J. Sci. I. R. Iran

Vol.7 No.3
Summer 1996

THE EFFECTS OF SALT STRESS ON THE SHOOT APICAL MERISTEM AND LEAF GENERATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

A. Majd and M. Shahbazi

Teacher Training University, Tehran 15, Islamic Republic of Iran

Abstract

Sunflower (*Helianthus annuus* L. cvs Mehr Shafagh) seedlings were grown in nutrient solutions containing 20.5 to 70.4 mM NaCl and 0 as the control. The effects of salinity on developmental changes occurring in the shoot apices of plants were studied. At 20.5 mM NaCl concentration, the leaf emergence rate and plastochron index of Shafagh increased significantly, but this phenomenon was not observed in Mehr. These parameters decreased in 41 mM and higher salt concentrations. In Shafagh plants, anatomical changes in shoot apical meristem at 20.5 mM NaCl consisted of the meristem and increased density and chromophilly in meristem cells, which are indicators of stimulated mitosis, particularly in the peripheral zone. No changes were observed in Mehr. In both cultivars, 41 mM and greater NaCl concentrations led to an increase in the width of meristems, uniform chromophilly of cells and dispersed mitotic activity. These phenomena are assumed to be signs of the transformation of a vegetative apical meristem into a reproductive one. The leaf segments shortened and xylem differentiation was seen nearer to the apex. The shoot apical meristem of Shafagh cultivar plants at 20.5 mM NaCl appeared to be activated for leaf generation. The effect of high salt concentrations on precocious flower formation of sunflower is also discussed.

Introduction

Progressive increase in soil salinity is a serious problem in arid and semi-arid regions of the world. Approximately one third of the earth's surface is arid or semi-arid (4.8×10 ha), of which one-half is estimated to be affected by salinity [1, 4].

Soil salinity, specially if caused by NaCl, adversely affects the growth of glycophyte plants and their yield. These adverse effects are due to the reduction of photosynthesis, hence a decrease in the biomass [, 19] and leaf emergence rate followed by a reduction in leaf area and total number of leaves [6, 11, 17, 25].

Anatomical changes in different parts of the plants in saline conditions have long been investigated [10, 17, 20, 21, 22, 23, 25, 26]. Environmental factors affecting the characteristics of the meristem and photoperiod have been studied comprehensively and there are also a few reports on the effects of calcium deficiency on anatomical characteristics [12]. But there are no reports on the likely changes in the anatomical structure of the shoot apical meristem and the site of leaf generation brought about by saline conditions.

The sunflower appears to be moderately salt tolerant, and the plants sprouted in soils with 2-4 mmho. cm^{-1} salinity [3]. Salinity reduces sunflower shoot growth, height, vegetative biomass, leaf number and leaf area considerably [9]. In this paper, structural and

Keywords: Mehr; Meristem; Plastochron; Shafagh

cytohistological changes in sunflower shoot apex and its plastochron caused through salinity (NaCl) are discussed.

Materials and Methods

The seeds of sunflower (*Helianthus annuus* L.) hybrid cultivars namely Mehr (ME) and Shafagh (SH) were obtained from the Karaj Seed and Plant Improvement Institute. The seeds were germinated in sand beds with distilled water for five days. Seedlings were then transferred to 800 ml containers of half-strength modified Hoagland solution. Nutrient solution contained: 2.5 mM Ca $(No_3)_2$, 1 mM $MgSO_4$, 2.5 mM KNO_3, 0.5 mM KH_2PO_3, 45 uM Fe EDTA, 23 uM, H_3BO_3, 1.79 uM $CuSO_4$, 0.75 uM $MnSO_4$, 0.38 uM $ZnSO_4$, 0.05 uM Na_2MoO_4, with 20.5, 26.4, 35.2, 41, 52.8 and 70.4 mM NaCl and 0 as control. The solution was renewed every five days and aerated continuously by aquarium pump. The pH of the nutrient solution was adjusted to 5.8 by adding HCl. Day /night temperatures were approximately: 30/17°C, relative humidity: 30/70% and plants were exposed to 80 Klx brightness in the middle of the day. The plants were kept in a greenhouse.

