SDRP Journal of Plant Science (ISSN: 2573-7988)

Alleviating Effects of Ascorbic acid and Glutathione for Faba Bean Plants Irrigated with Saline Water.

DOI: 10.25177/JPS.2.2.3

Research

Received Date: 09th Mar 2017
Accepted Date: 05th Apr 2017
Published Date:18th Apr 2017

Safi-naz S. Zaki[1]*, Gamal Farag Mohamed[2]

[1]Department of Water Relations and Field Irrigation, National Research Centre, Dokki, Cairo, Egypt.

[2]Botany Department, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt .

CORRESPONDENCE AUTHOR
Safi-naz S. Zaki
E-mail address: safinsab@gmail.com

CONFLICTS OF INTEREST
There are no conflicts of interest for any of the authors.

CITATION
Safi-naz S. Zaki, Alleviating Effects of Ascorbic acid and Glutathione for Faba Bean Plants Irrigated with Saline Water. (2018)SDRP Journal of Plant Science 2(2)

ABSTRACT

The plant, throughout its life cycle, has to cope with several abiotic stresses including salinity, affecting its growth and entire metabolism. Antioxidants help plant to beat such stresses. Therefore, effects of ascorbate (AsA) and glutathione (GSH) applied, as seed soaking and foliar spray solutions, alternatively on *Vicia faba* plant growth, physio-biochemical attributes and antioxidant defense system activity were studied under 150 mM NaCl stress. Irrigation of faba bean plants with saline water reduced growth and yield, photosynthetic efficiency, relative water content, membrane stability index and nutrient contents, while increased the activity of defense systems (non-enzymatic and enzymatic antioxidants), electrolyte leakage, malondialdehyde, hydrogen peroxide and NaCl contents. Exogenous AsA and GSH applied as seed soaking and foliar spray alternatively had no significant effects on the all above mentioned parameters under normal conditions. However under salt stress, they significantly improved plant growth and productivity, antioxidant defense systems activity (enzymatic; superoxide dismutase, catalase, glutathione peroxidase and ascorbate peroxidase, and non-enzymatic; AsA, GSH and α-tocopherol antioxidants) and nutrient contents. Application of AsA and GSH as seed soaking and foliar spray, respectively was the best treatment of which this study recommends to use for growing faba bean plants under NaCl stress.

Keywords: Faba bean, AsA, GSH, ROS, salinity.

1. INTRODUCTION

Salt stress is one of the world wide abiotic factors which caused inhibition of soil microorganisms' vitality and cause damage in plant growth and its yield in arid and semi-arid regions. Plant growth and its productivity in many crops can be reduced by osmotic stress, nutritional imbalance and specific ion toxicity (Alam, 1999; Gunes, et al., 1996; Cordovilla, et al., 1994). Salinity causes inhibition in metabolic processes and reduction in growth rate by reducing the uptake of water by plants (Munns, 1993; Munns, 2002). Many reports available on various plants reveal that

growth reduction under salinity stress conditions is mostly related to osmotic and specific ion effects (Khan,et al., 2000; Salim, 1991).

Investigated the responses of plant growth to salinity stress is important during the plant life cycle. Water deficit, excess ions, and nutrient imbalance are the major constraints for plants grown in salinity conditions (Koyro and Huchzermeyer, 1999). Many reports on the effect of salt stress on plants have focused on plant growth and its parts development as well as nutrient changes (Bernstein et al., 2001; Rodriguez et al., 2005; Zhu, 2003; Cordovilla et al., 1999). NaCl is the most important constituent stated for saline environments (Jacoby, 1999). However, Na^+ and Cl^- are thought to be necessary for many plants such as salt tolerant plants (Tucker, 1999).

Under certain experimental conditions salinity may inhibitor promote nutrient uptake by different plant species. The response of plant nutrient content to salinity changes with plant species and organs. Synergistic and antagonistic effects may increase or decrease the intensity of nutrient uptake by plants. Alam (1999), considered that cell membranes were protected from the adverse effects of Na+ by Ca2+ which also minimized the leakage of cytosolic K+. In Citrus spp. Ca2+ was effective in reducing of the transport of Na+ and Cl- from roots to the leaves thereby decreasing foliar injury (Zekri and Parsons, 1992).

Salt tolerant plants have active mechanisms to scavenge the ROS. These mechanisms include enzymatic antioxidants such as ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD), and non-enzymatic antioxidants like ascorbic acid (AsA), reduced glutathione (GSH), α-tocopherol (TOC) and carotenoids (Parida and Das, 2005; Younis et al., 2010). Mitigation of the adverse effects of salinity can be managed by the exogenous application of antioxidants and plant growth regulators solutions (Gunes et al., 2007; Khattab, 2007; Younis et al., 2010; Rady, 2011; Rady and Hemida, 2015; Rady and Mohamed, 2015; Rady and Hemida, 2016). Low molecular weight antioxidants such as ascorbate and glutathione which synthesized within the chloroplast are played an important role as redox buffers to influence the modulation processes in plant growth and development from mitosis and cell elongation to senescence and death (Sharma et al., 2012; Kasote et al., 2015). In the same trend, these compounds can activate the gene expression associated with biotic and abiotic stress conditions responses to maximize defense of sensitive plants.

Ascorbic acid (AsA) generated during aerobic metabolism and it is known as the best molecule for H_2O_2 detoxification, especially as a substrate of ascorbate peroxidase (APX), an essential enzyme of the ascorbate-glutathione cycle, found in most compartments of the plant cell [Smirnoff, N.; Wheeler, G.L., 2000]. AsA also helps to regenerate antioxidant pigments, carotenoids and α-tocopherol.

Reduced glutathione (GSH) is a tripeptide (γ-glutamyl-cysteinyl-glycine) and the most abundant LMW thiol of plants (Noctor et al., 2012). The sulphhydryl or thiol group (as indicated by the –SH) of GSH can donate an electron to free radicals. Due to its abundance and negative redox potential, GSH strongly contributes to the redox environment" (Schaffer and Beutner, 2001), allowing cells to maintain a healthy reduced redox homeostasis (Noctor et al., 2012). Reduced glutathione is oxidised to a glutathiyl anion radical (GS•). Two GS• can spontaneously bond to form glutathione disulphide (GSSG), which can be recycled back to GSH by the enzyme glutathione reductase, which requires NADPH as the reducing power (Foyer and Halliwell, 1976). Glutathione disulphide is able to form mixed disulfides with thiol-containing proteins. In this way, protein-bound glutathione protects the protein thiol groups from autooxidation to sulphonic acids (Kranner and Grill, 1996). The GSH acts as an antioxidant by quenching the ROS and is involved in the ascorbate–glutathione cycle, which eliminates damaging peroxides (Kasote et al., 2015).

Due to the considerable evidence of the adverse effects of salinity on plant growth, it was proved, from previous works, that exogenous application of low molecule antioxidants can enhance plant tissues to mitigate the adverse salt stress effects (Aly-Salama and Al-Mutawa, 2009; Azzedine et al., 2011; Semida et al., 2014; Rady and Mohamed, 2015; Rady et al., 2016).

Studies on plants of the family *Fabaceae* have suggested that, although shoot growth may be inhibited, salinity levels may stimulate root growth. The shorter stems are mainly due to shortening of the internodes in pea plants (Poljakoff-Mayber and Lerner, 1999). Broad bean, an important nutritious vegetable all over the world, contains 20-36% protein for human and animal consumption.

