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Glycinebetaine and ascorbic acid can alleviate the harmful effects of NaCl salinity in sweet pepper

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Abstract

Salinity stress decreased plant height, root length, fresh and dry weights of shoot, chlorophyll concentration, as well as K^+ concentration as compared to control. While, Na^+ concentration was increased. Low salinity level 1500 ppm (4.84 dSm^{-1}) increased plant height and chlorophyll a concentration. High reduction in this parameters occurred under high salinity level 6000 ppm NaCl (11.88 dSm^{-1}). Pre-soaking of sweet pepper seeds in either glycinebetaine or ascorbic acid partially counteracted the harmful effect of NaCl salinity. Low salinity level increased thickness of either midrib region or leaf blade (palisade and spongy parenchyma) as well as the main vascular bundle dimensions. Whereas, moderate and high salinity levels led to a decrease in these parameters. Pre-treatment with glycinebetaine or ascorbic acid mitigated the affect of salinity on thickness of the midrib region and mesophyll tissue of leaf blade. However, all studied anatomical characters were increased as compared to untreated plants grown under such salinity levels.

***Key words**:* Antioxidants, *Capsicum annuum*, leaf structure, osmoregulators, salt stress, seed germination.

Abbreviations: AsA_Ascorbic acid; GB_Glycinebetaine; LE_Lower Epidermis; Me_Mesophyll; Pa_Palisade Parenchyma; Ph_Phloem; Sp_ Spongy Parenchyma; UE_Upper Epidermis; Xy_Xylem.

Introduction

Salinity is one of the most important factors limiting plant growth and delaying seed germination as well as final germination percentage (Rahman *et al*., 2000). Moreover, plant growth cab be-severely affected by salt through inhibition of growth, decrease in photosynthetic activity, water deficit, ion uptake and salt-specific damages or oxidative stress (Zhu, 2001). Osmoregulators (e.g., glycinebetaine; GB) or antioxidant (e.g., ascorbic acid; AsA) are accumulated in plants as an adaptive mechanism to environmental stress such as salinity (Thomas *et al*., 1992). In plants that synthesize GB, it is accumulated in leaves in response to water deficit and salt stress (Rhodes and Hanson, 1993). In addition to its role as osmoprotectant, GB has been reported to stabilize photosynthetic reactions, the structure of extrinsic proteins of the PSII complex and ATP synthesis (Mamedov *et al*., 1991) as well as cell membranes (Jolivet *et al*., 1982) and activation of enzymes (Gorham, 1995). Sweet pepper is a moderate sensitive plant to salt stress. Salinity is known to affect many aspects of metabolism of plants and induce changes in their anatomy and morphology. Moreover, Chartzoulakis and Klapaki (2000) reported that Na^+ concentration in roots of sweet pepper increased with increasing salinity as compared to leaves. These changes are often considered to be adaptive, thus increasing the chances of survival during salinity stress. Salt stress caused alterations in plant cell structure and functions, and induced morphological and anatomical changes (Mitsuya *et al.* 2000).

The aim of this experiment was to study the effect of glycinebetaine (GB) or ascorbic acid (AsA) on number of morphological, physiological and anatomical aspects under normal or NaCl salt stress conditions.

Table 1. 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	$Ca(NO_3)_2.\ 4H_2O$	1003
Magnesium Sulphate	$MgSO_4.\ 7H_2O$	513
EDTA Iron	$[CH_2.N(CH_2.COO)_2]_2$ Fe Na	79
Manganous Sulphate	$MnSO_4.H_2O$	6.1
Boric Acid	H_3BO_3	1.7
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

Materials and Methods

Experiment was carried out in the glasshouse of the Agric. Bot. Dept., Fac. of Agric., Mansoura Univ. during the winter season 2005, with 10/14 light/dark period at 800–1100 µmol $m^{-2}\ s^{-1}$ PPFD, a day/night average temperature cycle of 26/15 ^oC and 65±5% relative humidity.

The following experiment was conducted to study the harmful effects of salinity on sweet pepper plants growing in nutrient film technique (NFT), through pre-soaking seeds in glycinebetaine at 2000 or 4000 ppm or ascorbic acid at 50 or 100 ppm under normal or saline conditions.

Plant materials

Sweet pepper (*Capsicum annuum* L. cv. Orlando, a ''California Wonder''-type pepper) seeds were secured from the Gohara Co. Cairo, Egypt.

