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MORPHOLOGICAL AND ANATOMICAL STUDIES ON LEUCAENA (*Leucaena leucocephala*) PLANTS GROWN UNDER STRESS OF DIFFERENT LEVELS OF SALINITY IN IRRIGATION WATER

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By

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ABSTRACT

An experiment was conducted in the laboratory of Seed Technology Research Department, Field Crops Research Institute, Agricultural Research Centre, Giza during February 1998 to determine the effect of different levels of salinity (0, 2000, 4000, 6000, 8000, 10000 and 12000 ppm of the salt mixture NaCl and $CaCl_2$, 1:1 w/w) on germination percentage of leucaena seeds. The second experiment was a pot experiment which was carried out at the Nursery of the Forestry Department, Horticulture Research Institute, Agricultural Research Centre, Giza during the two successive seasons of 1998 and 1999 to study the morphological and anatomical characters of vegetative growth of *Leucaena leucocephala* plants grown under stress of different levels of salinity (0, 2000, 4000 and 8000 ppm of the salt mixture NaCl and $CaCl_2$, 1 : 1 w/w). Moreover, the effect of salinity on crude protein and free proline contents in leaves of leucaena plants were also investigated.

Results indicated that leucaena plants can grow well under salinity level of 2000 ppm without significant negative effect on

their morphological characters of vegetative growth.However, increasing salt concentration in irrigation water significantly decreased plant height, stem diameter, fresh and dry weights of stem, number of compound leaves per plant and fresh and dry weights of the leaves per plant and the rate of reduction increased steadily as the salinity level increased and reached its maximum with salinity level of 8000 ppm. Thus, it could be concluded that leucaena plants could tolerate salinity up to 8000 ppm, but with significant reduction in vegetative growth.

Key words: *anatomy, leucaena, morphology, salinity.*

1. INTRODUCTION

Most of the newly reclaimed Egyptian soils are sandy, calcareous and some of them are salty. The area considered salt-affected to different degrees is approximately two million feddans (El-Gabaly, 1975). Most of this area is located in the northern part of the Nile Delta and newly reclaimed lands at Noubaria, Fayoum and Sinai. Such area could be devoted to crops and woody trees that are tolerant to salts. Moreover, expansion of the agricultural area requires an enormous amount of irrigation water, which is not currently sufficient to meet all the expected demand. Therefore, the possibility of using saline water for irrigation specially from underground or drainage water is expected specially when the River Nile flood is low as it was in some recent years. The application of saline water for irrigation is dependent upon the concentration, composition of dissolved salts and the degree to which the plant species are salt tolerant.

It is well established that salinity inhibits growth and reduces yield in many crop plants. The damage of salinity differs in different plant species, depending on the organ of the plant being harvested, and in many cases the shoot is affected more than the root. One of the new strategy in facing the salinity problem in Egypt is the use of salt-tolerant species, specially woody plants, for cultivation in newly reclaimed soils. *Leucaena leucocephala* is one of the most promising trees in this respect. It is a promising candidate for sandy soils as it is characterized by longer tap root and high rate of annual

leaflet drop. The strong, deep root system allows leucaena to combat erosion and tolerate drought. Moreover, leucaena offers a wide assortment of uses. It can produce firewood, timber, nitrogenous and rich organic fertilizers. Its diverse uses include providing wind break, shade, soil improvement and ornamentation (Ayensu, 1981). It is a nitrogen-fixing tree, which has been recently introduced in subtropical regions. Although, not widely known in Egypt, its importance increases as a fodder plant and for sand-dune fixation. Hence, it could be cultivated in newly-reclaimed areas of Egypt to support animal production and fix sand-dunes.

In this respect, Hyder *et al.,* (1984) studied the effect of salt stress at concentrations ranging from 0 to 11000 ppm of NaCl, KCl, $MgCl_2$ or $CaCl_2$ on germination and seedling development in *Leucaena leucocephala.* The obtained results indicated that germination was more inhibited by monovalent than by divalent cations and germination percentage decreased to 20% at salinity level of 11000 ppm NaCl. Seedling dry weight decreased linearly with increasing NaCl concentration in irrigation water. Likewise, Niazi *et al.,* (1985) stated that increasing salt concentration adversely affected seed germination and plant growth (plant height and shoot fresh weight) of *Leucaena leucocephala.* A reduction of 50% in plant growth occurred at salinity level of 8000 ppm NaCl. Also, Hansen and Munns (1988) found that NaCl at concentrations of 1500, 3000 and 6000 ppm reduced plant growth (plant height, leaf number and biomass) of leucaena seedlings. Moreover, Panchaban *et al.,* (1989) using NaCl at concentrations of 0, 2000, 4000 and 6000 ppm on leucaena seedlings found that salinity decreased the percentage of plant survival and the rate of reduction increased steadily as the salinity level increased. Again, Rab *et al.,* (1989) reported that germination of leucaena seeds was progressively reduced by increasing salinity from 59% in the control to 7% at salinity level of 10000 ppm of salt mixture containing NaCl / Na_2So_4 / $CaCl_2$ / $MgSo_4$ at 5 : 9 : 5 : 1 . Likewise, Cavalcante and Perez (1995) studied the effect of salt stress at concentrations ranging from 0 to 12000 ppm NaCl on germination of leucaena seeds. It was found that the percentage of germination was decreased progressively as salinity level increased.

