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Modification of Photosynthetic Pigments, Osmotic Solutes and Ions Accumulation in *Chlorella vulgaris* and Wheat Cv. Sds-1 Seedlings under the Influence of NaCl with Salicylic Acids

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ABSTRACT

Previous studies have shown that salicylic Acid (SA) plays an important role in the response of plants to salt and osmotic stresses. Therefore this experiment was conducted to investigate the impact of exogenous salicylic acid (0.5 mM) on some metabolic activities of *Chlorella vulgaris* and wheat cv. Sds-1 seedlings under 0, 50, 100, 150, 200, 400 and 600 NaCl mM. There are a highly significant decrease in chlorophyll a (at all salinity levels) in wheat seedlings and chlorophyll b (above 50 mM NaCl) in tested *Chlorella* and wheat plants. Soluble carbohydrates in *Chlorella* and wheat reached a maximum value (53.833 and 78.190 mg, respectively) when treated with 0.5 mM SA. Under treatment of SA, the highest concentration of soluble protein in salinized *Chlorella* and wheat was (76.658 and 194.360 mg, respectively) and total free amino acids value was (16.353 mg) in *Chlorella*. Na⁺ concentration in *Chlorella* and wheat increased (up to 390 and 200%, respectively) higher than control at the highest NaCl concentration. An opposite trend for K⁺, Ca⁺⁺ and Mg⁺⁺ contents was obtained. The lowest value of Ca⁺⁺ (61.5% reduction of the control) in two tested plants was observed at the highest salinity levels. K⁺ content in treated plants with SA increased (at all salinity levels) than control. The plants with SA may produce the required factors for plant protection that may reduce the harmful effects of NaCl.

Key words: Carbohydrates, *Chlorella vulgaris* protein, photosynthetic pigments, salicylic acid, salinity

INTRODUCTION

Salinity have an effect on the growth and productivity of plants (Heidari, 2009; Amirjani, 2010; Joseph and Jini, 2010). Salinity stress leads to osmotic stress, ion toxicity and mineral deficiencies (Musyimi *et al.*, 2008; Shaaban *et al.*, 2008). SA is both phenol compound and plant hormone which had a vital role in tolerance of abiotic stress (Al-Hakimi, 2006; Saleh *et al.*, 2007; Azooz and Youssef, 2010; Kazemi *et al.*, 2011).

Singh and Kshatriya (2002) and Srivastava *et al.* (2008) reported that salinity induced a decrease in pigmentation and photosynthetic capacity in cyanobacteria. Salt stress reduced photosynthetic pigments in leaves. Agastian *et al.* (2000) found that the oldest leaves start to develop chlorosis and fall with prolonged period of salt stress. Similar inhibition effect of salinity was also obtained in leaves of *B. parviflora* (Parida and Das, 2005).

Czepak *et al.* (2002) showed that SA induced a stimulation in photosynthesis process. NaCl stress had effect on carbohydrates metabolism (Shaddad *et al.*, 2005). In citrus, salinity reduces net

photosynthetic rate and carbohydrate accumulation (Lopez-Climent *et al.*, 2008). Desouky (1995) found that the carbohydrates content in stressed algal cell was reduced with the rise of salinization treatments. Kukreja *et al.* (2005) and Rai and Sharma (2006) reported that soluble sugar content was increased under salt stress. Szepesi (2006) stated that in the presence of SA, the leaves accumulated more osmolytes (e.g., glucose and fructose).

Protein degradation under salinity stress is caused by decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acid and denaturation of enzyme involved in protein synthesis (Manivannan *et al.*, 2008). While, Patel and Pandey (2007) reported that protein level under salinization increases as a result of the increased synthesis of pre-existing and certain new sets of proteins. Czerpak *et al.* (2002) found that, SA acid alone in the culture media increased the number of *Chlorella vulgaris* Beijerinck cells (about 40%) and content of proteins (about 60%) and its secretion to the medium. While, under the salt stress, salicylic acid treatment induced a significant decrease in soluble protein content as well as the amino acids of *Scenedesmus* sp. Thus, the reduction in protein content is not at the expense of amino acids (Galal, 2007).