The present study was initiated to evaluate the potential increase of salt tolerance in faba bean plants by using technique of a sequenced application of AsA and GSH, used as seed soaking and foliar spraying. Plant growth, membrane stability and cell turgid, osmoprotectants, low molecular weight antioxidants and enzymatic antioxidants were calculated to see which of these parameters were improved, by using the AsA and GSH sequencing, to elevate the antioxidative mechanisms of faba bean plants to increase their tolerance to salinity conditions.

2. MATERIAL AND METHODS

Growth conditions and treatments

A greenhouse pot experiment was conducted using faba bean (*Vicia faba* L., cv. "Giza 716") seeds. Seeds were surface sterilized in 0.1% $HgCl_2$ for 1 min, and were then washed in sterilize-deionized water. Plastic pots (35-cm diameter, 30-cm depth) were filled with equal sand amounts. Using commercial acid, sand was previously washed several times to remove all anions and cations, and was then washed with distilled water several times to remove the acid. In each pot, 6 seeds were sown, and pots (n = 90) were arranged for growing plants in an open greenhouse for 3-repeated pot experiment.

An average of 19 ± 3/10 ± 2 °C was the day/night temperatures, an average of 62.0 – 65.1% was the relative humidity, and an average of 10 – 11 h was the day length. A ½-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938) was supplied at 100% field capacity (FC) every 2 days to all pots until plants reached 20 days in old. Pots were then divided into 2 groups each of 45 pots, one of them was irrigated with pure nutrient solution, and other group was irrigated with nutrient solution salinized with NaCl up to 150 mM starting 21 days after sowing (DAS). - Each group was divided into 3 sub-groups (n = 15) that represent 3 treatments, to obtain 6 treatments and their details as follows:

Treatments					Description of treatments	
Group	Sub-group	Irrigation water	Antioxidants		Seeds were soaked in	Plants were sprayed with
First	1	Non-saline	Dist. water		Distilled water	Distilled water
	2		AsA-GSH		1 mM Ascorbic acid	1 mM Glutathione
	3		GSH-AsA		1 mM Glutathione	1 mM Ascorbic acid
Second	4	Saline (150 mM NaCl)	Dist. water		Distilled water	Distilled water
	5		AsA-GSH		1 mM Ascorbic acid	1 mM Glutathione
	6		GSH-AsA		1 mM Glutathione	1 mM Ascorbic acid

Irrigation with Hoagland's nutrient solution contained NaCl (150 mM) for stress treatments were started at 21 DAS up to 50 DAS. Thereafter, plants of all treatment including stress one were irrigated with pure nutrient solution up to obtaining the yield, this means that stressed plants were received 15 saline irrigations then provided with pure nutrient solution up to the end of the experiments.

The concentrations of AsA (1 mM) and GSH (1 mM) were selected for this study because they were greatly induced the best response of faba bean seedling growth, and the selection of 150 mM NaCl was selected because it was greatly affected faba bean seedling growth based on our preliminary studies (data not shown). All pots were arranged in a completely randomized design. Soil pH was adjusted back to the control pH of 6.0–

6.2 with diluted H_2SO_4. The experiment was repeated three times. 75-d-old seedlings from each treatment were collected for various growth, physio-biochemical and antioxidative defense systems determinations. At harvesting (135 DAS), pods on all plants of each treatment were collected to assess pods and seeds yields components.

Material harvesting, growth and yields, and photosynthesis efficiency analyses

The plant samples were harvested at 75 DAS, and a group of shoots of each treatment (n = 3) were separated to measure their lengths, No. of leaves and leaves area per plant. Shoots were then oven-dried on 70 °C for 48 h or up to a constant weight to record dry weights. At harvest, pods on all plants of each treatment were collected and No. of pods and No. of seeds per plant, 100-seed weight and seed yield per plant were assessed.

The upper third leaf was separated from another group of shoots of each treatment (n = 3) and immediately frozen in liquid nitrogen. Thereafter, leaves were pulverized in a mortar and stored at −25 °C until analysis.

Concentrations of leaf chlorophylls and carotenoid were assessed (Arnon, 1949) in acetone extract by measuring with a UV-160A UV–vis Recording Spectrometer (Shimadzu, Japan) at 663, 645 and 470 nm. Maximum quantum yield of PSII and Fv/Fm was calculated as; Fv/Fm= (Fm–F0)/Fm (Maxwell and Johnson, 2000). Performance index (PI) of photosynthesis (chlorophyll a fluorescence) based on the equal absorption (PIABS) was calculated (Clark et al., 2000).

Assessments of relative water content, membrane stability index and electrolyte leakage

Assessments of relative water content [RWC; Weatherly (1950) with some modifications by Osman and Rady (2014)], membrane stability index [MSI; Premchandra et al. (1990) with some modifications by Rady (2011)] and electrolyte leakage [EL; Sullivan and Ross (1979)], using fresh fully-expanded leaves excluding the midrib were done.

Assessments of proline, soluble sugars, ascorbic acid and glutathione contents

To extract and assess total soluble sugar content, the method of Irigoyen et al. (1992) was utilized. Homogenization for a dried leaf sample (0.2 g) was done in 5 ml of 96% (v/v) ethanol, and then washing in 5 ml 70% (v/v) ethanol was exercised. Thereafter and prior to measurements, extract centrifugation was done at 3500 × g for 10 min and supernatant storage was applied at 4 °C. To assess soluble sugar content, 0.1 ml of the ethanolic extract was reacted with 3 ml of reagent of freshly-prepared anthrone [150 mg anthrone + 100 ml of 72% (v/v) sulphuric acid] utilizing a boiling water bath for 10 min. Absorbances were read, after cooling, at 625 nm with a Bauschand Lomb-2000 Spectronic Spectrophotometer (Thermo Spectronic, Mercers Row, Cambridge, UK).

The rapid colorimetric Bates et al. (1973) method was applied to assess contents of proline in 0.5 g-dried leaf samples. Extraction in 10 ml of 3% (v/v) sulphosalicylic acid was done, and then extract centrifugation was conducted at 10.000 × g for 10 min. Supernatant (2 ml) was received 2 ml of freshly prepared acid-ninhydrin solution into a test-tube, and then the incubation was applied in a water bath at 90 °C for 30 min. Using an ice-bath, the reaction was terminated and the extraction was done with 5 ml of toluene by vortex-mixed for 15 s. In dark at room temperature, separation of the toluene and aqueous phases was allowed to occur for 20 min. With care, collection of the upper toluene phase was carried out and absorbance was read at 520 nm.

The method of Mukherjee and Choudhari (1983) was utilized to assess contents of ascorbic acid (AsA). Extraction was done in 10 ml of 6% (w/v) trichloroacetic acid, and the extract was mixed with 2% (w/v) dinitrophenylhydrazine, and then one drop of 10% (w/v) thiourea in 70% (v/v) ethanol was added. Using a water-bath, the mixture was boiled for 15 min. After cooling, 5 ml of 80% (v/v) H_2SO_4 was added and absorbances were read at 530 nm to calculate the contents of AsA from a standard curve.

