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INTERACTIVE EFFECTS OF CERTAIN VITAMINS, BIOREGULATOR AND YEAST EXTRACT ON SWEET PEPPER STEM AND LEAF ANATOMY UNDER TWO TYPES OF SALINITY.

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ABSTRACT

All salinity types at 2000 mg/L increased stem diameter due to an increase in pith diameter, cortex thickness, width of epidermis cell and vascular bundles dimensions (length as well as metaxylem vessel diameter). In addition, $CaCl_2$ and NaCl+$CaCl_2$ 1:1 (w/w) were the most effective in this respect. In addition, high salinity level (4000 mg/L) decreased most of the studied anatomical parameters. While, the pith diameter and number of vascular bundles were decreased only under NaCl at 4000 mg/L. On the other hand, pre-soaking seeds in selected chemicals used, in most cases, showed a positive effect on the stem structure and AsA at 50 mg/L or SA at 75 mg/L was the most effective in this respect.

Low level of all salinity types (2000 mg/L) increased midrib region thickness due to increasing the length of main vascular bundle. While, the highest salinity level (4000 mg/L) led to a decrease in this respect due to the decrease in length of main vascular bundle. In addition, NaCl was more effective in this respect followed by NaCl+$CaCl_2$ (1:1). On the other hand, the leaf blade (lamina) thickness was also decreased in plants grown under NaCl at 4000 mg/L followed by NaCl+$CaCl_2$ (1:1) due to a decrease in the thickness of palisade and spongy tissues as well as upper and lower epidermis width. Moreover, the application of chemicals used led to an increase in the thickness of midrib region as compared with untreated plants. In addition, SA (75 mg/L), AsA (50 mg/L) and α-tocopherol (100 mg/L) were more effective. In most cases, AsA at 50 mg/L or SA at 75 mg/L alleviated the harmful effect of salinity level (4000 mg/L) on midrib region and lamina thickness as well as the main vascular bundle dimensions when compared with untreated plants. Furthermore, AsA at 50 mg/L was more effective than the remaining treatments.

INTRODUCTION

The effect of salinity on plant caused various physiological and biological changes in plants. It damaged photosynthetic components, i.e. lipid peroxidation (Winston, 1990) and injuries to plant metabolism (Meneguzzo and Navarilzzo, 1999) and/or water deficit, ion uptake, salt-specific damages (Cumming and Elliot, 1991) and oxidative stress in plants (Xiong *et al.*, 2002). Salinity also induces water deficit, even in well-watered soils by decreasing the osmotic potential of soil solutes, thus making it difficult for roots to extract water from their surrounding media (Koca *et al.*, 2007; Sankar *et al.*, 2007). Excessive sodium (Na^+) inhibits the growth of many salt-sensitive plants and glycophytes, which include most crop plants. High concentrations of salt in soil enhanced generation of reactive oxygen species (ROS) including $^{\cdot}O_2$, H_2O_2, and $^{\cdot}OH$ (Wang *et al.*, 2008; Li, 2009). To prevent damage to cellular components by ROS, plants have developed a complex antioxidant system.

Many components of this antioxidant defense system can be found in various sub-cellular compartments (Hernandez *et al.,* 2000). The primary components of this system include carotenoids, ascorbate, glutathione and tocopherols, in addition to enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidases, and the enzymes involved in the ascorbate–glutathione cycle (Prochazkova and Wilhelmova, 2007).

Therefore, the present study aimed to clarify and alleviate the harmful effect of salinity on stem and leaf anatomy of sweet pepper plant growing in nutrient film technique (NFT) through application of certain vitamins and bio-regulator as well as yeast extract.

MATEREIALS AND METHODS

The experiment was carried out in the glasshouse of the Agricultural Botany Dept., Fac. of Agriculture, Mansoura Univ. during the growing season of 2008, to study the response of stem and leaf anatomy of sweet pepper to different sources of salinity i.e. NaCl, $CaCl_2$ and their combination (1:1 w/w); and how to minimize its harmful effects through pre-soaking seeds in vitamins (Ascorbic acid or α-tocopherol) or bio-regulator (Salicylic acid) or Yeast extract.

Plant materials

The seeds of sweet pepper (*Capsicum annuum* L. cv. Orlando), a hybrid 'California Wonder' used in this investigation were secured from the Gohara Co. Cairo, Egypt.

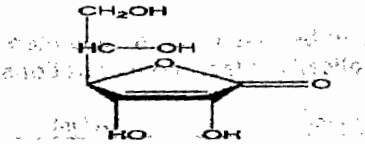
Chemicals:-

1. Vitamins, ascorbic acid Vit. C (AsA) and α-tocopherol Vit. E (α-toco.) were supplied by Sigma Chemicals Co., USA and used at the concentration of 50 or 100 mg/L each.
2. Bio-regulator, salicylic acid (SA) (2-hydroxybenzoic acid) was obtained from Sigma Chemicals, Co., USA. and initially dissolved in 100 µL dimethyl sulfoxide (Khan et al., 2003) and used at the concentrations of 75 and 150 mg/L,
3. Yeast extract, active dry yeast (*Saccharomyces cervisiae*) was applied at the concentration of 1000 or 2000 mg/L.
4. Salts:

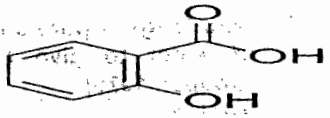
4.1. Sodium Chloride (NaCl) from EL-Gomhoria Co., Egypt and was used at the concentrations of 2000 and 4000 mg/L.

4.2. Calcium Chloride (CaCl2) from EL-Gomhoria Co., Egypt and was used at the concentrations of 2000 and 4000 mg/L.

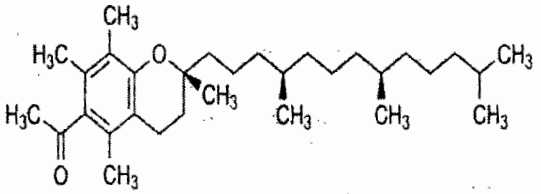
4.3. Their combination, NaCl: CaCl2 1:1 (w/w) was used at the concentrations of 2000 and 4000 mg/L.



Ascorbic acid (Vit. C)



Salicylic acid



Alpha-tocopherol (Vit. E)

Fig. (1): Structural formula of vitamins and bio-regulator used in this investigation.

Table (1): The Molarity (Mol), Electrical Conductivity (E.C.) and pH values for different nutrient solutions.

Nutrient solution (N.S.) mg/L	N.S.	N.S.+ NaCl		N.S.+ $CaCl_2$		N.S.+ {NaCl+$CaCl_2$} (1:1) w/w			
		2000 NaCl	4000 NaCl	2000 $CaCl_2$	4000 $CaCl_2$	2000(NaCl+$CaCl_2$)		4000 (NaCl+$CaCl_2$)	
						1000 NaCl	1000 $CaCl_2$	2000 NaCl	2000 $CaCl_2$
Mol (M)	0 (Control)	3.4×10^{-2}	6.9×10^{-2}	2.0×10^{-2}	3.6×10^{-2}	1.7×10^{-2}	0.9×10^{-2}	3.4×10^{-2}	2.0×10^{-2}
Ec dSm^{-1}	2.00	5.42	8.42	4.59	7.60	5.08		8.08	
pH	5.50	5.77	5.80	5.19	5.30	5.45		5.34	

After soaking, the sterilized seeds (25 seeds/dish) were placed in glass Petri dishes (11 cm) with a double layer of Whatman No. 1 filter paper. The dishes were left in an incubator in the dark for seed germination at 25 ± 2^0C and 90% relative humidity, and then dishes were covered with aluminum foils for darkness. In order to avoid water losses, 5 ml of the nutrient solution were added to Petri dishes, every 5 days. Thiram was added to the solution at a concentration of 2% (w/v) to control the fungi infection.

