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The effect of glycinebetaine or ascorbic acid on grain germination and leaf structure of sorghum plants grown under salinity stress

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Abstract

The effects of exogenously applied glycinebetaine or ascorbic acid (pre-soaking or pre-soaking plus spraying) on sorghum seedlings grown under salt stress were investigated. The seedlings were grown in hydroponic culture containing nutrient solution for 3 weeks and treated with NaCl at 1500, 3000 and 6000 ppm. Salinity increased significantly the germination percentage of sorghum grains when applied at low level but tended to decrease as the concentration of salt was raised. Ascorbic acid or glycinebetaine either alone or in combination with salinity increased germination percentage and ascorbic acid was more effective in this respect. Concerning leaf anatomy it was found that low salinity level (1500 ppm NaCl) increased the blade thickness, xylem and phloem tissues thickness and metaxylem vessel 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. However, 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. On the other hand, salinity induced leaf cell damages in leaf sorghum plant as compared to control plants. It could be concluded that glycinebetaine or ascorbic acid applications could minimize the harmful effects of salt and pre-soaking plus spraying was found to be more effective than that of pre-soaking application.

Key words: Sorghum, salt stress, nutrient solutions, antioxidants, osmoregulators, leaf anatomy

Abbreviations: AsA_Ascorbic acid; Ch_Chloroplast; CW_Cell Wall; GB_Glycinebetaine; L_lipid droplets; LE_ Lower Epidermis; M_Myelin-Figures; Me_Mesophyll; Mt_Mitochondria; MV_Membrane vesicles; N_Nucleus; Nu_Nucleolus; Pd_Plasmodesmata; Pg_Plastoglobuli; Ph_Phloem; PM_Plasma membrane; S_Starch grain; Th_ Thylakoid; UE_Upper Epidermis; V_Vacuole; Xy_Xylem

Introduction

Plants are exposed to many stress factors, such as drought, high salinity or pathogens, which reduce the yield of the cultivated plants or affect the quality of the harvested products. Salt stress, in general, reduces the water uptake capacity of the plant, thus reduces growth rate and metabolic activity. The initial growth reduction could be due to hormonal signals generated by the roots encountering salinity (Munns, 2002). As a more long term impact of salinity, the excessive salt toxicity levels lead to senescence and reduce the photosynthetic capacity due to the closure of stomata and limited carbon dioxide uptake, which cannot sustain proper growth (Zhu, 2001; Munns, 2002). In addition, Salinity is known to affect many aspects of metabolism, anatomy and ultra structure of plant cells (Rahman *et al.*, 2000). These reactions are often considered to be adaptive strategies, being helpful to sustain NaCl salinity. Salt stress even delays germination of seeds as well as the final germination percentage (Zeinali *et al.,* 2002). Salt stress causes a number of changes in plant metabolism. Of them, ion toxicity, osmotic stress and production of reactive oxygen species (ROS) such as singlet oxygen ($^1o_2$), superoxide radical ($O^-{}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are most prominent (Mittler, 2002). ROS are generally produced in mitochondria and chloroplasts. In chloroplasts, ROS are generated by direct transfer of excitation energy

from chlorophyll to produce singlet oxygen, or by univalent oxygen reduction of photosystem I in the Mehler reaction (Asada, 1999). ROS are highly reactive and in the absence of any protective mechanism they can seriously cripple normal metabolism through oxidative damage to lipids, proteins and nucleic acids. For example, H_2O_2 can down-regulate CO_2 assimilation by inhibiting several Calvin cycle enzymes (Asada, 1999). ROS are also known to serve as signaling intermediates in guard cells to promote stomatal closure (Foyer and Noctor, 2003), cause damage to cell membranes or even provoke apoptosis (Loreto *et al*., 2001). The generation of ROS could be limited or scavenged by an antioxidant system including antioxidant compounds ascorbate, salicylate, glutathione, tochopherols, etc. and antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (Foyer and Noctor, 2003). Salt tolerant plants, in addition to regulating the ion and water homeostasis should also have a better antioxidant system for the effective removal of ROS (Mittler, 2002). In view of a number of studies, salt tolerance is often correlated well with a more efficient oxidative system (Mittova *et al*., 2002; Bor *et al*., 2003).

It has been well established that plants accumulate a variety of osmoregulators solutes such as glycinebetaine (GB) as a number of quaternary ammonium compounds "betains" and tertiary sulphonium compounds (Rhodes and Hanson, 1993). In addition, betaine is considered to be involved in scavenging free radicals and in protecting enzymes in addition to their well-established roles as a simple osmolyte (Okuma *et al*., 2004). It is reported that betaine act as enzyme protectants against abiotic stresses (Sharma and Dubey, 2005) and protect higher plants against salt/osmotic stresses by stabilizing many functional units such as complex II electron transport (Hamilton and Heckathorn, 2001), membranes and proteins (Holmström *et al*., 2000), and enzymes such as RUBISCO (Mäkelä *et al*., 2000). Moreover, exogenous betaine improves stress tolerance by preventing photoinhibition (Ma *et al*., 2006) and reducing oxidation of lipid membranes (Demiral and Türkan, 2004) in a wide variety of accumulator/non-accumulator plants. These ROS are necessary for inter- and intracellular signaling (Foyer and Noctor, 1999) but at high concentrations they seriously disrupt normal metabolism of plants through oxidation of membrane lipids, proteins, and nucleic acids in the absence of any protective mechanism (Noctor and Foyer, 1998; Hernández *et al*., 2001). Plants possess both enzymatic and non-enzymatic antioxidant defense systems to protect their cells against ROS.

Ascorbic acid (AsA) in plants, the main source of vitamin C for humans, is also an essential compound for plants, with important roles as an antioxidant and as a modulator of plant development through hormone signalling (Pastori, *et al*., 2003). Although ascorbic acid 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 (Athar, *et al.,* 2008).In addition, Ascorbic acid plays important roles in plants, such as protective role against reactive oxygen species that are formed from photosynthetic and respiratory processes. AsA is linked to cell growth, being involved in the cell cycle and other mechanisms of plant cell growth and division, as well as acting as a co-factor for many enzymes (Lee and Kader, 2000).

