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Effects of applying various levels of nitrogen on parent plants on the resistance to salinity stress in achieved seeds in *Triticum aestivum* L. cv. Gaskojen at germination period

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In order to investigate the effects of nitrogen rates that were applied on parent plants, on the germination and early seedling growth of harvested seeds of wheat cv. Gascogen in the salinity stress, both field and laboratory experiments were carried out. Salinity reduced significant percentage and rate of germination, root and shoot length and dry weights. The effect of nitrogen rates on the germination characteristics in salinity was negative, to some extent. Interaction results showed that, the effects of nitrogen rates at low salinity levels was positive or ineffective, while at high levels of salinity the seeds produced at high nitrogen levels on the parent plants were more sensitive to salinity stress.

Key Words: germination, early seedling growth, nitrogen rates, salinity, wheat.

Introduction

Wheat is a major food crop in most parts of the world which suffer saline soils, hence increasing salinity tolerance in wheat is a major task on wheat production (Tuna *et al.*, 2008). Despite the great emphasis placed on the importance of wheat for nutritional and industrial uses, there is a scarcity of information on the effects of nutrition conditions of parents plant ,for example nitrogen, on germination parameters, at harvested seeds.

Salinity is one of the main abiotic stresses and a reducing factor on yield of many crops in the world (Tester and Davenport, 2003; Meloni *et al.*, 2008). Germination is one of the most critical phases of plant life in which greatly influenced by salinity (Misra and Dwivedi, 2004), and consequently induces a reduction in final germination and a delay in the initiation of the germination

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and seedling establishment. Germination and early seedling growth are frequently confronted by much higher salinities than vigorously growing plants. Germination occurs in the surface of soils which accumulate soluble salts as a result of evaporation and capillary rise of water (Almansouri *et al*., 2001). Thus, it is worthwhile to study the mechanisms to develop suitable measures to alleviate the negative effects of salinity on seed germination and thereby crop establishment on saline soils (Zheng *et al*., 2009).

Previous study showed that characteristics of germination decreased due to salinity in the wheat (Begum *et al*., 1992; Iqbal *et al*., 1998; Almansouri *et al*., 2001). However, the effect of nitrogen application on the produced seeds vigour has not been fully investigated (Hara and Toriyama, 1998). But up to now the combination investigation on the nitrogen rates and salinity has not been under attention. Therefore, the objective of this research was to study the effects of rates of nitrogen utilized on mother plants on the salinity resistant in wheat at germination phase in laboratory conditions.

Material and methods

Field experiment

Field experiment was conducted in a Complete Randomized Design with four replicates and four levels of 0, 120, 240 and 360 kg/ha urea. Experimental design in laboratory was two factors factorial (4×5) arranged in a Completely Randomized Design with 4 replicates of 25 seeds. First factor consists levels of salinity (0, 121.5, 243, 364.5 and 486 mMolal NaCl that gives 0, -0.4, -0.8, -1.2, -1.6 MPa water potential respectively) and the second one levels of nitrogen on parent plants. Germination rate, germination percentages, root and shoot length and dry weight, root number and abnormal seedlings were studied. Significant effect of salinity stress and nitrogen was observed.

In order to study the effects of nitrogen rates that applied in wheat mother plants on the resistant to salinity in harvested seeds at germination period, an experiment was conducted at Research Station, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in year 2008. Macronutrients amount of the soil were determined before the experiment (Table 1).

These treatments were applied at two stages of mother plants growth, first part was applied at late tillering phase and the second one at anthesis phase of wheat mother plants growth (Table 2).

Table 1. Macronutrient amount of the soil used for growing wheat

Soil depth (cm)	N (mg/kg)	K (mg/kg)	P (mg/kg)
0-20	840	125	9
20-40	700	100	6.4

Table 2. Nitrogen treatments in the field experiment and nitrogen rates at each applied time

Nitrogen treatments based on pure N (kg/ha)	Nitrogen applied based on Urea (kg/ha)	First part applied (Pure N, kg/ha)	Second part applied (Pure N, kg/ha)
0	0	0	0
55.2	120	36.8	18.4
110.4	240	73.6	36.8
165.6	360	110.4	55.2

Seeds derevied from each treatment were used to study the germination characteristics under salinity stress at laboratory conditions. The seed total nitrogen was determined by the standard Micro-Kjeldahl method (Bremner and Breitenbeck, 1983). Then the following formula used to calculate the crude protein of the seeds (Cox and Cherney, 2001): $CP=N \times 6.25$, in which CP is crude protein and N is the seed total nitrogen.

