

RESPONSE OF *Hibiscus rosa-sinensis* L. TO THE INTERACTIVE EFFECT OF SEAWATER SALINITY AND ASCORBIC ACID

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ABSTRACT

A pot experiment was carried out at the Experimental Station and in the Laboratory of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the two successive seasons of 2010 and 2011 to investigate the effective role of ascorbic acid at 50 and 100 ppm on alleviation the harmful effect of irrigation by diluted seawater (10, 20, 30 and 40%) on plant growth and biochemical constituents of *Hibiscus* (*Hibiscus rosa-sinensis* L.).

The results showed that shoot height, number of branches, number of leaves, leaf area, root length as well as fresh and dry weights of shoots and roots were increased at the low levels of salinity of diluted seawater irrigation (10%), and then decreased gradually with increasing irrigation water salinity from 10 to 40% as compared to non-salinized (control). The shortest plants resulted from the highest irrigation water salinity (40%) in both seasons. Most criteria of plant growth were significantly affected by application of ascorbic acid separately or collectively as promoted all the aforementioned characters in this study. In addition, salinity stress decreased photosynthetic pigments, N, P and K %, while increased proline and total soluble sugars. Moreover, AsA increased photosynthetic pigments, proline, total soluble sugars, N, P and K concentration. It could be noticed that the AsA at both levels could partially alleviate the harmful effect of salinity stress on plant growth and biochemical constituents of *Hibiscus* plant.

From the obtained results, It could be recommended to spray *Hibiscus* plants irrigated by diluted seawater with ascorbic at 100 ppm to overcome the destructive effect of salinity.

Keywords: Ascorbic Acid, Soil Salinity, Growth, Biochemical Constituents, *Hibiscus*.

INTRODUCTION

Water shortage and poor water quality are critical issues in many areas of the world. Nearly every country in the world experiences suffer from regular short-term water shortages, while more than 80 countries now suffer from serious water shortage (Jin *et al.*, 2007). Due to the rapid increase in population, industry development and expansion of agriculture, water supply continues to be a critical issue in Egypt. There is an urgent need to use alternative water sources for irrigating urban landscapes in order to conserve fresh water. However, the elevated seawater salinity may cause foliage damage of sensitive plant species and thus affect the aesthetic appearance. Therefore, identifying salt-sensitive plant species and categorizing salt tolerance may aid the selection of plant species for landscapes where diluted seawater may be used for irrigation. Salinity tolerance of commonly planted

landscape plants has been investigated extensively in the recent years where water supply is limited (Niu and Rodriguez, 2006 a&b; Cassaniti, 2009 and Valdez-Aguilar *et al.*, 2011) and a wide range of salinity tolerances were found among the tested plant species. Generally, salt tolerance of landscape plants has similar mechanisms of salt tolerance to agricultural crops, most of which are non-halophyte, vary widely with species and environmental conditions. Saline water for irrigation reduces plant size and growth resulting in smaller leaves, shorter stem, sometimes fewer green leaves (may be reducing aesthetic value), roots length and diameter (thinner or thicker)) and changes in physiological and metabolic processes including chlorophyll content, ion uptake and accumulation in tissues. The previous necessary parameters are often assessed based on growth reduction for salt tolerance of landscape plants but should be based primarily on aesthetic value. This study evaluated the growth responses of ornamental shrub (*Hibiscus rosa-sinensis* L.) commonly planted widely in gardens and landscapes (Cassaniti, 2008) as were irrigated with diluted seawater at five levels of salinity.

Hibiscus rosa sineses L. is a evergreen flowering shrub in the family Malvaceae. There are many reports implied that exogenous application of non-enzymatic antioxidants (ascorbic acid), could alleviate injurious effects of salinity on plants under saline conditions (Sayed and Gadallah, 2002; El Tohamy and El Gready, 2007 and Bassuony *et al.*, 2008). So, it can be considered as a successful approach to increase salinity tolerance and improve plant growth. Ascorbic acid (AsA) is reviewed as a natural antioxidant compound which may be accumulated in all plants in either photosynthetic or non-photosynthetic tissues, under normal and stress conditions (Smirnoff and Wheeler, 2000). Thus, high endogenous AsA in plants is necessary to counteract the oxidative stress in addition to regulate other processes of plant metabolism as a foliar spray (Chen and Gallie, 2004). From the earlier mentioned reports it is evident that exogenous application of ascorbic acid to plants plays a key role in the regulation of a number of metabolic processes and has positive effects in overcoming the adverse effects of salt stress in plants. So, the major objectives of the present study were to counteract the harmful effects of diluted seawater irrigation on landscape plants of *Hibiscus rosa-sinensis* L. by foliar application with ascorbic acid. It is further hoped that the results of this work may finally lead to the utilization of AsA for large scale irrigation coastal gardens and landscape plants.

MATERIALS AND METHODS

This experiment was carried out at the Experimental Station and in the Laboratory of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the two successive seasons of 2010 and 2011 (from April 1st to October 31st of every season) to investigate the effective role of ascorbic acid on alleviation the harmful effect of irrigation by diluted seawater on growth of *Hibiscus rosa-sinensis* L. plants. Six-month old *Hibiscus rosa-sinensis* L. transplants of a uniform height (25 ± 2 cm in length) were obtained from a local nursery and transplanted on April 1st of each

season, as one transplant/bag (17 cm diameter black polyethylene bags) filled with 5 kg sandy clay soil (sand:clay, 1:1 w/w) and remained in open field. Plants were equally irrigated with well water for three weeks. Initial soil samples were taken before the establishment of the study for determination of some physical and chemical properties (Table, 1). The physiochemical analysis of soil used in this experiment was done according to Piper (1950), Black (1965) and Page (1982).

Table (1): Physiochemical analysis of soil used in the experiment

Size distribution %			Anions and cations (meq /100g soil)							EC (mm/cm)	pH (1:1)
Clay	Silt	Sand	So ₄ ⁼	HCO ₃	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Cl ⁻		
30.6	13.4	56.0	0.877	0.51	0.36	1.0	1.30	0.04	1.31	1.92	7.66

Treatments were as follows:

1- Salinity concentrations: Control (fresh non saline tap water) and four dilutions of seawater (10, 20, 30 and 40%). Saline water for each treatment was prepared by mixing seawater with fresh non saline tap water at different ratios (v/v) under room temperature. The chemical analyses of the employed seawater, collected from the Mediterranean Sea in City Gamasa are shown in Tables (2).

Table (2): Properties of seawater used in the two seasons of 2010 and 2011

E.c. (ds/m)	Salts (TDS: total dissolved solids)	Anions and cations (ppm)							pH
48	30,720	So ₄ ⁼	HCO ₃	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Cl ⁻	8.28
		2772.29	297.92	79.2	5.47	10790	270	14816.28	

2- Ascorbic acid, Vit. C (AsA) was supplied by Sigma Chemicals Co., USA and used at the concentration of 50 or 100 mg/l each.