Moreover, is one of the most important antioxidants abundantly occurring in plants (Smirnoff, 2000) and it is present in all plants grown under "normal" and/or under "stressed" conditions. Generally, its concentration is higher in leaves as compared to other plant parts and it is 5–10 times higher than that of glutathione (GSH) (Smirnoff, 2005).

Sorghum is one of the most important for human beings and considered to be one of the moderately salt tolerant plants (Bernstein *et al*., 1995). The aim of subsequent experiments was to study the effects of Glycinebetaine (GB) or Ascorbic acid (AsA) on seed germination, ultra structure and anatomy of sorghum seedlings grown on nutrient solutions with or without salt supply.

Materials and methods

The experiment was carried out in the glasshouse of the Agric. Bot. Dept., Fac. of Agric., Mansoura Univ., El-Mansoura city, Egypt during the summer season 2005.

The following experiment was conducted to study the harmful effects of salinity on sorghum plants growing in NFT, through pre-soaking compared to pre-soaking plus spraying sorghum plants with osmoregulator (glycinebetaine) or antioxidant (ascorbic acid) under normal or saline conditions.

Plant materials

Sorghum (*Sorghum bicolor* (L.) Moench. var. *bicolor*) grains were secured from the Agricultural Research Centre, Giza, Cairo, Egypt.

Chemicals

1. GB was supplied by Sigma Chemical Co., USA and used at the concentration of 2000 or 4000 ppm.

2. AsA was obtained from EL-Gomhoria Co., Egypt and was used at the concentration of 50 or 100 ppm.
3. NaCl from EL-Gomhoria Co., Egypt and was used at the concentrations of 1500, 3000 and 6000 ppm.

Germination experiment

A homogenous lot of sorghum grains was surface sterilized by soaking in 0.001 %5 $HgCl_2$ for one min. and thoroughly washed with distilled water. Sorghum grains were then divided into 5 groups. The first group was soaked in distilled water and the remaining groups were separately soaked for 12 h in aqueous solution of GB at 2000 or 4000 ppm or AsA at 50 or 100 ppm.

Every group was divided into four sub-groups. Each sub-group contained 20 grains. The first sub-group was moistened with 10 ml nutrient Cooper solution serving as a control group (EC 2.5 dSm^{-1}) (Cooper, 1979). The remainder sub-groups were salinized with 10 ml nutrient cooper solution adding NaCl at 1500, 3000 and 6000 ppm. The treatments were designated as S0 (2.5 dSm^{-1}) control, S1 (4.84 dSm^{-1}), S2 (7.19 dSm^{-1}), and S3 (11.88 dSm^{-1}). The grains were allowed to germinate in an incubator at 25 ± 2^0C between discs of filter paper (Whatman No.1) placed in 11 cm glass Petri dishes. After 5 days from sowing the germination percentage was recorded.

Glass house experiment

The experiment layout consisted of 8 plastic channels (4 m long and 10 cm diameter). Every two channels were provided by an electric pump representing four groups (0, 1500, 3000 and 6000 ppm). Each channel had 40 pores (6 cm diameter). After 5 days from pre-soaking, 5 uniform sorghum seedlings with 3-5 seminal roots were transferred into 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, GB2, AsA1, AsA2. Each set contained 8 seedlings (one seedling/pot) spaced 10 cm representing a Nutrient Film Technique (NFT).

Each group of channels was divided into two subgroups i.e. pre-soaking and pre-soaking plus spraying. The seedlings assigned for pre-soaking plus spraying were sprayed twice (7 and 14 days from transplanting) with the same levels previously applied in the first group (pre-soaking method).

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 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 one liter per minute from a reservoir containing 10 liters.

For the effect of salinity, GB, AsA, and their interactions on mesophyll ultrastructure. Small pieces ($5mm^2$) from the right midrib region of the second leaf of sorghum were taken at the age 21 days after transferring plants into channels. Double fixation in Glutaraladehyde (2.5%) and osmium tetroxide (1%) was used. The fixative solutions were prepared in 0.01 M phosphate buffer of pH6.5. Glutaraladehyde was used first for overnight in the refrigerator and replaced with cold buffer for 15 min, and then the buffer was replaced with osmium tetroxide for 1h. Most of the osmium tetroxide was removed and replaced with two changes of buffer for 15 min each. The materials were passed along the dehydration gradient by substituting the buffer with 50, 70, 80, 95 and 100 % acetone, 10-15 min in each except 100% for which two changes of 30 min were made. The pure acetone was replaced gradually with a mixture of acetone and epoxy resin the 100% acetone was replaced with 2:1, 1:1 and 1:2 mixtures of acetone/epoxy resin for 15 minutes each. The dilute resin was then replaced with pure resin for overnight and was replaced again with fresh resin for 2hr and the materials were thus ready for embedding. Thick sections were made first to select the suitable area for ultrathin section (50-100µ) using LKB ultratone III microtome. Sections were collected on copper grids, and double stained with saturated uranyl acetate in 70% ethanol and Reynolds lead citrate for 15 min each. Sections were viewed, investigated and photographed, using transmission electron micros-copy (JEOL 100s TEM).

The samples were killed and fixed in formalin-acetic-alcohol (FAA), washed, dehydrated, cleared, embedded in paraffin wax (52-54°C melting points), sectioned, and double stained with saffranin-light green combination and cleared in clove oil (Gerlach, 1977). The sections were examined microscopically. Data were subjected to statistical analysis of variance according to Gomez and Gomez (1984).

