



GROWTH AND MINRAL STATUS OF COTTON PLANTS AS AFFECTED BY ABSICISIC ACID AND SALT STRESS

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Abstract:

Application of antioxidant materials like abscisic acid to alleviate salinity stress and promote cotton growth is high effectiveness target, whereas cotton plant is an attractive industrial crop. Pot experiment was conducted to evaluate the effect of salinity stress and abscisic acid (antioxidant materials to alleviate salinity stress) on cotton growth and macro nutrients status in shoots of cotton plants. Plants subjected to two salinity levels (2500 and 5000 ppm as diluted sea water), and tap water (250ppm) as control, sprayed abscisic acid (ABA) with two concentrations (20 and 40 ppm of ABA) and distilled water as a control. Salinity decreased stem and leaves dry weight compare to the control treatment. The lower concentrations of ABA (20 and 40 ppm as a foliar spray) improve dry weight of stem and leaves. Reversely, leaves/stem ratio decreased with both concentrations of the abscisic acid. The increment in dry weight of leaves and stem or their sum showed its higher values by application 40 ppm from ABA under the 5000 ppm salinity level and also under fresh water treatment but under the 2500 ppm treatment the highest values were by 20 ppm of growth regulator. Nevertheless, L/S ratio decreased by ABA treatment, whereas, the high concentration of ABA (40ppm) was super than lower concentration (20ppm) under both salinity levels. Generally, it can be used diluted seawater in irrigation of cotton plant with spraying abscisic acid to alleviate the harmful effect of salinity.

Keywords: Cotton (*Gosypiumbarbadense* L)-Salinity-Abscisic; Acid-Growth-Macronutrients Status.

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1. Introduction

Salinity is one of the major constraints that limit crop productivity in many important agricultural areas. It is anticipated that about 50% of arable lands of the world will be affected by salt stress by 2050 (Blumwald and Grober 2006). Soil salinity adversely affects crop productivity and agricultural sustainability in many areas of the world, especially in arid and semi-arid regions (Abdel latef 2010). Plant growth can be inhibited by high salt concentrations through osmotic

stress, nutritional imbalance, and specific ion toxicity (Munns and Tester 2008). Also irrigation by saline water led to adverse effects on soil and plants grown in it (Hussein et al., 2014 & 2015 and Min et al., 2017).

ABA plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins which create cellular dehydration tolerance in field crops (Luan 2002). This suggested that ABA is helpful to regulate stomata movement under normal conditions due to changes in transpiration under non stressed conditions, or that ABA's role is magnified after the soil drying has led to the very low transpiration (Hussain et al., 2013). Water stress increases accumulation of ABA in leaves (Davies and Jones, 1991). It is well documented that ABA acts as a stress signal, which triggers adaptive changes in physiology and morphology of plants. In addition to these functions in leaves, ABA plays an important regulatory role in root systems. Root growth is usually less inhibited than shoot growth under water deficit conditions (Sharp et al., 2004).

ABA is known to play an important role in enhancing plant water use efficiency under environmental stress. ABA exhibits both fast effects (modulation of ion flows resulting in stomatal closure) and relatively long-term effects on gene expression pattern (Jamalian et al., 2013). There are numerous processes that stimulate the development and growth of plants. Such processes are continuously governed by the hormones released by plants (known as phytohormones). Out of five characteristic phytohormones, one is ABA which helps in controlling many development and growth characteristics of plants such as leaf abscission, inhibition of fruit ripening, etc. ABA is commonly known as the "stress hormone" that responds to variety of environmental stresses including both biotic and abiotic stress (Zhang, 2012).

It is known that the growth inhibition and the adverse effects induced by salinity can be alleviated by many useful things such as fertilizers and water management, depending on plant species, salinity level, and environmental conditions (Tuna et al., 2007 and Hussein et al., 2017). Salt stress induced ABA accumulation in maize root tissues was compared with that in leaf tissues. While salt stress with NaCl resulted in a significant ABA accumulation in root tissues (up to 10-fold), the same stress only led to a small ABA accumulation in leaf tissues (Jia et al., 2002). Under saline conditions mutual effects of ions on their absorption are of particular interest. Ions in high concentration in the external solution (i.e. Na, Cl or Ca) are taken up at high rates, which may lead to excessive accumulation in the tissue (Gadallah, 1996).