The harvested seeds produced at different levels of nitrogen were used in the laboratory experiment.

Laboratory experiment

Experimental design

An experiment was conducted at Seed Research Laboratory of Ferdowsi University of Mashhad, Iran. The experimental design was two factors factorial (5×4) arranged in a Completely Randomized Design; with four replicates of 25 seeds. The first factor was salinity levels (0, 121.5, 243, 364.5 and 486 mMolal NaCl that gives 0, -0.4, -0.8, -1.2, -1.6 MPa water potential respectively), and the second one, was nitrogen levels on the mother plants (0, 120, 240, and 360 kg/ha urea).

Germination tests

Seeds were germinated in 9 cm diameter Petri-dishes with one Whatman No. 1 filter papers moistened with 2.5 ml of distilled water or the appropriate solutions at 21.5±2 ^{o}C. The trays containing Petri-dishes were supplied with ample water and were put into sealed plastic bags to prevent evaporation and therefore maintain approximately 100% relative humidity within each tray throughout the germination test. Seeds were considered to be germinated when the radicle length was 2-3 mm. Germinated seeds were counted daily for 8 days. On the final day germination percentages, number of roots in seedlings, root length, shoots length and abnormal seedlings were determined. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally seedlings (ISTA, 2003). Coleoptiles and radicles were isolated and dried in oven at 72 ^{o}C for 48 hours to determine seedling dry weights. Germination rate was calculated using the following formula (Maguire, 1962):

$Rs = \sum_{i=1}^{n} \frac{S_i}{D_i}$ In which Rs is germination rate, S_i is daily seed germination, D_i is number of day to n computation and n is number of days computation.

Statistical analysis

The statistical analysis of the results of both field and laboratory experiments was made by the Duncan multiple ranges at the 5% level, using SAS software, version 9.1.

Results

Field experiment

Effect of nitrogen rates on weight and volume of one thousand seeds (STW and STV) was significant (P≤0.001). Highest and lowest amounts of these factors were obtained at the 120 and 360 kg/ha treatments respectively (Fig.1a and 1b). The results of table 2 and 3 indicated that no existence any important relation between STW and STV with germination characteristics.

Result of analysis of variance indicated that nitrogen rates had a significant effect on the seed nitrogen and protein (P≤0.001). The percentages of seed nitrogen and protein, along with increasing at nitrogen rates that applied on the parent plants were increased (Fig. 2a and 2b).

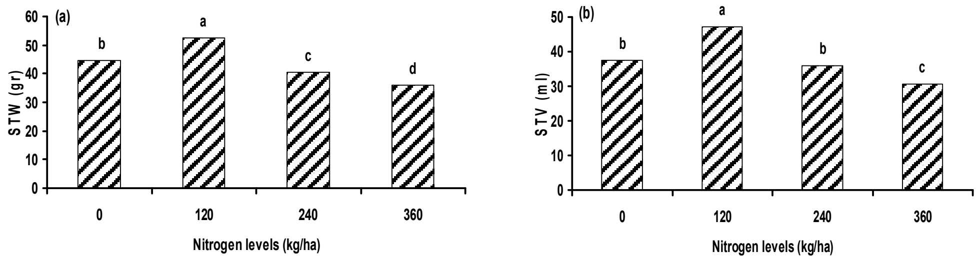

Fig. 1. Relationship between (a) 1000 seed weight (STW) and (b) 1000 seed volume (STV) of wheat with nitrogen rates.