Table (2): Weights (g) of pure substances to be dissolved in 1000 liters of water to give the theoretically ideal concentrations (Cooper, 1979).

Substance	Formula	Weight
Potassium dihydrogen Phosphate	KH_2PO_4	263
Potassium Nitrate	KNO_3	583
Calcium Nitrate	$Ca(NO_3)_2$. 4H2O	1003
Magnesium Sulphate	$MgSO_4$. $7H_2O$	513
EDTA Iron	$CH_2.N(CH_2.COO)_2]_2$ Fe Na	79.0
Manganous Sulphate	$MnSO_4.H_2O$	6.10
Boric Acid	H_3BO_3	1.70
Copper Sulphate	$CuSO_4.5H_2O$	0.39
Ammonium Molybdate	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.37
Zinc Sulphate	$ZnSO_4.7H_2O$	0.44

Table (3): Composition of yeast extract (according to, Nagodawithana, 1991)

Constituents	Value (%)
Protein	47
Carbohydrates	33
Minerals	8
Nucleic acids	8
Lipids	4

Approximate composition of vitamins

Vitamines	Value (µg/g)
Cholin	4000
Niacin	300-500
Thiamine (B_1)	60-100
Pantorhenate (B_5)	70
Riboflavin (B_2)	35-50
Pyridoxine HCL (B_6)	28
Folic acid	5-13
Biotin	1.3
Vit. B_{12}	0.001

Approximate composition of minerals

Minerals	Value (mg/g)	Minerals	Value (µg/g)
K	21	Cu	8.00
P	13.50	Ni	3.00
S	3.90	Sn	3.00
Mg	1.65	Cr	2.20
Ca	0.75	Mo	0.40
Zn	0.17	Se	0.10
Na	0.12	Li	0.17
Si	0.03	Va	0.04
Fe	0.02	Mn	0.02

The following experiment was carried out in the glasshouse of the Agric. Bot. Dept., Fac. of Agric., Mansoura Univ. during the spring–summer period of 2008 in a glasshouse under conditions of ambient light during winter, spring and early summer, with 10/14 light/dark period at 800–1100 µmol m^{-2s-1} PPFD, a day/night average temperature cycle of 26/15 °C and 65±5% relative humidity.

The focus of the current experiment was to provide fundamental biological understanding and knowledge on sweet pepper plants growing in nutrient film technique (NFT), under different sources of salinity NaCl, $CaCl_2$ and their combinations 1:1 (w/w); and how to minimizing the harmful effects through pre-soaking seeds in vitamins (Ascorbic acid, α-tocopherol) or bio-regulators (Salicylic acid), or Yeast extract. The seeds of sweet pepper were sown on Jan, 13, 2008. A homogenous sweet pepper seeds were placed in

100 ml beakers and 20 ml of 1% sodium hypochlorite was added for sterilization. These were left in the solution for 5 min followed by washing under running tap water and ionized water twice. Then divided into 9 sets. The first set was soaked (24hours) in distilled water as control and the remaining sets (8) were separately soaked for 24 h in aqueous solution of AsA or α–toco. at (50 or 100 mg/L) each or SA at (75 or 150 mg/L) or Yeast extract at (1000 or 2000 mg/L). Then germinated in seedling trays (209 eye) containing peat moss and perlite (1:1) as a rooting medium moistured by nutrient cooper solution (Cooper, 1979). Trays containing the seeds were placed in a glasshouse at 28 $\pm 2^0$C to germinate.

The experimental layout consisted of 7 automatic hydroponic units (groups) (experimental plots). Each hydroponic unit (Figure, 2) comprised of two plastic channels (4 m long * 10 cm in diameter) placed on the one side of the holder (4m length * 1.5 m height). Each channel had 40 pores (6 cm diameter). Every unit was provided by an electric pump representing seven groups (Table, 1) nutrient solution (2.0 dSm^{-1} as a control), 2000 mg/L NaCl (5.42 dSm^{-1}), 4000 mg/L NaCl (8.42 dSm^{-1}), 2000 mg/L $CaCl_2$ (4.59 dSm^{-1}), 4000 mg/L $CaCl_2$ (7.60 dSm^{-1}), 2000 mg/L NaCl+$CaCl_2$ (1:1) (5.08 dSm^{-1}) and 4000 mg/L NaCl+$CaCl_2$ (1:1) (8.08 dSm^{-1}).

The seedlings were transplanted to the experimental installation on Feb, 26 , 2008 (after 45 days from pre-soaking) at the stage of four/five true leaves. Two uniform seedlings were transplanted to 6 cm perforated pots (reticulated) containing peat moss and perlite (1:1) as a rooting medium.

Every two channels was divided into 9 sets, the first set was soaked in distilled water (control), AsA, α–toco. at (50 or 100 mg/L) each, SA at (75 or 170 mg/L), and Yeast extract at (1000 or 2000 mg/L). Each set contained (8 replicates) 16 seedlings (two seedling/pot) spaced 10 cm representing a Nutrient Film Technique (NFT).

To keep the concentrations of sodium chloride and mineral nutrients constant, the solution was changed every 7 to 10 days and the volume of the solution was maintained by adding distilled water as required after measuring the electrical conductivity by digital conductivity meter Lutron CD-4301. A nutrient solution was pumped into the channels at a flow rate of one liter per minute from a reservoir containing 10 liters.

Sampling dates:

Stem and leaf structure:

After 30 days from transplanting specimens were taken from the midrib region at the middle part of the 3rd completely developed foliage leaf as well as from the middle part of the 3rd internode (0.5 cm) of the main stem below the shoot tip. The obtained materials were killed and fixed in formalin-acetic-alcohol solution (FAA), then washed and dehydrated in series of ethanol (50%, 70%, 80%, 90% and 100%), cleared in ethanol: xylene (3:1-1:1- 1:3 and 100% xylene) and embedded in paraffin wax (52-54°C melting point). Sections were done at 15-20 µm thick using rotary microtome and double stained with saffranin-light green 1:1 (v/v) combination, cleared in clove oil and mounted in Canada balsam (Gerlach, 1977). The sections were examined by light microscope (ten sections for each treatment).

Stem structure:
The following measurements were recorded:
Stem diameter (μ).
Epidermal cell dimensions (length and width) (μ).
Cortex thickness (μ).
Dimensions of large vascular bundle (length and width) (μ).
Pith diameter (μ).
Number of vascular bundles (large and small).

Leaf blade structure:
The following measurements were recorded:
Thickness of midrib region (μ).
Thickness of leaf blade (μ).
Width of upper and lower epidermis (μ).
Thickness of palisade and spongy tissue (μ).
Main vascular bundle dimensions (length and width) (μ).

