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Effect of Zinc on Seed Germination of Barley (*Hordeum vulgare* L.) Genotypes under Salinity Stress

Amir Kazemi and Mehrab Yadegari

Department of Agronomy and Medicinal Plants, Faculty of Agriculture, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

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ABSTRACT

Laboratory experiment was carried out to determine the effects of zinc fertilizers on seed germination characteristics of different genotypes of barley under different levels of salinity stress. Experiment was conducted as factorial based on completely randomized design with four replications. The first factor of the experiment was barley genotypes (Morocco, Nosrat, and line 4), the second factor was salinity stress levels (2, 10 and 18 ds/m) and the another factor of experiment was zinc fertilizer levels (control, 0.5, 1.0 and 1.5 ppm of zinc nano and 0.5, 1.0 and 1.5 ppm of zinc chelate). Results of this study showed that there were significant differences between germination characteristics of three evaluated genotypes. Mostly line 4 showed the highest germination indexes compared to other genotypes. According to our results all three evaluated genotypes can be classified as relatively salt-resistant genotypes in germination stages. However salinity more than 18 ds/m decreased significantly germination ability of seeds and germination indexes in evaluated genotypes.

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INTRODUCTION

In arid and semi-arid regions, salinity is from the major abiotic stresses that substantially can reduce the yield of crops by more than 50%. Salinity affects about 7% of the world's land area for around 930 million ha [24]. It is one of the most serious factors limiting crops production, especially the sensitive ones [35. Salinity effects on the growth and survival of microorganisms, plants and soil animals [17,34]. According to several reports, high soil salinity currently affects the agricultural production in a large proportion worldwide [36].

Although salt stress affects all growth stages of plant, seed germination and seedling growth stages are the most sensitive stages in most plant species. Furthermore, germination and seedling stage are predictive of plant growth responses to salinity [9]. The ability of seed to germinate at high salt concentration in the soil is therefore of crucial importance for the survival and perpetuation of a species [6]. Despite the importance of seed germination under salt stress, mechanism/s of salt tolerance in seeds is no properly understood, especially when compared with the amount of information currently available about salt tolerance physiology and biochemistry in plants [18]. Different results were dedicated from the effect of salinity on different parameters of plants.

Zinc is an essential heavy metal [15], however this metal is highly toxic and biologically active at high concentration [19]. Zinc deficiency is common throughout the developed and developing world and lack of Zn can limit the growth and productivity of a wide range of crops [14].

Barley (*Hordeum vulgare* L.) is a major staple food in many regions of world. Barley is generally a suitable cereal for regions where other cereals do not grow well due to soil salinity, altitude or low rainfall. It is the most viable option in dry areas (<300 mm of rainfall). Food barley is used either for bread making (usually mixed with bread wheat) or for specific recipes [11]. Objectives of this research were the study of the effects of different concentrations of NaCl in the presence of different dosages of zinc fertilizers on germination indexes of three genotypes of barley.

Corresponding Author: Mehrab Yadegari, Department of Agronomy and Medicinal Plants, Faculty of Agriculture, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
Tel: +98 9133814318; Fax: +98 3813361093; E-mail: mehrabyadegari@gmail.com

Advances in Environmental Biology, 9(2) January 2015, Pages: 30-37

MATERIAL AND METHODS

To evaluate the effects of zinc element on seed germination characteristics and salinity tolerance in barley, a laboratory experiment was carried out in the Isfahan Center for Research of Agricultural Science and Natural Resources. The study was conducted in a factorial experiment based on the completely randomized blocks design by four replications. The experiment had four factors of barley genotype (Morocco, Nosrat, and line 4), zinc fertilizer (control, 0.5 1.0 and 1.5 ppm of zinc nano and 0.5 1.0 and 1.5 ppm of zinc chelate), and salinity stress (2, 10 and 18 ds/m). Salinity levels were prepared with NaCl. Seeds were sterilized at 1.5% sodium hypochlorite solution for three minutes and were rinsed completely using distilled water for several times. Afterward seeds were put separately in the fertilizer treatments for three hours and were dried for one hour. Twenty sterilized uniform seeds were placed in a petri dish (9 cm diameter) on aseptic filter paper (Whatman 42) using a forceps. Petri dishes had previously been disinfected. Petri dishes were placed in germinator at a temperature of 25°C, relative humidity of 70%, and lightness of 1300 lux. The number of germinated seeds were measured daily for seven days after germination. The seedlings with form short, thick and spiral hypocotyl are considered in abnormal germination. Germination percentage and germination speed were calculated using equations of 1 and 2 [20].

$$\text{Germination percentage (\%)} = \frac{n}{N} \text{ x } 100 \quad (1)$$

Where **n** is the number of germinated seed on the seventh day and **N** is total number of seeds.

$$\text{Germination speed} = \frac{\sum n_i}{\sum d} \quad (2)$$

Where $\mathbf{n_i}$ is number of germinated seed on the day and **d** is days after first recording.

