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Attenuation of negative effects of saline stress in wheat plant by chitosan and calcium carbonate

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Abstract

Background: Chitosan and Ca^+ are natural signal molecules that can be used in agriculture as biostimulants and elicitors. They enhance different physiological responses and mitigate the negative effects of salinity. So, this investigation was done to study the effect of soaking wheat grains in chitosan and CaCO_3 (20 and 40 mg/L) on alleviating the adverse effect of salinity stress (0.0 and 5000 mg/L) on growth, some biochemical and physiological and yields of wheat plant.

Results: Shoot length (cm), leaves no/tiller, shoot dry weight (g), root fresh weight (g) and root dry weight (g) were significantly decreased as a result of salt stress. Soaking wheat grains in Chitosan or CaCO_3 significantly promoted plant growth under normal and stressed conditions. Irrigation of wheat plants with saline water significantly decreased photosynthetic pigments (*Chlo-a*, *Chlo-b*, carotenoids and total pigments) in addition to *Chlo-a/Chlo-b* ratio, indole acetic acid content in the plant leaves. Meanwhile, saline water significantly increased phenolics, total soluble sugars (TSS) and proline content. H_2O_2 and lipid peroxidation expressed by malondialdehyde (MDA) content clearly showed significant increases under salinity stress compared with untreated control. Soaking wheat grains in chitosan or CaCO_3 before sowing significantly increased the accumulation of H_2O_2 and MDA in the leaves of wheat plants. Treatment of wheat grains with chitosan or CaCO_3 significantly promoted the activity of various antioxidant enzymes (SOD and POX) as compared to the control. CAT activity was significantly decreased as a result of chitosan or CaCO_3 treatments. The highest CAT activity was recorded in plants irrigated with 5000 mg/L saline water followed by control plants which recoded 36.40 and 24.82 U/min/g FW, respectively. On the other hand, irrigation of wheat plants with 5000 mg/L saline water significantly decreased spike length (cm), spikelets no/spike, grains wt/plant (g), 1000-grains wt (g), yield and biomass/plant (g) as well as, carbohydrate % and protein % compared with the control. However, treating wheat plants either with Chitosan or calcium carbonate resulted in obvious significant increases in carbohydrates and protein contents, especially in plants treated with 40 mg/L chitosan followed by 40 mg/L calcium carbonate. Soaking wheat grains in chitosan, especially at 40 mg/L, exhibited the strongest scavenging potential (2,2-diphenyl-1-picryl-hydrazyl-hydrate assay (DPPH%)) followed by treatment with 40 mg/L CaCO_3 .

Conclusion: In conclusion, the used treatment enhanced the protective parameters such as antioxidant enzymes, total phenols and free radical scavengers and consequently helped the plants to decrease lipid peroxidation, increased their tolerance and improved yield and spike quality. Application of 40 mg/L chitosan recorded the highest increment in the scavenging ability of the natural antioxidants of the plant extract toward the stable free radical DPPH.

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Background

Wheat plant (*Triticum aestivum* L.) is considered one of the most important crops. Salinity stress is a major limiting factor which negatively affects the growth and production of several crops all over the world. Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes, such as photosynthesis, antioxidant phenomena, nitrogen metabolism, ion homeostasis and osmolyte accumulation (Ashraf 2004). Thus, salinity exerts its undesirable effects through osmotic inhibition and ionic toxicity and by disturbing the uptake and translocation of nutritional ions (Misra and Dwivedi 2004).

Chitosan is a natural biopolymer derived from chitin, a polysaccharide found in exoskeleton of crustaceans, insects as well as cell wall of fungi and some algae (Boonlertnirum et al. 2010). It is low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture. Chitosan has been widely used in agricultural applications mainly for stimulation of plant immunity, to protect plants and food products against microorganisms (bacteria and fungi) (Hadwiger et al. 2002; ChunYan et al. 2003; Devlieghere et al. 2004; Patkowska et al. 2006; No et al. 2007). Also, many efforts were done to study the effect of chitosan on plant growth, development and productivity. A positive effect of chitosan was observed on the growth of roots, shoots and leaves of various plant species. Foliar application of chitosan increased growth and yield in sweet pepper, radish and sunflower (Ghonomie et al. 2010; Farouk et al. 2011; Bakhoum et al. 2020). Similar results were also observed in grapevine and strawberry (Gornik et al. 2008; Abdel-Mawgoud et al. 2010). Recently, Sheikha and Al-Malki (2011) indicated that application of different concentrations of chitosan enhanced bean shoot and root length, fresh and dry weights of shoots, root and leaf area. In addition, foliar applications with chitosan resulted in higher vegetative growth and improvement in fruit quality of cucumber (Farouk et al. 2008). For other cultivated plants, Bittelli et al. (2001) reported that foliar application of chitosan decreased transpiration in pepper plants, and reduced water use by 26–43% while maintaining biomass production and yield.

