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Research Article
Regulation of Antioxidant System in Wheat Cultivars by Using Chitosan or Salicylic Acid to Improve Growth and Yield under Salinity Stress

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Abstract

Background and Objective: Chitosan (CHT) is a natural molecule that stimulates many biological responses in plants. Salicylic acid (SA) is endogenous growth bioregulator in plants. Applications of chitosan and salicylic acid have defensive effects on plants in improving salinity stress. This study designed to evaluate the response of wheat cultivars for CHT or SA in alleviating salinity stress. **Materials and Methods:** Field experiment was carried out at the experimental station, Wadi El-Natrun district El-Behera Governorate, Egypt, during the two winter seasons of 2017/2018 and 2018/2019. To study the effect of spraying CHT and SA on wheat (*Triticum aestivum* L.) cultivars (Sakha 94 and Gemmieza 9) grown in saline soil. **Results:** Grains priming with different treatments of CHT and SA increased all studied morphological parameters as compared with the corresponding controls. Both spraying treatment materials increased photosynthetic pigments, osmoprotectant substances in both wheat cultivars. Treated plants with both materials increased in antioxidant enzymes, antioxidant compounds and decreased lipid peroxidation in both wheat cultivars. Applications of CHT or SA increased all studied yield parameters as well as the content of carbohydrates and protein of the yielded grains in both cultivars. **Conclusion:** Foliar spraying with CHT and SA improved the yield parameters of wheat cultivars grown in saline soil. High level of SA on both cultivars proved to be the most effective and Gemmieza 9 cultivar surpassed Sakha 94 in grain yield.

Key words: Chitosan, salicylic acid, plant growth, enzymes, antioxidant compounds, wheat, yield

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil salinization is a major limitation factor contributing to the loss of productivity of cultivation[1]. Salinity is one of the most determinant problems in arid and semi-arid regions especially with low amounts of irrigation water, high evapotranspiration rate and shortage of rainfall, which has a harmful influence on crop production. In Egypt, the great extent land reclamation requires great amounts of water for irrigation in order that undertaking powerful plant growth and high production. This has made it essential to use different sources of irrigation water that have relatively high salinity levels like well water. Sairam and Tyagi[2] stated that salt stress is believed one of the most important abiotic stress limiting plant growth and productivity as the result of producing the reactive oxygen species.

Chitosan (CHT) is a natural molecule that stimulates many biological responses in plants. The CHT was the initial shown to improve defense responses to abiotic and biotic stresses[3]. CHT is an initial oxidative rupture with hydrogen peroxide (H_2O_2) accumulation in different plants[4] as well as in plant cell cultures[5]. It is noticed that H_2O_2 accumulation could result to the induction of plant defense antioxidant enzymes and to the synthesis of secondary metabolites (polyphenolics and flavonoids) observed in different plant when treated with CHT[6]. Other biochemical and molecular changes observed in plants fed with CHT include callose apposition[7], increases in cytosolic Caion[8], plasma membrane H^+-ATPase inhibition[9], chromatin alterations, synthesis of alkaloids[10] and phyto-regulators jasmonic acid and abscisic acid[11]. Moreover, CHT stimulated ABA activity which plays a key role in the regulation of stomatal aperture and decreased the rate of transpiration when the plant is exposed to stress[12]. Therefore, it had been suggested that CHT may be a prospective anti-transparent that helps different crops to overcome drought stress.

Salicylic acid (SA) is a phenolic endogenous growth bioregulator in plants works as antioxidant compound, which contributes in the regulation of physiological processes in plants. Al-Hakimi[13] and Hayat *et al.*[14] reported that, SA plays an essential role in the defense mechanisms against abiotic stress. The SA plays an essential role in the regulation of ROS and antioxidant enzymes[15]. It is an endogenous signal molecule for the activation of plant growth and plant defense responses to systemic acquired. It has been shown that SA can markedly improve germination under salt stress[16]. Also, exogenously applied SA can significantly increase plant growth under both saline and non-saline conditions[17]. While the concentration of SA is between 1 and 10 mM significantly reduces transpiration in leaves of kidney bean (*Phaseolus vulgaris*) via regulating the behavior of stomata[18].

The objective of this work was to evaluate the protective role of chitosan (CHT) or salicylic acid (SA) in relation to growth, compatible solute and antioxidant defense system (antioxidant enzyme and non-enzymatic antioxidants) as well as yield and nutritional value in both wheat cultivars (Sakha 94 and Gemmieza-9) grown in saline soil.

MATERIALS AND METHODS

Study area: The field experiment was carried out in the experimental station at Wadi El-Natrun District, El-Behera Governorate, Egypt, North Africa Sahara (arid or semi-arid region) during the two winter seasons of 2017/2018 and 2018/2019. Grains of wheat cultivars (Sakha 94 and Gemmieza 9) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The soil texture of the experimental site was sandy. Soil analysis was performed according to the method described by Chapman and Pratt[19]. Some physical and chemical properties of a representative soil sample were listed in Table 1. Analysis of irrigation water was presented in Table 2.

Research procedure: The experimental design was split plot design with four replicates. The two cultivars (Sakha 94 and Gemmieza 9) occupy the main plots and the treatments of (CHT and SA) were allocated at random in the sub plots. The grains were soaked in different treatments with CHT (10 and 20 mg L^{-1}) and SA (25 and 50 mg L^{-1}) for 12 h before sowing.