Statistical analysis:

The obtained data were subjected to statistical analysis of variance according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Stem Structure

It could be observed from data presented in Table (4) and illustrated in Fig.(2) , that the stem diameter was increased under low salinity level (2000 mg/L) at all applied salinity types. This increase may be due to a promotive effect in increasing pith diameter, cortex thickness, width of the epidermal cell and vascular bundles dimensions (length and width as well as metaxylem diameter) .

In addition, $CaCl_2$ and NaCl+$CaCl_2$ 1:1 (w/w) were the most effective in this respect. On the other hand, the cortex thickness was decreased only under NaCl (Fig.2). On the other hand, high salinity level (4000 mg/L) of NaCl, $CaCl_2$ and their combination decreased most of the anatomical parameters i.e. stem diameter by (16%, 13% and 14%) Table (4), the cortex thickness by (28%, 34% and 38%), the width of epidermal cell by (33%, 36% and 33% respectively), the length of vascular bundle by (32%, 19% and 19% respectively). In addition, the pith diameter and number of vascular bundles were decreased only under NaCl at 4000 mg/L by 3% and 8% respectively. While, under $CaCl_2$ and NaCl+$CaCl_2$ at 4000 mg/L they increased by (3% and 4%) as well as (25% and 38%) respectively. Moreover, the metaxylem diameter under NaCl and NaCl+$CaCl_2$ was decreased by 21% and 23% respectively. But, the increase recorded under $CaCl_2$ at 4000 mg/L was about 4% only. The results obtained in this work are consistent with Casenave *et al.* (1999) who observed that cotton plants had smaller cortex and a decrease in the development of the xylem under higher salinity levels.

Table (4) Effect of NaCl, CaCl2 and their mixture or SA, AsA, α-toco. or Yeast extract as well as their combinations on sweet pepper stem structure.

Parameters / Treatment (mg/L)		Stem diameter		Pith diameter		Cortex Thickness		Epidermis dimensions				Vascular bundle dimensions (Large)				Metax-ylem diameter		Number of Vascular bundles	
								Length		Width		Length		Width					
		µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	Mm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±
Nutrient solution	Water	3807	0	1805	0	585	0	28	0	38	0	388	0	803	0	43	0	8	0
	SA 75	4174	+10	2021	+12	686	+17	26	-6	26	-31	367	-5	785	-2	42	-2	7	-13
	SA 150	3892	+2	2190	+21	439	-25	23	-16	21	-45	389	0	767	-4	42	-2	8	0
	AsA 50	4239	+11	1979	+10	706	+21	26	-6	27	-29	403	+4	867	+8	43	0	10	+25
	AsA100	4042	+6	1858	+3	667	+14	34	+23	28	-26	396	+2	685	-15	41	-6	7	-13
	α- 50	3863	+1	2218	+23	417	-29	27	-3	24	-36	385	-1	663	-17	40	-8	7	-8
	α-100	3948	+4	2124	+18	530	-9	29	+3	27	-29	346	-11	753	-6	44	+2	9	+13
	Yeast 1	3854	+1	2014	+12	517	-12	20	-29	23	-40	383	-1	731	-9	53	+23	11	+38
	Yeast 2	3920	+3	2134	+18	480	-18	23	-16	23	-38	399	+3	770	-4	36	-17	9	+13
NaCl 2000	Water	4258	+12	2350	+30	489	-16	32	+16	32	-14	432	+11	817	+2	50	+15	12	+50
	SA 75	4644	+22	2603	+44	553	-5	34	+23	33	-12	435	+12	835	+4	47	+8	7	-13
	AsA 50	4559	+20	2547	+41	539	-8	28	0	24	-36	417	+8	856	+7	46	+6	11	+38
NaCl 4000	Water	3196	-16	1749	-3	421	-28	26	-6	25	-33	264	-32	482	-40	34	-21	7	-8
	SA 75	3581	-6	1955	+8	471	-19	24	-13	24	-36	321	-17	603	-25	41	-4	9	+13
	AsA 50	3497	-8	1859	+3	460	-21	24	-13	27	-29	339	-13	681	-15	48	+10	11	+38
$CaCl_2$ 2000	Water	4418	+16	2113	+17	649	+11	28	0	29	-24	482	+24	845	+5	48	+10	11	+38
	SA 75	4926	+29	2492	+38	613	+5	28	0	32	-17	574	+48	877	+9	62	+44	11	+38
	AsA 50	5161	+36	2650	+47	663	+13	30	+6	41	+7	564	+45	981	+22	57	+31	12	+46
$CaCl_2$ 4000	Water	3316	-13	1862	+3	385	-34	23	-16	24	-36	314	-19	703	-12	45	+4	10	+25
	SA 75	3769	-1	1881	+4	564	-4	23	-16	24	-36	357	-8	660	-18	39	-10	9	+13
	AsA 50	3807	0	2174	+20	424	-27	23	-16	23	-38	367	-5	860	+7	33	-23	12	+50
$CaCl_2$ 2000	Water	4794	+26	2474	+37	521	-11	29	+3	32	-17	613	+58	1088	+36	59	+38	11	+38
	SA 75	5302	+39	2596	+44	703	+20	38	+35	35	-7	613	+58	992	+24	64	+48	12	+50
	AsA 50	5386	+41	2473	+37	728	+24	34	+23	25	-33	692	+78	1124	+40	70	+63	10	+25