Results and Discussions

1- Seed germination

Data illustrated in Figure (1) indicated that sorghum is sensitive to salinity. The low level of NaCl increased significantly sorghum germination percent-age whereas decreased gradually with increasing NaCl levels. The great reduction occurred under high salinity levels (6000 ppm NaCl). On the other hand, pre-soaking sorghum grains in either GB or AsA

increased germination percentage compared to control. Furthermore, AsA was more effective than GB in increasing germination percentage of sorghum grains. GB or AsA combined with low salinity level (1500 ppm) increased germination percentage as compared to non-salinized plants. On the other hand, both GB and AsA increased germination percentage under moderate and high salinity levels (3000 and 6000 ppm) as compared to the untreated plants under such salinity level. The results proved that GB and AsA partially counteracted the harmful effect of NaCl salinity.

Stimulating effect of low salinity level on plant growth may be resulted from the beneficial effect of low concentration of chloride on many physiological processes such as osmoregulators, photosynthesis and enzyme activities, chloride acts a cofactor of an NH_2oH sensitive, Mn-containing, O_2^-evolving enzyme (Kelley and Izawa, 1978). Moreover, - Critchley (1983) suggested that chloride facilitates electron transport by reversible ionic binding to the O_2^-evolving complex or to the thylakoid membranes. In addition, some enzymes are known to be stimulated by Cl^- such as ATPase (Churchli and Sze, 1984) and alpha-amylase hydrolysize starch to sugars require chloride for activation (Metzler, 1977).

On the other hand, salinity stress can affect seed germination through reduction of water uptake leading to moisture stress (osmotic effect), by ion toxicity and/or ionic imbalance, or by the accumulation of Na^+ and Cl^- ions and inhibition of the uptake of several essential nutrients such as K^+ causing nutritional imbalance in the plants or accumulation of these factors (Taamalli *et al,* 2004) and/or decreasing the activity of certain enzymes by either decreasing the rate of transcription or translation, which lead to decreasing both cell division and cell elongation (Dodd and Donovan, 1999).

Moreover, the inhibiting effect of salinity on seed germination percentage may be due to hormonal imbalance represented by increasing ABA and decreasing cytokinins, GA_3 and IAA (Roy *et al,* 1995). In addition, Uperty and Sarin (1976) found that salt effects at early stages of germination involved changes in the protein metabolism of the seeds, i.e. stimulation of proteolytic activity.

Certain plants accumulate significant amounts of GB, in response to high levels of salinity. It's likely that betaine is involved in the protection of macro-components of plant cell, such as protein complex and membranes under salt stress conditions (Sakamoto and Murata, 2002). It is known that GB is to maintain water content in plant cells by lowering solute potential under osmotic stress, i.e., to act osmotic adjustment (Mäkelä, 2004).

AsA counteracted the adverse effects of salinity on seedling growth as well as metabolic mechanisms and metabolic activities in the plants and promoted lipase and catalase activities of soybean, peroxidase isoenzymes, protease, peroxidase, catalase and invertase activities (Shaddad *et al,* 1999)

2. Leaf blade structure

Most vital processes of plants such as dry matter production, respiration and transpiration occur in leaves. The accumulation of dry matter by the seeds or grains requires the production of assimilates in the leaves, their translocation to the fruit, movement into the organs of the seeds or grains and the synthesis of materials to be stored, leaf anatomy seems to be of great importance in this regard.

Histologically, sorghum leaf blade is composed of three types of tissue: epidermis of the two surfaces of the leaf, mesophyll tissue undifferentiated into palisade and spongy parenchyma and closed collateral vascular bundles of the veins (Bendre and Pande, 2004).

Data presented in table (1) and illustrated in Figure (2) showed that low salinity level (1500 ppm NaCl) increased the blade thickness, xylem and phloem tissues thickness and metaxylem vessel diameter as well as main vascular bundle dimensions. On the other hand, moderate and high salinity levels (3000 and 6000 ppm NaCl) decreased all these parameters. The great reduction was observed under high salinity level.

This reduction may be due to a decrease in the dimensions of vascular bundle. The reduction in the dimensions of vascular bundle in sorghum leaf (Table 1) may affect the flow of water and minerals from the root to the shoot as well as the product of photosynthesis.

Concerning the effect of GB and AsA, it was observed in the same table that the blade thickness, xylem and phloem tissues thickness increased markedly with this treatment. The most effective in this respect, was GB at 2000, 4000 ppm (pre-soaking plus spraying). In addition, AsA (pre-soaking plus spraying) at two levels increased the main vascular bundle dimensions. Generally, GB proved to be more effective in this respect.

In additions, all interactions between low salinity level and GB or AsA increased markedly thickness of either the blade, xylem and phloem tissues and metaxylem vessel diameter as well as the main vascular bundle dimensions. Moreover, the thickness of blade decreased under moderate salinity level with application of GB or AsA except, GB (pre-soaking plus spraying). On the other hand, the thicknesses of xylem and phloem tissues, diameter of metaxylem

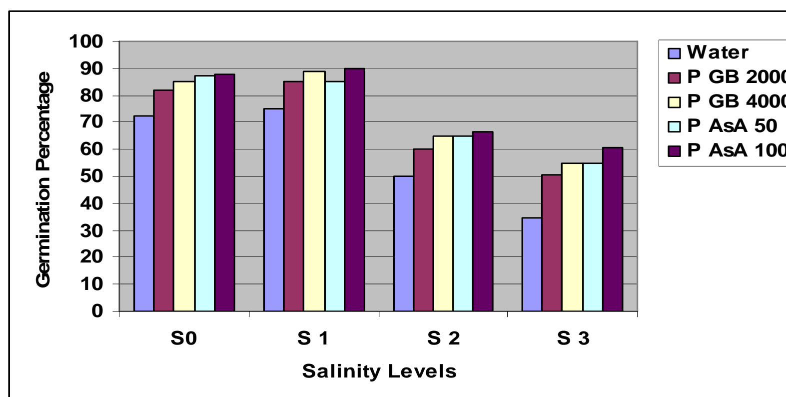


Fig 1. Effect of pre-soaking in GB or AsA on Germination percentage of sorghum grains grown under normal and NaCl conditions at 5 days from sowing.

vessel as well as length of the main vascular bundle were markedly increased under the same conditions. GB at two levels (pre-soaking plus spraying) was the most effective in this respect.

