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Effect of soaking with chitosan and proline under salinity on germination and physiological characters of wheat

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Abstract

A laboratory experiment was carried out at Central Laboratories in Central Administration for Seed Testing and Certification, Ministry of Agriculture, Egypt, during January 2019 to investigate the effect of soaking with chitosan and proline levels under salinity stress on wheat 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 (0.00, 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.* without salinity (0), 4, 8 and 12dSm^{-1} of NaCl. The results indicated that the highest germination and physiological characters were resulted from soaking in chitosan at 0.75%, followed by soaking in chitosan at 0.50%. The highest values of germination and physiological characters were produced from soaking wheat seeds in proline at 13mM, followed soaking in proline at 9mM. Increasing salinity levels from 0 to 4, 8 and 12dSm^{-1} of NaCl significantly reduced germination characters and physiological indices of wheat. It could be concluded that for maximizing germination and physiological parameters 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 considered the main food and major source for human nutrition and a part of daily dietary. Wheat 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. Although wheat is useful as a livestock feed. In Egypt, wheat is the main cereal winter crop covering an area reached about 3.196 million feddan in 2018 season and the total production exceeded 8.800 million tons with an average of 18.36 ardab/fed (FAO, 2020). However, 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 a natural polymer derived from deacetylation of chitin. Chitin is readily available from shellfish waste from food processing. As a high molecular polymer, nontoxic, bioactive agent, chitosan has become a useful appreciated compound due to its fungicidal effects and elicitation of defense mechanisms in plant tissues (Terry and Joyce, 2004). Chitosan forms a semi-permeable film that regulates gas exchange, reduces respiration and transpiration rates and slows down the ripening processes (Shehata *et al.*, 2012). Zeng and Luo (2012) showed that wheat seeds treated with chitosan significantly improved germination rate and impacted physiological indices. Hameed *et al.* (2013) reported that wheat seed priming with chitosan at 0.50% improved the final germination in terms of its rate, percentage, energy and index, while shortened the mean germination time. Hameed *et al.* (2014) found that the final germination percentage, germination rate, germination energy, germination index of wheat were markedly improved by chitosan priming treatments compared with the control. However, chitosan priming treatments reduced the mean germination time. Orzali *et al.* (2014) revealed that the chitosan seed treatment induced a decrease in disease severity and enhanced germination parameters, suggesting the possibility of the use of chitosan as a wheat seed treatment in crop protection in order to improve the plant defense response and germination parameters. Wang *et al.* (2016) showed that chitosan application had a positive effect on germination parameters. Aboonlertnirun and Sirikesorn (2018) decided that application of chitosan as seed soaking reduced seed injury and increased Peroxidase activity during seed germination. Peykani and Sepehr (2018) reported that significant differences in relation with germination percentage 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 final germination percentage of wheat crop. Li *et al.* (2019) suggested chitosan nanoparticles have positive effect on seed germination of wheat at a lower concentration (5µg/ml) than chitosan at 50µg/mL due to higher adsorption on the surface of wheat seeds.

Proline amino acid plays an adaptive role in the tolerance of plant cells to salinity by increasing the concentration of cultural osmotic components in order to equalize the osmotic potential of the cytoplasm (Wated *et al.,* 1983).The increase in proline content in plant tissues with the increase in salinity retards protein synthesis, and consequently accumulates free amino acids, including proline (El-Leboudi *et al.,* 1997). Ghaffari and Tadayon (2017) reported that soaking with exogenous proline at 10mM improved the germination percent, coefficient of germination, rate and index of germination and decreased mean germination time of sugar beet. Singh *et al.* (2018) stated that that seed treatment with various concentration of proline (1, 5, and 10mM) significantly increased the germination (%) and α-amylase activity of rice.