Chemicals

1. Glycinebetaine (GB) was supplied by Sigma Chemical Co., USA and used at the concentration of 2000 or 4000 ppm.
2. Ascorbic acid (AsA) was obtained from EL-Gomhoria Co., Egypt and was used at the concentration of 50 or 100 ppm.
3. Sodium Chloride (NaCl) from EL-Gomhoria Co., Egypt and was used at the concentration of 1500, 3000 and 6000 ppm.

Glass house experiment

A homogenous sweet pepper seeds lot was surface-sterilized by soaking in 0.001 %5 $HgCl_2$ for 1 min., and washed with distilled water then divided into 5 groups and each group individually presoaked for 24 h in distilled water (control), GB (2000 or 4000 ppm) or AsA (50 or 100 ppm) respectively. Seeds were germinated in small plastic boxes (5*5*20 cm) containing peat moss and perlite (1:1) as a rooting medium moistured by nutrient cooper solution (Cooper, 1979). Boxes containing the seeds were placed in an incubator at 25 $\pm 2^0C$ in the dark for seed to germinate.

The experimental layout consisted of 4 plastic channels (4 m long and 10 cm in diameter). Every channel was provided by an electric pump represent-ting four groups 0 ppm NaCl (2.5 dSm^{-1} as a control), 1500 ppm NaCl (4.84 dSm^{-1}), 3000 ppm NaCl (7.19 dSm^{-1}) and 6000 ppm NaCl (11.88 dSm^{-1}).

Each channel had 40 pores (6 cm diameter). After 40 days from pre-soaking, tow uniform seedlings were transplanted to 6 cm perforated pots (reticulated) containing peat moss and perlite (1:1) as a rooting medium. Every channel was divided into 5 sets i.e. water, GB1 (2000 ppm), GB2 (4000 ppm), AsA1 (50 ppm), AsA2 (100 ppm). Each set contained 8 seedlings (one 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 5 to 7 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. Morphological measurements were made at 30 days after transplanting to channels. The following variables were recorded: plant height, root length and fresh and dry weights of shoots. Leaf samples were extracted by methanol for 24 hour at laboratory temperature after adding a trace from sodium carbonate (Robinson *et al*., 1983), then Chlorophyll a and b were determined spectrophotometrically (Spekol 11). The quantities of Chlorophyll a and b in leaves were determined by the equation proposed by Mackiny (1941).

Table 2. Effect of sodium chloride salinity and pre-soaking in GB or AsA, as well as their interactions on plant height and root length of sweet pepper at 30 days from sowing.

Treatment / Salinity	Water	Glycinebetaine (ppm)		Ascorbic acid (ppm)		Mean
		2000	4000	50	100	
Plant height (cm)						
Control	9.6	11.6	10.4	9.4	10.5	10.5
1500 ppm NaCl	10.1	12.3	11.5	11.1	12.8	11.9
3000 ppm NaCl	6.7	10.4	10.3	10.4	8.7	10.0
6000 ppm NaCl	5.2	7.5	9.5	7.4	6.7	7.8
Mean	7.9	10.4	10.4	9.5	9.7	===
L.S.D. 0.05	Salinity 0.15		Materials 0.23		Salinity * Materials 0.45	
Root length (cm)						
Control	25.1	30.3	24.2	27.2	29.9	27.9
1500 ppm NaCl	24.2	24.1	24.2	25.3	23.1	23.1
3000 ppm NaCl	21.9	21.2	23.2	24.1	23.5	23.0
6000 ppm NaCl	14.9	20.2	21.4	21.3	22.2	21.3
Mean	21.5	23.9	23.3	24.5	24.7	===
L.S.D. 0.05	Salinity 0.2		Materials 0.2		Salinity * Materials 0.5	

The oven dry shoot and root of plant samples were digested by sulphoric-percloric acid mixture as described by Peterburgski (1968). The total Na^+ and K^+ were determined.

Specimens ($5mm^2$) including the midrib region were taken from the blade of the second leaf lamina from apex of sweet pepper plant after 30 days from transferring plants into the channels. The samples were killed and fixed in formalin-acetic-alcohol (FAA), then washed and dehydrated in series of ethanol (50%, 70%, 80%, 90% and 100%), cleared in series of ethanol: xylene (3:1- 1:1- 1:3 and 100% xylene) and embedded in paraffin wax (52-54^oC 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). Data were subjected to statistical analysis of variance according to Gomez and Gomez (1984).