Table 1: Physical and chemical analysis of the experimental site soil in the two seasons

Soil analysis	2017/2018	2018/2019
Physical properties		
Sand (%)	92.27	92.55
Silt (%)	5.20	5.15
Clay (%)	2.53	2.30
Texture class	Sandy loam	Sandy loam
Chemical properties		
$pH_{(1:1)}$	7.29	7.23
$EC_{(1:1)}$ (dS m^{-1})	5.22	5.14
Organic matter (%)	0.62	0.65
Total $CaCo_3$ (%)	5.91	4.74
Available N (mg kg^{-1})	8.90	8.40
Available P (mg kg^{-1})	2.04	2.15
Available K (mg kg^{-1})	187	178
Irrigation system	Drip irrigation	Drip irrigation

Table 2: Chemical analysis of irrigation water

		Electric conductivity		Ions concentration (milliequivalents L^{-1})						
Seasons	pH	dS m^{-1}	ppm	HCO_3^-	Cl^-	SO_4^-	Ca^{++}	Mg^{++}	Na^+	K^+
2017/2018	7.5	4.2	2688	3.2	29.1	7.9	5.3	4.6	32.5	0.55
2018/2019	7.6	4.3	2752	3.3	29.8	7.4	5.1	4.4	33.1	0.59

Wheat (*Triticum aestivum* L.) grains were sown at the end of November in both seasons in rows, 4 m long, a distance of 25 cm among rows. Plot area was 12 m (3.0 m width '4.0 m length). The recommended agricultural practices of growing wheat grains were applied; the seeding rate was (144 kg grains ha^{-1}). Pre-sowing, 360 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate (33.5% N) at rate of 180 kg ha^{-1} was applied at 5 equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52% K_2O) was added at two equal doses of 120 kg ha^{-1}, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Plant samples were taken after 75 days from sowing for measurements of growth characters (shoot length, number of leaves/tiller, fresh and dry weight of tiller). Chemical analysis measured were photosynthetic pigments, compatible solutes, lipid peroxidation, antioxidant enzymes (POX, SOD and CAT) and antioxidant compounds. At harvest, the following characters were recorded on random samples of 10 plants to estimate the following parameters: Plant height (cm), 1000 grains weight (g), grain yield/spike (g), straw yield, biological yield (ton ha^{-1}) and grain yield (ton ha^{-1}). Some chemical parameters were measured on the harvested grains as proteins (%), carbohydrates (%) and some macro-elements (N, P, K, Na and Ca).

Irrigation water requirements: Three irrigation water requirements calculated using Penman Monteith equation and crop coefficient according to Allen *et al.*[20]. The average amount of irrigation water applied with sprinkler irrigation system was 6000 m^3/ha/season for both seasons of the experimental work.

The amounts of irrigation water were calculated according to the following equation:

$$IWR = \left[\frac{ET0 \times Kc \times Kr \times I}{Ea} + LR\right] \times 4.2$$

where, IWR is the irrigation water requirement m^3/ha/irrigation, ET_0 is the reference evaporation-transpiration (mm/day), Kc is the crop coefficient, Kr is the reduction factor[21], I is the irrigation interval (day), Ea is the irrigation efficiency, 90%, LR is the leaching requirement = 10% of the total water amount delivered to the treatment.

Water-use efficiency (WUE): The WUE values calculated with the fowling equation[22]:

$$WUE = \frac{E_y}{E_t} \times 100$$

where, WUE is the water use efficiency (kg m^{-3}), E_y is the economical yield (kg/ha/season), E_t is the total applied of irrigation water (m^3/ha/season).

Chemical analysis

Photosynthetic pigments: Chlorophyll a, chlorophyll b and carotenoids were determined using spectrophotometric method described by Lichtenthaler and Buschmann[23]. Total soluble sugars were extracted by the method of Prud'homme *et al.*[24] and analyzed according to Yemm and Willis[25]. Free amino acids and proline were extracted according to the method described by Vartanian *et al.*[26]. Free amino acids were determined with the ninhydrin reagent method of Yemm *et al.*[27]. Proline was assayed according to the method described by Bates *et al.*[28]. Total carbohydrate was determined according to DuBois *et al.*[29]. The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges *et al.*[30]. Enzyme extracts were prepared according to method of Chen and Wang[31]. Catalase (CAT, EC 1.11.1.6) activity was determined by following the decrease in absorbance using spectrophotometer at 240 nm. Superoxide dismutase (SOD, EC 1.12.1.1) activity was spectrophotometrically assayed at 560 nm by nitro-blue-tetrazolium (NBT) reduction method by Chen and Wang[31]. Peroxidase (POX, EC 1.11.1.7) activity was evaluated according to Kumar and Khan[32]. Total N was determined by using micro-Kjeldahl method as described in AOAC[33]. β-carotene and lycopene were determined according to the method of Nagata and Yamashita[34]. The total flavonoids content was determined following the spectrophotometric method of Dewanto *et al.*[35]. Anthocyanin was extracted and measured according to Mirecki and Teramura[36]. P, K, Na and Ca, was determined by the method described by Chapman and Pratt[19].

Statistical analysis: The data were statistically analyzed on complete randomized design under split plot system using MSTAT-C[37] software. Means were compared by using least significant difference (LSD) at 5% level of probability.