Salinity is one of the most important abiotic stresses and it limits the productivity and geographical distribution of plants. According to the FAO report, over 6% of the world's land (about 800 million ha) is affected by salinity. Most of this salinity affected land has arisen from natural causes *i.e.* accumulation of

salts over long periods of time in arid and semiarid zones, and also by human activities such as irrigation (Munns and Tester, 2008). The adverse impacts imposed by salt stress are osmotic stress, ionic stress, nutrient imbalance, and the production of reactive oxygen species; then, the plants would display declining growth and photosynthesis rate, even death in the end. Salinity affects many aspects of plant metabolism and the accumulation of various organic solutes that provide the turgor necessary for cell expansion. Among them, the accumulation of low molecular weight solutes and compatible osmolytes, such as proline and glycine betaine, function as osmoprotectants (Wu *et al*., 2014). Kashem *et al.* (2000) demonstrated that increasing NaCl levels significantly reduced speed of germination of wheat. Afzal *et al.* (2006) pointed out that wheat germination percentage was significantly decreased due to salt stress at level of 15 dS cm^{-1}. Cox (2006) showed that wheat germination was delayed with increasing salinity stress. Saboora *et al.* (2006) reported that germination percentage and rate of wheat significantly decreased by different salt treatments. Mujeeb *et al.* (2008) accomplished that increasing concentrations of salinity (8.52 and 9.67dSm^{-1}) significantly reduced germination parameters of wheat.

Rahman *et al.* (2008) confirmed that there was a decrease in water uptake and wheat germination by increasing in salt concentration. Kandil *et al.* (2012) created that all germination characters of wheat were significantly varied under different salinity concentrations. Averages of germination characters of wheat were gradually decreased with increasing salinity concentrations from 0 to 14 dS m^{-1}. Kausar *et al.* (2012) indicated that physiological parameters of germination stress tolerance index, shoot length stress tolerance index, root length stress tolerance index were valuable to screen great quantity of sorghum germplasm for salt tolerance under salinity condition. Hussain *et al.* (2013) revealed that a gradual reduction in seed germination with increasing concentrations of NaCl solution from 6.8 to 13.2 and 19.0dSm^{-1}. Kandil *et al.* (2013) showed that percentages of seed germination was decreased with increasing salinity levels. Mahmoodzadeh *et al.* (2013) found a significant variation related to seed germination due to salinity stress. The salinity levels of 4dSm^{-1} and 8dSm^{-1} significantly overdue the initiation of seed germination. Fercha and Gherroucha (2014) indicated that salinity significantly decreased germination traits of wheat. Al-Saady (2015) indicated that increases in NaCl levels gradually decreased percentage of germination. Salinity at concentration of 160mM/L recorded gradual and significant decreases in rate of germination. Alom *et al.* (2016) point out that salinity levels significantly affected percentage of germination of wheat. Kandil *et al.* (2017) showed that increasing salinity levels to 15dSm^{-1} decreased percentage of germination, germination rate, index of germination, germination energy and seedling vigor index by 15.9, 15.0, 30.0, 35.9 and 37.6%, respectively compared without salinity. Ibrahim *et al.* (2019) found that germination rate and germination percentage were significantly reduced due to increasing salinity levels from 0 to 100, 200, and 300mM NaCl.

Therefore, the objective of this investigation was to study the effect of soaking with chitosan and proline levels on germination and physiological characters under salinity stress 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).

NL	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 (12 cm 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).

Data recorded

Germination characters

1. Final germination percentage (FG%). Normal seedlings of each replicate were counted after 8 days from planting (final count) and expressed as percentage according to the following equation described by ISTA (1996):

$$\text{FG \%} = \frac{\text{Number of normal seedlings}}{\text{Total number of tested seeds}}$$

2. Germination index (GI). It was calculated according to the following equation (Karim *et al.,* 1992):

$$\text{GI} = \frac{\textbf{Germination percentage in each treatment}}{\textbf{Germination percentage in control treatment}}$$

3. Germination rate (GR). It was calculated by the following formula (ISTA, 1996):

$$\text{GR} = \frac{\text{No. of germinated seeds}}{\text{Days to first count}} + \frac{\dots\dots\dots\dots\dots\dots\dots}{\dots\dots\dots\dots\dots\dots\dots} + \frac{\text{No. of germinated seeds}}{\text{Days to final count}}$$

4. Mean germination time (MGT, day). It was calculated based on the following equation of Ellis and Roberts (1981):

$$\text{MGT} = \frac{\sum \text{Dn}}{\sum \text{n}}$$

Where (n) is the number of seeds, which were germinated on day, D is number of days counted from the beginning of germination.

