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Seedlings characters of wheat as affected by soaking with chitosan and proline under salinity stress

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Abstract

To investigate the effect of soaking with chitosan and proline levels under salinity stress on seedlings characters of wheat, a laboratory experiment was carried out at Central Laboratories in Central Administration for Seed Testing and Certification, Ministry of Agriculture, Egypt, during January 2019. The experiment was conducted in factorial experiment in randomized complete block design (RCBD) with four replications. The first factor included four levels of soaking with chitosan (0.00, 0.25, 0.50 and 0.75%). The second factor integrated with five levels of soaking with proline (0, 1, 5, 9 and 13mM). The third factor incorporated with four levels of salinity *i.e.* 0, 4, 8 and 12dSm^{-1} of NaCl. The results indicated that soaking in chitosan at 0.75% recorded highest values of seedlings characters, followed by soaking in chitosan at 0.50%. The highest values of seedlings characters were produced from soaking in highest level of proline (13mM), followed soaking in proline at 9mM. The highest values of seedlings characters were obtained from the control treatment (without salinity stress), followed by salinity stress at the level of 4dSm^{-1} of NaCl and then salinity stress at the level of 8dSm^{-1} of NaCl. It could be concluded that for maximizing seedlings characters of bread wheat Shandaweel 1 cultivar under salinity stress, it could be recommended to soaking with the mixture of chitosan at the rate of 0.75 or 0.50% and proline at the rates of 13 or 9mM for 6 h.

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Introduction

Wheat (*Triticum aestivum* L.) is used mainly as a human food due to its nutritious, concentrated, easily stored and transported and easily processed into various types of foods like; bread, macaroni, biscuit and sweets. In Egypt, wheat production is not sufficient for local consumption, where Egypt still imports about 50% from bread wheat consumption to overcome the shortage due to excessive population. It is necessary to look for ways to increase wheat crop production and the cultivated area, especially in new reclaimed soils, to improve crop growth in the first stages of growth in saline affected areas by soaking seeds with mixture of chitosan and proline.

Chitosan is an abundant and comparatively cheap organic compound. It is a large cationic polysaccharide mainly obtained from waste materials from seafood processing. Chitosan have many special properties through amine and –OH groups which making it applicable in many areas and easily available for chemical reactions. Chitosan is non-toxic material which interacts with poly anions to form complexes and gels (Se and Niranjan, 2005).

Ma *et al.* (2012) indicated that 0.0625% oligochitosan pretreatment increased root length, shoot length, dry weight and chlorophyll content of wheat seedlings. Zeng and Luo (2012) showed that wheat seeds treated with chitosan significantly improved dry weight and root length. Hameed *et al.* (2013) reported that wheat seed priming with chitosan at 0.50% improved the vigour index, seedling shoot and root length. Hameed *et al.* (2014) found that shoot and root lengths of wheat were markedly improved by chitosan priming treatments compared with the control. Wang *et al.* (2016) showed that chitosan application had a positive effect on seedling growth and its content of chlorophyll. Behboudi *et al.* (2017) showed that wheat seed priming with chitosan had significant effects on shoot, seedling, root length, fresh and dry weight, as well as vigor indexes as compared to the control treatment. Peykani and Sepehr (2018) reported that significant differences in relation with root and shoot growth as well as seedling weight were observed at high concentrations of chitosan (75%) in comparison with the control plants. Rawat *et al.* (2018) showed that seed soaking with chitosan proved to enhance seedling growth indices (root and shoot lengths, shoot and root fresh and dry weights and seedling vigour index) of wheat crop. Li *et al.* (2019) suggested chitosan nanoparticles have positive effect on seedling growth of wheat at a lower concentration (5µg/ml) than chitosan at 50µg/mL due to higher adsorption on the surface of wheat seeds.

Proline is water soluble amino acid and the most important in the protection of plant facing stress. Proline could be turned as a signaling molecule to modify plant physiological functions in terms of osmotic adjustment, upgrade photosynthetic, enhanced ion uptake, antioxidant activity and also effects on cell explosion or cell death and cause gene expression, that can be vital for plant rescue from osmotic stress (Ali *et al.*, 2013).

Ghaffari and Tadayon (2017) reported that soaking with exogenous proline at 10mM improved seedlings lengths, weights and vigor index of sugar beet. Rady *et al.* (2018) indicated that seed soaking with proline (12mM), was more effective in improving wheat seedlings growth (shoot and root lengths, fresh and dry weights). Singh *et al.* (2018) stated that that seed treatment with various concentration of proline (1, 5, and 10mM) significantly increased seed vigour index and α-amylase activity of rice. Qamar *et al.* (2019) found that pre-soaked maize seeds with proline at 400 ppm enhanced the chlorophylls, total protein and amino acids in shoot.

