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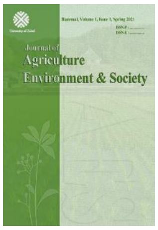

Contrasting effects of chelated zinc and nanoscale zinc oxide on barley growth and salinity tolerance

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ABSTRACT

An experiment was carried out to determine the effects of chelated zinc and nanoscale zinc oxide particles on tolerance salinity of barley. In the experiment, barley seeds were treated with different concentrations of chelated zinc (Zn-Chelated) and nanoscale zinc oxide (Nano-ZnO), and the effects of these treatments on seed germination, seedling vigor, plant growth, grain filling, and yield were studied. The inhibitory effect of nanoparticles and chelated zinc (1.5 ppm) was discovered. The results emphasize that water can be supplied to the barley followed by Zn-Chelated application with 0.5 ppm to get the desired results. With increasing salinity stress, seed germination and seedling vigor decreased sharply, so the highest obtained from control treatment and the lowest obtained from a salinity level of 18 dS m^{-1}. The genotypes respond differently to salinity levels and alkaline soils. It seems that the Khatam genotype has more tolerance to salinity conditions. Consequently, an experiment was conducted in a strip-plot design with three replications. Based on the correlation coefficients, the kernel number per spike (KNS) showed the highest correlation with the grain yield in barley genotypes, followed by grain filling rate (GFR), maximum grain weight (MGW), thousand-kernel weight (TKW), number of spikes (NS), and saturation water deficit (SWD), respectively. Thus, not only a higher KNS and TKW, but also GFR, MGW, and proline in aboveground plant parts are crucial for successful tolerance in barley. These findings indicate that these agrophysiological traits could be key factors and useful tools for screening many samples in a short time.

Highlights

- Barley seed germination and seedling vigor were significantly reduced by increasing salinity levels.
- Water followed by low-dose chelated zinc significantly enhanced barley germination, shoot and root length, and seedling vigor compared to other treatments.
- Chelated zinc application reduced the negative effects of salinity on barley growth and yield.
- Higher nanoscale zinc oxide concentrations inhibited plant growth, emphasizing the need for proper zinc source and concentration.
- Salinity increased barley leaf proline content, suggesting a stress response mechanism.
- Chelated zinc application improved plant water status under salinity stress by reducing saturation water deficit.

1. Introduction

More than 50% of the cultivated area might be salinized, and it is estimated to reach 9 billion people by 2050. Salinity is a major stressor on crop production in the world (Poustini et al., 2020), affecting 19.5% and 2.1% of irrigated land and dry land in the world, respectively (Sonia et al., 2019). Also, the total salt soil land is 932 m ha (sodic and saline are 581 m ha and 351 m ha, respectively) (Hasanuzzaman et al., 2014). Salt stress causes a nutritional imbalance through lowering phosphorus (Evelin et al., 2009), nitrate and calcium (Hu and Schmidhalter, 2005), zinc absorption, and the accumulating of sodium and chloride ions (Khoshgoftarmanesh et al., 2004). Plant

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growth is not increased by increasing nutrient concentration in saline soils because uptake is low (Mahlooji and Pessarakli, 2017).

Zinc is required for chlorophyll production, germination (Pandey et al., 2006; Cakmak, 2008), biomass production (Kaya and Higgs, 2002), crop productivity (Signorell et al., 2019) and seedling vigor (Rashid et al., 2019). The ability of a seed to germinate at a high salt concentration is important for the survival of a plant (Bojović et al., 2010). It is imperative to keep salinity low during germination and development. Furthermore, responses to salinity vary with germination, seedling vigor (Cuartero et al., 2006), agronomic factors, soil and water management, climate, genotypes, and elements of nutrition such as zinc (Tao et al., 2018). Measurement of traits, including germination (Atak et al., 2006), seedling vigor, relative water content (RWC), saturation water deficit (SWD), grain filling, yield, and yield component, can be used to monitor plant responses to salt stress (Izadi et al., 2014; Mahlooji and Pessarakli, 2017). Salinity was shown to decrease RWC and increase SWD (Ebrahimian and Bybordi, 2011). Trials can be rapidly screened for genotypes which maintain high leaf RWC and low leaf SWD values during stress (Gholinezhad et al., 2009).