Physiological indices

To calculate all physiological indices, it were estimated using following formulas, according to Ashraf *et al.* (2008).

1. Promptness index (PI). It was calculated according the following equation:

$PI = nd_1 (1.00) + nd_2 (0.75) + nd_3 (0.50) + nd_4 (0.25)$

Where nd_1, nd_2, nd_3 and nd_4 = Number of germinated seeds on the 1st, 2nd, 3rd and 4th days, respectively.

2. Germination stress tolerance index (GSTI). It was calculated using following formula:

$$\text{GSTI} = \frac{\text{PI of stress seeds}}{\text{PI of control seeds}} \times 100$$

3. Shoot length stress index (SLSI). It was calculated using following formula:

$$\text{SLSI} = \frac{\text{Shoot length of stressed seeds}}{\text{Shoot length of the control}} \times 100$$

4. Root length stress index (RLSI). It was calculated using following formula:

$$\text{RLSI} = \frac{\text{Root length of stressed seeds}}{\text{Root length of the control}} \times 100$$

5. Shoot fresh stress index (SFSI). It was calculated using following formula:

$$\text{SFSI} = \frac{\text{Shoot fresh of stressed seeds}}{\text{Shoot fresh of the control}} \times 100$$

6. Root fresh stress index (RFSI). It was calculated using following formula:

$$\text{RFSI} = \frac{\text{Root fresh of stressed seeds}}{\text{Root fresh of the control}} \times 100$$

7. Shoot dry stress index (SDSI). It was calculated using following formula:

$$\text{SDSI} = \frac{\text{Shoot dry of stressed seeds}}{\text{Shoot dry of the control}} \times 100$$

8. Root dry stress index (RDSI). It was calculated using following formula:

$$\text{RDSI} = \frac{\text{Root dry of stressed seeds}}{\text{Root dry of the control}} \times 100$$

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

Effect of chitosan levels

The obtained results in Tables 2, 3 and 4 indicated that germination characters (final germination percentage, germination index and germination rate) and physiological indices (promptness index, germination stress tolerance index, shoot length stress index, root length stress index, shoot fresh stress index, root fresh stress index, shoot dry stress index and root dry stress index) were significantly affected by soaking wheat seeds with chitosan levels (0.00, 0.25, 0.50 and 0.75%) before starting germination test. While, mean germination time only insignificantly affected by levels of soaking before starting germination test with chitosan.

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 final germination percentage, germination index and rate, promptness index, shoot and root length stress index, shoot and root fresh and dry stress index, 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.

The results obviously showed that the highest mean germination time value was resulted from control treatment (without soaking with chitosan) or soaking wheat seeds in chitosan at the rates of 0.25 or 0.50% and the lowest value was produced from soaking wheat seeds in chitosan at the rate of 0.75%. While, soaking wheat seeds in chitosan at 0.25% recorded the lowest germination stress tolerance index, followed by soaking in chitosan at the rate of 0.50% and then soaking in chitosan at the rate of 0.75%. On the other hand, control treatment (without soaking with chitosan) produced the highest germination stress tolerance index.

These increases in germination and physiological parameters 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

Table 2. Final germination percentage (FG%), germination index (GI), germination rate (GR) and mean germination time (MGT) of wheat seed as affected by the levels of soaking with chitosan and proline under salinity stress as well as their interactions.

