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Growth, Photosynthetic Pigments and Mineral Status of Cotton Plants as Affected by Salicylic Acid and Salt Stress.

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ABSTRACT

A pot experiment was conducted in the greenhouse of the National Research Centre (NRC), Cairo, Egypt during two successive seasons to evaluate the response of growth, photosynthetic pigments and mineral status of cotton plants sprayed by 200 and 400 ppm salicylic acid (SA) and grown under salt stress (irrigation with 3.9 $dSm^{-1} \approx$ 2500 ppm and 7.8 $dSm^{-1} \approx$ 5000 ppm more than the tape water 0.47 $dSm^{-1} \approx$ 300 ppm as a control). Data showed that dry matter, area of leaves, yield/plant, chlorophyll b concentration and uptake of N, P, K, Na and Ca were decreased by salt stress. Carotenoids increased by irrigation plants with 2500 ppm and tended to decrease but still more than the control. Chlorophyll a showed the same response but the value with 5000 ppm less than the control. Na/K ratio increased as salt concentration increased but Ca: (Na+K) showed the opposite response. Salicylic acid application of 200 ppm showed the highest improve in growth criteria, uptake of the macronutrients and Ca:(K+Na) compare to the control plants. Salicylic acid application lowered the adverse effect of salt stress and can use for amelioration of salt stress in cotton plants.

Key words: cotton (*Gossypium barbadense* L.) – salt stress – salicylic acid – growth – chlorophyll – carotenoids – mineral status

Introduction

Cotton (*Gossypium barbadense* L.) is first and the main fiber crop in Egypt. In the last decades, the cultivated area decline from year to another, from 1.2 million fed to 200,000 fed, and intern the national production lowered as well as the imported quantities raised and increased the fund needed to the importation process. While imported cotton is priced at between LE400 to LE500 per qentar, the locally produced cotton is over LE1,000 per qentar. The result is that farmers are unable to sell their cotton harvest and complain about it to the government. In the meantime, the government is obliged to keep its market open to imports according to its commitments to the World Trade Organization (WTO).

Soil salinity is a major constraint limiting agricultural productivity on nearly 20 % of the cultivated area and half of the irrigated area worldwide (Zhu, 2001). Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species (Meloni, *et al*. 2003 and Pal, *et al*. 2004).

As one of main abiotic stresses in nature, salt stress with high concentration of saline ions in soil does remarkable harm to plants, and seriously affects the growth and development of plants. Soil salinity is a major threat to cotton production worldwide. Excessive salt in saline soils leads to a series of physiological and biochemical decompensation in cotton plants mainly from osmotic effects (dehydration), nutritional imbalance and toxicity of salt ions (Ye, *et al*. 2003 and Zhenhuai, *et al*. 2005). Cotton is considered to be moderately tolerant to salinity, ranked second behind barley (Soltanpour and Follett, 1995).

Cotton and salt stress was studied by many authors: Cramer, *et al*. (1987); Khorsandi, and Anagholi. (2009), Hussein, *et al*. (2010) and Yao, *et al*. (2011).

Salicylic acid (SA) is part of a signaling pathway induced by several biotic and abiotic stresses (Ashraf, *et al*. 2011). It has been identified as an endogenous regulatory signal in mediating plant's defense against pathogens (Anosheh, *et al*. 2012), and it is also a natural signal molecule for the activation of plant's general defense mechanisms (Wang and Li, 2006). Exogenous application of SA has been shown to induce plant stress tolerance. Aplethora of research demonstrates protective effects of exogenous SA on plants against salinity (Wang and Li, 2006, and Hussein, *et al*. 2007), drought (Singh and Usha, 2003), and high temperatures (Ashraf, *et al*. 2010). Therefore, this work aimed to investigate the effect of salicylic acid application and irrigation by diluted seawater on growth, photosynthetic pigments, mineral status and yield of cotton plants.

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Materials and Methods

A pot experiment carried out in greenhouse of the National Research Centre. A study aimed to evaluate the influences of salicylic foliar application and irrigation by diluted seawater on growth and some chemical constituents of cotton plants. The treatments were as follows:

Salinity:

Irrigation by diluted seawater with 3.91dSm^{-1} (2500 ppm), 7.8 dSm^{-1} (5000 ppm) and fresh water 0.47 dSm^{-1} (3ooppm) as a control. Seawater dilution ratios were 1:12 and 1:6.