As detailed in Griffth (1980) method, contents of glutathione (GSH) was determined. Homogenization of fresh leaf tissue (50 mg) was exercised in 2 ml

of 2% (v/v) metaphosphoric acid, and then centrifugation was applied at 17,000 × g for 10 min. Neutralization of supernatant (0.9 ml) was done with 0.6 ml of 10% (w/v) sodium citrate. Assessments of 3 replicates were made for each sample. A composition of 700 µl of 0.3 mM NADPH, 100 µl of 6 mM 5,5′-dithio-bis-2-nitrobenzoic acid, 100 µl distilled water and 100 µl of extract was of each assay (1.0 ml) that was stabilized at 25 °C for 3 – 4 min, and then GSH reductase (10 µl of 50 Units ml^{-1}) was added. Absorbances were read at 412 nm to calculate GSH contents from a standard curve.

Determination of H_2O_2, lipid peroxidation and α-tocopherol contents

To determine the H_2O_2 content, 250 mg of fresh leaf and root material was homogenized with 5 mL of 5% trichloroacetic acid (TCA). The homogenates were then centrifuged at 12,000 ×*g* for 15 min at 4 °C, and the supernatants were collected. Aliquots of the supernatants were added to a reaction medium composed of 10 mM potassium phosphate buffer at pH 7.0 and 1 M KI. The H_2O_2 content was quantified spectrophotometrically at 390 nm by reference to a standard curve prepared with H_2O_2 solutions (Velikova et al., 2000), and it was expressed as µmol g^{-1} fresh mass (FW). Lipid peroxidation was estimated by quantifying the malondialdehyde (MDA) content in the same extracts used for the determination of H_2O_2 content (Heath and Packer, 1968). The MDA content was calculated using its coefficient of molar extinction (0.155 × 10−3 M^{-1} cm^{-1}) and expressed as µmol g^{-1} FW.

Content of α-tocopherol (α-TOC) was assessed by dissolving 20 mg of butylated hydroxytoluene (BHT) in a solvent mixture [900 ml of extraction solvent (nhexane -ethyl acetate, n-hexane) mixed with 100 ml of ethyl acetate]. Using R-TOC, standard solutions (20–200 µg/ml) were prepared by using stock solution (50 mg/100 ml n-hexane). According to the Konings et al. (1996) method, samples were prepared and saponified. Leaf tissue was sliced and dried in an oven at 40°C and homogenized, and then suspended in water in a 0.5-l conical flask, in which 21 g of KOH dissolved in 100 ml of ethanol was added and then 0.25 g of AsA per gram test portion was added. Saponification was done at 80°C for 40 min and cooling was immediately done. Water was added to bring the ethanol/water ratio to 0.3 and then n-hexane/ethyl acetate [9:1 (3 × 100 ml)] was added, and the mixtures were then extracted three times. Organic phases were combined, washed with water and filtered through anhydrous sodium sulphate into a beaker. The filtrates were evaporated to dryness and the residues were dissolved in n-hexane (HPLC grade) and stored in a freezer at –20°C. The TOC was determined on a HPLC system using a Waters Bondapak C18 reverse-phase column. The mobile phase (methanol/water 94:6) was used at a flow rate of 1.5 ml min−1 and the UV detector was set at 292 nm (Ching and Mohamed, 2001).

Antioxidant enzymes

Enzyme extracts were prepared by homogenizing 200 mg of lyophilized powder of leaves and roots in a cold mortar with 2 mL of 100 mM potassium phosphate buffer at pH 7.0, containing 0.1 mM EDTA (ethylenediaminetetraacetic acid). For APX activity estimation, 2 mM AsA was added to the extraction buffer. The homogenate was filtered through a nylon cloth and centrifuged at 12,000 *g* for 15 min. All procedures were conducted at 4 °C and the supernatant (extract) was stored at −25 °C until analysis. Protein content in the extracts was measured according to Bradford (1976). CAT (EC 1.11.1.6) activity was determined according to Harvir and MacHale (1987), by monitoring the decrease in absorbance at 240 nm due to H_2O_2 breakdown (ε = 36 M^{-1} cm^{-1}). The CAT activity was expressed as µmol H_2O_2 min^{-1} mg^{-1} protein.

SOD (EC 1.15.1.1) activitiy was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) chloride, as described previously by Beauchamp and Fridovich (1971). One SOD activity unit was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate, and the results were expressed as U mg^{-1} protein. APX (1.11.1.11) activity was determined according to the method described by Nakano and Asada (1981), by monitoring the oxidation of AsA, which was measured as the decrease in absorbance at 290 nm (ε = 2.8 × 10^{-3} M^{-1} cm^{-1}). GPX (EC 1.11.1.9) was assayed according to Kar and Mishra

(1976), by monitoring the increase in absorbance at 470 nm due to formation of tetraguaiacol (ε = 26.6 × 10^{-3} M^{-1} cm^{-1}). APX and GPX activity was expressed as μmol H_2O_2 min^{-1} mg^{-1} protein.

Determinations of nutrients and Na contents

The wet digestion of 0.1 g of fine dried material of plants was conducted using sulphuric and perchloric acid mixture as mentioned by Piper (1947). Nitrogen (N, %) was determined in powdery dried leaf by Orange-G dye colorimetric method according to Hafez and Mikkelsen (1981). Phosphorus (P, %) was calorimetrically determined using chlorostannusmolybdophosphoric blue color method in sulphuric acid system as described by Jackson (1967). The contents of K^+, Ca^{2+} and Na^+ (%) were determined using flame photometry (Williams and Twine, 1960).

Statistical analysis

The experimental design was completely randomized design. The results were subjected to one-way analysis of variance (ANOVA) to evaluate the significance of differences between treatments ($P \leq 0.05$), (Gomez and Gomez 1984).

3. RESULTS AND DISCUSSION

Growth Characters:

Data shown in Table 1 revealed that saline water irrigation affected all growth traits of faba bean plants in terms shoot length, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ and shoot dry weights $plant^{-1}$. Irrigated plants with saline water significantly decreased all investigated growth characters. Seed soaking and foliar spray with ascorbic acid and glutathione significantly increased all saline water irrigation affected growth parameters. Plants which grown from seeds soaked with 1mM AsA and sprayed with 1mM GSH showed the maximum value of growth parameters and plants enable to generate growth characteristics more than plants grown under saline water irrigation conditions (NaCl 150 mM).

Table 1. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on some growth traits of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters			
Irrigation water	Antioxidants		Shoot length (cm)	Number of leaves $plant^{-1}$	Leaf area $plant^{-1}$ (dm^2)	Shoot dry weight (g)
Non-saline	Dist. water		40.8±4.2a	56.3±6.7a	18.7±2.7a	40.6±3.2a
	AsA-GSH		41.3±4.9a	56.8±6.8a	18.8±2.7a	41.0±3.4a
	GSH-AsA		41.2±4.2a	56.9±6.5a	18.7±2.8a	40.8±3.4a
Saline	Dist. water		30.4±3.8c	30.7±4.7c	10.9±2.0c	25.4±2.2c
	AsA-GSH		39.2±4.3b	44.7±5.6b	15.1±2.6b	36.7±3.3b
	GSH-AsA		38.8±4.4b	42.8±5.8b	13.8±2.5b	34.8±3.2b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

Data of this study showed that, NaCl caused significantly reduction of some growth traits of faba bean (shoot length, number of $leaves^{-1}$, leaf area $plant^{-1}$and shoot dry weight; Table 1). This may be due to changes in osmotic potential caused by reduction in available water (Mousavi et al., 2013). However, application of AsA and GSH generate the best significant results for mitigation of the adverse effects of NaCl. Exogenous applications of various antioxidants have been reported to alleviate the adverse effects of salinity stress on plant growth parameters (Khan et al., 2013; Rady and Hemida, 2015; Rady et al., 2016). These antioxidants such as AsA and GSH have been found to alleviate and repair the damage caused by reactive oxygen species (ROS), enabling plants to develop an antioxidant mechanisms to increase the cellular defense strategy against NaCl-induced oxidative stress (Hemida et al., 2014; Wutipraditkul et al., 2015; Rady et al., 2016).