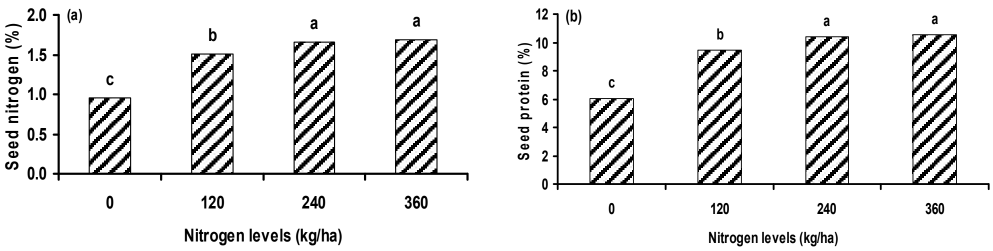

Fig. 2. Relationship between Nitrogen levels used in the field with nitrogen (a) and protein (b) of produced seeds.

Laboratory experiment

Germination percentages (GP) and germination rate (GR)

The germination percentages and rate indicated significant differences among nitrogen rate, salinity levels, and their interactions (Table 3). Results showed that nitrogen and salinity levels induced a negative impact on this criteria's as shown in Table 4. Interaction results showed that enhancement in nitrogen rates at low levels of salinity had no important effect on GP and GR, but at high level of salinity high nitrogen rate exert a negative impact on these indicators, in the other words, high nitrogen rates increased sensitivity of seeds to salinity stress (Fig. 3a and 3b).

Table 3. Summary of analysis of variance for germination percentages (GP), germination rate (GR), abnormal seedlings (AS) and root number (RN) attributes in salinity and nitrogen levels on mother plant experiments

Sources of Variance	**df**	**GP**	**GR**	**AS**	**RN**
Nitrogen	3	397.53^{***}	0.16^{***}	0.008^{ns}	0.013^{ns}
Salinity	4	24894.7^{***}	19.49^{***}	0.242^{***}	0.576^{***}
Nitrogen x Salinity	12	287.36^{**}	0.08^{***}	0.012^{ns}	0.013^{*}
Error	60	20.73	0.009	0.009	0.007
C.V.(%)	-	6.03	3.54	21.74	14.29

* Significant at P < 0.05., ** Significant at P < 0.01., *** Significant at P < 0.001.

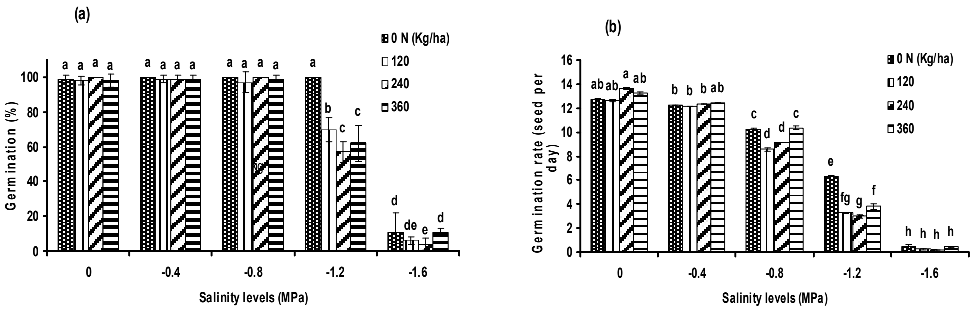

Fig. 3. Effect of nitrogen (Kg/ha) and salinity on germination percentage (a) and germination rate (b) of wheat vc. Gaskogen. Values with the same letter are not significantly different.

Abnormal seedlings percentage (ASP) and roots number (RN)

Salinity had a significant effect on the ASP and RN. The interaction between nitrogen and salinity was also significant in term of RN (Table 3). With increasing of salt concentration, the ASP was increased, while the number of roots was reduced. Moreover nitrogen rates exert a negative effect on the number of roots, to some extent (Table 4).

Table 4. The effects of applying various levels of nitrogen on mother plants and salinity on germination percentage (GP), germination rate (GR), abnormal seedling percentage (ASP) and root number (RN) of *Triticum aestivum* vc. Gaskojen under salinity stress

Treatment	GP (%)	GR (seed per day)	ASP (%)	RN
Nitrogen levels experiment				
0 N (kg /ha)	82[a]	8.3[a]	4.2[a]	3.34[ab]
120	74[b]	7.3[c]	4.2[a]	3.58[a]
240	72[b]	7.6[c]	3.4[a]	3.21[b]
360	73[b]	8.0[b]	3.2[a]	3.21[b]
Salinity experiment				
0.0 (MPa)	99[a]	13.0[a]	2.00[b]	4.74[a]
-0.4	99[a]	12.3[b]	1.50[b]	4.76[a]
-0.8	99[a]	9.5[c]	8.50[a]	3.89[b]
-1.2	72[b]	4.1[d]	6.75[a]	1.90[c]
-1.6	8[c]	0.3[e]	No seedlings	1.38[d]

Values followed by the same letter are not significantly different at $P < 0.05$.