These ions may inhibit the uptake of other ions into the root (i.e. K) and their transport to the shoot through the xylem, eventually leading to a deficiency in the tissue. Thus, there is the potential for many nutrient interactions in salt stressed plants which may lead to important consequences for growth. Salt stress inhibits the uptake and transport of K and Ca (Lynch J and Läuchli, 1984). High salinity, however, has ion specific effects in addition to osmotic effects, i.e. ion toxicity, nutrient imbalance or a combination of these factors (Lüchli and Epstein, 1990). Evidence of toxic effects of salt on plant membranes have been well documented (Leopold, 1984). Role of ABA in salt response may consist in: (1) PI signaling and Ca²⁺ release due to EF1 α regulation and cytoskeletal reorganization, (2) increase in cell wall resistance due to changes in hemicelluloses, cellulose, and lignin synthesis, (3) cytoskeleton remodeling due to interactions with ABP and GAPDH, (4) ROS scavenging by antioxidant enzymes (GST, CAT, and POX), (5) chromatin relaxation due to changes in HAC2, (6) translation regulation due to improved mRNA stabilization, (7) dehydration

tolerance, mainly due to synthesis of LEA proteins in RER, and (8) osmoprotectant synthesis (DMSP, betaine). The regulated metabolic pathways are marked with bold arrows (Szypulska, et al., 2017). Hypothetical model of ABA's influence on salt tolerance in higher plants. The all of the examined parameters were low in SS (stressed seedlings), but the stressed sprouts had a characteristic morphology. The roots of stressed seedlings had more root hairs than control roots. The inhibition of seedling growth under ABA pretreatment was manifested by primary root growth (by around 48%). Despite the above, changes in the length of coleoptiles or dry weight were not observed in the ABAS sample. However, the average fresh weight of sprouts in ABAS was approximately 28% higher than in SS (Szypulska, et al (2017).

In addition, many attempts have been made to overcome this disorder, including proper management and exogenous application of plant growth regulators. There is significant evidence that ABA acts as the root-to-shoot stress signal. A study has demonstrated that ABA contributes to the increase of xylem water potential as well as water uptake to the plant in the presence of salt (Fricke et al., 2004).

Therefore, this work aimed to evaluate the growth and mineral status of cotton plants responses to exogenous application of ABA and salt stress.

2. Materials and Methods

A pot experiment was conducted in the greenhouse of the National research centre at Dokki, Cairo Egypt during the winter growing season 2016-2017 (April - month) using clay loamy soil. soil was taken from Giza governorate and its particles size distribution was as follows, 7.0 coarse sand, 21.1% fine sand, 35.4% silt and 36.4% clay. The soil chemical analysis was as follows as pH 7.82, EC (dSm^{-1}) 1.3 and 0.5, 7.2, 3.1, 2.2, 0.4, 9.4 and 3.2 (m eqL^{-1}) for potassium, sodium, calcium, magnesium and chloride, respectively.

Polyethylene pots (50 × 35 cm) were filled with 30 kg soil. Five uniform seeds of cotton (*Gossypium barbadense L.*) were sown in April, 1st, 2016/2017 winter season; plants were thinned twice, the 1st at one week after sowing and the 2nd two weeks later to leave three plants /pot. Calcium super phosphate (15.5 % P_2O_5) and potassium sulfate (48.5 % K_2O) in the rate of 3.0 and 1.50 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot was added in two equal portions, the 1st after two weeks and the 2nd two weeks later. Three water salinity levels i. e. tap water (250 ppm), 2500 and 5000 mg/L, where, the two higher salinity treatments were obtained by diluting Red Sea water (Table 1) and used for irrigation by maintaining the levels of 70% of field capacity. Abscisic acid was sprayed at the rate of 20 and 40 ppm and sprayed twice, 21 and 36 days after sowing, respectively. The control plants were sprayed by distilling water.

The cotton plants were harvested after 60 days of sowing. All samples were dried in 70°C⁰ oven for 48 h. and weighed to determine absolute growth rate. After being collected, the ground leaf sample materials were wet digested with H_2SO_4 in the presence of H_2O for analysis of total N by the micro-Kjeldahl method (Bremner and Mulvaney, 1982), K, Ca, P and Na were determined as described by (cotton et al 1982).

Data collected were subjected to the proper statistical analysis with the methods described by (Gomez and Gomez1984).

Table 1: The chemical analysis of irrigation water used in the experiments.