Experimental design:

The layout of this experiment during both seasons was split plot design with three replicates (Mead *et al.*, 1993). The main plots included five salinity concentrations (0, 10, 20, 30 and 40% of seawater), while, the sub-plots were assigned for ascorbic acid at the concentration of 0, 50 and 100 mg/l. Five irrigation treatments with either fresh or diluted seawater were combined with three ascorbic acid concentration treatments to form 15 interaction treatments.

On April 20th of both seasons, the plants were divided into three sets. The 1st set (serving as control) was sprayed with tap water three times (once a week) before the start of irrigation with diluted seawater and the fourth time one month after salinization, whereas the 2nd and 3rd ones were sprayed with ascorbic acid at 50 and 100 mg/l (AsA1 and AsA2, receptively) three times (once a week) before the start of irrigation with diluted seawater and the fourth time a month later. After 40 days from transplanting, each set was divided into

five groups. The first group was still irrigated with normal tap water serving as control (S0). The remainder groups were irrigated with diluted seawater at the rate of 10, 20, 30 and 40% (S1, S2, S3 and S4, receptively). Plants were irrigated with 350 ml/pot fresh or diluted saline water twice per week to maintain soil water content at the range 60-65% of water holding capacity (WHC) and salts were washed once every two weeks by fresh water. Treatments were continued up to 6 months. The plants were fertilized with kristalon (17:17:17) at the rate of 2 g/bag three times; the first time after one month from transplanting (May 1st), whereas the second and third ones were added each 45 days afterwards (June, 15th and July 30th , respectively). Other agricultural processes were performed according to normal practices.

Data analysis:

Growth characters: Three plants from each treatment after 70 and 120 days from transplanting were taken to detremine the shoot height (cm), root length (cm), number of branches and leaves/plant, leaf area (cm²) roots and shoots fresh as well as dry weights (g/plant).

Photosynthetic pigments (mg/g fw): After 70 days from transplanting fresh leaf samples (the 3rd completely developed foliage leaf from the shoot tip) of 0.05 g were extracted by methanol for 24 h at laboratory temperature after adding a trace from sodium carbonate (Robinson *et al.* 1983), then chlorophyll a, b and carotenoids were determined spectrophotometrically (Spekol II) at wave lengths 452, 650 and 665 nm. The quantities of total chlorophylls, chlorophyll a, b and carotenoids concentration (mg/g) in leaves were determined by the equations proposed by Mackinny (1941).

Mineral composition: After 70 days from transplanting, the shoots of three plants in each replicate were dried at 70 °C for 48 hours and finally ground. The digestion of samples was undertaken according to Peterburgski (1968). Nitrogen was estimated by semikjeldahle method (Black, 1965). Phosphorus was determined colorimetrically at wave length 725 nm as described by Jackson (1967). Potassium was determined by flame photometer using Corning 400-flame photometer as described by Peterburgski (1968).

Total soluble sugars: After 70 days from transplanting, total soluble sugars were extracted from 0.1 g dry matter by ethanol 80% for overnight at laboratory temperature according to Kayani *et al.* (1990) then filtered through Whatmann No1 filter paper and were determined according to Sadasivam and Manickam (1996).

Proline determination: Proline was determined after 70 days from transplanting using the modified ninhydrine method of Troll and Lindsley (1955).

Statistical analysis:

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

RESULTS AND DISCUSSION

Morphological characters:

Data presented in Figures (1, 2, 3, 4, 5, 6, 7, 8 and 9) indicated that *Hibiscus rosa-sinensis* L shoot height (cm), root length (cm), number of branches/plant, number of leaves/plant, leaf area (cm²), as well as fresh and dry weights of shoot and roots (g/plant) were increased at low levels of salinity of diluted seawater irrigation (10%), then decreased gradually with increasing irrigation water salinity from 10 to 40% as compared to non-salinized (control). The shortest plants resulted from the highest irrigation water salinity (40%) in both seasons. The inhibitory effects of salinity on plant growth in this investigation are in agreement with the findings of Houimli *et al.* (2008); Khafagy *et al.* (2009); Afifi *et al.* (2010) and Hakim *et al.* (2010), they concluded that high salinity stress decrease plant growth in different plant species.

In the present investigation, the stimulatory effect of low diluted seawater irrigation (10%) on shoot height and root length may be resulted from the beneficial effects of low concentration of chloride on many physiological processes as photosynthesis and enzymes activity and osmoregulators, which allows cell enlargement at low water availability caused by the presence of ions especially Na⁺ and Cl⁻ (Khan *et al.*, 1997) and may increase calcium uptake, which plays a critical role in improving plant growth under saline conditions (Cramer *et al.*, 1987) and/or may be due to reducing permeability of Na⁺ through the plasma membrane and prevents loss of K⁺/Na⁺ ratio (He and Cramer, 1992) and/or defined as "free cytosolic calcium" which participates in regulation of metabolism and growth of plants. Moreover, Ca²⁺ is taken up by plants passively and only by root tips constituent of the middle lamella of cell walls where it helps to bind adjacent cells together and strengthen overall construction (Marschner, 1995) and influences membrane structure and function, stabilizing membranes and influencing permeability by binding phosphate and carboxylate groups of membrane phospholipids and protein (Davies and Monk-Talbot, 1990) and Ca²⁺ serves as an important second messenger in the perception and transduction of environmental and stress signals (Roos, 2000). The depressive effect of salinity on plant growth may be attributed to inhibition of cell division and enlargement, reduction in cell size (Ayala and O'Leary, 1995) and inhibition of cambial cell activity (Iraqi *et al.*, 1988). In addition, Hatung (2004) suggested that the bad effects of salinity on plant height and root length may be due to the bad effects of salinity on meristematic cell division and elongation as well as root penetration. Also, Zidan *et al.* (1990) suggested that the inhibition of root growth by salinity stress may be due to the reduction in the length of root tip elongation zone. Moreover, Azaizeh *et al.* (1992) revealed that the reduction in the root extension rates might come from the marked lowering of root radial hydraulic conductivity that occurs with salinization. In addition, Roy *et al.* (1995) revealed that salinity stress caused an increase in endogenous ethylene concentrations in plant tissues, which inhibits shoot elongation as a result of reduced cell elongation.