Under high salinity level (6000 ppm NaCl), applications of GB or AsA counteracted the harmful effect of salinity on the thickness of xylem tissue, phloem tissue and metaxylem vessel diameter as well as dimensions of the main vascular bundle, except pre-soaking sorghum grains in AsA at 50 ppm. Moreover, these treatments were not able to decrease the blade thickness under salinity level as compared to non-salinized plants.

The promotive effect of low salinity level on sorghum 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.

The inhibiting effects of high salinity level in leaf structure may be due to suppressed cell division and cell enlargement proportionally (Nieman, 1965), and 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.

From the above mentioned results it could be mentioned that GB or AsA application gave the best results related to leaf structure as compared to the untreated plants under all salinity levels. The promotive effect of GB or AsA on seedling, stem and root lengths may be the result of increasing cell division and cell enlargement due to water uptake caused by a decrease in the osmotic potential by increasing soluble sugars which serve as a substrate for increasing initiation of leaf primordia. Furthermore, the present results indicate that GB or AsA applications counteracted the deleterious effects of NaCl salt stress on leaf structure which may be due to a reduction in stomatal opening leading to decrease in the transpiration. This reduction may be due to a decrease in the dimensions of vascular bundle. In the present investigation, the reduction in the dimensions of vascular bundle in sorghum leaf (Table 1) may affect the flow of water and minerals from the root to the shoot as well as the product of photosynthesis.

Concerning the effect of GB and AsA it was observed in the same table that, the blade thickness, xylem and phloem tissues thickness increased markedly with this treatment. The most effective in this respect was GB at 2000, 4000 ppm (pre-soaking plus spraying). In addition, AsA (pre-soaking plus spraying) at two levels increased the main vascular bundle dimensions. Generally, GB proved to be more effective in this respect.

Data presented in the same table showed that all interactions between low salinity level and GB or AsA increased markedly thickness of blade, xylem and phloem tissues, metaxylem vessel diameter as well as the main vascular bundle dimensions. Moreover, the thickness of blade decreased under moderate salinity level with application of GB or AsA except, GB (pre-soaking plus spraying). On the other hand, the thickness of xylem and phloem tissues, diameter of metaxylem vessel as well as length of main vascular bundle was markedly increased under the same conditions. GB at two levels (pre-soaking plus spraying) was the most effective in this respect. Under high salinity level (6000 ppm NaCl), application of GB or AsA counteracted the harmful effect of salinity on the thickness of xylem tissue, phloem tissue and metaxylem vessel diameter

Table 1. Effect of sodium chloride salinity and GB or AsA applications (pre-soaking or pre-soaking plus spraying) and their interactions on sorghum leaf structure

Salinity (ppm)	Treatment (ppm)	Blade thickness (µm)		Xylem tissue thickness (µm)		Phloem tissue thickness (µm)		Metaxylem vessel diameter (µm)		Main vascular bundle dimensions (µ)			
										L.		W.	
Control	Water	36.0	%100.0	5.0	%100.0	2.0	%100.0	2.0	%100.0	7.0	%100.0	8.0	%100.0
	GB 2000 (Pre-soaking)	38.0	105.6	7.0	140.0	3.0	150.0	3.0	150.0	10.0	142.9	9.0	112.5
	GB 4000 (Pre-soaking)	42.0	116.7	8.0	160.0	3.0	150.0	3.0	150.0	11.0	157.1	10.0	125.0
	AsA 50 (Pre-soaking)	39.0	108.3	6.0	120.0	3.0	150.0	3.0	150.0	9.0	128.6	9.0	112.5
	AsA 100 (Pre-soaking)	41.0	113.9	6.0	120.0	3.0	150.0	3.0	150.0	9.0	128.6	8.0	100.0
	GB 2000 (Pre-soaking plus spraying)	50.0	138.9	7.0	140.0	4.0	200.0	3.5	175.0	11.0	157.1	10.0	125.0
	GB 4000 (Pre-soaking plus spraying)	55.0	152.8	8.0	160.0	3.0	150.0	4.0	200.0	11.0	157.1	10.0	125.0
	AsA 50 (Pre-soaking plus spraying)	42.0	116.7	7.0	140.0	2.0	100.0	3.0	150.0	9.0	128.6	10.0	125.0
	AsA 100 (Pre-soaking plus spraying)	44.0	122.2	7.0	140.0	3.0	150.0	3.0	150.0	10.0	142.9	10.0	125.0
1500 ppm	Water	38.0	105.6	6.0	120.0	2.0	100.0	3.0	150.0	8.0	114.3	9.0	112.5
	GB 2000 (Pre-soaking)	52.0	144.4	6.0	120.0	3.0	150.0	3.0	150.0	9.0	128.6	9.0	112.5
	GB 4000 (Pre-soaking)	54.0	150.0	6.0	120.0	3.0	150.0	3.0	150.0	9.0	128.6	10.0	125.0
	AsA 50 (Pre-soaking)	40.0	111.1	6.0	120.0	2.0	100.0	3.0	150.0	8.0	114.3	9.0	112.5
	AsA 100 (Pre-soaking)	41.0	113.9	6.0	120.0	3.0	150.0	3.0	150.0	9.0	128.6	10.0	125.0
	GB 2000 (Pre-soaking plus spraying)	57.0	158.3	7.0	140.0	3.0	150.0	3.0	150.0	10.0	142.9	11.0	137.5
	GB 4000 (Pre-soaking plus spraying)	64.0	177.8	6.0	120.0	4.0	200.0	3.0	150.0	10.0	142.9	12.0	150.0
	AsA 50 (Pre-soaking plus spraying)	50.0	138.9	6.0	120.0	3.0	150.0	3.0	150.0	9.0	128.6	10.0	125.0
	AsA 100 (Pre-soaking plus spraying)	50.0	138.9	7.0	140.0	3.0	150.0	3.0	150.0	10.0	142.9	12.0	150.0
3000 ppm	Water	30.0	83.3	4.0	80.0	2.0	100.0	1.5	75.0	6.0	85.7	7.0	87.5
	GB 2000 (Pre-soaking)	35.0	97.2	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0
	GB 4000 (Pre-soaking)	35.0	97.2	5.0	100.0	4.0	200.0	2.0	100.0	9.0	128.6	8.0	100.0
	AsA 50 (Pre-soaking)	32.0	88.9	5.0	100.0	3.0	150.0	2.5	125.0	8.0	114.3	8.0	100.0
	AsA 100 (Pre-soaking)	34.0	94.4	5.0	100.0	3.0	150.0	2.5	125.0	9.0	128.6	8.0	100.0
	GB 2000 (Pre-soaking plus spraying)	36.0	100.0	6.0	120.0	4.0	200.0	3.0	150.0	10.0	142.9	8.0	100.0
	GB 4000 (Pre-soaking plus spraying)	36.0	100.0	6.0	120.0	4.0	200.0	3.0	150.0	10.0	142.9	8.0	100.0
	AsA 50 (Pre-soaking plus spraying)	35.0	97.2	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0
	AsA 100 (Pre-soaking plus spraying)	35.0	97.2	5.0	100.0	4.0	200.0	2.5	125.0	9.0	128.6	8.0	100.0
6000 ppm	Water	22.0	61.1	3.0	60.0	2.0	100.0	1.0	50.0	6.0	85.7	6.0	75.0
	GB 2000 (Pre-soaking)	26.0	72.2	4.0	80.0	3.0	150.0	2.0	100.0	7.0	100.0	8.0	100.0
	GB 4000 (Pre-soaking)	29.0	80.6	5.0	100.0	2.0	100.0	2.0	100.0	7.0	100.0	8.0	100.0
	AsA 50 (Pre-soaking)	24.0	66.7	4.0	80.0	2.0	100.0	2.0	100.0	6.0	85.7	7.0	87.5
	AsA 100 (Pre-soaking)	25.0	69.4	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0
	GB 2000 (Pre-soaking plus spraying)	32.0	88.9	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0
	GB 4000 (Pre-soaking plus spraying)	32.0	88.9	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0
	As A50 (Pre-soaking plus spraying)	29.0	80.6	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0
	AsA 100 (Pre-soaking plus spraying)	30.0	83.3	5.0	100.0	3.0	150.0	2.0	100.0	8.0	114.3	8.0	100.0

