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Root Yield and Quality of Sugar Beet (*Beta vulgaris* L.) In Response to Ascorbic Acid and Saline Irrigation Water

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Abstract: This study was carried out during winter seasons 2005/2006 and 2006/2007 in the greenhouse of the National Research Center, Dokki, Giza, Egypt in order to investigate the response of growth, root yield and quality of sugar beet (*Beta vulgaris L*) to ascorbic acid (0, 200 and 400 ppm) and irrigation with different concentrations of saline water (control (tap water), 2500 and 5000 ppm). The results indicated that increasing of salt concentration in irrigation water decreased the root length, diameter and fresh and dry weight, while the root weight was increased with moderate saline water (2500 ppm) in comparison to high salinity level or control plants. Sucrose % was less affected up to low salinity, whereas it was slightly increased with high salinity level. TSS% was also increased with high saline water, while the purity % was only increased with well irrigated plants. Proline accumulation was gradually increased by increasing salinity level up to 5000 ppm. Application of ascorbic acid (ASA) as foliar spraying resulted in an increase of all growth characters and also root yield and quality as well as proline accumulation in comparison to untreated plants.Na and K contents in sugar beet juice were less affected by irrigation with saline water and ASA application, however Na and K gradually decreased with high salt concentration and ASA. Antioxidant enzymes glutathione reductase (GR) and ascorbate peroxidase (APX) were increased gradually up to high salinity and ASA application.

Key words: Sugar beet · Ascorbic acid · Salinity · Root quality · Proline · GR · APX

INTRODUCTION

The fresh water resources available for agriculture are declining quantitatively and qualitatively. Therefore, the use of lower quality supplies will inevitably be practiced for irrigation purposes to maintain economically viable agriculture. A biotic environmental stresses among which salt stress is considered as one of the most prevalent are the main cause of yield reduction in plants. In saline environments plants are directly exposed to osmotic stress resulting from a low external water potential induced by high salt concentration in the soil [1]. Therefore, plant show accumulation of osmotically active substances to high levels to create a water potential to facilitate inward water movement, either by uptake of inorganic ions from the external medium or by synthesis of organic solutes [2].

The main effects of salinity also has been attributed to the decrease in soil water potential or the increase of ion concentration in plant tissues to the levels that interfere with metabolism [3]. However, Newmann [4] indicated that the response of plant species to salinity stress vary with salt concentrations, type of salt, length of exposure, variety tested growth stage and development. The decreasing availability of fresh water for agriculture use,while the need for production of food and fuel from plants is increasing, nowadays a problem common to many countries under such conditions of fresh water scarcity, agriculture is forced to use more and more water of poorer quality or saline ones.

To achieve our goals, new technologies and management practices for use saline water in irrigation must be developed and implemented for sustainable production or permanent economic basis. Sugar beet (*Beta vulgaris* L.) ranks the second important sugar crops after sugar cane, producing annually about 40% of sugar production all over the world. In Egypt, it has been a large importance where there are wide newly reclaimed sandy soils at the northern and southern parts of Egypt, that could be cultivated sugar beet without competition with other winter crops due to its tolerance to salinity and ability to produce high sugar yield under saline

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conditions and limited water requirements in comparison with the other traditional winter crops. In recent years, the use of salt tolerance crops has been recognized as a successful method to overcome the salinity problem [5,6,7].

Roads and Loveday [8] indicated that sugar yield in sugar beet was not affected by salinity up to a soil- paste conducting value of 7 dS/m.

Ascorbic acid (Vitamin C) plays an important role in cell growth and division [9]. Moreover, ascorbic acid is the most important hydrophilic antioxidants in higher plants cells that scavenge many types of the damaging free radicals under environmental stresses [10].

Therefore, the main objective of this study was to evaluate the response of root yield and quality of sugar beet (*Beta vulgaris* L.) to ascorbic acid and irrigation with saline water.

MATERIALS AND METHODS

Pot experiments were carried out during the winter seasons 2005/2006 and 2006/2007 in the greenhouse of the National Research Center, Dokki, Giza, Egypt in order to investigate the effect of different concentrations of ascorbic acid (zero, 200 and 400 ppm) on root yield and quality of sugar beet (*Beta vulgaris* L.) plants grown under different levels of salinity (control (tap water), 2500 and 5000 ppm).

