

**URGING COTTON PLANTS TO OVERCOME THE SALT STRESS CONDITIONS
IN THE RECLAIMED LAND USING A COMBINATION OF
INNOVATIVE AGRICULTURAL FACTORS**

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ABSTRACT

The possibility of inducing cotton plants towards better growth and yielding under reclaimed soil with salinity level of about 7000 ppm was investigated in El-Tahrir Province, El-Behera Governorate, Egypt, during the two successive seasons of 2009 and 2010. For verifying this aim, the plants produced from calcium paste treated-seeds of cotton (seeds covered with the mixture of calcium nitrate, humic acid and wheat bran at the ratio of 1:1:3, respectively by weight) were sprayed with ascorbic acid at the rates of 0 (control), 200, 400 and 600 mgL⁻¹. Significant positive influences of calcium paste applied solely or in combination with all ascorbic acid applications were observed on growth traits (number of leaf plant⁻¹, total leaf area plant⁻¹ and dry weight of leaf plant⁻¹), chemical constituents (photosynthetic pigments, some photosynthates, some macro-and micronutrients) and yield (seed cotton yield plant⁻¹ and feddan⁻¹ as well as lint%) in comparison with the control (without calcium paste and ascorbic acid). As for ascorbic acid, plants sprayed with all studied rates represented significant increases in all aforementioned parameters with the best results obtained from the rate of 400 mgL⁻¹ as compared with untreated plants. Thereon, it has been recommended that spraying cotton plants (cultivar Giza 90), produced from calcium paste treated-seeds, with 400 mgL⁻¹ ascorbic acid solution may overcome the adverse conditions of reclaimed soils particularly, salinity up to 7000 ppm and consequently, economic yield is obtainable.

Key words: *ascorbic acid, cotton, reclaimed land, salinity, stress.*

1. INTRODUCTION

Cotton (*Gossypium barbadense* L.) is one of the most important fiber crops in the world due to its properties and uses. In Egypt, it is well known that the cultivated area is continually on the decrease. Thus, experimental attempts of the enlargement in and/or render a judgement on cultivating cotton crop in success must be done in new reclaimed soils with some assistant factors beside, its cultivation in the old soils to face the world's increasing demand of Egyptian cottons. It is worth mentioning that most of the new reclaimed areas in Egypt are salinity affected soils. Saline conditions disrupt several physiological processes in plants leading to a general reduction in growth and yield (Greenway and Munns, 1980, El-Saidi, 1997, Qadir and Shams 1997). The drastic influence of salinity on plant growth and metabolism was attributed, principally, to the enhanced Na⁺ uptake which causes ion excess in plant tissues (Abbas *et al.*,

1991, Ella and Shlaby 1993 and Leidi and Saize 1997) beside, the inhibition of K⁺, Ca²⁺ and NO₃⁻ uptake by plant roots (Maas, 1986 and Gorham and Bridges 1997). In addition, it is well established that salinity stress damages plant cells through production of reactive oxygen species including superoxide, hydrogen peroxide, hydroxyl anions and singlet oxygen (Scandalios, 1997). Efforts have been made to control salinity by technological means; reclamation, drainage, use of high leaching fractions and application of soil amendments (Abdel-Naby *et al.*, 2001). On the other hand, some trials have been made to alleviate the disturbances in plant metabolism excreted by salinity stress by using natural and safety substances among them ascorbic acid which may help to overcome some of these inhibitory effects (Rady and El-Sawah, 2009) beside, humic acids.

Ascorbic acid is an important antioxidant defense in plant cells (Foyer and Halliwell, 1976)

to protect them by scavenging the reactive oxygen species. It also stimulates respiration activities, cell division and many enzyme activities (Rautenkranz *et al.*, 1994). It has synergistic effects on growth, yield and its components as well as chemical composition of several crops under favorable and unfavorable environmental conditions *i.e.*, salinity (Rady and El-Sawah, 2009). It has been proved that the application of humic acid, as an organic soil amendment used either individually or in combination with others, resulted in a significant increase in crop yield and its components in the sandy soils through its improving hydrophysical properties and nutrient availability (Osman and Ewees, 2008). Elevated Ca^{++} concentrations in the nutrient solution mitigated the adverse effects of salinity by inhibition of Na^+ uptake (Greenway and Munns, 1980). LaHaye and Epstein (1969) clearly postulated that the $\text{Ca}^{++}/\text{Na}^+$ interaction takes place at the plasma lemma. They suggested that Na^+ acted by displacing Ca^{++} from membranes, leading to increased membrane permeability and intracellular Na^+ concentrations. After our preliminary pot studies, calcium nitrate, humic acid and wheat bran at the ratios of 1:1:3 by weight, respectively as the components of calcium paste were found to be the most significant in ameliorating salinity effects on cotton plants at a level not more than 7000 ppm (data not shown). Therefore, the mentioned components of calcium paste and the salinity level of 7000 ppm were used in this study.