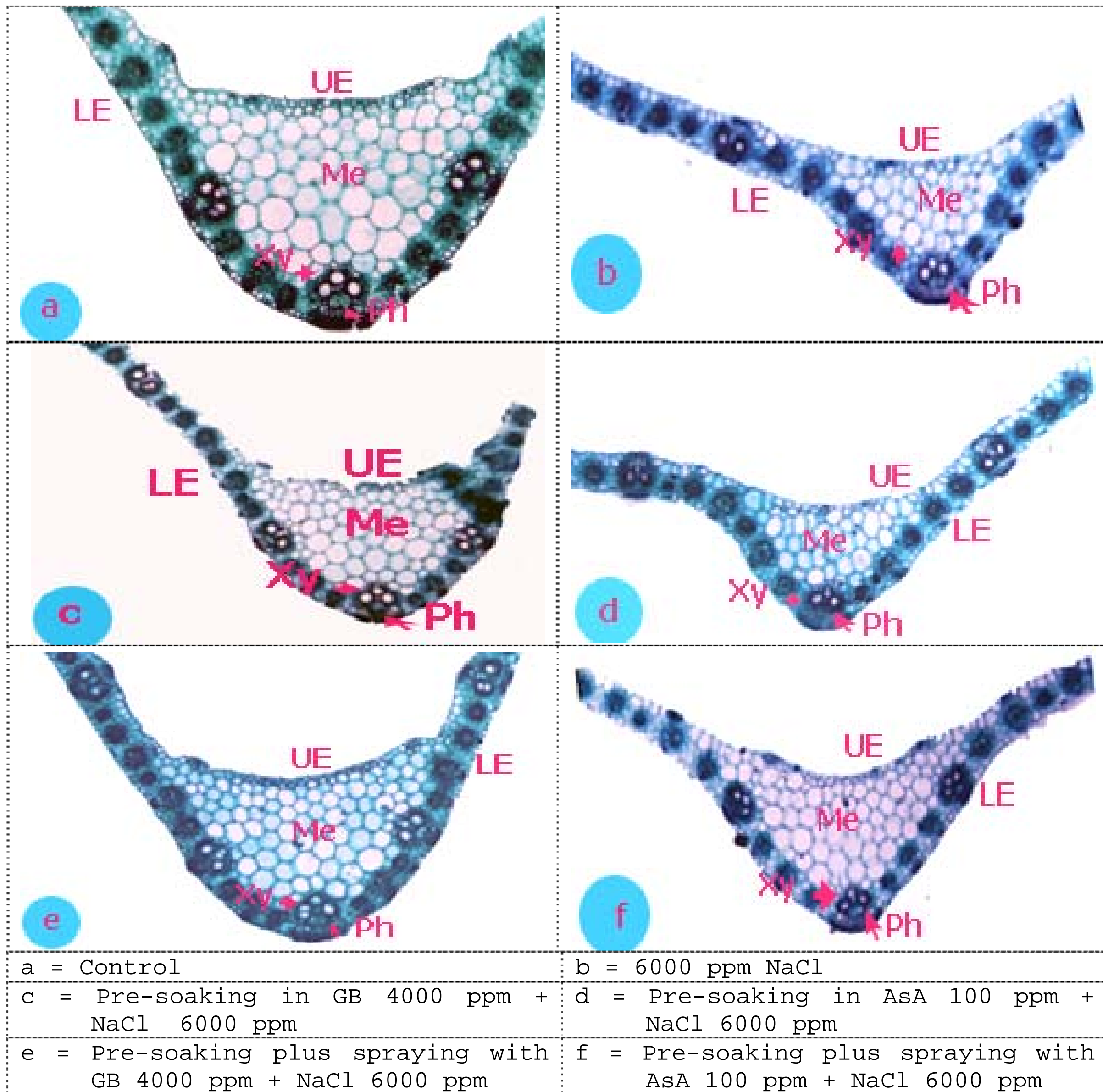


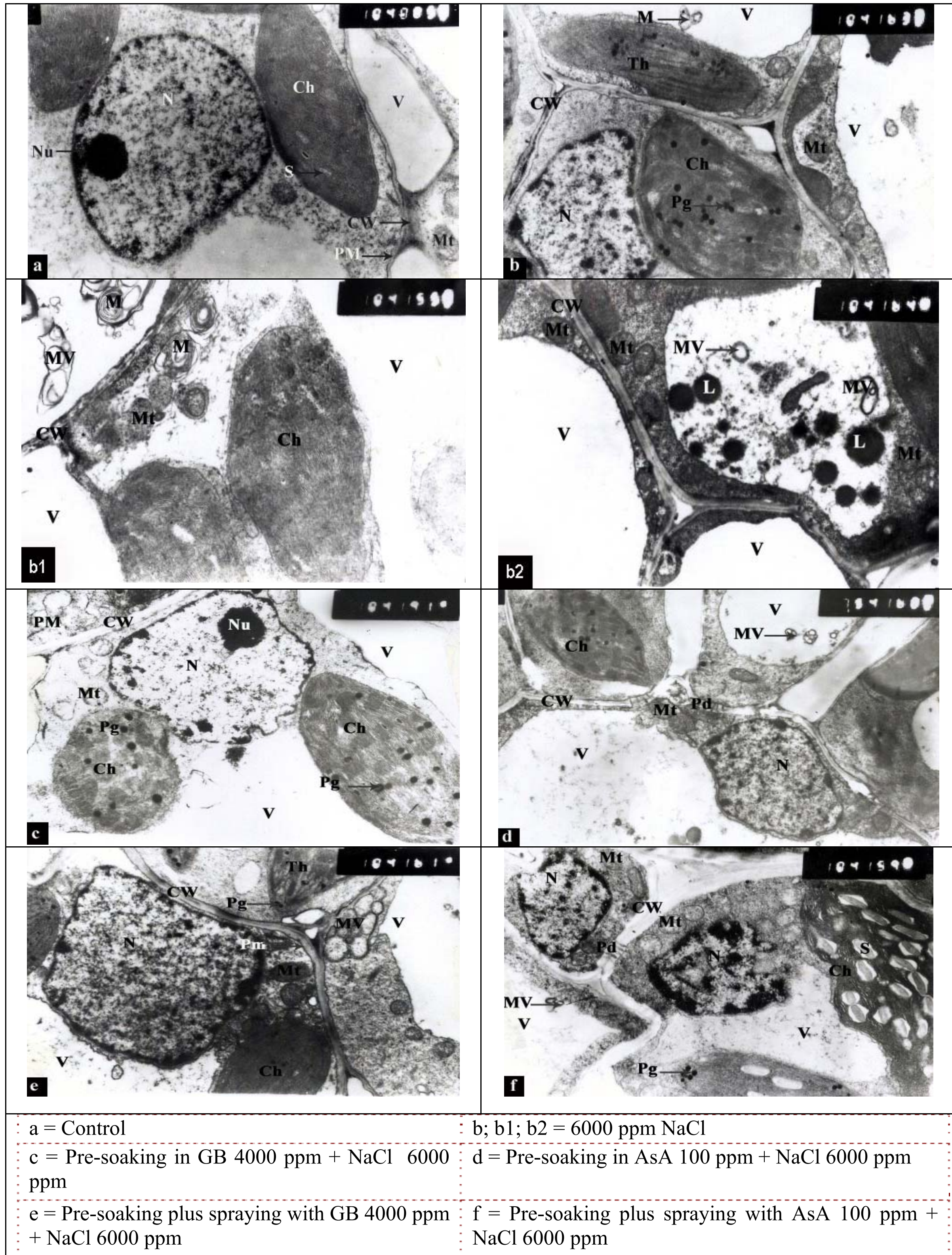
Fig 2. Cross sections of sorghum leaf plants showing that effect of pre-soaking or pre-soaking plus spraying with GB and AsA under saline conditions (x100). (also refer to abbreviations)

as well as dimensions of the main vascular bundle, except pre-soaking sorghum grains in AsA at 50 ppm. Moreover, these treatments were not able to decrease the blade thickness under salinity level as compared to non-salinized plants.

3. Leaf blade ultrastructure

The ultrastructure of sorghum leaf mesophyll nucleus of plants grown under normal conditions shows that the nucleus was regular, and the nuclear membrane, nucleolus, nuclear chromatin and nuclear envelope were intact (Fig 3-a). On the other hand, the mesophyll nucleus of sorghum plants grown under NaCl salinity vanished the nucleolus. The most notable effects of salinity are represented by smaller nucleus, condense the nuclear chromatin, and the nuclear envelop was shrinked.

The nucleus ultrastructure of sorghum leaf was taken from plants pre-soaked in GB at 4000 ppm or AsA at 100 ppm growing under salinity conditions at 6000 ppm NaCl showed that, the nucleus appeared normal, but GB caused shrinkage of the nuclear envelop in some positions. While, the nuclear envelop was normal in plants treated with AsA at 100 ppm but the nucleolus was vanished (Fig 3-c, -d).



a = Control	b; b1; b2 = 6000 ppm NaCl
c = Pre-soaking in GB 4000 ppm + NaCl 6000 ppm	d = Pre-soaking in AsA 100 ppm + NaCl 6000 ppm
e = Pre-soaking plus spraying with GB 4000 ppm + NaCl 6000 ppm	f = Pre-soaking plus spraying with AsA 100 ppm + NaCl 6000 ppm

Fig 3. Transmission electron micrographs of mesophyll cells of the leaf sorghum ultra-structure (also refer to abbreviations)

Pre-soaking plus spraying with GB at 4000 ppm or AsA at 100 ppm combined with NaCl showed that GB was more effective in reducing the harmful effects of salinity on nucleus ultrastructure than AsA (Fig 3-e, -f).