Results and Discussion

Morphological aspects

Table (2, 3) reveled that increasing salinity levels up to 6000 ppm NaCl (11.88 dSm^{-1}) decreased plant height, root length and shoot fresh as well as dry weights. This effect increased consistently and rapidly with increasing salinity level, as compared to non-stressed plants.

Data in tables 2 and 3 indicate that GB and AsA increased plant height, root length, fresh and dry weights of shoot. Pre-soaking with GB (2000 ppm) or AsA (100 ppm) proved to be more effective in this respect.

Concerning the interactions between salinity levels and pre-soaking in GB or AsA, it was found that these treatments decreased plant height and root length of sweet pepper when compared with untreated plants. GB at 2000 ppm proved to be more effective than AsA to reduce the harmful effect of salinity. Generally, GB and AsA counteracted the harmful effects of salinity on plant height and root length at all salinity levels used.

The stimulating effect of low salinity level on plant height may be attributed to the beneficial effect of low concentration of chloride on many physiological processes as photosynthesis and osmoregulators. Turgor reduction results in stomatal closure followed by reduction in gas exchanges "transpiration and photosynthesis" (Neumann *et al*., 1988). In addition, Hartung (2004) suggested that the adverse effects of salinity on plant height and root length may be due to the diverse effects of salinity on meristimatic cell division and elongation as well as root penetration.

In some plants, osmotic adjustment results from synthesis in the cytoplasm of compatible organic solutes (including proline, GB in addition to amino acids and sugars). Also, Younis *et al*. (2003) reported that the reduction in growth caused by salinity stress is due to inhibited apical growth in plants as well as internal hormonal imbalance. In both cases, reduction could have been caused by the toxic effects of ions (Na^+ and Cl^-) on metabolism or from adverse water relations. Moreover, the retardation in plant growth caused by salinity may be attributed mainly to the osmotic stress, which reduced availability and uptake of water and essential nutrients (Neumann, 1997), as

Table 3. Effect of sodium chloride salinity and pre-soaking in GB or AsA as well as their interactions on shoots fresh and dry weight of sweet pepper at 30 days from sowing.

Treatment / Salinity	Water	Glycinebetaine (ppm)		Ascorbic acid (ppm)		Mean
		2000	4000	50	100	
				Fresh weight (g/plant)		
Control	1.9	3.8	3.0	2.7	2.8	3.1
1500 ppm NaCl	1.6	3.0	2.5	2.5	2. 1	2.5
3000 ppm NaCl	1.0	2.4	2.3	2.6	2.1	2.4
6000 ppm NaCl	0.8	1.4	2.1	1.8	1.1	1.6
Mean	1.3	2.7	2.5	2.4	2.0	===
L.S.D. 0.05	Salinity 0.1		Materials 0.1		Salinity * Materials 0.2	
				Dry weight (g/plant)		
Control	0.24	0.44	0.59	0.35	0.42	0.45
1500 ppm NaCl	0.14	0.42	0.38	0.36	0.33	0.37
3000 ppm NaCl	0.13	0.39	0.34	0.26	0.25	0.31
6000 ppm NaCl	0.10	0.24	0.24	0.25	0.18	0.23
Mean	0.15	0.37	0.39	0.31	0.30	===
L.S.D. 0.05	Salinity 0.01		Materials 0.01		Salinity * Materials 0.11	

well as the excessive accumulation of both toxic ions i.e. Na^+ and intermediate compounds such as reactive oxygen species (Rodriguez *et al*., 2004) which cause damage to DNA, lipid and proteins and consequently a decrease in plant growth. In this respect, Mohamed *et al*., (1998) found that root morphology descriptors can be used as salt-sensitivy indicators which are affected by salt stress. Thus, roots are reported to be among the first organs affected by salt stress and are most sensitive.

The main inhibitory effect of salinity on plant growth has been attributed to osmotic inhibition of the absorption of available water, specific ion effect causing excessive accumulation of Na^+ or Cl^- or inadequate uptake of an essential nutrient, hormonal imbalance and accumulation of toxic intermediate products as free radical oxygen (Roy *et al*., 1995).Salinity stress increased ABA and ethylene concentration in plant tissue and decreased endogenous level of IAA and GA_3 content (Fricke *et al*., 2004).