Accordingly, this work was planned for studying the influence of seed cotton application with calcium paste then, foliar application of the produced plants with ascorbic acid on growth, some chemical constituents and yield of cotton plants (cultivar Giza 90) grown under saline reclaimed soil conditions.

2. MATERIALS AND METHODS

During the two successive summer seasons of 2009 and 2010, a field trial was carried out at El-Tahrir Province, El-Behera Governorate, Egypt, to realize the aforementioned aim. Before sowing, soil samples to 30 cm depth from the experimental site were collected and analyzed by the standard procedures of Jackson (1967). Analysis results of the soil samples are presented in Table (1).

Seeds of cotton (cultivar Giza 90) obtained from the Agricultural Research Center, Giza, Egypt were sown (20 cm between hills) on March 21, 2009 and 2010. Thinning was done 30 days after sowing leaving two plants hill⁻¹. A seasonal

total of 200, 250 and 100 kg feddan⁻¹ calcium superphosphate (15.5% P_2O_5), ammonium nitrate (33.5% N) and potassium sulphate (48% K_2O) were applied as recommended, respectively. treatments comprised 4 ascorbic acid rates; 0 (control), 200, 400 and 600 mgL⁻¹. These treatments were applied alone or in combination with seed treatment with calcium paste.

Table (1): Physical and chemical properties of the selected site in both seasons.

Property	2009	2010
Physical:		
Clay%	24.70	23.50
Silt%	22.10	23.90
Sand%	53.20	52.60
Soil texture	Loamy sand	Loamy sand
Chemical:		
pH (1:2.5)	7.900	7.750
ECe (dS m ⁻¹)	10.930	10.970
Organic matter%	1.080	1.100
CaCO ₃ %	6.050	6.090
Total N%	0.070	0.074
Available nutrients (mg Kg⁻¹ soil):		
K	70.15	72.10
P	19.05	18.80
Fe	06.52	06.10
Mn	05.45	05.00
Zn	01.15	01.05
Cu	01.60	01.75

2.1. Method of seed treatment with calcium paste

2.1.1. Preparation of calcium paste

Calcium nitrate, humic acid and wheat bran (a by-product of wheat grain) at the ratio of 1:1:3 (by weight), respectively were mixed and kneaded together by using Arabic Gum solution (8%) as a sticking agent to obtain paste of calcium able to remaining around seeds for a long time.

2.1.2. Treatment of seeds with calcium paste

Before sowing, cotton seeds were covered with calcium paste through the better mixing between them. Treated seeds were allowed to dry.

Chemical analysis of the most important components of humic acid and wheat bran was determined (Table 2) as outlined by A.O.A.C. (1995).

2.2. Method of ascorbic acid application

Ascorbic acid at the above mentioned concentrations was sprayed on the plant shoots to run off, three times; 40, 60 and 80 days after sowing. Few drops of Tween-20 were added to the spraying solution as a wetting agent.

The experimental design used was a split-plot with four replicates. The main plots were calcium paste treated-seeds or untreated seeds and ascorbic

acid treatments occupied the sub-main plots. The Experimental unit was 21 m² (3 x 7m). The cultural practices were applied as recommended for cotton production except for the variables under study.

2.3. Growth characters

Three months after sowing, plants of four hills were randomly chosen from each experimental unit for determining the number of leaf plant⁻¹, total leaf area plant⁻¹ (dm²) and dry weight of leaf plant⁻¹ (g).

2.4. Chemical constituents

Leaf of the plants submitted to vegetative growth traits were also subjected to chemical. Chlorophyll a, chlorophyll b and total carotenoids were extracted by acetone (80%) then, their concentrations were determined (mg g⁻¹ fresh leaf) using colorimetric method as described by Arnon (1949). Ascorbic acid was determined (mg g⁻¹ fresh leaf) using the dye 2,6-dichlorophenol indophenol method as outlined by A.O.A.C. (1995). Total soluble sugars were colorimetrically determined (mg g⁻¹ dry matter) using phosphomolybdic acid reagent according to Dubois *et al.* (1956). Free proline was extracted by 5-sulphosalicylic acid (3%) then, determined (mg g⁻¹ dry matter) colorimetrically using acid ninhydrin reagent as outlined by Bates *et al.* (1973). Nitrogen was colorimetrically determined (mg g⁻¹ dry matter) by using the Orange G dye according to the method of Hafez and Mikkelsen (1981). For P, K, Ca, Na, Fe, Mn and Zn determinations; the wet digestion of 0.1 g of fine dry material of leaf of each treatment was done with sulphuric and perchloric acids mixture as mentioned by Piper (1947). Phosphorus (%) were colorimetrically estimated using chlorostannus molybdo-phosphoric blue color method in sulphuric acid system as described by Jackson (1967). Potassium and sodium (%) were determined using a Perkin-Elmer, Flame Photometer (Page *et al.*, 1982). Calcium (%), iron, manganese and zinc (ppm) were determined using a Perkin-Elmer, Model 3300, Atomic absorption Spectrophotometer (Chapman and Pratt, 1961).