Calcium is a universal signaling molecule and the calcium-sensing (CaS) receptor is of fundamental importance for extracellular calcium signaling and calcium homeostasis (Bouschet et al. 2008). Calcium is an important second messenger in signal transduction pathways (Achary et al. 2013), mediating various defense responses

to the action under environmental stresses (Pan et al. 2012). Calcium (Ca²⁺) is micro and multifunctional element in plants used in different biochemical and physiological processes (Barker and Pilbeam 2007). Calcium is an essential nutrient for growth and development of plants which involved in various important functions as in stability of plant membrane and stabilization of cell wall as well as, increases a huge number of key enzymes activities and interacting with phytohormones (White and Broadley 2003). Moreover, it serves in the signaling network pathways as a secondary messenger under different abiotic stress (Sadak 2016). In addition, calcium appears to play a central role in many defense mechanisms which are induced by stress and calcium signaling is required for the acquisition of stress tolerance or resistance (Cousson 2009).

The aim of this study was to study the effect of chitosan and Ca²⁺ on growth, some physiological and biochemical aspects and yield of wheat plant irrigated with saline water.

Methods

Experimental procedures

Two pot experiments were conducted at the greenhouse of National Research Centre, Dokki, Cairo, Egypt, at the winter seasons of 2018/2019 and 2019/2020. Grains of wheat Sakha 94 were obtained from Agricultural Research Centre, Giza, Egypt. Chitosan and calcium carbonate used in the present work were supplied from Sigma-Aldrich. Wheat grains were soaked in different concentrations of Chitosan and CaCO₃ (0.0, 20 and 40 mg/L) for 12 h before sowing. The experimental design was complete randomized block design (CRBD) with 4 replicates. Grains of wheat were sown at November 25th; grains were selected for uniformity by choosing those of equal size and with the same color. Ten uniform air-dried wheat grains were sown along a central row in each pot at 30-mm depth in plastic pots, each filled with about 7 kg clay soil from Giza, consisting of the upper 10 cm of soil collected from an area of undisturbed native vegetation. To reduce compaction and improve drainage, the soil was mixed with yellow sand in a proportion of 3:1(v/v). Ten days after sowing, the seedlings were thinned to 5 seedlings per pot. Granular ammonium sulfate 20.5% N at a rate of 40 kg N ha⁻¹, and single superphosphate (15% P₂O₅) at a rate of 60 kg P₂O₅ ha⁻¹ were added to each pot. The N and P fertilizers were mixed thoroughly into soil of each pot immediately before sowing. Soil field capacity in the pots was estimated by saturating the soil

in the pots with water and weighing them after they had drained for 48 h. Field water capacity was 0.36. The two saline water levels used were 0.0, 5000 mg/L, respectively. The preparation of salt mixture according to Stroganov (1962) equation is shown in Table 1. The pots were irrigated with equal volumes of the various salinity levels.

The component of specific anions and cations in chloride mixture is expressed as percentage of total mill equivalents.

Plant samples were taken after 75 days from sowing for measurements of growth characters as shoot length (cm), leaves no/tiller, shoot, root fresh and dry weight (g/plant). Plant samples were taken for chemical analysis as photosynthetic pigments, indole acetic acid (IAA $\mu\text{g}/100$ g fresh weight), hydrogen peroxide (H_2O_2 , $\mu\text{g}/100$ g fresh weight), lipid peroxidation (MDA, $\mu\text{g}/100$ g fresh weight) contents and some antioxidant enzymes as super oxide dismutase (SOD), peroxidase (POX) and catalase (CAT) (U/min/g fresh wt). After drying of plant samples, total soluble sugars (TSS, mg/g dry weight) proline (mg/100 g dry wt) were determined. At harvest: spike length (cm), spike weight (g), spikelet's number/spike, grains number/spike, grains weight/plant (g), 1000-grains weight (g) and biomass yield/plant (g). Some nutritional values of the yielded grains were determined as carbohydrate%, protein%, phenolics (mg/100 g dry weight), flavonoids (mg/100 g dry weight) and DPPH%.

Measurements

Photosynthetic pigments: Chlorophyll *a*, chlorophyll *b* and carotenoids were determined using spectrophotometric method described by Lichtenthaler and Buschmann (2001). Indole acetic acid content was extracted and analyzed by the method of Larsen et al. (1962). Total soluble sugars (TSS) were extracted by the method of (Homme et al. 1992). TSS were analyzed according to (Yemm and Willis 1954). Proline was extracted and assayed according to the method described by Bates et al. (1973). The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges et al. (1999). Hydrogen peroxide content was determined using the method of Velikova et al. (2000). Enzyme extracts were collected following the method described by Chen and

Wang (2006). Superoxide dismutase (SOD, EC 1.12.1.1) activity was spectrophotometrically assayed at 560 nm by nitro-blue-tetrazolium (NBT) reduction method (Chen and Wang 2006). Peroxidase (POX, EC 1.11.1.7) activity assayed according to the method of Bergmeyer (1974). Catalase (CAT) (EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm (Chen and Wang 2006). The enzyme activities were calculated by Kong et al. (1999). Total carbohydrate determination was carried out according to Herbert et al. (1971). The protein content was determined by micro-Kjeldahl method according to A.O.A.C. (1990). Total phenol content was measured as described by Danil and George (1972). Flavonoid content of crude extract was determined by the aluminum chloride colorimetric method (Chang et al. 2002). The free radical scavenging activity by grain extracts was done according to the method reported by Gyamfi et al. (2002).