In calcareous soils, zinc precipitates in unavailable forms for plants (Degryse et al., 2020). Soil salinity is also associated with zinc efficiency in alkaline conditions (Morshedi and Farahbakhsh, 2012). The high pH (Rengel, 2015; Mueller et al., 2012) and $CaCO_3$ content of these soils are usually considered the reasons for the low availability of Zn (Mahlooji, 2017). By reducing the amount of soil moisture in this area, Zn and Fe in soil solution reduced mobility. The lack of these elements in plants can be compensated by spraying with a solution (Cakmak et al., 2017). Many studies suggest that foliar micronutrient fertilizer could increase plant productivity (Schjoerring et al., 2019), plant resistance to environmental stresses (Dwivedi et al., 2016), shoot growth (Phuphong et al., 2020), nutrient uptake (El-Fouly et al., 2010), yields (Sarkar et al., 2007) of wheat and barley (Morshedi and Farahbakhsh, 2012; Keshavarz and saadat, 2016), and reduce the effect of salinity on yield (Mahlooji et al., 2018), Na concentration on roots and leaves (Thalooth et al., 2006), and nutritional disorders (El-Fouly et al., 2002). Nanomaterials are proposed to be the building materials for the new millennium. It indicates that different plants have different responses to the same nanoparticles and nanofoliar applications. Nanoparticles generate both additive effects (Hong et al., 2005; Yang et al., 2006; Lu et al., 2002; Prasad et al., 2012), and inhibitory effects (Nel et al., 2006; Qiang et al., 2008; Lin and Xing, 2008; Yang and Watts, 2005; Lin and Xing, 2007; Doshi et al., 2008) or not change yield (Knijnenburg et al., 2018) and need to be explored.

About two billion (Chen et al., 2017) or one-third (Zou et al., 2019) of people suffer from zinc malnutrition (Chen et al., 2017). Zn is an essential micronutrient, which is deficient in many regions worldwide (low solubility of Zn in soils rather than low total amount of Zn), such as in calcareous and salt-affected soils of central Iran (Khoshgoftarmanesh et al., 2004), the fourth most important yield-limiting nutrient in India (Prasad et al., 2012), half of the cultivated soils (Phuphong et al., 2020) and 30% of the global soils (Babaeian *et al*., 2011). Zinc is an essential micronutrient for humans, animals, and plants, which acts either as the metal component of enzymes or as a functional structural or regulatory co-factor of a large number of enzymes. Zn is typically the second most abundant transition metal in organisms after iron and is the only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) (Auld, 2001). In the developing world, cereal crops play an important role in nutrition, but zinc concentration in cereal grains is very low, particularly when grown on Zn-deficient soils. As a major solution to Zn deficiency, enrichment of cereal grains with zinc foliar application (biofortification) is the most sustainable, with low cost, and useful in improving Zn concentrations in grain. Farmers are using both sulfates and chelated Zn (with ethylenediamminetetraacetic acid, EDTA) for soil and foliar applications. Therefore, this study was conducted to find out the role of zinc fertilizer application in reducing salinity stress and to determine the agrophysiological traits of barley genotypes to selecting and screening. Two aspects were studied in this investigation: 1) the seed treatment with chelated Zn and nanoscale ZnO and study of seed characteristics; and 2) a field experiment with foliar application of chelated Zn and nanoscale ZnO.

2. Materials and methods

2.1. Preparation of Zn-chelate and ZnO nanoparticles and seed treatment

Zinc oxide nanoparticles were produced by China's Neutrino Company with a purity of 99%. The average particle diameter was less than 30 nanometers and the specific surface area was more than 30 m^2gr^{-1}. Due to the fact that Nano-ZnO is not soluble in water, first the Nano-ZnO was suspended directly in deionized water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. To avoid aggregation of the particles, magnetic bars were placed in the suspensions for stirring before use. Because ZnO will not dissolve in water and plants cannot absorb it, farmers are widely using Zn-chelate (EDTA-Zn). EDTA-Zn was produced by the Spanish Company TradCorp and had a 14% zinc element. Both chelated and Nano-ZnO suspensions were prepared at concentrations of 0.5, 1.0, and 1.5 ppm. A control was also maintained, corresponding to pure water.