Yield and Yield Components

Table (2) shows that, seed soaking and foliar spray with AsA and GSH at all treatments significantly increased yield and its components particularly the treatment of AsA-GSH which induced significant increases surpassed the plant which irrigated with saline water and treated with distilled water by 58.3% in pods number plant-1, 96.7% in seeds number plant-1, 41.5% in weight of 1000-seed and 178.2% in seed yield plant-1.

Table 2. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on yield components of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters			
Irrigation water	Antioxidants		Pods No. plant^{-1}	Seeds No. plant^{-1}	100-seed weight (g)	Seed yield plant^{-1} (g)
Non-saline	Dist. water		12.6±0.8a	41.6±3.9a	70.4±5.5a	29.3±2.4a
	AsA-GSH		13.0±0.9a	42.4±3.9a	71.0±5.6a	30.1±2.4a
	GSH-AsA		12.9±0.9a	42.1±4.0a	70.8±5.6a	29.8±2.6a
Saline	Dist. water		7.2±0.6c	18.2±2.4c	48.2±3.4b	8.77±1.1c
	AsA-GSH		11.2±0.7b	35.8±3.4b	68.2±6.4a	24.4±2.3b
	GSH-AsA		11.0±0.7b	35.4±3.3b	68.0±6.6a	24.1±2.4b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

Leaf photosynthetic pigments and photosynthetic efficiency

Photosynthetic pigments components were significantly reduced as a result to the irrigation with saline water, so Table 3 shows decreased concentrations of total chlorophylls, total carotenoids, and photosynthetic efficiency (*Fv/Fm* and performance index; PI) of faba bean leaves. However, seed soaking and foliar application of AsA and GSH have been shown to increase total chlorophylls and carotenoids concentrations in compare with plant treated with distilled water. Irrigation of faba bean plants with saline water showed a negative effect on *Fv/Fm* and PI%. While, the exogenous application of AsA and GSH have been shown to elevate the adverse effect of saline water.

Table 3. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on leaf photosynthetic pigments (mg g^{-1} fresh weight) and photosynthetic efficiency (*Fv/Fm* and performance index; PI) of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters			
Irrigation water	Antioxidants		Total chloro-phylls	Total carotenoids	*Fv/Fm*	PI (%)
Non-saline	Dist. water		2.04±0.03a	0.38±0.01a	0.83±0.02a	9.42±0.31a
	AsA-GSH		2.10±0.04a	0.38±0.01a	0.84±0.02a	9.50±0.32a
	GSH-AsA		2.08±0.03a	0.37±0.01a	0.83±0.02a	9.48±0.30a
Saline	Dist. water		1.09±0.02d	0.25±0.00c	0.65±0.01c	5.26±0.18c
	AsA-GSH		1.89±0.03b	0.35±0.01b	0.80±0.02ab	8.74±0.25b
	GSH-AsA		1.70±0.03c	0.34±0.01b	0.78±0.02b	8.55±0.24b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

The pronounced positive effects of the sequenced application of AsA- GSH under saline water conditions compared to normal conditions explain the significant differences in growth characteristics of faba bean plants obtained under both distilled and saline water irrigation (Table 3). Total chlorophyll, total carotenoids, F_v/F_m, and PI of salinity-stressed plants were significantly reduced, while the activity of the antioxidant defense systems (i.e., enzymatic and non-enzymatic antioxidants), and were significantly higher than those of unstressed transplants. This may be explained the important role of antioxidants as a main mechanism in alleviating the salinity stress effects on plants (Singh et al., 2016).

The obtained data in Table (4) report significant increases in RWC % and MSI%, and reduction of EL%, for faba bean plants irrigated with either H_2O or 150 mM NaCl compared to irrigated plants with distilled water. The reported data of RWC, MSI and EL shows variation among all treatments of seed soaking and foliar spraying with distilled water or AsA and GSH as an integrated treatment, when plant were irrigated with H_2O or 150 mM NaCl as shown in Table (4). The combination between soaking in AsA and spraying with GSH as integrated treatments and irrigation with H_2O gave maximum RWC% and MSI% and minimum EL% of plant tissues compared to all the other treatments.

Table 4. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on relative water content (RWC %), electrolyte leakage (EL %) and membrane stability index (MSI %) of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters		
Irrigation	Antioxidants		RWC (%)	EL (%)	MSI (%)
Non-saline	Dist. water		86.2±2.8a	6.24±0.32c	78.4±2.6a
	AsA-GSH		86.3±2.7a	6.20±0.30c	78.5±2.9a
	GSH-AsA		86.2±2.8a	6.26±0.36c	78.3±2.5a
Saline	Dist. water		62.1±2.0c	18.06±0.89a	43.8±2.0c
	AsA-GSH		80.2±2.4ab	8.18±0.38b	68.2±2.3b
	GSH-AsA		78.0±2.3b	8.46±0.42b	63.7±2.2b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

RWC and MSI in faba bean plants treated with antioxidants and irrigated with H_2O or NaCl showed great response to the application of antioxidant sequenced of AsA_{1mM}- GSH_{1mM} as an integrated treatment, in relation to control levels. The changes in cell membranes under salinity were being measured as electrolyte leakage (EL), where salt stress has been repeatedly reported to cause peroxidative damage to plasma membrane (Koca et al., 2007; Younis and Tourky, 2015). Seed soaking with AsA or GSH significantly increased the RWC in faba bean plants compared with untreated ones. Furthermore, as a consequence of salinity stress EL% in tomato (Shalata and Neumann, 2001), tobacco (Okuma et al., 2004) and canola (Khattab, 2007) plants was decreased by the exogenous application of individual AsA or GSH, respectively (Rady and Hemida, 2016).

Effects of seed soaking and foliar spray with ascorbic acid and glutathione on leaf contents of total soluble sugars, free proline, AsA and GSH of faba bean plants

Table 5 reports increased concentrations of total soluble sugars, free proline, ascorbic acid, and glutathione in faba bean leaves as a result to the adverse effects of seed soaking and foliar spraying of AsA and GSH in plant which irrigated with saline water. These previously components were significantly or insignificantly increased gradually with irrigation with saline water. However, exogenous AsA and GSH applications have been shown to increase total soluble sugars, free proline, and ascorbic acid and further increase glutathione concentrations. The sequencing of AsA-GSH treatment generated faba bean plants with highest values of soluble sugars, free proline and GSH concentrations, while the highest AsA concentration reported with GSH-AsA sequence.