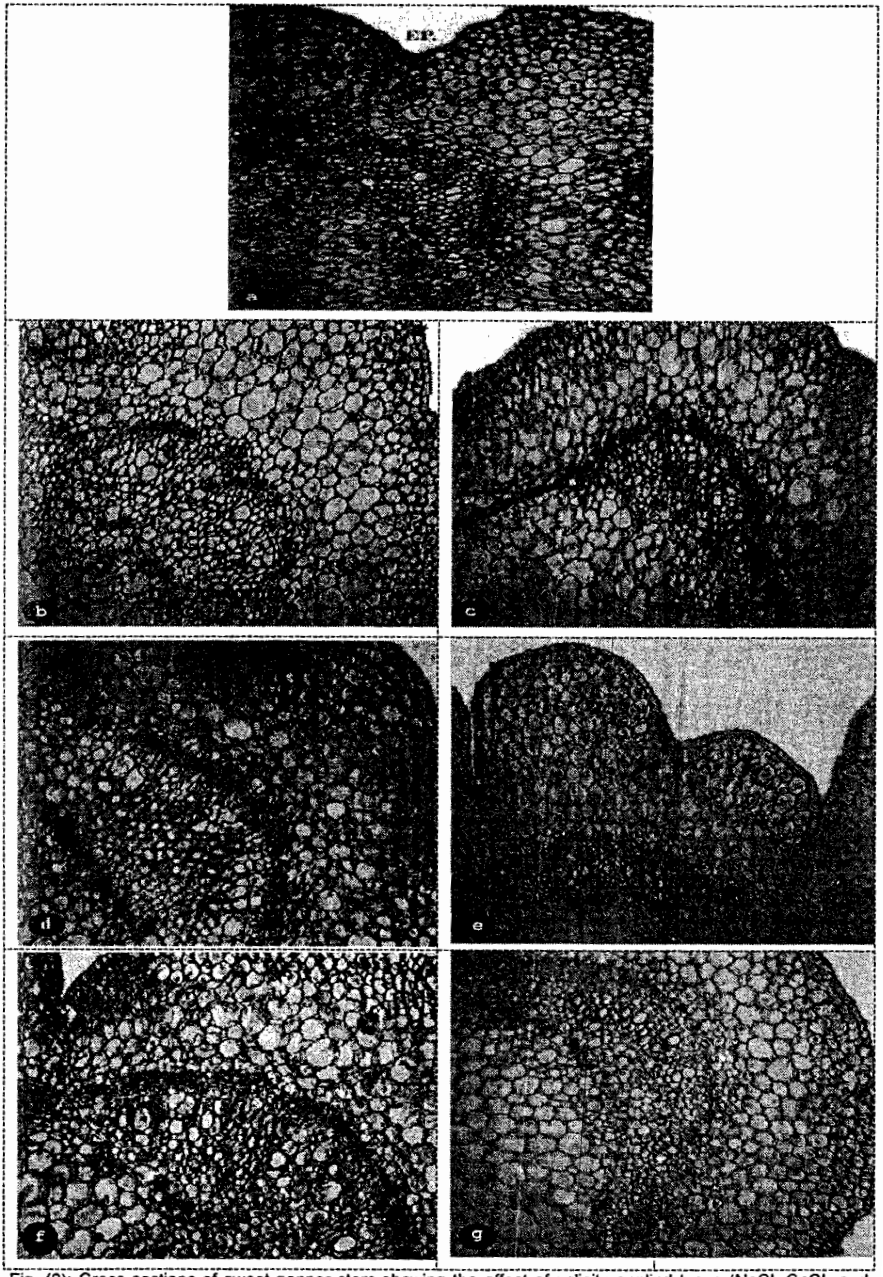


Fig. (2): Cross sections of sweet pepper stem showing the effect of salinity applied types (NaCl, $CaCl_2$ and their combination 1:1) (x100).

a = Control ; b, c = NaCl at 2000 and 4000 mg/L

d, e = $CaCl_2$ at 2000 and 4000 mg/L ; f, g = NaCl+$CaCl_2$ 1:1 at 2000 and 4000 mg/L

Abbreviations: Ep= Epidermis; Co= Cortex, L.V.B= Large Vascular Bundle, Pi= Pith, M.Xy.= Metaxylem.

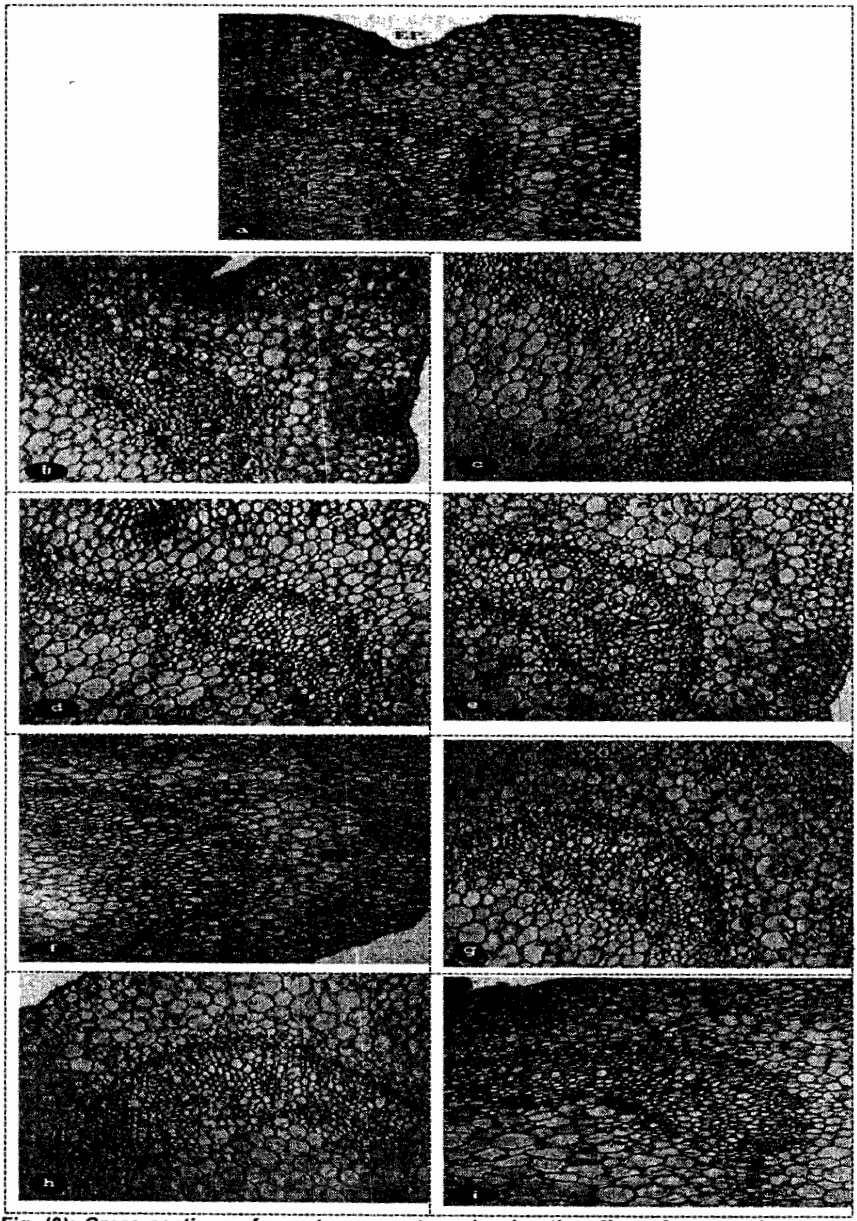


Fig. (3): Cross sections of sweet pepper stem showing the effect of pre-soaking in SA, AsA, α-toco and yeast extract under non-saline conditions (x100).

a = Control	
b, c = SA at 75 and 150 mg/L	d, e = AsA at 50 and 100 mg/L
f, g = α-toco. at 50 and 100 mg/L	h, i = Yeast extract at 1000 and 2000 mg/L

Abbreviations: Ep= Epidermis; Co= Cortex, L.V.B= Large Vascular Bundle; Pi= Pith; M.Xy.= Metaxylem.