2.2. Seeds

Barley genotype seeds (Morocco, Nosrat, and Khatam) were procured from the Isfahan Agricultural Research and Natural Resources Center, Iran. The average germination rate of the seeds was 99%. The seeds selected were of uniform size to minimize errors in seed germination and seedling vigor.

2.3. Laboratory experiments

An experiment was performed in the laboratory of the Isfahan Agricultural and Natural Resources Research

Center to investigate the effect of zinc compounds on germination and salinity tolerance indices. The experiment was conducted as a factorial experiment with a completely randomized design and three replications. The first factor was three barley genotypes, including Morocco, Nosrat, and Khatam. The second factor was seven zinc concentrations, including Nano-ZnO at three levels of 0.5, 1.0, and 1.5 ppm, Chelate-Zn at three levels of 0.5, 1.0, and 1.5 ppm, and control (without fertilizer consumption). The third factor was water quality, including 2, 10 and 18 dS m^1.

2.4. Seedling Vigor Index

First, petri dishes (100 x 15 mm) were disinfected. Also, in order to prevent contamination, the seeds were soaked in 1.5% sodium hypochlorite solution for 3 minutes and then washed several times with completely "distilled water". Then the seeds were placed separately in a petri dish with a fertilizer treatment with different concentrations for 3 hours, and the treated barley seeds were shade-dried for 1 hour. One piece of sterilized filter paper and five mL of quality water were added (as per the recommendations of the International Seed Testing Association, 2011). The Petri dishes were covered and placed in an incubator at 25 ± $1^{\circ C}$ for eight days. Seedlings were counted every day for 7 days after the start of the experiment. On the eighth day, the length of the radicle and the plumule and the percentage of germination were measured. Germination was calculated based on the number of seeds germinated in a petri plate and expressed as a germination percentage. The seedling Length Vigor Index (SLVI) was calculated by the formula described by Abdul-Baki and Anderson (1973).

Seed Length Vigor Index (SLVI) = Germination Rate (root length + shoot length)

2.5. Field experiment

The field experiment was conducted during 2013-14 at Esfahan Kaboutarabad Research Station. The experiment was conducted in a strip-plot design with three replications. Each plot consisted of six rows, each 4 m in length, and were spaced 20 cm apart. Three water irrigation quality, including W_1 = 1-2 dS m^{-1} (low salinity) as a check, W_2 = 10 dS m^{-1} (common salinity in the region), and W_3 = 18 dS m^{-1} (high salinity), were evaluated in vertical factors. The horizontal factors were spraying, including Nano-ZnO, Zn-Chelate and water spraying as a check. Three different barley genotypes, including Morocco (salt-sensitive), Nosrat (semi-salt-tolerant), and Khatam (salt-tolerant), are spilt within vertical factors. On November 5, Seeds were sown with a density rate of 450 seeds m^{-2} by a cereal row planting machine (Wintersteiger Plotman). To irrigate the plots, water was delivered from the channel (S_1 = 2 dS m^{-1}), a local well (S_2 = 10 dS m^{-1}), and mixed drainage water and local water well (S_3 = 18 dS m^{-1}). The application rates of Nano-ZnO and Zn-chelate were 100 and 1000 g ha^{-1}, respectively. Grain yield was measured in plots of 0.4 4 m^2. At the maturity stage, grain yield samples are harvested and weighed. Developmental stages were determined according to the method suggested by Zadoks et al. (1974). Saturation water deficit of the flag leaves was measured as described by Pask et al. (2012). In order to estimate, analyze, and interpret the parameters related to grain filling, a linear (two-piece) regression model based on the DUD procedure and Proc Nlin guidelines of SAS software was used as follows (Rondanini et al., 2004).

2.6. Statistical analysis

Data were subjected to analysis of variance by SAS (SAS Institute 2007). Means of treatments were compared by the least significant differences (LSD) test at ($P \leq 0.05$). The Pearson correlation between traits is determined by correlation analysis.

