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Effect of antioxidants on some morphological and anatomical features of maize grown under salinity conditions

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Abstract: The present investigation was conducted at the greenhouse of the National Research Center, Dokki, Giza, Egypt, studies were conducted to evaluate the effect of salinity and some antioxidants on some morphological and anatomical characters of *Zea mays* L. cv. single hybrid 10. The treatments of salinity were by irrigation with diluted Red Sea water {0 (tap water), 2500 and 5000} ppm. Treatments with [Ascorbic acid (Vitamin C), Citric acid and Tochopherols (Vitamin E)] as antioxidants were at the concentration of 200 ppm, and their interaction with salinity treatments .The results showed that, a significant decrease of plant height, stem, leaves and top dry weight, with the increase of salt concentration in the water of irrigation. Also, an increased of plant height, number of leaves, stem diameter, area of leaves, dry weight of leaves and leaves and stems were recognized with spraying of antioxidants. However, Ascorbic acid gave the highest values of plant height, stem diameter, number and area of leaves as well as dry weight of stem, leaves and top under irrigation by 2500 ppm saline water similar results were obtained as increment of stem diameter, number of leaves, dry weight of stem and leaves and top under irrigation by saline water at 5000 ppm and foliar application with Tocopherols at 200 ppm as comparing to the other treatment with antioxidants. From anatomical point view, an increase of both leaf blade thickness and length of vascular bundles under low salinity level, were recognized whereas, a reduction in all studied characters under high salinity level was observed as comparing to the control. Antioxidants led to increase in thickness of mesophyllic tissue as well as length and width of leaves vascular bundles as comparing to the control. Also, antioxidants interacted with high salinity level and caused increment in thickness of leaf blade, mesophyllic tissue, length and width of vascular bundles in maize leaves as comparing to salinity treatments alone.
Keywords: *Zea mays* L. salinity, antioxidants, growth and leaf anatomy.

Introduction

Maize (*Zea mays* L.) is one from the main important crop in the summer cropping system in Egypt for its importance as a fodder crop, in oil and starch industries and recently as a biofuel crop. Salt stress adversely affected the growth metabolic processes and yield of maize as mentioned by different authors among of them **[1]** and **[2]**. Antioxidants application considered as one from the recent ways for enhancing the salt tolerance in different plants. Citric acid also, used in control of diseases and to improve growth of plants through its effect on scavenging of free radicals and photosynthetic activity **[3]** and **[4].**

Ascorbic acid functions as antioxidant, enzyme cofactor, and a precursor for oxalate and tartarate synthesis in plants. It participates in a variety of processes, including photosynthesis, photoprotection, of cell cycle, cell-wall growth and cell expansion, synthesis of ethylene, gibberellins, anthocyanins, and hydroxyproline. It also play an important role in resistance to environmental stresses, **[5].** Ascorbic acid is

synthesized partly in the cytosol and the mitochondria, and it is also found in the apoplast, vacuoles, peroxisomes and chloroplasts **[6]** mentioned that, although Ascorbic acid (ASA) is one of the most important and abundantly occurring water soluble antioxidants in plants, relatively little is known about its role in counteracting the adverse effects of salt stress on plant growth.

Many studies were carried out on the relationship between Tocopherols and a biotic stresses: **[7]**, **[8]** and **[9]**. Therefore, this study was designed to investigate the effect of antioxidants in growth and anatomical structure of leaves of maize plants grown under salinity conditions.

Material and Methods

A Pot experiment was conduced in the greenhouse of the National Research Center at Dokki, Giza, Egypt. During the summer season of 2013 to evaluate the effect of salinity and some antioxidants on growth and anatomical structure of maize plants cv. single hybrid 10. The treatments of salinity by irrigation with diluted Red Sea water {0 (tap water), 2500 and 5000} ppm.

The experiment included two levels of salinity in combination with three antioxidants more than the control treatment i.e. 12 treatments in 5 replicates arranged in completely randomized design. Metallic tin pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 30 k.g. of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2 kg of gravel, (particles about 2-3 cm. in diameter) was used to cover the bottom of the pot. Irrigation water was poured through a vertical tube (2.5 cm. In diameter), so the movement of water was from the base to upward.

Grains of maize (*Zea mays*, L. cv. single hybrid 10) were sown in 15 July, plants were thinned twice the 1st 20 days after sowing and the 2nd two weeks 35 days from sowing to three plants/pot. Calcium super phosphate (16% $P_2 05$) and potassium sulfate (48.5 % $K_2 0$) in the rate of 2.29 and 1.14 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot was added in two equal portion the 1st after two weeks from sowing and the 2nd two weeks latter. The antioxidants (Ascorbic acid, Citric acid and Tocopherols) were sprayed twice , the 1st at 21 days from sowing and the 2nd two weeks later. Control plants received the same amount of fresh water.

The data were statistically analyzed as described by [10].