Each treatment and measurement was applied to 8-12 plants and repeated three times. The statistic studies were done by T test.

Plastochron Index (PI) Measurement

Plastochron index was measured eight days after transplanting the seedlings in nutrient solutions containing different NaCl concentrations, and was continued for ten days. The PI was calculated using Erickson and Michelini's index [7].

Growth Measurements

The plants were harvested 18 days after applying salt treatments. Leaf area was measured by Hitachi Delta-T Devices. Leaves, stems and roots were dried by exposing at laboratory temperature.

Anatomical Experiments

The seedlings were harvested seven days after the plants were transplanted in nutrient solutions containing different NaCl concentrations. Shoot apices were fixed for 12 h in FAA (in the presence of 0.1 mM $CaCl_2$) and dehydrated in a graded ethanol series and embedded in paraffin. Tissue blocks were sectioned to 6-8 uM sections by a rotary microtome. The sections were stained with either methyl green-Pyronin or Hematoxilin-Eosin. The sections were photographed using a Zeiss photomicroscope M3.

Results

Leaf area was reduced significantly ($P<0.05$) at all NaCl concentrations, the reduction being greatest in the highest salt concentrations (Fig. 1).

The effect of NaCl stress on the plant's dry matter production is shown in Figure 2. In SH, the differences in leaf dry matter between control and all stressed plants were significant ($P<0.05$), but reductions in stem and root dry weight were found to be significant only at the highest salt concentrations. In ME, the effects of different salt levels on leaf and root dry weights were statistically significant but stem dry weight did not change significantly.

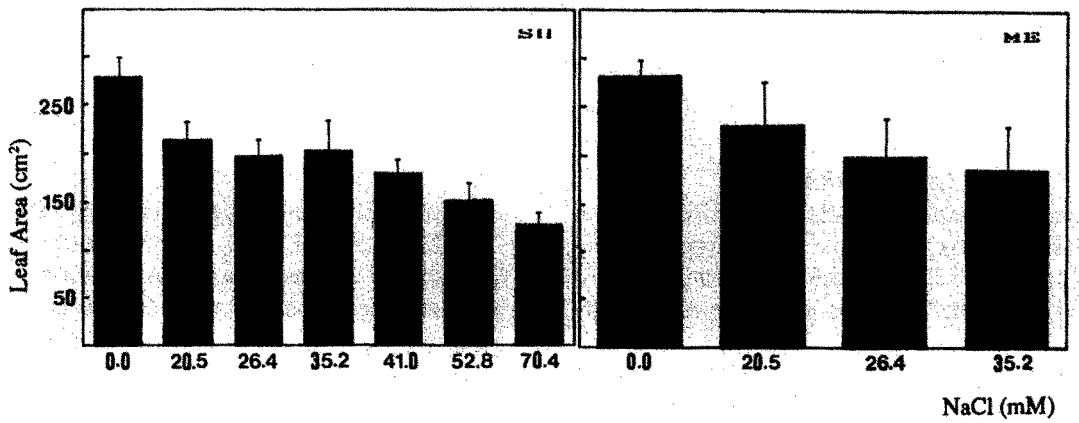


Figure 1. Effects of different NaCl concentrations on leaf area of sunflower plants; ME and SH cultivars. The data are the means of four replicates and error bars represent SD values.