The present investigation is an attempt to bring to light more information about the effect of salinity on seed germination and plant survival as well as on the morphology and anatomy of vegetative growth of leucaena plants. Moreover, the effect of salinity on crude protein and proline contents in leaves of leucaena plants were also investigated.

2. MATERIALS AND METHODS

Two independent experiments were used in this investigation. The first was conducted in the laboratory of Seed Technology Research Department, Field Crops Research Institute, Agricultural Research Centre, Giza during February 1998 to determine the effect of different levels of salinity (0.0, 2000, 4000, 6000, 8000, 10000 and 12000 ppm) on germination percentage of leucaena seeds according to the Rules for Seed Testing (Anonymous, 1985). The germination experiment could be considered an indication for the maximum level of tolerable salinity of the investigated species. The results obtained opened the door for choosing the different levels of salinity used in the second experiment. Levels of salinity which decreased germination percentage of leucaena seeds more than 50% were excluded. The second experiment was a pot experiment which was carried out at the Nursery of the Forestry Department, Horticulture Research Institute, Agricultural Research Centre, Giza during the two successive seasons of 1998 and 1999.

Seeds of leucaena were treated by boiling water and then soaked in tap water for 48 hours before either germination on sterilized cotton pads in petri-dishes (10 cm diameter) in the first experiment or sowing in pots (30 cm diameter) filled with fixed weight of 8 kg of loam soil and sand (1:1 w/w) mixture in the second experiment.

2.1. Laboratory experiment

Artificial salinized water was prepared by dissolving the salt mixture (1:1 w/w) of sodium chloride (NaCl) and calcium chloride ($CaCl_2$). Seven levels of salinity; namely, 0, 2000, 4000, 6000, 8000, 10000 and 12000 ppm were used, in petri-dishes experiment, to test

their effect on germination percentage of leucaena seeds. For this purpose, 100 seeds were assigned for testing each of the saline treatments. The experiment was made in a randomized complete block design with five replicates. The replicate contained seven perti-dishes, each was assigned for one treatment where 20 seeds were placed on cotton pads saturated with the tested solution and the pads were kept moist throughout the experiment. The dishes were incubated at 25°C and germination percentage was recorded after 14 days.

2.2. Pot experiment

The experiment was made in a randomized complete block design with four replicates. The replicate contained 40 pots, each 10 pots were assigned for one treatment. The treatments were four levels of salinity in irrigation water; namely, 0.0 (using tap water as a control), 2000, 4000 and 8000 ppm of salt mixture (NaCl : $CaCl_2$, 1 : 1 w/w).

Leucaena seeds were sown on 8th March, 1998 in the first season, and replicated on 10th March, 1999 in the second season to provide the experimental plant materials. For this purpose, black plastic pots of 30 cm diameter were filled with about 8 kg of a mixture containing loam soil and sand (1 : 1 w/w) for each pot. Five seeds were sown in each pot and the pots were irrigated immediately with the assigned concentrations of salinity. Each level of salinity in irrigation water was added regularly (500 ml/pot/week) during the whole period of the experiment (eight months from sowing date). Irrigation treatments were applied four times with salt-water followed by one irrigation with tap - water (for leaching the accumulated salts) and then repeated in the same manner till the end of the experiment. After three months from sowing, plant survival was recorded and plants were thinned to one plant per pot.

2.3. Recording of data

2.3.1. Morphological characters of vegetative growth

For all determined characters, 20 plants from each treatment, five from each replicate, were used for this purpose. In each growing

season, data were recorded on individual plants at the age of eight months from sowing date. The morphological characters includes: plant height (cm), stem diameter (mm), fresh and dry weights of the stem per plant (g), number of remained compound leaves per plant and fresh and dry weights of leaves per plant (g). Data on morphological characters were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (L.S. D.) of each character was calculated.

2.3.2. Anatomical studies

Specimens were taken from the median internode of the main stem as well as from leaflets of the corresponding leaf. Plants used for examination were taken throughout the first season, 1998, at the age of five months from sowing date.

Specimens were killed and fixed for one week in F. A. A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 microns, double stained with safranin-light green, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of noticeable responses resulting from investigated treatments.

2.3.3. Chemical studies

Chemical analysis was performed on leaves of treated and untreated plants, in the first season of 1998 at the age of eight months from sowing date. The following determinations were made.

Free proline content

Free proline was determined in fresh leaves according to the method described by Bates *et al.,* (1973). Bush and Lomb spectrophotometer (model spectranic 2000) was used, the absorbance was measured at 520 nm. Free proline content was estimated as mg/g fresh weight (FW).

Crude protein

Total nitrogen content was determined in dry leaves using the

modified micro-kjeldhahl method described by Pregl (1945). Nitrogen content was multiplied by 6.25 to calculate the crude protein (Anonymous, 1975).