The anatomical studies were carried out to investigate the changes occurred in leaves of maize (*Zea mays*, L. cv. single hybrid 10) plants as affected by antioxidants, salinity, and their interaction. In laboratory, the sections of samples were prepared by the method suggested by **[11]**. Samples were taken of one square centimeter from middle of the flag leaf of maize plants. Plant materials were immediately kept and preserved on F.A.A. solution, samples were dehydrated in series of solutions of ascending concentrations of ethyl alcohol varying from 50% to 100% ethyl alcohol. The samples were then embedded in paraffin wax m.p.58- 61^c using xylol as solvent. By using rotary microtome, sections were cut at the thickness of 15 microns and then mounted on slides with the aid of egg-albumin as an adhesive. Wax dissolved in xylol and the slides were passed through descending series of ethyl alcohol solutions varying from 100% to 50% ethyl alcohol concentrations in descending order. The sections on the slides were stained with safranin and light green, then the colored sections were kept as permanent preparations on the slides with Canada balsam as mounting medium. All photographs were prepared by Nikon Camera on a Carl Zeiss Jena microscope photographs.

Results and Discussion

Morphological studies :

Effect of salinity on growth characters:

The effect of salinity on the growth of maize plants presented in Table (1) the data showed that, generally, all growth characters i.e. plant height, stem diameter, number and area of green leaves, dry weight of stem, leaves and top were significantly differ only for stem diameter, area of leaves, stem, leaves and top dry weights, which, continuously decrease with the increase of salt concentration in the water of irrigation. This may be due to the adverse effect of salt stress in one or more of metabolic processes; photosynthetic activity

[12], enzymes and anti-oxidant activity, **[13],** protein metabolism **[14]**, Water adjustment **[15]**, mineral disruption **[16] and [15]** and growth regulators **[1]** and **[17].**

Effect of antioxidants on growth characters:

Data in Table (1) pointed out that, various Antioxidants (Citric acid, Ascorbic acid and Tocopherols) increased plant height without significant differences. A positive relationship between spraying Tocopherols and stem diameter, area of leaves, dry weight of leaves, and stems and top were also detected.

Data also showed that, the highest values were in plant height number and area of leaves and stem, leaves and top dry weight when plants sprayed by Tocoperols. In this regard **[6]** reported the favorable effect of exogenous Ascorbic acid application on two wheat varieties (one sensitive and the other tolerate to salt stress). **[9]** revealed that, spraying Tocophrols increased the dry weight of top and bulb of onion plants. Also, **[18]** revealed that, Tocopherols synthesis is regulated in plant responses to environmental stress and "stress hormones" such as Jasmonic acid, Salicylic acid and Abscisic acid. Such type of results were recently accepted by **[19]** they reported that, Cinnamic acid induced changes in reactive oxygen species scavenging enzymes and protein profile in maize (*Zea mays* L.) plants grown under salt stress.

The interaction between salinity levels and foliar application with Antioxidants on growth characters:

The interactive effects of salinity and antioxidant on the growth presented in Table (1). Such interaction was significantly affected in stem diameter, area of leaves as well as stems, leaves and top dry weights. However, differences in plant height and number of green leaves were non-significantly affected. Application of Ascorbic acid under low salinity level resulted in highest value of plant height, area of leaves and stems, leaves and top dry weights. Tocopherols surpassed the other antioxidant used for stem diameter, number of leaves, stems and leaves and top dry weights under salt stress irrigation by 5000 ppm saline water. However, Citric acid treatment was superior in the effect on plant height, number and area of leaves.

From data presented in Table (1) it could be concluded that, Ascorbic acid was more affective under the low salinity level. However, Tocopherols was more affective under the high salinity level and under irrigation by tap water.

Table (1). Effect of Antioxidants on some growth features of maize plant grown under salinity conditions.

Characters / Treatments (ppm)	Plant height (cm)	Stem diameter (cm)	Number of leaves	Area of leaves	Dry weight (g)		
					Stem	leaves	Top
Control (Tap water)	118	1.83	9.5	3108	7.37	14.32	21.69
Salinity at level (2500)	109	2.00	8.0	2268	7.02	6.71	13.77
Salinity at level (5000)	92	1.57	7.3	1692	5.59	6.66	12.25
Ascorbic acid (200)	132	2.27	9.0	3857	7.81	13 14	20.95
Citric acid (200)	133	2.20	9.0	3752	7.83	12.25	20.08
Tocopherols (200)	139	2.23	10.0	4114	10.49	15.31	25.80
Salinity at level (2500) + Ascorbic acid (200)	136	1.90	9.8	3752	8.01	14.81	28.85
Salinity at level (2500) + Citric acid (200)	128	2.13	8.8	3228	6.10	12.85	18.95
Salinity at level (2500)+ Tocopherols (200)	122	1.83	8.5	3307	6.79	12.82	1964
Salinity at level (5000)+ Ascorbic acid (200)	102	1.93	8.3	2744	5.42	6.52	11.94
Salinity at level (5000)+ Citric acid (200)	108	1.80	9.0	3498	5.42	9.51	14.93
Salinity at level (5000)+ Tocopherols (200)	105	2.00	9.0	3342	7.07	9.76	16.83
L.S.D at 5%	N.S	0.33	N.S	1301	2.34	8.29	7.52