EC (ppm)	Soluble cations (meq/L)				Soluble anions (meq/L)		
	Ca	Mg	Na	K	HCO ₃ +CO ₃	Cl	SO ₄
250	1.1	0.7	2.0	0.1	0.1	1.2	1.0
2500	13.0	9.3	16.7	2.0	1.5	27	12.5
5000	18.0	15.1	42.0	3.5	1.9	55	22.4

3. Results and Discussion

Growth Response: Data in Table 2 revealed that generally, high salinity level negatively affected growth parameters. Regardless the effect of ABA, salinity treatment especially the highest level decreased stem, leaves weight. Significant increments were noticed when stems and leaves plants were subjected to the high level of salinization (5000 ppm). For instance, the reductions in stems (g/plant) were 18.6 and 26.0 % and the reductions in leaves yield were 21 and 28.7 % in two higher saline water compared with control (fresh water), respectively. These results are well supported by those published by several authors concerning the effect of salinity on tuber and leaves yields of table beet plants (Kandil, et al., 1999 ; Mekki and El-Gazzar1999 and El-Etreiby,2000).The high salinity level used sharply decreased chlorophyll a, chlorophyll b, carotenoids and total chlorophyll concentrations in comparable with that irrigated regularly with fresh water (Hussien et al., 2017). The depressive effect of salinity on bulb and leaves yield is probably due to osmotic inhibition of water absorption, accumulation of certain ions in high concentration in plant tissues and alteration of the mineral balance of plants (Khafagi and El-Lawandy, 1996), and/or due to the reduction in photosynthetic activity and carbohydrates metabolism (Heuer and Plaut, 1989).The suppression of shoot growth under salinity may either be refer to osmotic reduction in water availability or to excessive accumulation of ions, known as specific ion effect.(Abdel Latef2010 and Marschner1995).

The decrease in leaves and stems accumulation is mainly due to increase in Na⁺ and Cl under high salt stress causing a reduction in the activity of CO₂ –fixation during photosynthesis and a decrease in the enzymatic activity of the metabolic processes. Significant increase in leaves and stems yields of cotton plants applied with ABA application. ABA application caused growth enhancement at all salinity levels. Possibly due to the beneficial effect of ABA on the physicochemical properties affecting plant growth. ABA plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins which create cellular dehydration tolerance in field crops (Luan, 2002).

Leaves yield of cotton was responded well to ABA application, which increased to 24.1, 60.8 % at irrigation with saline water 2500 ppm and 27.7, 42.9 % at irrigation with saline water 5000 ppm for 20 and 40 ppm of ABA level, respectively. Also, there are progressive increases in stems and leaves yield with increasing rates of ABA application. The highest stems (23.87 g/plant) and leaves (15.17 g/plant) weights were obtained under fresh water with 40 ppm ABA, in addition , Szypulska, et al (2017) also demonstrated that the treatment of barley caryopses with 100 IM ABA induced proteomic changes similar to those caused by salinity, excluding the JIP protein. The

observed reduction in the salt-responsive proteome could indicate that ABA pretreatment plays a role in the acquisition of tolerance to salinity stress.

Both salinity and Abscisic acid (ABA) rate affected leaves and stem of cotton and an interaction was detected between these factors (Table 2) & Fig. (1,2,3). At lower salinity level of 250ppm, increasing ABA supply promoted leaves and stem of cotton and its components (39.04g/pot) was obtained at 40ppm ABA, confirming the impact of higher ABA input on the aboveground production. Increase in yield was due to improvement in growth parameter reflected in dry matter production of the vegetative growth. In field, Li-Xing Weia et al., (2017) stated that a 6% increase in plant height Priming of rice (*Oryza sativa L.*) ($P < 0.05$) was observed following ABA treatment at 10 μM . Both panicle weight (70% and 100%) and stem weight (79% and 109%) were markedly increased by ABA treatment ($P < 0.05$) (1 μM and 10 μM), as compared to the control treatment. It has been well established that abscisic acid (ABA) is a vital cellular signal that mediates the expression of a number of salt and water deficit responsive genes. The basis for ABA as an important signal is that both salt stress and water deficit can induce a rapid and massive accumulation of ABA in plant tissues.

Table 2: Dry weight of leaves and stems of cotton plants response to spray with ABA under salinity conditions.