Ascorbic acid plays an important role in preserving the activity of enzymes (Padh, 1990). Smirnoff (1996) pointed out that the beneficial effect of AsA on root length may be attributed to the fact that AsA is involved in the regulation of root elongation, cell vacuolation and cell expansion.

Glycinebetaine is one of the osmoregulators solutes naturally accumulate in the plants as an adaptive mechanism to environmental stress as salinity, and accumulated and synthesized in certain members of the Solanaceae (Mäkelä, 2004). In addition, the stimulating effect of GB on plant growth may be attributed to an increase in the viability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells (Tao and Gao, 2003), and reducing the accumulation of harmful sodium and chloride in plant tissues (Rahman *et al*., 2002). In addition, it alleviates oxidative stream caused by salinity through its effects on increasing the antioxidant enzymes activities and protects higher plants against salt stress, by stabilizing many function units, like oxygen-evolving PSII complex, and ATP synthesis, membrane integrity, and enzyme activity (Tao and Gao, 2003).

Physiological aspects

Photosynthetic pigments

Data in table 4 indicated that salinity level at 6000 ppm NaCl (11.88 dSm^{-1}) led to a decrease in chlorophylls a and b in leaves, and this effect increased consistently and rapidly with increasing salinity level as compared to non-stressed treatment. The great reduction was observed under high salinity level. Chlorophyll a and b concentrations significantly increased with GB and AsA application as compared to the untreated plants under non-stressed conditions. Pre-soaking with AsA was the most effective in increasing chlorophyll a and b concentrations. In most cases, all interactions between salinity levels and both GB and AsA resulted in a decreased chlorophyll a and b concentrations as compared to non- stressed plants.

Glycinebetaine and ascorbic acid at the two applied levels reduced the harmful effect of salinity on chlorophyll a and b concentrations; this reduction in photosynthetic pigments was attributed to enhance

Table 4. Effect of sodium chloride salinity and pre-soaking in GB or AsA as well as their interactions on chlorophyll a and b concentration mg/g FW of sweet pepper at 30 days from sowing.

Treatment / Salinity	Water	Glycinebetaine (ppm)		Ascorbic acid (ppm)		Mean
		2000	4000	50	100	
Chlorophyll a						
Control	1.56	1.61	2.13	1.84	2.15	1.93
1500 ppm NaCl	1.60	1.30	1.70	1.60	1.40	1.50
3000 ppm NaCl	1.50	1.30	1.30	1.50	1.30	1.35
6000 ppm NaCl	0.98	1.20	1.40	1.20	0.89	1.17
Mean	1.41	1.33	1.63	1.52	1.43	===
L.S.D. 0.05	Salinity 0.01		Materials 0.02		Salinity * Materials 0.04	
Chlorophyll b						
Control	1.43	1.30	1.21	1.33	1.64	1.37
1500 ppm NaCl	1.20	0.87	0.94	1.12	1.20	1.03
3000 ppm NaCl	0.42	0.80	0.85	1.10	0.84	0.90
6000 ppm NaCl	0.86	0.84	0.76	1.02	0.62	0.81
Mean	0.98	0.96	0.94	1.15	1.07	===
L.S.D. 0.05	Salinity 0.01		Materials 0.01		Salinity * Materials 0.03	

activity of the chlorophyll degrading enzyme chlorophyllase (Mishra and Sharma, 1994).

Moreover, the high salinity caused a disturbed chloroplast structure, number and size which affected chlorophyll content (El-Banna and Attia, 1999) and/or caused disruption of chloroplasts by oxidative stress which cause a decrease in chlorophyll content as well as decreased the photosynthetic reactions (Rahman *et al*., 2000).

Glycinebetaine increased cytokinin concentration which retards chlorophyll degradation (Shetty *et al*., 1992). However, GB and AsA decreased the toxic ion especially sodium concentration, while, increased some ions as Mg^2 which needed for chlorophyll synthesis (Shaddad, 1999), and increased potassium concentration, which increased leaf photosynthetic efficiency possible by increasing the number of chloroplasts per cell, number of cells per leaf and consequently leaf area (Possingham, 1980).