Salinity is an abiotic stress which causes low productivity in most crops around the world. Such stress decreases germination and provokes an uneven emergence of seedlings, thereby reducing population density, an aspect that affects crop establishment. On the other hand, salinity is known to inhibit plant growth caused, at a first phase, by water uptake reduction in roots, which is called osmotic stress or water stress phase (Munns and Tester, 2008). Afzal *et al.* (2006) pointed out that wheat seedling developments were significantly decreased due to salt stress at level of 15 $dScm^{-1}$.

Roots and shoots fresh and dry weights were reduced increasingly due to salinity compared to the control treatment. Saboora *et al.* (2006) reported that total dry weight and shoot and root dry weights of wheat significantly decreased by different salt treatments. Kaydan *et al.* (2007) reported that shoot and root dry weights of wheat seedlings were reduced with increasing level of NaCl. Mujeeb *et al.* (2008) accomplished that increasing concentrations of salinity (8.52 and 9.67dSm^{-1}) significantly reduced length and dry weight of wheat shoot. Haidarizadeh and Zarei (2009) designated that salinity more affected total weight, radicle weight, leaf weight and leaf length of wheat at 6 days. Akbarimoghaddam *et al.* (2011) stated that there is adverse effect in wheat shoot dry weight due to increases in NaCl concentrations. Increasing salinity concentrations decreased dry matter of seedling. Kandil *et al.* (2012) created that seedlings characters of wheat were significantly varied under different salinity concentrations, except shoot dry weight. Averages of seedling characters of wheat were gradually decreased with increasing salinity concentrations from 0 to 14 dS m^{-1}.

Ahmad *et al.* (2013) showed that the wheat shoot development significantly affected more than radicle development at higher salinity levels. Ghafiyehsanj *et al.* (2013) showed that with increasing of salinity, the amount of protein, insoluble sugar, shoot and root fresh weight were reduced, but soluble sugar, proline and malondialdehyde amounts were increased. Kandil *et al.* (2013) showed that root and shoot weights were decreased with increasing salinity levels. They added that increases in Na and Cl concentration in shoots maybe due to the reverse relationship between the salinity. Fercha and Gherroucha (2014) indicated that salinity significantly decreased seedling traits of wheat. Al-Saady (2015) indicated that increases in NaCl levels gradually decreased fresh and dry weights of seedlings and level of chlorophyll. Alom *et al.* (2016) point out that salinity levels significantly affected shoot length, root length and seedling dry weight of wheat. Kandil *et al.* (2017) revealed that increasing salinity levels from 3, 6, 9, 12 and 15dSm^{-1} significantly decreased shoot and root lengths, fresh and dry weights and all physiological indices. Ibrahim *et al.* (2019) found that shoot and root lengths, fresh and dry weights and seedling vigor index were significantly reduced due to increasing salinity levels from 0 to 100, 200, and 300mM NaCl.

Hence, the purpose of this investigation was to study the effect of soaking with chitosan and proline levels under salinity stress on seedlings characters under conditions of laboratory experiment.

Materials and methods

A laboratory experiment was carried out at Central Laboratories in Central Administration for Seed Testing and Certification, Ministry of Agriculture and Land Reclamation, Egypt, during January 2019. The objective of this investigation was to investigate the effect of soaking with chitosan and proline levels under salinity stress on wheat Shandaweel 1 cultivar germination and physiological characters.

The experiment was conducted in factorial experiment in randomized complete block design (RCBD) with four replications. The experiment included three factors.

The first factor included four levels of soaking with chitosan (0.25, 0.50 and 0.75% "w/v"), besides control treatment (without soaking with chitosan). The second factor integrated with five levels of soaking with proline (1, 5, 9 and 13mM), in addition control treatment (without soaking with proline). The soaking treatments with chitosan and proline were done for 6 hours.

Chitosan powder (Poly-(1.4-B-D-glucopyranosamine); 2-Amino-2-deoxy-(1-4)-B-D-glucopyranan) was prepared by dissolving a proper amount in 5% acetic acid solution. Chitosan and proline amino acid were produced by El-Nasr Pharmaceutical Chemicals Co., Egypt, which was obtained from El-Gomhouria Company for Trading Pharmaceutical Chemical & Medical.

The third factor incorporated with four levels of salinity as sodium chloride (NaCl) *i.e.* without salinity (0), 4, 8 and 12dSm^{-1} of NaCl. The concentrations of salinity are prepared from NaCl as showed in Table 1.

Table 1. Concentration of salinity stress (mM) and weight of NaCl (g).

No.	Concentration (dSm^{-1})	Weight of NaCl (g)	EC (dSm^{-1})
1	0 (control treatment)	Without salt (distilled water only)	0.001
2	4	2.560 g NaCl/L distilled water	3.530
3	8	5.120 g NaCl/L distilled water	8.390
4	12	7.680 g NaCl/L distilled water	11.960

Random sample of 400 seeds per each treatment were allowed to germinate during, January 2019, as the rules of International Seed Testing Association (ISTA, 2013) in Central Laboratories in Central Administration for Seed Testing and Certification, Ministry of Agriculture and Land Reclamation on top filter paper in sterilized Petri-dishes (12cm diameter). Each Petri-dish contains 25 seeds, and four Petri-dishes kept close together and assessed as though they were one 100-seed replication.