Statistical analysis

The data were statistically analyzed on complete randomized design system (CRBD) with four replicates. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability (Snedecor and Cochran 1980).

Results

Data presented in Table 2 indicated that irrigation of wheat plants with 5000 mg/L saline water markedly decreased plant growth parameters (shoot length, leaves number per tiller, shoot fresh and dry weights, and root fresh and dry weights). Meanwhile, soaking wheat grains in chitosan or calcium carbonate (either at 20 or 40 mg/L) before sowing significantly promoted all the above-mentioned growth criteria, especially in plants treated with 40 mg/L chitosan, followed by plants treated with CaCO_3 .

Data presented in Table 3 indicated that irrigation of wheat plants with 5000 mg/L saline water significantly decreased yield and yield components under study (i.e., spike length (cm), spike weight (g), spikelet's number/spike, grains number/spike, grains weight/plant, 1000 grains weight, yield and biomass/ plant (g)). On the

Table 1 The components of the salt mixture used for chloride salinization expressed as % of the total salt content

MgSO_4	CaSO_4	NaCl	MgCl_2	CaCO_3	
10	1	78	2	9	
Na^+	Mg^{+2}	Ca^{+2}	SO^{-2}	Cl^-	CO^{-2}
38	6	6	5	40	5

Table 2 Effect of chitosan and calcium carbonate (0, 20 and 40 mg/L) on morphological characters of wheat plant grown under different salinity levels (0, 5000 mg/L)

Treatments	Shoot length (cm)	Leaves no/tiller	Shoot fresh wt (g)	Shoot dry wt (g)	Root fresh wt (g)	Root dry weight (g)
Control	32.00	4.33	1.90	0.61	0.41	0.10
0 mg/L saline water						
20 mg/L chitosan	34.00	5.00	2.31	0.76	0.58	0.12
40 mg/L chitosan	36.00	6.00	2.79	0.94	0.70	0.14
20 mg/L CaCO ₃	34.33	5.33	2.25	0.72	0.57	0.11
40 mg/L CaCO ₃	38.67	6.00	2.43	0.90	0.63	0.14
5000 mg/l saline water	29.67	3.67	1.44	0.54	0.36	0.07
5000 mg/L saline water						
20 mg/L chitosan	32.00	4.33	1.62	0.65	0.43	0.09
40 mg/L chitosan	34.00	5.00	2.04	0.73	0.43	0.10
20 mg/L CaCO ₃	33.00	4.33	1.53	0.56	0.37	0.09
40 mg/L CaCO ₃	33.67	4.67	1.81	0.65	0.42	0.10
LSD (5%)	2.70	0.81	0.21	0.07	0.06	0.01

Data are means of two seasons

Table 3 Effect of chitosan and calcium carbonate (0, 20 and 40 mg/L) on yield and its components of wheat plant grown under different salinity levels (0, 5000 mg/L)

Treatments	Spike length (cm)	Spike wt (g)	Spikelets no/spike	Grains no/spike	Grains wt/plant (g)	1000 grains wt (g)	Biomass/plant (g)
Control	9.50	1.13	13.33	30.20	2.11	30.21	3.52
0 mg/L saline water							
20 mg/L chitosan	10.77	1.28	14.67	42.73	2.31	61.00	3.79
40 mg/L chitosan	11.90	1.40	16.00	46.73	2.87	66.67	3.87
20 mg/L CaCO ₃	10.33	1.21	14.33	42.20	2.30	60.67	3.72
40 mg/L CaCO ₃	11.73	1.33	15.33	44.33	2.33	63.67	3.81
5000 mg/l saline water	8.00	1.06	9.00	25.41	1.82	25.41	3.08
5000 mg/L saline water							
20 mg/L chitosan	9.33	1.16	10.33	28.05	1.95	55.67	3.35
40 mg/L chitosan	10.33	1.21	11.67	31.99	2.12	59.00	3.56
20 mg/L CaCO ₃	8.33	1.09	10.00	28.15	1.92	52.33	3.18
40 mg/L CaCO ₃	9.00	1.15	10.33	34.47	1.93	54.67	3.29
LSD (5%)	0.62	0.05	0.92	1.74	0.05	0.69	0.06

Data are means of two seasons

other hand, exogenous treatment of chitosan and CaCO₃ increased significantly yield and its components of wheat plants grown either under normal irrigation water or salinity stress conditions as compared with their corresponding untreated controls. Meanwhile, treating plants with CaCO₃ resulted in similar results, but to a lesser degree (Table 3). 40 mg/L chitosan and 40 mg/L CaCO₃ treatments were more effective than 20 mg/L of either chitosan or CaCO₃ treatments.