Characters / Treatments	Final germination percentage (FG%)	Germination index (GI)	Germination rate (GR)	Mean germination time (MGT)
A- Chitosan levels:				
0.00% (control)	84.30	0.843	5.269	8.114
0.25%	84.93	0.849	5.308	8.114
0.50%	86.60	0.866	5.413	8.114
0.75%	87.93	0.879	5.496	8.081
LSD $_{(0.05\%)}$	1.7	0.017	0.108	NS
B- Proline levels:				
0mM (control)	79.12	0.791	4.945	8.338
1mM	83.62	0.836	5.227	8.237
5mM	86.29	0.863	5.393	8.149
9mM	89.41	0.894	5.589	8.046
13mM	91.25	0.913	5.703	7.759
LSD $_{(0.05\%)}$	1.9	0.019	0.120	0.105
C- Salinity levels:				
0dSm^{-1} (control)	100.00	1.000	6.250	8.060
4dSm^{-1}	97.40	0.974	6.088	8.087
8dSm^{-1}	80.26	0.803	5.017	8.135
12dSm^{-1}	66.10	0.661	4.131	8.140
LSD $_{(0.05\%)}$	1.7	0.017	0.108	0.074
D- Interactions (F. test):				
A ×B	NS	NS	NS	NS
A ×C	*	*	*	NS
B ×C	*	*	*	*
A ×B × C	*	*	*	NS

Table 3. Promptness index (PI), germination stress tolerance index (GSTI), shoot length stress index (SLSI) and root length stress index (RLSI)of wheat seed as affected by the levels of soaking with chitosan and proline under salinity stress as well as their interactions.

Characters / Treatments	Promptness index (PI)	Germination stress tolerance index (GSTI)	Shoot length stress index (SLSI)	Root length stress index (RLSI)
A- Chitosan levels:				
0.00% (control)	10.62	170.1	78.23	71.60
0.25%	10.96	81.8	84.16	75.91
0.50%	11.27	83.0	89.59	78.12
0.75%	11.54	83.5	89.63	79.38
LSD $_{(0.05\%)}$	0.38	20.9	4.07	2.90
B- Proline levels:				
0mM (control)	10.39	93.9	68.87	70.59
1mM	10.25	97.1	84.89	73.38
5mM	11.34	105.9	85.96	76.05
9mM	11.65	113.4	91.23	77.60
13mM	11.87	112.8	96.06	83.64
LSD $_{(0.05\%)}$	0.42	18.0	4.55	3.14
C- Salinity levels:				
0dSm^{-1} (control)	12.85	119.7	112.06	107.14
4dSm^{-1}	12.59	118.6	99.67	92.69
8dSm^{-1}	10.55	99.1	87.28	74.98
12dSm^{-1}	8.41	81.0	42.60	30.21
LSD $_{(0.05\%)}$	0.38	20.9	4.07	2.90
D- Interactions (F. test):				
A ×B	NS	NS	NS	NS
A ×C	NS	NS	*	*
B ×C	*	NS	*	*
A ×B × C	*	NS	*	*

Table 4. Shoot fresh stress index (SFSI), root fresh stress index (RFSI), shoot dry stress index (SDSI) and root dry stress index (RDSI) of wheat seed as affected by the levels of soaking with chitosan and proline under salinity stress as well as their interactions.

Characters / Treatments	Shoot fresh stress index (SFSI)	Root fresh stress index (RFSI)	Shoot dry stress index (SDSI)	Root dry stress index (RDSI)
A- Chitosan levels:				
0.00% (control)	73.28	89.07	83.11	80.31
0.25%	83.47	93.70	83.65	81.81
0.50%	100.44	127.40	111.94	89.42
0.75%	114.45	134.12	139.95	93.30
LSD $_{(0.05\%)}$	1.68	0.79	0.60	0.18
B- Proline levels:				
0mM (control)	66.33	62.54	70.38	70.46
1mM	82.41	84.18	89.28	76.96
5mM	103.37	112.30	99.53	84.78
9mM	140.27	137.27	123.73	86.11
13mM	159.66	171.59	140.39	112.73
LSD $_{(0.05\%)}$	1.88	0.88	0.79	0.21
C- Salinity levels:				
0dSm^{-1} (control)	161.84	191.67	161.64	119.53
4dSm^{-1}	116.26	115.67	114.65	98.11
8dSm^{-1}	99.32	87.54	92.45	84.28
12dSm^{-1}	64.22	59.42	49.91	42.92
LSD $_{(0.05\%)}$	1.68	0.79	0.60	0.18
D- Interactions (F. test):				
A ×B	*	*	*	*
A ×C	*	*	*	*
B ×C	*	*	*	*
A ×B × C	*	*	*	*