3. RESULTS AND DISCUSSION

3.1. Percentages of seed germination

Germination percentages of leucaena seeds as affected by different levels of salinity are shown in Table (1).

Table (1): The effect of different levels of salinity on germination percentages of *Leucaena leucocephala* seeds.

Salt concentration (ppm)	0	2000	4000	6000	8000	10000	12000
Germination percentages	89	86	75	61	52	17	0

It is realized that the used low concentration of 2000 ppm artificial salinized water (NaCl:$CaCl_2$, 1:1 w/w) induced a slight decrease of 3.4 % in germination percentage of leucaena seeds from the control. Whereas, the used high concentration of 12000 ppm salinized water completely inhibited seed germination of leucaena. Data also indicated that the percentage of germination was decreased progressively as salinity level increased. The percentage of germination was 89 for the control treatment and decreased to 86, 75, 61, 52, 17 and 0 under salinity stress of 2000, 4000, 6000, 8000, 10000 and 12000 ppm, respectively.

Similar results were recorded by Hyder *et al.*, (1984), Niazi *et al.*, (1985), Rab *et al.*, (1989) and Cavalcante and Perez (1995). They stated that germination percentage of leucaena seeds was progressively reduced by increasing salinity level, being in agreement with the present findings.

3.2. Percentages of plant survival

The percentages of plant survivals of three months old leucaena plants grown under different levels of salinity stress in two seasons and the results of their statistical analysis are given in Table (2).

Table (2): Percentages of plant survival of three month old leucaena plants as affected by different levels of salinity in two successive seasons.

Salt concentrations (ppm)	Percentages of plant survival	
	First season of 1998	Second season of 1999
0	88	92
2000	84	87
4000	75	72
8000	41	39
L. S. D. (0.05)	7.18 %	6.05 %

Data presented in Table (2) clearly show that all assigned concentrations of artificial salinized water significantly decreased the percentage of plant survival in both seasons except that of 2000 ppm where the difference was insignificant. Such reduction increased progressively with increasing salt concentration in irrigation water and reached its maximum with salinity level of 8000 ppm, which reduced the percentage of plant survival by 53.4% in the first season and by 57.6% in the second one less than the control. It is worthy to note that, leucaena plants grown under salinity stress of 4000 ppm showed significant reduction in the percentage of plant survivals by 14.8 and 21.7 % less than the control in the first and second season, respectively.

The previous report of Panchaban *et al.*, (1989) using NaCl at concentrations of 0, 2000, 4000 and 6000 ppm on leucaena seedlings found that salinity decreased the percentage of plant survivals and the rate of reduction increased steadily as the salinity level increased, being in accordance with the present findings. Such decrease in the percentage of plant survivals due to salinity stress could be attributed to the toxicity of one or more specific ions, osmotic inhibition of water absorption under relatively high level of salinity and / or the combination of the previous two factors as mentioned by Seatz *et al.*, (1958).

3.3. Morphological characters of vegetative growth

Data on morphological characters are presented in Table (3).

Table (3): Morphological characters of vegetative growth of leucaena plants, 8 months old, as affected by different levels of salinity in two successive seasons.

Salt Concentrations (ppm)	Plant height (cm)	Stem diameter (mm)	Fresh weight of stems (g)	Dry weight of stems (g)	No. of leaves per plant	Fresh weight of leaves (g)	Dry weight of leaves (g)
First season of 1998							
0	133.6	10.2	41.4	19.8	21.4	51.1	18.4
2000	128.5	9.6	37.6	18.1	20.1	45.8	16.9
4000	103.2	7.8	28.3	14.5	16.7	33.5	13.2
8000	79.9	6.7	18.4	9.1	13.9	23.3	10.1
L. S. D. (0.05)	11.57	0.91	4.93	1.85	1.91	6.67	1.74
Second season of 1999							
0	150.2	11.	46.5	20.9	22.1	53.2	18.1
2000	139.7	10.4	41.7	19.5	21.2	48.9	17.1
4000	112.5	8.3	32.6	15.7	17.5	38.1	13.7
8000	87.3	7.1	23.7	11.3	14.6	30.6	11.6
L. S. D. (0.05)	14.19	0.98	5.16	1.91	1.95	6.82	1.65

3.3.1. Plant height

The mean values of plant height of leucaena plants grown under different levels of salinity stress in two seasons and the results of their statistical analysis are shown in Table (3).

It is obvious that the control plants recorded a mean plant height of 133.6 cm in the first season and of 150.2 cm in the second one, which proved significant difference with all treatments of salinity, in both seasons, except that of plants grown under salinity stress of 2000 ppm where the difference was insignificant. It is evident that increasing salt concentration in irrigation water significantly retarded plant height. The significant decrease in plant height of leucaena plants due to the effect of 4000 ppm salinized water was 22.75% less than the control plants in the first season and 25.1% less than the control in the second one. Whereas, the significant decrease in plant height due to the effect of 8000 ppm salinized water was 40.2 and 41.9% less than the control plants in the first and second season, respectively.