2.5. Seed cotton yield

At harvest, plants of the four hills were randomly marked in each experimental unit to determine seed cotton yield hill⁻¹ (g) and lint percentage (% of seed cotton). Seed cotton yield feddan⁻¹ (kentar) was determined using all plants of each experimental plot.

2.6. Statistical analysis

All data obtained in both seasons of this study were subjected to the analysis of variance. LSD at

0.05 was used to differentiate means according to Snedecor and Cochran (1980). Test of homogeneity, commonly known as the Bartlett's test was made according to Gomez and Gomez (1984).

3. RESULTS AND DISCUSSION

3.1. Growth characters

Number of leaf plant⁻¹, total leaf area plant⁻¹ and leaf dry weight plant⁻¹ presented in Table (3) were significantly increased by 29.0, 36.2 and 29.4%, respectively in the plants produced from seeds treated with calcium paste as compared with the parameters of the plants of the untreated seeds (combined data). These improving effects on studied parameters may be due to that Ca²⁺ of calcium paste reduced the harmful effects of salinity on seeds and consequently positively reflected on germination and emergence percentages. In addition, it has been explained in a way that root hairs of plants were able to maintain high levels of membrane-associated Ca²⁺ when exposed to high concentrations of NaCl (Cramer *et al.*, 1985; Jafri and Ahmed 1995; Reinhardt and Roast 1995 a and b). In fact, it is essential to have Ca⁺ of selective permeability *i.e.*, membrane integrity (Poovaiah and Reddy, 1993). This helps in improving growth traits through improving plant growth. In addition, the mechanism of humic acid as one of calcium paste components on stimulating growth may be similar to that of plant growth regulators as humic substances function as auxins and thus affect plant metabolism in a positive manner (Osman and Ewees, 2008). Humic acid improves chemical properties of the soil because it increases soil microorganisms which enhance nutrient cycling positively reflecting on photosynthesis and growth (Sayed *et al.*, 2007). It also promotes plant growth by its effects on ion transfer at the root level by activating the oxidation-reduction state of the plant growth medium and so increased absorption of nutrients especially, micronutrients by preventing precipitation in the nutrient solution. Furthermore, it enhances cell permeability, which in turn makes more rapid entry of nutrients into root cells and so results in higher uptake of plant nutrients. This effect was associated with the function of hydroxyls and carboxyls in these compounds (Osman and Ewees, 2008). Wheat bran as one of calcium paste components is able to maintain the water (Table 2) in rhizosphere and consequently dilution of salinity around the seeds. Regarding the results of this study, increasing macro-and microelements (N, P, K, Ca, Fe, Mn

and Zn) as shown in Tables (6-8) and the decrease in Na⁺ (Table 7) induced by seed treatment with calcium paste led to the significant increase in growth characters.

All sub-main treatments; ascorbic acid at the rates of 0, 200, 400 and 600 mgL⁻¹ (Table 3) revealed a gradual significant increase in all studied vegetative growth traits, *i.e.*, number of leaf plant⁻¹, total leaf area plant⁻¹ and leaf dry weight plant⁻¹ which was observed as a result in raising ascorbic acid rate gradually applied up to 400 mgL⁻¹. The rate of 600 mgL⁻¹ ascorbic acid proved to slightly decrease the mentioned parameters as compared with the rate of 400 mgL⁻¹. The maximum results were recorded with ascorbic acid foliar application at the rate 400 mgL⁻¹. This rate surpassed the results of water foliar spray (the rate of zero mgL⁻¹ ascorbic acid) by 27.7, 33.9 and 27.6% for the number of leaf plant⁻¹, total leaf area plant⁻¹ and leaf dry weight plant⁻¹, respectively (combined data). These results indicate that the most pronounced counteracted effects of the studied soil salinity (Ca 6000 ppm) on vegetative growth traits under study existed by the exogenous application of ascorbic acid which led to the increase in endogenous level of this substance (Table 5) and consequently protect plant cells including the photosynthetic apparatus by scavenging reactive oxygen species (Zhang and Schmidt, 2000) thus, vigorous plant growth will be obtained under salinity stress. In this connection, Elade (1992) stated a positive action for antioxidants especially, ascorbic acid on growth and attributed this finding to their effects on counteracting drought, salinity and disease stresses and protecting plant cells against free radicals responsible for plant senescence as well as to their auxinic action and consequently enhancing growth characters. In addition, ascorbic acid might regulate cell wall expansion, cell division and cell elongation through its action in cell vacuolarization (Cordoba-Pedregosa *et al.*, 1996), improves the nutritional status (Tables 6-8) and absorbing phenolic compounds which lead to save the growing tissues from toxic effects of the oxidized phenols (Gupta *et al.*, 1980) and/or enhances the biosynthesis of soluble sugars (Table 5). These findings are in coincidence with those obtained by Ahmed *et al.* (1998); Rady and El-Sawah (2009).