Mäkelä *et al.*, (1999) suggested that GB application enhanced photosynthetic efficiency by reducing photorespiration in plants grown under salt-stress. In addition, exogenous application of GB not only alleviated oxidative stress by salinity but also inhibited the degradation of chlorophyll and proteins caused by salinity (Guo *et al*., 2004).

Glycinebetaine may play a specific role in delaying the salt stress-induced senescence in leaves and/or photoprotection (Demiral and Tűrkan, 2006).

Mineral composition

Tables (5, 6) showed that there was a gradual increase in Na^+ concentration in either shoots or roots with increasing salinity levels in nutrient solution.

On the other hand, significant decrease in K^+ concentration occurred with increasing salinity levels. In this respect, high salinity level 6000 ppm NaCl (11.88 dSm^{-1}) was the most effective in this concern as compared to control plants. Moreover, root system accumulated high concentration of Na^+ with increasing salinity levels in nutrient solution as compared to shoot. However, K^+ concentration decreased in the root system more than shoot one.

The same tables revealed that Na^+ concentration significantly decreased, while, K^+ concentration increased with application of GB or AsA as compared to non-salinized plants. In most cases, Pre-soaking with GB at 4000 ppm proved to be more effective in decreasing Na^+ concentration in the shoot and root systems. AsA (pre-soaking) at 100 ppm was the most effective in increasing K^+ concentration in the shoots and roots. Moreover, all interactions between salinity and both GB or AsA decreased Na^+ concentration and/or increased K^+ concentration as compared to salinized plants.

The preferential accumulation of sodium in root over shoots may be interpreted as a mechanism of tolerance in at least two ways, firstly: maintenance of a substantial potential for osmotic water uptake into the roots, secondly: restricting the spread of Na^+ to the shoots (Renault *et al*., 2001).

In the present study, sodium accumulated in the roots more than shoots. High sodium concentration strongly inhibited uptake and accumulation of K^+ by roots. Because K^+ is a macronutrient involved in turgor control, inhibition of potassium uptake should stunt growth (Renault *et al*. 2001).

Moreover, Hu and Schmidhalter (1998) revealed that Na^+ and Cl^- are the two ions most frequently implicated with toxicity in plants, because both are

Table 5. Effect of sodium chloride salinity and pre-soaking in GB or AsA as well as their interactions on sodium concentration (mg/g DW) in sweet pepper shoot and root at 30 days from sowing

Treatment / Salinity	Water	Glycinebetaine (ppm)		Ascorbic acid (ppm)		Mean
		2000	4000	50	100	
			Shoot			
Control	45.0	20.7	31.0	30.7	25.0	26.9
1500 ppm NaCl	68.7	27.7	54.0	31.0	33.7	36.6
3000 ppm NaCl	82.7	58.0	56.0	48.3	38.7	50.3
6000 ppm NaCl	93.0	62.7	61.0	76.0	44.7	61.1
Mean	72.3	42.3	50.5	46.5	35.5	===
L.S.D. 0.05	Salinity 1.4		Materials 2.2		Salinity * Materials 4.3	
			Root			
Control	52.0	36.3	31.7	48.0	34.0	37.9
1500 ppm NaCl	59.7	69.0	67.0	62.7	44.0	60.7
3000 ppm NaCl	86.7	78.0	77.0	81.7	50.0	71.8
6000 ppm NaCl	95.7	81.3	79.3	86.3	77.0	81.0
Mean	73.3	66.3	63.8	69.7	51.3	===
L.S.D. 0.05	Salinity 3.8		Materials 5.7		Salinity * Materials 11.3	

Table 6. Effect of sodium chloride salinity and pre-soaking in GB or AsA as well as their interactions on potassium concentration (mg/g DW) in sweet pepper shoot and root at 30 days from sowing.