Salicylic acid:

The rates of 200 and 400 ppm salicylic acid were sprayed (21and 35 days).

The experiment included 9 treatments which were the combination of three treatments of irrigation by diluted seawater and three treatments of salicylic acid foliar spray. The experimental design was two way randomized blocks (2WRB) in 8 replicates. Metallic ten pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 30 Kg. of air dried clay soil. Initial soil sample were air dried, crushed, sieved to pass through 2mm sieve and analyzed as described by Page *et al*., (1982). Some chemical and physical properties of the studied soil and sea water are shown in Tables (1 & 2). The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2 kg of gravel (Particles about 2-3 cm in diameter), so the movement of water from the base upward.

Table 1: Some physical and chemical characteristics of the investigated soil

Characteristics	Values
Physical properties (%):	
Organic matter	1.30
Calcium carbonate	2.53
Sand	21.5
Silt	30.2
Clay	48.3
Chemical properties	
pH (1 : 2.5 soil : water ratio)	7.15
EC (Soil paste extraction) dSm^{-1}	1.30
Soluble cations (me/100g):	
Calcium	2.38
Magnesium	1.27
Potassium	0.23
Sodium	1.82
Soluble anions (me/100g):	
Carbonate	-
Bicarbonate	0.91
Chloride	1.90
Sulphate	1.89

Table 2: Some chemical characteristics of the used water

Characteristics	Values
pH	8.4
EC (dSm^{-1})	50
Total salinity (g/L)	36
Cations (g/L):	
Calcium	0.42
Magnesium	1.31
Potassium	0.42
Sodium	11.02
Anions (g/L):	
Bicarbonate	0.12
Chloride	19.88
Sulphate	2.74

Seeds of cotton (*Gossypium barbadence* L.) cv Giza 86 were sown at May, 20 and seedlings were thinned twice 15 and 30 days to left two 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 first at 21 days from sowing and the second two weeks later. Irrigation with diluted seawater in different concentrations were started 30 days after sowing (One irrigation by salt water and the next was by fresh water alternatively). At the end of cotton season, some growth parameters were determined such as, plant height (cm), root length (cm), No. of leaves/plant, dry weight of leaves, steam, root and whole plant (g/plant), leaves area (m^2) and cotton yield (g/plant). Cholorophylls and carotenoids were determined according to the method described by Von Wetistien, (1957).

Leaves samples of three plants from every treatment were collected, cleaned, dried in an electric oven muffle at 70 °C. The dry matter was ground in a stainless steel mill. Chemical analysis was carried out according to the methods which described by Cottenie, *et al*. (1982). All collected data were subjected to the proper statistical analysis as described by Snedecor and Cochran (1989).

Results and Discussion

Growth:

Salt stress effect:

Significant response was obtained in plant height and number of green leaves as a result of irrigation by diluted seawater (Table 3). The root length and area of green leaves showed no significant difference to this salt treatment. Irrigation cotton plants with 5000 ppm diluted seawater decreased root, stem, leaves and whole plant dry weight by 40.24, 48.84, 58.00 and 49.97 %, respectively compare to the control treatment. This means that salt stress affected leaves dry weight more than the other plant organs.

Cramer, *et al*. (1987) previously reported that high Na^+ concentrations may interfere with growth of many plant species, cotton (*Gossypium hirsutum* L.) included. Basal (2010) stated that the most general effect of salinity on plant is a reduction in growth (total biomass production) and growth rate. Total (shoot + root) dry weight decreased progressively as the salinity level increased from 0 to 125 and 250 mM NaCl salinity levels. Mean shoot fresh and dry weight (stem + leaves) values of cotton genotypes were significantly affected by salinity stress. A decrease in shoot fresh weight and dry weight of 15 cotton cultivars was observed with increase in salt concentration of the growth medium. The spotty pattern in crop stand at maturity under saline soil conditions is actually initiated at the time of germination and vegetative growth phase. Moreover, build up of certain ions in the cytoplasm may affect certain metabolic processes (Ali, *et al*. 2005). The cotton roots became thinner in response to increasing nutrient solution Na:Ca ratio, especially at the highest Na concentration (Dodd and Guppy, 2010).