RESULTS

Growth parameters: The growth parameters of both wheat cultivars (Sakha 94 and Gemmieza 9) grown under saline soil in response to treatment with different concentrations of CHT or SA are presented in Table 3. Treatment of wheat plants with different concentrations of CHT or SA enhanced all studied morphological parameter (plant height (cm), leaves number/tiller, tiller fresh and dry weight (g) as well as water content percentages as compared with the corresponding controls. The most pronounced effect noticed on the plants treated with SA than CHT in both cultivars as compared with the corresponding controls.

Photosynthetic pigments: Treatment of both wheat cultivars with different concentrations of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) increased photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) as compared with the corresponding controls as shown in Table 4. The maximum increases in total pigments were achieved by using SA as compared with the corresponding control in both cultivars. Total pigments increase from 22.51-29 and 30.41 mg g^{-1} fresh weight (~29 and 35%) in Sakha 94 compared to its control plants and from 23.86-30.30 and 33.94 mg g^{-1} fresh weight (~27 and 42%) in Gemmieza 9 treated with SA25 and 50 mg L^{-1}, respectively compared to its control plants. Gemmieza 9 cultivar surpassed Sakha 94 in photosynthetic pigments in all used treatments.

Changes in compatible solutes: Data in Table 5 showed the effect of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) on compatible solutes (TSS, proline and free AA) of the two experimental wheat cultivars. Treatment of wheat cultivars with different concentrations of CHT or SA increased TSS, proline and free AA. In both cultivars, the maximum increases in proline and free AA were obtained by using the lowest concentrations (CHT 10 and SA 25 mg L^{-1}), meanwhile the same trend for TSS obtained using high concentrations (CHT 20 and SA25 mg L^{-1}) of both CHT and SA as compared with the corresponding control.

Table 3: Effect of chitosan or salicylic acid at different concentrations on morphological criteria of wheat cultivars at 75 days from sowing under saline soil

Treatments (mg L^{-1})	Plant height (cm)	Leaves number/tiller	Tiller fresh weight (g)	Tiller dry weight (g)	Water content (%)	Root fresh weight (g)	Root dry weight (g)
Sakha 94*							
Control (0.0)	40.52±0.29	6.30±0.34	6.49±0.16	1.52±0.03	76.58±0.10	1.80±0.06	0.83±0.03
Chitosan (10)	41.69±0.14	7.25±0.32	7.94±0.19	1.55±0.05	80.48±1.35	2.21±0.05	1.16±0.07
Chitosan (20)	42.83±0.04	7.00±0.27	7.58±0.19	1.59±0.04	79.02±0.12	2.52±0.04	1.43±0.06
Salicylic acid (25)	52.09±0.06	7.67±0.33	8.10±0.29	1.69±0.09	79.14±1.44	2.65±0.05	1.56±0.05
Salicylic acid (50)	46.46±0.32	7.56±0.31	8.21±0.24	1.73±0.05	78.93±1.35	2.82±0.04	1.62±0.05
Gemmieza 9*							
Control (0.0)	43.53±0.48	6.72±0.29	6.03±0.28	1.57±0.06	73.96±2.19	1.70±0.01	0.98±0.07
Chitosan (10)	45.23±0.24	7.53±0.34	6.93±0.01	1.67±0.03	75.90±0.49	2.07±0.03	1.43±0.10
Chitosan (20)	46.45±0.29	7.63±0.31	7.70±0.05	1.69±0.03	78.05±0.26	2.10±0.06	1.47±0.01
Salicylic acid (25)	49.35±0.13	7.33±0.29	7.98±0.05	1.88±0.07	76.44±0.73	2.05±0.003	1.51±0.01
Salicylic acid (50)	47.09±0.35	7.33±0.28	7.64±0.09	1.75±0.05	77.09±0.74	2.00±0.04	1.47±0.04
LSD 5%	0.73	1.03	0.50	0.15	2.34	0.13	0.09

Each value represents the mean±standard error (n = 3), *Cultivars

Table 4: Effect of chitosan or salicylic acid at different concentrations on photosynthetic pigments (mg g^{-1} fresh weight) of wheat cultivars at 75 days from sowing

Treatments (mg L^{-1})	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Sakha 94*				
Control (0.0)	14.88±0.31	4.42±0.11	3.21±0.01	22.51±0.18
Chitosan (10)	15.60±0.14	4.89±0.01	3.74±0.08	24.22±0.21
Chitosan (20)	15.44±0.27	5.00±0.05	4.38±0.15	24.82±0.16
Salicylic acid (25)	18.77±0.28	5.46±0.09	4.78±0.01	29.00±0.21
Salicylic acid (50)	19.94±0.11	5.56±0.10	4.91±0.05	30.41±0.07
Gemmieza 9*				
Control (0.0)	15.85±0.10	4.34±0.05	3.67±0.02	23.86±0.07
Chitosan (10)	17.38±0.24	5.04±0.19	4.10±0.02	26.52±0.03
Chitosan (20)	16.03±0.34	4.47±0.12	4.33±0.08	24.83±0.38
Salicylic acid (25)	20.13±0.19	4.61±0.12	5.55±0.03	30.30±0.11
Salicylic acid (50)	21.62±0.16	6.40±0.11	5.92±0.13	33.94±0.18
LSD 5%	0.72	0.32	0.23	0.01

Each value represents the mean±standard error (n = 3), *Cultivars

Lipid peroxidation: The presented data (Table 6) showed that lipid peroxidation (Malondialdehyde content) decreased in wheat cultivars (Sakha 94 and Gemmieza 9) plants in response to treatment with different concentrations of CHT or SA. There is a gradual reduction with increasing concentration of both materials.