Figure Abbreviations: T, Tunica; C, Corpus; Ir, Initiation ring; Mm, Medullary meristem; If, Leaf initium; Ef, Ebauche foliar; ME, Mehr; SH, Shafagh

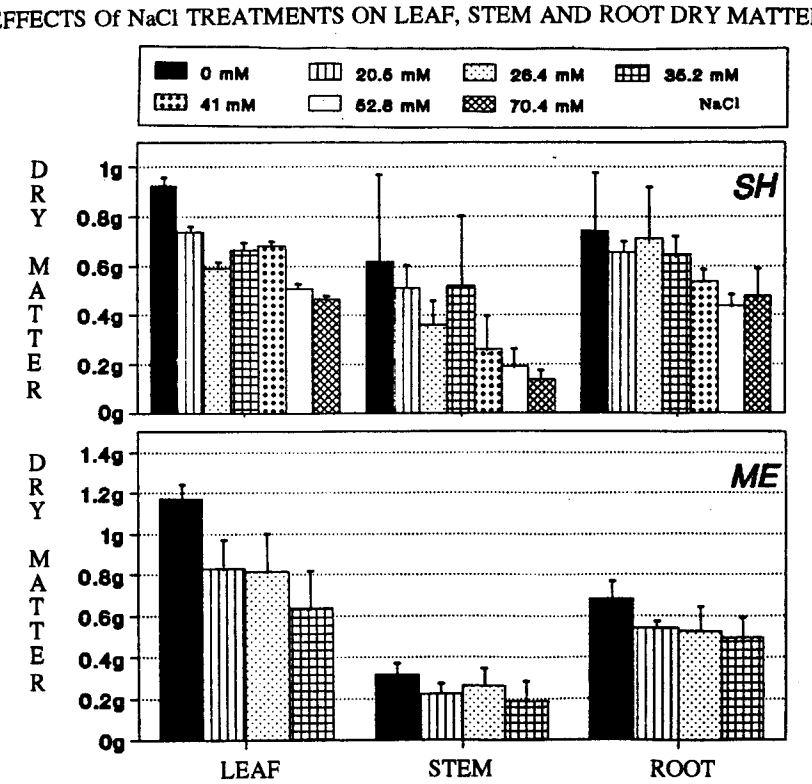


Figure 2. Effects of different NaCl concentrations on matter production of sunflower plants (leaf, stem and root); ME and SH cultivars. The data are the means of four replicates and error bars represent SD values.

Plastochron Index

The effect of salt stress shown in the regressions is depicted in Figure 3. In SH the slope of the regression line increased in 20.5 mM NaCl, but the slope decreased at greater NaCl concentrations (Fig. 3). The duration of the plastochron for SH control plants was 73.4 h, but the period was shortened by low salt concentration to 63.1 h. The plants grown in higher salt concentrations had greater plastochron duration, (for example, 97.4 h in 70.4 mM NaCl). In ME, the slope of PI: time relationship line decreased in all NaCl concentrations (Fig. 3). The mean plastochron was 90.4 h in control plants and increased in all of the salt treatments reaching 113 h in 35.2 mM NaCl.

Anatomical Examination

The SH apical meristem was small and flat (Fig. 4). The meristem of ME was also small, but slightly convex (Fig. 5). The tunica consisted of two cellular layers, which surrounded the inner meristem (T, Figs. 4,5). The central cells of the tunica were relatively large and stained more slightly and had less pyroninophilly. Chromocenters were easily distinguished in higher magnification, which was an indication of the cells being less active mitotically.