Earthenware pots (40cm diameter and 40 cm depth) were filled by 30 kg sand soil and arranged in factorial experiments in complete randomize design with 10 replicates for each treatment. The analysis of soil used was carried out following the methods described by Jackson [11] and data presented in Table 1.

Seeds of sugar beet (Montarosa cv.) mainly obtained from the Sugar Research Institute, Agricultural Research Center, Giza, Egypt were sown at November 20 in the two successive winter seasons. Thinning was practiced at 30 days after planting to leave one plant/pot till harvest. Phosphorus and potassium fertilizers were added before sowing at a rate of 6.0 and 3.0 g/pot of calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48-50% K_2O), respectively. Nitrogen fertilizer was applied as two equal portions at a rate of 0.60 g/pot for each in a form of ammonium nitrate (33.5%N) at 30 and 60 days after planting. At 45 days after planting irrigation with saline water was started. Portable water available in the experimental site was used in order to get water with different salt concentrations. The salt type used in irrigation water was mainly the chloride mixture, which suggested by Strogonov [12], the components of salt mixture are shown in Table 2.

Table 1: Physical and chemical properties of the soil

Properties	1st season (0-30 cm)	2nd season (0-30cm)
Mechanical analysis		
Coarse sand (%)	62.50	65.30
Fine sand (%)	32.30	30.80
Total sand (%)	94.80	96.00
Silt (%)	4.50	3.30
Clay (%)	0.70	0.70
Soil texture	Sandy	Sandy
Chemical analysis		
pH	7.70	7.60
E.C (mmhos/cm^2)	0.11	0.11
Organic matter (%)	0.50	0.50
Calcium carbonate (%)	3.20	3.40
Total N (ppm)	40.00	37.50
Available P (ppm)	4.80	3.90
Cations (meq/100 g soil)		
Ca^{+2}	2.50	2.30
Mg^{+2}	4.00	3.50
Na^{+}	1.54	1.74
Anions (meq/100 g soil)		
HCO_3^-	1.72	1.60
Cl^-	0.70	0.70

Table 2: The components of salt mixture used for chloride stalinization

% of total salts contents				
$MgSO_4$	$CaSO_4$	NaCl	$MgCO_3$	$CaCO_3$
10	1	78	2	9
% of specific anions from total				
Na^+	Mg^{++}	Ca^{++}	SO_4^-	Cl^-	CO_3^-
38	6	6	5	40	5

At 45 days after planting (DAP) sugar beet plants were subjected to irrigation with two levels of salt concentrations till harvest (2500 and 5000 ppm) in addition to tap water (served as control). At the same time of irrigation with saline water, plants were sprayed with different concentrations of ascorbic acid (0, 200 and 400 ppm). The irrigation whether with tap water or saline water must reach the level of 65% of the total maximum holding capacity of the soil by weighting the pots and the needed amount of water was added and after three consecutively irrigations with saline water, extra amount of tap water was added for leaching purpose.

At 90 DAP proline accumulation (µ mole/g fresh weight) in fresh leaves was estimated according to Bates *et al.* [13]. At the same time, a representative leaves samples were taken and immediately deep frozen for estimation the activity of antioxidant enzymes Glutathione Reductase (GR) and Ascorbate Peroxidase (APX). The extraction of the antioxidant enzymes GR and APX were determined, 5 g. of frozen leaves tissues were homogenized in pre- chilled mortar in presence of 10 ml of 50 mM potassium phosphate buffer (pH 7) with 1% (w/v) insoluble polyvinylpyrrolidone (PVP) and 0.1 mM EDTA. The extraction procedures were repeated twice and supernatants were pooled, raised to a certain volume, referred as crude enzyme extract, all operations were carried out at - 4 ^{0}C for further analysis. The activity of GR was determined according to the method described by Zanetti [14], and APX activity according to Nakano and Asada [15]. The activity was expressed as change in the optical density per gram fresh weight per minute under the experimental conditions.

At harvest time, the following criteria were recorded, root yield/plant (g), root length (cm), root diameter (cm), fresh and dry weights of top (g). Sucrose % was determined according to Le-docte [16], total soluble solids (TSS %) were measured in fresh roots using hand refractmeter, juice purity % was also determined as a ratio between sucrose % and TSS % according to Carruthers and Oldfield [17]. Sodium and potassium % were determined in the juice by using Flame photometry. Sugar yield (g/plant) was calculated as follows:

Sugar yield (g/plant) = Root yield (g) × Sucrose % × purity %.