Antioxidant enzymes: Foliar spray of wheat cultivars with CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) increased various antioxidant enzyme activities (POX, CAT and SOD) compared to the corresponding control (Table 6). Data clearly show that, CHT (10 mg L^{-1}) was more effective in POX and CAT activities than other treatments in Sakha 94 as well as, SA (50 mg L^{-1}) was more effective than other treatments in Gemmieza 9. While in SOD the maximum increased was obtained with the SA (25 mg L^{-1}) and CHT (20 mg L^{-1}) treatments in Sakha 94 and Gimmeiza 9, respectively.

Antioxidant compounds: Table 7 shows the influence of different concentrations of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) on antioxidant compounds (Phenols,

Table 5: Effect of chitosan or salicylic acid at different concentrations on total soluble sugar (TSS), free amino acids (FAA) and proline (mg/100 g dry weight) of wheat cultivars at 75 days from sowing

Treatments (mg L^{-1})	TSS	Proline	FAA
Sakha 94*			
Control (0.0)	1323±46.19	176.58±6.23	445.78±14.63
Chitosan (10)	2047±15.06	342.58±17.28	579.73±8.69
Chitosan (20)	2816±74.80	263.85±4.28	467.16±4.04
Salicylic acid (25)	2243±2.51	571.50±6.03	556.75±1.41
Salicylic acid (50)	4298±26.61	531.92±7.89	472.50±3.91
Gemmieza 9*			
Control (0.0)	2490±40.84	208.14±5.11	506.89±1.73
Chitosan (10)	2807±44.18	249.14±10.31	542.55±1.51
Chitosan (20)	2940±170.61	210.84±1.28	523.55±10.42
Salicylic acid (25)	2649±54.52	323.65±9.21	667.54±11.42
Salicylic acid (50)	4352±4.52	264.50±4.84	590.28±7.09
LSD 5%	46.19	24.05	23.95

Each value represents the mean±standard error (n = 3), *Cultivars

Table 6: Effect of chitosan or salicylic acid at different concentrations on lipid peroxidation and antioxidant enzymes activity (g fresh weight/h) of wheat cultivars at 75 days from sowing

		Antioxidant enzymes		
Treatments (mg L^{-1})	Lipid peroxidation	POX	CAT	SOD
Sakha 94*				
Control (0.0)	10.31±0.19	16.33±0.13	392.7±2.97	71.56±0.27
Chitosan (10)	7.87±0.17	25.25±0.14	432.2±0.30	92.92±0.21
Chitosan (20)	7.36±0.09	22.54±0.12	420.8±0.23	99.00±0.31
Salicylic acid (25)	8.36±0.07	16.71±0.24	425.6±0.25	113.50±0.20
Salicylic acid (50)	8.14±0.04	20.62±0.25	412.6±0.34	93.20±0.16
Gemmieza 9*				
Control (0.0)	12.81±0.28	18.97±0.25	373.1±0.24	74.40±0.23
Chitosan (10)	10.26±0.08	24.56±0.30	401.5±0.54	151.74±0.66
Chitosan (20)	9.56±0.33	37.03±0.12	412.3±0.34	131.50±0.55
Salicylic acid (25)	8.33±0.16	27.66±0.29	407.7±2.11	113.50±0.17
Salicylic acid (50)	8.29±0.10	48.68±0.27	452.2±0.27	112.40±0.28
LSD 5%	0.51	0.69	0.13	2.97

Each value represents the mean±standard error (n = 3), *Cultivars

Table 7: Effect of chitosan or salicylic acid at different concentrations on antioxidant compounds of wheat cultivars at 75 days from sowing

Treatments (mg L^{-1})	Phenols (mg/100 g dry weight)	Flavonoids (mg/100 g dry weight)	Lycobine (µg g^{-1} dry weight)	β-carotene (µg g^{-1} dry weight)	Anthocyanin (mg g^{-1} dry weight)
Sakha 94*					
Control (0.0)	21.28±0.30	4.15±0.09	0.198±0.01	1.866±0.08	0.1402±0.003
Chitosan (10)	24.04±0.37	4.29±0.14	0.414±0.01	5.366±0.11	0.1424±0.001
Chitosan (20)	19.82±0.47	4.61±0.22	0.439±0.03	8.021±0.05	0.1686±0.002
Salicylic acid (25)	24.29±0.28	4.76±0.04	0.474±0.04	8.531±0.08	0.1580±0.003
Salicylic acid (50)	21.91±0.34	4.20±0.14	0.268±0.02	7.788±0.29	0.1917±0.003
Gemmieza 9*					
Control (0.0)	18.92±0.27	3.83±0.08	0.179±0.01	1.739±0.07	0.1413±0.001
Chitosan (10)	20.85±0.16	4.32±0.10	0.292±0.03	3.369±0.17	0.1663±0.004
Chitosan (20)	23.82±0.24	5.32±0.07	0.340±0.02	4.700±0.27	0.2507±0.003
Salicylic acid (25)	20.11±0.26	4.26±0.11	0.434±0.03	5.890±0.25	0.1831±0.002
Salicylic acid (50)	17.13±0.22	3.92±0.15	0.499±0.02	7.867±0.06	0.1779±0.004
LSD 5%	0.93	0.33	0.01	0.51	0.01