Photograph shown in Figure (1) illustrates the retarding effect of salinity on height of five month old leucaena plants.

Fig. (1): Habit of *Leucaena leucocephala* plants, five month old, grown under stress of different levels of salinity.

3.3.2. Diameter of the main stem

Data on stem diameter taken at the base of the main stem of leucaena plants, eight month old, grown under stress of different levels of salinity in two seasons are given in Table (3).

It is clear that the assigned low concentration of 2000 ppm artificial salinized water induced insignificant decrease in stem

diameter of leucaena plants by 5.88% less than the control in the first season and by 7.97% less than the control in the second one. At the same time, the medium concentration of 4000 ppm salinized water as well as the high concentration of 8000 ppm salinized water significantly retarded stem diameter. It is worthy to note that, the significant decrease in stem diameter was more pronounced as the salinity level increased and expressed its maximum with salinity level of 8000 ppm, being 34.31% less than the control in the first season and 37.17% less than the control in the second one.

3.3.3. Fresh weight of the stem

Data presented in Table (3) clearly show that all tested concentrations of salinity in irrigation water decreased fresh weight of leucaena stem in both studied seasons, and the rate of reduction increased steadily as the salinity level increased. The significant decrease in fresh weight of leucaena stem was observed at salinity levels of 4000 and 8000 ppm, being 31.64 and 55.56% less than the control in the first season against 29.89% and 49.03% less than the control in the second one, respectively.

3.3.4. Dry weight of the stem

It is realized from Table (3) that the low concentration of 2000 ppm salinized water caused insignificant reduction in dry weight of leucaena stem in both seasons. Whereas, the medium and high concentrations of 4000 and 8000 ppm salinized water decreased significantly such trait in both seasons, and the decrease was more pronounced as the salinity level increased; being 26.77 and 54.04% less than the control in the first season and 24.88 and 45.93% less than the control in the second one, respectively.

3.3.5. Number of compound leaves per plant

The mean values of number of compound leaves per plant of leucaena grown under different levels of salinity stress in two seasons and the results of their statistical analysis are shown in Table (3).

It is obvious that the control plants recorded a mean number of 21.4 leaves per plant in the first season and of 22.1 leaves per plant in the second one, which proved significant increase over any of the investigated treatments expect that of plants grown under salinity

stress of 2000 ppm where the difference was insignificant. Data also indicated that all assigned concentrations of salinized water decreased number of leaves per leucaena plant and the rate of reduction increased steadily as the salinity level increased. The significant decrease was detected at salinity levels of 4000 and 8000 ppm, being 21.96 and 35.05% less than the control in the first season against 20.82 and 33.94% less than the control in the second season, respectively.

3. 3. 6. Fresh weight of leaves per plant

It is clear from Table (3) that the low concentration of 2000 ppm salinized water showed no significant effect on fresh weight of leaves per leucaena plant, eight month old, in both seasons although a decrement of 10.37% in the first season and of 8.08% in the second one less than the control was observed in this respect. At the same time, the medium concentration of 4000 ppm salinized water as well as the high concentration of 8000 ppm salinized water decreased significantly fresh weight of leaves per leucaena plant in both seasons. Moreover, the rate of reduction increased proportionally with increasing salt concentration in irrigation water and expressed its maximum with salinity level of 8000 ppm, which reduced fresh weight of leaves per leucaena plant by 54.4% less than the control in the first season and by 42.48% less than the control in the second one.

3.3.7. Dry weight of leaves per plant

Data presented in Table (3) show that all tested concentrations of salinity in irrigation water decreased dry weight of leaves per leucaena plant, eight month old, in both seasons, and the rate of reduction increased steadily as the salinity level increased. It is worthy to note that the significant decrease in dry weight of leaves per leucaena plant was detected at salinity levels of 4000 and 8000 ppm, being 28.26 and 45.1% less than the control in the first season against 24.31 and 35.91% less than the control in the second season, respectively.

From the aforementioned results, it could be stated that all tested concentrations of salinity (2000, 4000, and 8000 ppm) in irrigation water decreased the investigated morphological characters

(plant height, stem diameter, fresh and dry weights of stem, number of compound leaves per plant and fresh and dry weights of leaves per plant) of vegetative growth of leucaena plants, eight month old, in both seasons (1998 and 1999), and the rate of reduction increased steadily as the salinity level increased and reached its maximum with salinity level of 8000 ppm. It is worthy to note that salinity level of 2000 ppm showed insignificant decrease in all studied characters in both seasons, whereas, the significant decrease was detected at salinity levels of 4000 and 8000 ppm for all morphological characters under investigation in both seasons.

In this connection, Hyder *et al.*, (1984) using NaCl at concentrations of 0 to 11000 ppm on leucaena stated that symptoms of salt injury appeared at NaCl concentration of 4000 ppm and seedling dry weight decreased linearly with increasing NaCl concentration. Likewise, Niazi *et al.*, (1985) concluded that increasing salt concentration adversely affected plant growth. A 50% reduction in plant growth occurred at salinity level of 8000 ppm NaCl. Also, Hansen and Munns (1988) found that NaCl at concentrations of 1500, 3000 and 6000 ppm reduced growth of leucaena plants (plant height, leaf number and biomass). These results are in accordance with the present findings.