Salinity (ppm)	ABA foliar (ppm)	Stems (S)	Leaves (L)	S+L	L/S
TW (250ppm)	0	16.78	13.2	29.98	0.78
	20	20.13	14.83	34.96	0.74
	40	33.87	15.17	39.04	0.64
Means		20.26	14.4	34.66	0.71
SW (2500ppm)	0	13.27	10.4	23.67	0.78
	20	17.53	10.73	28.26	0.61
	40	18.77	13.0	31.77	0.69
Means		16.5	11.37	27.87	0.69
SW (5000ppm)	0	13.2	10.43	23.63	0.79
	20	16.27	9.47	25.74	0.58
	40	17.27	10.93	28.20	0.63
Means		15.0	10.27	25.77	0.66
LSD (salinity)					
LSD(ABA)		1.4	0.5		
LSD(S*ABA)		0.4	0.49		
		0.96	0.63		

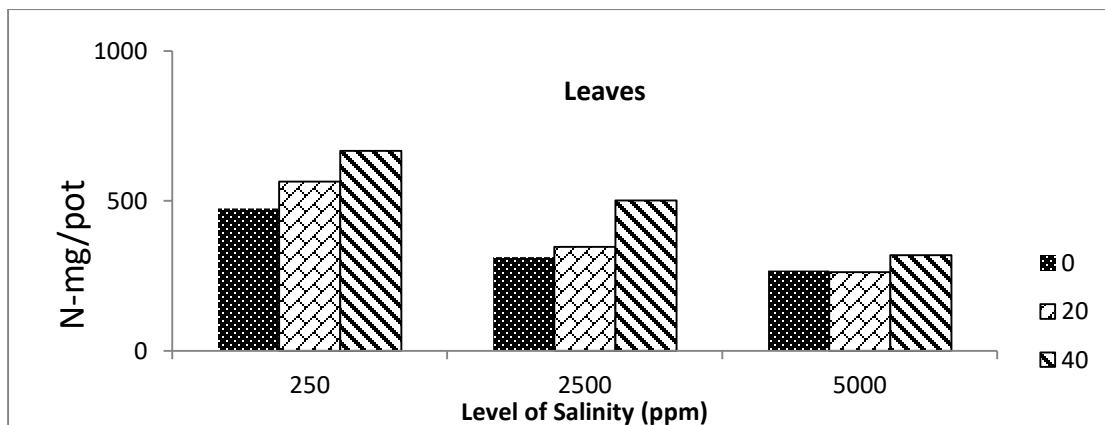
Concentration and Uptake of Some Minerals

The mineral nutrition of the leaves and seeds was assessed (Table 3). Each level of salinity stress affected the nutrient status of the explants distinctively. It can be observed that N in shoots decreased as salinity increased but reversely abscisic acid increased it under different salinity treatments. The degree of increases was more under freshwater treatment and lesser under high salinity.

Table 3: some macronutrients concentration (%) of cotton plants as affected by salinity and ABA spraying

Salinity (ppm)	ABA foliar (ppm)	N		P		K	
		Leaves	Stems	Leaves	Stems	Leaves	Stems
250 ppm	0	3.6	1.7	0.23	0.22	2.7	3.2
	20	3.8	1.9	0.25	0.24	3.3	4.0
	40	4.4	2.4	0.29	0.28	3.8	4.8
Means		3.9	2.0	0.25	0.24	3.2	4.0
2500 ppm	0	3.0	1.5	0.22	0.19	2.2	2.4
	20	3.2	1.6	0.22	0.21	2.5	2.8
	40	3.9	2.2	0.26	0.25	3.2	3.8
Means		3.3	1.8	0.22	0.21	2.6	2.8
5000 ppm	0	2.5	1.2	0.15	0.14	1.6	1.8
	20	2.8	1.4	0.17	0.16	2.0	2.5
	40	2.9	1.8	0.20	0.19	2.8	3.1
Means		3.7	1.5	0.17	0.16	2.1	2.4
ABA means	0	3.0	1.4	0.19	0.18	2.1	2.4
	20	3.3	1.6	0.21	0.20	2.6	3.1
	40	3.7	2.1	0.25	0.24	3.2	3.9