Anatomical studies :

Effect of salinity on epidermal layers:

Both leaves abaxial and adaxial epidermal layers of *Zea mays* cv. single hybrid 10, consists of uniseriate layer of epidermal cells as presented in Figs. (1, 2 and 3). The data presented in Table (2) clearly revealed that, both salinity levels were reduced thickness of the adaxial epidermal layer by – 20.00% less than the control.

Morever, increasing salinity level from 2500 ppm to 5000 ppm was depressed thickness of both adaxial and abaxial epidermal layers by -20% less than the control as presented in (Table 2 and Figs. 1, 2 and 3). Such response could be a common effect of salinity stress that reduced blade expansion and total transpiring area. Similar results were observed as an effect of salinity stress on barely **[20]** and **[21].**

Effect of antioxidants on epidermal layers:

The effect of foliar application with the antioxidants (Ascorbic acid, Citric acid and Tocopherols) on the thickness of the epidermal layers were inconstant as presented in Table (2) and (Figs. 4, 5 and 6). Foliar application of Ascorbic acid enhanced thickness of the abaxial epidermal layer by +20% more than the control, while thickness of the adaxial epidermal layer was similar to the control. Furthermore, the epidermal cells appeared more regular and clear in shape (Fig. 4) as comparing to the control (Fig. 1). This effect could be due to the effect of Ascorbic acid on cell wall expansion and cell enlargement through it's action in vaculatization.

This was also in agreement with the results obtained by **[22],** who recorded increase in thickness of the epidermal layers of tomato plants treated with 200 ppm of Ascorbic acid.

Foliar application of Citric acid resulted in thickness of the epidermal layers similar to that obtained under salinity level of 2500 ppm. However, the epidermal cells appeared, regular, developed and clear in shape (Fig. 5) as comparing to the control (Fig. 1). On the contrast, foliar application with Tocopherol not only resulted in thickness of epidermal layers similar to that recorded under high salinity level (5000 ppm), but also, the epidermal cells were confused and depressed in shape (Fig. 6) as comparing to the control (Fig. 1).

The interaction between salinity levels and foliar application with Antioxidants on epidermal layers:

The data presented in Table (2) and illustrated in (Figs. 7, 8 and 9) showed that, under low salinity level of 2500 ppm, foliar application with antioxidants was protected and improved shape of the epidermal layers, however, their thickness was reduced as comparing to both the control and low salinity level of 2500 ppm .

On the other side of view, the interaction treatments between high salinity level of 5000 ppm was resulted in distressed shape of epidermal layers as comparing to the control, such response was associated with reduction on thickness of both upper and lower epidermal layers. Such effect mean's that under higher level of salinity (5000 ppm) the effect of antioxidants were slightly as comparing to both the control (Fig. 1) and high salinity level of 5000 ppm (Figs.10,11 and 12).

Effect of salinity on mesophyllic tissue :

The mesophyllic tissue of plants irrigated with low salinity level appeared swollen in shape as comparing to the control. Such effect was increased thickness of mesophyllic tissue by + 27.27% more than the control whereas, increasing salinity level from 2500 ppm to 5000 ppm was reduced mesophyllic tissue by – 27.27% less than the control. This response appeared to be due to the compacted, pressed and dark colored of parenchymatous cells in mesophyllic tissue (Figs. 2 and 3) as comparing to the control (Fig. 1). This mean's that such level of salinity hardly affected parenchymatous cells in mesophyllic tissue as well as intercellular spaces between parenchymatous cells in mesophyllic tissue.

Effect of antioxidants on mesophyllic tissue:

The effect of foliar application with antioxidants on mesophyllic tissue**,** showed that Ascorbic acid, Citric acid and Tocopherols were clearly improved shape and size of mesophyllic tissue as presented in Table (2) and illustrated in Figs (4, 5 and 6) as comparing to the control (Fig. 1). The thickness of mesophyllic tissue was increased as a result of foliar application with Ascorbic acid and Tocopherols by (+36.36% and +9.09%) respectively more than the control. While treatment with Citric acid was resulted in thickness of mesophyllic tissue similar to the control.

Also, more regular and develop shapes of parenchymatous cells in mesophyllic tissue was observed under treatments of foliar application of Ascorbic acid and Citric acid as in (Figs. 4 and 5) and comparing to the control (Fig. 1).