It is obvious that irrigation of wheat plants with saline water significantly decreased photosynthetic pigments

(chlorophyll-*a*, chlorophyll-*b*, carotenoids and total pigments) in addition to chlorophyll-*a*/chlorophyll-*b* ratio and endogenous indole acetic acid contents in the plant leaves (Table 4). On the other hand, soaking wheat grains in chitosan at 40 mg/L significantly increased chlorophyll-*a* content followed by treating grains with 40 mg/L CaCO₃ (Table 4). Chlorophyll-*b* and carotenoids contents as well as IAA contents followed the same trend. On the other hand, the ratio of chlorophyll-*a*/chlorophyll-*b* (*a/b*) was stable in all treatments irrigated with 5000 mg/L saline water.

Table 4 Effect of chitosan and calcium carbonate (0, 20 and 40 mg/l) on photosynthetic pigments and IAA ($\mu\text{g}/100$ g fresh wt) of wheat plant growing under different salinity levels (0, 5000 mg/l)

Treatments (mg/l)	Chlo-a	Chlo-b	Chlo-a/Chlo-b	Carotenoids	Total pigments	IAA ($\mu\text{g}/100$ g fresh wt)
Control	12.44	7.52	1.65	2.50	22.46	26.97
0 mg/L saline water						
20 mg/L chitosan	13.52	8.08	1.67	3.05	13.52	34.07
40 mg/L chitosan	14.57	8.60	1.69	3.75	14.60	44.07
20 mg/L CaCO_3	13.36	7.96	1.68	2.64	13.23	31.53
40 mg/L CaCO_3	13.89	8.27	1.68	3.38	13.83	40.04
5000 mg/l saline water	10.41	6.67	1.56	2.60	19.67	19.79
5000 mg/L saline water						
20 mg/L chitosan	11.71	7.20	1.63	3.33	13.20	25.16
40 mg/L chitosan	12.47	7.63	1.63	3.85	13.76	27.81
20 mg/L CaCO_3	11.30	6.95	1.63	3.28	12.14	25.95
40 mg/L CaCO_3	12.04	7.38	1.63	3.80	13.00	30.21
LSD (5%)	0.11	0.14	0.04	0.14	0.25	1.55

Table 5 Effect of chitosan and calcium carbonate (0, 20 and 40 mg/L) on osmo-protectants (TSS mg/g fresh weight, proline mg/100 g fresh weight and phenolics mg/100 g dry weight) contents of wheat plant growing under different salinity levels (0, 5000 mg/L)

Treatments	TSS (mg/g fresh wt)	Proline (mg/100 g fresh wt)	Phenolics (mg/100 g dry wt)
Control	11.37	33.05	27.07
0 mg/L saline water			
20 mg/L chitosan	38.08	63.04	37.95
40 mg/L chitosan	43.74	68.70	30.12
20 mg/L CaCO_3	36.48	61.49	47.15
40 mg/L CaCO_3	41.29	65.64	56.60
5000 mg/l saline water	13.27	43.03	32.89
5000 mg/L saline water			
20 mg/L chitosan	47.73	72.58	38.91
40 mg/L chitosan	51.40	80.75	63.14
20 mg/L CaCO_3	42.74	63.14	34.17
40 mg/L CaCO_3	45.76	70.25	50.26
LSD (5%)	0.30	1.19	1.24

Data presented in Table 5 indicated that irrigation of wheat plants with saline water significantly increased total soluble sugars (TSS) (mg/g fresh weight), proline content (mg/100 g dry weight) and phenolics (mg/100 g dry wt). Moreover, treating wheat plants either with chitosan or calcium carbonate resulted in obvious significant increases in the above-mentioned parameters, especially in plants treated with 40 mg/L chitosan followed by 40 mg/L calcium carbonate (Table 5).

Data presented in Table 5 also indicated that soaking wheat grains in 20 mg/L chitosan or CaCO_3 caused significant increases in phenolics content. Furthermore, soaking wheat grains in 40 mg/L chitosan or CaCO_3 resulted in significant increases in total phenolics.

Lipid peroxidation expressed by malondialdehyde (MDA) content and H_2O_2 content clearly showed significant increases under salinity stress compared with control plants. Meanwhile, soaking wheat grains in chitosan or CaCO_3 before sowing significantly decreased the accumulation of H_2O_2 and MDA in the leaves of wheat plants as compared with their corresponding untreated controls either at normal irrigated plants or saline treated plants as shown in Table 6.