Each value represents the mean±standard error (n = 3), *Cultivars

Table 8: Effect of chitosan or salicylic acid at different concentrations on yield components of wheat cultivar under saline soil

Treatments (mg L^{-1})	Plant height (cm)	Spike length (cm)	Spikelet number/spike	Spike weight (g)	Grains weight/spike	Grains number/spike	1000 grains weight	Straw yield (t ha^{-1})	Grain yield (t ha^{-1})	Biological yield (t ha^{-1})
Sakha 94*										
Control (0.0)	55.36±0.09	8.91±0.42	13.19±0.57	1.60±0.01	1.48±0.17	39.8±1.56	39.68±0.68	5.00±0.31	2.84±0.18	7.84±0.25
Chitosan (10)	57.98±0.52	9.21±0.47	13.42±0.37	1.99±0.03	1.81±0.01	42.2±1.50	42.71±1.12	5.33±0.12	3.94±0.14	9.26±0.58
Chitosan (20)	56.40±0.49	9.35±0.55	14.53±0.13	1.99±0.06	1.73±0.02	43.7±0.58	42.13±4.63	5.69±0.28	3.96±0.05	9.64±0.26
Salicylic acid (25)	62.88±0.05	10.48±0.23	15.57±0.07	2.19±0.03	2.01±0.06	43.9±2.54	45.31±0.21	6.34±0.23	4.04±0.13	10.38±0.33
Salicylic acid (50)	56.94±0.77	10.57±0.44	15.59±0.27	1.99±0.09	1.83±0.10	43.4±3.58	46.79±0.47	6.20±0.15	4.26±0.13	10.46±0.29
Gemmieza 9*										
Control (0.0)	57.23±0.03	9.49±0.07	13.60±0.09	1.87±0.05	1.52±0.11	39.5±3.64	41.18±2.27	5.10±0.12	2.89±0.14	8.00±0.09
Chitosan (10)	59.63±0.32	9.85±0.36	13.96±0.31	1.89±0.10	1.69±0.03	44.3±1.39	42.60±2.25	5.86±0.12	3.55±0.12	9.46±0.27
Chitosan (20)	59.89±0.19	9.92±0.47	14.99±0.44	1.89±0.16	1.71±0.04	45.0±0.58	45.88±2.88	6.07±0.05	4.20±0.23	10.50±0.42
Salicylic acid (25)	64.93±0.31	10.54±0.27	14.84±0.23	1.92±0.15	1.99±0.02	43.2±0.64	43.99±2.89	6.47±0.23	4.31±0.21	10.78±0.62
Salicylic acid (50)	65.98±0.57	10.75±0.09	14.91±0.16	1.98±0.18	1.95±0.08	44.6±2.02	46.69±2.06	6.65±0.10	4.42±0.13	11.06±0.06
LSD 5%	1.21	1.15	0.88	0.25	0.22	5.58	6.13	1.17	0.37	0.69

Each value represents the mean±standard error (n = 3), *Cultivars

Table 9: Effect of chitosan or salicylic acid at different concentrations on the percentage of carbohydrates and protein of wheat cultivars yielded grains

Treatments (mg L^{-1})	Carbohydrates (%)	Protein (%)
Sakha 94*		
Control (0.0)	52.14±0.53	11.98±0.25
Chitosan (10)	65.33±0.18	12.22±0.09
Chitosan (20)	64.63±0.38	14.15±0.14
Salicylic acid (25)	59.89±0.24	12.32±0.36
Salicylic acid (50)	63.01±0.17	13.08±0.13
Gemmieza 9*		
Control (0.0)	49.29±0.18	10.18±0.26
Chitosan (10)	56.92±0.18	11.50±0.61
Chitosan (20)	58.69±0.30	11.24±0.14
Salicylic acid (25)	61.93±0.20	11.35±0.19
Salicylic acid (50)	63.74±0.16	12.15±0.17
LSD 5%	0.89	0.66

Each value represents the mean±standard error (n = 3), *Cultivars

Flavonoids, lycobine, β-carotene and anthocyanin) of both wheat plants cultivars. Application of CHT or SA led to a marked increase in all antioxidant studied compounds as compared with corresponding control. The maximum increased in antioxidant compounds was obtained by 25 mg L^{-1} SA except anthocyanin at 50 mg L^{-1} SA in Sakha 94. In Gemmeiza 9 the most pronounced increased in total phenols, flavonoids and anthocyanin was obtained at 20 mg L^{-1} CHT, while Lycobine and β-carotene was obtained at 25 mg L^{-1} SA.

Yield components: Table 8 shows the influence of different concentrations of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) on yield parameters of both wheat plants cultivars. Application of CHT or SA led to a marked increase in all yield parameters studied (spike length, spike weight, spikelet number/spike, grain number/spike, grain weight/spike, weight of 1000 grain, straw yield ton/ha, biological yield ton/ha and grain yield ton/ha) in both cultivars when compared to corresponding controls. High level of SA (50 mg L^{-1}) on both cultivars proved to be the most effective. Gemmeiza 9 cultivar treated with SA surpassed Sakha 94 in grain yield (t ha^{-1}).