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 Hameed *et al.* (2013), Hameed *et al.* (2014), Orzali *et al.* (2014), Wang *et al.* (2016), Peykani and Sepehr (2018), Rawat *et al.* (2018) and Li *et al.* (2019).

Effect of proline levels

The obtained results in Tables 2, 3 and 4 showed that the studied soaking with proline levels (control treatment "without soaking with proline", 1, 5, 9 and 13mM) significantly affected germination characters (final germination percentage, germination index, germination rate and mean germination time) and physiological indices (promptness index, germination stress tolerance index, shoot length stress index, root length stress index, shoot fresh stress index, root fresh stress index, shoot dry stress index and root dry stress index).

It could be observed that the highest values of final germination percentage, germination index and rate, promptness index, germination stress tolerance index, shoot and root length stress index, shoot and root fresh and dry stress index 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).

Soaking wheat seeds in the highest level of proline (13mM) resulted in the lowest mean germination time, followed soaking in proline at the rate of 9mM without significant differences between them, then soaking in proline at the rate of 5mM and soaking in proline at the rate of 1mM. While, germinating wheat seeds without soaking with proline (the control treatment) resulted in the highest mean germination time.

These increases results may be due to proline content plays a vital role to protect cellular membranes by

damaging effect of reactive oxygen species and maintain proteins contents (Errabii *et al.*, 2006). In addition, proline was effective in promoting germination and increasing α-amylase expression. Also, these results suggest that proline exhibit positive effects on stress alleviation through the stimulation of α -amylase expression (Sultana *et al.* (2000). Ghaffari and Tadayon (2017) and Singh *et al.* (2018) confirmed these results.

Effect of salinity levels

The achieved results in Tables 2, 3 and 4 clearly showed that salinity levels significantly affected germination characters (final germination percentage, germination index, germination rate and mean germination time) and physiological indices (promptness index, germination stress tolerance index, shoot length stress index, root length stress index, shoot fresh stress index, root fresh stress index, shoot dry stress index and root dry stress index).

Increasing salinity levels from 0 to 4, 8 and 12dSm^{-1} of NaCl significantly reduced germination characters and physiological indices of wheat, with exception mean germination time that had a reverse trend.

It could be detected that highest values of final germination percentage, germination index and rate, promptness index, germination stress tolerance index, shoot and root length stress index, shoot and root fresh and dry stress index 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.

In general, increasing salinity levels from 0 to 4, 8 and 12dSm^{-1} of NaCl significantly increased mean germination time. Where, the control treatment (without salinity stress) recorded the lowest mean of germination time. However, the highest mean germination time was produced from salinity levels at the rate 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), Habibi and Abdoli (2013), Kandil *et al.* (2013), Mahmoodzadeh *et al.* (2013), Zadeh *et al.* (2013), Oproi and Madosa (2014), Al-Saady (2015), Alom *et al.* (2016), Kandil *et al.* (2017 a) and Ibrahim *et al.* (2019).

Effect of interactions

The effect of the interaction among three studied factors (levels of chitosan, proline and salinity) on final germination percentage, germination index, germination rate, promptness index, shoot and root length stress index, shoot and root fresh and dry stress index was significant. While, mean germination time and germination stress tolerance index insignificant affected by the interaction among three studied factors (levels of chitosan, proline and salinity) as shown in Tables 2, 3 and 4. The author will mention and discuss only the significant interaction among the levels of soaking with chitosan and proline under salinity stress on final germination percentage and promptness index.