The effect of the interactions between the main treatments (untreated or treated seeds with calcium paste) and the sub-main treatments (ascorbic acid foliar application) was significant. The highest values of the number of leaf plant⁻¹,

total leaf area plant⁻¹ and leaf dry weight plant⁻¹ were recorded from the treatment of 400 mgL⁻¹ ascorbic acid under seed treatment with calcium paste which recorded 21.4, 29.2 and 24.0% increases, respectively as compared to the treatment of zero mgL⁻¹ ascorbic acid under seed treatment with calcium paste and recorded 70.1, 87.5 and 69.1% increases, respectively above the treatment of zero mgL⁻¹ ascorbic acid under untreated seeds (combined data). The superiority of the treatment having the highest values might come from improving the nutritional status of the plants of this treatment (Tables 6-8), the shortage of Na⁺ (Table 7) and the increase in total soluble sugars and free proline (Table 5) saving more osmotic solutes which enable plant cells to maintain more water against salinity.

3.2. Chemical constituents

3.2.1. Leaf photosynthetic pigments, soluble sugars, free proline and ascorbic acid

Results in Tables (4 and 5) reveal that chlorophyll a, chlorophyll b, total carotenoids, total soluble sugars, free proline and ascorbic acid in plant leaf were significantly increased by 29.8, 32.1, 30.6, 19.4, 23.6 and 19.3%, respectively as a result of treating the seeds with calcium paste as compared with the untreated seeds (combined data). These pronounced increments may be due to the increase in ascorbic acid in plant leaf (Table 5) which had an auxinic action and also synergistic effects on biosynthesis of sugars and carbohydrates (Al-Qubaie, 2002). Besides, the effective role of Ca⁺⁺ and wheat bran in Na⁺ driving away and more water retention around seeds, respectively. In addition, humic acid (as one of the calcium paste components) enhanced the chlorophyll content reflecting from its role in enhancing leaf nutritional status (Tables 6-8) especially, N as an important part of chlorophyll molecule. Moreover, humic acids led to uptake K element leading to a corresponding increase in the chlorophyll fluorescence which can serve as an indicator of stress induced accompanied with alteration of the endogenous hormone balance (Marschner, 1995).

Our results indicated that chlorophyll a, chlorophyll b, total carotenoids, total soluble sugars, free proline and ascorbic acid concentrations in plant leaf, were gradually increased by significant quantities as a result of foliar application with ascorbic acid up to 400 mgL⁻¹ then negligibly decreased (combined data, tables 4 and 5). The previous characters were increased by 23.5, 26.3, 27.8, 31.3, 28.3 and 26.3%, respectively with spraying ascorbic acid at

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Table (2): The most important constituents of humic acid and wheat bran used in the experiment of both seasons.

Humic acid analysis			Wheat bran analysis		
Main constituent	2009	2010	Main constituent	2009	2010
Humic acid%	18.5	18.5	Moisture%	4.50	4.30
Total Fe%	0.5	0.5	Ash%	5.20	5.15
Total Mn%	0.5	0.5	Total fibers%	35.45	36.25
Total Zn%	0.5	0.5	Water holding capacity (g g ⁻¹)	3.35	3.40
Total Cu%	0.5	0.5			

Table (3): Influence of calcium paste and ascorbic acid on some growth traits of cotton plants under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B
	Number of leaves plant ⁻¹			Total leaves area plant ⁻¹ (dm ²)			Dry weight of leaves plant ⁻¹ (g)		
0	27.4	38.4	32.9	33.7	48.9	41.3	16.2	22.1	19.2
200	32.2	41.9	37.1	39.9	55.2	47.6	18.9	24.2	21.6
400	37.3	46.6	42.0	47.3	63.2	55.3	21.5	27.4	24.5
600	37.0	45.8	41.4	45.9	60.0	53.0	20.9	26.8	23.9
Mean A	33.5	43.2		41.7	56.8		19.4	25.1	
LSD_{0.05} A	3.6			4.7			2.7		
B	2.5			3.4			1.8		
A×B	5.6			6.7			3.9		

+Ca=Seed covered with calcium paste (Calcium nitrate: Humic acid: Wheat bran 1:1:3 by weight).

0Ca=Seed without calcium paste.

A denotes Ca treatments, B denotes ascorbic acid treatments and A×B denotes the interaction.