3.4. Anatomical studies

Referring to the aforementioned morphological characters; namely, plant height, diameter of the main stem, fresh and dry weights of stem, number of compound leaves per plant and fresh and dry weights of leaves per plant of *Leucaena leucocephala* grown under salinity stress of 2000, 4000 and 8000 ppm, it was found that all tested concentrations of salinity in irrigation water decreased all studied morphological characters and the rate of reduction was higher as salinity level increased. The significant reduction was detected at 4000 ppm as well as at 8000 ppm.

This may justify a further study on the internal structure of the main stem and the leaves of leucaena plants grown under salinity stress of 4000 in comparison with those of control.

3.4.1 Anatomy of the main stem

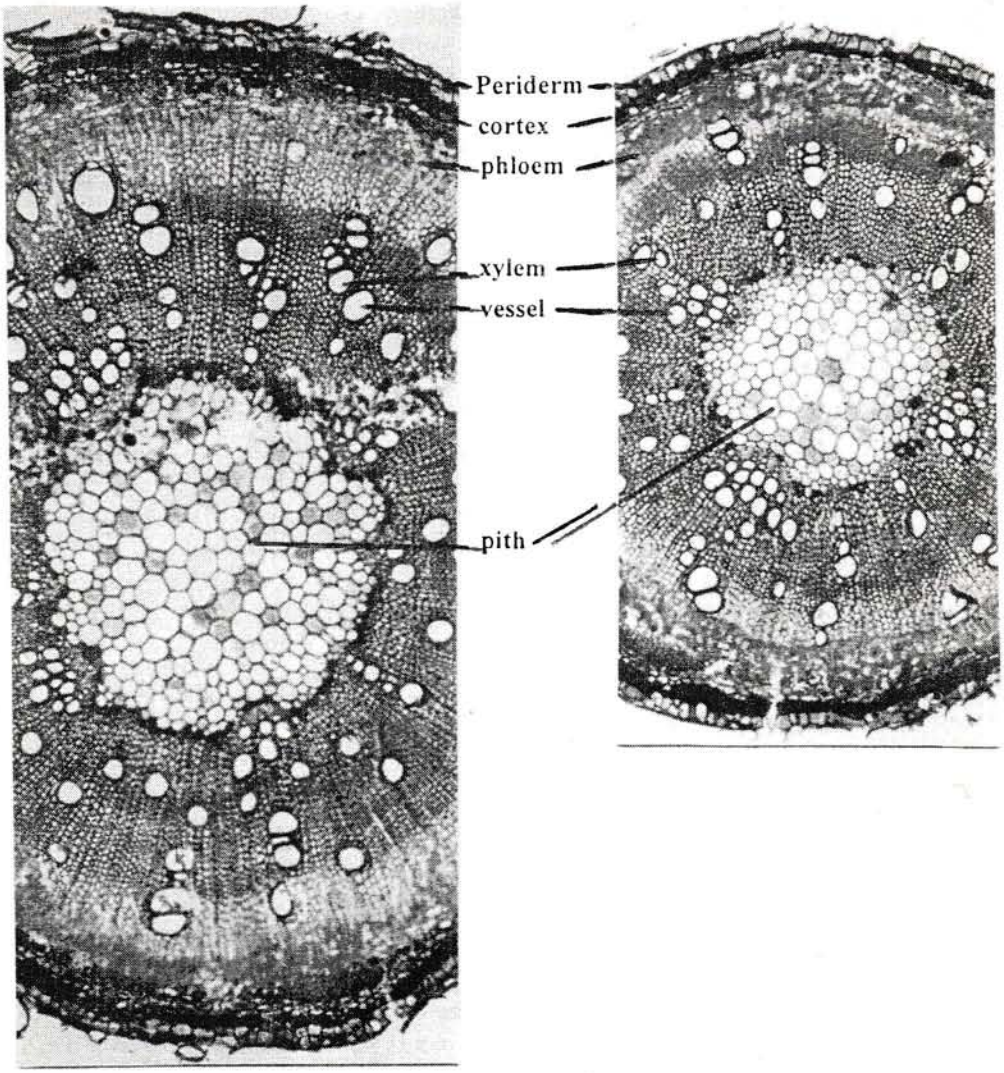
Microscopical measurements of certain characters in transverse

sections through the median internode of the main stem of leucaena plants grown under salinity stress of 4000 ppm and those of control are given in Table (4). Likewise, microphotographs illustrating salinity and control treatments are shown in Figure (2).

Table (4) : Measurements in micron of certain histological features in transverse sections through the median internode of the main stem of normal leucaena plants and of those grown under salinity stress of 4000 ppm at the age of five months from sowing date. (Means of three specimens).