An increase in leaves and stems N, P and K concentration and uptake were observed with addition of abscisic acid (ABA) (Fig. 1,2,3). They differed significantly due to salinity levels irrigation water and ABA application. At the lowest salinity (250ppm) there was a significant increased N in leaves and stems associated with increasing ABA concentration. Total N uptake by leaves and stems of plants were affected by salinity-ABA interactions. Since, for plant grown in soil when water saline \ were applied, the growth limiting factor was the salinity rather than ABA, the total N uptake at these levels of salinity was significantly lower compared with the N uptake at lowest salinity (250 ppm). The decrease in total N uptake by increasing salinity, apart from the effects of salinity on cotton growth, has been partly attributed to a probable substitution of Cl⁻ for NO₃⁻ (Tuil and Van, 1965). Papadopoulos and Rendig, (1983) found that leaf Cl concentration was significantly correlated with the average electrical conductivity of five soil solution samplings during the growing period. Thomas (1980) noted the same effect of increasing salinity on Cl⁻ content of leaves and postulated that the osmotic adjustment of plants may be due to the rapid absorption of Cl⁻.



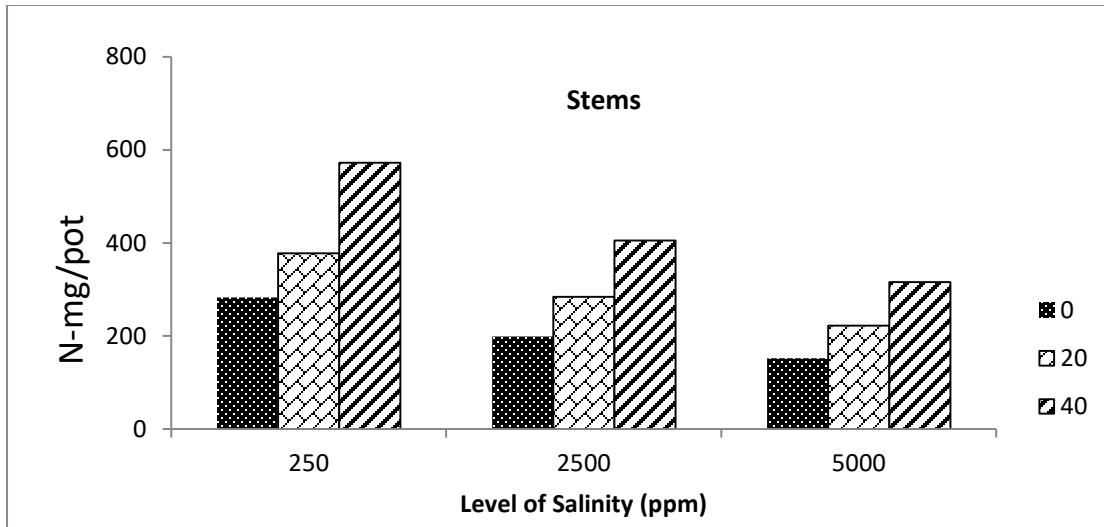


Figure 1: Nitrogen uptake (mg/pot) of cotton plants as affected by salinity and ABA spraying