Table (4): Influence of calcium paste and ascorbic acid on leaf photosynthetic pigments of cotton plants under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca+2	+ Ca+2	Mean B	0 Ca+2	+ Ca+2	Mean B	0 Ca+2	+ Ca+2	Mean B
	Chlorophyll a (mg g ⁻¹ fresh weight)			Chlorophyll b (mg g ⁻¹ fresh weight)			Total carotenoids (mg g ⁻¹ fresh weight)		
0	0.73	0.96	0.85	0.48	0.66	0.57	0.31	0.41	0.36
200	0.81	1.05	0.93	0.53	0.72	0.63	0.34	0.45	0.40
400	0.91	1.19	1.05	0.63	0.81	0.72	0.40	0.51	0.46
600	0.89	1.15	1.02	0.61	0.77	0.69	0.39	0.50	0.45
Mean A	0.84	1.09		0.56	0.74		0.36	0.47	
LSD0.05 A	0.11			0.07			0.05		
B	0.08			0.05			0.03		
A×B	0.17			0.10			0.08		

the rate of 400 mgL⁻¹ as compared with the treatment free from ascorbic acid (tap water). The promotive effect of ascorbic acid on chlorophylls and carotenoids and the other components under study might be attributed to the enhancing effects of this antioxidant on the nutritional status of cotton plants (Tables 6-8). N is one of the essential chlorophyll components. Besides, Fe and Mn are necessary for the biosynthesis of chlorophyll and Zn is necessary for the biosynthesis of tryptophan which is the precursor of auxin biosynthesis and consequently more biosynthesis of these components in the face of cell elongation. Furthermore, the role of ascorbic acid as an antioxidant, directly involved in the regulation and protection of photosynthetic processes (Farago and Brunhold, 1994) could lead to the enhancing effect on pigments and the components under study. This treatment having the highest values of the constituents under study bestowed the tested plants the ability to satisfactorily overcome soil salinity. This may be due to their containing sufficient amount of soluble sugars, free proline (Table 5) which form sufficient cellular solutes able to sustain of cell turgor. This, maintains metabolic activities in the plant cells and/or protect plants against adverse conditions *i.e.* salinity and drought. The enhancing effect of ascorbic acid on tested soluble sugars might be attributed to its promotive effect on studied pigments (Table 4) leading to the enhancement of photosynthesis and consequently, the increase in the photosynthates. The positive effects of ascorbic acid on pigments and photosynthates obtained in this study are in agreement with those obtained by Rady and El-Sawah (2009).

The results of the combination between treated or untreated seeds with calcium paste and ascorbic acid foliar application exhibited in Tables (4 and 5) revealed that the combination between calcium paste treated-seeds and spraying plant shoots with ascorbic acid at 400 mgL⁻¹ increased chlorophyll a, chlorophyll b, total carotenoids, total soluble sugars, free proline and ascorbic acid by 24.0, 22.7, 24.4, 29.5, 27.1 and 28.0%, respectively as compared to calcium paste treated-seeds interacted with foliar spray with tap water, and by 63.0, 68.8, 64.5, 59.7, 59.6 and 52.2% when compared with the combined treatment of zero rate ascorbic acid under calcium paste untreated-seeds it scored increments, respectively. These significant increases scored as a result of the application with Ca⁺⁺, wheat bran as a fibrous source having high water holding capacity and ascorbic acid. The

former may reduce the harmful effects of salinity by its taking the place of Na⁺ in rhizosphere and may on membranes in absorbing roots. The second; wheat bran saves more water to overcome drought caused by salinity in the soil. The latter; ascorbic acid as one of antioxidants prevent enzyme inactivation, prevent the generation of more dangerous radicals and allow flexibility in the production of photosynthetic assimilatory power. Moreover, electron transfer to O₂ prevented over reduction of electron transports chain, which reduced the risk of harmful back reaction within the photosystem (Foyer *et al.*, 1990). In addition, Elade (1992) proved that most antioxidants were responsible for accelerating the biosynthesis of various pigments and consequently more photosynthesis producing more quantities of photosynthates. Besides, Shahidi and Wanasundara (1992) stated that, phenolic antioxidants play important roles as free radical terminators and sometimes, as a metal chelators.

3.2.2. Macro-and micronutrients

It could be stated from the combined data in Tables (6-8) that N, P, K, Ca, Ca/Na ratio, Fe, Mn and Zn were significantly increased with the treatment of calcium paste covered-seeds as compared with the treatment of calcium paste untreated-seeds. These increases were 23.4, 40.0, 25.7, 22.8, 82.9, 13.5, 10.8 and 14.0% for N, P, K, Ca, Ca/Na ratio, Fe, Mn and Zn, respectively. On the other side, Na showed a reversed behavior. It decreased with increasing other elements at 34.7% in plant leaf. The shortage occurred in Na⁺ and the increase in Ca⁺⁺; the increase in Ca/Na. In addition, the high water retention capacity of wheat bran (Table 2) and having some minerals such as Fe, Mn, Zn and Cu of humic acid (Table 2) might explain these results. Humic acid as one of the calcium paste components plays an important role for increasing the supplying power of soil capacity against nutrient loss and deficiency. The principal physiological function of humic acids may be that they reduce oxygen deficiency in plants, which results in better uptake of nutrients (Osman and Ewees, 2008).