Characters	Salinity level		
	0.0 (Control)	4000 ppm	± % to control
Stem diameter	3773	2507	- 33.6
Thickness of periderm	128	96	- 25.0
Thickness of cortex	94	113	+ 20.2
Thickness of vascular tissue	1088	691	- 36.5
Thickness of phloem tissue	269	179	- 33.5
Thickness of xylem tissue	819	512	- 37.5
Vessel diameter	70	50	- 28.6
Pith diameter	1152	704	- 38.9

It is clear that the salinity level of 4000 ppm reduced the diameter of the main stem by 33.6% less than the control. The decrease in stem diameter, due to salinity stress, could be attributed mainly to the prominent decrease in all included tissues except that of cortex which showed an increase of 20.2% more than the control. It is obvious that salinity treatment decreased the thickness of periderm and of vascular tissue of the main stem by 25.0 and 36.5% less than control, respectively. The prominent reduction which was observed in the thickness of vascular tissue of the main stem, due to salinity stress at 4000 ppm, could be attributed mainly to the decrease in the thickness of phloem tissue and xylem tissue by 33.5 and 37.5% less than the control, respectively. Also, vessel diameter was decreased by 28.6% from the control. Moreover, pith diameter was decreased by 38.9% from the control.



Control

Salinity level of 4000 ppm

Fig.(2):Transverse sections through the median internode of the main stem of leucaena plants, five month old, as affected by salinity stress. (x 40)

3. 4. 2. Anatomy of the leaf

Microscopical counts and measurements of certain characters in transverse sections through leaflets blades of the median compound leaf on the main stem of normal leucaena plants and of those grown under salinity stress of 4000 ppm, at the age of five months from sowing date, are presented in Table (5). Likewise, microphotographs illustrating these treatments in transverse sections are shown in Figure (3).

Table (5): Counts and measurements in micron of certain histological features in transverse sections through leaflets blades of the median compound leaf on the main stem of normal leucaena plants and of those grown under salinity stress of 4000 ppm at the age of five months from sowing date (Means of three specimens).

Characters	Salinity level		
	0.0 (Control)	4000 ppm	± % to control
Thickness of lamina	204	181	- 11.3
Thickness of epidermis	24	21	- 12.5
Thickness of palisade tissue	97	84	- 13.4
Thickness of spongy tissue	60	54	- 10.0
Thickness of midvein	264	192	- 27.3
Dimensions of midvein bundle Length	168	108	- 35.7
Width	150	102	- 32.0
No. of vessels / midvein bundle	29	22	- 24.1

It is realized that salinity stress at the level of 4000 ppm reduced the thickness of lamina and midvein of leaflets of the median compound leaf on the main stem of leucaena plant, five month old, by 11.3 and 27.3% less than the control, respectively. The thinner leaflets induced by salinity stress could be attributed to the decrease in thickness of epidermis as well as in thickness of mesophyll tissues (palisade and spongy). The decrements below the control were 12.5, 13.4 and 10.0% for the thickness of epidermis, palisade tissue and spongy tissue, respectively. Such treatment decreased the dimensions of midvein bundle below the control by 35.7% in length and by 32.0% in width. Moreover, the number of vessels per midvein bundle was decreased by 24.1% less than the control.

As far as the authors are aware, information concerning anatomical structure of the main stem and leaves of leucaena plants grown under stress of salinity are not available in the literature.