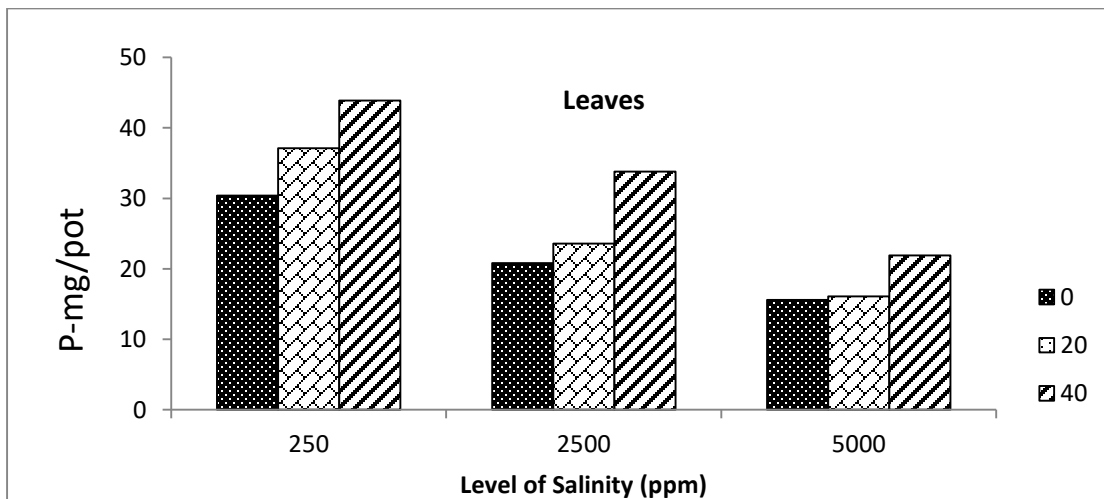
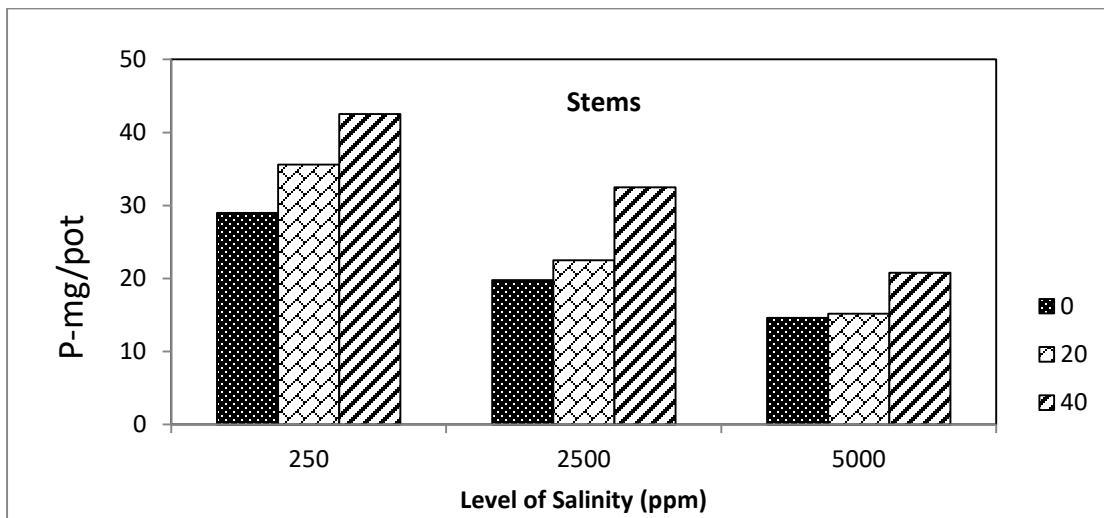


Figure 2: Phosphours uptake (mg/pot) of cotton plants as affected by salinity and ABA spraying