The corpus consisted of cells smaller than those found

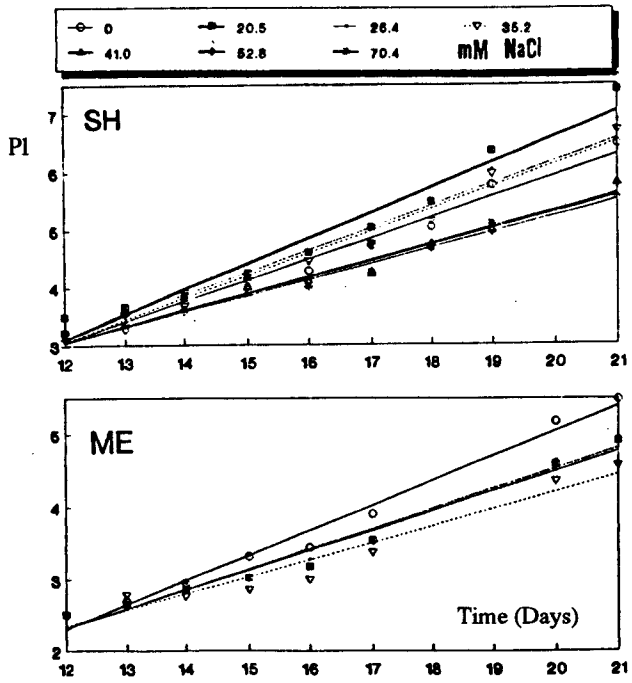
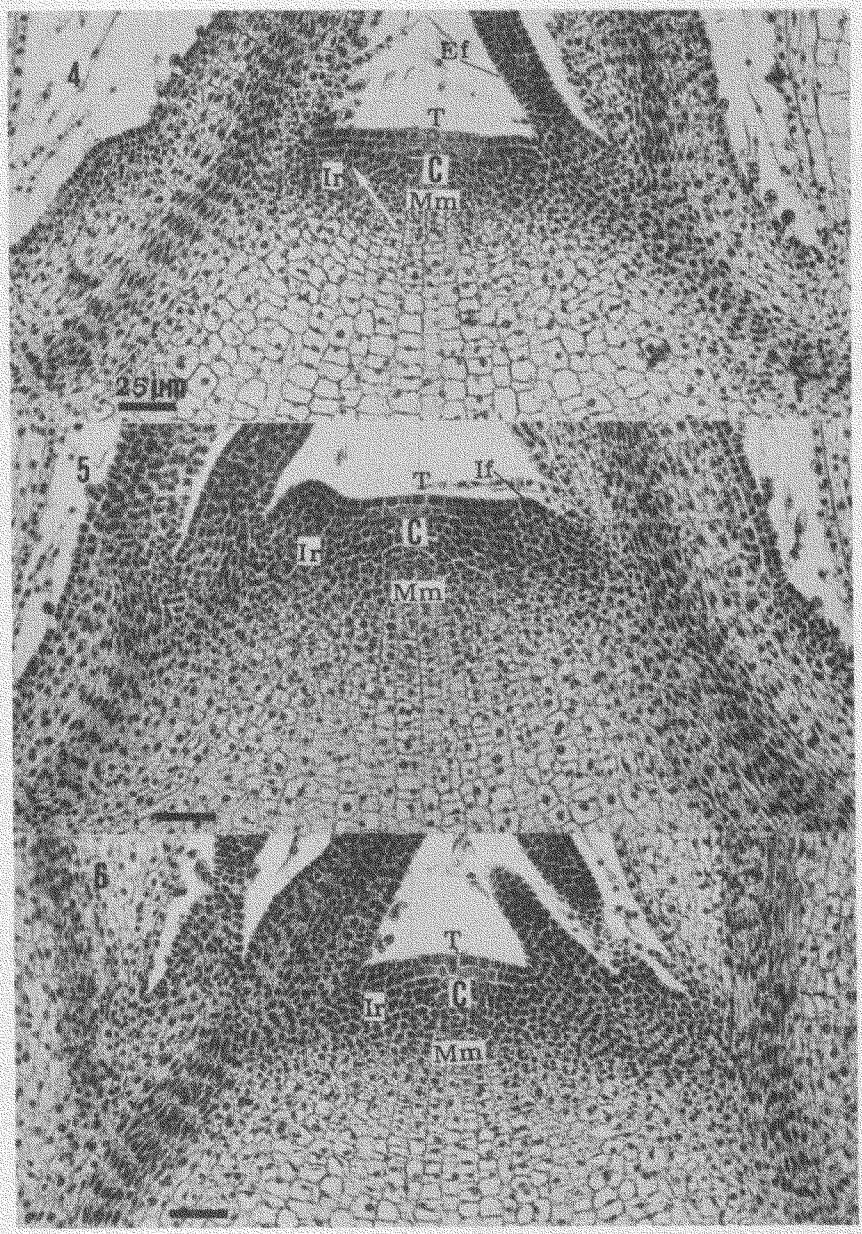


Figure 3. Plastochron index as a function of plants age (days) for sunflower plants growing at different NaCl concentrations; ME and SH cultivars. The regression lines are strictly linear ($r>0.98$).