Treatment / Salinity	Water	Glycinebetaine (ppm)		Ascorbic acid (ppm)		Mean
		2000	4000	50	100	
			Shoot			
Control	55.3	71.0	76.0	80.0	87.0	78.3
1500 ppm NaCl	50.0	70.0	61.7	72.0	75.0	69.7
3000 ppm NaCl	41.0	67.7	60.0	63.0	65.0	63.9
6000 ppm NaCl	23.0	43.0	50.0	40.0	54.0	46.8
Mean	42.3	62.9	61.9	63.8	70.3	===
L.S.D. 0.05	Salinity 1.2		Materials 1.8		Salinity * Materials 3.5	
			Root			
Control	62.0	70.0	72.0	61.7	68.7	68.1
1500 ppm NaCl	59.3	63.0	61.0	57.0	58.7	59.0
3000 ppm NaCl	48.7	60.0	52.0	54.0	51.0	54.3
6000 ppm NaCl	19.0	38.0	41.0	40.3	42.0	40.3
Mean	47.3	57.7	56.5	53.3	55.1	===
L.S.D. 0.05	Salinity 1.3		Materials 1.9		Salinity * Materials 3.9	

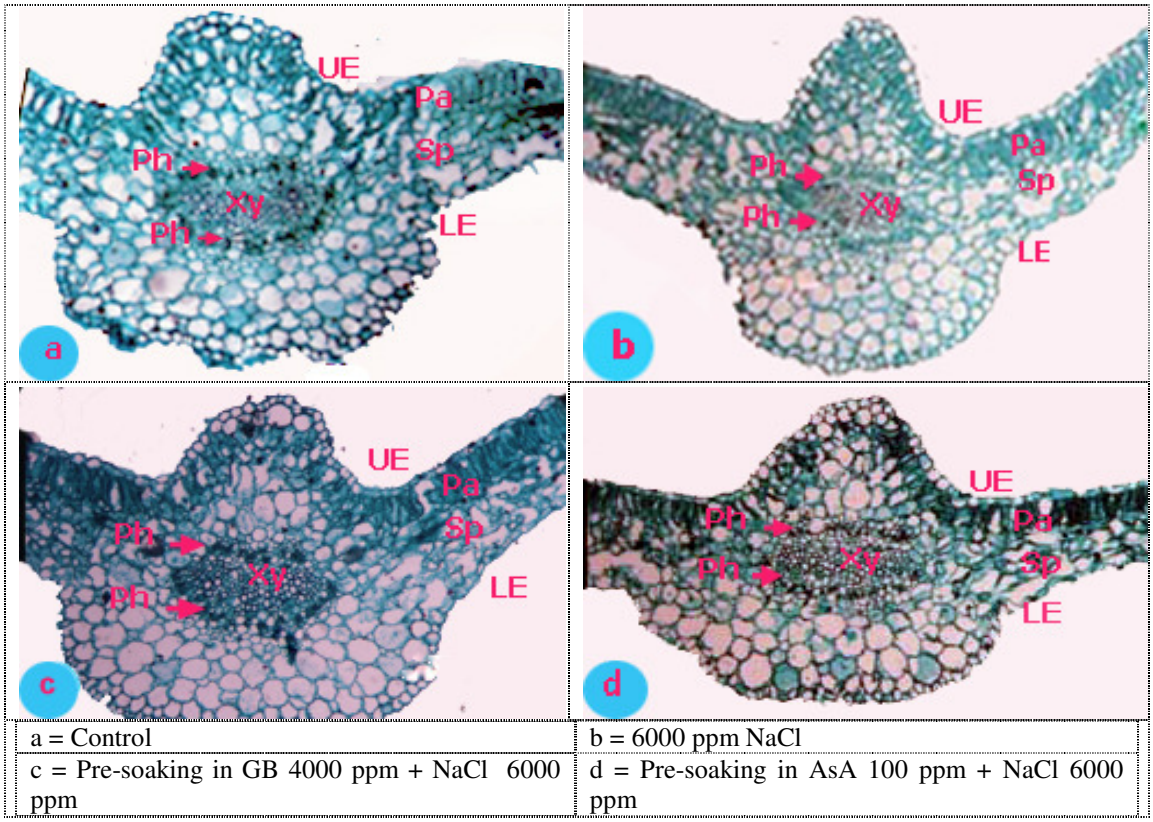
highly water soluble, readily taken up, and transported to the shoots in the transpiration stream and salt stress inhibits the uptake and transport of K^+. In this context, the high accumulation of sodium in plant root may be due to the result of the ability to avoid Na^+ toxicity reducing the transport of Na^+ to the shoot where it may disturb ion charge balance causing specific toxicity and/or a regulatory mechanisms located within the roots that prevent translocation of cations such as Na+ from the root to shoot, and/or the high mobility of Na^+ in the phloem (Adams, 1994).