The interaction effect, between salinity and nitrogen in term of number of roots per seedling was significant, as with enhancement of nitrogen level, the number of roots decreased at high levels of salinity (Fig. 4).

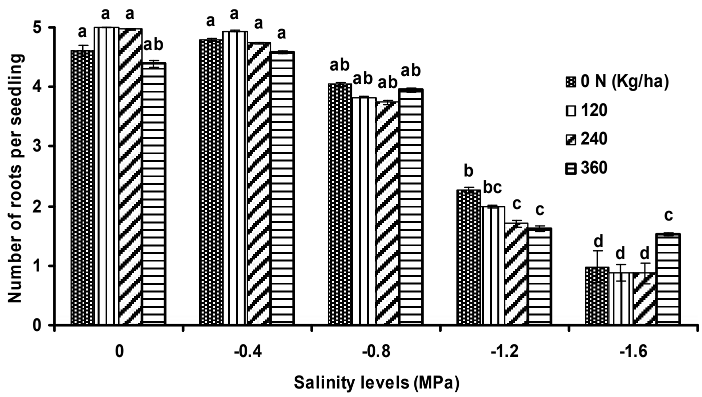

Fig. 4. Effect of nitrogen and salinity on root number of wheat vc. Gaskogen. Values with the same letter are not significantly different.

Root length (RL), shoot length (SL), root shoot length ratio

Nitrogen rates affected the shoot length; likewise effects of salinity levels and interaction between salinity and nitrogen were significant (Table 5). Mean comparison result showed that nitrogen rates had a positive effect on the shoot length. Moreover, salinity treatments decreased the root and shoot length compared to their respective control values. Further more, the proportion of dry weight allocated to the roots increased with increasing NaCl levels, as shown by the root/shoot dry weight ratio (Table 6). Interaction results showed that nitrogen at low levels of salinity exerts a positive effect on these factors while at high levels of salinity was negative (Fig 5a and 5b). Root shoot length ratio increased with enhance in nitrogen rate, in high level of salinity (Fig 5c).

Table 5. Summary of analysis of variance for root length (RL), shoot length (SL), root length to shoot length (RL/SH) root weight (RW), shoot weight and root weight to shoot weight (RW/SW) attributes in salinity and nitrogen levels on mother plant experiments

AOV	df	RL	SL	RL/SL	RW	SW	RW/SW
Nitrogen	3	0.03[ns]	0.10[*]	0.12[ns]	0.000001[ns]	0.0000001[ns]	0.07[ns]
Salinity	4	13.5[***]	8.86[***]	1.09[***]	0.00002[***]	0.00002[***]	0.87[***]
Nitrogen x Salinity	12	0.06[*]	0.07[*]	0.18*	0.000002[ns]	0.0000002[ns]	0.09[ns]
Error	60	0.032	0.03	0.10	0.000001	0.0000002	0.12
C.V.(%)	-	8.88	10.6	17.50	0.13	0.04	23.46

*Significant at P < 0.05., **Significant at P < 0.01., ***Significant at P < 0.001.

Table 6. The effects of applying various levels of nitrogen on mother plants on root length (RL), shoot length (SL), root length to shoot length (RL/SH) root weight (RW), shoot weight and root weight to shoot weight (RW/SW) of *Triticum aestevum* var. Gaskojen under salinity stress