Data were analyzed using SAS statistical software. Mean separations were performed by Duncan's multiple range test (DMRT) at 5% level.

Results:

Effect of treatments on germination percentage:

Germination speed was significantly affected at 1% level of probability by fertilizer and genotype treatments and also their interactive effects. Interactive effects of fertilizer treatments and genotype on the germination speed was significant at 5% level. Salinity effect, interactive effect of salinity and genotype treatments and the interactive effect of all three treatments (salinity, genotype and fertilizer) were significant on germination percentage (Table 1).

Table 1: Results of variance analysis of evaluated indexes

Source of Variations	Degree of Freedom	Germination Percentage	Germination Speed
Fertilizer treatment	6	1022.833**	19.036 **
Genotype treatment	2	1598.101**	40.079**
Salinity treatment	2	227.214 ^{ns}	4.800 ^{ns}
Fertilizer× Salinity	12	205.414**	2.123 ^{ns}
Fertilizer× Genotype	12	614.848**	15.143**
Genotype× Salinity	4	48.383 ^{ns}	1.973 ^{ns}
Genotype× Salinity× Fertilizer	24	92.342 ^{ns}	2.877 ^{ns}
Error	189	89.524	1.785

*and ** indicate significant difference at 5% and 1% probability level, respectively, ns is not significant.

Totally, between fertilizer treatments, the highest germination percentage (28.58%) was recorded in control treatment. Other fertilizer treatments (different dosages of zinc nano and zinc chelate) did not present significant different (Figure 1). Several reasons may be presented for reduction of germination percentage in treatments primed with zinc Nano and zinc chelate. Metabolic and enzyme disorders caused by ionic components may be one of the main reasons for decreasing germination percentage in fertilizer treatments compared to control treatment. It is also probably that osmotic and ionic effects increased the antigerminative ingredients in plants and consequently reduced the germination percentage [2]. Also the increasing ions accumulation in the root environment may be another factor for decreasing the germination percentage in fertilizer treatments. Since in present study all treatments were exposed to different dosages of sodium chloride, it can be said that presence of zinc fertilizers intensified the ionic accumulation in root environment and increased the salinity effects on plants. However in some studies the increasing effects of fertilizers on the germination percentage was reported [23,29], in different studies increasing fertilizers consumption was reported as an antigermination factor [26]. In a laboratory experiment, Semeniuk [30] also reported reduction of germination in *Matthiola incana* as nitrogen, phosphor and potassium fertilizers consumption dosage increased. In similar study, Ashagre et al. [3] reported

that presence of zinc in seed environment of tomato caused the phytotoxicity effects and reduced seed germination.

From all genotype treatments, the highest germination percentage was found in line 4 that had statistically a significant difference with other genotypes. Other genotypes showed had similar group (Figure 2). Totally, increasing salinity decreased the germination percentage and the highest germination percentage was observed in salinity level of 2 ds/m as 15.70 % and the lowest germination rate was measured in salinity rate of 18 ds/m as 11.62% (Figure 3). Significant reduction of germination with increasing salinity was reported (1, 6, and 8). Since all cultivated genotypes in this experiment found to be salt-resistant genotypes, salinity could not considerably affect the germination of evaluated genotypes [32].

Response of different genotypes to fertilizer treatments was statistically significant. The highest germination percentage (56.64 %) was obtained from line 4 in control treatment of fertilizers (no fertilizer consumption) and the lowest germination percentage (7.08%) was obtained from Nosrat genotype in both fertilizer treatments of 1 ppm zinc Nano and 1.5 ppm zinc chelate (Figure 4).