Figures 4-6. Shoot apex meristem of sunflower plants: 4-Shafagh apical meristem, which is small and flat. 5-Mehr apical meristem, which is small and slightly convex. 6-Apical meristem of Shafagh's plant treated with 20.5 mM NaCl, which is relatively convex and prominent. Divisions of subterminal layers in initiating ring (/). 4,5,6, Scale bar =25 um.

in the tunica, which have further chromophilly (C, Figs. 4,5). The central apical zone (summit of meristem) was made up of corpus and the central region of the tunica.

The peripheral meristem or initiating ring (Ir, Figs. 4,5) was characterized by small cells which are strongly pyroninophill. This region had regular and periodic activation (plastochron functioning) that produced three-four opposite leaf pairs at first and then alternate leaves on two foliar helics. Periclinal divisions of the initiating ring and anticlinal divisions of tunical lateral cells participate to form a primordium.

The medullary meristem region (Mm, Figs. 4,5) was situated in the central region of the apex below the surface layer. The cells were usually large and contained laterally located nuclei. Their Nucleo-Cytoplasmic (N/C) ratios, pyroninophilly and chromophilly were between those of the apical zone and initiating ring cells.

Anatomical Changes of Shoot Apex Affected by Salinity

In SH, apical meristem of plants treated with 20.5 mM NaCl became relatively convex and prominent (Figs. 6,7). Density and chromophilly of meristematic cells were increased, however, their size was reduced (regardless of central cells of tunica).

Anticlinal division in tunica, particularly in surface layers, and periclinal division of sub-tunica layers were stimulated. These changes appear to be the signs of cell mitotic stimulation caused by salinity. In 20.5 mM NaCl, corpus was more obvious than in control (C, Fig. 7).

Homogeneous chromophilly and basophilly appeared in the whole meristem at higher salt treatments (Fig. 8). The central apical zone was not distinguishable from the other zones. The central cells of tunica were smaller and had more chromophilly and basophilly than those of control. Most nuclei were in prophase to telophase state, an indication of mitotic stimulation in this region.

The apex zone was not precisely defined in high salt concentrations .and the meristem mass appeared to be made up of several layers (Fig. 8). Moderate and high salt concentrations increased the width of apical meristem from about 100 um in control to about 138 um and 130 um in 35.2 and 41.0 mM NaCl (seven days after salinization) (Figs. 4, 8, 9). The whole volume of apical meristem and distance of medullary meristem from apex was increased (Fig. 9).

In control plants, periclinal divisions of subterminal layers in the initiating ring were concentrated at a few points for initial leaf formation (/Fig. 4). The continuation of these divisions and anticlinal divisions of tunica layers together resulted in leaf initiation (If, Fig. 5), leaf primordium and finally ebauche foliar (Ef, Fig. 4) formation. At high salt concentrations (50 mM NaCl and more) cell division was dispersed, with no signs of concentration, and corpus, the more or less passive region in the vegetative state, was activated (//, Fig. 9).

Salt treatment also accelerated the differentiation of conducting tissues. In control plants, long and narrow cells of procambial strands were distinguishable in young ebauche foliar (Ef, Fig. 4).

Anatomical examination showed that in treated plants, the initial proconducting strands appeared in the younger and smaller ebauche foliar and their distance from the apex was shorter than those of the control.

Total leaf number (the youngest ebauche foliar was counted) of control and salt treated plants on the seventh day after salinization was 13 in control plants and 16 and 11 in 20.5 and 70.4 mM NaCl respectively. As compared with the control, leaf segments in salt treated plants were shorter and young leaves were settled densely.

The apical meristem of ME plants did not clearly respond to salinity. The homogeneous basophilly and chromophilly was only obvious in the whole meristem at 41 mM NaCl and higher concentrations. The meristem was partly made of several layers, and the length of leaf segments was decreased.