It could be concluded from the results that salinity stress caused vanished nucleolus, and irregularity in nuclear shape, and the nuclear envelop was shrinked in some nucleus and chromatin was found to be dense. Similar results were also obtained by Rahman *et al.,* (2000) and Sam *et al.,* (2003/2004). In addition, Strogonov (1974) found that the nucleus was the most sensitive component of the pea cell to NaCl, while nucleus size and shape was relatively stable.

Furthermore, the Myelin-Figures were also found from plasma membrane (Fig 3-b1), which were absent in control plants (Fig 3-a). Myelin-Figures as observed in the present study are considered as artifacts due to double fixation with gluteraldehyde and osmium tetroxide (Bowers and Maser, 1988). These artifacts were absent in the control plants but they were observed in the NaCl-treated plants and thus were considered as a reflection of the membrane changes due to the NaCl treatment (Rahman *et al.,* 2001). Pre-soaking sorghum grains in GB at 4000 ppm or AsA at 100 ppm and grown under NaCl at 6000 ppm, maintained the plasmamembrane and cell wall structure, and no shrinkage or detachment of plasma membrane was detected.

Moreover, a large amount of lipid droplets in cytoplasm was accumulated in sorghum leaf cells (Fig 3-b2). The lipid droplets which were not found in control plant leaves, observed in the cytoplasm of salt treated-plants may be due to lose of lipid in the cell membrane (Olmos and Hellin, 1996). The accumulation of lipid droplets observed in salt-treated cell has also been reported in other plant tissues exposed to salt or water stress (Rahman *et al.,* 2000). The accumulation of lipid droplets disappears when the stress condition ends (Poljakoff-Mayber, 1981). The accumulation of lipid droplets is also considered as a reserve of energy to be used by the cell to cover the increased demand in metabolic energy required to tolerant salinity in selected cells (Rahman *et al.,* 2000). Moreover, Foyer (1993) recorded that plants subject to harmful stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, protein and nucleic acids.

In order to defend themselves against oxidants, plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen. Plants with high levels of antioxidants have been reported as having greater resistance to this oxidative damage.

Moreover, some chloroplasts contained a large number of plastoglobuli (Fig 3-b) as compared to control (Fig 3-a). Nazarenko and Serebryakova (1990) reported that an increase in the number and size of chloroplast plastoglobuli produced by NaCl treatment as observed in the present investigation. Plastoglobuli are ubiquitous in chloroplast and chromoplasts, and their probable role in salt tolerance has been proposed (Burguess, 1985).

In the present study, pre-soaking sorghum grains in GB or AsA or pre-soaking plus spraying minimized the harmful effects of salinity on the ultrastructure of leaf mesophyll cells as compared to non-treated plants grown under saline conditions. However, GB applied as pre-soaking plus spraying showed the most beneficial effect in this respect.

References

Aloni R (1987) Differentiation of vascular tissues. Ann Rev Plant Physiol 38: 179-204.

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50: 601–639.

Athar HR, Khan A and Ashraf M (2008) Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Enviro and Exp Botany 63: 224-231

Bendre AM and Pande PC (2004) Introductory Botany. 4th ed. Pp 323-339.

Bernstein N, Silk WK and Läuchli A (1995) Growth and development of sorghum leaves under condition of NaCl stresses: Possible role of some mineral elements in growth inhibition. Planta 196: 699-705.

Bor M, Özdemir F and Türkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet (*Beta vulgaris* L.) and wild beet (*Beta maritime* L.). Plant Sci 164: 77–84.

Bowers B and Maser M (1988) Artifacts in fixation for transmission electron microscopy. In R.F.E. Crang and Klomparens eds., Artifacts in Biological Electron Microscopy. Plenum Press, New York. 13-42.

Burguess J (1985) An Introduction to Plant Cell Development. First esition. Cambridge University Press, Cambridge, UK. 42-47.

Churchli KA and Sze H (1984) Anion-sensitive, H^+ pumping ATPase of oat roots. Plant Physiol 76: 490-497.

Cooper A (1979) The ABC of NFT. Growers Books, London, P. 59.

Cramer GR, Lynch J, läuchli A and Epstein E (1987) Influx of Na^+, K^+ and Ca^{2+} into roots of salt-stressed cotton seedling. Plant Physiol 83: 510-516.

Critchley C (1983) Further studies on the role of chloride in photosynthetic O_2 evolution in higher plants. Biochim Biophys Acta 724: 1-5.

Demiral T and Türkan I (2004) Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment? J Plant Physiol 161: 1089–100.

Dodd GL and Donovan LA (1999) Water potential and ionic effects on germination and seeding growth of two cold desert shrubs. Amer J Bot 86: 1146-1153.

Foyer CH (1993) Ascorbic acid. In R.G. Alscher & J. L. Hess, (Eds), Antioxidants in higher plants (pp. 51-58). Boca Raton, FL: CRC Press.

Foyer CH and Noctor G (1999) Leaves in the dark see the light. Science 284: 599–601.

Foyer CH and Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119: 355–364.

Gerlach D (1977) Botanische Mikroteenk. Eine einfuhrung, Thieme verlage, Stuttgart, BRD.

Gomez KA and Gomez AA (1984) Statistical Procedures for Agriculture Research. John Wiley and Sons, Inc. New York.

Hamilton III EW and Heckathorn SA (2001) Mitochondrial adaptation to NaCl. Complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. Plant Physiol 126: 1266–74.