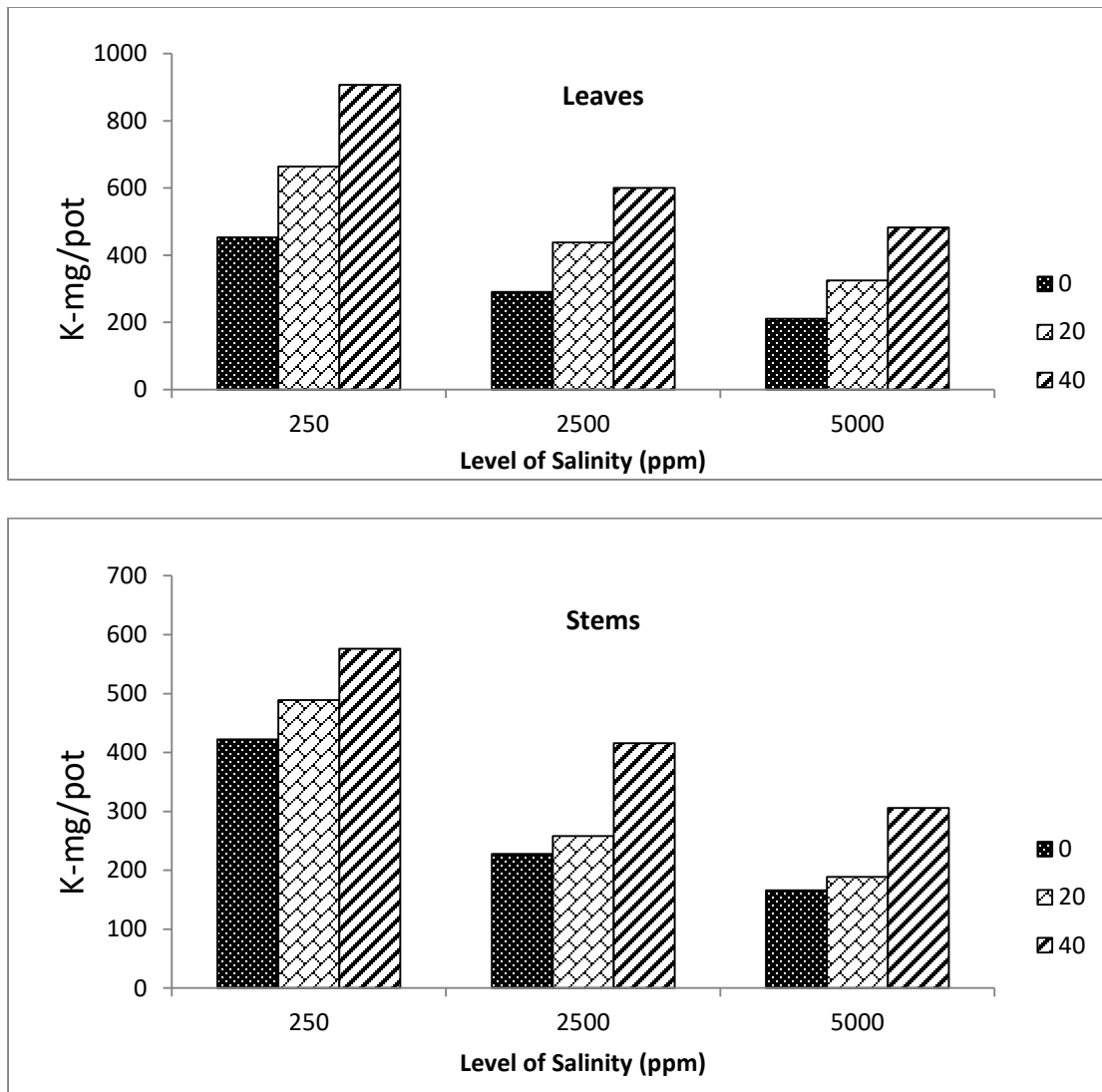


Figure 3: Potassium uptake (mg/pot) of cotton plants as affected by salinity and ABA spraying.

Increasing the salinity level of irrigation water decreased P concentration in leaves and stems of cotton plant. The decrease of plant P concentration may be explained on the premise of competition between Cl⁻ and phosphate ions in the blub zone or perhaps due to the restricted blub growth (khalil et al., 1967). At the salinity levels of irrigation water, there was a significant increased P in the plant associated with the increasing ABA applied. Phosphorus concentration in leaves tended to increase from 0.22 to 0.24 and 0.28 % as the ABA level was raised from control to 20 and 40ppm ABA, respectively. The obtained data revealed that increasing the salinity levels of irrigation water decreased K concentration and its uptake by leaves and stems of cotton plant (Fig. 3). Potassium concentration tended to decrease from 3.2 to 2.6 and 2.1% in leaves and from 4 to 2.8 and 2.4 in stems as the salinity level was raised from 250 ppm to 2500 and 5000 ppm, respectively. The reduction of the concentration of K at the highest salinity levels (2500, 5000 ppm), this may be explained by competition between Na⁺ and K⁺ ions, these results confirmed the depression of plant K concentration under the highest levels of salinity. At the salinity levels of irrigation water, there was a significant increased K uptake by leaves and cotton of the plant associated with the increasing ABA applied. Generally, decreasing nutrient concentrations under

the interaction between irrigation with tap water and spraying abscisic acid may be attributed to the dilution effect resulted by increasing growth (Abou-Baker et al.,2011). This claim confirmed by the growth data in Table 2. While decreasing nutrient concentration under salinity treatments is a logic trend and refer to Osmotic pressures and chemical induced drought, nutrient imbalance, toxicity of Na and Cl and death of some useful microorganisms as concluded by (Abou-Baker and El-Dardiry 2015). Salinity can directly affect on nutrient uptake, such as Na⁺ reducing K⁺ uptake. Salinity can also cause a combination of complex interactions that affect plant metabolism (Hussein, et al., 2012).

Na Ratios with Some Other Macronutrients Concentration

The Na/K, Na/Ca and Ca : (Na+ K) sharply depressed with ABA under fresh water especially with 40ppm of ABA. the Na/K and Na/Ca ratio increased by increase the salinity of water irrigation while the Ca/(Na+K) decreasing .from table (),it is clear that the Ca/(Na+K)ration is lower in stem than in leaves and high salinity (500ppm) gave the highest ratio of Ca(Na+K).