Discussion

The sunflowers displayed a uniform pattern in the emergence of successive leaves, and the relationship between plastochron index and the plant age was strictly linear. Salinity considerably affected the slopes of regression lines.

The increasing leaf generation at low salt concentrations and its decrease in moderate and high NaCl concentrations was due to the shortening and lengthening of the PI respectively. These were the other noticeable responses to NaCl of hybrid Shafagh.

In SH, light salt treatment increased leaf emergence rate (LER) and plastochron index, but in ME, this increase was not observed, which corresponds to their genetic differences; SH is presumably more tolerant to salinity than ME.

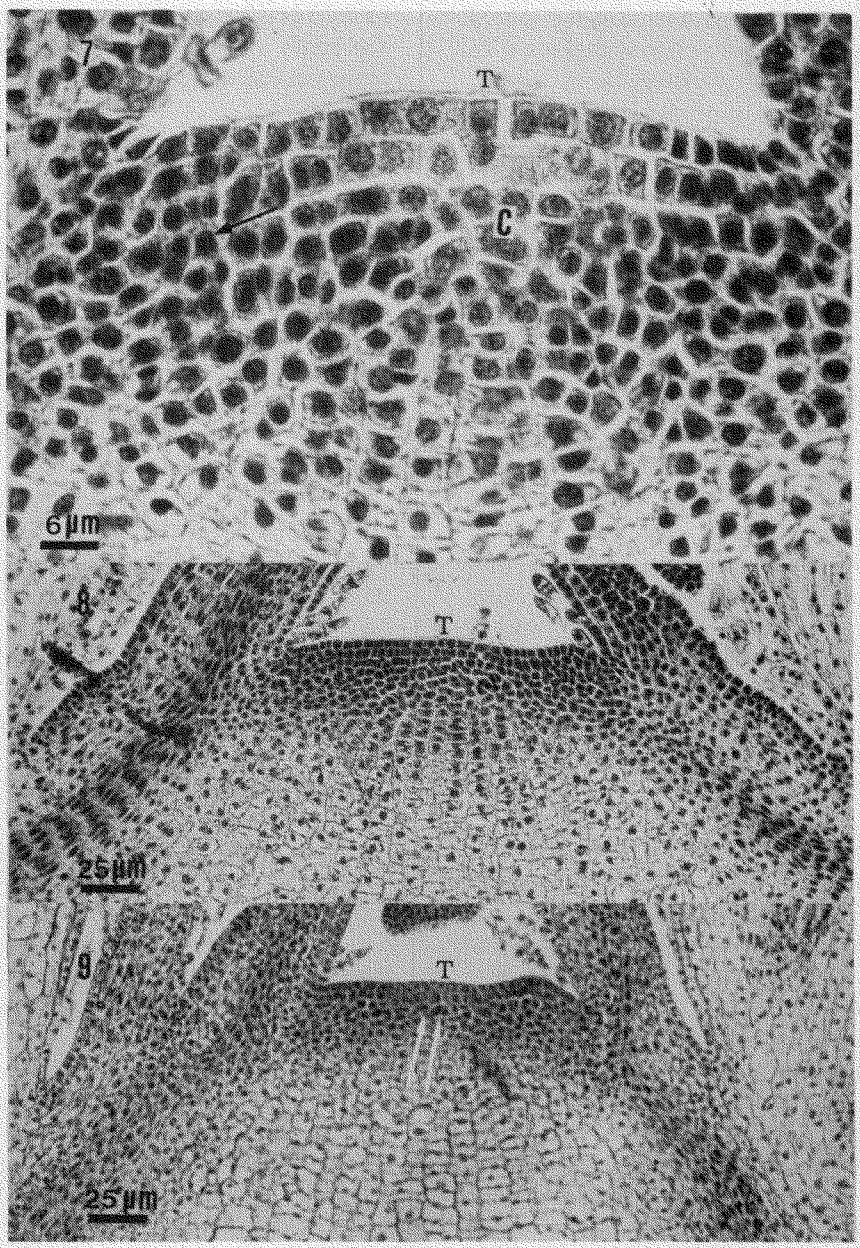
In the presence of 40 mM NaCl and higher concentrations, LER and PI reduction become more severe by lengthening the treatment duration. As a whole, the lengthening effects of plastochron were determined by fully declined weight and maximum size of leaves.