Gomez *et al*. (1996) found an increase in Na^+ concentration and a decrease in K^+ concentration in leaves, this result may be due to a possible antagonism between K^+ and Na^+.

Compatible solute such as GB is known to play a role in the process of osmotic adjustment in many crops accumulated under environmental stress and the main role is probably, due to insulating plant cells against the ravages of salt by preserving the osmotic balance, by stabilizing the structure of key protein such as Rubisco, by protecting the photosynthetic apparatus and by functioning as oxygen free radical (Heuer, 2003). Moreover, Bohnert and Jernsen (1996) revealed that GB preferentially excludes inorganic ions such as sodium from the hydration sphere of proteins and thus protects enzymes from denaturation. Glycinebetaine may be considered as an additional N-source to the plant; it is possible that GB in the external medium binds and "protects" ion channels (Ca^{++} and K^+ channels and / or aquaporins) and

Table7. Effect of sodium chloride salinity and pre-soaking in GB or AsA as well as their interactions on sweet pepper leaf structure.

Salinity	Treatments (ppm)	Thickness of midrib (µm)		Thickness of leaf blade (µm)		Thickness of palisade tissue (µm)		Thickness of spongy tissue (µm)		Main vascular bundle dimensions (µ)			
										L		W	
Control	Water	80.0	%100.0	22.0	%100.0	6.0	%100.0	12.0	%100.0	18.0	%100.0	27.0	%100.0
	GB 2000	92.0	115.0	25.0	113.6	10.0	166.7	13.0	108.3	22.0	122.2	45.0	166.7
	GB 4000	93.0	116.3	26.0	118.2	8.0	133.3	14.0	116.7	24.0	133.3	51.0	188.9
	AsA 50	90.0	112.5	22.0	100.0	6.0	100.0	13.0	108.3	21.0	116.7	40.0	148.1
	AsA 100	95.0	118.8	25.0	113.6	8.0	133.3	15.0	125.0	22.0	122.2	41.0	151.9
1500 ppm NaCl	Water	88.0	110.0	24.0	109.1	8.0	133.3	12.0	100.0	19.0	105.6	30.0	111.1
	GB 2000	89.0	111.3	30.0	136.4	11.0	183.3	16.0	133.3	21.0	116.7	48.0	177.8
	GB 4000	90.0	112.5	33.0	150.0	12.0	200.0	18.0	150.0	22.0	122.2	52.0	192.6
	AsA 50	80.0	100.0	29.0	131.8	9.0	150.0	12.0	100.0	20.0	111.1	40.0	148.1
	AsA 100	82.0	102.5	32.0	145.5	9.0	150.0	17.0	141.7	21.0	116.7	42.0	155.6
3000 ppm NaCl	Water	70.0	87.5	22.0	100.0	8.0	133.3	11.0	91.7	16.0	88.9	25.0	92.6
	GB 2000	80.0	100.0	26.0	118.2	11.0	183.3	13.0	108.3	18.0	100.0	35.0	129.6
	GB 4000	85.0	106.3	28.0	127.3	11.0	183.3	15.0	125.0	20.0	111.1	35.0	129.6
	AsA 50	78.0	97.5	25.0	113.6	7.0	116.7	14.0	116.7	16.0	88.9	32.0	118.5
	AsA 100	82.0	102.5	27.0	122.7	10.0	166.7	14.0	116.7	17.0	94.4	34.0	125.9
6000 ppm NaCl	Water	62.0	77.5	14.0	63.6	5.0	83.3	6.0	50.0	14.0	77.8	22.0	81.5
	GB 2000	75.0	93.8	22.0	100.0	8.0	133.3	11.0	91.7	16.0	88.9	26.0	96.3
	GB 4000	85.0	106.3	20.0	90.9	5.0	83.3	12.0	100.0	26.0	144.4	27.0	100.0
	AsA 50	65.0	81.3	15.0	68.2	6.0	100.0	7.0	58.3	15.0	83.3	25.0	92.6
	AsA 100	67.0	83.8	15.0	68.2	5.0	83.3	7.0	58.3	16.0	88.9	25.0	92.6



a = Control	b = 6000 ppm NaCl
c = Pre-soaking in GB 4000 ppm + NaCl 6000 ppm	d = Pre-soaking in AsA 100 ppm + NaCl 6000 ppm

Fig 1. Cross section of sweet pepper leaf blade showing the effect of pre-soaking with GB and AsA under saline condition (magnification 100X). LE: Lower Epidermis, Me: Mesophyll, Pa: Palisade Parenchyma, Ph: Phloem, Sp: Spongy Parenchyma, UE: Upper Epidermis, Xy: Xylem.

influences properties of the cell membrane; an additional table with cell sap concentrations would be helpful to get behind the effects of the supplied compounds on selectivities of K^+ over Na^+, where it is highly possible that calcium is also involved in that game.