Carbohydrates and protein contents in grains yield: In the present study data in Table 9 shows that the effect of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) on carbohydrates and protein contents in grains yield of two wheat plants Sakha 94 and Gemmieza 9. Treatment of wheat plants with different concentrations of CHT or SA increased the contents of carbohydrates and protein in grains yield. A marked increase in carbohydrate than protein contents was observed in grain yielded.

Table 10: Effect of chitosan or salicylic acid at different concentrations on the percentage of N, P, K, Na and Ca of both wheat cultivars in the yielded grains

Treatments (mg L^{-1})	N (%)	P (%)	K (%)	Na (%)	Ca (%)
Sakha 94*					
Control (0.0)	2.08±0.04	0.37±0.03	0.30±0.01	0.13±0.02	0.012±0.001
Chitosan (10)	2.13±0.03	0.40±0.05	0.31±0.03	0.12±0.02	0.015±0.003
Chitosan (20)	2.46±0.09	0.41±0.02	0.40±0.06	0.12±0.01	0.016±0.002
Salicylic acid (25)	2.14±0.06	0.39±0.01	0.31±0.07	0.10±0.01	0.015±0.001
Salicylic acid (50)	2.28±0.01	0.41±0.07	0.35±0.07	0.10±0.01	0.013±0.001
Gemmieza 9*					
Control (0.0)	1.77±0.09	0.36±0.04	0.33±0.04	0.12±0.04	0.007±0.002
Chitosan (10)	2.00±0.03	0.40±0.07	0.33±0.07	0.09±0.02	0.010±0.003
Chitosan (20)	1.95±0.03	0.42±0.03	0.34±0.13	0.08±0.01	0.012±0.001
Salicylic acid (25)	1.97±0.02	0.41±0.05	0.33±0.03	0.09±0.04	0.013±0.001
Salicylic acid (50)	2.14±0.06	0.42±0.02	0.30±0.04	0.10±0.01	0.090±0.001
LSD 5%		0.12	0.013	0.03	0.010.02

Each value represents the mean±standard error (n = 3), *Cultivars

Table 11: Effect of chitosan or salicylic acid at different concentrations on water use efficiency (kg grain yield/m^3) of wheat cultivars under saline soil

	Cultivars	
Treatments (mg L^{-1})	Sakha 94	Gemmieza 9
Control (0.0)	0.474±0.08	0.482±0.06
Chitosan (10)	0.656±0.04	0.659±0.02
Chitosan (20)	0.659±0.08	0.700±0.07
Salicylic acid (25)	0.674±0.07	0.719±0.10
Salicylic acid (50)	0.710±0.09	0.736±0.10
LSD 5%		0.059

Each value represents the mean±standard error (n = 3)

Minerals nutrition in grains yield: Application of various concentrations of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) induced significant increases in macro element (N, P, K and Ca), while Na ion contents decreased in wheat cultivars plants (Sakha 94 and Gemmieza 9) as compared to those of control plants (Table 10). Generally, CHT 20 mg L^{-1} was the most effective treatment in increasing the most studied minerals with other treatments in Sakha 94. While the highest concentrations of both tested materials (CHT and SA) were the most effective in increasing N, P and K contents in both cultivars as compared to the corresponding controls.

Water use efficiency (WUE): Table 11 shows that, the influence of different concentrations of CHT (10 and 20 mg L^{-1}) or SA (25 and 50 mg L^{-1}) on water use efficiency (WUE) of both wheat plant cultivars. Treatment of wheat plants with different concentrations of CHT or SA increased significantly WUE as compared with the corresponding control in both cultivars. The most pronounced increase was observed at SA (50 mg L^{-1}) treatment in both cultivars (Gemmieza 9 and Sakha 94). Gemmieza 9 cultivar surpassed Sakha 94 in WUE as compared with the corresponding treatment.

DISCUSSION

Treatment of wheat plants with different concentrations of CHT or SA increased all morphological parameter and water content (%) (Table 3).The positive effect of CHT on plant growth might be due to its effect on enhancing nutrient uptake like nitrogen, phosphorous and potassium (Table 10). In this concern, Guan *et al.*[38] proved that the exciting effect of CHT on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure. Moreover, such effect accompanied by decreasing the accumulation of harmful free radicals, increasing antioxidants compounds and enzyme activities. This trend supports the obtained results in Table 6-7. Moreover, the mechanism of CHT in counteracting the harmful effect of water stress might be because of its influence on pathways involving jasmonic acid which plays a key role in the regulation of water use by plants[39]. They reported the effects of CHT on stomatal aperture suggest the possibility that it might be a valuable anti-transparent with useful agricultural applications. Choudhary *et al.*[40] found that foliar-applied chitosan improved plant growth physiological components in plant growth under water stress in maize and common bean plants. The increase in water content may be due to the role of chitosan in increase of organic solutes in plants. The same results obtained by Abd El-Gawad and Bondok[41] on tomato.