Table 3: Effect of saline water on the growth parameters of cotton crop.

Saline water, ppm	Plant height, cm	Root length, cm	No. of leaves/ plant	Dry weight , g/plant				Leaves area, m^2	Cotton yield, g/plant
				Leaves	Stem	Root	Whole plant		
5000	101.61[a]	18.22	11.78[b]	4.25[b]	11.29[b]	3.49[c]	19.03[c]	0.087	6.79[c]
2500	116.55[ab]	20.44	13.22[ab]	6.12[a]	13.75[b]	4.07[b]	23.94[b]	0.126	9.39[b]
Control	131.22[a]	19.67	15.00[a]	10.12[b]	22.07[a]	5.84[a]	38.04[a]	0.137	11.97[a]
L.S.D. at 5%	19.82	N.S.	3.05	2.29	3.19	0.55	2.48	N.S.	2.35

Considerable reduction in yield of cotton plants as irrigated by both concentrations of salts in diluted seawater. The reduction amounted by 43.27 and 21.55% in comparable 5000 and 2500 ppm with plants received fresh water (Table 3). In general, cotton plants were capable of producing seed cotton under salt stress, as well as, after salt stress relief. However, as the salt stress severity increased the ability of cotton to compensate yield loss decreased. Khorsandi and Anagholi, (2009) showed that irrigation of cotton at vegetative stage Gemination 1 with either moderate (10 dSm^{-1}) or high (20 dSm^{-1}) salinity waters should be avoided. Moderate saline water could be applied either at reproductive stage Gemination 2 and boll development stage Gemination 3. High salinity water can be used for irrigation only at Gemination 3 stage to produce acceptable cotton seed yield.

Salicylic acid effect:

It is clearly shown from Data in Table (4) that the spraying 200 ppm SA more effective than the others. The highest effect of SA on plant growth criteria was in leaves dry weight (44.63%) followed by in whole plant dry weight (34.83%) and the lowest was in green leaves (4.88%) area followed by in that in plant height (18.69%). Arfan, *et al*. (2005) revealed that salt stress reduced the growth and grain yield of both cultivars of wheat. The highest values of shoot dry weight were obtained by SA treatments. The maximum number of nodules per plant was achieved by the 333 application of SA at 10 M (Hegazi and El-Shraiy, 2007). The highest yield occurred in 0.50 mM SA treatment. Applications of 0.50 mM SA should be recommended in order to improve yield (Yieldrim and Dursun, 2009).

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Table 4: Effect of salicylic acid on growth and yield of cotton plants

Salicylic acid, ppm	Plant height, cm	Root length, cm	No. of leaves/ plant	Dry weight , g/plant				Leaves area, m^2	Cotton yield/ plant, g/plant
				Leaves	Stem	Root	Whole plant		
Controrol	110.28^b	18.67b	12.67^b	6.05^b	14.00^b	4.18^b	24.23^b	0.123	9.49^b
200	130.89^a	22.00^a	16.56^a	8.75^a	18.70^a	5.22^a	32.67^a	0.129	11.33^a
400	108.22^b	17.67^b	11.67^b	5.69^b	14.41^b	4.01^b	24.11^b	0.098	7.32^c
L.S.D. at 5% level	10.61	1.67	2.79	2.29	3.40	0.83	5.87	N.S.	1.76

Salicylic acid X Salt stress:

Table (5) and Fig. (1) present the effect of the interaction between the experimental treatments on the growth parameters and the yield/plant of cotton. The highest values of them were gave by applying 200 ppm SA under all saline water treatments and control. The lowest values of plant height, number of green leaves and roots and stem dry weight were by spraying distilled water (control) and irrigation by 5000 ppm salts, however, values of yield/plant and root length, area of leaves/plant, stem and whole plant dry weight were the lowest values in plants received 400 ppm SA irrigation. by 5000 ppm salts in diluted seawater used in its irrigation. Arfan, *et al.* (2006) pointed out that the most effective levels for promoting growth and grain yield were 0.75 and 0.25 mM SA under normal and saline conditions, respectively. The improvement in growth and grain yield of wheat plant (S-24 cultivar) due to SA application was associated with improved photosynthetic capacity. Changes in photosynthetic rate due to SA application were not due to stomatal limitations, but were associated with metabolic factors, other than photosynthetic pigments and leaf carotenoids. Hussein, *et al.* (2007) found that growth of maize plants showed improve with SA spraying under different salinity levels. Hayat, *et al.* (2012) observed that the salinity significantly reduced the plant growth, gas exchange parameters but increased proline content and electrolyte leakage in the leaves. The effects were more pronounced at 30 days after salicylic acid application (DAS) than 45 DAS. Moreover, reverse effect on growth of plants was overcome by application via leaves.