The interaction effect among the levels of soaking with chitosan and proline under salinity stress on final germination percentage, the results presented in Table 5 clearly recorded that this interaction significantly affected final germination percentage. The highest percentage of final germination (100.00%) was recorded from all levels of soaking with chitosan and proline under without salinity stress, or soaking in the mixture of chitosan and proline at the highest level of them (0.75% and 13mM, respectively) under salinity level of 4dSm^{-1} of NaCl.

The second best interaction treatment which recorded (99.33%) was soaking with chitosan at the rate of 0.75% and proline at the rate of 9mM under salinity level of 4dSm^{-1} of NaCl, followed by soaking with the mixture of chitosan (0.75%) and proline (5mM) or soaking with the mixture of chitosan (0.50%) and proline (9 or 13mM) or soaking with the mixture of chitosan (0.25%) and proline (13mM) or soaking with the mixture of chitosan (0.00%) and proline (13mM) under salinity level of 4dSm^{-1} of NaCl without significant differences among them. However, the lowest percentage of final germination (38.66%) 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. Final germination percentage (FG%) of wheat seed 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	100.00	94.66	61.33	38.66
	1mM	100.00	95.33	66.66	52.66
	5mM	100.00	96.66	77.33	64.66
	9mM	100.00	97.33	80.66	70.00
	13mM	100.00	98.66	90.66	76.66
0.25%	0mM	100.00	94.66	69.33	49.33
	1mM	100.00	95.33	66.66	54.66
	5mM	100.00	96.66	77.33	64.66
	9mM	100.00	98.00	80.66	70.00
	13mM	100.00	98.66	90.66	76.66
0.50%	0mM	100.00	96.66	76.66	52.00
	1mM	100.00	97.33	83.33	62.00
	5mM	100.00	97.33	86.66	74.66
	9mM	100.00	98.66	86.00	76.00
	13mM	100.00	98.66	88.00	80.00
0.75%	0mM	100.00	97.33	76.66	56.66
	1mM	100.00	98.00	75.33	62.00
	5mM	100.00	98.66	90.66	80.66
	9mM	100.00	99.33	92.00	78.00
	13mM	100.00	100.00	88.66	82.00
LSD (0.05%)				7.7	

The interaction among the three studied factors (levels of soaking with chitosan and proline under salinity levels) significantly affected promptness index as results accessible in Table 6. The highest promptness index (13.83) was obtained from soaking in the mixture of chitosan (at 0.75%) and proline (at 9 or 13mM) under without salinity stress of (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 or soaking with chitosan at the rate of 0.75% and proline at the rate of 5mM under without salinity stress was the second best interaction treatment, which recorded promptness index at 13.66. However the third best interaction treatment was soaking with the mixture of chitosan (0.50%) and proline (13mM) without salinity stress without 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 PI (5.41).

Table 6. Promptness index (PI) of wheat 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	9.75	10.75	7.75	5.41
	1mM	11.58	12.00	8.50	6.58
	5mM	12.66	12.16	10.50	8.25
	9mM	13.33	12.83	10.91	9.33
	13mM	13.33	13.25	11.50	9.41
0.25%	0mM	11.58	11.50	8.50	6.50
	1mM	12.66	12.00	8.75	7.00
	5mM	13.33	12.33	10.50	8.25
	9mM	13.41	13.08	10.91	9.33
	13mM	13.56	13.25	11.75	9.41
0.50%	0mM	12.16	11.66	9.66	6.66
	1mM	12.66	12.41	10.83	7.83
	5mM	13.33	12.91	11.16	9.08
	9mM	13.58	13.33	11.33	9.75
	13mM	13.60	13.33	11.75	9.83
0.75%	0mM	12.41	12.41	10.25	7.25
	1mM	12.91	12.58	10.83	7.83
	5mM	13.66	13.08	11.75	10.08
	9mM	13.83	13.16	11.75	10.08
	13mM	13.83	13.66	12.25	10.33
LSD (0.05%)				1.71	

Conclusion

From obtained results in this study it could be concluded that for maximizing germination and physiological parameters 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 6h.

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