The obtained data were statistically analyzed as factorial experiments in complete randomize design according to Snedecor and Cochran [18] and the combined analysis was done according to Steel and Torrie [19], the treatments means were compared using LSD test and 5% of probability.

RESULTS

Data presented in Table 3 indicated that the root diameter, fresh and dry weight of sugar beet foliage were significantly decreased by irrigation with saline water (2500 and 5000 ppm) in comparison with the treatment received normal irrigation (control plants), however the root length was not significantly affected under the same condition of irrigation with saline water. The reduction of both fresh and dry weights estimated by 4.82, 38.49, 10.34 and 21.17% when salt concentration was increased in irrigation water from zero to 2500 and/or 5000 ppm, respectively.

Root yield (g/plant) was also significantly affected by increasing salinity levels in irrigation water, however the plants irrigated with moderate saline water (2500 ppm) produced the highest root yield (301.89) in comparison with the treatments received the highest salinity level (5000 ppm) or the treatment received tap water. Such increases in root yield under moderate saline water estimated by 9.65 and 17.28% over the control plants and the treatment received high saline water (5000 ppm), respectively.

Sugar yield (g/plant) was decreased with increasing salinity levels in irrigation water, however irrigation with moderate saline water resulted in an increase of sugar yield in comparison with high saline water or control plants, but the differences between them don't reach to significant level (Table 3).

Data presented in Table 3 also indicated that the sucrose percent in sugar beet juice was less affected due to irrigation with different concentration of saline water, the plants exposed to moderate saline water produced the same values of treatment received well water (control), however, the sucrose % was only increase to 19.33% when the plants irrigated with high salinity. Total soluble

Table 3: Effect of irrigation with saline water on root yield and quality of sugar beet

Salinity (ppm)	Root length (cm)	Root diameter (cm)	Fresh top weight (g)	Dry top weight (g)	Root weight (g)	Sucrose (%)	Sugar yield (g)	TSS (%)	Purity (%)	Proline (µ mole/g FW)
Zero	23.33	6.04	217.25	38.36	275.32	18.65	44.70	21.31	0.87	4.44
2500	24.25	6.58	206.78	34.60	301.89	18.69	45.95	22.51	0.83	8.30
5000	24.33	5.63	133.63	30.24	257.41	19.33	41.23	23.40	0.83	13.85
LSD 5%	NS	0.37	24.52	4.69	35.23	--	NS	0.57	0.02	1.42

Table 4: Effect of Ascorbic acid on root yield and quality of sugar beet

Ascorbic (ppm)	Root length (cm)	Root diameter (cm)	Fresh top weight (g)	Dry top weight (g)	Root weight (g)	Sucrose (%)	Sugar yield (g)	TSS (%)	Purity (%)	Proline (µ mole/g FW)
Zero	20.42	5.59	135.87	29.60	242.79	18.65	37.50	22.18	0.84	5.48
200	23.08	6.04	194.18	34.43	267.29	19.55	44.49	22.54	0.86	8.47
400	28.42	6.62	227.79	39.16	324.54	18.62	49.89	22.49	0.83	12.63
LSD 5%	2.59	0.37	24.52	4.69	35.23	--	5.51	NS	0.02	1.42

solids (TSS %) was gradually increased by increasing salt concentration in irrigation water, while the purity % was less affected under the same conditions and the differences between moderate and high salinity were insignificant, only the control plants recorded the highest purity %.

In this regard, the proline accumulation in fresh green leaves was more accumulated and significantly increased due to using moderate or high salinity in irrigation water in comparison with the control plants. The highest proline accumulation in the green leaves resulted in by using high saline water and the lowest with well watered.

Concerning, the application of ASA (Vitamin C) as foliar spraying significantly increased the all studied traits (Table 4). Root length, diameter, weight and also the fresh and dry foliage were gradually increased by increasing the ASA concentration from 0-200 and/or 400 ppm. The maximum fresh, dry weights as well as root weight were recorded by using high ASA concentration, such increases estimated by 67.65, 32.30 and 33.67%, respectively.