Table 5: Effect of the interaction between saline water and salicylic acid on the growth parameters of cotton plants

Saline water, ppm	Salicylic acid, ppm	Plant height, cm	Root length, cm	No. of leaves/ plant	Leaves area, m^2	Cotton yield/ plant, g
5000	Control	86.83^e	17.67^{cd}	10.67^b	0.097	6.57^b
	200	116.67^{cd}	20.67^{abc}	13.67^b	0.107	8.58^b
	400	101.33^{de}	16.33^d	11.00^b	0.058	5.21^b
2500	Control	108.33^d	20.33^{abc}	13.00^b	0.136	8.31^b
	200	137.00^{ab}	22.00ab	15.00^b	0.136	11.69^a
	400	104.33^{de}	19.00^{bcd}	11.67^b	0.105	8.19^b
Control	Control	135.67^{abc}	18.00^{cd}	14.33^b	0.136	13.60^a
	200	139.00^a	23.33^a	21.00^a	0.143	13.75^a
	400	119.00^{bcd}	17.67^{cd}	12.33^b	0.132	8.56^b
L.S.D. at 5% level		18.37	4.84	2.89	N.S.	3.05

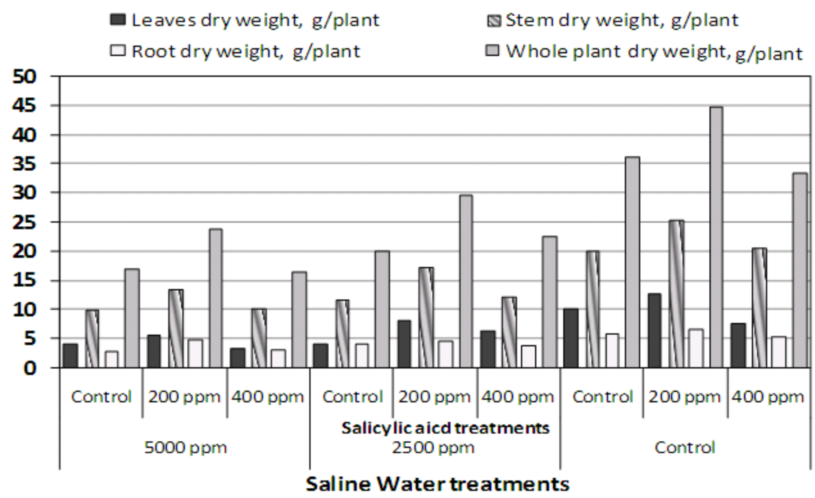

Fig. 1: Effect of the interaction between saline water and salicylic acid treatments on leaves, stem, root and whole plant dry weights (g/plant).

Mineral content:

Salt stress effect:

Significant decrease was detected in K concentration in leaves of cotton plants with the increase in salt concentration in irrigation water (Table 6).Uptake of different elements were significantly affected with salt stress. This may be due to that effect of salinity was more clearly on dry weight so that, data of uptake take the same trend of dry weight. The lower effect was continuous with increase in salt concentration in the diluted seawater in K and Ca concentrations. However, the differences in N, P and Na concentration resulted from the two salt stress treatments were not significant. Grattan and Grieve (1998) emphasized that salinity dominated by Na^+ salts not only reduces Ca^{2+} availability but reduces Ca^{2+} transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs. Salinity can directly affect nutrient uptake, such as Na^+ reducing K^+ uptake or by Cl^- reducing NO_3^- uptake. Salinity can also cause a combination of complex interactions that affect plant metabolism, susceptibility to injury or internal nutrient requirement. Yildirim, *et al.* (2006) revealed that the most effective biological increased in the K^+/Na^+ ratio, which was positively correlated with plant growth. Alteration of mineral uptake may be one mechanism for the alleviation of salt stress. Hussein, *et al.* (2010) reported that salt stress affected the content of nutrients in sorghum plants. Similarly, Dodd *et al*. (2010) showed that increasing of EC increased Na concentration and decreased K and P concentrations in cotton shoot.