Table 5. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on leaf contents of total soluble sugars, free proline, AsA and GSH of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters			
Irrigation water	Antioxidants		Sugars	Free proline	AsA	GSH
Non-saline	Dist. water		10.9 ± 0.2b	2.61 ± 0.03b	1.19 ± 0.01d	0.79 ± 0.01c
	AsA-GSH		11.1 ± 0.2b	2.68 ± 0.04b	1.48 ± 0.02c	0.91 ± 0.01b
	GSH-AsA		11.1 ± 0.3b	2.64 ± 0.04b	1.67 ± 0.02b	0.87 ± 0.01b
Saline	Dist. water		17.7 ± 0.5a	5.32 ± 0.07a	1.68 ± 0.02b	0.90 ± 0.03b
	AsA-GSH		18.2 ± 0.5a	5.38 ± 0.07a	2.38 ± 0.03a	1.78 ± 0.02a
	GSH-AsA		17.8 ± 0.6a	5.36 ± 0.05a	2.46 ± 0.03a	1.76 ± 0.02a

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

As indicated also in Table (5), AsA and GSH leaf contents in pretreated plants were consistently higher than those plant treated with distilled water. Pretreatment with each of two antioxidant compounds in sequenced application as seed soaking and foliar application induced significant increase in both concentrations of AsA and GSH under irrigation with 150 mM NaCl. The highest concentration was noticed as a result to the treated with sequenced application of AsA_{1Mm}-GSH_{1Mm} for AsA, and with GSH_{1Mm} - AsA_{1mM} for GSH, that results are a accordance with the results obtained by Chen et al. (2012), Hemida et al. (2014) and Younis and Tourky (2015). Also, Table (5) pointed that saline water irrigation (150 mM NaCl) significantly increased the concentrations of total soluble sugars and free proline concentrations in faba bean plants. The highly significant increases in total soluble sugar and free proline concentrations were observed with the sequenced application of AsA_{1Mm}-GSH_{1Mm} treatments. This alleviated effect of AsA and GSH on more accumulation of soluble sugars and proline probably, attributed to their ameliorative effects on the photosynthetic systems and their protective role played in salt tolerance due to the redox status in plants (Ameer et al., 2006; Eid et al., 2011).

Effects of seed soaking and foliar spray with AsA and GSH on leaf contents of α-TOC, MDA and H2O2 of faba bean plants.

Measurements of α-tocopherol (α-TOC), malodialdehyde (MDA) and hydrogen peroxide (H_2O_2) showed differences among all treatments of seed soaking and foliar spraying with distilled water, AsA and GSH, individually, as well as in sequence of AsA_{1mM}-GSH_{1mM} as an elevating treatment, when plant were irrigated with distilled water or 150 mM. The obtained data shows significant increases in α-TOC and a considerable decrease of MDA and H_2O_2, in comparison to control plant. The seed soaking in AsA_{1mM} and foliar spraying with GSH_{1mM} as integrated treatments and irrigation with distilled water gave highest concentration of α-TOC and minimum MDA and H_2O_2 of faba bean tissues compared to all the other treatments.

Salt stress cause lipid peroxidation in several species, which has often been used as an indicator of stress induced damage at the cellular level (Hernández and Almansa, 2002). However, our results demonstrate that MDA accumulation, and therefore enhanced lipid peroxidation, in salt-stressed plants does not necessarily indicate oxidative damage in leaves. MDA accumulation decreased transiently in plants receiving 150 mM NaCl; for distilled water MDA accumulation was significantly observed. Tolerant species have an excellent capacity for protecting themselves from salt-induced oxidative stress through mechanisms of photoprotection and antioxidant protection (Azevedo-Neto et al., 2006; Yazici et al., 2007). Our results demonstrate that faba bean plants irrigated with NaCl (150 mM NaCl) will produce signs of oxidative stress in the early stages (as reflected by MDA accumulation). The highest MDA accumulation was observed with 150mM NaCl-irrigated plants without soaking or foliar spraying with AsA and GSH. (Kyparissis et al., 1995; Munné-Bosch and Alegre, 2000). Furthermore, MDA has been shown to participate in the activation of early defense responses to abiotic stress by triggering relevant gene expression (Weber et al., 2004), thus it is likely that early and transient MDA accumulation

in faba bean leaves exposed to 150 mM NaCl activates defense mechanisms at the transcript level, an aspect that warrants further investigation.

α-tocopherol, in conjunction with other antioxidants, contributes to the preservation of an adequate redox state in chloroplasts and to maintaining thylakoid membrane structure and function during plant responses to stress by inhibiting the propagation of lipid peroxidation and preventing 1O2 accumulation in chloroplasts (Munné-Bosch, 2005). Furthermore, α-tocopherol (vitamin E) levels increased in plants treated with the high dose of NaCl (150 mM), which is especially interesting in terms of increasing the levels of antioxidant vitamins in plants without the need to use genetically-modified plants (Asensi-Fabado and Munné-Bosch, 2010). This may be useful for increasing the levels of antioxidants in faba bean fruits, which are well known for their usage as human food sciences.

Table 6. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on leaf contents of α-tocopherol (α-TOC), malodialdehyde (MDA; lipid peroxidation) and hydrogen peroxide (H_2O_2) of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters		
Irrigation water	Antioxidants		α-TOC (μmol g^{-1} DW)	MDA (μmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)
Non-saline	Dist. water		1.64 ± 0.04c	24.7 ± 0.6c	6.12 ± 0.14c
	AsA-GSH		1.66 ± 0.04c	24.4 ± 0.5c	6.04 ± 0.15c
	GSH-AsA		1.66 ± 0.04c	24.6 ± 0.5c	6.10 ± 0.14c
Saline	Dist. water		2.46 ± 0.06b	42.3 ± 0.9a	16.24 ± 0.32a
	AsA-GSH		2.96 ± 0.08a	32.2 ± 0.7b	8.46 ± 0.18b
	GSH-AsA		2.93 ± 0.08a	33.6 ± 0.7b	8.78 ± 0.20b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

Table 7. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on leaf enzyme; superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX) activities in faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters			
Irrigation water	Antioxidants		SOD (UA g^{-1} protein)	CAT (μmol H_2O_2 min^{-1} g^{-1} protein)	APX (μmol H_2O_2 min^{-1} g^{-1} protein)	GPX (μmol H_2O_2 min^{-1} g^{-1} protein)
Non-saline	Dist. water		3259 ± 63c	159.7 ± 3.0b	11.7 ± 0.2d	19.9 ± 0.4d
	AsA-GSH		3321 ± 67c	163.4 ± 3.0b	12.0 ± 0.2d	20.0 ± 0.4d
	GSH-AsA		3286 ± 65c	165.2 ± 3.4b	12.1 ± 0.2d	20.0 ± 0.4d
Saline	Dist. water		5026 ± 93b	108.5 ± 2.1c	18.5 ± 0.4c	27.8 ± 0.5c
	AsA-GSH		6198 ± 110a	182.4 ± 3.6a	27.0 ± 0.5a	35.9 ± 0.6a
	GSH-AsA		5841 ± 103a	169.6 ± 3.4b	23.7 ± 0.4b	33.4 ± 0.5b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

Results of **Table 7** show increases in the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX) activity and in the leaf contents in faba bean plants treated with 150 mM NaCl compared to the controls. Results also reveal that there are significant variations among the activities of SOD, CAT, APX and GPX in faba bean plants when they were pretreated in AsA and GSH sequences. The activities of SOD, CAT, APX and GPX in faba bean plants irrigated with saline water increased with seed soaking in sequenced applications of AsA_{1mM}-GSH_{1mM} or GSH_{1mM}-AsA_{1Mm} significantly exceeded those of planted growing from seed soaking in AsA or GSH singly. The interaction between the sequenced AsA_{1mM}-GSH_{1mM} or GSH_{1mM}-AsA_{1Mm} as integrated treatments and irrigation with 150 Mm NaCl showed maximum values of SOD, CAT, APX and GPX activity in faba bean plants compared to the other treatments.