Each filter paper was moistened as required with a water solution of different NaCl concentrations, except the control. The papers belong to each dish were replaced every two days to prevent accumulation of salt.

The whole experiment comprised 80 Petri dishes for each replication, arranged in Factorial Experiment in Randomized Complete Block Design (RCBD).

Seedling characters

After 8 days from planting (final count), five seedlings were randomly selected from each replication and each treatment for evaluating the followed parameters:

1. Shoot length (cm). It was recorded from the seed to the tip of the bade in centimeters (cm).

2. Root length (cm). It was recorded from the seed to the tip of the root in centimeters (cm).

3. Seedling vigor index (SVI). It was calculated according to the formula of Abdul Baki and Anderson (1973):

$$(SVI) = \frac{(\text{Shoot length} + \text{Root length}) \times \text{Germination percentage}}{100}$$

4. Total chlorophylls (SPAD). Total chlorophyll content was assessed in wide leaf of plume by SPAD-502 (Minolta Co. Ltd., Osaka, Japan).

5. Shoot fresh weight (g). The weight of five seedling shoots were weighted in gram (g).

6. Root fresh weight (g). The weight of five seedling roots were weighted in gram (g).

7. Shoot dry weight (g). The weight of five seedling shoots were recorded after oven drying at 70 º C until constant weight (Agrawal, 1986).

8. Root dry weight (g). Weight of five seedling roots were recorded after oven drying at 70 ºC until constant weight (Agrawal, 1986).

Regarding to the system of analysis of variance (ANOVA), it was used for the Factorial Experiment in Randomized Complete Block Design (RCBD) as published by Gomez and Gomez (1984) of the subjected data. LSD method was used as defined by Snedecor and Cochran (1980) to compare the differences among treatment means at 5% level of probability. The data as Russel (1986) method was statistically analyzed using RCBD design by MSTAT-C computer package.

Results and discussion

A. Effect of chitosan levels

The obtained results in Tables 2 and 3 indicated that seedling characters (shoot length, root length, seedling vigor index, total chlorophylls, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight) were significantly affected by soaking wheat seeds with chitosan levels (0.00, 0.25, 0.50 and 0.75%) before starting germination test.

It could be noticed that soaking wheat seeds in chitosan at the highest rate of (0.75%) surpassed the other studied levels of soaking with chitosan and recorded the highest values of shoot and lengths, seedling vigor index, total chlorophylls, shoot and root fresh and dry weights, followed by soaking in chitosan at the rate of 0.50% without significant

differences between them in most cases and then soaking in chitosan at the rate of 0.25%. However, control treatment (without soaking with chitosan) recorded the lowest values of these characters.

Table 2. Shoot and root lengths, seedling vigor index (SVI) and total chlorophylls in wheat seedlings as affected by the levels of soaking with chitosan and proline under salinity stress as well as their interactions.

Characters / Treatments	Shoot length (cm)	Root length (cm)	Seedling vigor index (SVI)	Total chlorophylls (SPAD)
A- Chitosan levels:				
0.00% (control)	7.63	8.13	14.06	1.255
0.25%	9.14	9.99	17.22	1.281
0.50%	9.69	10.22	18.24	1.300
0.75%	11.23	12.18	21.62	1.341
LSD (0.05%)	0.54	0.71	1.14	0.015
B- Proline levels:				
0mM (control)	7.69	9.39	14.76	1.227
1mM	9.31	9.71	17.24	1.252
5mM	9.46	10.10	17.59	1.289
9mM	10.05	10.35	19.01	1.327
13mM	10.59	11.09	20.33	1.375
LSD (0.05%)	0.60	0.79	1.27	0.022
C- Salinity levels:				
0dSm^{-1} (control)	12.28	14.26	26.54	1.300
4dSm^{-1}	11.01	12.33	22.77	1.300
8dSm^{-1}	9.63	9.95	15.90	1.292
12dSm^{-1}	4.77	3.98	5.93	1.284
LSD (0.05%)	0.54	0.71	1.14	0.015
D- Interactions (F. test):				
A ×B	NS	NS	NS	NS
A ×C	*	*	*	*
B ×C	*	NS	NS	NS
A ×B × C	*	*	*	NS

Table 3. Shoot and root fresh and dry weights of wheat seedlings as affected by the levels of soaking with chitosan and proline under salinity stress as well as their interactions.