The amino acids level under salt stress conditions are directly related to the alterations of the enzymatic activities caused by feedback and/or depressive mechanisms (El-Bassiouny and Bakheta, 2005). In *Cladophora vagabunda* (Chlorophyceae) there were concomitant increases in the acidic amino acids: aspartate and the glutamate and the basic amino acids: lysine, histidine and arginine in response to salinity stress (Rao *et al.*, 2007). Salicylic acid causes a significant increase in total soluble sugars and the total free amino acids of onion plants (Amin *et al.*, 2007). The changes in ion accumulation like sodium, potassium, calcium and magnesium were different according variation in the external environment also have been investigated in a large number of plants (Abou Al-Hamd, 2007). Gaber and Dohdoh (1999) showed that saline irrigation water increased nitrogen and sodium content, while decrease the other elements such as K⁺ and Ca⁺².

The purpose of this work was to provide additional information on the role of salicylic acid in alleviation the inhibitory effect of NaCl salt in both *Chlorella vulgaris* and wheat cv. Sds-1 seedlings.

MATERIALS AND METHODS

Our experiment performed at 2007. *Chlorella vulgaris* and wheat cv. Sds-1 were the tested plants in this experiment.

Isolation and treatment of *Chlorella vulgaris* cultures: One selected chlorophycean namely *Chlorella vulgaris* (unicellular, non-motile green microalgae) was isolated from soil of Qena, Egypt and used in this investigation. *Chlorella vulgaris* was grown for 7 days in Bold's basal medium (Bischoff and Bold, 1963). This medium containing 0, 50, 100, 150, 200, 400 and 600 NaCl mM and/or SA (0.5 mM). SA is added one day prior to the addition of NaCl and pH of the nutrient solution was maintained at pH 5.5±0.2. Three replicates were prepared for each treatment.

Growth conditions and treatment of wheat cv. Sds-1 seedlings: Seeds of wheat cultivar (cv. Sds-1) were obtained from the breeding program of Agriculture Research, Dokky and Cairo, Egypt. Seeds of the tested wheat cultivar surface sterilized with mercuric chloride (0.1%) for 5 min then rinsed 3 times with distilled water. Wheat seeds were soaked for 7 h in the dark at 22°C, either in 0.5 mM SA or in distilled water as control and left to dry for 15 h. Wheat seeds (20 seed) were germinated in Petri dishes (9 cm diameter) provided with two filter papers saturated with 20 mL

distilled water containing 0 (control), 50, 100, 150 and 200 mM NaCl. The pH of the solution was maintained at pH 5.5±0.2. Three replicates were prepared for each treatment. The plants were left to grow for 15 days in a growth chamber maintained at 25/19°C day/night temperature cycles.

Estimation of photosynthetic pigments: The photosynthetic pigments (Chlorophyll a, b and carotenoids) were determined according to Metzner *et al.* (1965). The pigments extract was measured against a blank of pure 85% acetone at wavelengths of 452.5, 644 and 663 nm. While net photosynthesis and dark respiration were determined using the oxygen meter (G867 mitt O₂ electrode).

Estimation of soluble carbohydrates: The water-soluble carbohydrates and total carbohydrates were quantified by the anthrone sulphuric acid method which was carried out by Fales (1951) and Schlegel (1956) and adapted by Badour (1959).

Estimation of soluble protein: To estimate soluble proteins and water insoluble protein according to the method of Lowry *et al.* (1951).

Estimation of total free amino acids: Free amino acids were extracted from algal cells or wheat seedlings and were determined according to the method of Moore and Stein (1948).

Estimation of mineral elements: A known weight (0.1 g) of the dried algae material or dried tissue material was ground into a fine powder by a micro mill and assayed for mineral determinations.

The wet digestion method (Schlegel, 1956) using perchloric acid was used. After complete charring by 1 mL conc. Sulphuric acid, 1 mL of sulphuric-perchloric mixture (1:1 by volume) of 50% perchloric was added. The flask was further left on hot plate till the sample become colorless and the majority of fumes developed. After cooling the solution was filtered and then diluted to a definite volume. Samples of this solution were taken for Na⁺ and K⁺ determination by atomic absorption spectrometer and the versene (disodium dihydrogen ethylene-diamine-tetra acetic acid) titration method (Schwarzenbach and Biedermann, 1948) was employed for calcium and magnesium determination.