Ascorbic acid increased the potassium concentration in shoot and root of sweet pepper plants growing under normal and saline conditions. In this respect Neveen, Shawky (2003) suggested that the protection of sweet pepper plants against salt stress by an exogenous supply of AsA is believed to be caused indirectly as a result of its effect on K^+ uptake which plays an essential role in many metabolic processes; such as, may be induce the synthesis of stress proteins as a "messenger" (chitinase, glucanase, peroxidase, peroxidismutase) in general is it the induction of PR-proteins.

Leaf blade structure

Data presented in table (7) and illustrated in Figure (1) indicates that low level of salinity 1500 ppm (4.84 dSm^{-1}) increased thickness of midrib region due to increasing the dimensions of the vascular bundle. In addition, thickness of the leaf blade was increased due to a corresponding increase in the palisade and spongy parenchyma (Figure 1). While, salinity at 3000 (7.19 dSm^{-1}) and 6000 ppm (11.88 dSm^{-1}) decreased the thickness of midrib region, due to the decrease in the dimensions of the main vascular bundle. In addition, the high salinity level markedly decreased leaf blade thickness. This reduction was due to a decrease in thickness of both palisade and spongy tissues.

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

Furthermore, the decreases in the diameter of vascular bundle in the leaf blade result in lowering

the accumulation of necessary water required for photosynthesis.

The promotive effect of low salinity level on sweet pepper leaf thickness may be due to an increase in thickness of mesophyll tissue. Strogonov (1964) 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.

Furthermore, Aloni (1987) suggested that increase or decrease in the vessel diameter might increase or decrease the efficiency of water conduction, owing to increase or decrease in the resistance to flow. Numerous structural changes may be induced under saline conditions in both halophytes and glycophytes. These include: a) increased succulence. b) Inhibition of differentiation and changes in diameter and number of xylem vessels. c) Reduction in thickness of lamina and mesophyll. d) Thickening of the cuticle. e) Changes in the number and size of stomata. f) Extensive development of tyloses. g) Earlier occurrence of lignifications (Strogonov 1964).

The inhibiting effects of high salinity level on leaf structure may be due to inhibition the growth of vascular elements (Rashid *et al*. 2004), 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.

Regarding, the effect of GB or AsA, it was showed in the same table that thickness of midrib region was markedly increased. This result is in agreement with those reported by Ali (2001). He reported that the palisade thickness in tomato leaf increased with AsA at 200 and 400 ppm but spongy tissue thickness was not/or slightly affected.

Regarding the dimensions of vascular bundles, it is clear from the same table that the length and width of vascular bundle were increased in sweet pepper plants treated with GB or AsA. GB at 4000 ppm and AsA at 100 ppm were the most effective in increasing length of the vascular bundle.

Meanwhile, GB at 2000 ppm or 4000 ppm was the most effective in increasing the width of vascular bundle. Furthermore, Ali (2001) revealed that AsA affected xylem vessel differentiation and development. This effect may be due to the effect of AsA on the growth rate stimulating cell expansion, vacuolation and fluid uptake (Gonzalez-Reyes *et al*. 1994) and cell division (Conklin, 2001) as well as the effect on membrane hyper polarization of the cells (Gonzalez-Reyes *et al*. 1995).

Data in the same table indicate that interaction effect between low and moderate salinity levels and GB or AsA, tended to increase the thickness of midrib region, dimensions of vascular bundle (length and width), as well as leaf blade thickness corresponding to an increase in the thickness of palisade and spongy tissues.

Generally, presoaking of sweet pepper seeds in glycinebetaine at 4000 ppm or ascorbic acid at 100 ppm help to sustain salt effects on thickness of the midrib region and mesophyll tissues of leaf blade induced some anatomical characters as compared to untreated plants.

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