Characters / Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
A- Chitosan levels:				
0.00% (control)	0.050	0.012	0.005	0.005
0.25%	0.085	0.067	0.006	0.006
0.50%	0.109	0.091	0.007	0.007
0.75%	0.133	0.134	0.008	0.007
LSD (0.05%)	0.001	0.001	0.001	0.001
B- Proline levels:				
0mM (control)	0.067	0.042	0.005	0.005
1mM	0.081	0.061	0.006	0.006
5mM	0.093	0.071	0.006	0.006
9mM	0.111	0.086	0.008	0.006
13mM	0.119	0.120	0.008	0.008
LSD (0.05%)	0.002	0.001	0.001	0.001
C- Salinity levels:				
0dSm^{-1} (control)	0.131	0.109	0.010	0.009
4dSm^{-1}	0.098	0.088	0.007	0.007
8dSm^{-1}	0.084	0.064	0.006	0.006
12dSm^{-1}	0.063	0.043	0.003	0.003
LSD (0.05%)	0.001	0.001	0.001	0.001
D- Interactions (F. test):				
A ×B	*	*	*	*
A ×C	*	*	*	*
B ×C	*	*	*	*
A ×B × C	*	*	*	*

These increases in seedlings characters as a result of soaking wheat seeds with chitosan may be due to chitosan application enhances the physiological response and mitigates the adverse effect of abiotic stresses through stress transduction pathway via secondary messengers. Chitosan treatment stimulates photosynthetic rate, stomatal closure through ABA synthesis, enhances antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways and induces production of organic acids, sugars, amino acids and other metabolites which are required for the osmotic adjustment, stress signaling and energy metabolism under stresses (Hidangmayum *et al.*, 2019). These results in good accordance with those reported by Ma *et al.* (2012), Hameed *et al.* (2013), Hameed *et al.* (2014), Behboudi *et al.* (2017), Rawat *et al.* (2018) and Li *et al.* (2019).

B. Effect of proline levels

The obtained results in Tables 2 and 3 showed that the studied soaking with proline levels (control treatment "without soaking with proline", 1, 5, 9 and 13mM) significantly affected seedling characters (shoot length, root length, seedling vigor index, total chlorophylls, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight).

It could be observed that the highest values of shoot and lengths, seedling vigor index, total chlorophylls, shoot and root fresh and dry weights were produced from soaking wheat seeds in the highest level of proline (13mM), followed soaking in proline at the rate of 9mM without significant differences between them in most cases, then soaking in proline at the rate of 5mM and soaking in proline at the rate of 1mM. While, the lowest values of these characters were produced from control treatment (without soaking with proline).

These increases results may be due to increasing activity of α-amylase which in turn has resulted in better mobilization of stored carbohydrate reserves resulted in improvement of seedlings parameters (Kata *et al.*, 2014). In addition, proline exhibit positive effects on stress alleviation through the stimulation of α -amylase expression (Sultana *et al.* (2000). Ghaffari and Tadayon (2017) and Rady *et al.* (2018) confirmed these results.

C. Effect of salinity levels

The achieved results in Tables 2 and 3 clearly showed that salinity levels significantly affected seedling characters (shoot length, root length, seedling vigor index, total chlorophylls, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight). Increasing salinity levels from 0 to 4, 8 and 12dSm^{-1} of NaCl significantly reduced seedling characters of wheat.

It could be detected that highest values of shoot and lengths, seedling vigor index, total chlorophylls, shoot and root fresh and dry weights were obtained from the control treatment (without salinity stress), followed by salinity stress at the level of 4dSm^{-1} of NaCl and then salinity stress at the level of 8dSm^{-1} of NaCl. While, the lowest values of these germination and seedlings and also physiological indices were produced from the highest salinity level of 12dSm^{-1} of NaCl.

The reduction in germination and physiological parameters due to salinity stress concentration may influence the germination of bread wheat seed either by creating an osmotic potential external to the seed preventing water uptake, or the toxic effect of Na^+ and Cl^- ions on the germination seeds (Munns and Tester, 2008). Beside, salinity stress may be owing to salinity stress reduces cell turgor pressure and inhibits root and shoot growth. When the content of Na and Cl is high in plant cells, cells fail to divide normally, especially at the germination phase (Datta *et al.*, 2009). These results in good agreement with those found by Kandil *et al.* (2012), Ma *et al.* (2012), Biabani *et al.* (2013), Habibi and Abdoli (2013), Ayed *et al.* (2014), Alom *et al.* (2016), Kandil *et al.* (2017) and Ibrahim *et al.* (2019).

D. Effect of interactions

The effect of the interaction among three studied factors (levels of chitosan, proline and salinity) on shoot and root lengths, seedling vigor index, shoot and root fresh and dry weights was significant. While, total chlorophylls in wheat seedlings insignificant affected by the interaction among three studied factors (levels of chitosan, proline and salinity) as shown in Tables 2 and 3. The author will mention and discuss only the significant interaction among the levels of soaking with chitosan and proline under salinity stress on shoot and root lengths and shoot and root fresh weights.