Regarding to antioxidant enzymes, Table 6 clearly shows that irrigation of wheat plants with 5000 mg/l saline water caused significant increases in different enzymes superoxide dismutase SOD, peroxidase POX and catalase CAT as compared to those plants irrigated with tap water. Treatment of wheat grains with chitosan or CaCO_3 significantly promoted the activity of various antioxidant enzymes (SOD and POX) as compared to the control (Table 6). On the other hand, CAT activity was significantly decreased as a result of chitosan or CaCO_3 treatments. The highest CAT activity (U/min/g FW) was recorded in plants irrigated with 5000 mg/l saline water followed by control plants which recoded 36.40 and 24.82 U/min/g FW, respectively.

Data presented in Table 7 indicated that irrigation of wheat plants with saline water significantly decreased carbohydrate % and protein % compared with the control plants. Meanwhile, it increased significantly flavonoids

Table 6 Effect of chitosan and calcium carbonate (0, 20 and 40 mg/L) on H₂O₂, MDA (µg/100 g fresh wt), and antioxidant enzymes (SOD, POX and CAT U/min/g FW) activities of wheat plant growing under different salinity levels (0, 5000 mg/L)

Treatments	H ₂ O ₂	MDA	SOD	POX	CAT
Control	4.46	7.76	28.53	62.99	24.82
0 mg/L saline water					
20 mg/L chitosan	4.14	7.43	32.68	66.59	11.72
40 mg/L chitosan	4.07	7.31	35.52	68.75	12.27
20 mg/L CaCO ₃	4.24	7.62	31.45	65.54	11.69
40 mg/L CaCO ₃	4.14	7.54	33.87	67.77	12.29
5000 mg/l saline water	6.38	10.95	31.75	65.97	36.40
5000 mg/L saline water					
20 mg/L chitosan	5.43	9.39	36.24	68.23	14.62
40 mg/L chitosan	5.04	8.11	37.90	72.30	15.55
20 mg/L CaCO ₃	5.53	9.61	35.49	67.62	13.94
40 mg/L CaCO ₃	4.90	8.68	36.85	69.55	14.85
LSD (5%)	0.13	0.17	0.30	0.54	0.49

content and DPPH activities of the yielded grains of wheat plant as compared with control plants. Treating wheat plants either with chitosan or calcium carbonate with different concentrations resulted in obvious significant increases in carbohydrates, protein content, flavonoids contents and DPPH activities especially in plants treated with 40 mg/l chitosan followed by 40 mg/l calcium carbonate (Table 6). Data in Table 7 showed that salinity stress caused significant increases in the antioxidant activity (as DPPH radical scavenging capacity) of wheat grain. Also, foliar treatment of wheat plant with chitosan and CaCO₃ with different concentrations (20

and 40 mM) caused significant increases in the antioxidant activity as compared with control plants.

Discussion

Data presented in Tables 2 and 3 indicated that irrigation of wheat plants with 5000 mg/l saline water significantly decreased plant growth parameters and yield attributes. The obtained results of salinity stress are in agreement with those recorded by Abdel-Mawgoud et al. (2010), Sheikha and Al-Malki (2011) and Zehra et al. (2012), Rady et al. (2015) and Sadak (2016) on different plant species. In this concern, salinity stress limits plant growth and yield by adversely affecting various physiological and biochemical processes, such as photosynthesis, antioxidant phenomena, nitrogen metabolism, ion homeostasis and osmolyte accumulation (Ashraf 2004). Thus, salinity exerts its undesirable effects through osmotic inhibition and ionic toxicity and by disturbing the uptake and translocation of nutritional ions (Misra and Dwivedi 2004). Moreover, the reductions in yield and yield components of wheat plant under salinity stress might be through reductions in growth (Table 2) and photosynthetic pigments (Table 4) contents, thus reducing the output of photosynthesis (Anjum et al. 2003) and diminishing activities of Calvin cycle enzymes (Ashraf et al. 2013).

A positive effect of chitosan was observed on the growth and yield of various plant species. Foliar application of chitosan increased growth and yield in chilli and maize plants (Chookhongkha et al. 2012; Mondal et al. (2013). Similar results were also observed in grapevine and strawberry (Gornik et al. 2008; Abdel-Mawgoud et al. 2010). Sheikha and Al-Malki (2011) and Khan et al. (2018) indicated that application of different

Table 7 Effect of chitosan and calcium carbonate (0, 20 and 40 mg/L) on carbohydrates%, protein%, flavonoids (mg/100 g dry weight) contents and DPPH% of wheat plant grains growing under different salinity levels (0, 5000 mg/L)