Interactive effect of fertilizer treatment and salinity stress was significant (Figure 5). The highest rate of germination (43.58%) was measured in salinity treatment of 2 ds/m under control treatment of fertilizer. This rate was significantly higher than other measured values.

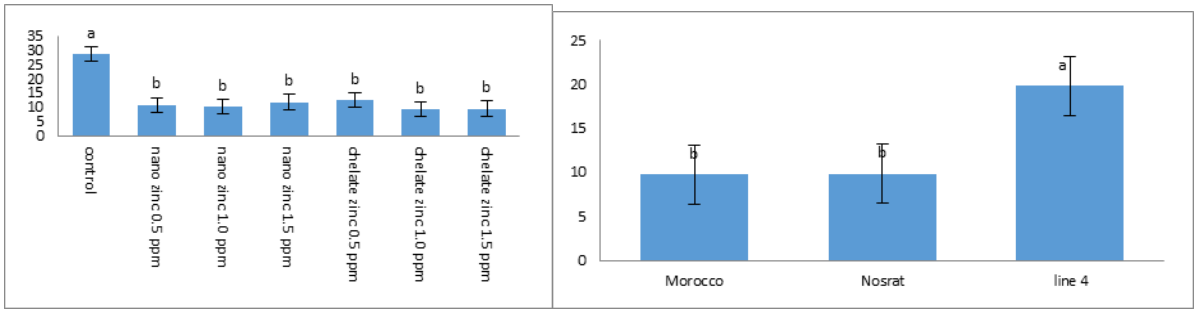

Fig. 1: Effect of fertilizer treatments on germination percentage._____Figure 2- Effect of genotype treatments on germination percentage.

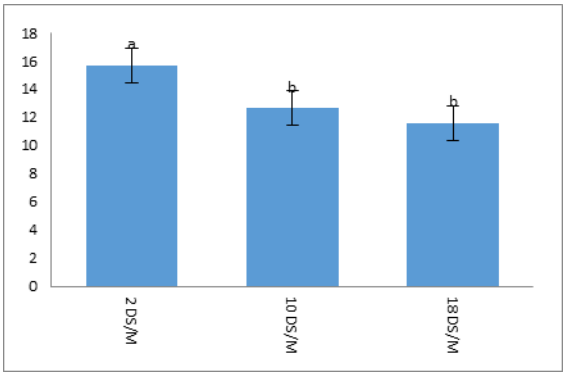

Fig. 3: Effect of salinity treatment on germination percentage.

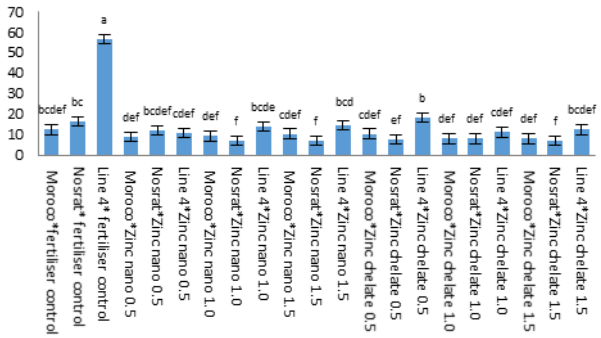

Fig. 4: Interactive effect of fertilizers treatment and genotype on germination percentage.

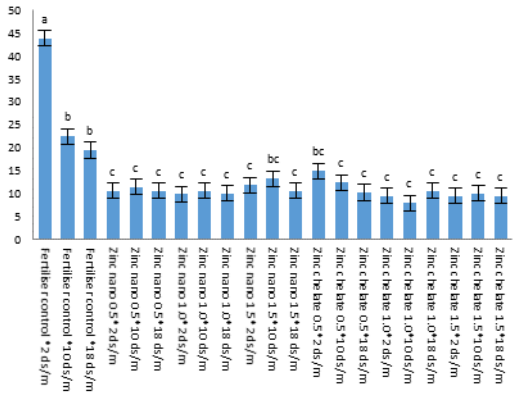

Fig. 5: Interactive effects of fertilizer treatments and salinity on germination percentage.

Effect of treatments on germination speed:

Response of germination speed to different treatments of fertilizer, genotype and their interactive effects was significant in level of 1%. Nevertheless, germination speed and its interactive effect with fertilizer treatment and genotype treatment did not show any significant response to salinity treatment. Also, interactive effect of all three treatments on the germination speed was not significant (Table, 1).