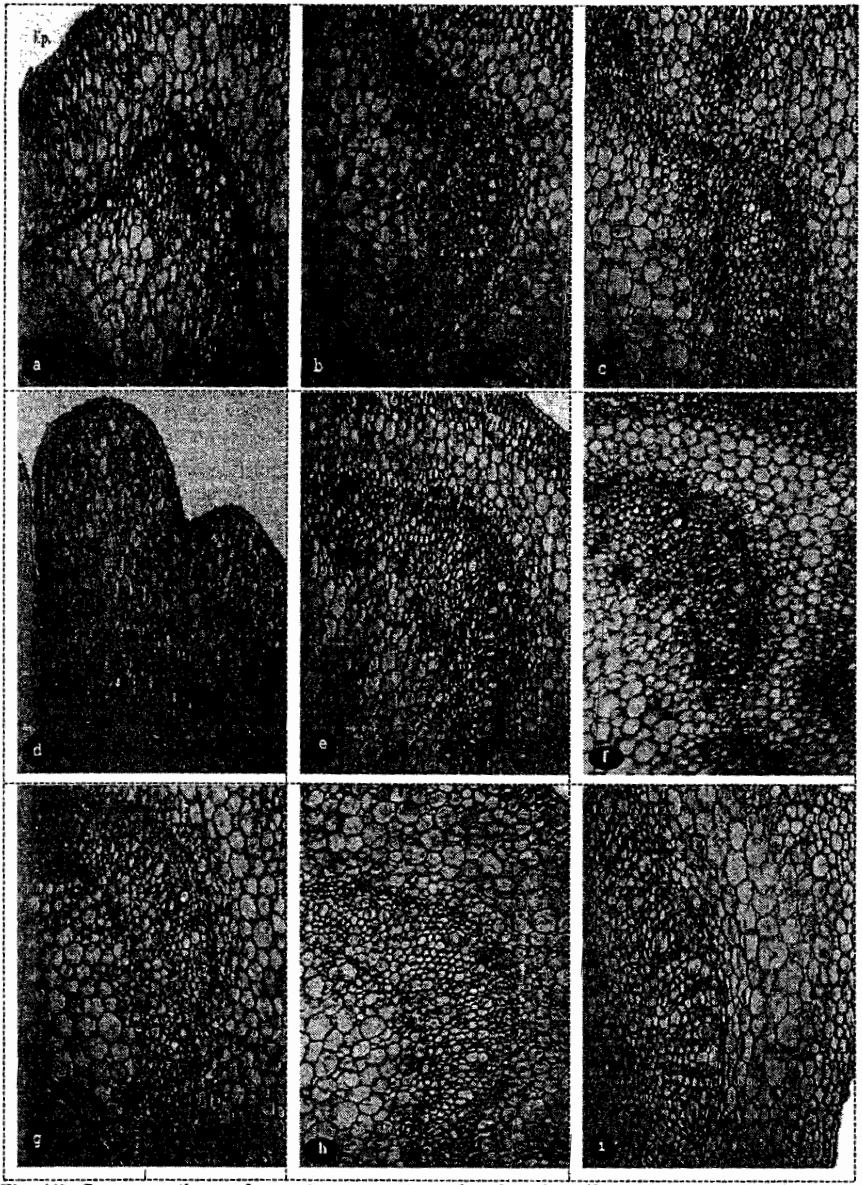


Fig. (4): Cross sections of sweet pepper stem showing the effect of pre-soaking in SA at 75 mg/L and AsA at 50 mg/L under high salinity level (4000 mg/L) of NaCl, $CaCl_2$ and their combination 1:1 (x100).

a = NaCl at 4000 mg/L	b = NaCl + SA at 75mg/L	C = NaCl + AsA at 50 mg/L
d = $CaCl_2$ at 4000 mg/L	e = $CaCl_2$ + SA at 75 mg/L	f = $CaCl_2$ + AsA at 50 mg/L
g = NaCl+$CaCl_2$ at 4000 mg/L	h = (NaCl+$CaCl_2$)+SA at 75 mg/L	i = (NaCl+ $CaCl_2$)+AsA at 50 mg/L

Abbreviations: Ep= Epidermis; Co= Cortex, L.V.B= Large Vascular Bundle; Pi= Pith; M.Xy.= Metaxylem.

Furthermore, Pimmongkol *et al.* (2002) stated that the diameters of stems and the width of vascular bundles were decreased in rice grown under NaCl. Akram *et al.*, (2002) showed that salinity (10, 15, or 20 dS/m^{-1}) reduced the size of the largest metaxylem and cortex thickness in the stem *of Triticum aestivum* L.

Concerning the effect of salicylic acid, ascorbic acid, α-tocopherol and yeast extract used on sweet pepper stem structure, data presented in the same table and illustrated in Fig. (3) revealed that pre-soaking seeds in selected chemicals used, in most cases, gave a positive effect on the stem diameter. In this concern, AsA at 50 mg/L and SA at 75 mg/L gave a high value (11% and 10% respectively) as compared to control and remaining treatments. In addition, pre-soaking seeds in AsA at 50 mg/L led to an increase in the pith and cortex diameter as well as number and vascular bundles dimensions (length and width), while deceased the thickness of epidermal cells as compared to remaining treatments. On the other hand, pre-soaking in α-tocopherol and yeast extract at two levels decreased the most of these anatomical parameters (Table, 4) and (Fig.3).

Regarding the interactions between salinity levels and pre-soaking sweet pepper seeds in AsA and SA, it could be showed from Table, 4 and (Fig.4) that, the treatment with AsA or SA led to an increase in the most anatomical characters under low salinity level (2000 mg/L) of all salinity types. Meanwhile, the cortex thickness was decreased under NaCl at 2000 mg/L. On the other hand, the interactions at high level 4000 mg/L, in mo st cases increased stem diameter as compared to untreated plants under high salinity level. The existed increase in the stem diameter was mainly due to the increase in pith thickness, cortex thickness and number of vascular bundles as well as vascular bundle dimensions (Table,4). As a matter of fact, the cambial activity was stimulated since more vessels per bundle were initiated.

Leaf blade Structure

Data presented in Table (5) and illustrated in (Fig.5) indicate that the low level of all salinity types (2000 mg/L) increased midrib region thickness due to increasing the length of main vascular bundle. In addition, the thickness of leaf blade (lamina) was increased due to a corresponding increase in the spongy parenchyma. While, the high salinity level (4000 mg/L) led to a decrease in this respect due to a decrease in length of main vascular bundle.

However, NaCl was more effective in this respect followed by NaCl+$CaCl_2$ (1:1). On the other hand, the leaf blade (lamina) thickness was also decreased in pepper plants grown under the high salinity level due to a decrease in the thickness of palisade and spongy tissues as well as that upper and lower epidermis (Fig.5). Furthermore, the highest reduction of leaf blade thickness was observed in NaCl treatment, followed by NaCl+$CaCl_2$ (Fig.5).

This reduction was probably due to a decrease in cell size of both palisade and spongy tissues. Similar results were previously reported by El-Banna, 2006 and Arafa *et al.*, 2009.These results are disagreement with (Wignarajah *et al.*, 1975 and Poljakoff-Mayber, 1975). They suggested that

the increase in blade thickness is a remarkable response to salinity and succulence involves development of large cells in the spongy mesophyll and sometimes multilayer palisade tissue.

The inhibiting effects of high salinity level on leaf structure may be due to inhibition the growth of vascular elements and/or correlation with an inhibition of the procambial activity which form primary vascular tissues and/or decrease in the number and size of mesophyll cells (Rashid *et al.*, 2004). Therefore, it could be concluded that salinity may have an inhibition effect on the activity of the various initial cells forming the leaf blade with regard to cell division and enlargement.

Furthermore, the decreases in the dimensions of vascular bundle in the leaf blade result in lowering the accumulation of necessary water required for photosynthesis. In addition, the promotive effect of low salinity level on sweet pepper leaves thickness may be due to an increase in thickness of mesophyll tissue. In addition, Aloni (1987) suggested that increase or decrease in the vessel diameter might increase cr decrease the efficiency of water conduction, owing to increase or decrease in the resistance to flow.

Generally, the highest level of salinity caused a reduction in the conductive tissues of sweet pepper plant. The decrease in mesophyll tissue, xylem and phloem thickness leads to a slow rate in the translocation of photoassimlates towards the developing seeds.