3. Results

3.1. Seed germination and seedling vigor

According to the mean comparison result, germination percentage (GE), length of shoot (LS), length of root (LR) and seedling length vigor index (SLVI) were significantly (p0.01) affected by Zn concentration, genotype and water quality, but no significant differences in LR were found among the water quality. The results showed that barley seeds responded positively towards the treatment at various concentrations of both Chelated-Zn and Nano-ZnO particles. However, the control treatment (Table 1) produced the highest germination percentage (GE = 63.28%), shoot length (LS = 23.19 mm), radicle length (LR = 22.23 mm) and seedling length vigor index (SLVI = 2968.26). Among the different Chelated-Zn and Nano-ZnO particle concentrations, 0.5 ppm showed the maximum and an increased concentration (1.5 ppm) showed decreased GE, LS, LR, and SLVI. Chelated-Zn showed more GE and SLVI and larger LR compared to Nano-ZnO, though Nano-ZnO had the least LS. Seed treated with 0.5 ppm Chelated-Zn, more GE (57.89%), LS (20.91 mm), LR (21.22 mm) and SLVI (2425.41) was obtained compared to other various Zn concentrations (1.5 ppm) (Table 1). Moreover, examination of the correlation coefficients of the studied traits showed that there was a significant and positive correlation was found between the percentage of germination with the SLVI (r = 0.95, p = 0.01), LS (r = 0.61, p = 0.01), and LR (r = 0.39, p = 0.01) under different salinity levels (Table 2). Despite of non-significant effect of water quality on LR (Figure 1), the highest GE, LS, LR and SLVI were observed in Khatam genotype and minimum salinity, whereas the lowest these traits were noted in Nosrat and high saline water quality (Figures 1-3).

Germination is the stage in a plant's development cycle most adversely affected by salinity. Barley, the fourth most important cereal crop, has prominent salinity tolerance relative to other cereal crops, but its salinity tolerance diverges among genotypes (Mwando et al., 2020). As the quality of water salinity levels increased, the seed germination characteristics decreased as well. The results showed that different levels of salinity had significant effects on germination percent and longitudinal traits of seed (root length, shoot length, and seedling vigor index).

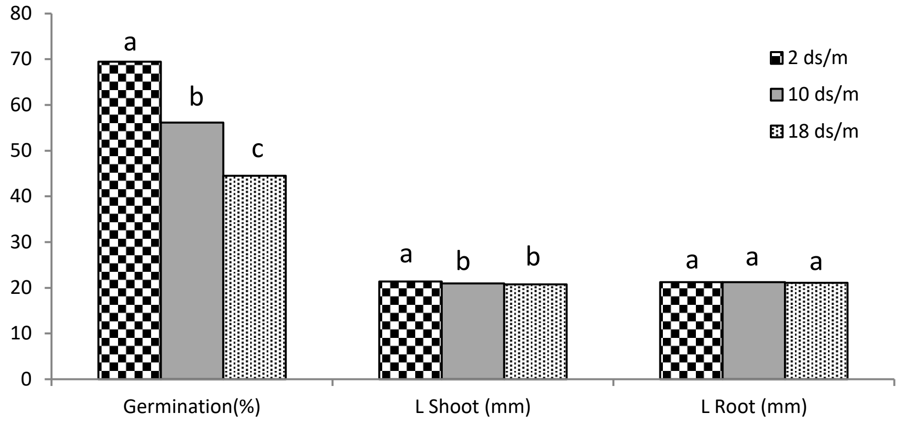

Figure 1. Effect of water quality on germination percentage, length of shoot and root

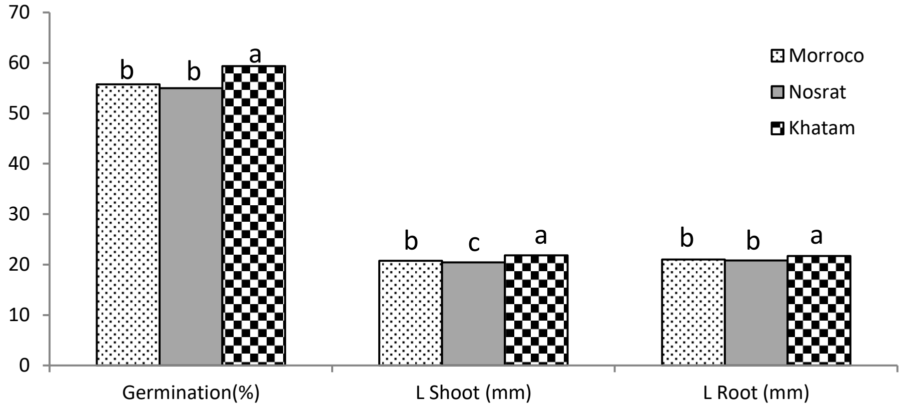

Figure 2. Effect of three barley genotypes on germination percentage, length of shoot and root