The interaction between salinity levels and foliar application with Antioxidants on mesophyllic tissue:

The effect of interaction between salinity levels and foliar application with antioxidants on mesophyllic tissue, showed variable anatomical changes which could be detected on shape and size of mesophyllic tissue as presented in (Table 2) and Figs. (7-12) and comparing to the control (Fig. 1). Ascorbic acid treatments under low and high salinity levels were slightly reduced thickness of mesophyllic tissue by -9.09% less than the control, such response was associated with disorder and scattered shape of parenchymatous cells in mesophyllic tissue. However, interaction treatments between low salinity level and foliar application of Citric acid was resulted in thickness of mesophyllic tissue similar to the control. This response was associated with more clear develop and regular shapes of mesophyllic tissue parenchymatous cells under low salinity level. Whereas, under high salinity level it was enhanced mesophyllic tissue thickness by + 18.18% more than the control and by + 62.5% more than the high salinity level of 5000 ppm alone (Figs.1, 8 and 11). Under high salinity level Citric acid treatment could not ameliorate the effect of high salinity level on shape of parenchymatous cells in mesophyllic tissue which appeared disorder in shape.

Under low salinity level, the interaction treatment between low salinity level of 2500 ppm and foliar application with Tocopherols was enhanced thickness of mesophyllic tissue by +36.36% more than the control. Furthermore, the interaction treatment between high salinity level of 5000 ppm and foliar application with Tocopherols the thickness of mesophyllic tissue was constant and similar to the control .

Effect of salinity on vascular bundles :-

Low salinity level (2500 ppm) was enhanced both length and width of the leaves vascular bundles Table (2) as comparing to the control. Such response was accompanied with large metaxylem vessels and extension occurred on area occupied by phloem and sclerenchymatous tissue .

On the other side of view, high salinity level (5000 ppm) was reduced both length and width of the leaves vascular bundles, such response was run parallel with the reduction occurred in extension of metaxylem vessels and area occupied by phloem and sclerenhcymatous tissues which appeared compacted in shape.

The leaves vascular bundles appeared pressed, compacted and darked in colour. Such effects leads to reduce the transport of assimilates from the leaves in this regard **[23]** demonstrated that, reduced area of xylem vessels resulted in more resistance flow of water which required more energy to transport any quantity of water from root to the leaves.

The effect of foliar application with Antioxidants on vascular bundle:

Ascorbic acid, Citric acid and Tocopherols were resulted in improved shape and size of vascular tissues as presented in Table (2) and illustrated in Figs. (4, 5 and 6) as comparing to the control (Fig. (1). Length and width of vascular bundles were increased as a result of foliar application with Ascorbic acid and Tocopherols as comparing to the control. While treatment with Citric acid led to increase in length of vascular bundle but, width of vascular bundle was similar to the control.

The interaction between salinity levels and foliar application with Antioxidants on vascular bundles:

The interaction between salinity levels of 2500 ppm and foliar application with the growth factors (Ascorbic acid, Citric acid and Tocopherols) were decreased length and width of vascular bundles except vascular bundle in plants treated with Ascorbic acid and Citric acid which increased in length and width of vascular bundle by +10.00% and + 8.70% respectively more than the control.

Anatomical examination of the flag leaves/cross–sections revealed clear destruction of the vascular system in interaction treatments with salinity level of 5000 ppm and foliar application with Ascorbic acid, Citric acid and Tocopherols as presented in (Figs. 10, 11 and 12). However, the interaction between high salinity level of 5000 ppm and foliar application of Ascorbic acid, Citric acid and Tocopherol was resulted in increasing length of vascular bundles as comparing to plants treated with high saline water alone. similar response was also detected in width of vascular bundles except in case of interaction treatment of high salinity level with Ascorbic acid treatment which decreased width by -4.34% less than the control.