Regarding the effect of chemical used on sweet pepper leaf structure data presented in Table (5) and illustrated in Fig.(6) show that pre-soaking seeds in SA, AsA, α-toco and yeast extract at both levels led to an increase in the thickness of midrib region as compared with untreated plants. This increase was proportional to the type and concentration of these chemicals. In this respect, SA (75 mg/L), AsA (50 mg/L) or α-toco. (100 mg/L) were more effective in this respect. This increase may be due to an increase in the main vascular bundle length. While, the remain treatments caused a slight increase, but yeast at 2000 mg/L had no effect in this concern. Furthermore, the same table and figures revealed that most chemical used, in most cases, increased the lamina thickness except yeast at 2000 mg/L. The promotive effect may be due to an increase in the spongy tissue thickness as well as upper epidermis thickness and decreased the palisade tissue thickness. This result is in agreement with those reported by Arafa and Harb (1989) who revealed that AsA had no effect on the non-saline structure of pea leaflets. In the contrary, Ali (2001) reported that the palisade thickness in tomato leaf was increased with AsA but spongy tissue thickness was not/or slightly affected and ascorbic acid affected xylem vessels differentiation and development. This effect may be due to the effect of ascorbic acid on the growth rate stimulating cell expansion, vacuolation and fluid uptake (Gonzalez-Reyes *et al.*, 1994) and cell division (Conklin, 2001). In addition, El-Banna (2006) found that application of AsA increased markedly thickness of sweet pepper midrib region, leaf blade thickness and main vascular bundle dimensions (length and width).

Table (5) Effect of NaCl, $CaCl_2$ and their mixture or SA, AsA, α-toco. or Yeast extract as well as their combinations on sweet pepper Leaf Blade structure.

Parameters / Treatment (mg/L)			*Midrib Thickness*		*Lamina Thick-ness*		*Palisade Tissue Thickness*		*Spongy Tissue Thick-ness*		*Upper Epider-mis Width*		*Lower Epidermis Width*		*Main Vascular Bundle Dimensions*			
															Length		*Width*	
			µm	100 % ±	µm	100 % ±	µm	100% ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±
Nutrient solution		Water	1249	0	230	0	68	0	115	0	25	0	22	0	270	0	894	0
		SA 75	1303	+4	245	+6	72	+5	115	0	32	+29	25	+17	292	+8	994	+11
		SA 150	1278	+2	234	+2	40	-42	158	+38	22	-14	14	-33	292	+8	959	+7
		AsA 50	1303	+4	245	+6	61	-11	133	+16	29	+14	22	0	223	-17	1328	+49
		AsA100	1289	+3	238	+3	50	-26	144	+25	22	-14	22	0	274	+1	871	-3
		α- 50	1264	+1	234	+2	61	-11	133	+16	22	-14	18	-17	274	+1	1001	+12
		α-100	1296	+4	245	+6	72	+5	112	-3	32	+29	29	+33	263	-3	1588	+78
		Yeast 1	1253	0	230	0	50	-26	133	+16	25	0	22	0	248	-8	1192	+33
		Yeast 2	1289	+3	234	+2	54	-21	126	+9	29	+14	25	+17	277	+3	533	-40
NaCl	2000	Water	1310	+5	245	+6	29	-58	158	+38	40	+57	32	+50	284	+5	1134	+27
		SA 75	1357	+9	263	+14	76	+11	130	+13	36	+43	22	0	284	+5	1210	+35
		AsA 50	1354	+8	259	+13	50	-26	155	+34	22	-14	25	+17	266	-1	1112	+24
	4000	Water	979	-22	155	-33	32	-53	90	-22	22	-14	11	-50	220	-19	1102	+23
		SA 75	1109	-11	180	-22	47	-32	86	-25	25	0	22	0	241	-11	1321	+48
		AsA 50	1094	-12	180	-22	50	-26	86	-25	22	-14	22	0	256	-5	1076	+20
$CaCl_2$	2000	Water	1372	+10	263	+14	79	+16	126	+9	32	+29	25	+17	335	+24	756	-15
		SA 75	1444	+16	277	+20	68	0	151	+31	32	+29	25	+17	349	+29	1390	+55
		AsA 50	1436	+15	277	+20	72	+5	151	+31	32	+29	22	0	241	-11	1699	+90
	4000	Water	1217	-3	212	-8	61	-11	112	-3	22	+25	18	-17	241	-11	1019	+14
		SA 75	1242	-1	227	-2	76	+11	101	-13	25	+22	25	+17	241	-11	1458	+63
		AsA 50	1238	-1	227	-2	68	0	101	-13	32	+22	25	+17	194	-28	1224	+37
NI : $CaCl_2$ (1:1)	2000	Water	1451	+16	281	+22	76	+11	148	+28	29	+40	29	+33	306	+13	1616	+81
		SA 75	1602	+28	382	+66	61	-11	252	+119	29	+25	40	+83	310	+15	1246	+39
		AsA 50	1595	+28	313	+36	72	+5	194	+69	25	+25	22	0	331	+23	1825	+104
	4000	Water	1130	-10	184	-20	47	-32	94	-19	22	+22	22	0	256	-5	810	-9
		SA 75	1210	-3	212	-8	43	-37	119	+3	29	+22	22	0	288	+7	526	-41
		AsA 50	1210	-3	212	-8	43	-37	122	+6	25	+25	22	0	252	-7	1152	+29

Regarding the interactions between salinity levels and types and selected chemicals used it could be showed in the same Table (5) and Fig.(7) that the SA, AsA, α-toco. And Yeast extract used, in most cases, increased the thickness of midrib region, dimensions of vascular bundle as well as leaf blade thickness with corresponding to an increase in the thickness of palisade and spongy tissues under the low salinity level (2000 mg/L) as compared to control.