Figure (1): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on shoot and root length (cm) of *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting

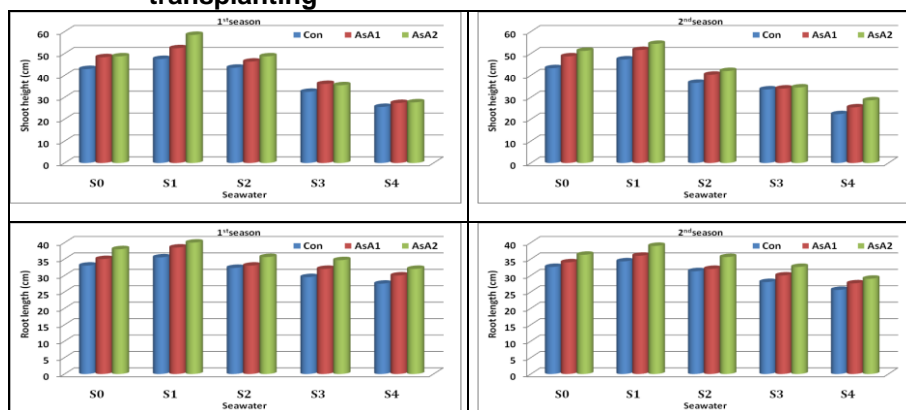
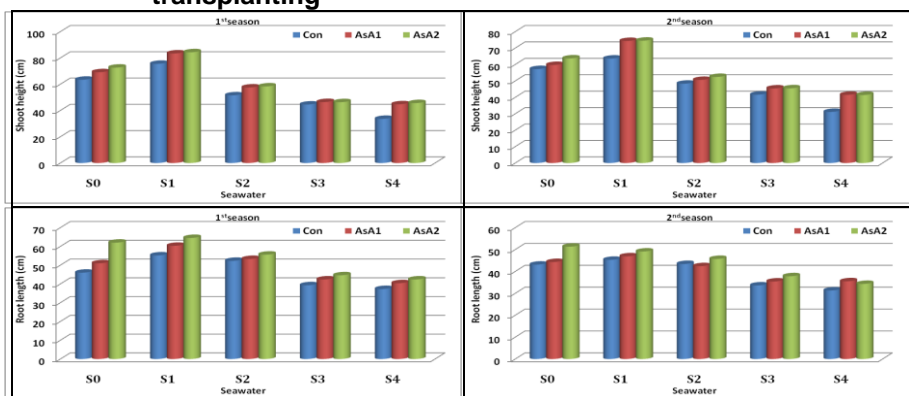


Figure (2): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on shoot and root length (cm) of *Hibiscus rosa-sinensis* L. plant after 120 days from transplanting



The inhibitory effects of salt stress on growth are conformity with the findings published by Cicek and Cakirlar (2002), Abraham and Kiran (2003), Shawky (2003) and Da Silva *et al.* (2003). They reported that salinity reduced shoot length, fresh and dry weight. Moreover, Shannon and Grieve (1999) indicated that salinity reduced fresh weight of some vegetables. Furthermore, diluted seawater irrigation decreases stomatal conductance and consequently less CO₂ is available for carboxylation reactions in the photosynthesis apparatus (Yadav *et al.*, 1996), which leads to reduction of photosynthetic capacity resulting less net assimilation and relative growth rates (Kingsbury *et al.*, 1984) and finally decreased in fresh and dry weights of plants. Moreover, the reduction in fresh and dry weight of *Hibiscus rosa-*

sinensis L. plant might be attributed to a decrease in either leaf number or leaf area per plant (Fig. 5, 6, 7 and 8). Furthermore, under salinity, net photosynthetic and CO₂ uptake decrease mainly because sodium chloride treatment decreases stomatal conductance (Al-Khafaf *et al.*, 1990), and consequently less CO₂ is available for carboxylation reaction in the photosynthetic apparatus and ribulose- 1,5 - bisphosphate carboxylase / oxygenase activity decreases (Fedina and Tsonev,1997), and photochemical reactions are inhibited. In addition, salinity changes thylakoid membrane structure (Sato *et al.*, 1992), and decreases the chlorophyll and carotenoids (Fig. 10). One possible reason for the observed inhibition of salinity could be the inhibitory effect of salinity stress on protein synthesis, including synthesis of RUBPC. On the other hand, the reduction in fresh and dry weights may be resulted from a reduction in phosphorous content in plant tissues (Fig. 11) which is implicated in carbohydrates metabolism, regulates the partitioning of carbon and utilization of photo assimilates (Younis *et al.*,1987) and smaller the leaf area (Fig. 7 and 8) lowers the ability of the plant to capture light and thereby adversely affects the process of photosynthesis (Cramer *et al.*,1994), which ultimately has a direct effect on biomass production.

Figure (3): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on number of branches per plant of *Hibiscus rosa-sinensis* L. after 70 days from transplanting

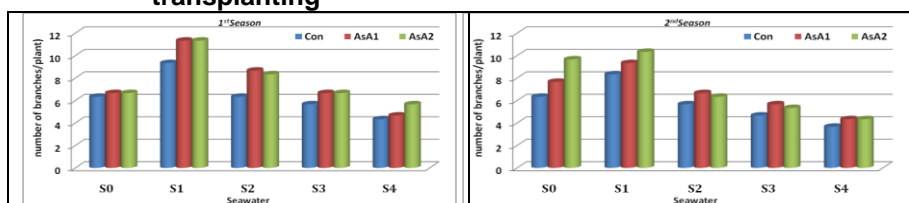
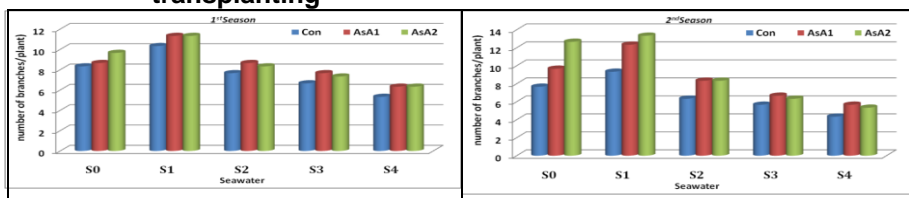


Figure (4): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on number of branches per plant of *Hibiscus rosa-sinensis* L. after 120 days from transplanting



Concerning the effects of AsA, data in Figures (1) to (9) revealed that spraying plants by ascorbic acid increased significantly plant growth under diluted seawater irrigation (10%) when compared with untreated one. On the other hand, under moderate and high salinity irrigation seawater application of AsA, in most cases, counteracted the harmful effect of diluted seawater salinity on plant growth. Generally, application AsA at 100 ppm proved

optimum values for plant growth. These results could be supported by Bassiem and Anton (1998) and Faissal and Hassan (2004).