Plants suffered from salt stress shows dissimilarity between the ROS production and quenching activity of the antioxidant system, often resulting in oxidative stress (Apel and Hirt, 2004; Parvaiz and Satyawati, 2008). To mitigate the adverse effects of salinity-induced stress, plants make use of complex antioxidive defense mechanisms. Thus, tolerance to NaCl stress in higher plants is correlated to the levels of antioxidant enzymes and substrates (Athar et al., 2008). It is also evident that the antioxidant enzymes determined in this study have specific roles in alleviating the oxidative stress induced by NaCl-salinity. The activity of the investigated antioxidant enzymes in faba bean under irrigation with either distilled water or 150 mM NaCl in response to AsA or GSH and their sequenced applications as integrated treatments. Superoxide dismutase is the first defense agent against ROS as it is the major scavenger of $O2^{-\bullet}$ (Mekki et al., 2015). Results reported in Table 7 showed that all applications markedly enhanced SOD activity in faba bean plants, especially the sequenced application ones (i.e., AsA_{1mM}-GSH_{1mM}) compared to their corresponding controls. Stress enhanced the accumulation of the ROS including H_2O_2 in plant cells. The metabolism of H_2O_2 depends on various functionally interrelated antioxidant enzymes such as CAT, APX and GPX. These enzymes are involved in elimination of H_2O_2 from stressed cells (Kim et al., 2005). The enzyme CAT is the most effective one that scavenges H_2O_2 in cells to preventing oxidative damage. Our results demonstrated that CAT activity was significantly increased in all treatments. This is may be due to the increases occurred in the activity of the enzymes APX and GPX in plants due to antioxidants application (AsA1mM-GSH1mM), acting on H2O2 to remove it. These results are also supported by finding of Khattab (2007) Abedi and Pakniyat (2010) and Rady and Hemida (2016). They concluded that the reduction of CAT activity was supposedly due to the inhibition of enzyme synthesis, change in the assembly of enzyme subunits, or protein degradation under stress. Moreover, increase in the GPX activity under various stress conditions has been linked with protection from oxidative damage, lignification and cross-linking of cell wall to prevent the adverse effects of such condition (Moussa and Abdelaziz , 2008).

Effects of seed soaking and foliar spray with AsA and GSH on contents of (N, P, K+ and Ca2+) and (Na+) and its ratios of faba bean plants.

Contents macro-nutrients (i.e., N, P, K+ and Ca+2) and sodium (Na+) and its ratio (i.e., K+/ Na+ , K+/ Ca+2and Na+/ Ca+2), of salt-stressed faba bean plants were positively affected by ascorbic acid (AsA) and/or glutathione (GSH) as shown in **Table 8 and 9**. AsA or GSH application lonely, used as seed soaking or foliar spraying was significantly or insignificantly increased all macro-nutrients contents compared to the controls (seed soaking or foliar spray with distilled water). Combined treatment applications of AsA and GSH (i.e., seed soaking in AsA + foliar spray with GSH, seed soaking in GSH + foliar spray with AsA) significantly increased all investigated characteristics compared to the control (seed soaking + foliar spray with distilled water). Among all combined treatments, the combined treatment of seed soaking in AsA + foliar spray with GSH found to be the best, increasing N%, P%, K+% and Ca+2%, by 27.8%, 20.0%, 133.7%, 27.27%, also K+/ Na+ , K+/ Ca+2and Na+/ Ca+2 ratio were increased in the same trend, while Na+% was decreased, in compared to the un treated plants.

Table 8. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on contents of macro-nutrients (N, P, K^+ and Ca^{2+}) and sodium (Na^+) of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters				
Irrigation	Antioxidants		N (%)	P (%)	K^+ (%)	Ca^{2+} (%)	Na^+ (%)
Non-saline	Dist. water		2.83±0.03a	0.34±0.02a	2.50±0.03a	1.28±0.02a	0.21±0.01c
	AsA-GSH		2.89±0.04a	0.35±0.02a	2.58±0.03a	1.32±0.02a	0.21±0.01c
	GSH-AsA		2.84±0.03a	0.34±0.02a	2.52±0.04a	1.28±0.02a	0.20±0.01c
Saline	Dist. water		2.05±0.02c	0.25±0.01c	0.92±0.02c	0.88±0.01c	1.22±0.05a
	AsA-GSH		2.62±0.03b	0.30±0.01b	2.15±0.03b	1.12±0.01b	0.49±0.02b
	GSH-AsA		2.54±0.03b	0.30±0.01b	2.10±0.03b	1.17±0.02b	0.51±0.02b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

Table 9. Effects of seed soaking and foliar spray with ascorbic acid (AsA) and glutathione (GSH) on ratios of K^+ and Ca^{2+} in relation to Na^+ of faba bean cv. "Giza 716" plants irrigated with saline water

Treatments			Parameters		
Irrigation water	Antioxidants		K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	$K^+ + Ca^{2+}/Na^+$ ratio
Non-saline	Dist. water		11.90±0.43a	6.10±0.26a	18.00±0.01c
	AsA-GSH		12.29±0.48a	6.29±0.23a	18.57±0.01c
	GSH-AsA		12.05±0.45a	6.14±0.20a	18.19±0.01c
Saline	Dist. water		0.75±0.03c	0.72±0.03c	1.48±0.05a
	AsA-GSH		4.39±0.18b	2.29±0.09b	6.67±0.02b
	GSH-AsA		4.12±0.13b	2.25±0.09b	6.37±0.02b

Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

4. CONCLUSION:

Seed soaking and foliar spraying with AsA and GSH individually or in sequenced integration, enhanced plant growth, antioxidants and osmoprotectants such as AsA, GSH, proline and soluble sugars under 150 mM NaCl stress conditions. The alleviating effects of the treatment of sequenced integrations were more pronounced under NaCl stress than those of the individual application of AsA or GSH. The sequenced integration of AsA_{1mM}-GSH_{1mM} was found to be more effective than GSH_{1mM}-AsA_{1mM}.

REFERENCES:

1. Abedi, T., Pakniyat, H., 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (Brassica napus L.). Czech. J. Genet. Plant Breed. 46, 27–34.
2. Alam, S. M. (1999) Nutrient uptake by plants under stress conditions. p. 285–313. In M. Pessarakli (ed.) Handbook of plant and crop stress. Second ed. rev. and exp. Marcel Dekker, New York.
3. Aly-Salama, K.H., Al-Mutawa, M.M., 2009. Glutathione-triggered mitigation in salt-induced alterations in plasmalemma of onion epidermal cells. Int. J. Agric. Biol. 11(5), 639–642.