The data presented in Tables (6-8) reveal that when Na significantly reduced, all tested nutrients represented significant gradual increases with increasing ascorbic acid rate. Spraying of ascorbic acid at the rate of 400 mgL⁻¹ proved to be the best and exhibited, in general, the most pronounced counteracted effect on soil salinity. The treatment of 400 mg L⁻¹ ascorbic acid surpassed the treatment of tap water by 19.1, 36.7, 21.4, 25.3,

Table (5): Influence of calcium paste and ascorbic acid on some leaf photosynthates of cotton plants under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B
	Total soluble sugars (mg g ⁻¹ dry weight)			Free proline (mg g ⁻¹ dry weight)			Ascorbic acid (mg g ⁻¹ fresh weight)		
0	20.6	25.4	23.0	0.47	0.59	0.53	0.69	0.82	0.76
200	23.8	28.1	26.0	0.53	0.67	0.60	0.78	0.92	0.85
400	27.5	32.9	30.2	0.60	0.75	0.68	0.87	1.05	0.96
600	27.0	31.5	29.3	0.58	0.71	0.65	0.97	1.18	1.08
Mean A	24.7	29.5		0.55	0.68		0.83	0.99	
LSD _{0.05} A	3.3			0.08			0.10		
B	2.2			0.06			0.07		
A×B	4.8			0.11			0.14		

Table (6): Influence of calcium paste and ascorbic acid on some macronutrients of cotton leaves under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B
	Nitrogen (% dry weight)			Phosphorus (% dry weight)			Potassium (% dry weight)		
0	1.88	2.42	2.15	0.24	0.36	0.30	1.88	2.41	2.15
200	2.06	2.58	2.32	0.29	0.41	0.35	2.08	2.61	2.35
400	2.31	2.80	2.56	0.34	0.47	0.41	2.32	2.89	2.61
600	2.29	2.74	2.52	0.32	0.44	0.38	2.27	2.83	2.55
Mean A	2.14	2.64		0.30	0.42		2.14	2.69	
LSD _{0.05} A	0.23			0.05			0.23		
B	0.15			0.04			0.16		
A×B	0.34			0.07			0.32		

Table (7): Influence of calcium paste and ascorbic acid on calcium, sodium an Ca/Na ratio of cotton leaves under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B
	Calcium (% dry weight)			Sodium (% dry weight)			Calcium/Sodium ratio		
0	0.90	1.07	0.99	1.20	0.70	0.95	0.75	1.53	1.14
200	0.96	1.20	1.08	0.99	0.63	0.81	0.97	1.90	1.44
400	1.10	1.37	1.24	0.79	0.56	0.68	1.40	2.46	1.93
600	1.09	1.31	1.20	0.82	0.59	0.71	1.33	2.22	1.78
Mean A	1.01	1.24		0.95	0.62		1.11	2.03	
LSD _{0.05} A	0.13			0.09			0.16		
B	0.08			0.06			0.11		
A×B	0.20			0.12			0.23		

Table (8): Influence of calcium paste and ascorbic acid on some micronutrients of cotton leaves under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B
	Iron (ppm)			Manganese (ppm)			Zinc (ppm)		
0	416	464	440	246	274	260	166	188	177
200	437	495	466	263	292	278	181	204	193
400	466	535	501	285	315	300	199	229	214
600	459	527	493	280	311	296	198	225	212
Mean A	445	505		269	298		186	212	
LSD_{0.05} A	36			26			22		
B	23			17			15		
A×B	48			34			30		

69.3, 13.9, 15.4 and 20.9% for N, P, K, Ca, Ca/Na ratio, Fe, Mn and Zn, respectively. Contrary, Na reduced up to 28.4%. Ascorbic acid increased the tolerance of cotton plants, especially at the rate of 400 mgL⁻¹ under studied soil salinity. This reflected on improving vegetative growth traits (Table 3), photosynthetic pigments (Table 4), photosynthates among them endogenous ascorbic acid (Table 5) and seed cotton yield (Table 9), surely reflected also on stimulating the nutritional status of plants. These results are supported by the results of Ahmed and Abd El-Hameed (2004),

shoots with 400 mgL⁻¹ ascorbic acid solution. This treatment scored 15.7, 30.6, 19.9, 28.0, 60.8, 15.3, 15.0 and 21.8% for N, P, K, Ca, Ca/Na ratio, Fe, Mn and Zn, respectively compared with the treatment of Ca⁺⁺ past treated seeds under tap water spray. Contrary, Na decreased down to 20.0%. In addition, the treatment of Ca⁺⁺ past treated seeds under 400mg L⁻¹ ascorbic acid granted increases at 48.9, 95.8, 53.7, 52.2, 228.0, 28.6, 28.0 and 38.0% for the same parameters, respectively as compared with the treatment of the interaction of calcium paste untreated-seeds under

Table (9): Influence of calcium paste and ascorbic acid on seed cotton yield and lint% of cotton plants under saline reclaimed soil conditions (Data are combined across seasons).