With reference to the interaction effect among the levels of soaking with chitosan and proline under salinity stress on shoot length, the results presented in Table 4 clearly indicated that this interaction significantly affected shoot length. The longest wheat shoot (15.00cm) was recorded from soaking wheat seeds in the mixture of chitosan at 0.75% and proline at 9 or 13mM under without salinity stress (0dSm^{-1} of NaCl). The second best interaction treatment which recorded shoot length of 13.96cm was soaking with chitosan at the rate of 0.75% and proline at the rates of 9 or 13mM under salinity level of 4dSm^{-1} of NaCl, followed by soaking with the mixture of chitosan (0.50%) and proline (13mM) under salinity level of 4dSm^{-1} of NaCl without significant differences among them. However, the shortest wheat shoots (2.43cm%) was produced from control treatment of both soaking in chitosan and proline (without soaking with chitosan and proline) under highest salinity level of 12dSm^{-1} of NaCl.

Table 4. Shoot length (cm) of wheat seedlings as affected by the interaction among the levels of soaking with chitosan and proline under salinity stress.

Chitosan levels	Proline levels	Salinity levels			
		0dSm^{-1}	4dSm^{-1}	8dSm^{-1}	12dSm^{-1}
0.00%	0mM	9.03	6.66	4.53	2.43
	1mM	10.53	8.80	7.26	2.73
	5mM	11.13	9.36	7.60	3.10
	9mM	11.40	9.53	8.13	3.96
	13mM	11.56	9.70	8.20	5.60
0.25%	0mM	10.43	8.66	5.60	2.52
	1mM	11.66	11.10	9.06	3.10
	5mM	12.36	11.26	9.96	3.50
	9mM	12.50	11.63	10.46	5.50
	13mM	12.90	11.70	10.96	5.66
0.50%	0mM	11.66	9.63	6.93	2.70
	1mM	12.40	11.40	10.23	3.60
	5mM	12.43	11.43	10.80	4.50
	9mM	12.53	11.73	11.16	6.23
	13mM	13.76	12.33	11.46	7.60
0.75%	0mM	12.53	11.20	10.70	4.10
	1mM	13.16	13.11	11.43	5.26
	5mM	13.56	13.13	12.46	5.90
	9mM	15.00	13.96	12.80	6.43
	13mM	15.00	13.96	12.80	11.13
LSD (0.05%)		1.41			

Regarding the triple interaction among the levels of soaking with chitosan and proline under salinity stress, it had significant effect on root length as accessible in Table 5. The longest root of wheat seedlings (18.83cm) was recorded soaking in the mixture of chitosan (0.75%) and proline (13mM) under without salinity stress (0dSm^{-1} of NaCl). The second best interaction treatment which recorded root length of wheat seedlings of 18.13cm was soaking with chitosan at the rate of 0.75% and proline at the rate of 9mM under without salinity stress (control treatment), followed by soaking with the mixture of chitosan (0.75%) and proline (5mM) without salinity stress (0dSm^{-1} of NaCl) or soaking with the mixture of chitosan (0.75%) and proline (13mM) under salinity level of 4dSm^{-1} of NaCl without significant differences among them. However, the shortest root of wheat seedlings (2.16cm) was produced from control treatment of both soaking in chitosan and proline (without soaking with chitosan and proline) under highest salinity level of 12dSm^{-1} of NaCl.

Table 5. Root length (cm) of wheat seedlings as affected by the interaction among the levels of soaking with chitosan and proline under salinity stress.

Chitosan levels	Proline levels	Salinity levels			
		0dSm^{-1}	4dSm^{-1}	8dSm^{-1}	12dSm^{-1}
0.00%	0mM	9.96	9.20	5.80	2.16
	1mM	10.20	9.20	7.16	3.33
	5mM	11.33	9.83	7.95	3.83
	9mM	11.34	9.93	9.06	4.06
	13mM	11.93	11.03	10.76	4.33
0.25%	0mM	13.50	10.10	7.33	2.96
	1mM	13.53	10.46	8.13	3.30
	5mM	13.60	12.33	9.50	3.63
	9mM	14.50	12.96	10.76	4.20
	13mM	15.16	13.16	10.80	4.63
0.50%	0mM	13.93	10.70	9.03	3.10
	1mM	14.00	12.46	9.26	3.66
	5mM	14.46	12.90	10.63	4.10
	9mM	15.36	13.26	10.96	4.26
	13mM	15.76	13.50	12.96	5.50
0.75%	0mM	15.63	13.36	11.03	3.23
	1mM	16.93	13.63	11.23	4.03
	5mM	17.16	15.00	11.46	4.23
	9mM	18.13	16.53	11.80	4.73
	13mM	18.83	17.16	13.33	6.26
LSD (0.05%)		2.18			

Regarding the triple interaction among the levels of soaking with chitosan and proline under salinity stress, it had significant effect on shoot fresh weight as accessible in Table 6. The highest shoot fresh weight (0.184g) was recorded from soaking in the mixture of chitosan (0.75%) and proline (13mM) under without salinity stress of (0dSm^{-1} of NaCl). The second best interaction treatment, which recorded shoot fresh weight of 0.177 g was soaking with chitosan at the rate of 0.75% and proline at the rate of

9mM under without salinity stress (control treatment), followed by soaking with the mixture of chitosan (0.75%) and proline (13mM) under salinity level of 4dSm^{-1} of NaCl without significant differences among them. However, the lowest shoot fresh weight (0.010 g) was produced from control treatment of both soaking in chitosan and proline (without soaking with chitosan and proline) under highest salinity level of 12dSm^{-1} of NaCl.