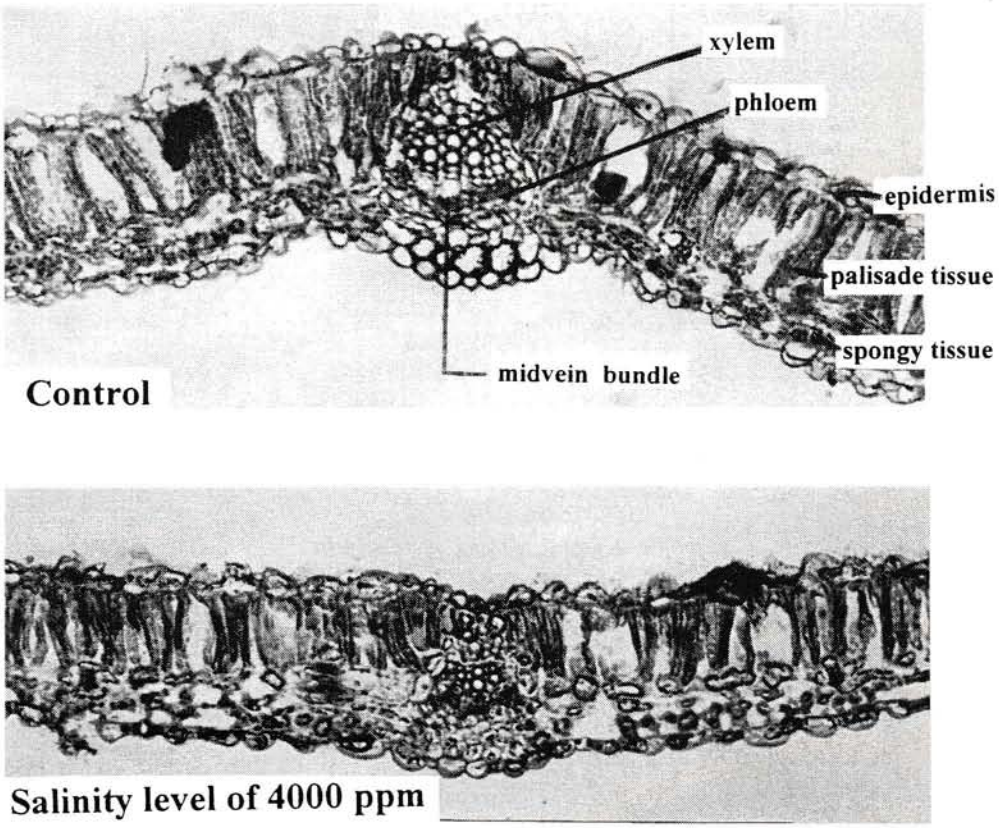


Fig. (3):Transverse sections through leaflet blades of the median compound leaf on the main stem of leucaena plants, five month old, as affected by salinity stress.

(x 150)

3.5. Chemical studies

Free proline and crude protein contents were determined in leaves of *Leucaena leucocephala* plants, eight month old, grown under different levels of salinity stress in the first season of 1998. Data on chemical analysis are presented in Table (6).

Table (6): The effect of salinity on free proline and crude protein contents in leaves of *Leucaena leucocephala* plants eight month old.

Salt concentrations (ppm)	Free proline(mg / g FW)	Crude protein %
0	1.18	23.40
2000	1.22	23.18
4000	1.58	21.62
8000	1.64	21.31

3.5.1. Free proline

It is realized from Table (6) that the content of free proline in leaves of leucaena plants was relatively low under control treatment and increased with increasing salt concentration in irrigation water.

The recorded values were 1.18 milligram free proline per gram leaf fresh weight for control plants and increased to 1.22, 1.58 and 1.64 milligram per gram leaf fresh weight for plants grown under salinity stress of 2000, 4000 and 8000 ppm, respectively. It is clear that salinity stress at 2000 ppm caused a negligible increase of 3.4% in proline content more than the control. Whereas, plants grown under salinity stress of 4000 and 8000 ppm showed a prominent increase of 33.9 and 39.0% in proline content more than the control, respectively.

3.5.2. Crude protein

Data presented in Table (6) clearly show that the percentage of crude protein was 23.4 in leaves of control plants and it was 23.18, 21.62 and 21.31 in leaves of plants grown under salinity stress of 2000, 4000 and 8000 ppm, respectively. Such finding indicated that the assigned low concentration of 2000 ppm salinized water showed a negligible decrease of 1% in protein content less than the control Whereas, the other two concentrations of 4000 and 8000 ppm salinized water decreased protein content by 7.6 and 8.9% less than

the control, respectively.

From the above mentioned results of the chemical analysis, it could be stated that increasing salt concentration in irrigation water from 2000 ppm up to 8000 ppm increased proline content and decreased protein percentage, to a less extent, in leaves of leucaena plants eight month old in comparison with control plants. This could be attributed to the breakdown of protein from its precursors and the conversion of some amino acids resulted from the degradation of protein such as glutamic and asparatic acids to proline (Morris *et al.*, 1969 and Reid and Wample, 1985).

In this connection, Pessarakli (1994) stated that the accumulation of soluble proline in leaves of many higher plant species could be induced by environmental stresses such as light, temperature, drought and salinity. Also, it was found that the amount of proline accumulation correlates with the degree of salinity, being in agreement with the present findings. Flowers *et al.*, (1977) postulated that proline may function as a compatible solute which had an important role of balancing cytoplasmic and vacular water potentials. Ridge *et al.*, (1993) pointed out that proline may serve as a substrate for respiration, an energy source and a storage compound for the recovering plant following stress.

4. REFERENCES

Anonymous (1975). Official Methods of Analysis of the Association of the Official Analytical Chemists (A.O.A.C.). 12th Edit., Published by A.O.A.C., Washington D.C.

Anonymous (1985). International Rules for Seed Testing. International Seed Testing Association, Seed Sci. and Technol., 13 : 299-355.

Ayensu E. S. (1981). Firewood Crops, Shrubs and Tree Species for Energy Production. National Academy of Sciences, National Academy Press, Washington D.C., USA.

Bates L. S., Waldren R. P. and Teare I. D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, *39* : 205-207.

Cavalcante A. and Perez A. (1995). Effects of water and salt stresses on germination of *Leucaena leucocephala* (Lam.) de Wit. seeds. Pesquisa-Agropecuaria-Brasileira, 30 (2) : 281-289.

El-Gabaly M. M. (1975). Reclamation and mangement of salt affected soil. Intern. Symposium on new developments in the field of salt affected soils. Dec. 4-9, p. 401-434, Ministry of Agriculture, Egypt.

Flowers T.J., Troke P.F. and Yeo A. R. (1977). The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol., *28* : 89-121.

Hansen E. H. and Munns D. N. (1988). Effects of $CaSO_4$ and NaCl on growth and nitrogen fixation of *Leucaena leucocephala*. Plant and Soil, *107* (1): 95-99.