Figure (5): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on number of leaves per plant of *Hibiscus rosa-sinensis* L. after 70 days from transplanting

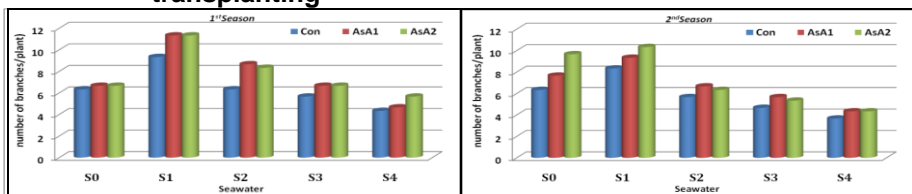


Figure (6): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on number of leaves per plant of *Hibiscus rosa-sinensis* L. after 120 days from transplanting

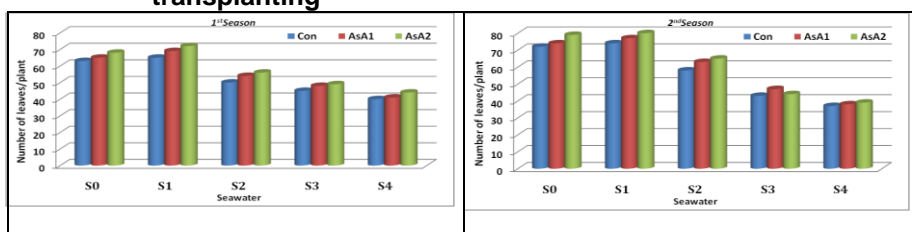


Figure (7): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on leaf area (cm²) of *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting

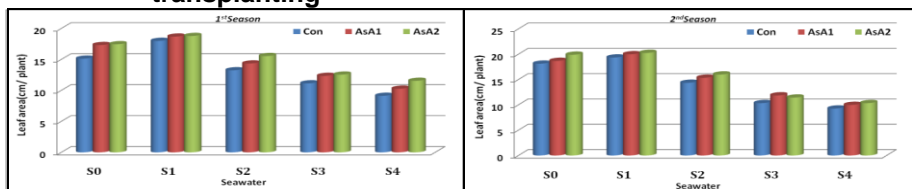
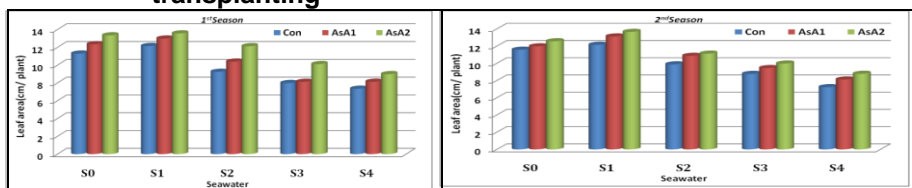
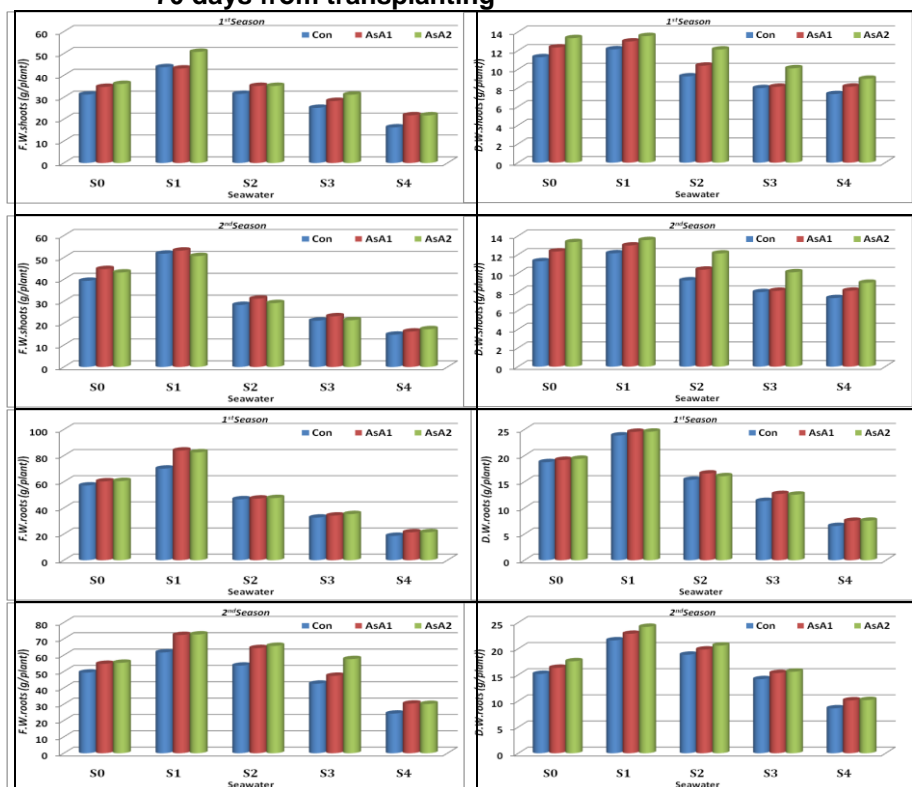


Figure (8): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on leaf area (cm²) of *Hibiscus rosa-sinensis* L. plant after 120 days from transplanting



The enhancing effect of AsA on plant growth may be due to that AsA is a major primary antioxidant (Nijs and Kelley, 1991), plays an important role in preserving the activity of enzymes (Padh, 1990), implicated in the regulation of the cell division, cell cycle progression (Smirnov, 1996), cell elongation (De Tullio *et al.*, 1999) and is the major antioxidant that scavenges H₂O₂ (Chen and Gallie, 2004), which may be cause deterioration of membrane lipids, leading to increased leakage of solutes from membranes (Mishra and Choudhuri, 1999).

Figure (9): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on shoot and root fresh and dry weights (g/plant) of *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting



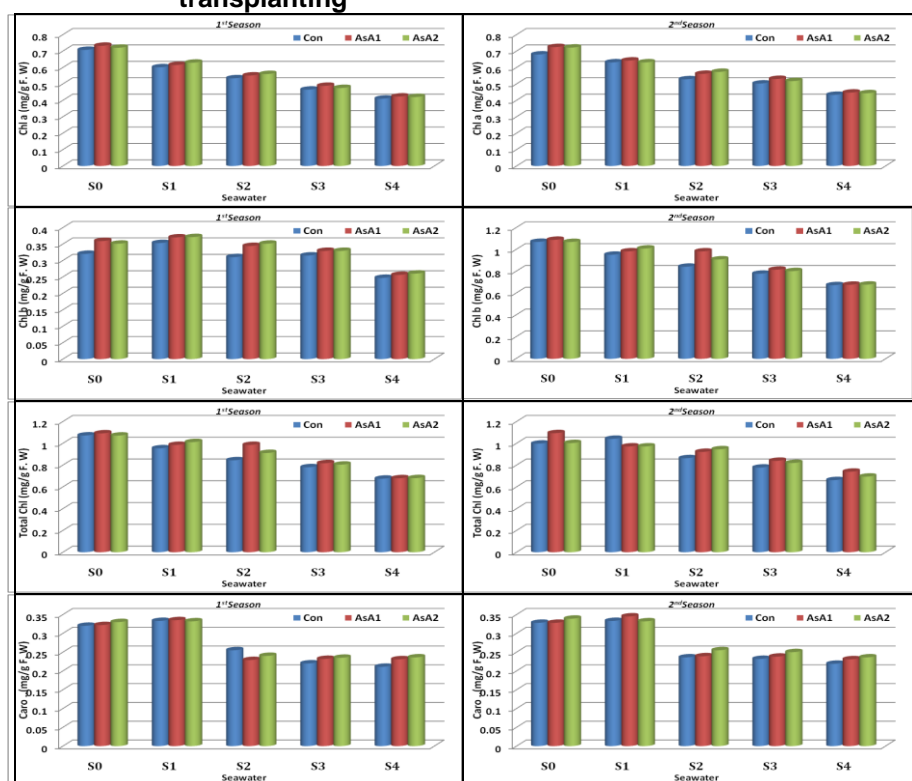
These results are in agreement with those also obtained in other different plants by El-Tohamy *et al.* (2008) and Khafagy *et al.* (2009). Moreover, Talaat (1998) found that AsA increased the vegetative growth of lavender plants. Furthermore, Tarraf (1999) recorded that AsA increased significantly lemongrass plant height as well as fresh and dry weights. Youssef *et al.* (2005) revealed that AsA at 0, 100 and 200 mg/l had a significant effect in increasing *Matricaria chamomilla* L. plant growth. Generally, it could be shown that any of each applied plant vitamins treatment could be partially

counteracted the harmful effect of high salinity levels (30 and 40% seawater used for irrigation) which enhanced all growth parameters. This increasing effect of plant vitamins used on growth parameters comparing with the effect of salinity stress levels only but this increase nearly but less control.

Photosynthetic pigments:

It could be clearly shown from data in Figure (10) that the highest chlorophyll a, b and its total as well as carotenoids concentration was extracted from the leaves of the control plants and then decreased gradually with increasing irrigation water salinity. The lowest values were recorded from plants irrigated with the highest seawater salinity (40% diluted) in both seasons. These results were similar and statistical in the two seasons of the experiment.

Figure (10): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on chlorophyll a, b and its total as well as carotenoids content (mg/g fresh weight) of *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting



From the above mentioned results it could be showed that there was clear effect of irrigation with saline water on the leaf pigment contents (chlorophyll a, b and its total, as well as carotenoids). It can be observed that

the high levels of salinity irrigation water (30 and 40% diluted)) induced a significant decrease in the contents of pigment fractions (chlorophyll a and b) and consequently of the total chlorophyll concentrations as compared with control plants. The total chlorophyll concentration of the leaves of both plants exhibited a little increase when irrigation with the lowest seawater salinity (10%). These results are in agreement with those of Parida *et al.* (2004) who reported that photosynthetic rate increased at low salinity and decreased at high salinity. In addition, the stimulatory effects of low salinity level may be resulted from the effect of low concentration of Cl^- on photosynthesis due to that Cl^- facilitates electron transport by reversible ionic binding to the O_2 -evolving complex or to the thylakoid membranes (Critchley *et al.*, 1982). Generally, chlorophyll contents were reduced markedly when irrigation with the highest seawater salinity (40%). It may be due to the reason that the total chlorophyll and the proportion of its components depend on the biological process and concentration of the salt. Lopez *et al.* (2002), Panda and Khan (2003) and El-Banna (2006) also obtained similar findings. The decrease in chlorophyll content under stress is a commonly reported phenomenon and in various studies, this may be due to membrane deterioration (Ashraf and Bhatti, 2000) and/ or suppressed the specific enzyme, which is responsible for the synthesis of green pigments due to its effect on preserving certain essential ions for chlorophyll synthesis (Mitsuya *et al.*, 2003) and/or enhancement in the activity of chlorophyll degradation enzyme chlorophyllase (Mishra and Sharma, 1994) and/or the inhibitory effect of Cl^- on the activity of Fe-containing enzymes cytochrome oxidase, which in turn may decrease the rate of chlorophyll biosynthesis as well as an increase chlorophyll degradation (Santos and Ross, 2000) and/or increased ABA content resulting in promoting chlorophyll breakdown (Hall and McWha, 1981) and/or caused a disturbed chloroplast structure, number and size which affected chlorophyll content (Arafa *et al.*, 2009) and/or caused disruption of chloroplasts (Rahman *et al.*, 2000) and/or decreased mineral absorption needed for chlorophyll biosynthesis such as iron and manganese, and stability of chlorophyll or pigment-protein complex, which leads to oxidation of chlorophyll and decreases its concentration. The primitive effect of AsA on photosynthetic pigments may be attributed to stimulation the biosynthesis of chlorophylls and delay leaf senescence (El-Gabas, 2006) and the fact that AsA activates some enzymes such as: protease, peroxidase, catalase and invertase activities which are important in regulating photosynthetic carbon reduction and delayed leaf senescence, thus producing more photo pigments (Rautenkranz *et al.*, 1994) and stimulates respiration activities, affects nutritional cycles activity and plays an important role in the electron transport system (Givan, 1979) and related to its central role in photosynthesis (Foyer and Lelandais, 1993) and/or antioxidant and protects plants against damage resulting stress (Mehdy, 1994) and/or photoprotection (Conklin, 2001).

In addition, it is generally known that photosynthetic efficiency depends on photosynthetic pigments such as chlorophylls 'a' and 'b', which play an important role in photochemical reactions of photosynthesis (Taiz and Zeiger, 2006). Generally, this result means that AsA applications could be used to mitigate the harmful effects of salinity on chlorophyll a, b and total

chlorophyll concentrations. On the other hand, AsA (100 ppm) was more effective to reduce the harmful effect of salinity on chlorophyll a, b and total chlorophyll as well as carotenoids concentration.