4. Ameer, K.H., Muhammad, S.A.A., Habib, U.R.A., Muhammed, A., 2006. Interactive effect of foliarly applied ascorbic acid and salt stress on wheat at the seedling stage. Pak. J. Bot. 38(5), 1407–1414.
5. Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Ann. Rev. Plant Biol. 55, 373–399.
6. Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris L. Plant Physiol.1949; 24: 1–5.
7. Asensi-Fabado MA, Munne´-Bosch S (2010) Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends Plant Sci. 15:582–592.
8. Athar, H., Khan, A., Ashraf, M., 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot. 63, 224–231.
9. Azevedo Neto A.D., Prico J.T., Eneas-Filho J., Braga de Abreu C.E., Gomes-Filho E. (2006): Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot., 56: 235–241.
10. Azzedine, F., Gherroucha, H., Baka, M., 2011. Improvement of salt tolerance in durum wheat by ascorbic acid application. J. Stress Physiol. Biochem. 7(1), 27–37.
11. Bates, L.S., Waldren, R.P., Teare, I.D. Rapid determination of free proline for water-stress studies. Plant Soil. 1973; 39: 205–207.
12. Beauchamp, C., Fricovich, I. Superoxide dismutase: improved assays and na assay applicable to acrylamide gels. Anal. Biochem.1971; 44: 276–287.
13. Bernstein, N., Ioffe, M. and Zilberstaine, M. (2001) Salt stress effects on avocado rootstock growth. I. Establishing criteria for determination of shoot growth sensitivity to the stress. Plant and Soil, 233: 1-11.
14. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem.1976; 72: 248–254.
15. Chen, J.H., Jiang, H.W., Hsieh, E.J., Chen, H.Y., Chien, C.T., Hsieh, H.L., Lin, T.P., 2012. Drought and salt stress tolerance of an Arabidopsis glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. Plant Physiol. 158, 340–351.
16. Ching, L.S., Mohamed, S. Alpha-tocopherol content in 62 edible tropical pants. J Agric Food Chem. 2001; 49: 3101–3105.
17. Clark, A.J., Landolt, W., Bucher, J.B., Strasser, R.J. Beech (Fagus sylvatica)response to ozone exposure assessed with a chlorophyll a fluorescence performance index. Environ. Pollut. 2000; 109: 501–507.
18. Cordovilla, M. P., Ligero, F. and Lluch, C. (1994) The effect of salinity on N fixation and assimilation in Vicia faba. J. Exp. Bot., 45: 1483-1488.
19. Cordovilla, M. P., Ligero, F. and Lluch, C. (1999) Effect of salinity on growth nodulation and nitrogen assimilation in nodules of faba bean (Vicia faba L.) App. Soil Ecol., 11: 1-7.
20. Eid, R.A., Taha, L.S., Ibrahiem, S.M.M., 2011. Alleviation of adverse effects of salinity on growth, and chemical constituents of marigold plants by using glutathione and ascorbate. J. Appl. Sci. Res. 7(5), 714–721.
21. Foyer, C.H. and Halliwell, B. (1976) The Presence of Glutathione and Glutathione Reductase in Chloroplasts: A Proposed Role in Ascorbic Acid Metabolism. Planta, 133, 21-25.
22. Foyer, C.H., Noctor, G., 2000. Oxygen processing in photosynthesis: regulation and signaling. New Phytol. 146, 359–388.
23. Gomez, K.A., Gomez, A.A., 1984. Statistical Analysis Procedures for Agricultural Research. John Wiley and Sons, New York, NY, USA.
24. Griffth, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106, 207–212.
25. Gunes, A., Inal, A. and Alpaslan, M. (1996) Effect of salinity on stomatal resistance, proline, and mineral composition of pepper. J. Plant Nutr., 19: 389–396.
26. Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G., Cicek, N., 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity. J. Plant Physiol. 164, 728–736.
27. Gupta, B., Huang, B., 2014. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. Int. J. Genom. Vol. 2014, Article ID 701596, 18 pages.
28. Hafez, A., Mikkelsen, D.S. Colorimetric determination of nitrogen for evaluating the nutritional status of rice. Commun. Soil Sci. Plant Anal.1981; 12: 61-69.
29. Havir, E.A., McHale, N.A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 1987; 84: 450–455.
30. Heath, R.L., Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 1968; 125: 189-198.
31. Hemida, Kh.A., Ali, R.M., Ibrahim, W.M., Sayed, M.A., 2014. Ameliorative role of some antioxidant compounds on physiological parameters and antioxidants response of wheat (Triticum aestivum L.) seedlings under salinity stress. Life Sci. J. 11(7), 324–342.
32. Hernández JA, Almansa MS. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiologia Plantarum 115: 251–257.
33. Hoagland, D.R. and Arnon, D.I. (1938) The water culture method for growing plants without soil. California Agricultural Experiment Station Circulation, 347, 32.
34. Irigoyen, J.J., Emerich, D.W., Sanchez-Diaz, M., 1992. Water stress induced changes in the concentrations of proline and total soluble sugers in nodulated alfalfa (Medicago sativa) plants. Physiol. Plant. 8, 455–460.
35. Jacoby, B. (1999) Mechanism involved in salt tolerance of plants. pp. 97-123. In M. Pessarakli (ed.) Handbook of plant and crop stress. Second ed. rev. and exp. Marcel Dekker,
36. Jakson, M.L. Soil Chemical Analysis. Prentice Hall of India Private limited, New Delhi, India. 1967.
37. Kar, M., Mishra, D. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 1976; 57: 315–319.
38. Kasote, D.M., Katyare, S.S., Hegde, M.V., Bae, H., 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int. J. Biol. Sci. 11(8), 982–991.
39. Khan, A., Ahmad, I., Shah, A., Ahmad, F., Ghani, A., Nawaz, M., Shaheen, F., Fatima, H.U., Pervaiz, F., Javed, S., Hayat, F., Nawaz, H., Zubair, R., 2013. Amelioration of salinity stress in wheat (Triticum aestivum L.) by foliar application of phosphorus. Int. J. Exp. Bot. 82, 281–287.
40. Khan, M. A., Ungar, I. A. and Showalter, A. M. (2000) Effects of Salinity on Growth, Water Relations and Ion Accumulation of the Subtropical Perennial Halophyte, Atriplex griffithii var. Stocksii. Annals of Botany, 85: 225-232.
41. Khattab, H., 2007. Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline condition. Aust. J. Basic Appl. Sci. 1, 323–334.
42. Kim, S.Y., Lim, J.H., Park, M.R., Kim, Y.J., Park, T.I.I., Seo,

Y.W., 2005. Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under salt stress. J. Biochem. Mol. Biol. 38, 218–224.