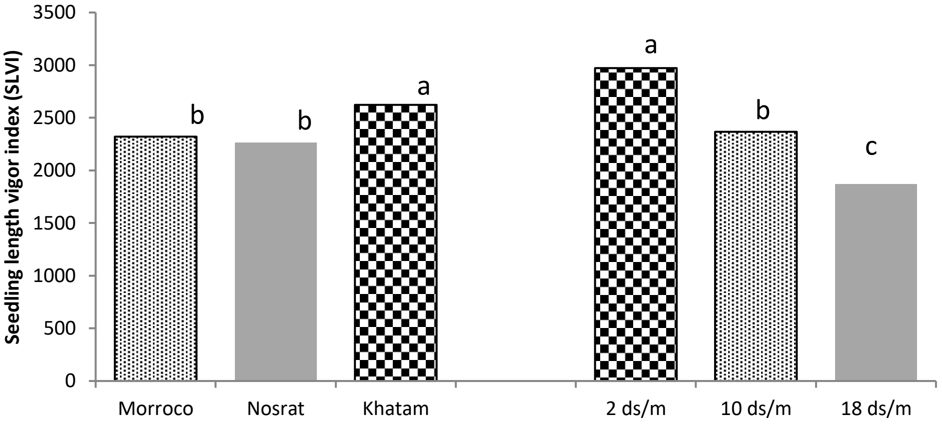

Figure 3. Effect of three barley genotypes and water quality on seedling length vigor index

With increasing salinity stress, all of the traits decreased sharply, so the highest obtained from control treatment and the lowest obtained from a salinity level of 18 dS m^{-1}. These results were generally consistent with findings by Naeem et al., (2017) and Khodarahmpour et al., (2012). The genotype differences also contributed to the observed differential response to the salinity treatments. It seems that the Khatam genotype has more tolerance to salinity conditions. Genotypes of Nosrat and Morocco were sensitive to salinity stress (Figure 2). Thus, salinity tolerance at the germination and seedling stages is an indicator for screening tolerant genotypes. This also agrees with the reports from Shahid et al., 2012; Ravelombola et al., 2017. The negative effects of salinity on germination may be due to reducing osmotic potential (Somani, 2007), increasing ionic concentration of ions on metabolism (Khodarahmpour et al., 2012), interfering with certain aspects of metabolism, such as changing the balance of growth regulators (Khan and Ungar, 2001), which results in limited water uptake by seeds (Chachar et al., 2008).

Table 1. Mean comparison for germination percentage, length of shoot and root, and seedling length vigor index of three barley genotypes in three concentrations of nanoscale ZnO and Chelated-Zinc.

Concentration (ppm)	Germination (%) (GE)		Shoot length (mm) (LS)		Root length (mm) (LR)		Seedling length vigor index (SLVI)	
	Chelated Zinc	Nano ZnO	Chelated Zinc	Nano ZnO	Chelated Zinc	Nano ZnO	Chelated Zinc	Nano ZnO
0.5	57.89 b	55.11 cde	20.91 b	20.89 b	21.22 b	20.92 bc	2425.41 b	2311.58 bcd
1.0	57.11 bc	54.22 de	20.51 bc	20.62 bc	21.19 b	20.86 bc	2353.74 bc	2252.22 cd
1.5	55.89 bcd	53.44 e	20.33 c	20.72 bc	21.11 b	20.58 c	2291.78 bcd	2215.06 d
Control	63.28 a		23.19 a		22.33 a		2968.27 a	
LSD 5%	2.11		0.48		0.45		134.36	

Different lowercase letters indicate significant differences between treatment based on LSD test at $p<0.05$.

Table 2. Correlation coefficients between traits of three barley genotypes grown under different salinity levels.