The SA considers endogenous signal molecule that activate plant growth and plant defense responses to systemic acquired. Kovacik *et al.*[17] and Ma *et al.*[42] showed that, SA improved germination and plant growth and development under salinity stress in *Matricaria chamomilla* and *D. superbus* plants. The present study showed that SA treatments induced an increase in water contents of the salt

stressed plants as compared to the control plants on the two tested wheat cultivars (Table 3). Kordi *et al*.[43] reported that relative water content increased in sweet basil with SA treatment under water stress. Increases in water contents of wheat plants treated with SA may be attributed to lower transpiration rates[44].

CHT or SA with various concentrations increased photosynthetic pigments in both cultivars as compared with the corresponding controls (Table 4). The abiotic stress that impact the plant growth and also induced a negative response on photosynthesis. Choudhary *et al*.[40] found that *Zea mays* L. plants treated with Cu-chitosan enhanced chlorophyll content. Moreover, Varamin *et al*.[45] reported that chitosan alleviates the water stress effect on photosynthetic pigments. Farouk and Amany[46] mentioned that there are different ways to improve photosynthetic pigment contents, e.g. enhancing endogenous levels of cytokinins, which stimulate chlorophyll synthesis or prevent the decline in the light-harvesting pigment protein complexes (chlorophyll 'a' or 'b'). This action protects the photosynthetic apparatus and oxidative damage of chloroplast lipids, pigments and proteins[47]. Moreover, it is possible that the increase in supplying magnesium (crucial ion at the center of photosynthetic pigment) may improve the synthesis of chlorophyll[48]. It could be suggested that exogenous chitosan might alleviate abiotic stresses by increment in chlorophyll concentration, decreasing the stomatal and non-stomatal transpiration as well as improving the water use efficiency. In this respect, Bittelli *et al*.[49] proposed that chitosan might be an effective anti-transpiring tool to preserve water resources.

Khodary[50] attributed the increasing effect of SA in photosynthetic capacity to its stimulatory effects on Rubisco activity and pigment contents of *Zea maize* plants under salinity stress when treated with salicylic acid. Moreover, exogenously SA effectively improved the growth, photosynthesis, stomata and chloroplast development of *Dianthus superbus*[42]. Also, SA is antioxidant compound intense in the chloroplast and protects the photosynthetic apparatus when a plant is exposed to drought stress, by scavenging the excessively ROS[51].

Treatment of wheat cultivars with different concentrations of CHT or SA increased TSS, proline and FAA (Table 5). In this connection, El-Bassiouny and Abdel-Monem[52] stated that increasing the organic solutes, in terms of total soluble sugars, proline and free amino acids, improves plant cells tolerance to salt stress throughout rising osmotic pressure in the cytoplasm and relative water contents vital for plant growth as well. Moreover, soluble sugars enhanced membrane stabilization, which might act as scavengers of ROS[53]. In addition, Abdallah *et al*.[54] stated that proline vital roles from harmful effects of osmotic stress by osmotic adjustment, stabilization and protection of enzymes, proteins and membranes as well.

Chibu and Shibayama[55] referred back these positive effects to the greater availability of amino compounds released from CHT. Moreover, It appears that CHT increased the concentration of simple organic molecules, like sugar, free amino acids and total soluble phenols, that play a role in regulating plant osmosis and consequently better growth and yield under un-favorable environmental conditions[6] in white clover. Iriti *et al*.[56] stated that CHT was capable to increase osmoprotectants compounds, such as TSS, FAA and soluble phenols, that may improve plant tolerance to environmental stress conditions.

SA treatment raises proline content of *Ocimum basilicucm* plant under salt stress[57]. In this concern, Abdallah *et al*.[58] demonstrated that, in plants exhibited to stress, the osmotic adjustment occurs by the accumulation of high concentrations of compatible solutes, e.g., proline, soluble sugars and free amino acids in quinoa plants.

The presented data (Table 6) shows that lipid peroxidation decreased in wheat cultivars (Sakha 94 and Gemmieza 9) plants in response to treatment with different concentrations of CHT or SA. These results are in agreement with the result obtained by Farouk *et al*.[59] who stated that CHT application decreased hydrogen peroxide (H_2O_2) accumulation by 37%, lipid peroxidation by 57% and membrane permeability by 16% in cowpea plants under high water deficit stress. Chitosan affects the stabilizing of cellular membranes through increasing antioxidants compounds, saving cell membranes from oxidative stress and hence improving plant cell permeability. This observation is supported by the results of Guan *et al*.[38].

Mostofa *et al*.[60] reported that pretreatment of rice seedlings with SA significantly decreased the salt-induced malondialdehyde level in comparison to the control. SA improved the plant tolerant and decreased lipid peroxidation through regulated antioxidant machinery. Rasool *et al*.[61] confirmed that, plants, to overcome the adverse effects of oxidative stress, are well-regulated antioxidant machinery that able of protecting biomolecules from further damages.

Foliar spraying of wheat cultivars with CHT or SA enhanced stress tolerance by increasing CAT, SOD and POX activities. In this concern, Sharma and Dubey[62] revealed that

this antioxidant system creates protection versus oxidative harm who found to increase the lifetime of active oxygen species within the cellular environment. The present study found that CHT sprayed plants can eliminate reactive oxygen species through induction of higher SOD and CATactivities[63].

Regarding effect of SA treatment, Horvath *et al.*[64] demonstrated that, in different plants species, pre-treatment with low concentrations of SA enhances tolerance toward most kinds of abiotic stress due to an enhanced antioxidant capacity. Ma *et al.*[42] proved that, SA significantly increased the antioxidant enzyme activities of *D. superbus*.