Ascorbic acid (mgL ⁻¹)	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B	0 Ca ⁺²	+ Ca ⁺²	Mean B
	Seed cotton yield hill ⁻¹ (g)			Seed cotton yield feddan ⁻¹ (kentar)			Lint (% seed cotton)		
0	13.7	42.2	28.0	2.62	8.01	5.32	32.2	36.0	34.1
200	19.4	48.7	34.1	3.68	9.25	6.47	32.7	36.2	34.5
400	40.1	58.2	49.2	7.66	11.04	9.35	34.0	37.4	35.7
600	39.4	57.1	48.3	7.48	10.83	9.16	33.5	37.0	35.3
Mean A	28.2	51.6		5.36	9.78		33.1	36.7	
LSD_{0.05} A	4.3			0.80			3.1		
B	3.0			0.54			NS		
A×B	6.4			1.18			4.6		

Rady and El-Sawah (2009) who reported that the effect of antioxidants, especially ascorbic acid on producing healthy plants leads to enhancing the plants to have a great ability for uptake of elements. Moreover, Gonzalez-Reyes *et al.* (1994) concluded that ascorbate free radical caused hyper polarization of plasma membranes, and this energization could then facilitate transport processes across such membranes. Most of the previous results are consistent with those of Ali (2000), Rady and El-Sawah (2009).

The data presented in Tables (6-8) show that the best treatment in which plant leaf collected the highest amounts of nutrients, (except with the reverse regarding Na,) was covering the seeds with calcium paste interacted with spraying plant

tap water, whereas Na reduced up to 53.3%. The increments obtained from the above mentioned best treatment may be explained with the increasing value of Ca/Na ratio which reveal that Ca⁺⁺ behave when takes out Na⁺ at significant amounts from rhizosphere and consequently from plant leaf. (Table 7) reflected on increasing nutrients in plants. Besides, the water imbibing properties of wheat bran fibers (Table 2), and their ability to absorb water and/or organic compound (Mongeau and Brassard, 1982) support the rhizosphere by water to face drought and salinity. The acidity of humic acid facilitate more solubility and absorption of nutrients. As for ascorbic acid, Wise and Naylor (1987) stated that antioxidants such as ascorbate, glutathione and α-tocopherol

are directly correlated with the ability to defend plant cells against oxidative damage resulting from salinity stress and consequently producing healthy plants having a great ability for nutrients uptake.

3.3. Seed cotton yield

Combined data in Table (9) indicate that seed cotton yield hill⁻¹ and feddan⁻¹ as well as lint % were significantly increased by 83.0, 82.5 and 10.9% as a result of covering the seeds before sowing with calcium paste, respectively as compared with calcium paste untreated seeds. The improvement in seed cotton yield and lint % may be due to the existence of Ca⁺⁺ to take the place of Na⁺ in the rhizosphere and in plant leaf as shown in Table (7). This may be positively reflected on plant growth (Table 3) and leaf photosynthetic pigments (Table 4) as well as macro- and microelements (Tables 6-8) and other components, such as total soluble sugars and free proline (Table 5) as osmotic substances and consequently the increase in seed cotton yield and lint %. Moreover, the better water retention capacity of wheat bran (Table 2) and humic acids partially capable to retain water and nutrients (Osman and Ewees, 2008) in the rhizosphere led to overcoming the harmful effect of salinity. Raviv (1998) stated that humic acids may exert direct enzymatic or hormonal effects on plant growth and yield.

The sub-main treatment of ascorbic acid foliar application at the rate of 400 mgL⁻¹ gave the highest increases for seed cotton yield hill⁻¹ and feddan⁻¹ as compared with the other ascorbic acid rates. These increases were significant comparing with the rates of 0 (control) and 200 mgL⁻¹ whereas, they were insignificant as compared to 600 mgL⁻¹. Estimation of Lint% revealed insignificant values among all ascorbic acid rates. The increases in seed cotton yield hill⁻¹ and feddan⁻¹ scored at 400 mgL⁻¹ ascorbic acid solution were 75.7 and 75.8%, respectively as compared to the treatment of zero rate ascorbic acid. The improving effect of ascorbic acid on seed cotton yield was mainly attributed to its positive action on enhancing growth traits (Table 3), photosynthetic pigments of plant leaf (Table 4), cellular solutes *i.e.* total soluble sugars and free proline (Table 5) for sustenance of cells turgor leading to maintenance of metabolic activities in plants and plant nutritional status (Tables 6-8). In this respect, Al-Qubaie (2002) stated that ascorbic acid as an antioxidant compound has an auxinic action and also synergistic effect on the biosynthesis of carbohydrates and controlling the

incidence of most fungi on plants. This stimulates them and reflects on seed cotton yield. Besides, the induced effect of ascorbic acid as one of vitamins on growth and yield may be due to that vitamins are recognized to be coenzymes involved in specific biochemical reactions in plants such as oxidative and non-oxidative decarboxylations (Robinson, 1973). The results regarding the beneficial effect of ascorbic acid on seed cotton yield are confirmed with those reported by Rady and El-Sawah (2009).