To our knowledge, this report is the first description of salinity effects on shortening the plastochron duration. However, shoot development of sunflower was slowed by increased salinity, similar to the results of Curtis and Lauchli [5,6]. Our results concerning the shoot apex anatomy of sunflower agrees with reports from Knowels [13] and Lyndon [16]. Shoot apical meristem of sunflower is usually the type Dicots.

Anatomical changes in SH plants under saline conditions consist of the promination of the apical meristem and the increased density of basophilly and chromophilly of cells. These are all indications of salt stimulation on mitotic activity, particularly in the peripheral zone at 20.5 mM NaCl. The increasing LER and PI in this treatment corresponded well with anatomical changes.

Moderate and high salt concentrations increased the width of apical meristem by reducing zonation of the whole meristem. Also the increased and relatively uniform chromophilly of the cells could be a sign of meristem transformation from vegetative to reproductive-like state, which is similar to the preflowering phase characteristics of sunflower meristem [13]. In excess salt concentrations (50 mM NaCl and more), the mitotic activity was dispersed throughout the whole meristem and the passive initiating ring could be due to the lowered LER and the decreased PI.

Acceleration in formation of procambial strands and differentiation conducting tissues correspond with apical



Figures 7-9. Apical meristem of sunflower hybrid Shafagh's plant treated with different concentrations of NaCl. 7-20.5 mM salinity stimulates cell division in tunica and subtunica layers (/).Scale bar = 6 um. 8-35.2 mM there is homogeneous chromophilly and basophilly in the whole meristem and central apical zone is not distinguishable from the other zone and the meristem mass appears to be made up of several layers. 9-41 mM, the whole volume of dispersed, with no signs of concentration and corpus was activated (//). 8,9, Scale bar = 25 um.

inducing vascular differentiation according to Aloni [2]. The lower LER can be a reason for the decrease in vascular tissue in sunflower shoot at high salt concentrations (authors, unpublished data).

Some authors believe that apical meristems possess a considerable degree of autonomy [8, 18, 24]. Apical meristem responses are largely independent of other processes within the plant.

To our knowledge, there are no published findings about salinity effects on shoot apical meristem and

plastochron function. Such studies are necessary for understanding the structural and functional changes of meristems treated with NaCl and for determining the tolerant cultivars and establishing the precocious or late crops.