Table 6. Shoot fresh weight (g) of wheat seedlings as affected by the interaction among the levels of soaking with chitosan and proline under salinity stress.

Chitosan levels	Proline levels	Salinity levels			
		0dSm^{-1}	4dSm^{-1}	8dSm^{-1}	12dSm^{-1}
0.00%	0mM	0.027	0.021	0.016	0.010
	1mM	0.058	0.021	0.019	0.012
	5mM	0.101	0.026	0.023	0.014
	9mM	0.102	0.102	0.065	0.018
	13mM	0.105	0.102	0.102	0.048
0.25%	0mM	0.116	0.022	0.020	0.011
	1mM	0.124	0.047	0.021	0.019
	5mM	0.130	0.080	0.056	0.048
	9mM	0.132	0.118	0.094	0.078
	13mM	0.140	0.118	0.111	0.080
0.50%	0mM	0.122	0.101	0.106	0.024
	1mM	0.136	0.109	0.107	0.088
	5mM	0.160	0.116	0.107	0.101
	9mM	0.170	0.118	0.107	0.104
	13mM	0.170	0.126	0.107	0.106
0.75%	0mM	0.132	0.125	0.112	0.045
	1mM	0.160	0.130	0.112	0.100
	5mM	0.168	0.150	0.120	0.102
	9mM	0.177	0.162	0.137	0.117
	13mM	0.184	0.176	0.140	0.138
LSD (0.05%)		0.004			

The interaction among the three studied factors (levels of soaking with chitosan and proline under salinity levels) significantly affected root fresh weight as results accessible in Table 7. The highest root fresh weight (0.306 g) was obtained from soaking in the mixture of high level of chitosan (0.75%) and proline (13mM) under without salinity stress (0dSm^{-1} of NaCl). Soaking with chitosan at the rate of 0.75% and proline at the rate of 13mM under salinity level of 4dSm^{-1} of NaCl was the second best interaction treatment, which recorded root fresh weight of 0.303 g. However, the third best interaction treatment was soaking with the mixture of chitosan (0.75%) and proline (9mM) under without salinity stress with significant differences among them. While, control treatment of both soaking in chitosan and proline (without soaking with chitosan and proline) under highest salinity level (12dSm^{-1} of NaCl) produced the lowest root fresh weight (0.003 g).

Table 7. Root fresh weight (g) of wheat seedlings as affected by the interaction among the levels of soaking with chitosan and proline under salinity stress.

Chitosan levels	Proline levels	Salinity levels			
		0dSm^{-1}	4dSm^{-1}	8dSm^{-1}	12dSm^{-1}
0.00%	0mM	0.008	0.007	0.006	0.003
	1mM	0.009	0.008	0.006	0.004
	5mM	0.028	0.008	0.007	0.004
	9mM	0.046	0.008	0.007	0.005
	13mM	0.044	0.009	0.007	0.007
0.25%	0mM	0.072	0.055	0.011	0.006
	1mM	0.074	0.064	0.052	0.029
	5mM	0.086	0.076	0.068	0.032
	9mM	0.094	0.080	0.078	0.056
	13mM	0.094	0.090	0.090	0.060
0.50%	0mM	0.102	0.061	0.047	0.010
	1mM	0.102	0.075	0.054	0.043
	5mM	0.107	0.104	0.097	0.048
	9mM	0.110	0.105	0.102	0.101
	13mM	0.183	0.179	0.108	0.103
0.75%	0mM	0.105	0.090	0.050	0.032
	1mM	0.160	0.101	0.059	0.044
	5mM	0.213	0.138	0.090	0.058
	9mM	0.237	0.200	0.120	0.087
	13mM	0.306	0.303	0.213	0.135
LSD (0.05%)		0.003			

Conclusion

From obtained results in this study it could be concluded that for maximizing seedlings characters of bread wheat Shandaweel 1 cultivar under salinity stress, it could be recommended to soaking with the mixture of chitosan at the rate of 0.75 or 0.50% and proline at the rates of 13 or 9mM for 6 h.

References

AbdulBaki AA, Anderson JD. 1973.Viability and leaching of sugars from germinating barley. Crops Sci **10,** 31-34.

Afzal I, Basra SMA, Farooq M, Nawaz A. 2006. Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. Int. J. Agri. Bio **8(1),** 23-28.

Agrawal PK. 1986. Seed vigor: Concepts and Measurements. In: Seed Production Technology (Ed. J.P. Srivastava and L.T. Simarsk), ICARDA, Aleppo, Syria pp, 190-198.