Treatment	RL (mm)	SL (mm)	RL/SL	RW (gr)	SW (gr)	RW/SW
Nitrogen levels experiment						
0 N (kg /ha)	3.46[a]	2.00[b]	2.35[a]	0.0030[a]	0.0025[a]	1.18[a]
120	3.78[a]	2.28[ab]	2.71[a]	0.0034[a]	0.0028[a]	1.65[a]
240	4.04[a]	2.58[ab]	2.09[a]	0.0028[a]	0.0028[a]	1.11[a]
360	3.97[a]	2.78[a]	2.70[a]	0.0039[a]	0.0029[a]	1.43[a]
Salinity experiment						
0.0 (MPa)	9.13[a]	5.76[a]	1.61[c]	0.0064[a]	0.0056[a]	1.15[b]
-0.4	6.62[b]	4.92[b]	1.38[c]	0.0045[ab]	0.0055[a]	0.84[c]
-0.8	2.65[c]	1.19[c]	2.50[b]	0.0039[b]	0.0019[b]	2.08[ab]
-1.2	0.48[d]	0.12[d]	3.94[a]	0.0011[c]	0.0005[c]	2.20[a]
-1.6	0.18[e]	0.05[d]	2.90[b]	0.0005[c]	0.0002[c]	2.50[a]

Values followed by the same letter are not significantly different at P < 0.05.

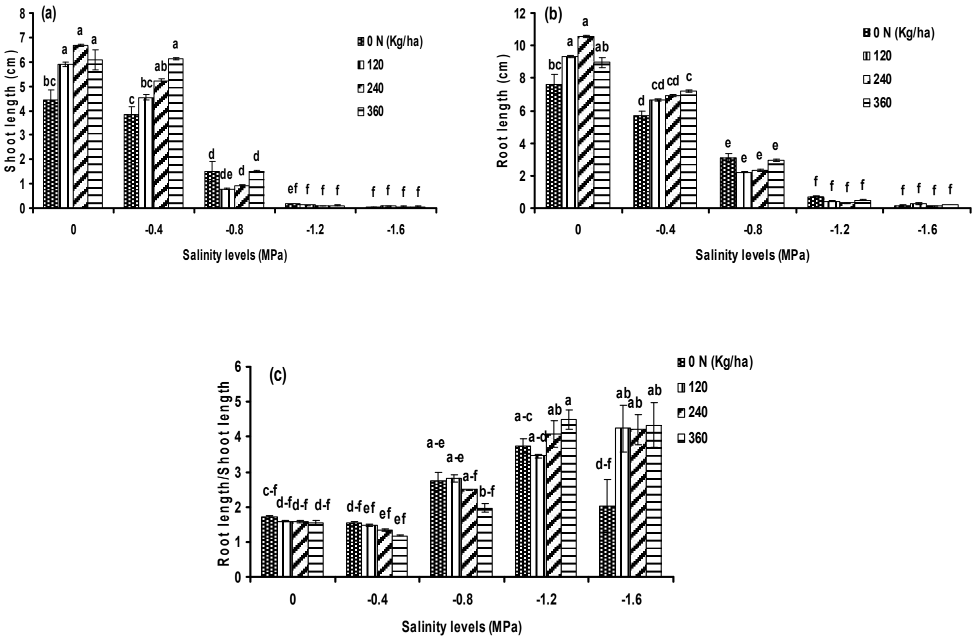

Fig. 5. Effect of nitrogen (Kg/ha) and salinity on the shoot length (a), root length (b) and root/shoot ratio (c) of wheat vc. Gaskogen. Values with the same letter are not significantly different.

Root weight, shoot weight and root shoot dry weight ratio

Salinity affected the root and shoot weight and root to shoot dry weight ratio (Table 5), as salinity increased, root and shoot weight decreased while the root shoot weight ratio increased (Table 6).

Discussion

Salinity reduced the percentage and rate of germination. The inhibitory effect of NaCl stress on seed germination is due to an osmotic effect and/or ion toxicity (Tobe *et al.*, 2004; Song *et al.*, 2008), which may change certain enzymatic or hormonal activities (Al-Harbi *et al.*, 2008). Salinity-induced declines in germination are usually due to only an osmotic effect for halophytes, but nonhalophytes such as wheat are more likely to exhibit additional ion toxicity (Song *et al.*, 2008; Salehi and Khajeh-Hosseini, 2008).