Between fertilizer treatments, the highest germination speed (3.414) was observed in control treatment. This treatment had significant different with other fertilizer treatments (Figure 6). Other fertilizer treatments (including different dosage of zinc nano and zinc chelate) had not any significant different. Negative effect of presence of ionic components on the germination indexes, especially germination speed, was reported by several authors. Semeniuk [30] on *Matthiola incana* plants showed that fertilizers had positive effectiveness on speed of germination. Presence of different ions in seed environment may affect germination and seedling growth by theirs toxic effects or by affecting osmotic pressure which disorder water absorption [31]. Excess accumulation of ions in the cell wall reduces the osmotic potential, modifies the metabolic activities negatively and limits the cell wall elasticity. These factors cause the stress situation in plant [25]. In some situations, these factors may considerably reduce photosynthesis, disturb physiological processes, inhibit growth of seedling and in critical situation may cause plant dead [21]. It seems that in this study the toxicity effects of presence of metal ions is the most important factor in decreasing germination speed.

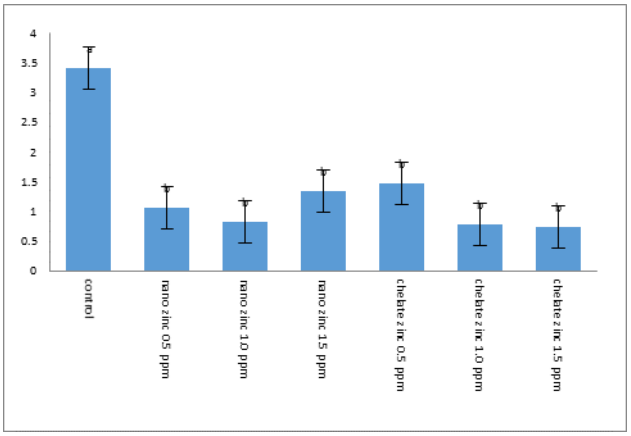

Fig. 6: Effect of fertilizer treatments on germination speed.

Germination speed in evaluated genotypes did not show any significant different. Line 4 showed the highest germination speed (2.39) (Figure 7). After that, Nosrat genotype showed the second highest germination speed (1.02) and Morocco showed the least germination speed (0.66).

However the effect of salinity treatment on the germination speed of evaluated plants was not significant, Duncan's test results showed a significant different between salinity treatment of 18 ds/m and other treatments. Totally increased in salinity decreased germination speed, however difference for salinity treatments of 2 and 10 ds/m was not significant (Figure 8).

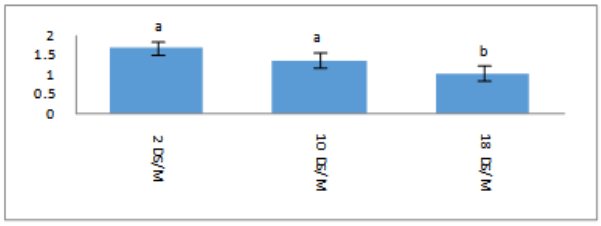

Fig. 7: Effect of salinity treatment on germination speed.

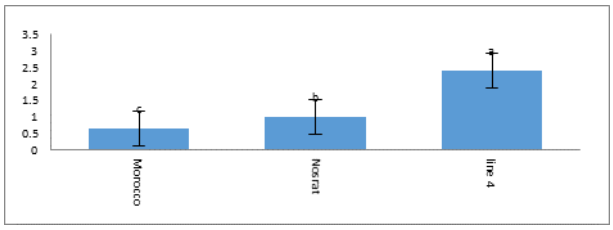

Fig. 8: Effect of genotype treatments on germination speed.

In several past studies, non-impressibility of germination speed from different level of salinity was reported. Gholami et al. [13] reported that germination speed in *Vicia monantha* was not influenced from salinity levels up to 150 mM. It may be concluded that salinity level up to 18 ds/m could not affect on germination speed of evaluated genotypes and theses genotypes are tolerant to this rate of salinity.
Response of different genotypes to fertilizer treatment was statistically significant (Figure 9). The highest germination speed (7.80) was obtained from line 4 in control treatment of fertilizer. This rate was significantly higher than other treatments. The lowest germination speed (0.35) was measured from Nosrat genotype under fertilizer treatments of 1.5 ppm zinc chelate. However this rat was not statistically different from the most of other treatments. (Figure 10).