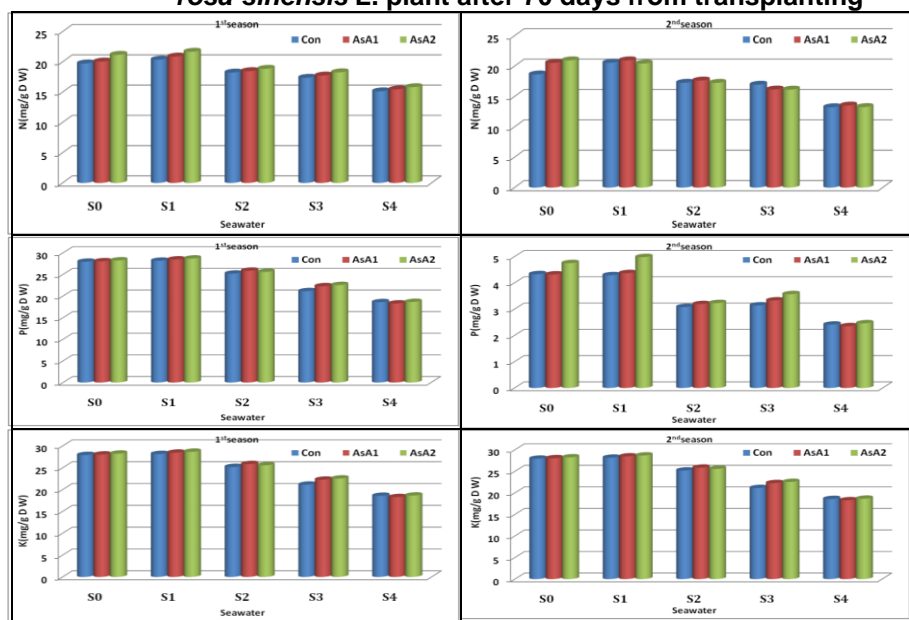
Ion uptake:

Data in Figure (11) indicated that irrigation *Hibiscus rosa-sinensis* L. with the low levels of diluted seawater (10%) caused a significant increase in nitrogen (N), phosphorus (P) and potassium (K) concentration of shoots after 70 days from transplanting, then a gradually decrease with increasing salinity in irrigation diluted seawater as compared to control. The lowest values of this parameter were recorded from plants irrigated with the highest water salinity (40%) in both seasons. These results were similar and statistical in the two seasons of the experiment. These results agree with the findings reported by Abd El-Fattah (2001) on *Adhatoda*, *Hibiscus* and *Phyllanthus* shrubs and Mazher *et al.* (2006) on *Sesbania aegyptiaca* and Abd El-Aziz *et al.* (2006) on *Khaya senegalensis*, Mazher *et al.* (2008) on *Taxodium disticum*, Abd el-Mawgoud *et al.* (2010) on green bean and Abd El-Aziz *et al.* (2011) on *Matthiola incana*, who found that saline water generally depressed uptake of nutrient ions and transport processes in the plant. In the present study, increasing salinity of seawater irrigation was associated with decreasing in N, P and K concentration in shoot. Ion uptake depends on species or genotype, salinity level and the chemical composition of the soil solution (Niu and Rodriguez, 2008). Salt-tolerant genotypes usually adapted to salinity are of three distinct types: osmotic stress tolerance, Na or Cl exclusion and the tolerance of tissue to accumulated Na or Cl (Munns and Tester, 2008). Exposure to salinity affects transport processes in the plant, the result of which can be an alteration of the nutritional status and tissue ion balance. In this concern, Tester and Daveport (2003) reported that high soil Na⁺ caused a deficiency of other nutrients by disrupting the uptake of nutrients directly by interfering with transporters in the root plasma membrane, such as K⁺-selective ion channels; and inhibiting root growth by the osmotic effects of Na⁺ and because of the detrimental effects of Na⁺ on soil structure. Potassium is most abundant inorganic cation in vacuoles, which plays a large part in maintaining cell turgor pressure and potassium homeostasis between cytoplasm and vacuole (Heelebust, 1976). Excess of sodium and chloride, the predominant ions in saline soils, creates high ionic balance that may improve the selectivity of root membrane (Bohra and Doerffling, 1993).

Sodium is frequently accumulated in the vacuoles where it can replace potassium both quantitatively and qualitatively (Storey *et al.*, 1983). Therefore, the uptake and transport of K⁺ was inhibited (Lynch and Läuchi, 1984). The decrease in K concentration with increasing in Na concentration in irrigation seawater may be due to a possible antagonism between K and Na (Rashid *et al.*, 2004). This antagonism could be due to the direct competition between K and Na at the site of ion uptake at plasma lemma (Epstein and Rains, 1987) and Na was to replace K in the activation of few enzymes which depend on a univalent action (Jeffrey, 1987). In this respect, Flowers *et al.* (1997) pointed out that osmotic adjustment is essential for a plant to survive in saline environments. The increase in Na concentration in

plants with the salinity may be a result of the ability of plants to use Na to maintain an adequate osmotic potential gradient between the plant tissues and the external solution (Glenn, 1987).

Figure (11): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on N, P and K concentration (mg/g dry weight) in shoots of *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting



The influence of salinity on phosphorous accumulation in crop plants is variable and depends upon the plant. In our work the results in Figure (11) indicated that increasing irrigation seawater salinity level promoted a reduction in phosphorous concentration in shoot plant tissues. In general, this close negative relationship between phosphorous and salinity level may be due to the postulation of Greenway *et al.* (1969) who attributed the reduction in phosphorous and its uptake by plants under saline conditions to a decrease in the root absorption potential and to a decrease in the translocation of phosphorous upward through the root as a result of the increase in the osmotic pressure of the root medium. Champagnol (1979) concluded that unlikely chloride and phosphate ions are competitive in terms of plant uptake. However, Papadopoulos and Rendig (1983) concluded that chloride may have suppressed phosphorous uptake and accumulation in tomato shoot. In other cases, reduction in plant phosphorous content may result from reduced activity of phosphorous in the soil solution due to the high ionic strength of the media (Awad *et al.*, 1990). Most of the studies show that salinity reduced phosphorous concentration in plant tissues. Phosphate availability in soil solution is tightly controlled by absorption process and by the low solubility of calcium-phosphorous mineral (Grattan and Grieve, 1994).

Therefore, it is understandable that phosphorous concentration in field grown agronomic crops decreased as salinity increased (Sharpley *et al.*, 1992). The reduction in nitrogen under saline conditions may be due to the suppressive effect of salinity on reducing water uptake (Lea-Cox and Syvertsen, 1993) and/or an increase in chloride uptake and accumulation accompanied by a decrease in shoot nitrate concentration of plants due to the competition between chloride and nitrate which decreases nitrate content (Khan and Srivastava, 1998). This conclusion is supported by Munns and Termaat (1986).

Saline conditions can influence the different steps of nitrogen metabolism, such as an uptake (EL-Nimr, 1986), reduction of protein synthesis (Cramer *et al.*, 1985), ammonium uptake (Hawkins and Lewis, 1993). On the other hand, the adverse effect of salinity on N concentration might be explained on the assumption that the excess Cl in saline water antagonized the uptake of nitrate by the affected plants (Talaat, 1995). Additionally, the reduction in P content under saline conditions (Bolanos *et al.*, 2006) should be explained on the fact that Na salts raised the pH of the soil, which in turn reduced the availability of P to the plant (Sonneveld and Voogt, 1983).