Treatments	Carbohydrate (%)	Protein (%)	Flavonoids (mg/100 g dry wt) (%)	DPPH (%)
Control	42.74	12.75	32.55	34.49
0 mg/L saline water				
20 mg/L chitosan	43.25	38.54	34.94	42.97
40 mg/L chitosan	43.77	52.72	36.92	52.70
20 mg/L CaCO ₃	43.25	37.75	35.95	41.51
40 mg/L CaCO ₃	43.51	49.20	35.54	47.30
5000 mg/l saline water	41.72	12.14	39.95	42.74
5000 mg/L saline water				
20 mg/L chitosan	41.97	42.87	42.23	54.55
40 mg/L chitosan	42.74	60.52	43.94	61.85
20 mg/L CaCO ₃	41.72	40.56	43.59	50.60
40 mg/L CaCO ₃	41.79	56.64	43.21	59.01
LSD (5%)	0.28	0.42	1.20	0.43

concentrations of chitosan enhanced bean and pea growth and yield through increasing plant immunity to different kinds of stress. Chitosan is considered as one of growth regulators and as a signal molecules in addition to its role as a high effective biomolecule (Górnik et al. 2008). Moreover, this promotive role resulted by enhancing activities of enzymes of nitrogen metabolism as well as improving the translocation of nitrogen in the leaves thus increased growth and development (Sultana et al. 2017). Chitosan treatment induces overexpression of genes involved in photosynthesis, changes in programming of protein metabolism with an enhancement of various storage proteins and hormone metabolism (Landi et al. 2017).

Similar findings were reported earlier concurrent with our obtained positive results of Ca^+ on different plant under abiotic stresses as Shores et al. (2011), Zehra et al. (2012), Xu et al. (2013), Ibrahim et al. (2016) and Haleema et al. (2018) and Sadak et al. (2020). The positive role of Ca^{2+} might be attributed to that Ca^{2+} was suggested to be a crucial secondary messenger that used in signaling-related processes to many defense mechanisms which are induced by salinity stress (Tuteja 2009). Ca^{2+} can increase membrane stability and protect them from lipid peroxidation and oxidative stress induced by salinity stress, thus improving water status of plant (Nayek et al. 1983 and Shao et al. 2008) as calcium is an important constituent of plant cell wall and plays an important role in cell division and enlargement.

It is obvious that irrigation of wheat plants with saline water significantly decreased photosynthetic pigments in addition to chlorophyll-*a*/chlorophyll-*b* ratio and endogenous indole acetic acid contents in the plant leaves (Table 4). These reductions in different photosynthetic pigments components in response to salt stress were confirmed on various plants Sakhonwasee and Phingkasana (2017) on tomato plant. These decreases might be referred to the increased levels of reactive oxygen species (ROS) production and oxidative stress that happened with salinity stress, thus damaging chloroplast membranes and the antagonistic effects of sodium ion on magnesium ion absorption (Smirnov 1993). As well as, these decreases could be considered as an important regulatory step to avoid the absorbance of high light and the over reduction of photosynthetic electron transport chain, thus increasing the production of ROS (Munne-Bosch and Alegre 2000; Mazars et al. 2010).

The obtained results showed that chitosan treatment increased photosynthetic pigments. These increases could be due to improving cytokinins contents that stimulated chlorophylls synthesis or to the increased availability of amino compounds released from chitosan (Chibu and Shibayama 2001). Farouk and Amany (2012) stated

that chlorophylls and carbohydrates of cowpea plant were reduced under water stress, whereas foliar application of chitosan significantly increased these parameters. Moreover, Khan et al. (2002) who reported that chitosan significantly increased photosynthetic pigments in maize and soybean plants. Pereira et al. (2017) found that chitosan treatment increased photosynthetic pigments of *Phaseolus vulgaris*. In another report, Behboudi et al. (2018) foliar treatment of chitosan increased photosynthetic pigments of barley plant. These results might be due to the increases of nitrogen and magnesium contents in the leaves because nitrogen and magnesium are the most important elements in the chemical composition of chlorophylls (Dzung et al. (2011).

The enhancement of photosynthetic pigment obtained in wheat leaves by foliar treatments of CaCO_3 had been proved previously on different species as pepper (Yang et al. 2016) and tung tree (Li et al. 2017). This promotive effect might be attributed to the effect of Ca^{2+} in preventing dehydration damage of cellular structures via maintaining the osmotic strength of cytoplasm in plants (Arshi et al. 2006).

Salinity stress decreased significantly IAA contents of wheat leaves (Table 4). In agreement with the obtained results, Khater et al. (2018) stated that subjecting cowpea plant to stress decreased IAA contents. Generally, the reduction of different phytohormones among them IAA caused by different abiotic stresses might be attributed the decrease in enzyme activity which participates in phytohormone synthesis and/or increases in enzymes participate in its degradation (Vaseva-Gemisheva et al. 2005). Moreover, different treatments could increase IAA contents of wheat plants. Muthukrishnan et al. (2019) confirmed the promoting role of chitosan on IAA contents of chickpea plant. These increases might be due to the induced effect of Chito on auxin-related gene expression, accelerated IAA biosynthesis and transport and reduced IAA oxidase activity increases (Li et al. 2019).