Traits	Germination	Length shoot spike	Length root	Seedling length vigor index (SLVI)
Germination	1			
Length shoot	0.61 **	1		
Length root	0.39 **	0.65 **	1	
SLVI	0.95 **	0.81 **	0.58**	1

**Significant at 0.01 level of probability.

3.2. Grain filling

Grain filling rate (GFR) and maximum grain weight (MGW) were significantly affected by salinity levels of irrigation water quality, zinc foliar applications, and genotypes (Table 3). The Result of the mean comparison of irrigation quality showed that the highest GFR (0.00148 g /day^{-1}) and MGW (0.0405 g) were at minimum saline water. In comparison to the control (2 dS m–^{1}), GFR decreased by about 16 and 23% in salinity levels of 10 and 18 dS m–1, respectively, and MGW decreased by about 15% and 20% in salinity levels of 10 and 18 dS m$^{–1}$, respectively. According to the mean comparison of zinc foliar applications, the highest GFR (0.00138 g day^{-1}) and the MGW (0.0356 g) belonged to the Zn-EDTA spraying solution and the lowest one was related to the control. Application of Zn-EDTA in GFR and MGW increased about 15 and 20% in comparison with check, respectively. A Comparison of averages showed that the maximum GFR (0.00148 g day^{-1}) and MGW (0.0367 g) belonged to the Khatam genotype, but Morocco (sensitive genotype) had the lowest GFR and MGW. In comparison with the Khatam genotype, GFR declined by about 5.4 and 33% in Nosrat and Morocco, and MGW was reduced by approximately 10.4 and 11% in Nosrat and Morocco, respectively. In addition, a significant and positive correlation was found between the grain yield and GFR (r = 0.72, p = 0.01) and MGW (r = 0.63, p = 0.01), which can be attributed to the importance and effective role of these two physiological traits in tolerance to salinity (Table 4).

Table 3. Effects of saline water quality and fertilizer application on agrophysiological parameters of barley genotypes

Treatments	Grain filling rate (GFR) (g.day^{-1})	Maximum grain weight (MGW) (gr)	Saturation water deficit (SWD) (%)	Proline (µg.g^{-1})	Number of spike (NS)	Kernel number per spike (KNS)	Thousand-kernel weight (gr)(TKW)	Grain yield (GY) (kg.ha^{-1})
S (Quality(dS m^{-1})								
S_1=2	0.00148 a	0.0405 a	14.17 a	196.87 b	333.72 a	27.38 a	33.00 a	3123.26 a
S_2=10	0.00124 b	0.0331 b	14.98 a	212.43 b	286.37 b	23.36 b	28.69 b	1737.85 b
S_3=18	0.00114 b	0.0281 c	15.23 a	255.12 a	259.81 c	20.57 c	22.85 c	1524.03 c
LSD 5%	0.0002	0.003	4.36	15.84	19.69	2.02	2.09	132.32
F (Fertilizer)								
F_N=Nano-ZnO	0.00113 a	0.0352 a	12.97 a	241.37 a	297.04 a	22.59 b	27.2 a	2069.81 b
F_Z=Zn-EDTA	0.00138 a	0.0356 a	12.63 a	183.52 b	318.37 a	24.15 a	29.13 a	2365.46 a
F_C=Check	0.00115 b	0.0309 b	17.77 a	239.54 a	264.50 b	24.56 a	27.59 a	1818.52 b
LSD 5%	0.0002	0.002	5.18	15.14	22.29	1.31	2.57	292.87
G (Genotype)								
G_1=Moroco	0.00099 b	0.0327 b	18.90 a	170.76 c	317.37 a	12.91 c	29.62 a	1381.94 c
G_2=Nosrat	0.00140 a	0.0329 b	11.89 b	230.86 b	294.22 b	27.74 b	26.08 b	2232.57 b
G_3=Khatam	0.00148 a	0.0367 a	11.57 b	262.80 a	268.31 c	30.66 a	29.84 a	2770.63 a
LSD 5%	0.0001	0.002	2.85	12.60	12.45	1.55	0.94	124.16
S	*	**	NS	**	**	**	**	**
F	*	**	NS	**	**	*	NS	*
S*F	NS	*	**	**	NS	NS	**	**
G	**	**	**	**	**	**	**	**
G*S	NS	NS	**	NS	NS	NS	**	**
G*F	NS	NS	NS	**	**	NS	**	**
G*S*F	NS	NS	NS	**	**	**	**	**
CV%	14.44	10.69	22.97	10.31	7.68	11.79	6.05	8.82

Means within similar letters in each column are not significantly different (LSD 5%). NS, non-significant, * $p<0.05$, ** $p<0.01$