Generally, the Na ratio in leaves or stem salinity increased by 20ppm and decreased with 40ppm ABA under all salinity treatment.

(Ali, et al., 2013) indicated that N, P, K and Ca were decreased with increasing salinity concentration while sodium was increased

Table 4: Na ratios with some other macronutrients concentration as affected by ABA spraying and salinity.

Salinity (ppm)	ABA foliar (ppm)	Na/K		Na/Ca		Ca/(Na+K)	
		Leaves	Stems	Leaves	Stems	Leaves	Stems
250 ppm	0	0.40	0.38	1.2	1.3	0.26	0.20
	20	0.48	0.45	1.16	1.36	0.34	0.22
	40	0.34	0.31	0.88	0.96	0.32	0.23
Means		0.4	0.38	1.08	1.2	0.70	0.22
2500 ppm	0	0.54	0.54	1.13	1.3	0.35	0.27
	20	0.70	0.69	1.34	1.96	0.30	0.24
	40	0.50	0.55	1.12	1.4	0.29	0.23
		0.58	0.74	1.59	1.55	0.31	0.25
5000 ppm	0	0.88	0.94	1.4	2.0	0.33	0.23
	20	1.1	0.96	1.8	2.7	0.28	0.18
	40	0.64	0.71	1.4	2.2	0.27	0.23
		0.87	0.87	1.5	2.3	0.29	0.21
ABA means	0	0.60	0.62	1.24	1.5	0.31	0.23
	20	0.76	0.61	1.43	2.00	0.30	0.21
	40	0.49	0.52	1.13	1.52	0.29	0.23

Data in Table (4) cleared that the growth regulator (ABA) increased the concentration of NPK in leaves under different salinity levels. NP concentration in stem followed the same response to the ABA concentrations. In this concern, contents of Ca and Cl increased on salinization in both shoot and root tissues overall temperatures because of the large increase in their activity in the external solution. In contrast, K and S contents decreased by salinity because (1) their ion activities in the

external solution reduced (2) there was interference with their uptake by the major saline actions. It was accepted that competition exists between Na and K leading to a reduced level of internal K at a high external NaCl concentration. In contrast the Ca/(Na +K) ratio increased that showed the opposite response. The K/Na ratio in the shoots and roots of plants decreased steadily with increasing salt addition. This is in agreement with Hussein and Abo Talb (2018) on cotton also, reveal that Na/K increased as the salinity level increased, but Na/Ca and K/Ca ratios increased with the moderate salinity level and tended to decrease, while it is still more than the control. However, Ca/(K +Na) decreased with the first level of salinity and tended to increase with the high level of salinity, but the values of this ratio are still less than those of the control.

ABA application reduced the toxicity of salt treatment and sometimes enhanced growth through reduction of inorganic ions accumulation. Also, ABA improved K uptake under salinity, effectively increasing K/Na ratios in the tissues. This effect is consider to be important in salt tolerance. Using fewer ions to be carried through the transpiration stream leading to the decrease in their accumulation. Another probability could be due to ABA effects on membrane leakage and membrane stability (Gadallah, 1996 and Pustovoitova, 1987).

The ratio of K/Na was decrease progressively on salinization. With stressed plants, ABA application reduced the toxicity of salt treatment, improved K uptake under salinity, effectively increased K/Na ratio and helped the plants to avoid Na toxicity and sometimes enhanced growth (Gadallah, 1996).

In addition, Wang, et al (2007) reported that cucumber seedlings treated with exogenous polyamines and combined with salinity exhibited a higher level of K⁺ accumulation and lower levels of Na⁺ and Cl⁻ accumulation compared with the seedlings treated only with salt stress.

4. Conclusion

The combined efforts of governments and farmers in finding alternative irrigation water resources (such as diluted seawater) should be taken seriously, especially after increasing climate change hazards like water shortage and increasing salinity as most hazards abiotic stresses affected plant production and the recent studying how to increase plant alleviation to abiotic stress is a good approach. From this study, it can be concluded that, it can be used diluted seawater (2500ppm) in irrigation of cotton plant with spraying abscisic acid to alleviate the harmful effect of salinity, abscisic acid application led to increasing leaves and stem weight of cotton plants and nutrient content compared to control (foliar with distilled water) and the high rate of abscisic acid (40ppm) was superior than low one (20 ppm).

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