Shoot was found to be more sensitive to salinity than that root, as shoot length and weight was more pronounced as compared to that of root. Therefore, root to shoot length and weight ratio increased as salinity increased (Table 6). Thus, an increased root/shoot ratio appears to be an adaptation to salinity, resulting in more efficient water and nutrient uptake under saline stress (Gorham *et al*., 1999). This indicated that, root growth is less sensitive to salinity compared to the shoot growth. This caused to plants tolerance stress conditions via up taking water by more extended subterranean organs for less extended part of upper organs (Fallahi *et al*., 2008). Moreover, it is suggested that decrease in seed germination and depression in seedling growth under saline conditions is attributed to decrease water uptake followed by limited hydrolysis of food reserves from storage tissues as well as due to impaired translocation of food reserves from storage tissue to developing embryo axis (Misra and Dwivedi, 2004).

There is a few studies on the effects of nitrogen on early establishment of plants under saline conditions, but nitrogen containing compounds accumulation is usually correlated with plant salt tolerance, even though this correlation is based on untested hypotheses (Mansour, 2000). For example result of Al-Harbi *et al*. (2008) showed that fertilization with N may be used to reduce some of the negative effects of salinity on tomato. However, their results indicated lack of a significant interaction between salinity and nitrogen levels on emergence percentages, emergence rate early, seedling growth, and leaf area characters. Their results also did not show any clear trend to indicate that N levels had a direct effect on salinity induced decreased growth. Results of Amiri *et al*. (2010), on wheat, Cerda and Martinez (1988) on tomato indicated that nitrogen had a positive effect on germination and emergence factors under saline stress. In our experiment, the effect of nitrogen rates on the germination factors was negative. Interaction results showed that nitrogen at low levels of salinity exerts a positive or ineffective effect on these factors while at high levels of salinity was negative. Phytohormones modulated by nitrogen nutrition, may also affect plant response to saline environment. For example, higher cytokinin content was found in nitrate treated plants compared with those receiving either ammonia or mixed nitrogen nutrition. Cytokinines can influence polyamines synthesis which may has protective function under salt stress (Mansour, 2000). As nitrogen rates increased in the field, the percentage of seed nitrogen was increased (Table 3), probably the amounts of nitrogen that have been existing in the soil without fertilization (Table 1) had been sufficient for biosynthesis of phytohormones, and exceeded nitrogen to soil exerts a negative impact on harmonic balance that being between phytohormones in seeds. On the other hand K^+ and Ca^{2+} ions have protective function for plant tissue from NaCl toxicity during germination and early seedling growth (Khajeh-Hosseini *et al*., 2002). High nitrogen levels on the mother plants possibly have reduced the concentrations of K^+ and Ca^{2+} in the produced seeds, hence the seeds more sensitive to salinity during germination and early seedling growth. As Morshed *et al.* (2008) in soybean and Karaaslan (2008) in oilseed rape were concluding that nitrogen fertilization had a negative effect on K^+ or Ca^{2+} concentrations of seeds.

Conclusions

From the present study it can be concluded that nitrogen management in seed production is important and the nitrogen rates on the mother plants should be low, when the produced seeds are sown in the saline conditions. Further work to determine the concentrations of K^+ and Ca^{2+} ions in the embryo tissues of seeds in different varieties of wheat will help to see if there is any relationship between concentrations of the ions and salinity tolerance of the seeds during germination and early seedling growth.

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References

Al-Harbi, A.R., Wahb-Allah, M.A. and Abu-Muriefah, S.S. (2008). Salinity and nitrogen level affects germination, emergence, and seedling growth of tomato. Journal of Vegetation Sciences 14(4): 380 – 392.

Almansouri, M., Kinet, J.M. and Lutts, S. (2001). Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil 231: 243–254.

Amiri, M.B., Rezvani-Moghaddam, P., Fallahi, J. and Yazdani, R. (2010). The study of nutritional management of parent plant in combination with seed priming by biofertolizers in toward to increase of salinity tolerance in wheat cv. Sayonez at germination period. Proceedings of the 1st Iranian Congress, Environmental Stresses in Agricultural Science. 28-29 January, Birjand, Iran, pp 150.

Begum, F., Karmoker, J.L., Fattah, Q.A. and Maniruzzaman, A.F.M. (1992). The Effect of salinity on germination and its correlation with K^+, Na^+, Cl^- accumulation in germinating seeds of *Triticum aestivum* L. cv. Akbar. Plant and Cell Physiology 33(7): 1009-1014.