Table 4. Coefficient correlations between traits of three barley genotypes grown under different salinity levels

Traits	Grain yield (GY)	Number of spike (NS)	Kernel number per spike(KNS)	Thousand-kernel weight (TKW)	Saturation Water deficit (SWD)	Proline	Grain filling rate(Slope)	Maximum grain weight (MGW)
GY	1							
NS	0.38 **	1						
KNS	0.74 **	- 0.12 ^{NS}	1					
TKW	0.46 **	0.53 **	0.12 ^{NS}	1				
SWD	- 0.26 *	0.01 ^{NS}	- 0.28 *	- 0.01 ^{NS}	1			
Proline	- 0.01 ^{NS}	- 0.43 **	0.24 *	- 0.34 ^{NS}	- 0.05 ^{NS}	1		
Slope	0.72 **	0.17 ^{NS}	0.69 **	0.24 *	- 0.31 **	0.16 ^{NS}	1	
MGW	0.63 **	0.41 **	0.38 **	0.61 **	- 0.14 ^{NS}	- 0.08 ^{NS}	0.71 **	1

^{NS}, non significant. ** and *, significant at 0.01 and 0.05 probability levels, respectively.

Our results indicated that the salinity declined at the rate of grain filling. Likewise, grain filling is the main stage in production, and the longer this period allows the transfer of photosynthetic production from source to sink, resulting in increased grain yield. Therefore, we can conclude that the cause can be disrupted by the process of photosynthesis due to ionic toxicity, reduction of GFR, and MGW. Reducing the MGW reduces the final yield by shortening the grain filling period and accelerating grain maturation and grain prematurity. Reducing the GFR could be due to destroying the chloroplasts, decreasing carbon dioxide, chlorophyll content, fluorescence, leaf area, and photosynthesis caused by ionic and osmotic stress. These results are in accordance with those reported by Azizipour et al. (2010), and Munns and James (2003).

Due to the essential role of the element zinc in the plant, which is directly involved in the biosynthesis of growth materials such as auxin, and the supply of nutrients, the period of grain filling is prolonged. Although plants need little zinc, if they do not have enough of this element, they will be affected by the physiological stresses caused by the dysfunction of various enzymatic systems and other zinc-related metabolic functions. It seems that consumption of zinc increases the total amount of carbohydrates, starches, and proteins made by the plant. The more carbohydrates, the faster and longer the filling period, and as a result, the kernel-grain-weight improves. Many researchers have suggested that in salinity conditions, the growth periods of vegetative and reproductive wheat genotypes decrease, which leads to their premature maturation (Munns and James, 2003). There are some reports that confirm the consumption of nutrients, increased growth hormone production, and the period of wheat grain filling in salinity stress (Hagh Bahari and Seyed Sharifi, 2014).

3.3. Saturation water deficit (SWD)

Despite the non-significant effects of quality water irrigation and Zn fertilizer on SWD, the effects of genotype were highly significant (p0.01). In addition, the lowest SWD of minimum saline water (2 dS m^{-1}) and Zn-EDTA were produced (Table 3). Compared to control, SWD (12.63%) of flag leaf decreased with Zn-EDTA. Furthermore, the Khatam genotype had the least SWD (11.57%). The results demonstrated that the tolerant genotype, rather than the sensitive genotype (Morocco), showed a lower SWD under salinity. SWD was decreased in both the Khatam (salt-tolerant) and Nosrat (semi-tolerant) genotypes. The results demonstrated a negative significant correlation between SWD (r = -0.26*) and grain yield (Table 4). Similarly, results have been reported by Kadkhodaei et al. (2014), Ram et al. (2015), and Maghsoodi and Razmjoo (2014).

Higher SWD was recorded when the temperature exceeded the normal range and was observed under water deficit stress. It seems that species having a low rate of water loss through their leaf cuticle are better adapted to abiotic stress. In line with our results, Kafi et al., (2011), Ullah et al., (2012), and Raza et al., (2017), reported that SWD gradually decreased under salt conditions. However, a greater reduction was observed in salinity tolerant varieties. Plants subjected to salinity stress showed signs of wilting. The leaf SWD indicates that the leaf water deficit is considered to be an important marker of tolerance in plants. It seems that the high Na^+ absorption under saline conditions was due to impaired water absorption and increased SWD. So, higher values of leaf SWD under tension may be due to the reduction in water, leaf area, leaf turgor, transpiration, stomatal conductance, absorption of radiation under leaf rolling, production of leaves, and yield. Moreover, leaf senescence, abscission, and plant canopy temperature were also higher in plants with high SWD. These results were generally consistent with the findings of Munns and Tester (2008), Ebrahimian and Bybordi (2011), Ardestani and Rad (2012), and Mahlooji et al. (2018).