Table 6: Effect of saline water on the concentration and content of macro nutrients of cotton plants.

Saline water, ppm	K%	Na%	P%	N%	Ca%	Uptake, mg/plant				
						K	Na	P	N	Ca
5000	1.75[b]	0.684	0.169	1.54	1.328	28.13[c]	19.62[b]	3.49[b]	5.78[b]	16.649[c]
2500	2.04[b]	0.656	0.194	1.64	1.638	42.20[b]	27.37[b]	5.48[b]	9.26[b]	18.518[b]
Control	2.53[a]	0.753	0.210	1.66	1.421	81.54[a]	61.88[a]	13.11[a]	22.29[a]	22.862[a]
L.S.D. at 5% level	0.39	N.S.	N.S.	N.S.	N.S.	6.85	8.95	3.14	4.69	7.766

Salicylic acid effect:

The concentration as well as content differences of different elements determined in this work were significant except Na concentration (Table 7). The highest responses of these nutrients were by application 200 ppm of salicylic acid compare with control or 400 ppm treatments. Nitrogen, P, K, Ca and Na concentrations and uptake increased with 200 ppm SA application, but decreased with increasing SA rate to 400 ppm. No significant difference between 400 ppm SA and control. Similar results were obtained by Khan *et al*. (2010) who found that the alleviation of salt stress effects on growth and photosynthesis with 0.5 mM SA was a result of increased content of leaf N, P, K, and Ca. The application of higher concentration of SA (1.0 mM) on plants grown with NaCl further enhanced the negative effects of NaCl on growth, photosynthesis, and yield. Further, the accumulation of Ca^{2+} in plants receiving SA possibly maintained membrane integrity and helped in reducing the toxic effects of Na^+ and Cl^- ions. Wang, *et al*. (2011) noticed that no significant alterations in Mn and Ca content were observed but content of Cu, Zn, Fe and P decreased in plants exposed to SA alone. Nazar, *et al*. (2011) found that it also helped in restricting Na^+ and Cl^- content in leaf, and maintaining higher efficiency of PSII, photosynthetic N-use efficiency (NUE) and water relations in Pusa Vishal. However, application of 1.0 mM SA resulted in inhibitory effects.

Table 7: Effect of salicylic acid (SA) rates on the concentration and content of macro nutrients of cotton plants.

Salicylic acid, ppm	K%	Na%	P%	N%	Ca%	Uptake, mg/plant				
						K	Na	P	N	Ca
Control	1.92[b]	0.660	0.150[b]	1.53[b]	1.364[b]	41.52[b]	28.59[b]	4.48[b]	7.04[b]	8.070[b]
200	2.49[a]	0.740	0.216[a]	1.81[a]	1.723[a]	70.15[a]	52.07[a]	11.48[a]	20.92[a]	36.213[a]
400	1.90[b]	0.693	0.207[a]	1.49[b]	1.299[b]	40.21[b]	28.21[b]	6.12[b]	9.37[b]	12.412[b]
L.S.D. at 5% level	0.41	N.S.	0.056	0.13	0.281	13.40	10.48	2.34	3.72	7.379

Salicylic acid X Salt stress:

All nutrients concentration except Na% affected significantly with the interaction of SA and salt stress. The highest concentration of K% in plants received distilled water via leaves and P and N by spraying 200 ppm SA when plants irrigated by fresh water. Calcium highest value by the same SA concentration and irrigated by 2500 ppm salt solution, however, spraying the same SA concentration (200 ppm) and irrigation by 5000 ppm diluted seawater resulted in higher percentage of Na. Concerning the content of macronutrients measured herein, spraying SA in the rate of 200 ppm and irrigated by fresh water showed the highest values of all nutrients (Table

8). Foliar application of SA had an ameliorative effect under both nonsaline and saline conditions (Khan *et al*., 2010), and had a protective role of SA in membrane integrity and regulation of ion uptake (Gunes *et al*., 2007).

Table 8: Effect of the interaction between saline water and salicylic acid on the concentration and content of macro nutrients of cotton plants.