Bremner, J.M. and Breitenbeck, G.A. (19830. A simple method for determining ammonium in semi-micro Kjeldahl analysis of soils and plant materials using block digester. Communications in Soil Science and Plant Analysis 14(10): 905– 913.

Cerda, A. and Martinez, V. (1988). Nitrogen fertilization under saline conditions in tomato and cucumber plants. Journal of Horticultural Sciences 63: 451–458.

Cox, W.J. and Cherney, D.J.R. (2001). Row spacing, plant density, and nitrogen effects on corn silage. Agronomy Journal 93: 597-602.

Fallahi, J., Ebadi, M.T. and Ghorbani, R. (2008). Effects of salinity and drought stresses on germination and seedling growth of Clary (*Salvia sclarea*). Iranian Journal of Environmental Stresses in Agricultural Sciences 1(1): 57-67.

Hara, Y. and Toriyama, K. (1998). Seed nitrogen accelerates the rates of germination, emergence, and establishment of rice plants. Soil Science and Plant Nutrition 44(3): 395-366.

Iqbal, N., Ashraf, H.Y., Javed, F., Iqbal, Z. and Shah, G.H. (1998). Effect of salinity on germination and seedling growth of wheat (*Triticum aestivum* L.). Pakistan Journal of Biological Sciences 3(1): 226-227.

Gorham, J., Wyn-Jones, R.G. and Mc-Donell, R. (1999). Some mechanisms of salt tolerance in crop plants. Plant and Soil 89: 15–40.

ISTA, (2003). International Seed Testing Association. ISTA Handbook on Seedling Evaluation, 3rd edition, Zurich, Switzerland.

Karaaslan, D. (2008). The effect of different nitrogen doses on seed yield, oil, protein and nutrient contents of spring rape. Pakistan Journal of Botany 40(2): 807-813.

Khajeh-Hosseini, M., A.Powell, A. and J.Bingham, I. (2002). Comparison of the seed germination and early seedling growth of soybean in saline conditions. Seed Science Research 12: 165-172.

Maguire, J.D. (1962). Speed of germination- Aid in selection and evaluation for seedling emergence and vigor. Crop Science 2: 176-177.

Mansour, M.M.F. (2000). Nitrogen containing compound and adaptation of plants to salinity stress. Plant Biology 43: 491–500.

Meloni, D.A., Gulotta, M.R. and Martınez, C.A. (2008). Salinity tolerance in Schinopsis quebracho Colorado: seed germination, growth, ion relations and metabolic responses. Journal of Arid Environment 72: 1785–1792.

Misra, N. and Dwivedi, U.N. (2004). Genotypic difference in salinity tolerance of green gram cultivars. Plant Science 166: 1135–1142.

Morshed, R.M., Rahman, M.M. and Rahman, M.A. (2008). Effect of nitrogen on seed yield, protein content and nutrient uptake of Soybean (*Glycine max* L.). Journal of Agriculture and Rural Development 6(1 and 2): 13-17.

Salehi, M. and Khajeh-Hosseini, M. (2008). The effect of seed size in wheat (*Triticum aestivum* L.) on germination and seedling growth in saline condition. Proceedings of the 1st National Congress of Seed Science and Technology. 13-14 November, Gorgan, Iran, pp: 26.

Song, J., Fan, H., Zhao, Y., Jia, Y., Du, X. and Wang, B. (2008). Effect of salinity on germination, seedling emergence, seedling growth and ion accumulation of a euhalophyte *Suaeda salsa* in an intertidal zone and on saline inland. Aquatic Botany 88(4): 331-337.

Tester, M. and Davenport, R. (2003). Na^+ tolerance and Na^+ transport in higher plants. Annals of Botany 91: 503–527.

Tobe, K., Li, X.M. and Omasa, K. (2004). Effects of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (Chenopodiaceae). Seed Science Resarch 14: 345–353.

Tuna, A.L., Kaya, C., Higgs, D., Murillo-Amador, B., Aydemir, A.S. and Girgin, R. (2008). Silicon improves salinity tolerance in wheat plants. Environmental and Experimental Botany 62: 10–16.

Zheng, C., Jiang, D., Liu, F., Dai, T., Liu, W., Jing, Q. and Cao, W. (2009). Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. Environmental and Experimental Botany 67: 222–227.

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