Hyder S. Z., Al-Thamari M. A. and Al-Eyyed S. (1984). Effect of salt stress on germination and seedling development in *Leucaena leucocephala*. Arab Gulf Journal of Scientific Research, 2 (2): 711-720.

Morris C. J., Thompson J. F. and Jonson C. M. (1969). Metabolism of glutamic and N-acetylglutamic acid in leaf discs and cell free extracts of higher plants. Plant Physiol., 44 : 1023-1026.

Nassar M. A. and El-Sahhar K. F. (1998). Botanical Preparations and Microscopy (Microtechnique) Academic Bookshop, Dokki, Giza, Egypt, 219 pp. (In Arabic).

Niazi M. L. K., Haq M. I. and Malik K. A. (1985). Salt tolerance studies on ipil ipil (*Leucaena leucocephala*) cv. K-8. Pakistan Journal of Botany, *17* (1) : 43-47.

Panchaban S., Katawatin R. and Srisataporn P. (1989). Effect of salinity on growth of fast growing trees. Kaen Kaset Khon Kaen Agriculture Journal, Thailand, *17* (2) : 91-99.

Pessarkli M. (1994). Handbook of Plant and Crop Stress. Marcel Dekker, Inc. New York, Basel, Hong Kong. 659 pp.

Pregl F. (1945). Quantitative Organic Microanalysis. 4th. Edit. J. and A. Churchill Ltd., London.

Rab N., Hussain A. and Makhdum M. I. (1989). Effects of sucrose on seed germination of ipil ipil (*Leucaena leucocephala*) at different salinity levels. Pakistan Journal Scientific and Industrial Research, *32* (1) : 55-56.

Reid D. M. and Wample R. L. (1985). Water relations and Plant hormones. In Encyclopedia of Plant Physiology. New Series, Volume II. Editors : A. Pirson, Gottingen and M. H. Zimmermann, Haruard springer - Verlag. Berlin, Heidelberg, New York, Tokyo.

Ridge I., Murphy P., Bell M. and Parker P. (1993). Plant Physiology. Biology form and function. Edit. Irene Ridge. Hodder and Stoughton Ltd. The open University, UK.

Seatz L. F., Sterges A. T. and Kramer S. R. (1958). Anion effect of plant growth and anion composition. Soil Sci. Soc. Amer. Proc., *22* (6) : 149-152.

Snedecor G. W. and Cochran W. G. (1982). Statistical Methods. The Iowa State University Press. 7th. Edit., 2nd. Printing. 507 pp.

دراسات مورفولوجية وتشريحية على نباتات اللوسينا النامية تحت إجهاد مستويات مختلفة من ملوحة ماء الرى

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ملخص

أجريت تجربتان مستقلتان، التجربة الأولى في معمل قسم بحوث تكنولوجيا البذور بمعهد بحوث المحاصيل الحقلية بمركز البحوث الزراعية بالجيزة خلال شهر فبراير 1998 بهدف دراسة تأثير مستويات مختلفة من الملوحة (صفر ،2000 ،4000 ، 6000 ، 8000 ، 10000، 12000 جزء فى المليون من مخلوط ملح كلوريد الصوديوم وملح كلوريد الكالسيوم بنسبة 1 : 1 بالوزن) على إنبات بذور اللوسينا. كانت التجربة الثانية تجربة أصص وأجريت فى مشتل قسم الغابات بمعهد بحوث البساتين بمركز البحوث الزراعية بالجيزة خلال موسمين متتاليين هما 1998 و 1999 لدراسة الصفات المورفولوجية والتشريحية للنمو الخضرى لنباتات اللوسينا النامية تحت إجهاد مستويات مختلفة من ملوحة ماء الري (صفر، 2000، 4000 ، 8000 جزء في المليون من مخلوط ملحي كلوريد الصوديوم و كلوريد الكالسيوم بنسبة 1 : 1 بالوزن). كما تم دراسة تأثير الملوحة على محتوى أوراق اللوسينا من البروتين الخام والبرولين الحر.

تشير أهم النتائج الرئيسية أن نباتات اللوسينا تستطيع النمو جيدا تحت مستوى ملوحة ماء ري 2000 جزء في المليون دون أي تأثير على الصفات المورفولوجية للنمو الخضري. وفي نفس الوقت يؤدى زيادة تركيز الملح فى ماء الري إلى حدوث نقص معنوي في جميع الصفات المورفولوجية تحت الدراسة (ارتفاع النبات، قطر الساق، الوزن الرطب والجاف للساق، عدد الأوراق المركبة المتكونة على النبات والوزن الرطب والجاف لأوراق النبات) ويزيد معدل النقص تدريجيا بزيادة تركيز الملح وكان أقصى نقص تحت مستوى ملوحة ماء ري 8000 جزء في المليون. لذا يمكن القول أن نباتات اللوسينا تستطيع أن تتحمل ملوحة ماء الري حتى 8000 جزء في المليون ولكن مع حدوث نقص معنوي في النمو الخضري.

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