Data presented in Table 5 indicated that irrigation of wheat plants with saline water significantly increased total soluble sugars (TSS), proline content and phenolics. Moreover, treating wheat plants either with chitosan or calcium carbonate resulted in obvious significant increases in the above-mentioned parameters. The obtained results on proline and TSS of wheat plants are confirmed in various plant species under stress Ku et al. (2012) on *Nicotiana benthamiana* plant, El-Bassiouny and Sadak (2015) on flax plant, Ibrahim et al. (2016) on sunflower plant. These increases in the two major organic osmolytes contents (proline and TSS) might help plants to regulate osmotic potential of cells which led to improve water absorbance and translocation under salinity stress (Oraki et al. 2012). As well as, proline is

involved in protection of cellular structures, different enzymes from oxidative damages and acts as scavenger of free radical (Rady et al. 2015). With respect to phenol contents, phenolic compounds are antioxidants which trigger a series of secondary metabolites formed via shikimic acid or malonic acid cycles, as well as it has a cellular signaling functions (Michalak 2006).

It is well known that free radical-induced peroxidation of membrane lipids is an indicator of damage induced by stress at the cellular level (Jain et al. 2001). Thus, MDA level produced during lipids membrane peroxidation is usually used as an indicator of oxidative damage. Guimaraes et al. (2011) showed that increased MDA content might result in electrolyte leakage, indicating a loss of membrane integrity. As well as, salinity stress increased effect on MDA and H_2O_2 contents could be attributed to the inadequate induction of antioxidant system (Hossain et al. 2013). The H_2O_2 may play a role as a secondary messenger in response to abiotic stress, leading to a tolerance increase toward these unfavorable conditions, by maintaining cellular homeostasis through the antioxidant enzymes SOD, POX and CAT. The role of Chitosan on decreasing MDA and H_2O_2 contents might be proposed that CHT receptors are present on the plasma membrane; however, through a signaling cascade, the chloroplast is the primary CHT action organelle (Hadwiger 2013). Charge–charge interactions between positively charged CHT amine groups and negatively charged phospholipids promote a signal that will lead to the octadecanoid pathway activation; this metabolic pathway is directly related to the decreased H_2O_2 formation (Pichyangkura and Chadchawan 2015; Almeida et al. 2020).

Meanwhile, 20 and 40 mg/L $CaCO_3$ decreased MDA contents. These results are similar to those obtained by Issam et al. 2012, Xu et al. (2013) and Kaur et al. (2017) on different plant species. Hydrogen peroxide is used in different biochemical processes and signaling cascades required for plant growth and development. Generally, H_2O_2 contents increased in various plants subjected to stress (Dawood et al. 2014; Sadak, 2016; Kaur et al. 2017). In our investigation, H_2O_2 content increased in wheat plant subjected to salinity stress, whereas calcium chloride treatment decreased H_2O_2 contents. These obtained results are in harmony with those obtained by Amor et al. (2010) and Sadak et al. (2020).

Regarding to antioxidant enzymes, Table 6 clearly showed that irrigation of wheat plants with 5000 mg/L saline water caused significant increases in different enzymes superoxide dismutase SOD, peroxidase POX and catalase CAT as compared to those plants irrigated with tap water. Treatment of wheat grains with chitosan or $CaCO_3$ significantly promoted the activity of various antioxidant enzymes (SOD and POX) as compared to the

control (Table 6). On the other hand, CAT activity was significantly decreased as a result of chitosan or $CaCO_3$ treatments. The highest CAT activity (U/min/g FW) was recorded in plants irrigated with 5000 mg/l saline water followed by control plants which recorded 36.40 and 24.82 U/min/g FW, respectively. Plants normally cope with oxidative damage by increasing the activity of antioxidant enzymes in which it indicates stress resistance in plants (Weng et al. 2015). The enhancing effect of salinity stress is confirmed earlier on different plant species Ibrahim et al. (2016) on sunflower and Kaur et al. (2017). Oxidative stress is an important sign of abiotic stress and the increased SOD, POX and CAT activities were correlated with increasing protection from destructive resulted from oxidative stress (Miller et al. 2010). Superoxide is converted by the SOD enzyme into H_2O_2 which is still toxic and must be removed via transformation to H_2O in the next step. H_2O_2 is then eliminated by conversion to H_2O by POX enzyme (Parida et al. 2004). Peroxidases are important enzymes in various biochemical processes in plant, either in response to biotic or abiotic stresses. Also in the scavenging of ROS and plant cells protection from H_2O_2 injury (Bowler et al. 1992). The present study demonstrated that chitosan pretreatment induces the activities of SOD, POX and CAT. It was also reported that chitosan alleviates the adverse effect of water stress by enhanced production of antioxidant enzymes (Hidangmayum et al. 2019). The result also corresponded to the previous studies, which also revealed that chitosan application increases SOD, POX and CAT activities in wheat under salinity stress in wheat and maize seedlings under salt stress condition (Peykani and Sepehr 2018).