Ahmad M, Shahzad A, Iqbal M, Asif M, Hirani AH. 2013. Morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. Australian. J. Cro. Sci **7(1),** 66-74.

Akbarimoghaddam H, Galavi M, Ghanbari A, Panjehkeh N. 2011. Salinity effects on seed germination and seedling growth of bread wheat cultivars. Trakia. J. of Sci **9 (1),** 43-50.

Ali Q, Anwar F, Ashraf M, Saari N, Parveen R. 2013. Ameliorating effects of exogenously applied proline on seed composition, seed oil quality and oil antioxidant activity of maize (*Zea mays* L.) under drought stress. Intern. J. of Molecular Sci **14,** 818-835.

Alom R, Abu Hasan M, Rabiul Islam M, Wang QF. 2016. Germination characters and early seedling growth of wheat (*Triticum aestivum* L.) genotypes under salt stress conditions. J. Crop Sci. Biot **19(5),** 383-392.

Al-Saady HAA. 2015. Germination and growth of wheat plants (*Triticum aestivum* L.) under salt stress. J. of Pharma. Chem. Bio. Sci **3(3),** 416-420.

Ayed S, Neila R, Zoubeir C, Saddreddine B, Feker K, Themir B, Mayada M, Mongi B. 2014. Effect of salt stress (sodium chloride) on germination and seedling growth of durum wheat (*Triticum durum* Desf.) genotypes. Int. J. of Bio and Cons **6(4),** 320-325.

Behboudi F, Sarvestani ZT, Kassaee MZ, Sanavi SAMM, Sorooshzadeh A. 2017. Phytotoxicity of chitosan and SiO_2 nanoparticles to seed germination of wheat (*Triticum aestivum* L.) and Barley (*Hordeum vulgare* L.) plants. Not. Sci. Bio **9(2),** 242-249. DOI: 10.15835/nsb9210075.

Biabani A, Heidari H, Vafaie-Tabar M. 2013. Salinity effect of stress on germination of wheat cultivars. Int. J. of Agric. and F. Sci. Tech **4(3),** 263-268.

Cox TD. 2006. Germination of five wheat varieties over a range of salinities. Centaurs **14,** 15-18.

Datta JK, Nag S, Banerjee A, Mondal NK. 2009. Impact of salt stress on five varieties of wheat (*Triticum aestivum* L.) cultivars under laboratory condition. J. Appl. Sci. Environ. Man **13(3),** 93-97.

Fercha A, Gherroucha H. 2014. The role of osmoprotectants and antioxidant enzymes in the differential response of durum wheat genotypes to salinity. J. of Appli. Bot. and food Quality **87,** 74-79.

Ghaffari H, Tadayon MR. 2017. Effect of proline on seed germination indices of castile sugar beet cultivar seed germination criteria under drought stress. Iranian J. of Seed Sci. and Tech **7(2),** 171-182.

Ghafiyehsanj E, Dilmaghani K, Shoar HH. 2013. The effects of salicylic acid on some of biochemical characteristics of wheat (*Triticum aestivum* L.) under salinity stress. Annals. Bio. Res **4(6),** 242-248.

Gomez KA, Gomez AA. 1984. Statistical Procedures for Agricultural Research. 2nd Ed., Jhon Wiley and Sons Inc., New York pp, 95-109.

Habibi A, Abdoli M. 2013. Influence of salicylic acid pre-treatment on germination, vigor and growth parameters of garden cress (*Lepidium sativum*) seedlings under water potential loss at salinity stress. Int. Res. J. Appl. Basic. Sci **4(6),** 1493-1499.

Haidarizadeh M, Zarei MA. 2009. Effect of different sodium chloride concentrations on early seedlings growth of wheat cultivar (*Triticum aestivum* L.). J. of Bio. Sci **9(2),** 188-191.

Hameed A, Sheikh MA, Hameed A, Farooq T, Basra SMA, Jamil A. 2013. Chitosan priming enhances the seed germination, antioxidants, hydrolytic enzymes, soluble proteins and sugars in wheat seeds. Agrochimica, Vol. LVII - N. **1,** 32-46.

Hameed A, Sheikh MA, Hameed A, Farooq T, Basra SMA, Jamil A. 2014. Chitosan seed priming improves seed germination and seedling growth in wheat (*Triticum aestivum* L.) under osmotic stress induced by polyethylene glycol. Philippines Agric. Sci **97(3),** 294-299.

Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. 2019. Application of chitosan on plant responses with special reference to abiotic stress. Physiol. Mol. Bio. Plants DOI.org/10. 1007/s12298-018-0633-1

Ibrahim MEH, Zhu X, Zhou G, Ali AYA, Elsiddig AMI, Farah GA. 2019. Response of some wheat varieties to gibberellic acid under saline conditions. Agrosyst. Geosci. Environ **2,** 190003, DOI,10.2134/age2019.01.0003.

ISTA Rules. 2013. International Seed Testing Association. ISTA Germination Sec., Chapter **5,** 55-44.