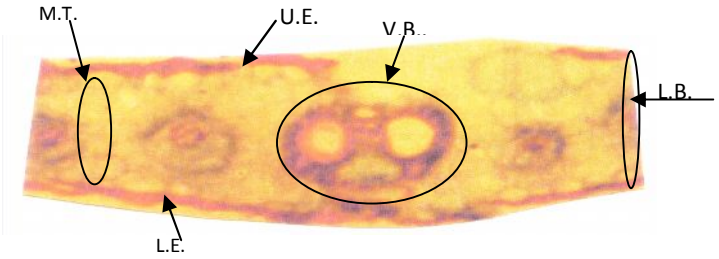


Fig.1 1

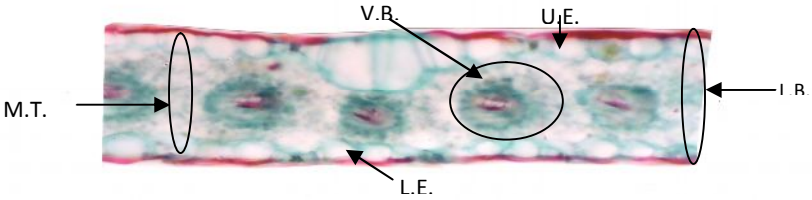


Fig.2

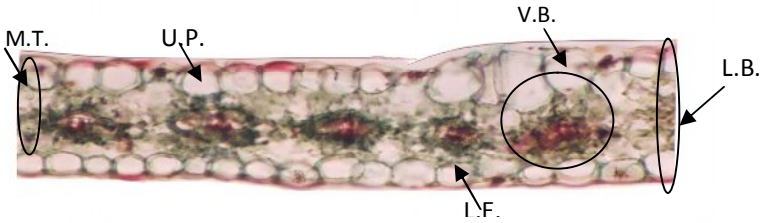


Fig.3

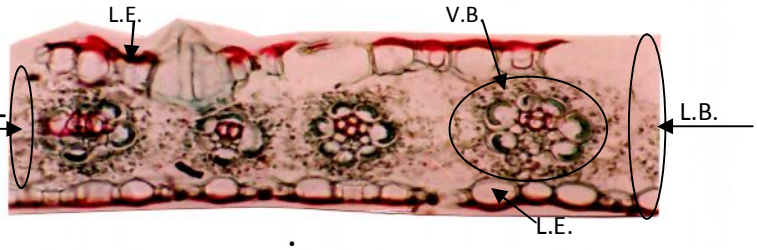


Fig.4

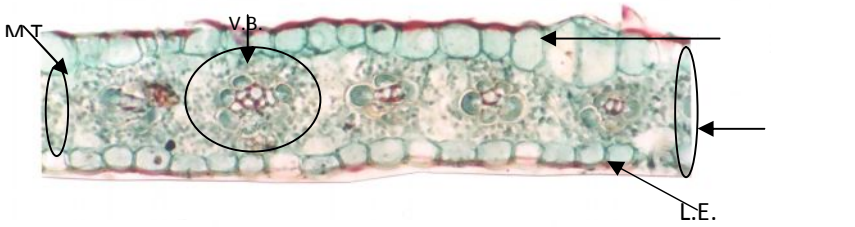


Fig. 5

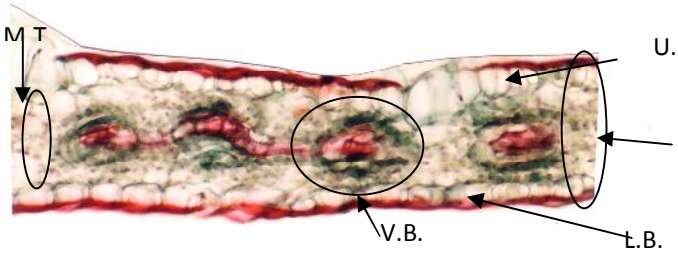
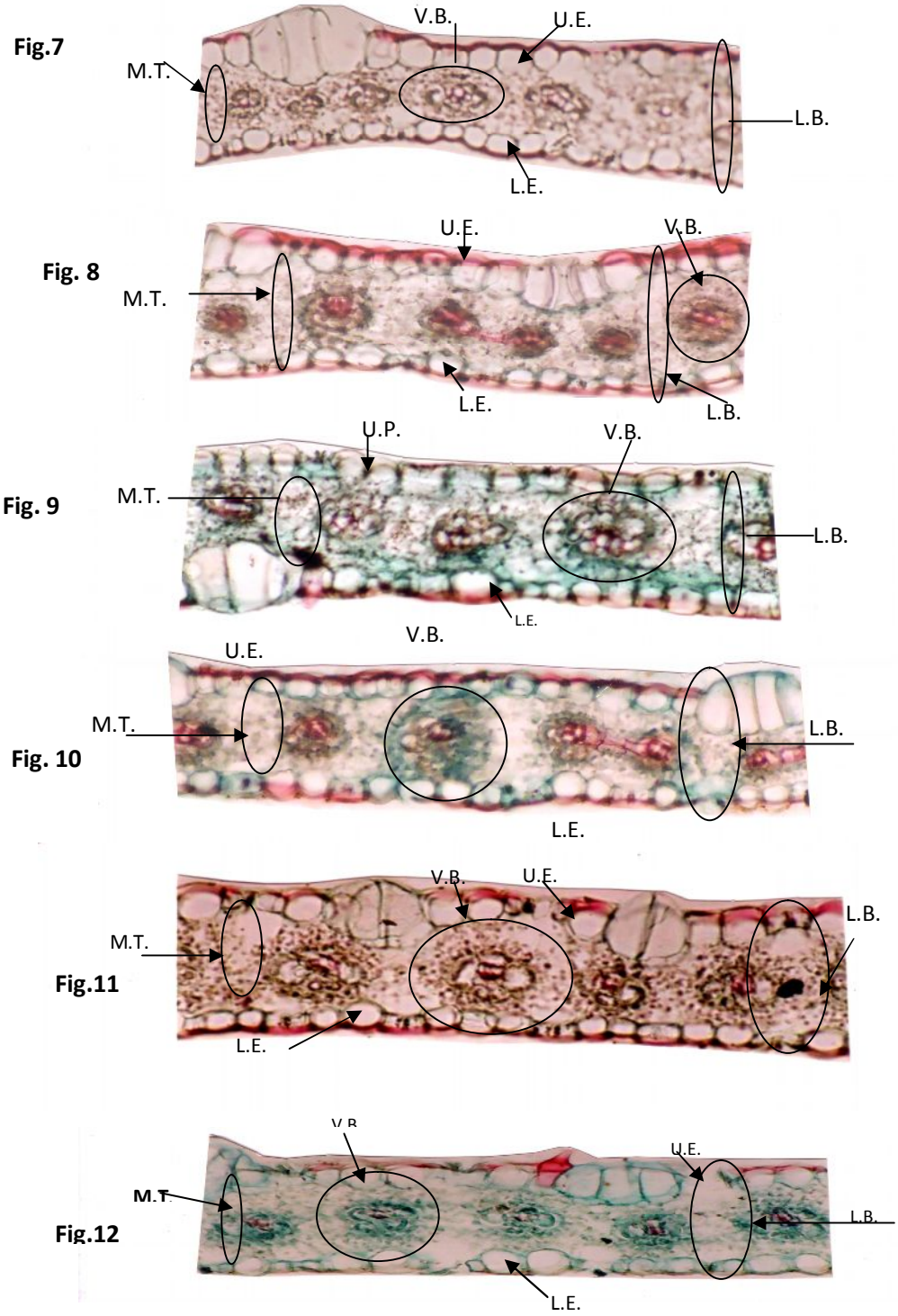