Generally, the effect of calcium carbonate on antioxidant enzymes of plant under salinity stress is in accordance with Amor et al. (2010), Xu et al. (2013) and Ibrahim et al. (2016). Eventually, the above-mentioned relationship confirmed that $CaCl_2$ has an important role in alleviation water stress in wheat plant by adjusting membrane integrity, cell wall structure and improving water status of plant or by its direct role on osmolytes and antioxidants. As well as, Ca is involved in the biosynthesis of cell wall and interacts with phytohormones (Barker and Pilbeam 2007). Therefore, calcium acts as a site-specific cofactor on peroxidase enzyme (Mac-laughlin and Wimmer 1999).

Data presented in Table 7 indicated that irrigation of wheat plants with saline water significantly decreased carbohydrate % and protein % compared with the control plants. Meanwhile, increased significantly flavonoids content and DPPH activities of the yielded grains of wheat plant as compared with control plants. Treating wheat plants either with chitosan or calcium carbonate with different concentrations resulted in obvious

significant increases in carbohydrates, protein content, flavonoids contents and DPPH activities especially in plants treated with 40 mg/L chitosan followed by 40 mg/L calcium carbonate (Table 7). These decreases on carbohydrates contents are mainly due to the reduction in growth parameters (Table 2) and photosynthetic pigments (Table 4). Carbohydrate and protein changes of the yielded grains are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration (Dubey and Pessarakli 2001). Salinity stress decreased chlorophyll contents in leaves, thus causing reduction of photosynthetic activity. So, decreased carbohydrates accumulation in mature leaves might reduce carbohydrate transport from leaves to the developing grains (Neslihan-Ozturk et al. 2002; Liu et al. 2004). On the other hand, the stimulating effect of chitosan and Ca⁺ treatments on carbohydrate contents of yielded grains might be due to the increases in growth parameters and photosynthetic pigments (Table 3). As well as, these increases in carbohydrate and proteins contents might be due to the increased photosynthetic output so decreased carbohydrates formation in leaves increased the translocation of carbohydrate from leaves to developing grains.

Flavonoids are plant secondary metabolites, the antioxidant activity (DPPH) of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo (Geetha et al. 2003). As this is the first report on the antioxidant activity of grains of wheat thorough phytochemical analyses should be done to identify the active phenolic and flavonoid components. The increased contents of flavonoid under saline conditions may reflect some kind of defense against stress conditions (i.e., oxidative burden) since salt stress was accompanied by increased production of reactive oxygen species (Rezazadeh et al. 2012). Exogenous treatment of wheat plants with chitosan or CaCO₃ resulted in a significant increase in flavonoids contents compared to untreated plants. These observations reveal that the bioactive molecule of treatments may be an inducer for the biosynthesis of secondary metabolites (flavonoids) which act as oxygen scavengers to reduce oxidative stress and, hence, increase the growth and yield wheat plant (Table 7).

Data in Table 7 showed that salinity stress caused significant increases the antioxidant activity (as DPPH-radical scavenging capacity) of wheat grain. Also, foliar treatment of wheat plant with chitosan and CaCO₃ with different concentrations (20 and 40 mM) caused significant increases in the antioxidant activity as compared with control plants. Yu et al. (2002) suggested that significant levels of antioxidant activities and phenolic

components have been detected in wheat and wheat-based food products and indicated that wheat may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion. The increase in the scavenging activity can be considered an advantage of treatment used. This could be attributed to the increases in total phenols and total flavonoids (Abd Allah et al. 2020; Orabi et al. 2015).

Conclusion

From the obtained data, it could be concluded that salinity stress adversely affected growth and biochemical parameters as compared with control plants. Meanwhile, soaking wheat grains in chitosan or calcium carbonate before sowing, especially in the higher dose (i.e., 40 mg/L) have a significant positive effect on plant growth, photosynthetic pigments, yield, yield components and bio-yield. It is obvious that application of chitosan or CaCO₃ significantly increased growth, yield quantity and quality via increasing photosynthetic pigments, indole acetic acid, total soluble sugars, proline, phenolics and the activity of some antioxidant enzymes. Moreover, wheat plants treated with chitosan or CaCO₃ gave higher technological characteristics of grain like carbohydrate%, protein%, flavonoids and antioxidant activities.

Abbreviations

Ca⁺: Calcium; CaCO₃: Calcium carbonate; *Chl*-a: Chlorophyll a; *Chl*-b: Chlorophyll b; IAA: Indole acetic acid; TSS: Total soluble sugars; MDA: Malondialdehyde; H₂O₂: Hydrogen peroxide; SOD: Superoxide dismutase; POX: Peroxidase; CAT: Catalase; DPPH: 2,2-Diphenyl-1-picryl-hydrazyl-hydrate assay.

Authors' contributions

MShS designed and performed the experiment, responsible of all the physiological and biochemical analysis and also wrote and reviewed the manuscript. IMT designed and performed the experiment, statistical analysis, responsible of all the physiological and biochemical analysis and also wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this published manuscript.

Declarations

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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