Regarding the effect of AsA, the same Figure revealed that spraying Hibiscus plants with AsA increased significantly nitrogen, phosphorus and potassium concentration in shoots at the two seasons compared with control plants. In addition, AsA (100 ppm) was the most effective on increasing N, P and K concentration. Applications of ascorbic acid help plants to limit toxic ion (Na^+) and increased potassium concentration which plays an essential role in many metabolic processes (Shawky, 2003) and/or might be attributed that increasing P concentration, which might be increase organic acids content excreted from roots into the soil and consequently increases the solubility of most nutrients which may be utilized by plants and/or is synthesized in plants and/or is product of D-glucose metabolism which affects some nutritional cycles activity in higher plants and play an important role in the electron transport system (El-Kobisy *et al.*, 2005) and/or is known as a growth regulating factor which influences many biological processes (Price, 1966) and/or acts as a co-enzymatic reactions by which carbohydrates and proteins are metabolized and involved in photosynthesis and respiration processes (Robinson, 1973). In addition, Hanafy-Ahmed *et al.* (1995) showed that ascorbic acid caused favorable effect on the content of N, P^+ and K^+ in the different faba bean plant organs. Ascorbic acid is mitigating partially or completely the adverse effects of salt stress which may be one aspect of the role of the vitamin C in the activation of some enzymatic reactions (Al-Hakimi and Hamada, 2001) and stabilizing and protecting the photosynthetic pigments and the photosynthetic apparatus from being oxidized (Hamada, 1998). Furthermore, the main function of anti-oxidants is their protective effect of cell membranes and their binding transport proteins (H^+ -ATP-ase membrane pumps), maintained their structure and function against the toxic destructive effect of ROS during stress, in turn more absorption and translocation of minerals (Dicknson *et al.*, 1991) and increased N, P and K contents in leaves (El-Shazly and El-Masri 2003 and El-Gabas, 2006). In

general, these results might be attributed to the effect of AsA producing healthy plants and enhancing the plants to have great ability for elements uptake as well as their roles on regulation ions and may modify the movement of nutrients within the plant tissues and play an important role to enhance the activity of enzymes (Cherki *et al.*, 2002). The interactions between different treatments of seawater irrigation and AsA were almost significant on mitigative partially or completely the adverse effects of salinity stress on ion uptake in plant shoots.

Total soluble sugars and proline:

It is shown from data in Figures (12) and (13) that increasing irrigation water salinity caused a significant increase in total soluble sugars and proline (mg/g dry weight) in plant shoots as compared to control. The lowest values in these parameters were recorded from plants irrigated with the lowest salinity in diluted seawater (10%) in both seasons. The maximum averages were achieved by increasing irrigation water salinity level (40%). These results were similar and statistical in the two seasons of the experiment. In view of the results obtained from this study, it is obvious that increasing irrigation water salinity caused an increase in total soluble sugars and proline (mg/g dry weight) in the shoot compared with control. In response to salinity stress, the wide variety of physiological and biochemical changes occurs in plants (Qasim *et al.*, 2003). Among them, sequestration of salt ions in vacuoles and accumulation of low molecular weight solutes in the cytosol decrease the osmotic potential (Burg *et al.*, 1996 and Jampeetong and Brix, 2009). Decreasing osmotic potential of plant cells may be due to the accumulation of inorganic and organic solutes including mainly proline (Singh *et al.*, 2000), sugars (Kerepesi and Galiba, 2000). In addition, compatible solutes protect cell membranes against destabilization (Jolivet *et al.*, 1983) which dilute salinity and increase salt tolerance (Ashraf and McNeilly, 1990). Sharma *et al.* (1996) concluded that sugars and proline accumulations were considered as a suitable screening parameter for salinity tolerance and it's mediated and concomitant through decline in protein synthesis, N and K concentrations and K/Na ratio.

Figure (12): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on total soluble sugars (mg/g dry weight) in *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting

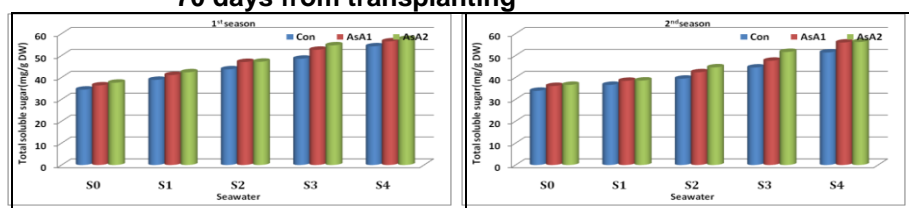
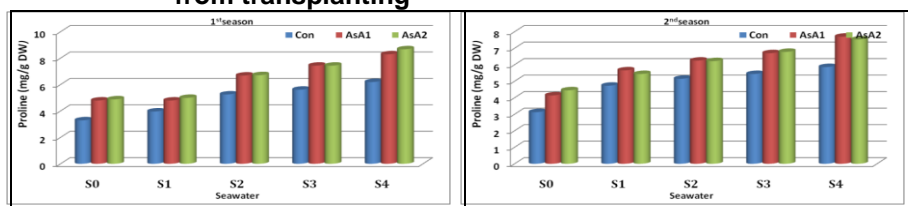


Figure (13): Effect of AsA at 50 and 100 ppm and irrigation by diluted seawater and their interactions on proline (mg/g dry weight) in *Hibiscus rosa-sinensis* L. plant after 70 days from transplanting



Several studies suggest that there is a strong correlation between sugar accumulation and osmotic stress tolerance (Abd-El Baki *et al.*, 2000; Gilmour *et al.*, 2000; Streeter *et al.*, 2001; Taji *et al.*, 2002; Bartels and Sunkar, 2005). Singh (2004) proved that a greater accumulation of sugars lowers the osmotic potential of cells. Another possible role of sugars may be as a readily available energy source. Moreover, Tajdost *et al.* (2007) suggested that the increment in soluble carbohydrates due to salinity may play an important role in increasing the osmotic pressure of the cytoplasm. The increasing of sugars under salinity stress was recognized as a result of starch degradation, sugars synthesis by non-photosynthesis pathways, non-converting of these components to other productions and decreasing of transporting from leaves (Premachandre *et al.*, 1991). The accumulation of carbohydrates might play an important role in increasing the internal osmotic pressure, which is widely regarded as response to salinity stress condition. In addition, the maintenance of the soluble sugars level in leaves could be associated with decreasing growth under salinity. Furthermore, Bartels and Sunkar (2005) found a strong correlation between sugars accumulation and osmotic tolerance. Hence, improvement of crop performance by increasing osmotic potential-adjusting ability might be more significant in increasing plant growth. On the other hand, Trivedi *et al.* (2004) reported that salinity stress decreased total sugars and starch of wheat plant. These reductions may be due to losses in the photosynthetic activity with a drop in the net formation of carbohydrates. Meloni *et al.* (2008) observed a slight but not significant increment in total soluble carbohydrates after salt stress. In general, the increment in soluble components among which total sugars due to saline conditions might, in turn, play an important role in increasing the osmotic pressure of the cytoplasm. In addition, sugars as osmolytes enable plants to keep better water relations under salt stress conditions and sugars concentration may be used as an indicator to the osmoprotectant levels in wheat plant and may contribute to salt tolerance in this system.