In this regard, sucrose per cent was only increased by the application of 200 ppm ASA in comparison with the treatments received 400 ppm or untreated plants (control). However, sugar yield (g/plant) was increased gradually up to high ASA level. The highest sugar yield (49.89) was recorded at the treatment received 400 ppm followed by 200 ppm (44.49), while the lowest (37.50) with control plants.

Data presented in Table 4 also indicated that TSS % was not significantly affected by ASA application and also the purity % seem to be less affected under the same conditions. Proline accumulation in the green leaves was significantly increased up to high ASA application resulted in the highest proline content, while the lowest was recorded with untreated plants (Table 4).

Data presented in Fig 1, 2 indicated that root length, fresh weight of foliage were significantly affected by the interaction between salinity levels x ASA concentrations. In general, increasing salt concentration up to 5000 ppm in irrigation water increased the root length and decreased fresh weight of sugar beet foliage when it received high ASA spraying. However, the lowest fresh weight was obtained by moderate salt concentration x unspraying with ASA, respectively.

Table 5: Effect of interaction between salinity levels and ascorbic acid on Na and K contents of sugar beet juice

Salinity (ppm)	Ascorbic (ppm)	Na (%)	K (%)
Zero	0	0.53	0.21
	200	0.39	0.15
	400	0.52	0.21
2500	0	0.51	0.20
	200	0.47	0.20
	400	0.49	0.19
5000	0	0.52	0.20
	200	0.47	0.16
	400	0.42	0.14

Figure 3 also indicated the well irrigated water produced the highest sugar yield (54.88) as plants received high ASA concentration followed by high saline water (48.31) under the same ASA spraying. However, well irrigated plants produced the highest purity per cent in sugar beet juice as plants received 200 ppm ASA (Fig. 4).

In this regard, the proline accumulation, in general it was increased gradually up to high ASA application with well irrigated plants and either irrigated by moderate and high saline water. The highest proline accumulation (22.35) was recorded as plants irrigated with high salinity and ASA levels (Fig. 5).

Concerning, the Na and K percentages in the sugar beet juice, data in Table 5 show that the Na and K contents were less affected due to irrigation with saline water or ASA application. Na and K contents were only gradually decreased by increasing ASA concentration up to 400 ppm when plants irrigated with high saline water.

Data presented in Table 6 indicated that the antioxidant enzymes Glutathione Reductase (GR) and Ascrobate Peroxidase (APX) were gradually increased by increasing salinity and ASA levels. In general, the plants received normal irrigation produced the lowest GR and APX contents, while using moderate or high salt concentration in irrigation water resulted in an increase of

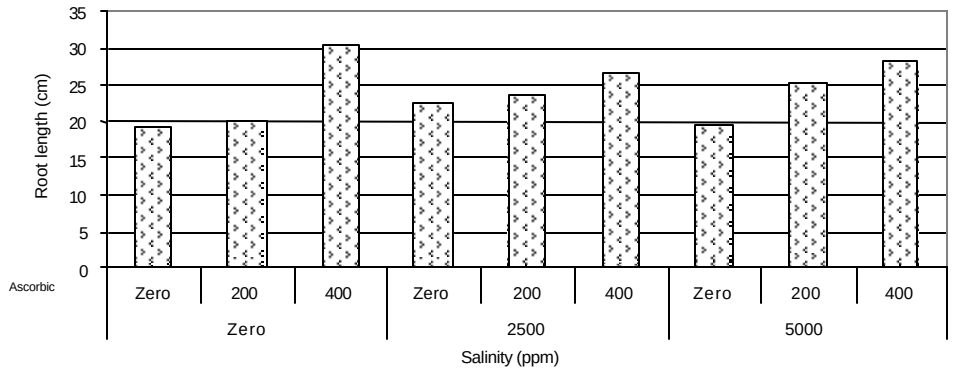

Fig. 1: Effect of irrigation with saline water and ascorbic acid application on root length (cm) of sugar beet plants

