2009) Anatomical changes induced by increasing NaCl salinity in three fodder shrubs *Nitraria retusa*, *Atriplex halimus* and *Medicago arborea*. Acta Physiologiae Plantarum, 31(5), 947-960. DOI: 10.1007/s11738-009-0310-7

Boughalleb, F., Haylaoui, H., Denden, M., (2012) Effect of salt stress on growth, water relations, solute composition and photosynthetic capacity of the xero-hallophyte *Nitraria retusa* (L.) Environment Research Journal, 6(1), 1-13. DOI: 10.3923/erj.2012.1.13

Bruns, S., Hecht-Buchholz, C., (1990) Light and electron microscope studies on the leaves of several potato cultivars after application of salt at various

development stages. Potato Researches, 33(1), 33-41. DOI: 10.1007/BF02358128

Carpita, N., Sabularse, D., Montezinos, D., Delmer, D.P., (1979) Determination of the pore size of cell walls of living plant cells. Science, 205(4411), 1144-1147.

Carter, J.M. and Nippert J.B., (2011) Physiological response of *Tamarix ramosissima* to extreme NaCl concentrations. American Journal of Plant Science, 2(6), 808-815. DOI: 10.4236/ajps.2011.26095

Chaparzadeh, N., D`Amico, M.L., Khavari-Nejad, R.A., Izzo, R., Navari-Izzo, F., (2004) Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiology and Biochemistry, 42(9), 695-701. DOI:10.1016/j.plaphy.2004.07.001

Debouba, M., Gouida, H., Suzuki, A., Ghorbel, M.H., (2006) NaCl stress effects on enzymes involved in nitrogen assimilation pathway tomato "*Lycopersicon esculentum*" seedlings. Journal of Plant Physiology, 163 (12), 1247-1258. DOI: 10.1016/j.jplph.2005.09.012

De Felipe, M.R., Sanchez Conde, M.P., (1984) Mineral composition and ultrastructure of leaves of tomato plants (*Lycopersicon esculentum* Mill.) subjected to different osmotic pressures and calcium levels. Anales de Edafologia y Agrobiologia, 43(1-2), 269-282.

De Lacerda, C. F., Asis Junior, J.O., Lemos Filho, L.C.A., de Oliveira, T.S., Guimeras, F.V.A., Gomes-Filho, E., Prisco, J.T., Bezzerra, M.A., (2006) Morpho-physiological responses of cowpea leaves to salt stress. Brazilian Journal of Plant Physiology, 18(4), 455-465. DOI: 10.1590/S1677-04202006000400003

Elavoumoottil, O.C., Martin, J.P., Moreno, M.L., (2003) Changes in sugars, sucrose synthase activity and protein in salinity tolerant callus and cell suspension culture of *Brassica oleracea* L. Biologia Plantarum, 46(1), 7-12. DOI:10.1023/A:1022389428782

FAO (2008): Land and Plant Nutrition Management Service. Available at: http://www.fao.org/ag/agl/agll/spush.

Fernandez, F.M., Arrabaca, M.C., Carvalho, L.M.M., (2004) Sucrose metabolism in Lupinus albus L. under salt stress. Biologia Plantarum, 48(2), 317-319. DOI: 1023/B:BIOP.0000033465.59361.d2

Fricke, W., Hinde, P.S., Leigh, R.A., Tomos, A.D., (1995) Vacuolar solutes in the upper epidermis of barley leaves. Intercellular differences follow patterns. Planta, 196, 40-49. DOI: 1007/BF00193215

Gapińska, M., Skłodowska, M., Gabara, B., (2007) The morphological, biophysical and biochemical changes in the salinity-stressed tomato plant roots. In: 7th International Conference Ecophysiological Aspects of Plant Responces to Stress Factors. Kraków, Poland, September 19-22. Acta Physiolgiae Plantarum, 29 (Supl. 1), 58. DOI:10.1007/s11738-007-0082-x

Giełwanowska, I., Szczuka, E., Bednara, J., Górecki, R., (2005) Anatomical feature and ultrastructure of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. Annals of Botany, 96(6), 1109-1119. DOI: 10.1093/aob/mci262

Ginbot, Z.G., Farrant, J.M., (2011) Physiological response of selected eragrostis species to water-deficit stress. African Journal of Biotechnology, 10(51): 10405-10417. DOI: 10.5897/AJB11.1124

Glińska, S., Gapińska, M., (2013) The effect of pre-incubation of *Allium cepa* L. roots in the ATH-rich extract on Pb uptake and localization. Protoplasma, 250(2), 601-611. DOI: 10.1007/s00709-012-0445-z

Gupta, S.D., (2007) Plasma membrane ultrastucture in embryogenic culture of orchardgrass during NaCl stress. Biologia Plantarum, 51(4), 759-763. DOI: 10.1007/s10535-007-0155-0