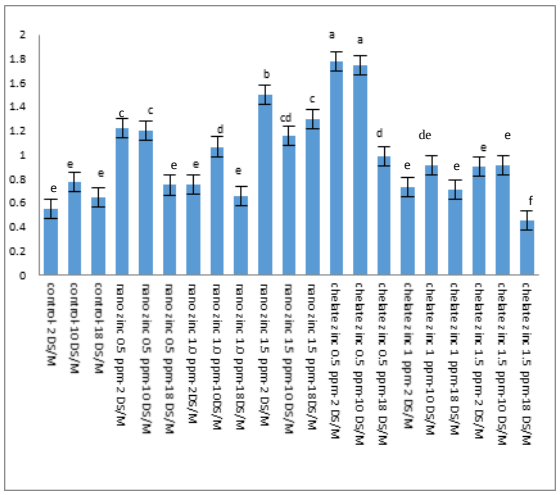

Fig. 9: Interactive effect of fertilizer treatments and salinity on germination speed.

Advances in Environmental Biology, 9(2) January 2015, Pages: 30-37

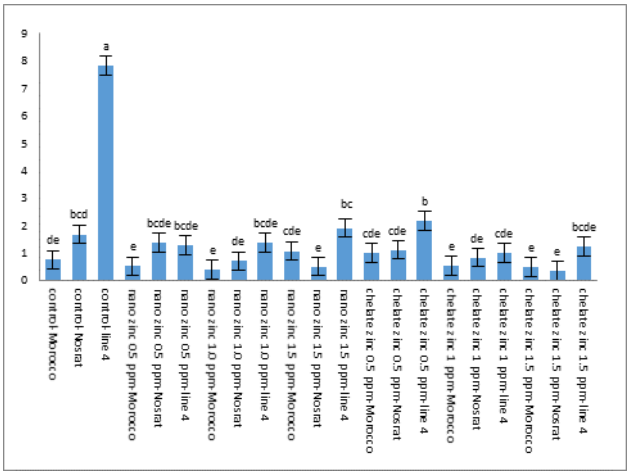

Fig. 10: Interactive effect of fertilizer treatments and genotypes on germination speed.

Discussion:

Results of this study showed, there is a significant differences between germination characteristics of three evaluated genotypes. The highest germination indexes were observed in new genotype of line 4. The germination indexes of these genotypes were higher than other two renowned genotypes (Nosrat and Morocco). This may show capability of this new genotype for survival and perpetuation. Germination indexes of Nosrat genotype were also mostly better than Morocco genotype. This experiment showed that all three evaluated genotypes have a relatively similar tolerant to presence of sodium chloride ions and can be classified as relatively salt-resistant genotypes. In laboratory situation and in early stages of plant growth, presence of sodium chloride ions up to 18 ds/m did not statistically affect germination speed and germination percentage. All three genotypes mostly showed similar responses to fertilizer treatments and their responses difference was insignificant. Totally the presence of ions of zinc chelate and zinc nano, caused considerable decrease in germination indexes. This research monitored the effects of NaCl on seed germination and the growth of plants to give the best tolerated barley plants in salinity and drought region. Many researchers reported the destructive effects of salinity and decrease growth parameters for example *Thymus broussonetii* Boiss [5], *Nigella sativa* [7], *Suaeda maritime* [12], *Artemisia annua* L. [16], *Schinopsis quebracho* [22], *Carthamus tinctorius* L. [27]), *Lallemantia iberica* [28], *Ocimum basilicum* [4], *Matricaria recutita* [10], *Plantago* sp L., *Alyssum* spp, *Portulaca oleracea, Sesamum indicum, Origanum majorana, Trigonella foenum, Anethum graveolens, Melilotus officinalis, Trachyspermum ammi, Cuminum cyminum, Lactuca sativa* and *Lallemantia royleana* [33].

Conclusion:

According to results of this study it can be consequence that in barley plant, presence of sodium chloride ions in the low dosages mostly do not affect germination indexes or even may increase the germination ability of plants but high dosages of sodium chloride decreases germination indexes. However the presence of metal ions of zinc in low dosages is considered as essential micronutrient for the growth of plants; in early stages of plant germination, especially in laboratory situations, the presence of zinc ions, even in low concentration, can reduce germination.

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