Statistical analysis: The data of all experiments were subjected to analysis by the least significant differences test (L.S.D) using PC-STATE program version 1A coded by Rao M; Blane K Zannenber M University of Georgia. The p<0.05 and p<0.01 were considered statistically significant and highly significant, respectively.

RESULTS

It has been confirmed that treatment of *Chlorella vulgaris* with SA under the effect of NaCl. Photosynthetic pigments showed different degrees (Table 1). For example, a highly significant decrease has been observed in ch. a (above 150 mM NaCl) and ch.b of *Chlorella vulgaris* (over 50 mM NaCl). Also, at the higher salinity levels, there is a highly significant reduction in carotenoids. Salinity stress induced a non significant decrease in the photosynthetic oxygen evolution of *Chlorella vulgaris* up to 100 mM NaCl, after that a highly significant decrease was obtained. On the contrary, salinity stress induced a non significant increased up to 150 mM in O₂-uptake, then it increased significantly at 200 mM. At 400 and 600 mM NaCl, O₂-uptake increased

highly significantly as in Table 1. Generally, treatments with SA resulted in a higher efficiency for plant characteristics and reduced the harmful effect produced by salinity (Table 1).

Table 2 revealed that there are a highly significant decrease in ch. a concentration (between NaCl untreated wheat seedlings (control) and NaCl treated seedlings) and ch.b (above 50 mM NaCl). As well as NaCl induced a significant decrease at 100 mM and a highly significant decrease in carotenoids at 150 and 200 mM NaCl. Under the level of 200 mM NaCl, the content of ch. b was 0.614 mg g⁻¹ fresh weight in wheat leaves treated with SA against 0.535 mg g⁻¹ fresh weight in leaves treated with only NaCl.

Table 1: Effect of salinity treatments and/or SA on photosynthesis (O₂-evolution) and respiration (dark O₂-uptake) and photosynthetic pigments of *Chlorella vulgaris*

Treatments	NaCl (mM)	Chl. a (µg mL ⁻¹ algal suspension)	Chl. b (µg mL ⁻¹ algal suspension)	Carotenoids (µg mL ⁻¹ algal suspension)	O ₂ -evolution (mgL ⁻¹ algal suspension)	O ₂ -uptake (mg L ⁻¹ algal suspension)
Reference control	0	8.033	4.701	2.128	7.317	3.475
	50	7.865	4.863	2.289	7.125	3.500
	100	7.422	4.248**	2.045	7.000	3.700
	150	7.486	4.251**	2.096	5.500**	3.850
	200	5.921**	3.018**	1.809*	5.000**	4.153*
	400	5.177**	2.688**	1.652**	4.667**	4.500**
	600	4.775**	2.425**	1.401**	4.167**	5.300**
NaCl+0.5 mM SA	0	9.858**	5.834**	2.620**	8.333*	3.367
	50	9.229**	4.822	2.814**	8.700**	2.950
	100	8.853*	4.608	2.237	8.000	3.267
	150	8.413	4.486	2.259	7.000	3.550
	200	7.341	3.916**	2.090	7.250	3.550
	400	7.068**	3.603**	2.044	6.500	3.210
	600	6.452**	3.068**	1.663**	6.167**	3.200
LSD						
	5%	0.711	0.323	0.247	0.822	0.533
	1%	0.960	0.435	0.333	1.109	0.720*

*Significant differences. **Highly significant differences as compared with absolute control

Table 2: Effect of salinity treatments and/or SA on photosynthetic pigments of wheat cultivar (cv. Sds-1)

Treatments	NaCl (mM)	Chl.a (mg g ⁻¹ fresh weight)	Chl.b (mg g ⁻¹ fresh weight)	Carotenoids (mg g ⁻¹ fresh weight)
Reference control	0	1.527	0.806	0.354
	50	1.398**	0.727	0.340
	100	1.341**	0.685**	0.313*
	150	1.225**	0.584**	0.302**
	200	1.110**	0.535**	0.273**
NaCl+0.5 mM SA	0	1.512	0.833	0.358
	50	1.438	0.775	0.345
	100	1.388**	0.752	0.329
	150	1.309**	0.704*	0.311*
	200	1.155**	0.614**	0.269**
LSD				
	5%	0.090	0.088	0.037
	1%	0.123	0.120	0.050*