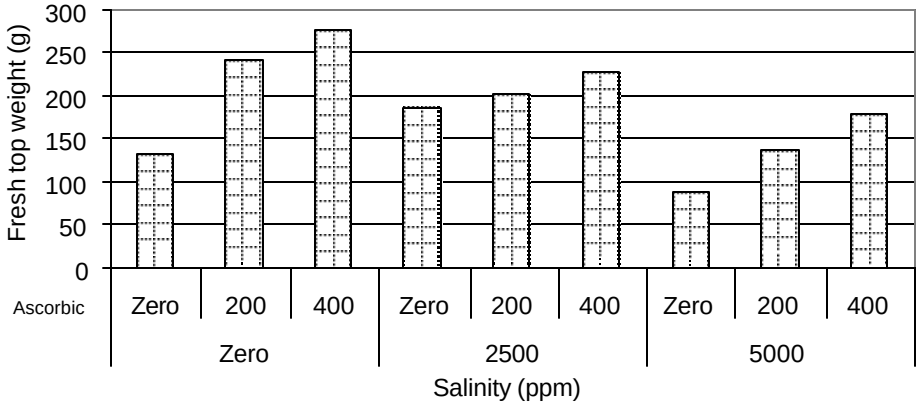

Fig. 2: Effect of irrigation with saline water and ascorbic acid application on fresh top weight (g) in sugar beet plants

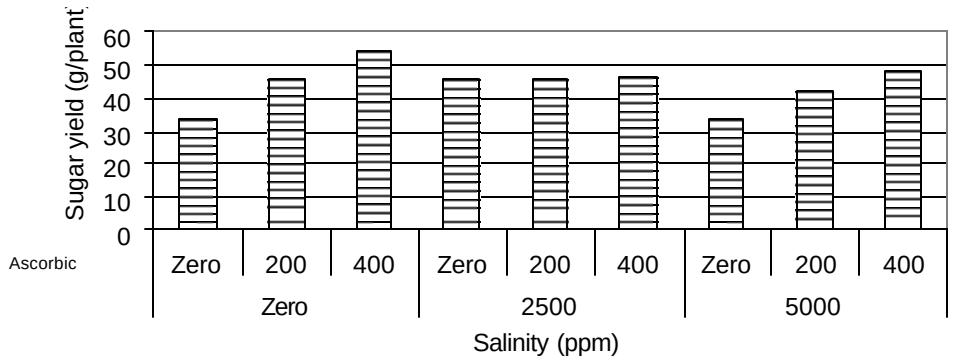

Fig.3: Effect of irrigation with saline water and ascorbic acid application on sugar yield (g /plant) of sugar beet plants

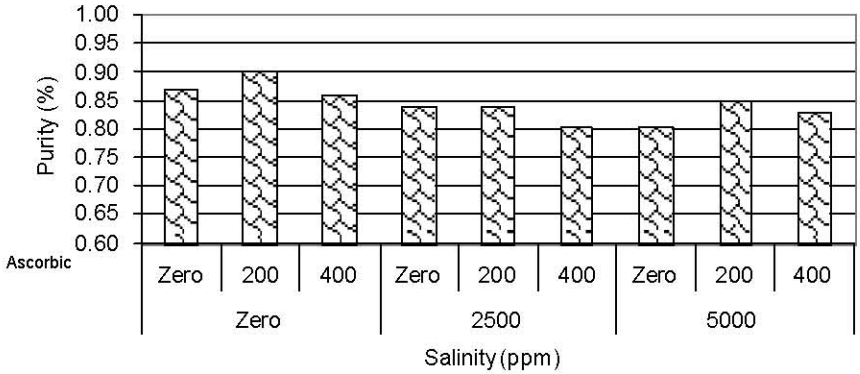

Fig. 4: Effect of irrigation with saline water and ascorbic acid application on purity % in sugar beet juice

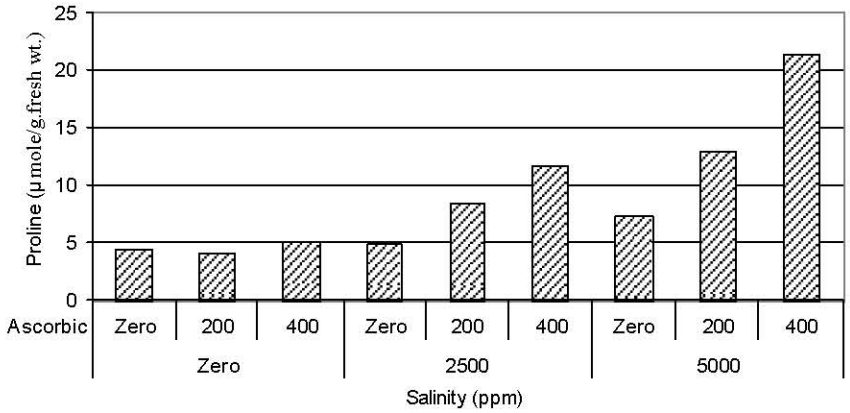

Fig. 5: Effect of irrigation with saline water and ascorbic acid application on proline accumulation of green leaves of sugar beet plants