Hernandez, J.A., Almansa, M.S., (2002) Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiologiae Plantarum, 115(2), 251-257. DOI:10.1034/j.1399-3054.2002.1150211.x

Hernandez, J.A., Ferrer, M.A., Jimenez, A., Ros-Barcelo, A., Sevilla, F., (2001) Antioxidant systems and $O_2^{\cdot-}/H_2O_2$ production in the apoplast of *Pisum sativum* L. leaves: its relation with NaCl-induced necrotic lesions in minor veins. Plant Physiology, 127(3), 817-831. DOI: 10.1104/pp.010188

Ivitis, E., Cherlet, M., Tóth, T., Lewińska, K.E., Tóth, G., (2013) Characterisation of productivity limitation of salt-affected lands in different climatic regions of Europe using remote sensing derived productivity indicators. Land Degradation and Development, 24(5), 438-452. DOI: 10.1002/ldr.1140

James, R.A., Munns, R., Caemmerer, S., Trejo, C., Miller, C., Condon, T., (2006) Photosynthetic capacity is related to the cellular and subcellular portioning of Na^+, K^+ and Cl^- in salt-affected barley and durum wheat. Plant Cell and Environmental, 29(12), 2185-2197. DOI: 10.1111/j.1365-3040.2006.01592.x

Khavari-Nejad, R.A. and Mostofi Y., (1998) Effects of NaCl and $CaCl_2$ on photosynthetic pigments, saccharides and chloroplast ultrastructure in leaves of tomato cultivars. Photosynthetica, 35(1), 151-154. DOI: 10.1023/A:1006846504261

Kholova, J., Sairam, R.K., Meena, R.C., (2010) Osmolytes and metal ions accumulation, oxidative stress and antioxidant enzymes activity as determinants of salinity stress tolerance in maize genotypes. Acta Physiolgiae Plantarum, 32(2), 477-486. DOI: 10.1007/s11738-009-0424-y

Mäkelä, P., Kärakkäinen, J., Somersalo, S., (2000) Effect of glycinebetaine on the chloroplast ultrastructure, chlorophyll and protein content and RuBPCO activities in tomato grown under drought or salinity. Biologia Plantarum, 43(3), 471-475. DOI: 10.1023/A:1026712426180

Meloni, D.A., Oliva, M.A., Ruiz, H.A., Martines, C.A., (2001) Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. Journal of Plant Nutrition, 24(3), 599-612. DOI: 10.1081/PLN-100104983

Mitsuya, S., Kawasaki, M., Taniguchi, M., Miyake, H., (2003) Relationship between salinity-induced damages and aging in rice leaf tissues. Plant Production Science, 6(3), 213-218. DOI: 10.1626/pps.6.213

Mitsuya, S., Yano, K., Kawasaki, M., Taniguchi, M., Miyake, H., (2002) Relationship between the distribution of Na and the damages caused by salinity in the

leaves of rice seedlings grown under saline condition. Plant Production Science, 5(4), 269-274.

Morales, M.A., Sanchez-Blanco, M.J., Olmos, E., Toreccilla, A., Alarcon, J.J., (1998) Changes in the growth, leaf water relations and cell ultrastructure in *Argyranthemum coronopifolium* plants under saline conditions. Journal of Plant Physiology, 153(1-2), 174-180. DOI: 10.1016/S0176-1617(98)80062-X

Munné-Bosch, S., Alegre, L., (2002) Plant aging increases oxidative stress in chloroplasts. Planta, 214(4), 608-615.DOI: 10.1007/s004250100646

Munns, R., (2002) Comparative physiology of salt and water stress. Plant Cell and Environment, 25(2), 239-250. DOI: 10.1046/j.0016-8025.2001.00808.x

Nandwal, A.S, Kukreja, S., Kumar, N., Sharma, P.K., Jain, M., Mann, A., Singh, S., (2007) Plant water status, ethylene evolution, N2-fixing efficiency, antioxidant activity and lipid peroxidation in *Cicer arietinum* L. nodules as affected by short-term salinization and desalinization. Journal of Plant Physiology, 164(9), 1161-1169. DOI: 10.1016/j.jplph.2006.05.017

Navarro, A., Bañon, S., Olmos, E., Sánchez-Blanco, M.J., (2007) Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. Plant Science, 172(3), 473-480. DOI: 10.1016/j.plantsci.2006.10.006

Panagos, P., Van Liedekerke, M., Jones, A., Montanarella L., (2012) European Soil Data Centre: Response to European policy support and public data requirements. Land Use Policy, 29(2), 329-338. DOI: 10.1016/jlandusepol.2011.07.003

Pareek, A., Singla, S.L., Grover, A., (1997) Short-term salinity and high temperature stress-associated ultrastructural alterations in young leaf cells of *Oryza sativa* L. Annals of Botany, 80 (5), 629-639. DOI: 10.1006/anbo.1997.0494

Parida, A,K., Das, A.B., Mitra, B., (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees, 18, 167-174. DOI:10.1007/s00468-003-0293-8

Rahman, S., Matsumuro, T., Miyake, H., Takeoka, Y., (2000) Salinity-induced ultrastructural alterations in leaf cells of rice (*Oryza sativa* L.). Plant Production Science, 3(4), 422-429.