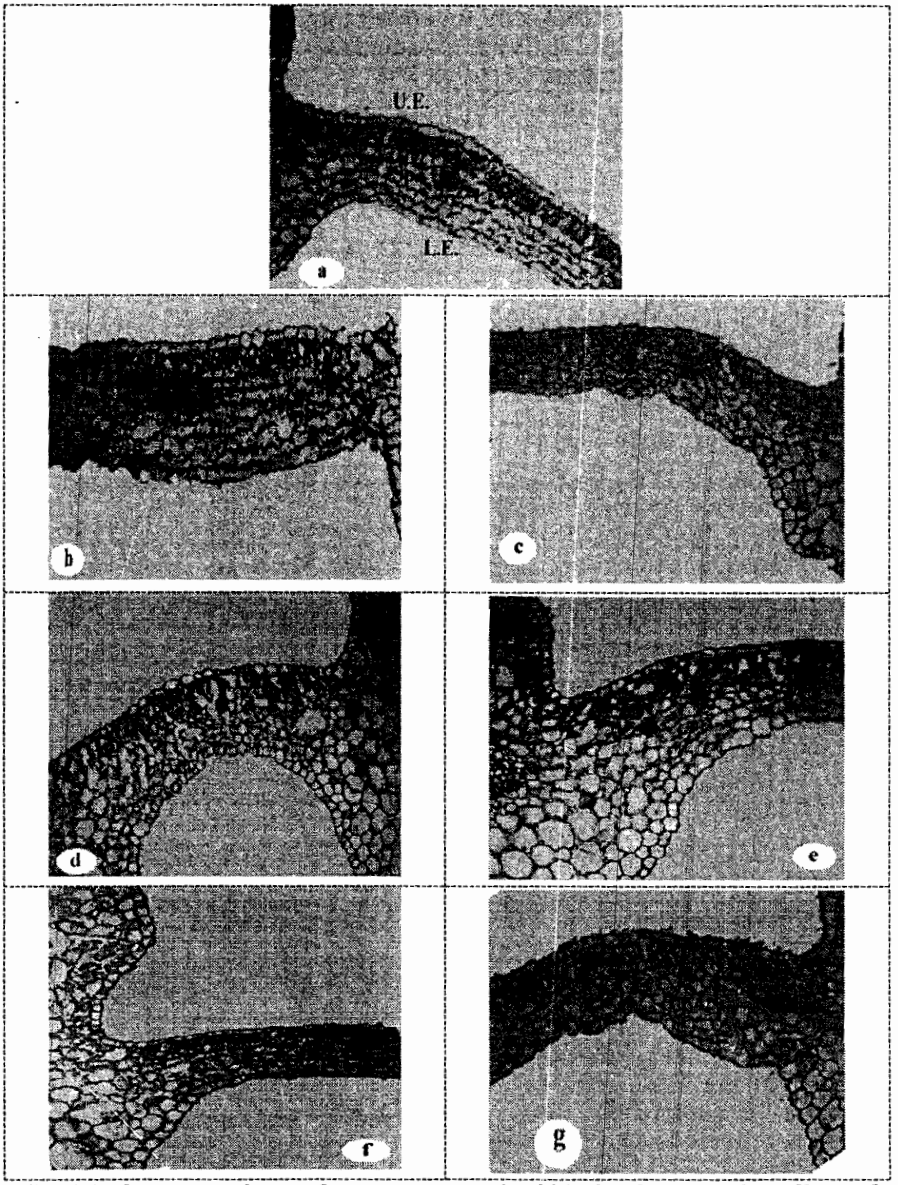


Fig. (5): Cross sections of sweet pepper leaf blade showing the effect of salinity applied types (NaCl, $CaCl_2$ and their combination 1:1) (x100).

a = Control	b, c = NaCl at 2000 and 4000 mg/L
d, e = $CaCl_2$ at 2000 and 4000 mg/L	f, g = NaCl+$CaCl_2$ 1:1 at 2000 and 4000 mg/L

Abbreviations: LE= Lower Epidermis; Pa= Palisade Parenchyma; Sp= Spongy Parenchyma; V.B.= Vascular Bundle; UE= Upper Epidermis.

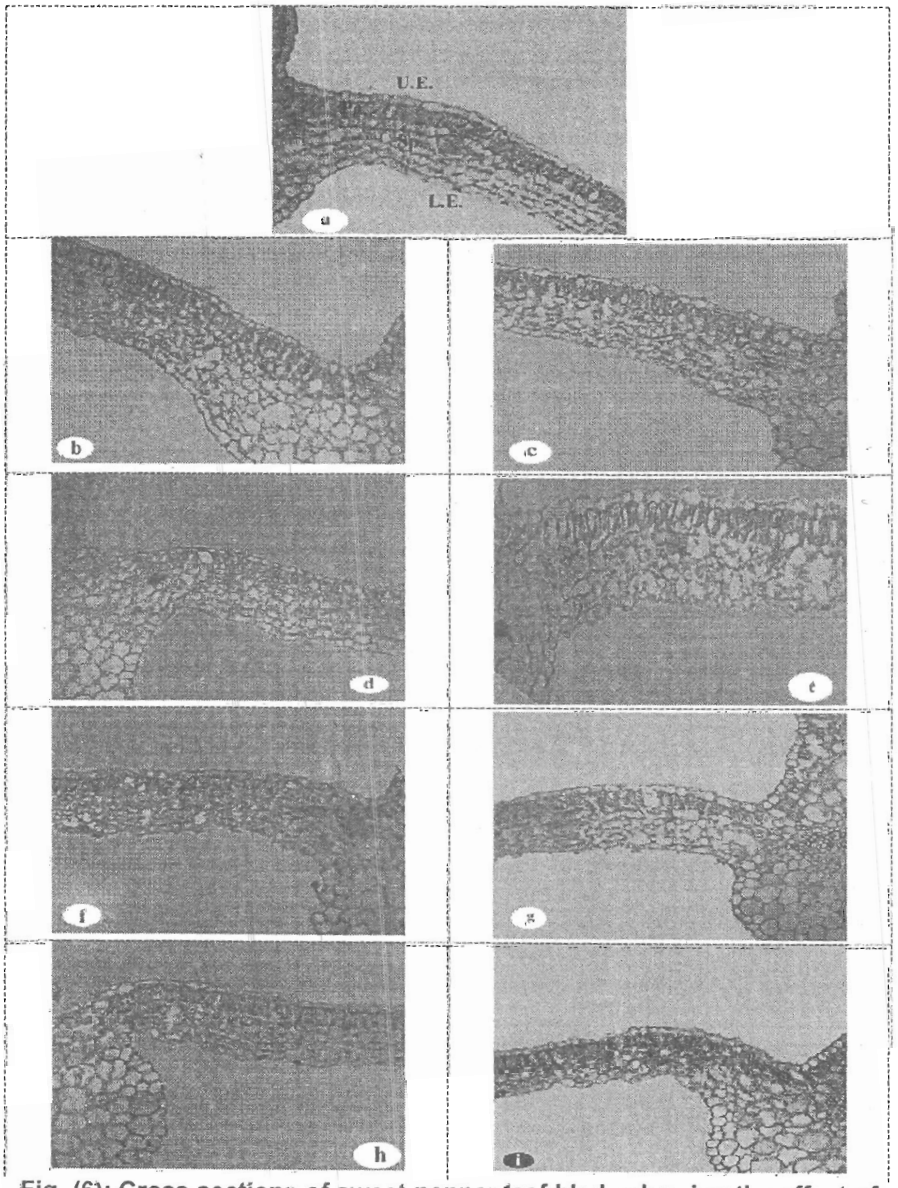


Fig. (6): Cross sections of sweet pepper leaf blade showing the effect of pre-soaking in SA, AsA, α-toco and yeast extract under non-saline conditions (x100).

a = Control	
b, c = SA at 75 and 150 mg/L	d, e = AsA at 50 and 100 mg/L
f, g = α-toco. at 50 and 100 mg/L	h, i = Yeast extract at 1000 and 2000 mg/L

Abbreviations; LE= Lower Epidermis; Pa= Palisade Parenchyma; Sp= Spongy Parenchyma; V.B.= Vascular Bundle; UE= Upper Epidermis.