Fig. 6



Abbreviations of maize flag leaves anatomical characters (*Zea mays* cv. Single hybrid 10).
L.B. = Leaf blade (µm.) , L.E. = Lower epidermis (µm.) , M.T. = Mesophyll tissue (µm.), U.E. = Upper epidermis (µm.) and V.B. = Vascular bundle (µm.).

Figures (1 — 12): Photomicrographs of the Cross-sections through blade of flag leaves of maize (*Zea mays* cv. single hybrid 10) plants. (All Figs. 20X)
Fig. (1): Control
Fig. (**2**): Plant leaves treated with salinity at 2500 ppm,
Fig. **(3):** Plant leaves treated with salinity at 5000 ppm.
Fig. (4): Plant leaves treated with Ascorbic acid (200 ppm).
Fig. **(5)**: Plant leaves treated with Citric acid (200 ppm).
Fig. (6): Plant leaves treated with Tochopherols (200 ppm).
Fig. (7): Plant leaves treated with salinity at 2500 ppm supplemented with Ascorbic acid (200) ppm.

Fig. (8): Plant leaves treated with salinity at 2500 ppm supplemented with Citric acid (200) ppm.
Fig. (9): Plant leaves treated with salinity at 2500 ppm supplemented with Tochopherols (200)ppm.
Fig. (10): Plant leaves treated with salinity at 5000 ppm supplemented with Ascorbic acid (200) ppm.
Fig. (11): Plant leaves treated with salinity at 5000 ppm supplemented with Citric acid (200) ppm.
Fig. (12): Plant leaves treated with salinity at 5000 ppm supplemented with Tochopherols (200) ppm.

Therefore, it could be concluded that, foliar application of the Ascorbic acid and Tocopherols under salinity level of 2500 ppm could not improved anatomical response of vascular tissues, but Citric acid treatment partially reduced harmful effect of salinity.

Effect of salinity on leaf blade:-

Effect of salinity levels on leaf blade thickness was presented in Table (2) the results showed that, salinity level of 2500 ppm was increased leaf blade thickness by (+9.52%) more than the control. Such response was associated with the increase in mesophyllic tissue but, salinity level of 5000 ppm, led to reduction of leaf blade thickness by – 23.81% less than the control. Such response was correlated with the inhibition occurred on thickness of the mesophyllic tissue and both adaxial and abaxial epidermal layers as presented in Figs. (2 and 3) and comparing to the control Fig.(1) .

The effect of foliar application with antioxidants on leaf blade:

The data in Table (2) showed that, foliar application with Citric acid and Tocopherols were reduced leaf blade thickness by – 4.76% less than the control. While foliar application with Ascorbic acid was increased leaf blade thickness by + 23.81% more than the control, this response was mainly due to the increase occurred on mesophyllic tissue and epidermal layers thickness as presented in Figs. (4, 5 and 6).

The interaction between salinity levels and foliar application with antioxidants on leaf blade.