Saline water, ppm	Salicylic acid, ppm	K%	Na%	P%	N%	Ca%	Uptake, mg/plant				
							K	Na	P	N	Ca
5000	Control	1.55^{c}	0.620	0.157^{ab}	1.46^{d}	1.417^{bc}	21.67^{d}	13.45^{e}	2.18^{d}	3.30c	4.579^{d}
	200	2.18^{abc}	0.773	0.184^{ab}	1.77^{abc}	1.430^{bc}	42.05^{cd}	31.82^{cde}	5.97^{cd}	10.75^{b}	15.574^{cd}
	400	1.52^{c}	0.660	0.165^{ab}	1.39^{d}	1.137^{c}	20.66^{d}	13.59^{e}	2.32^{d}	3.29^{c}	4.057^{d}
2500	Control	1.64^{c}	0.627	0.125^{b}	1.57^{bcd}	1.340^{bc}	26.22^{d}	16.38^{e}	1.94^{d}	3.06^{c}	4.129^{d}
	200	2.48^{ab}	0.653	0.227^{ab}	1.81^{ab}	2.020^{a}	62.81^{bc}	40.64^{bcd}	8.82^{bc}	15.69^{b}	32.043^{b}
	400	2.01^{bc}	0.687	0.228^{ab}	1.55^{cd}	1.553^{abc}	37.58^{d}	25.09^{de}	5.68^{cd}	9.01^{bc}	13.676^{cd}
Control	Control	2.57^{ab}	0.733	0.167a^{b}	1.58^{bcd}	1.336^{bc}	76.70^{b}	55.93^{b}	9.31^{bc}	14.76^{b}	19.498^{bc}
	200	2.83^{a}	0.793	0.237^{a}	1.96^{a}	1.720^{ab}	105.58^{a}	83.76^{a}	19.66^{a}	36.31^{a}	61.023^{a}
	400	2.19^{abc}	0.733	0.227^{ab}	1.54^{cd}	1.207^{bc}	62.38^{bc}	45.95^{bc}	10.36^{b}	15.80^{b}	19.504^{bc}
L.S.D. at 5% level		0.70	N.S.	0.097	0.23	48.6	0.486	18.16	4.06	6.45	1278.0

Salt stress effect:

Table (9) showed that Chl.a increased with salinity treatment but the increase with 2500 ppm salts solution was more than that caused from 5000 ppm salt treatment. Carotenoids increased with first salt treatments also but decreased with the higher salt treatment used to be less than the control. However, Chl.b did not significantly affected with salt stress. The increase in chlorophyll concentration was by Axler and Owen (1994). Mousava, *et al.* (2008) reported that under low level of sulphate the content of chl.a or b and carotenoids concentrations were increased while at high level of salinity insignificant increase was observed. Na:K and Na:Ca ratios seemed to be without effect, while, Ca:(K+Na) ratio lowered as the salt concentration increased in comparable with the control treatment. Hussein, *et al.* (2007) pointed out that salt stress led to a clear increase in chl.a, chl.b and chl.a+chl.b concentrations, however, carotenoids concentration was not affected significantly by such abiotic stress.Chl.a:chl.b was slightly affected while chl.a+chl.b: carotenoids ratio values were unchanged with the salinity treatment.

Table 9: The effect of saline water on macro nutrients ratios and chlorophyll of cotton plants.

Saline water, ppm	Chl.a	Chl.b	Carotene	Na : K	Na : Ca	Ca :(K+Na)
5000	4.52^{b}	4.51	0.64^{b}	0.414	0.5	3.293^{b}
2500	6.70^{a}	4.38	1.37^{a}	0.343	0.4	4.482a^{b}
Control	4.42^{b}	4.13	1.14^{a}	0.301	0.6	4.746^{a}
L.S.D. at 5% level	0.87	N.S.	0.44	N.S.	N.S.	1.247

Salicylic acid effect:

Czerpak , *et al.* (2002) noticed that SA strongly stimulated action on the content of chlorophylls *a* and *b* and carotenoids (especially β-carotene. Moharekar, *et al.* (2003) reported that both wheat and moong seedlings, chl *a/b* ratio decreased significantly only in wheat and remained constant in moong. On the other hand, total carotenoid content, size of xanthophyll pool, and de-epoxidation rate increased significantly with an increase in SA concentration in both plant species. Yildrim and Dursun (2009) showed the SA positive effect on chlorophyll content in leaves. Khandaker, *et al.* (2007) found that SA improved chlorophyll occurred in 10^{-5} M SA treatment. Ca:(K+Na ratio responded similarly, where spraying SA did not exert any significant effect on Na:K and Na:K ratios (Table 10).