*Significant differences. ** Highly significant differences as compared with absolute control

The data presented in Table 3 showed that soluble carbohydrates unaffected up to 150 mM, after that there is a significant increase at 200 mM and a highly significant at 400 and 600 mM. While, soluble protein in *Chlorella vulgaris* increased highly significantly with increasing salt in the medium. Salinity stress induced a non significant increase in total free amino acids up to 200 mM, then a significant increase was recorded.

There was a marked increase in soluble carbohydrates, soluble protein and total free amino acids under SA treatment compared to control. For example, soluble carbohydrates in *Chlorella vulgaris* reached a maximum value (53.833 mg g⁻¹ dry weight) when treated with SA and remained highly significantly higher than control at 100 up to 600 mM NaCl. Also, soluble protein and total free amino acids depended on the dose of NaCl in a similar to soluble carbohydrates manner. The data presented showed that the highest content of soluble protein and total free amino acids (76.658 mg g⁻¹ dry weight and 16.353 mg g⁻¹ dry weight respectively) in *Chlorella vulgaris* treated with SA observed at 600 mM NaCl.

Our results in Table 4 demonstrated that NaCl induced a highly significant increase in both soluble carbohydrates (at 150 and 200 mM NaCl) and soluble protein (at 200 mM NaCl) of wheat seedlings. While, total free amino acids remained more or less unchanged up to 100 mM. After that, there is a significant reduction at 150 mM and a highly significant at 200 mM. The lowest value of total amino acids (27.451-mg g⁻¹ dry weight) was recorded at 200 mM NaCl. SA treatment induced a noticeable enhancement in soluble carbohydrates and soluble protein under salinity stress. Since, soluble carbohydrates and soluble protein reached a maximum value (78.190 mg g⁻¹ dry weight and 194.360 mg g⁻¹ dry weight respectively) when treated with SA and remained highly significantly higher than control (from of 0.0 + 0.5 SA up to 200 mM NaCl+ 0.5 SA).

Salinity levels stimulated the accumulation of Na⁺ in *Chlorella vulgaris* and. The content of Na⁺ in *Chlorella* increased from 3.910-mg g⁻¹ dry weight to 19.120-mg g⁻¹ dry weight (Table 5). An

Table 3: Effect of salinity treatments and/or SA on soluble carbohydrates, soluble protein and total free amino acids of *Chlorella vulgaris*

Treatments	NaCl (mM)	Soluble carbohydrates (mg g ⁻¹ dry weight)	Soluble protein (mg g ⁻¹ dry weight)	Total free amino acids (mg g ⁻¹ dry weight)
Reference control	0	31.787	54.634	8.040
	50	27.806	65.520**	10.013
	100	27.588	65.923**	10.427
	150	33.541	66.301**	10.973
	200	37.486*	66.528**	11.197
	400	66.550**	68.040**	11.420*
	600	72.818**	76.910**	11.560*
NaCl+0.5 mM SA	0	32.779	55.339	9.480
	50	36.082	59.875**	13.780**
	100	40.293**	63.000**	14.600**
	150	38.551**	66.377**	15.307**
	200	52.369**	72.274**	16.013**
	400	53.833**	75.600**	12.867**
	600	48.424**	76.658**	16.353**
LSD				
	5%	4.528	3.265	3.321
	1%	6.109	4.405	4.480*

*Significant differences. **Highly significant differences as compared with absolute control

Table 4: Effect of salinity treatments and/or SA on soluble carbohydrates, soluble protein and total free amino acids of wheat cultivar (cv. Sds-1)