The data in Table (2) showed that, under salinity level of 2500 ppm and foliar application with Ascorbic acid and Citric acid, the leaf blade thickness was decreased by (- 19.05% and - 4.76%) respectively less than the control, but the effect of Tocopherols under the same salinity level led to increase in thickness of leaf blade by + 14.29% more than the control. Such response was corresponding with the increase recorded on mesophyllic tissue and length of vascular bundle as presented in Figs. 7,8 and 9.

Under salinity level of 5000 ppm and foliar application of Ascorbic acid and Tocopherols, the leaf blade thickness was reduced by (- 14.29% and – 4.76%) respectively less than the control. While foliar application with Citric acid under the same level of salinity led to increase in leaf blade thickness by (+ 4.76%) more than the control. Such response was corresponding with the increase in mesophyllic tissue thickness as presented in Table (2) and Figs. 10, 11 and 12. The present results was in complete accordance with the data obtained by **[24]** in sorgum plants, it was found that, low salinity level (1500 ppm NaCl) increased the blade thickness, xylem and phloem tissues thickness and metaxylem vessels diameter as well as main vascular bundle dimensions. At the same time, moderate and high salinity levels (3000 and 6000 ppm NaCl) decreased all these parameters. The great reduction was observed under high salinity level. It also concluded that, Ascorbic acid or glycinebetaine had a stimulating effect in this respect and glycinebetaine proved to be more effective in this respect particularly in case of pre-soaking plus spraying method. Also, **[25]** studied the effect of salinity on kallar grass leaves and stems which showed a significant decrease in midvein thickness, lamina thickness, mesophyllic thickness and mesophyllic area along leaf axis with increasing salinity level.

Table (2). Effect of antioxidants on anatomical characters of maize flag leaves under salinity conditions

Characters (µm.) / Treatments (ppm)	Leaf blade thickness			Epidermal layers thickness						Mesophyllic tissue thickness			Vascular bundles measurements					
				Adaxial			Abaxial						Length			Width		
	Absolute value	% ± of control	% ± of salinity levels	Absolute value	% ± of control	% ± of salinity levels	Absolute value	% ± of control	% ± of salinity levels	Absolute value	% ± of control	% ± of salinity levels	Absolute Value	% ± of control	% ± of salinity levels	Absolute Value	% ± of control	% ± of salinity levels
Control (Tap water)	168	00.00	*	40	00.00	*	40	00.00	*	88	00.00	*	160	00.00	*	184	00.00	*
Salinity at level (2500)	184	+9.52	00.00	32	-20.00	00.00	40	00.00	00.00	112	+27.27	00.00	200	+25.00	00.00	184	00.00	00.00
Salinity at level (5000)	128	-23.81	00.00	32	-20.00	00.00	32	-20.00	00.00	64	-27.27	00.00	144	-10	00.00	160	-13.04	00.00
Ascorbic acid (200)	208	+23.81		40	00.00		48	+20.00		120	+36.36		200	+25.00		240	+30.43	
Citric acid (200)	160	-4.76		32	-20.00		40	00.00		88	00.00		200	+25.00		184	00.00	
Tocopherols (200)	160	-4.76		32	-20.00		32	-20.00		96	+9.09		200	+25.00		200	+8.70	
Salinity at level (2500) + Ascorbic acid (200)	136	-19.05	-26.08	24	-40.00	-25.00	32	-20.00	-20.00	80	-09.09	-28.57	176	+10.00	-12.00	176	-4.34	-4.34
Salinity at level (2500) + Citric acid (200)	160	-04.76	-13.04	32	-20.00	00.00	40	00.00	00.00	88	00.00	-21.42	160	00.00	-20.00	200	+8.70	+8.70
Salinity at level (2500)+ Tocopherols (200)	192	+14.29	+4.34	32	-20.00	00.00	40	00.00	00.00	120	+36.36	+7.14	160	00.00	-20.00	160	-13.04	-13.04
Salinity at level (5000)+ Ascorbic acid (200)	144	-14.29	+21.73	32	-20.00	00.00	32	-20.00	00.00	80	-9.09	+25.00	176	+10.00	+22.22	176	-4.34	+10.00
Salinity at level (5000)+ Citric acid (200)	176	+4.76	-4.34	32	-20.00	00.00	40	00.00	+25.00	104	+18.18	+62.5	184	+15.00	+27.77	200	+8.70	+25.00
Salinity at level (5000)+ Tocopherols (200)	160	-4.76	-13.04	32	-20.00	00.00	32	-20.00	00.00	88	00.00	+37.5	200	+25.00	+38.88	200	+8.70	+25.00