Hernández JA, Ferrer MA, Jiménez A, Barceló AR and Sevilla F (2001) Antioxidant systems and O^-_2/H_2O_2 production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127: 817–31.

Holmström KO, Somersalo S, Mandal A, Palva TE and Welin B (2000) Improved tolerance to salinity and low temperature in transgenic tobacco producing glycinebetaine. J Exp Bot 51: 177–85.

kelley PM and Izawa S (1978) The role of chloride ion in photosystem II. I. Effects of chloride ion on photosystem II electron transport and on hydroxylamine inhibition. Biochem Biophys Acta 502: 198-210.

Lee SK and Kader AA (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Post Harv Biol Technol 20:207-220.

Loreto F, Velikova V and Di Marco G (2001) Respiration in the light measured by CO_2 emission in CO_2 atmosphere in maize leaves. Aust J Plant Physiol 28: 1103–1108.

Ma QQ, Wang W, Li YH, Li DQ and Zou Q (2006) Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum*) by foliar-applied glycinebetaine. J Plant Physiol 163: 165–75.

Mäkelä P (2004) Agro-industrial uses of glycinebetaine. Sugar Tech 6: 207-212.

Mäkelä P, Kärkkäinen J and Somersalo S (2000) Effect of glycinebetaine on chloroplast ultrastructure, chlorophyll and protein content, and RuBPCO activities in tomato grown under drought or salinity. Biol Plant 43:471-5.

Metzler DE (1977) Biochemistry- The chemical reactions of living cells, pp 357-370. Academic press, New York.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7: 405–410.

Mittova V, Guy M, Tal M and Volokita M, (2002) Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt dependent oxidative stress: increased activities of antioxidant enzymes in root plastids. Free Radical Res 36: 195–202.

Munns R (2002) Comparative physiology of salt and water stress. Plant, Cell and Environment 25: 239-250.

Nazarenko LV and Sererbryakova VN (1990) Ultrastructural changes in Euglena cells of different nutritional types under the influence of salinity. Fiziol Rast 37: 142-146.

Nieman RH (1965) Expansion of bean leaves and its suppression by salinity. Plant Physiol 40: 156-161.

Noctor G and Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49: 249–279.

Okuma E, Murakami Y, Shimoishi Y, Tada M and Murata Y (2004) Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. Soil Sci Plant Nutr 50: 1301–5.

Olmos E and Hellin E (1996) Cellular adaptation from a salt-tolerant cell line of *Pisum sativum*. J Plant Physiol 148: 727-734.

Pastori GM, Kiddle G, Antoniw J, Bernard S, Veljovic-Jovanovic S, Verrier PJ, Noctor G and Foyer CH (2003) Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell 15: 939–951

Poljakoff-Mayber A (1981) Ultrastructural consequence of drought. In L.G. Paleg and D. Aspinall eds., The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, New York. 389-403.

Rahman MS and Matsumuro T, Miyake H, Takeoka Y (2000) Salinity-induced ultrastructural alternations in leaf cells of rice (*Oryza sativa* L.). Plant Prod Sci 3: 422-429.

Rahman MS, Matsumuro T, Miyake H and Takeoka Y (2001) Effects of salinity stress on the seminal

root tip ultrastructure of rice seedlings (*Oryza sativa* L.). Plant Prod Sci 4: 103-111.

Rashid P, Karmoker JL, Chakrabortty S and Sarker BC (2004) The effect of salinity on ion accumulation and anatomical attributes in mungbean (*Phaseolus radiatus* L. cv. BARI-3) seedlings. Inter J of Agric and Biol 6: 495-498.

Rhodes D and Hanson AD (1993) Quaternary ammonium and tertiary sulfonium in higher plants. Annu Rev of Plant Physiol and Plant Molecular Biol 44: 357-384.

Roy D, Basu N, Marik R and Banerjee SK (1995) Changes in levels of endogenous gibberellins and abscisic acid in salt-sensitive germinating rice by NaCl-salinity. Plant Physiol Biochem New Delhi 22: 200-202.

Sakamoto A and Murata N (2002) The role of glycinebetaine in the protection of plants from stress: clues from transgenic plants. Plant, Cell and Environ 25: 163-171.

Sam O, Ramirez C, Coronado MJ, Testillano PS and Risueno MC (2003/2004) Changes in tomato leaves induced by NaCl stress: leaf organization and cell ultrastructure. Biologia Plant 47: 361-366.

Shaddad MA, Radi AF, Abdel-Rahman AM and Azooz MM (1999) Response of seeds of *lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine. Plant and Soil 122: 177-183.

Sharma P and Dubey RS (2005) Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. J Plant Physiol 162: 854–64.

Smirnoff N (2000) Ascorbate biosynthesis and function in photo protection. Biol Sci 355: 1455–1465.

Smirnoff N (2005) Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In: Smirnoff, N. (Ed.), Antioxidants and Reactive Oxygen Species in Plants. Blackwell Publishing Ltd., Oxford, UK, 53–86.

Strogonov BP (1964) Physiological basis of salt tolerance of plants. Jerusalem Isr Program for Sci Transl Pp. 279.

Strogonov BP (1974) Structure and Function of Plant Cells in Saline Habitats. New York, London: Israel Program for Scientific Translation, 284 Pp.

Taamalli W, Abz L, Youssef NB, Miled DDB and Zarrouk M (2004) Lipid breakdown in sunflower (*Helianthus annuus* L.) seeds during post germinative growth under salt-stress. Rivista Italian delle Sostanze Grasse 81: 90-97

Uperty DC and Sarin MN (1976) Physiological studies on salt tolerance in *Pisum sativum* L. IV. Ionic composition and nitrogen metabolism. Acta Agronomica Academia Scientiarum Hungaricae 24: 669-674.

Zeinali E, Soltani A and Galeshi S (2002) Response of germination components to salinity stress in oil seed rape (*Brassica napus*L.). Iranian J of Agric Sci 33: 137-145.

Zhu JK (2001) Plant salt tolerance. Trends in Plant Science 6: 66-71.