Treatments	NaCl (mM)	Soluble carbohydrates (mg g ⁻¹ dry weight)	Soluble protein (mg g ⁻¹ dry weight)	Total free amino acids (mg g ⁻¹ dry weight)
Reference control	0	35.247	119.146	38.496
	50	39.827	123.379	41.984
	100	44.407	132.57	36.944
	150	62.811**	139.860*	31.816*
	200	63.162**	145.314**	27.451**
NaCl+0.5 mM SA	0	62.025*	151.929**	43.456
	50	53.930**	148.309**	44.427
	100	57.354**	163.674**	44.672*
	150	62.811*	167.454**	41.184
	200	78.190**	194.360**	39.899
LSD				
	5%	9.166	18.122	6.016
	1%	12.501	24.716	8.205

*Significant differences. **Highly significant differences as compared with absolute control

Table 5: Effect of salinity treatments and/or SA on mineral elements [sodium (Na⁺) content, potassium (K⁺) content, calcium (Ca⁺⁺) content, magnesium (Mg⁺⁺)] content of *Chlorella vulgaris*

Treatments	NaCl (mM)	Na ⁺ (mg g ⁻¹ dry weight)	K ⁺ (mg g ⁻¹ dry weight)	Ca ⁺⁺ (mg g ⁻¹ dry weight)	Mg ⁺⁺ (mg g ⁻¹ dry weight)
Reference control	0	3.910	18.337	32.533	4.480
	50	4.961	17.844	31.467	4.160
	100	8.646**	17.103*	30.933	4.080
	150	9.804**	16.610**	30.400	3.840
	200	13.443**	15.869**	28.000	3.360*
	400	15.173**	15.129**	21.333**	3.120*
	600	19.120**	12.661**	20.000**	2.880**
NaCl+0.5 mM SA	0	3.694	21.299**	33.600	4.640
	50	4.065	20.682**	33.067	4.800
	100	6.561**	20.559**	32.800	4.320
	150	7.302**	20.312**	31.200	3.840
	200	13.196**	20.188**	31.467	3.840
	400	14.308**	19.942**	30.400	3.520
	600	17.421**	19.325	28.800	3.360*
LSD					
	5%	1.376	1.117	4.743	1.070
	1%	1.857	1.508	6.399	1.444*

*Significant differences. **Highly significant differences as compared with absolute control

opposite trend for K⁺, Ca⁺⁺ and Mg⁺⁺ contents was obtained. The lowest value of Ca⁺⁺ (20.000 mg) was observed at the highest salinity concentration. The content of K⁺ in treated *Chlorella* with SA increased at all salinity levels than control.

There is a highly significant increase in the accumulation of Na⁺ in wheat cv. Sds-1 seedlings under salinity stress. At the lowest NaCl concentration (50 mM NaCl), the value of Na⁺ was 16.154 mg g⁻¹ dry weight in salinized plant against 8.646 mg in the control plant. As well as the content of Na⁺ was 25.730 mg g⁻¹ dry weight at 200 mM NaCl (the highest NaCl concentration). While a highly significant reduction have been observed in K⁺ and Mg⁺⁺ (at 150 and 200 mM NaCl)

Table 6: Effect of salinity treatments and/or SA on mineral elements [sodium (Na⁺) content, potassium (K⁺) content, calcium (Ca⁺⁺) content, magnesium (Mg⁺⁺)] content of wheat cultivar (cv. Sds-1)

Treatments	NaCl (mM)	Na ⁺ (mg g ⁻¹ dry weight)	K ⁺ (mg g ⁻¹ dry weight)	Ca ⁺⁺ (mg g ⁻¹ dry weight)	Mg ⁺⁺ (mg g ⁻¹ dry weight)
Reference control	0	8.646	16.47	10.4	4.8
	50	16.154**	14.86	8.8	4.32
	100	21.097**	14.471*	7.200**	4.32
	150	22.796**	13.638**	6.933**	3.120**
	200	25.730**	12.361**	6.400**	2.880**
NaCl+0.5 mM SA	0	12.756**	26.710**	8.4	4.8
	50	14.610**	27.358**	7.600*	4.56
	100	18.780**	25.877**	8.4	4.56
	150	20.788**	25.785**	8.000*	4.32
	200	21.714**	25.414**	7.733*	3.84
LSD					
	5%	2.104	1.923	2.065	1.269
	1%	2.87	2.622	2.817	1.731

* Significant differences ** Highly significant differences as compared with absolute control.

and in Ca⁺⁺ (after 50 mM NaCl). SA increased K⁺ content in treated wheat cv. Sds-1 at all salinity levels than control. The maximum value of K⁺ was 27.358 mg g⁻¹ dry weight at 50 mM NaCl+ 0.5 mM SA (Table 6).