Rajashaker, C.B., Lafta, A., (1996) Cell-wall changes and cell tension in response to cold acclimation and exogenous abscisic acid in leaves and cell cultures. Plant Physiology, 111, 605-612. DOI: 10.1104/pp.111.2.605

Radić, S., Stefanić, P.P., Lepedus, H., Roje, V., Pevalek-Kozlina, B., (2013) Salt tolerance of *Centarea ragusina* L. associated with efficient osmotic adjustment and increased antioxidative capacity. Environmental and Experimental Botany, 87(, 39-48. DOI: 10.1016/j.envexpbot.2012.11.002

Romero-Aranda, R., Soria, T., Cuartero, J., (2001) Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Science, 160(2), 265-272. DOI: 10.1016/S0168-9452(00)00388-5

Sairam, R.K., Rao, V.K., Srivastava, G.C., (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress,

antioxidant activity and osmolyte. Plant Science, 163 (5), 1037-1046. DOI: 10.1016/S0168-9452(02)00278-9

Sam, O., Ramirez, C., Coronado, M.J., Testillano, P.S., Risueño, M.C., (2003/4) Changes in tomato leaves induced by NaCl stress: leaf organization and cell ultrastructure. Biologia Plantarum, 47(3), 361-366. DOI: 1023/B:BIOP.000002387.58899.88

Samardakiewicz, S., Suski, S., Gabryś, H., Woźny, A., (2013) Influence of lead (Pb^{2+}) on chloroplast distribution patterns in *Lemna trisulca* L. mesophyll cell in darkness. In: 6th Polish Society of Experimental Plant Biology Conference. Lodz, Poland, September 16-19. BioTechologia. Journal of Biotechnology, Computational Biology and Bionanotechnology, 94(3), 401-402.

Silva, C., Martinez, V., Carvajal, M., (2008) Osmotic versus toxic effect of NaCl on pepper plants. Biologia Plantarum, 52(1), 72-79. DOI: 1007/s10535-008-0010y

Snoussi, S.A., Abdul Hussain, F.H., Abdul Hussain, M.S., (2005) Dynamics of absorption of some biogenics salts at tomato and bean plant cultivated in saline medium. Journal of Central European Agriculture, 6(2), 151-156.

Stępień, P., Kłobus, G., (2006) Water relations and photosynthesis in *Cucumis sativus* L. leaves. Biologia Plantarum, 50(4), 610-616. DOI: 10.1007/s10535-006-0096-z

Stoeva, N., Kaymakanova, M., (2008) Effect of salt stress on the growth and photosynthesis rate of bean plants (*Phaseolus vulgaris* L.). Journal of Central European Agriculture, 9(3), 385-392.

Strogonov, B.P., (1964) Salt toxicity and adaptation of plants to salinity In: Poljakoff-Maber, A., Meyer, A.A (ed.): Physiological Basis of Salt Tolerance of Plants (as Affected by Various Types of Salinity). p.p. 130-162. Israel, Program for Scientific Translations, Jerusalem.

Tarchoune, I., Degl`Innocenti, E., Kadour, R., Guidi, L., Lachaal, M., Navari-Izzo, F., Ouerghi, Z., (2012) Effects of NaCl or Na_2SO_4 salinity on plant growth, ion content and photosynthetic activity in *Ocimum basilicum* L. Acta Physiolgiae Plantarum, 34(2), 607-615. DOI: 10.1007/s11738-011-0861-2

Trotta, A., Redondo-Gomez, S., Pagliano, C., Clemente, M.E.F., Rascio, N., La Rocca, N., Antonacci, A., Andreucci, F., Barbato, R., (2012) Chloroplast ultrastructure and thylakoid polypeptide composition are affected by different salt concentrations in the halophytic plant *Arthrocnemum macrostachyum*. Journal of Plant Physiology, 169(2), 111-116. DOI: 10.1016/j.jplph.2011.11.001

Yamane, K., Kawasaki, M., Taniguchi, M., Miyake, H., (2012) Salinity-induced subcellular accumulation of H_2O_2 in leaves of rice. Protoplasma, 249(2), 301-308. DOI: 10.1007/s00709-011-0280-7

Yang, F., Xiao, X., Zhang, S., Korpelainen, H., Li, Ch., (2009) Salt stress in response in *Populus cathayana* Rehder. Plant Science, 176(5), 669-677. DOI: 10.1016/j.plantsci.2009.02.008