Table 6: Glutathione Reductase (GR) and Ascrobate Peroxidase (APX) in response to irrigation with saline water and spraying with ascorbic acid

Salinity (ppm)	Ascorbic (ppm)	GR (n mole/g. fresh wt)	APX (µ mole/g. fresh wt.)
Zero	0	160.77±10.23	1.77±0.08
	200	180.87±6.70	1.96±0.09
	400	194.27±10.23	2.14±0.07
2500	0	174.17±3.87	1.87±0.06
	200	241.16±10.24	2.37±0.07
	400	279.11±11.82	2.79±0.12
5000	0	200.96±7.73	2.19±0.08
	200	288.05±10.23	3.11±0.15
	400	294.74±13.40	3.46±0.09

* Values are means of three replicates ± SE

both antioxidant enzymes, thereby the highest GR (294.74±13.397) and APX (3.46±0.091) were recorded as plants received high saline water and ASA spraying.

DISCUSSION

The present study indicated that, in general some growth characters namely root diameter and fresh and dry top weight of sugar beet plants were significantly affected by irrigation with saline water, increasing salt concentration in irrigation water decreased the both traits. The reduction in these traits due to high irrigation saline water mainly attributed to the enhancing Na^+ uptake, which cause ion excess in plant tissues [2, 20]. Moreover, Schwarz and Gale [21] pointed that prolonged saline stress caused a reduction in available assimilates which lead to growth reduction. This may be also attributed to the lowest plant growth which it's adversely effects of a specific ion concentration exceeds their thresholds and become toxic. Salts may also reduce plant growth by reducing the water potential or by interfering with nutrient uptake. These results are also in agreement with those obtained by Mekki and El Gazzar [6] and Ahmed [22]. Salinity increases the amount of work necessary to

counteract osmotic and ionic stresses for normal cellular maintenance, as a consequence, these are less energy left for growth requirements [23].

Our results are shown in Table 3 also indicated that using moderate saline water increased the root diameter and foliage fresh and dry weight in comparison with the treatment received high salinity level, this means that the sugar beet plants seem to be more tolerant under low level of salinity, but a harmful effect was detected due to increasing salinity to high level, this due to the increase in Na and Cl under high salt stress caused a reduction in the activity of CO_2-fixation in photosynthesis and decrease the enzymatic activity such as inhibition of chlorophyllase enzyme activity which is known in the metabolic processes [22,24].

In general, the plant biomass is dependent absolutely on the growth of plants; the stress caused by the ions concentrations allows the water gradient to decrease, making it more difficult for water and nutrients to move through the root membrane [25]. In this concern, accumulation of salts in the root zone affect plant performance through creation of water deficit and disruption of ions homeostasis [26], which in turn cause metabolic dysfunctions. For instance, high saline concentrations relation to other salts (Table 2) disrupt root permeability to ions by displacing calcium in the plasma membrane [27].These results were also confirmed by Mekki and El Gazzar[6] and Lingle *et al.* [28].

In this regard, the increase in root weight (g/plant) was also increased under moderate salinity level by 9.6 and 12.28% over the control plants and high salinity level, respectively. Such increase in root weight was attributed to the increase in total biomass under the same saline water condition and these findings are previously confirmed by Farley and Draycott [29] and Kandil [30]. Our results are shown in Table 3 show that sucrose % was increased with high salt stress up to 19.93%, while, no changes were noticed in that per cent with either moderate salinity or control treatments. The increase in sucrose % with high salinity level is mainly due to the increase in total carbohydrates accumulation under the same condition of salinization. The increase in total carbohydrates with increasing salinity was reported by Munns and Termat [1]. Also, Kandil [30] found that sucrose % was increased by using salt mixture of 50.2% NaCl, 6.6% $Na_2 SO_4$, 30.4% Ca SO_4 and 12.8% Mg Cl_2. On contrast, the reductions in sugar yield with high saline water resulted from the increase in TSS % and also lead to decrease Juice purity %. On the other word, the increase in TSS % with high salinity level is mainly due to high concentration of solutes. Wyse *et al.* [31] stated that sink tissues commonly store high concentrations of osmotically active compounds, this is particularly true for plants such as sugar beet and sugar cane in these tissues, turgor regulation is necessary to avoid very high cell turgor. Maintenance of cell turgor is an importance to salt or water stress [32]. This means that our obtained data indicated that under high saline irrigation water, the uptake of Na and K (Table 5) was decreased in juice and consequently decreased the total impurities in root juice. Similar findings were reported by Mekki and El-Gazzar [6] and Lingle *et al.* [28], they pointed that higher concentration of other salts increased either uptake of K in the roots or its accumulation in the juice.