References

1. Adams, P., Thomas, J.C., Vernon, D.M. and Bohnert, H.J. Distinct cellular and organismic responses to salt stress. *Plant Cell Physiology*, **33**, (8), 1215-23, (1992).
2. Aloni, R. The induction of vascular tissues by auxin. In *Plant hormones and their roles in plant growth and development*, (ed. P.J. Davies), pp. 363-374, Kluwer Academic Publishers, (1988).
3. Blamey, F.P.C., Edwards, D.G. and Asher, C.J. *Nutritional disorders of sunflower*. Queensland, Australia, (1987).
4. Bradbury, M. and Ahmad, R. The effect of silicon on the growth of *Prosopis juliflora* growing in saline soil. *Plant and Soil*, **125**, 71-74, (1990).
5. Curtis, P.S. and Lauchli, A. Responses of Kenaf (*Hibiscus cannabinus* L.) to salt stress germination and vegetative growth. *Crop Science*, **25**, 944-949, (1985).
6. Curtis, P.S. and Lauchli, A. The effects of moderate salt stress on leaf anatomy in *Hibiscus cannabinus* (Kenaf) and its relation to leaf area. *American Journal of Botany*, **75**, (1), 538-542, (1987).
7. Erickson, R.O. and Michelini, F.J. The plastochron index. *American Journal of Botany*, **44**, 297-305, (1957).
8. Feldman, L.R. and Torrey, J.G. The isolation and culture in vitro of the quiescent center of zea mays. *Ibid.*, **63**, 345-355, (1976).
9. Golan Goldhirsh, A., Hankamer, B. and Lips, S.H. Hydroxyprolin and prolin content of cell walls of sunflower, peanut and cotton growing under salt. *Plant Science*, **69**, (1) 27-32, (1990).
10. Hung, C.X. and Van Steveninck, R.F. M. Salinity induced structural changes in meristematic cells of barely roots. *New Phytologist*, **115**, 17-22, (1990).
11. Ivanitskaya, E.F. Characteristic features of the anatomic stucture of plants under various salinity conditions. *Fixiologiya Rastenii*, **9**, 199-209, (1962).
12. Kalra, G.S. Responses of the tomato plant to calcium deficiency. *Botanical Gazette*, **118**, 18-37, (1956).
13. Knowels, P.F. Morphology and Anatomy. In *Sunflower, Science and Technology*. No. 19 in the series Agronomy, pp 55-87. (ed. J.F. Carter). The American of Agronomy Inc. (1978).
14. Lazof, D., Bernstein, N. and Lauchli, A. Growth and development of *Lactuca sativa* shoot as affected by NaCl stress: consideration of leaf development stages. *Botanical Gazette*, **152**, (1), 72-76, (1991).
15. Longstreth, D.J., Bolanos, J.A. and Smith, J.E. Salinity effects on photosynthesis and growth in *Alternanthera philxeroides* (Mart). *Griseb. Plant Physiology*, **75**, 1044-1047, (1984).
16. Lyndon, R.F. Plant Development. The Cellular Basis. Academic Division of Unwin Hyman Ltd., (1990).
17. Poljakoff-Mayber, A. and Gale, J. (ed.) *Plants in saline environments, ecological studies 15*. Springer Verlag, Berlin, Heidelberg, New York, (1975).
18. Robertson, J.M., Yeung, E.C., Reid, D.M. and Hubick, K.T. Developmental responses to drought and abscissic acid in sunflower roots. II-Mitotic activity. *Journal of Experimental Botany*, **4**, (244), 339-356, (1990).
19. Robinson, S.P., Downton, W.J.S. and Millhouse, J.A. Photosynthesis and ion content of leaves and isolated chloroplasts of salt stressed spinach. *Plant Physiology*, **73**, 238-242, (1983).
20. Serrato-Valenti, G., Ferro, M., Ferraro, D. and Riveros, F. Anatomical changes in *Prosopis tamarugo* Phil. Seedling growing at different levels of NaCl salinity. *Annals of Botany*, **68**, 47-53, (1991).
21. Shaheen, M.A. Leaf anatomy of some grape cultivars in response to salinity. *Horticulture Science*, **25**, (9), 1172, (1990).
22. Solomon, M., Gedaiovich, E., Mayer A.M. and Poljakoffmayber, A. Changes induced by salinity to the anatomy and morphology of excised pea roots in culture. *Annals of Botany*, **57**, 311-318, (1986).
23. Tie, H. and Cramer, G.R. Cellular responses of two rapidcycling Brasica species *B. napus, B. carinata* to seawater salinity. *Plant Physiology*, **87**, (1), 54-60, (1993).
24. Torrey, J.G. and Feldman, L.J. The organization and function of the root apex. *American Scientist*, **65**, 334-344, (1977).
25. Wignarajah, K., Jennings, D.H. and Handley, J. F. The effects of salinity on growth of *Phaseolus vulgaris* L., I. Anatomical changes in the first trifoliate leaf. *Annals of Botany*, **39**, 1029-1038, (1975).
26. Valenti, G.S., Melone, L., Orsi, O. and Riveros, F. Anatomical changes in *Prosopis cineravia* (L.) druce seedlings growing at different levels of NaCl salinity. *Ibid.*, **70**, (5), 399-404, (1992).