43. Koca, H., Bor, M., Ozdemir, F., Turkan, I., 2007. Effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp. Bot. 60, 344–351.
44. Konings, E.J.M., Roomans, H.H.S., Beljaars, P.R. Liquid chromatographic determination of tocopherols and tocotrienols in margarine, infant foods, and vegetables. J AOAC Int.1996; 79: 902–906.
45. Koyro, H. W. and Huchzermeyer, B. (1999) Salt and drought stress effects on metabolic regulation in maize. pp. 843-878. In M. Pessarakli (ed.) Handbook of plant and crop stress. Second ed. rev. and exp. Marcel Dekker, New York.
46. Kranner, I. and D. Grill. 1996. Significance of thiol-disulphide exchange in resting stages of plant development. Bot. Acta 109: 8–14.
47. Kyparissis A, Petropoulou Y, Manetas Y. 1995. Summer survival of leaves in a soft-leaved shrub (Phlomis fruticosa L., Labiates) under Mediterranean field conditions: Avoidance of photoinhibitory damage through decreased chlorophyll contents. J. Exp. Bot., 46:1825-1831.
48. Maxwell, K., Johnson, G.N. Chlorophyll fluorescence—a practical guide. J.Exp. Bot. 2000; 51: 659–668.
49. Mekki, B.E-D., Hussien, H-A., Salem, H., 2015. Role of glutathione, ascorbic acid and α-tocopherol in alleviation of drought stress in cotton plants. Int. J. ChemTech Res. 8(4), 1573–1581.
50. Mousavi, S.A.R., Chauvin, A., Pascaud, F., Kellenberger, S., Farmer, E.E., 2013. GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. Nature 500, 422–426.
51. Moussa, H., Abdel-Aziz, S.M., 2008. Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust. J. Crop Sci. 1, 31–36.
52. Mukherjee, S.P., Choudhuri, M.A., 1983. Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol. Plant. 58, 166–170.
53. Munne-Bosch S, Alegre L. Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in Rosmarinus officinalis plants. Planta 2000;210:925–931.
54. Munne-Bosch, S. The role of α-tocopherol in plant stress tolerance. J. Plant Physiol. 2005, 162, 743–748.
55. Munns, R. (1993) Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. Plant Cell Environ., 16: 15-24.
56. Munns, R. (2002) Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239–250.
57. Nakano, Y., Asada, K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 1981; 22: 867–880.
58. Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., Queval, G., Foyer, C., 2012. Glutathione in plants: An integrated overview. Plant Cell Environ. 35 (2), 454–484.
59. Okuma, E., Murakami, Y., Shimoishi, Y., Tada, M., Murata, Y., 2004. The effects of exogenous proline and betaine on tobacco cultured cells under saline conditions. Soil Sci. Plant Nutr. 50, 1301–1305.
60. Osman, A., Rady, M.M. Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants. J. Hortic. Sci. Biotechnol. 2014; 89: 237–244.
61. Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effect on plants–a review. Ecotoxicol. Environ. Saf. 60, 324–349.
62. Parvaiz A., Satyawati S. (2008): Salt stress and phyto-biochemical responses of plants – a review. Plant, Soil and Environment, 54: 88–99.
63. Piper, C.S. Soil and plant analysis. New York: Interscience Publishers, Inc. NC, USA.1947.
64. Poljakoff-Mayber, A. and Lerner, H. R. (1999) Plants in saline environment. In M. Pessarakli (ed.), Handbook of Plant and Crop Stress, pp. 125-152. Marcel Dekker Press Inc. New York.
65. Premchandra, G.S., Saneoka, H., Ogata, S. Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. J. Agric. Sci. Camb. 1990; 115: 63–66.
66. Rady, M.M., 2011. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (Phaseolus vulgaris L.) plants under salinity and cadmium stress. Sci. Hortic. 129, 232–237.
67. Rady, M.M., Hemida, K.A., 2015. Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. Ecotoxic. Environ. Saf. 119, 178–185.
68. Rady, M.M., Hemida, K.A., 2016. Sequenced application of ascorbate-proline-glutathione improves salt tolerance in maize seedlings. Ecotoxic. Environ. Saf. 133, 252–259.
69. Rady, M.M., Mohamed, G.F., 2015. Modulation of salt stress effects on the growth, physio-chemical attributes and yields of Phaseolus vulgaris L. plants by the combined application of salicylic acid and Moringa oleifera leaf extract. Sci. Hortic. 193, 105–113.
70. Rady, M.M., Taha, R.S., Mahdi, A.H.A., 2016. Proline enhances growth, productivity and anatomy of two varieties of Lupinus termis L. grown under salt stress. S. Afr. J. Bot. 102, 221–227.
71. Rodriguez, P., Torrecillas, A., Morales, M. A., Ortuno, M. F. and Sanchez-Blanco, M. J. (2005) Effects of NaCl salinity and water stress on growth and leaf water Relations of Asteriscus maritimus plants, Environ. Exp. Bot., 53(2): 113-123.
72. Salim, M. (1991) Change in water conducting properties of plant roots by nutrition and salt stress. J. Agron. Crop Sci. 166: 285-287.
73. Schafer, F. Q., & Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/ glutathione couple. Free Radic. Biol. Med., 30, 1191–1212.
74. Semida, W.M., Taha, R.S., Abdelhamid, M.T., Rady, M.M., 2014. Foliar-applied α-tocopherol enhances salt-tolerance in Vicia faba L. plants grown under saline conditions. S. Afr. J. Bot. 95, 24–31.
75. Shalata, A., Neumann, P.M., 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Exp. Bot. 52, 2207–2211.
76. Sharma, P., Jha, A.B., Dubey, R.Sh., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot., Vol. 2012, Article ID 217037, 26 pages.
77. Singh, R., S. Singh, P. Parihar, R.K. Mishra and D.K. Tripathi et al., 2016. Reactive Oxygen Species (ROS): Beneficial companions of plant's developmental processes. Front. Plant Sci., Vol. 7. 10.3389/fpls.2016.01299.
78. Smirnoff, N.; Wheeler, G.L. Ascorbic acid in plants: Biosynthesis and function. Crit. Rev. Biochem. Mol. Biol. 2000, 35, 291–314.
79. Sullivan, C.Y., Ross, W.M. Selecting the drought and heat resistance in grain sorghum. In: Mussel, H., Staples, R.C. (Eds.), Stress Physiology in Crop Plants. John Wiley & Sons, New York. 1979; 263–281.

80. Tucker, M. R. (1999) Essential plant nutrients: Their presence in North Carolina soils and role in plant nutrition, North Carolina Department of Agriculture & Consumer Services, Miscellaneous Publication.
81. Velikova, V., Yordanov, I., Edreva, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci. 2000; 151: 59–66.
82. Weatherly, P.E. Studies in the water relations of cotton. 1. The field measurement of water deficits in leaves. New Phytol. 1950; 49: 81–97.
83. Weber, W.,Rimann, M., Spielmann, M., Keller, B.,Daoud-El Baba, M.,Aubel, D., Weber, C.C., and Fussenegger, M. (2004). Gas-inducible transgene expression in mammalian cells and mice. Nat. Biotechnol. 22, 1440–1444.
84. Williams, V., Twine, S. Flame photometric method for sodium, potassium and calcium. In: Modern Methods of Plant Analysis, eds K. Peach and M.V. Tracey (Berlin: Springer-Verlag). 1960; 3–5.
85. Wutipraditkul, N., Wongwean, P., Buaboocha, T., 2015. Alleviation of salt-induced oxidative stress in rice seedlings by proline and/or glycinebetaine. Biol. Plant. 59(3), 547–553.
86. Yazici I, Türkan I, Sekmen AH, Demiral T. Salinity tolerance of purslane (Portulaca oleraceae L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. Environ. Exp. Bot. 2007; 61: 49-57.
87. Younis, M.E., Hasaneen, M.N.A., Kazamel, A.M.S., 2010. Plant growth metabolism and adaptation in relation to stress conditions. XXVI. Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in Vicia faba seedlings. Protoplasma. 239, 39–48.
88. Younis, M.E., Tourky, S.M.N., 2015. Influence of salinity and adaptive compounds on oxidative stress and antioxidant system in broad bean cultivars contrasting in salt tolerance. Plant Know. J. 4(1), 25–32.
89. Zekri, M. and Parsons, L. R. (1992) Salinity tolerance of Citrus rootstocks: Effects of salt on root and leaf mineral concentrations. Plant Soil, 147: 171-181.
90. Zhu, J. K. (2003) Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol., 6: 441-445.

SIFT DESK , Deerpark Dr, #75, Fullerton,CA,92831,United States. Email: info@siftdesk.org