The interaction between treated or untreated seeds with calcium paste and ascorbic acid foliar application at various rates, had a significant effect on seed cotton yield hill and feddan and in significant effect on lint % Table (9). The highest values were obtained with the application of 400 mgL⁻¹ ascorbic acid. This treatment increased seed cotton yield / hill and feddan as well as lint % by 3.9 %, respectively by 324.8, 321.4 and 16.1%, respectively as compared to zero mgL⁻¹ ascorbic acid under untreated seeds with calcium paste. This favorable yielding may be due to the positive combined effect of calcium, wheat bran and humic acid. The former has an antagonistic effect to the harmful effects of Na⁺, the second has a high percentage of fibers (Table 2) having several physiological effects, depending upon the physical and chemical properties among them, the ability of fibers to retain water and to bind organic compounds (Schneeman, 1986) deluting salinity concentration and saving acidity effect with the latter; humic acid in rhizosphere and consequently more solubility and absorption of nutrients by plant roots.

In conclusion, within the experimental conditions studied, it has been concluded that the present results gave an evidence to the role of the calcium paste components; Ca⁺⁺ has an effective role in Na⁺ driving out from saline rhizosphere, wheat bran has a high water holding capacity due to its high contents of fibers to save more water in the rhizosphere against salinity and humic acid which increases useful soil microorganisms for plants, function as auxins affecting plant metabolism in a positive manner and acts as chelating agent through hydroxyls and carboxyls as active groups for micronutrients and water molecules. Thus, minimizes the loss of nutrients by leaching. Besides, it is considered as a storehouse with easily mobile or available to uptake by plant roots, and in turn is positively reflected on growth and yield through overcoming the harmful effects of soil salinity. In addition, the role of ascorbic acid as an antioxidant, especially at the concentration

of 400 mg L⁻¹, in inducing salinity tolerance of cotton plants cultivated in salt-affected reclaimed soils.

It has been recommended that cultivation of cotton crop can be accomplished in saline reclaimed soils possess salinity level up to 7000 ppm with using some assistant factors. They are covering the seeds before sowing with calcium paste (mixture consists of calcium nitrate, humic acid and wheat bran at the ratio of 1:1:3 by weight, respectively). Then, spraying the produced plants with ascorbic acid at the rate of 400 mgL⁻¹ three times at 40, 60 and 80 days after sowing.

4. REFERENCES

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دفع نباتات القطن للتغلب على ظروف الإجهاد الملحي في الأراضي المستصلحة باستخدام توليفة مستحدثة من العوامل الزراعية

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ملخص

أجرى هذا البحث بمديرية التحرير- محافظة البحيرة-مصر، خلال موسمي 2009 و2010 بهدف دراسة تأثير تغليف بذور القطن بعجينة الكالسيوم (مخلوط مكون من نترات الكالسيوم، حامض الهيوميك، وردة القمح بنسبة 1:1:3 بالوزن على الترتيب) ورش النباتات الناتجة بحامض الأسكوربيك بتركيز صفر (كنترول)، 200، 400، و600 ملجم/لتر على إمكانية تحسين النمو والقدرة المحصولية لنباتات القطن النامية تحت ظروف التربة المستصلحة المتأثرة بالأملاح (حوالي 7000 جزء في المليون).

لوحظ حدوث تأثيرات إيجابية معنوية لمعاملة البذور بعجينة الكالسيوم قبل الزراعة بمفردها أو مع جميع معاملات حامض الأسكوربيك على صفات النمو (عدد الأوراق/نبات، المساحة الكلية للأوراق/نبات، والوزن الجاف للأوراق/نبات)، المكونات الكيماوية (صبغات البناء الضوئي، بعض نواتج التمثيل الغذائي، وبعض المغذيات الكبرى والصغرى)، والمحصول (محصول القطن الزهر/نبات، محصول القطن الزهر قنطار/فدان، ونسبة القطن الشعر) وذلك مقارنة بالكنترول (المعاملة الخالية من عجينة الكالسيوم وحامض الأسكوربيك).

أظهرت النباتات التي رُشَّت بجميع تركيزات حامض الأسكوربيك (200، 400، و600 ملجم/لتر) زيادة معنوية في جميع القياسات (صفات النمو، المكونات الكيماوية والمحصول)، وكانت أفضل النتائج المتحصل عليها هي نتيجة رش النباتات بتركيز 400 ملجم/لتر مقارنة بالنباتات التي عُولمت رشاً بالماء.

يمكن في ضوء تلك النتائج إستنتاج أن رش نباتات القطن (صنف جيزة 90) الناتجة من بذور عُولمت قبل زراعتها بعجينة الكالسيوم وحامض الأسكوربيك بتركيز 400 ملجم/لتر، ربما تُمكن النباتات من التغلب على الظروف المعاكسة للأراضي المستصلحة حديثاً خاصةً الملحية حتى تركيز 7000 جزء في المليون، وبالتالي إمكانية الحصول على محصول إقتصادي في ظل هذه الظروف.

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