DISCUSSION

Our results showed that the photosynthetic pigments in *Chlorella vulgaris* markedly decreased especially at the higher salinity levels. El-Sheekh and Hafez (2002) showed that by increasing the time of exposure of *Chlorella vulgaris* to salinity, there was a gradual decrease in the chlorophyll content. Also in the present work, salt stress reduced the pigment biosynthesis in wheat plants. These results were in close agreement with those obtained by Teizera and Pereira (2007). Manivannan *et al.* (2008) reported that the reduction in leaf chlorophyll under salinity has been attributed to the destruction of the chlorophyll pigments and the instability of the pigment protein complex. Moreover, chloride may play an important role in inhibition of chloroplast reaction by inhibiting the synthesis of rubisco and chlorophyll or accelerate chlorophyll degradation (Musyimi *et al.*, 2007).

On contrast of our results, Anand *et al.* (1994) found that 3% salinity increased chlorophyll a of *Chroococcus minor* and *Oscillatoria salina*.

Our result revealed that SA caused a marked increase in the pigment fractions. The increase in pigment content after salicylic acid treatment was also obtained in soybean (Zhao *et al.*, 1995) and wheat (Singh and Usha, 2003) grown under normal or stress conditions. Enhancing effects of SA on photosynthetic capacity could be attributed to its stimulatory effects on Rubisco activity and pigment content (Khodary, 2004).

Also our data confirmed that the photosynthetic oxygen evolution of *Chlorella vulgaris* highly significantly decreased at the higher salinity levels. El-Sheekh (2004) found that the rate of oxygen evolution of *Chlorella vulgaris* under steady state and flash light conditions diminished by increasing salt concentrations. Wright and Reed (1985) have suggested the decline in photosynthesis under hyperosmotic stresses might be due to changed fine structure of the chloroplast causing a disruption of energy transfer between the 2 photosystems (Fu and Bell, 2003).

Salicylic acid treatments increased the photosynthetic oxygen evolution of tested *Chlorella vulgaris* and thus alleviated the effect of salt stress. These results agreed with those of Galal (2007), who reported that SA alleviated the inhibitory effect of salinity on the rate of photosynthesis and pigment contents in *Scenedesmus*. Also, Galal (2007) found that SA induced a slight increase in the rate of photosynthesis as compared with that of control treatment. In accordance with this, Tari *et al.* (2002) established that SA increased the rate of photosynthetic electron transport above the control level and increased the photochemical quenching parameter in the presence of Na⁺.

The results of respiration in this work was agreed with the results obtained by Tropin (2004), who stated that stress is associated with increased dark respiration and maintenance respiration. Schwarz and Gale (1984) mentioned that the increase in maintenance respiration under salt stress could be regarded as a characteristic feature of salt tolerance. This enhancement in respiration of salinized plants could be due to the need for a higher energy allocation for maintenance of osmotic adjustment, ion concentration gradient and active transport processes for the repair of tissues (Hamada and El-Enany, 1994).

Our results of interaction of salinity and SA were in close agreement with the results of Babalar *et al.* (2007), who demonstrated that SA in a concentration dependent manner effectively reduce respiration in plants and harvested fruits.

Alamgir and Ali (1999) found that sugar content increased in some genotypes of rice but also decrease in the others genotypes. Rao *et al.* (2007) reported that algae produced carbohydrates as an osmoprotectant at higher salinity. Abdel-Samad *et al.* (2004) also reported the role of soluble carbohydrate in osmotic adjustment.

Wheat cv. Sds-1 plants and *Chlorella vulgaris* increased soluble carbohydrates accumulation. This increased related to salt tolerance. In this respect, Mohammed (2007) stated that the sugar accumulation and its distribution in different plants might be availed trait to discriminate genotypes of different tolerance to saline and osmotic stresses.