Table 10: Effect of salicylic acid on macro nutrients ratios and chlorophyll of cotton plants.

Salicylic acid, ppm	Chl.a	Chl.b	Carotene	Na : K	Na : Ca	Ca :K+Na)
Control	5.50	4.12	1.01	0.363	0.5	3.488^{b}
200	4.92	4.39	1.04	0.309	0.5	5.598^{a}
400	5.24	4.52	1.09	0.386	0.6	3.434^{b}
L.S.D. at 5% level	N.S.	N.S.	N.S.	N.S.	N.S.	1.039

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Salicylic acid X Salt stress:

Examination Data in Table (11) showed that for the Chl.a concentration, the highest values with 400 ppm SA under control treatment irrigation and 5000 ppm salt treatment but with 2500 ppm salt treatment the reverse was true. Chl.b increased with the increase of SA concentration and irrigation by 5000 ppm salt solution but reversely responded under 2500 ppm salt in diluted seawater and sprayed by distilled water. Carotenoides showed increases with SA treatment under both salinity treatments. Moreover, chl.a+chl.b in ppm was decreased by SA application with high salt treatment and slightly affected using moderate salt treatment but lowered in plants irrigated by fresh water. Furthermore, considerable increases were detected chl.a:chl.b ratio by the increases in addition of SA when plants irrigated fresh water and moderate salinity treatments, meanwhile, it decreased when salt concentration in diluted seawater raised to be 5000 ppm.

Cramer, *et al.* (1987) concluded that potassium influx declined significantly with increasing salinity but calcium influx was complex and exhibited two different responses to salinity. At low salt concentrations, influx decreased curvilinearly with increasing salt concentration. At 150 to 250 millimolar NaCl, $^{45}Ca^{2+}$ influx increased in proportion to salt concentrations. Maas (1993) stated that in saline soil, Na^+, K^+ and Cl^- are the dominant ions affecting plant growth. Ali, *et al.* (2005) confirmed this finding. Under these conditions the activities of some essential nutrients may also be reduced (Gratten and Grieve, 1992) and plants may experience nutritional disorders. Under normal growth conditions, Na and K contents were about same in the WT and transgenic lines. After 14 days of 250 mM NaCl stress, cellular Na^+ contents increased while K^+ content decreased significantly in both but the transgenic plants accumulated more K^+ and less Na than the WT plants, and the K^+/Na^+ ratio was higher in the transgenic plants (Zhang, *et al*. 2011).

Table 11: The effect of the interaction between saline water and salicylic acid on macro nutrients ratios and chlorophyll of cotton plants.

Saline water, ppm	Salicylic acid, ppm	Chl.a	Chl.b	Carotene	Na : K	Na : Ca	Ca : (K+Na)
3000	Control	5.06^{ab}	2.11^{b}	0.24^{b}	0.399	0.4	3.071^{b}
	200	3.46^{b}	4.59^{ab}	0.73^{ab}	0.374	0.6	4.253^{b}
	400	5.04^{ab}	6.83^{a}	0.94^{ab}	0.468	0.6	2.553^{b}
1500	Control	6.63^{ab}	4.83^{ab}	1.26^{ab}	0.404	0.5	3.008^{b}
	200	7.14^{a}	4.60^{ab}	1.30^{ab}	0.271	0.3	6.252^{a}
	400	6.39^{ab}	3.72^{ab}	1.55^{a}	0.353	0.5	4.187^{b}
Control	Control	4.82^{ab}	5.43^{ab}	1.52^{a}	0.285	0.6	4.386^{b}
	200	4.14^{ab}	3.97^{ab}	1.09^{ab}	0.281	0.5	6.290^{a}
	400	$4.29a^{b}$	3.01^{b}	0.81^{ab}	0.338	0.6	3.562^{b}
L.S.D. at 5% level		3.02	3.31	1.02	N.S.	N.S.	197.9

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