References

1. Hussein, M.M., Abd EI-Khadr, A.A., Kady, K.A. and R.A. Youssef, (2007). Effect of salinity and supplementary phosphorus and potassium on growth, yield, nutrition development and anatomical structure of sorghum plants. 8th National Conference Egyptian Soil Science Society: Soils and water studies in sustainable agriculture systems. Nov. 25 – 26.
2. Navairo, A. Banon S., Olmos, E. and M.J. Sanchez-Blanco (2007). Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. Plant Science, 172 : 473-480.
3. Rivasseau , C.; Boisson, A.N.; Mongelard, G.; Couram, G.; Bastien, O. and R. Bligny, (2006). Rapid analysis of organic acids in plant extracts by capillary electrophoresis with indirect UV detection Directed metabolic analyses during metal stress. Journal of Chromatography A, 1129: 283-290.
4. Keutgen, A. and E. Pawelzik, (2007). Modifications of taste- relevant compounds in strawberry fruit under NaCI salinity. Food Chemistry, 105 : 1487-1494.
5. Smirnoff, N. and G.L. Wheeler, (2000). Ascorbic acid in plants: biosynthesis and function. Crit. Rev. Plant Sci., 19: 267-290.
6. Athar, H.R.; Khan, A. and M. Ashraf, (2007). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environmental and Experimental Botany, 63(1): 224-231.
7. Munne-Bosch S. and L. Alegre, (2002). Interplay between ascorbic acid and lipophilic antioxidant defenses in chloroplasts of water-stressed Arabidopsis plants. FEBS Lett, 524:145-148.
8. Sattler, S.E., Gillil, L.U., Magallanes, L.M., Pollard, M. and D. DellaPenna, (2004). Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. Plant Cell; 16: 1419-1432.
9. Hussein, M.M. and N.H. EI-Greatly, (2007). Inflounce of alpha-α-Tocopherol and potassium di-hydrogen phosphate on growth and endogenous phytohormones on onion plants grown under salinity stress. J. Agric. Sci., Mansoura Univ., 32(11): 9335 – 9345.
10. Snedecor, G.W. and W.G. Cochran, (1982): Statistical Methods. 7th Ed. Iowa State Univ., lowa, U.S.A.
11. Sass, J.E. (1958). Botanical Microtechnique. The lowa State University press, lowa P. 228.
12. Katerji, N.; Hoorn, J.W.;. Hamdy A. and. M. Mastrorilli, (2003). Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. Agricultural water Management Volume, 62, Issue 1: 37-66.
13. Rodriguez, A.A., Lascano HR, Bustos D, E. Taleisnik (2007). Salinity-induced decrease in NADPH oxidase activity in the maize leaf blade elongation zone. J. Plant Physiol 164, 223–230.
14. Frendo, P., Didierjean, L., Passelegue, E. And G. Burkard, (1992). A biotic stresses induce a thaumatin-like protein in maize; cDNA isolation and sequence analysis Plant Science, 85, Issue 1: 61-69.
15. Hu,Y. Zoltan Burucs, Z.; Tucher, S.V. and U. Schmidhalter, (2007). Short-term effects of drought and salinity on mineral nutrient distribution along growing leaves of maize seedlings .Environmental and Experimental Botany, June 60 Issue 2: 268-275.
16. Gunes, A. Inal, A. Alpasla ,M., Eraslan, F., Guneri, E., Bagci A. and N., Cicek, (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. Journal of Plant Physiology, 164: 728-736.
17. Tuna, A.L., Kaya; 0. Murat Dikilitas, M., and D. Higgs, (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environmental and Experimental Botany, 62, (1), 1-9.
18. Munne-Bosch S. and L. Alegre, (2005). The role of α-Tocopherol in plant stress tolerance . Journal of Plant Physiology, 162: 743-748.
19. Singh, P.K., Singh, R. and S. Singh, (2013). Cinnamic acid induced changes in reactive oxygen species scavenging enzymes and protein profile in maize (*Zea mays* L.) plants grown under salt stress. Physiol. Mol. Biol. Plants, 19 (1): 53–59.
20. Miteva, T. and S. Vaklinova,(1991). Photosynthesis photorespiration and respiration in young barley plants upon influence of NaCl. CR Acad. Bulg. Sci. 44 : 89-92.
21. Uzunova, A.N. and L.P. Popova, (2000). Effect of salicylic acid on leaf anatomy and chloroplast ultra structure of barley plants. Photosynthetic, 38 (2): 243- 250.
22. Ali, Z.A. (2001). Ascorbic acid induced anatomical changes in leaves and stems of tomato plants. Bull. NRC Egypt, 26, (3): 371-382.
23. Munns, R. and A. Termaat, (1986). Whole- plant responses to salinity Australian Journal of Plant Physiology 13, 143-160.

24. Arafa, A.A.; Khafagy, M. A. and M. F., El-Banna (2009). The effect of glycinebetaine or ascorbic acid on grain germination and leaf structure of Sorghum plants grown under salinity stress, Australian Journal of Crop Science; Vol. 3 (5): p. 294.
25. Ola, H.A.E., Reham E.F., Eisa S.S. and S.A. Habib (2012). Morpho-anatomical changes in salt stressed kallar grass (*Leptochloa fusca* L. Kunth). Res. J. Agric. Biol. Sci. 8(2): 158-166.

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