Kandil AA, Sharief AE, Elbadry, Doha EA. 2017. Seedling parameters as affected by soaking in humic acid, salinity stress and grain sorghum genotypes. Int. J. of Environ. Agri and Biotech., (IJEAB) **2(6),** 3213-3223.

Kandil AA, Sharief AE, Elokda MA. 2012. Germination and seedling characters of different wheat cultivars under salinity stress. J. of Basic & Appli. Sci **8,** 585-596.

Kandil EE, Schulz R, Muller T. 2013. Response of some wheat cultivars to salinity and water stress. J. of Appli. Sci. Res **9(8),** 4589-4596.

Kata LP, Bhaskaran M, Umarani R. 2014. Influence of priming treatments on stress tolerance during seed germination of rice. Intern. J. Agric. Environ. Biotech **7,** 225-232.

Kaydan D, Yagmur M, Okut N. 2007. Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). Tarim Bilimleri Dergisi **13(2),** 114-119.

Li R, He J, Xie H, Wang W, Bose SK, Sunb Y, Hu J, Yina H. 2019. Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). Intern. J. of Bio. Macromolecules **126,** 91-100.

Ma L, Li Y, Yu C, Wang Y, Li X, Li N, Chen Q, Bu N. 2012. Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. Protoplasma **249,** 393-399.

Mujeeb KA, Gul A, Farooq M, Rizwan S, Ahmad I. 2008. Rebirth of synthetic hexaploids with global implications for wheat improvement. Australian J. Agric. Res **59,** 391-398.

Munns RY, Tester M. 2008. Mechanisms of salinity tolerance. Ann. Rev. of Plant Bio **59,** 651-681.

Peykani LS, Sepehr MF. 2018. Effect of chitosan on antioxidant enzyme activity, proline, and malondialdehyde content in *Triticum aestivum* L. and *Zea maize* L. under salt stress condition. Iranian J. of Plant Physiol **9(1),** 2661-2670.

Qamar R, Noreen S, Safdar M, Babar ME. 2019. Influence of exogenous application of proline on some physio-biochemical parameters of maize (*Zea mays* L.) under drought stress. Intern. J. of Sci. and Res. Pub **9(8),** 858-869. DOI.org/10.29322 /IJSRP.9. 08.2019.p92117.www.ijsrp.org.

Radym M, Kuşvuran A, Alharby HF, Alzahrani Y, Kuşvuran S. 2018. Pretreatment with proline or an organic bio-stimulant induces salt tolerance in wheat plants by improving antioxidant redox state and enzymatic activities and reducing the oxidative stress. J. of Plant Growth Reg. DOI.org/10.1007/ s00344-018-9860-5.

Rawat PS, Kumar R, Ram P, Pandey P. 2018. Effect of nanoparticles on wheat seed germination and seedling growth. Intern. J. of Bio., Life and Agric. Sci **12(1),** 13-16.

Russell DF. 1986. MSTAT-C computer based data analysis software Crop and Soil Science Department, Michigan State University USA.

Saboora A, Kiarostami K, Behroozbayati F, Hajihashemi S. 2006. Salinity tolerance of wheat genotype at germination and early seedling growth. Pakistan J. Bio. Sci **9(11),** 2009-2021.

Se KK, Niranjan R. 2005. Enzymatic production and biological activities of chitosan oligosaccharides (COS): a review. Carbohydr. Polym **62,** 357-368.

Singh M, Singh AK, Nehal N, Sharma N. 2018. Effect of proline on germination and seedling growth of rice (*Oryza sativa* L.) under salt stress. J. of Pharmaco. and Phytochem **7(1),** 2449-2452.

Snedecor GW, Cochran WG. 1980. Statistical Methods. 7th Ed. Iowa State University Press, Iowa, USA pp. 507.

Sultana N, Ikeda T, Mitsui T. 2000. GA_3 and proline promote germination of wheat seeds by stimulating α-amylase at unfavorable temperatures. Plant Prod. Sci **3(3),** 232-237.

Wang Y, Yu D, Li C, Zhou X. 2016. Effect of chitosan on seed germination and seedling physiological characters of wheat under salt stress. Agric. Res. Arid Areas **34(1),** 180-185.

Wated A, Reinhard EL, Lerner HR. 1983.Comparison between a stable NaCl selected Nicotiana cell line and the wild type, Na, K and proline pools as a function of salinity. Plant Physiol **73,** 624-629.

Wu G, Zhou Z, Chen P, Tang X, Shao H, Wang H. 2014. Comparative ecophysiological study of salt stress for wild and cultivated soybean species from the yellow river delta, China. The Sci. World J **45,** 321-340.

Zadeh AK, Mousavi SH, Nejad ME. 2013. The effect of salinity stress on germination and seedling growth of native and breaded varieties of wheat. J. of Nov. Appl. Sci **2(12),** 703-709.

Zeng D, Luo X. 2012. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. J. of Soil Sci **2,** 282-288.