An important point should be taken in consideration that SA application increased the soluble carbohydrates content in *Chlorella vulgaris* above the absolute control value at all the salinity levels used. These results agreed with those of Tari *et al.* (2002) who showed that the reduction in total reducing sugar contents in tomato plants was partially reversed by SA providing a pool of compatible osmolytes in the presence of sodium. On the contrary, Khodary (2004) reported that the decrease in soluble carbohydrates content by reason of SA treatment might be assumed to inhibit polycarbohydrates-hydrolysing enzyme system on one hand and/or accelerate the incorporation of soluble sugar into polycarbohydrates.

The results of soluble protein in the present work were in close agreement with the findings of Abou Al-Hamd (2007) and Shanab and Galal (2007) who showed that salinity increased soluble protein. Conversely, Parida *et al.* (2002) found that soluble protein was decreased in response to salinity. The high soluble proteins of tested *Chlorella* and wheat might play an important role in increasing the osmotic pressure of the cytoplasm.

Our results showed that SA increased soluble proteins (as osmolytes) in salinized plants. It is therefore concluded that SA might alleviate the imposed salt stress via osmotic adjustment.

Our data revealed that the accumulation of amino acids was increased in *Chlorella*. While in wheat cv. Sds-1 seedlings was decreased under NaCl treatment. These results agreed with the results obtained by Abdel-Latef (2005).

In this work, SA stimulated the total free amino acids content. Hussein *et al.* (2007) found that all determined amino acids concentrations were increased with the application of SA.

Difference with our results, SA treatment induced a distinct decrease in soluble protein content as well as the amino acids of salinized *Scenedesmus* sp. Thus, the reduction in protein content is not at the expense of amino acids (Galal, 2007).

It is worthy to mention that the amount of Na⁺ in wheat plants at the level of 50 and 200 mM NaCl was twice as the amount of Na⁺ in *Chlorella vulgaris* at the same levels. It might be expected that the tolerance of *Chlorella vulgaris* was correlated to its high capacity to reduce the absorption of Na⁺ which minimizing the negative effect that those ions produce in the cells. In this respect, Murillo-Amador *et al.* (2006) reported that salt-tolerance in glycophytes is associated with the ability to limit uptake and/or transport of saline ions (mainly Na⁺ and Cl⁻) from the root to aerial parts. Our results demonstrated that salinity stress induced a distinct decrease in uptake of K⁺, Ca²⁺ and Mg²⁺ in both tested plants at higher Na⁺ concentration. It also reported by Hosseini and Thengane (2007) and Pagter *et al.* (2009), on the contrary, Yildirim *et al.* (2006) showed that an increase in concentration of K⁺ and Ca²⁺ in plants under salt stress could ameliorate the deleterious effects of salinity on growth and yield.

Decreasing the level of Na⁺ by SA in this work explain the ameliorating effect of SA likely due to increased antioxidant activity of SA on growth of NaCl stressed plants. Similarly, Al-Hakimi and Hamada (2001) found that SA decreased Na⁺ content of wheat shoot and root tissues under salinity. On the other hand, application of SA induced a marked stimulation in the contents of K⁺, Ca⁺⁺ and Mg⁺⁺ in tested *Chlorella* and wheat. Gunes *et al.* (2007) observed the similar effects of SA on Na⁺, K⁺, Ca⁺⁺ and Mg⁺⁺ content of wheat plants grown under salinity. The recorded increase in K⁺, Ca⁺⁺ and Mg⁺⁺ in both tested plants as a result of SA treatment might play a role in salt tolerance of them, which in turn increased the driving force for water and consequently maintaining tissue water content and growth of the two experimental plants. This might suggest that the deteriorate effects of salt stress on plant growth and some relevant physiological activities of tested plants might be alleviated and/or modified to some extent by the use of SA.

CONCLUSION

Salicylic acid treatment might stimulate salinity tolerance in *Chlorella vulgaris* and wheat cv. Sds-1 seedlings via accelerating their photosynthesis performance, which might be responsible for the improved growth in these plants. The accumulation of soluble carbohydrates, soluble protein, total free amino acids and cations (K⁺, Ca⁺⁺ and Mg⁺⁺) in SA-treated plants might participate in increasing their salt tolerance via increasing the internal osmotic pressure. Therefore, our results suggest that SA might be used for improving plant growth and yield in saline areas.

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