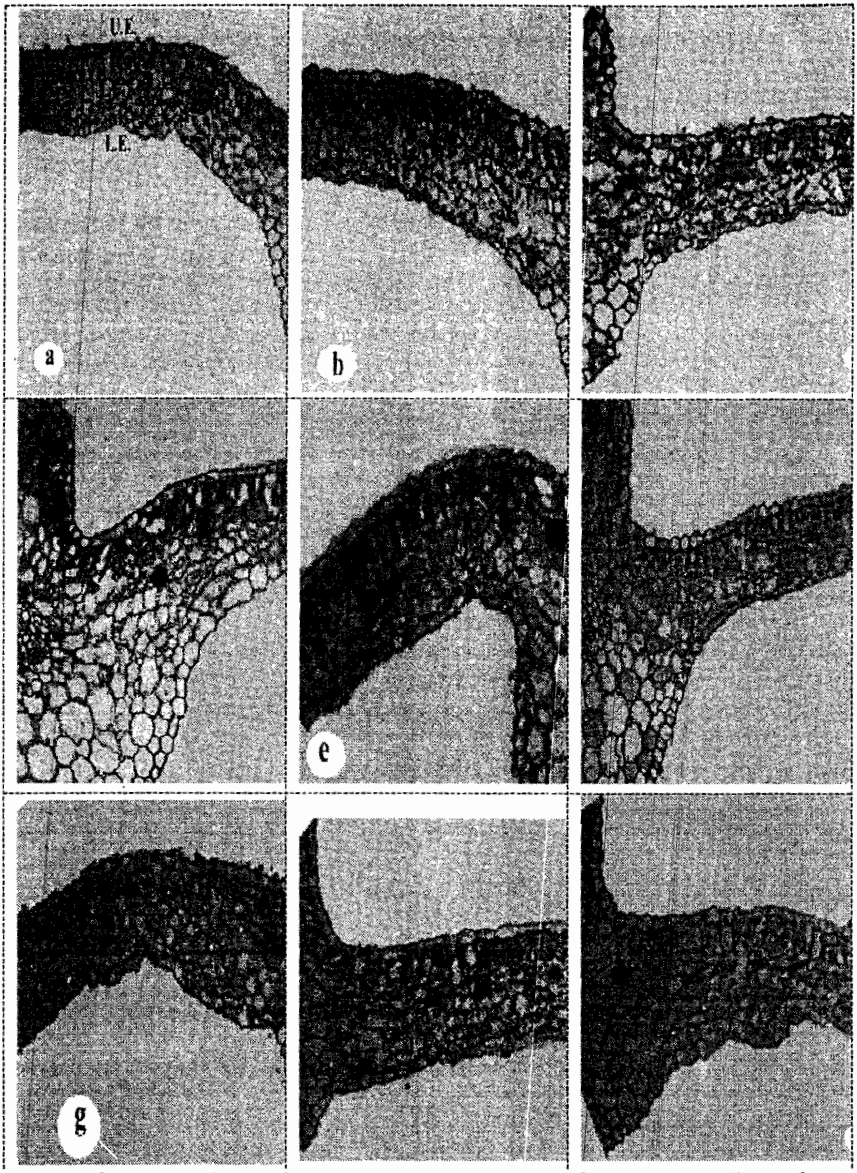


Fig. (7): Cross sections of sweet pepper leaf blade showing the effect of pre-soaking in SA at 75 mg/L and AsA at 50 mg/L under high salinity level of NaCl, $CaCl_2$ and their combination 1:1 (4000 mg/L) (x100).

a = NaCl at 4000 mg/L	b = NaCl + SA at 75mg/L	c = NaCl + AsA at 50 mg/L
d = $CaCl_2$ at 4000 mg/L	e = $CaCl_2$ + SA at 75 mg/L	f = $CaCl_2$ + AsA at 50 mg/L
g = NaCl+$CaCl_2$ at 4000 mg/L	h = (NaCl+$CaCl_2$)+SA at 75 mg/L	i = (NaCl+ $CaCl_2$)+AsA at 50 mg/L

Abbreviations: LE= Lower Epidermis; Pa= Palisade Parenchyma; Sp= Spongy Parenchyma; V.B.= Vascular Bundle; UE= Upper Epidermis.

Furthermore, the interactions SA at 75 mg/L or AsA at 50 mg/L with high salinity level (4000 mg/L) increased leaf blade thickness with corresponding to an increase in mesophyll tissue thickness and width of upper epidermal cells as compared to untreated plants under such salinity types. Moreover, AsA at 50 mg/L was more effective than SA at 75 mg/L. Under high salinity level (4000 mg/L), application of SA at 75 mg/L or AsA at 50 mg/L, in most cases, alleviated the harmful effect of salinity level (4000 mg/L) on midrib region and lamina thickness as well as main vascular bundle dimensions when compared with untreated plants and such salinity level. Furthermore, AsA 50 mg/L was more effective than the remaining treatments.

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التاثيرات التفاعلية لبعض الفيتامينات والمنظمات الحيوية ومستخلص الخميرة على تشريح ساق وورقة الفلفل الحلو النامى تحت ظروف نوعين من الملوحة .

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أدي التركيز المنخفض (٢٠٠٠ جزء في المليون) من جميع الأملاح المستخدمة إلي زيادة قطر الساق نتيجة لزيادة قطر النخاع، وسمك القشرة ، وعرض خلايا البشرة وكذلك طول الحزم الوعائية وسمك نسيج الخشب التالي. ولقد وجد أن كلوريد الكالسيوم ومخلوط الملحين الأكثر تأثيراً. لقد أدي التركيز المرتفع من الملوحة (٤٠٠٠ جزء في المليون) إلي نقص معظم الصفات التشريحية. بينما كلوريد الصوديوم (٤٠٠٠ جزء في المليون) أدي إلي نقص قطر النخاع وعدد الحزم الوعائية. علي العكس من ذلك أدي إستخدام أي من المواد السابقة إلي حدوث نتيجة إيجابية علي تركيب الساق، وكان كل من حمض الأسكوربيك بتركيز ٥٠ جزء في المليون أو حمض السالسليك بتركيز ٧٥ جزء في المليون الأكثر تأثيراً.

ولقد لوحظ أن التركيز المنخفض (٢٠٠٠ جزء في المليون) من الأملاح المستخدمة أدي إلي زيادة سمك العرق الوسطي وذلك نتيجة إلي زيادة طول الحزمة الوعائية الرئيسية. بينما أدي التركيز المرتفع (٤٠٠٠ جزء في المليون) إلي نقص سمك العرق الوسطي وذلك نتيجة نقص طول الحزمة الوعائية الرئيسية، بالإضافة إلي ذلك كان كلوريد الصوديوم الأكثر تأثيراً يليه مخلوط الملحين. ولقد وجد أن كلوريد الصوديوم أو مخلوط الملحين يؤدي إلي نقص سمك النصل نتيجة لنقص سمك النسيج العمادي والأسفنجي وكذلك سمك خلايا البشرة العليا والسفلي. كما أدي إستخدام حمض الأسكوربيك أو الألفاتوكوفيرول أو حمض السالسليك أو مستخلص الخميرة إلي زيادة سمك العرق الوسطي وذلك مقارنة بالنباتات الغير معاملة. ولقد لوحظ أن حمض الأسكوربيك بتركيز ٥٠ جزء في المليون أو حمض السالسليك بتركيز ٧٥ جزء في المليون أو الألفاتوكوفيرول بتركيز ١٠٠ جزء في المليون الأكثر تأثيراً. كما وجد أن إستخدام أي من حمض الأسكوربيك بتركيز ٥٠ جزء في المليون أو حمض السالسليك بتركيز ٧٥ جزء في المليون أدي إلي تقليل الأثار الضارة للتركيز المرتفع للملوحة (٤٠٠٠ جزء في المليون) علي سمك العرق الوسطي، وسمك النصل وكذلك أبعاد الحزمة الوعائية وذلك مقارنة بالنباتات الغير معاملة تحت الظروف العادية أو أي من مستويات الملوحة المستخدمة.

قام بتحكيم البحث

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