Application of CHT or SA led to a marked increase in antioxidant compounds (phenols, flavonoids, lycobine, β-carotene and anthocyanin) of both wheat plants cultivars as compared with corresponding control Table 7. Mathew and Sankar[65] recommended that CHT exogenous application raised phenolic compounds as well as antioxidant activities in *Ocimum* plant. Farouk *et al.*[59] established that application of CHT increased carotenoids, ascorbic acid and total phenolic content in cowpea leaf tissue accompanied with reducing the generation of free radicals and lipid peroxidation when plants are stressed. In this regard, phenolic compounds inhibit the oxidation of lipids and proteins by the transfer of phenolic hydrogen atoms to a radical[66].

Abdallah *et al.*[58] found that SA increased the flavonoids, phenolic content and antioxidant substances of Quinoa yielded seed. It could be expected that SA application on plants increase flavonoid, phenolic and antioxidant concentration as previously mentioned by Bakry *et al.*[51] in linseed and Bagherifard *et al.*[67] in artichoke. Aldesuquy and Ghanem[68] found that application SA induced increase in the flavonoid composition (phenolic compartment) anthocyanin, lycopene and β-carotene contents in wheat cultivars.

Under the present study conditions, it can be concluded that CHT may play an important role in the growth and productivity of wheat cultivars grown under salinity stress (Table 8). Perhaps, this because they be able to produce different metabolites which cause a reduction in transpiration and thus more water become available to plants for improved growth and production[69]. Some of these promoting effects of CHT on ear length and weight/plot and grain yield/plot of maize plants[40]. It is recommended that CHT might be a promising material used to decrease the harmful effect of salinity stress on the growth and yield of wheat plants.

The application of SA enhanced the growth and yield under salinity stress in different studies carried out by Morad *et al.*[44] on wheat and Jini and Joseph[70] on rice.

Treatment of wheat plants with different concentrations of CHT or SA increased the contents of carbohydrates and protein in the yielded grains (Table 9). The effects of CHT in rising photosynthetic pigments and total carbohydrate contents were established by Farouk *et al.*[71] in radish. Moreover, Mahdavi *et al.*[72] found that application of the lowest concentrations (0.05-0.4%) of CHT increased protein content in the stressed safflower seedlings.

Concerning the effect of SA, the values of carbohydrate, protein and oil were gradually increased with increasing concentrations of SA of the yielded quinoa seeds[58] and peanut seeds[73].

The results showed clearly that an increase in all measured elements (N, P, K and Ca, except for Na which decreased (Table 10). Metwaly and El-Shatoury[74] found that application of CHT increased the contents of N, P and K in cabbage under water stress. Also, Farouk and Amany[46] found that chitosan significantly increased N and K content in cowpea plant.

Al-Rubaye and Abd Atia *et al.*[75] found that SA increased significantly N, P and K contents in the Summer Squash fruit. This might be attributed to the role of SA to encourage plant growth, the absorption and transport of nutrients, membrane permeability, growth rate and photosynthesis[76]. Stevens *et al.*[77] agree with these results, who reported that SA helped the protection of membrane functions in tomato plants. This action initiates the antioxidant reactions and promoted Ca uptake that preserve the plant from the oxidative damage[78]. Jini and Joseph[70] found that the application of SA improved salinity tolerance of rice varieties by decreasing sodium and rising potassium content.

Wheat plants treated with various concentrations of CHT or SA increased water use efficiency under saline soil as compared with the corresponding control in both cultivars (Table 11). Metwaly and El-Shatoury[74] found that foliar application with chitosan increased water use efficiency of cabbage leaves under full, moderate and severe irrigation. Bittelli *et al.*[49] showed that CHT decreased transpiration by stimulating closure of stomata. The obtained results proposed that CHT may be an efficient anti-transpiration tool to preserve water use in agriculture.

As for the effect of SA capacity in affecting WUE, the obtained results (Table 11) came on line with the results of Hafez and Farig[79] on wheat. They concluded that the treatment of SA plus 70% depletion of available soil moisture could be a promising way for enhancing wheat productivity and water use efficiency in water deficient areas which suffer from saline soil condition.

CONCLUSION

Chitosan (CHT) and salicylic acid (SA) treatments enhanced the vegetative growth and bioactivity of wheat cultivars under salt stress. Interestingly, CHT and SA treatments mitigated salinity stress effects by increasing, chlorophyll, organic solutes (TSS, proline, free amino acids) and the antioxidant enzyme activities (catalase, superoxide dismutase and peroxidase) as well as increasing the non-enzymatic antioxidants of leaves. The increase in the produced secondary metabolites due to CHT or SA application reflected positively on enhancing all yield parameters and the nutritional value (grain quality) in both cultivars. Gemmieza 9 cultivar showed better response to SA treatment compared to Sakha 94 cultivar in grain yield, while both cultivars exhibited its high response with the high level of SA.

SIGNIFICANCE STATEMENT

This study discover the positive role of either chitosan or salicylic acid in alleviating the stress conditions of saline soils that can be beneficial for improving the performance of wheat plants to increase the productivity of land area unit. This study will help the researcher to uncover the critical areas of secondary metabolites defense causative agents of plants against environmental stress that many researchers were not able to explore. Thus, a new theory on new agricultural practices and recommendations may be arrived at.

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