In general, the phenomenon of free proline accumulation in plants exposed to diverse environmental stresses has considerable eco-physiological significance. Proline has also been known to accumulate in the leaves of many higher species subjected to salt stress [33]. The present data in Table 3 show that proline was more accumulated in green sugar beet leaves when it exposed to high saline water. In this concern, Delauney and Verma [34] pointed that the level of proline overproduction during salt stresses is assumed to be very important because it is recognized that it influences not only the osmotic potential but also minimizes the effect of salt damage. Also, the accumulation of proline was reported to serve as nitrogen storage compound and protecting of cellular structure [35].

In view to the effect of ASA application, the obtained results in Table 4 indicated a gradually increase in root growth and root weight due to increasing ASA concentration, this means that ASA fulfills many key functions in the plant biology as well as it participate in the regulation of mitosis and cell expansion [36,37]. Ascorbate is also a substrate for key enzymatic reactions, for example in the production of ethylene [38]. The increase of foliage biomass and root weight in sugars beet plants, mainly due to the effective role of ASA application. These results could be explained by the finding of Aberg [39], who reported that ascorbic acid could be involved the main metabolic processes, especially with energy transfer coenzymes of carbohydrates metabolism and improved photosynthetic activity. Similar finding was also reported by Gamal El Din [40] on sunflower plants.

In this regards, the sugar yield was also gradually increased by increasing ASA application, this means that the increase in carbohydrate metabolic and improving its translocation is reflected to the increase in sugar content and also sugar yield (Table 4). Data also showed that the proline accumulation was increased due to ASA application; this means that the ASA is a key compound of plant antioxidant system and it has a number of other physiological roles. It is a factor for violaxanthin deopoxide and hydroxylase enzymes involved the synthesis of hydroxyl porline and hydroxylysine [9, 41].

Concerning, the Na and K contents in sugar beet juice, in general Na and K were gradually decreased by increasing ASA and salinity levels (Table 5). The decrease of Na and K in sugar beet juice is very desirable for decreasing the total impurities, and then the quality was increased. However, K^+ is one of the inorganic solutes that play a key role in osmotic adjustment. Plants accumulate high levels of K^+ in cells for maintaining the osmotic equilibrium under salt stress, Na^+ competes with K^+, thereby plants can't accumulate potassium [42, 43]. On the other hand, the enhancement of mineral content in carton plants due to ASA application was also reported by Eid and Abou - Leila [44].

Our results presented in Table 6 indicated that, in general using the highest salt concentration in irrigation water produced an increase in both antioxidant enzymes (GR and APX) when plants were sprayed with high ASA concentration. Other studies pointed that the antioxidant metabolites as ascorbate and glutathione are present in chloroplasts in very high concentration [45,46] and apart from their obvious role as enzyme substrate, that can react chemically with almost all forms of activated O_2 [47]. Foyer *et al.* [48] stated that over expression of GRase in chloropiasts doubled the concentrations of ascorbate and glutathione in leaves and conferred increased resistance to oxidative stress, where it complete the ascorbate glutathione cycle by regenerating the reduction of glutathione using NADPH which in turn stabilize the ascorbate pool and increase the plant tolerance [46, 49]. According to this results drought and salt stress caused a decrease in the content of reduced glutathione, which is the main lipid soluble antioxidant of plant cells, while the increments of these antioxidant enzyme activities helps the plant to destroy H_2O_2 and maintenance the ascrobate pool which in turn to elevate the plant tolerance to salt stress and this is required for the proper protection of cells against photo inhibition [48].

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