Proline is a main osmolyte which accumulates under saline conditions in many plants and it is one of the adaptation mechanisms to salinity and water deficit (Kumar *et al.*, 2003). These results were in agreement with those previously reported by Ashraf and Akhtar (2004), Banu *et al.* (2009), Jampeetong and Brix (2009), Zhao *et al.* (2009), Chelli-Chaabounia *et al.* (2010) and Wang *et al.* (2011). Accumulation of proline

with increasing salinity might be attributed to adapt the strategies by plants to cope up with stress conditions. Proline accumulation in plant tissues may act as antioxidant substances which scavenges active oxygen species by enhancing photochemical electron transport activation (Alia *et al.*, 1991). Floyd and Nagy (1984) suggested that the accumulation of proline might contribute to the detoxification of the active oxygen species. Proline accumulation can be due to an increase in proline biosynthesis, via two different pathways from glutamate or ornithine/arginine (Kavi Kishor *et al.*, 2005), and a decrease in proline degradation. In this investigation, higher accumulation of proline in plant shoots was found with increasing irrigation water salinity. Because proline can provide protection for plants against osmotic stress, oxidative damage and membrane integrity (Ozden *et al.*, 2009 and Pei *et al.*, 2010), proline accumulation is believed to represent an important cellular mechanism for salinity tolerance (Kavi Kishor *et al.*, 2005). Hussein *et al.* (2007) found that among the different parameters responding to NaCl stress, rapid accumulation of free proline within the cell is the most significant one. As increase NaCl stress, endogenous free proline content of *Chrysanthemum morifolium* increased. Also, in cells of *Distichlis spicata* treated with 200 mM NaCl, the cytosolic proline concentration was estimated to be more than 230 mM (Ketchum *et al.*, 1991). More recently, a correlation between the proline accumulation and abiotic stress tolerance has been suggested in different halophytes and cell cultures (Lokhande *et al.*, 2010 and Sekmen *et al.*, 2012). However, in *Hordeum vulgare* L., proline content was not correlated with salt tolerance (Patterson *et al.*, 2009).

On the other hand, spraying plant with AsA at 50 or 100 ppm increased total soluble sugar and proline (mg/g dry weight) in the two growing seasons as compared to the untreated plants. In addition, AsA at 100 ppm gave the highest values of total soluble sugars and proline concentrations as compared with the other treatments. Regarding the interactions, data indicated that total soluble sugars and proline concentrations were significantly increased with increasing salinity level from 10 to 40% (diluted seawater). The maximum increase was recorded for plants grown under 40% diluted seawater. These findings can be related to some earlier studies in which it has been observed that exogenous application of AsA increased total soluble sugars by Talaat (1998) and Agwah (1990) and proline by Skriver and Mundy (1990) and Mattioni *et al.*, (1997). Ascorbic acid plays a role as activator or intermediate in the formation of carbohydrate during photosynthesis. It may be the transformative product of the sugar first found in photosynthesis (Ghourab and Wahdan, 2000). Moreover, Ahmed (2001) reported that ascorbic acid enhances the biosynthesis of carbohydrates and translocation of sugars. In addition, Hammam *et al.* (2001) revealed that ascorbic acid at 25-200 ppm increased the total soluble solids, total and reducing sugars compared with control in mango trees. Silveira *et al.*, (2003) pointed out that proline accumulation was due, at least in part, to the increase in the salt induced proteolysis associated with the increments in the glutamine synthetase activity and hence the increase in the concentration of glutamate precursor in the soluble amino acid pool. Ozdemir *et al.* (2004) reported that accumulation of proline has been considered as a carbon and

nitrogen source for rapid recovery from stress and stabilizer for membranes and some macro molecules and also a free radical scavenger. In the contrary, El-Lithy and El-Greadly (2001) found that ascorbic acid at 100 and 200 ppm had no effect on the sugar content of cantaloupe fruits.

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استجابة نبات الهيبسكس للمعاملة بحمض الأسكوربيك والرئ بماء البحر
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أجريت تجربة أصص بمزرعة ومعمل قسم الخضر والزينة - كلية الزراعة - جامعة المنصورة خلال العامين ٢٠١٠ و ٢٠١١ لدراسة تأثير رش حمض الأسكوربيك بتركيز ٥٠ و ١٠٠ جزء في المليون في تقليل الأثر الضار للرئ بتركيزات مختلفة من مياه البحر (١٠، ٢٠، ٣٠ و ٤٠%) على النمو والمكونات الكيميائية لنبات الهيبسكس.

ولقد أشارت النتائج المتحصل عليها إلى أن رئ النباتات بالتركيز المنخفض من ملوحة مياه البحر (١٠%) قد أدى إلى تحسين من صفات النمو (ارتفاع المجموع الخضرى، عدد الأفرع للنبات، عدد الأوراق، مساحة الورقة، طول الجذر، الوزن الطازج والجاف لكل من المجموع الخضرى والجذرى) ثم حدث نقص تدريجى فى جميع الصفات السابق ذكرها مع زيادة تركيز الملوحة فى مياه الرئ مقارنة بالنباتات التى رويت بمياه غير مالحة (الكنترول). وكانت أكثر النباتات تأثراً تلك التى تم رئها بالتركيز الأعلى من مياه البحر المخففة (٤٠%) فى كلا الموسمين. بالإضافة إلى ذلك أدت زيادة الملوحة إلى نقص فى الصبغات النباتية والنتروجين والبوتاسيوم والفوسفور بينما أدت إلى زيادة البرولين والسكريات الكلية الذاتية.

كان للرئ بحمض الاسكوربيك (٥٠ و ١٠٠ جزء فى المليون) تأثيراً محسناً على جميع صفات النمو السابق ذكرها سواء للنباتات التى رويت بمياه البحر المخففة أو بالمياه غير المالحة. وقد أدى أيضاً رش النباتات بحمض الاسكوربيك إلى زيادة تركيز الصبغات النباتية والنتروجين والبوتاسيوم والفوسفور والبرولين والسكريات الكلية الذاتية مقارنة بنباتات الكنترول (الرئ بدون حمض الاسكوربيك) فى كلا الموسمين.

ولقد اوضحت النتائج أيضاً أن الرش بحمض الاسكوربيك أدى إلى تقليل الأثر الضار للملوحة على صفات النمو والمحتويات الكيميائية لنباتات الهيبسكس التى رويت بمياه البحر المخففة. ولذلك نوصى برئ نباتات الهيبسكس بحمض الاسكوربيك بتركيز ١٠٠ جزء فى المليون عند رئها بمياه البحر المخففة.

قام بتحكيم البحث

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