3.4. Proline content

Data on proline measurement indicated that the differences in quality of water irrigation, zinc foliar applications, and genotype were significant (p0.01). In addition, the lowest proline content of minimum saline water (S_1: 2 dS m^{-1}) and Zn-EDTA were produced (Table 3). Salinity increased flag leaf proline content. In comparison with S_1, flag leaf proline content was inclined by about 8 and 30% in S_2 and S_3, respectively. Compared to control, the proline content (183.52 $\mu g.g^{-1}$) decreased with Zn-EDTA (approximately 24%). Furthermore, the Khatam genotype had the most proline content (262.8 $\mu g.g^{-1}$). Proline content increased in Nosrat (G_2 = semi-tolerant) and Khatam (G3 = salt-tolerant) by around 35 and 54%, respectively, as compared to G_1 (Table 3).

Additionally, the proline content increased with the rise of salinity. Through the accumulation of proline, barley plants may deal with cell dehydration and help to maintain survival. Under saline conditions, high concentrations of sodium and chloride ions reduced leaf area and may have deformed water absorption, anabolic enzymes, and -

glutamil kinase. These increased proline content. These findings agreed with those of Munns and Tester (2008); Ebrahimian and Bybordi (2011); Misra and Saxena (2009) and Aflaki Manjili et al., (2012). Also, a considerable increase in the proline content was observed in barley treated with Nano-ZnO and check (non-Zn fertilizer). It seems that the induction of proline accumulation in response to Nano may be due to an activation of proline synthesis through the glutamate pathway. In line with our results, Mohammadi et al. (2014, 2016); Karimi and Sepehri (2018) reported that proline content was promoted in plants treated with Nano. Notably, the results demonstrated that the tolerant genotype (Khatam) accumulates a markedly higher concentration of proline in leaves than the sensitive genotype (Morocco). It was observed that proline played a significant role in decreasing lipid peroxidation. These results are in accordance with those reported by Abbasi et al., (2016) and Ahmadi et al., (2009).

3.5. Yield and yield components

However, the effects of irrigation-water quality, zinc fertilizer and genotype were highly significant on yield and yield components, but no significant differences in thousand-kernel weight (TKW) were found among the zinc fertilizer. Number of spikes (NS) of approximately 15 and 22%, kernel number per spike (KNS) of approximately 15 and 25%, thousand-kernel weight (TKW) of approximately 13 and 31%, and grain yield (GY) of approximately 39 and 48% were reduced in S_2 (10 dS m^{-1}) and S_3 (18 dS $m–^1$), respectively, when compared to S_1 (2 dS $m–^1$).grain yield (GY) increased by about 30 and 13%, respectively, while kernel number per spike (KNS) decreased by about 2 and 8% in Zn-EDTA (F_2) and Nano-ZnO (F_1) compared to check (F3). Khatam (G_3 = tolerant) had the highest KNS (30.66), TKW (29.84 gr), GY (2770,63 kg/ha) and the lowest NS (268), but Morocco (G_1 = sensitive) had the lowest KNS (12.91), GY (1381.94 kg/ha) and the highest NS (317.37) and TKW (29.62 gr) (Table 3). Furthermore, Khatam had the highest GY, KNS, and TKW. This revealed that genotypic differences were markedly important. Moreover, a significant positive correlation (Table 4) was found between GY and NS (r = 38**), KNS (r = 0.74**) and TKW (r = 0.46**).

All yield components were adversely influenced by salt stress, among which KNS was the most sensitive component. This revealed that the pollen development processes are sensitive to salinity during the flowering stage, causing a lapse of viable pollen and floret abortion. These results, therefore, show that salinity inhibited grain filling, yield, and yield components. Similar consistent results were reported for barley (Steppuhn and Raney, 2005) and oats (Zhao et al., 2007).

It seems that low salinity conditions of irrigation water and zinc foliar application are advisable to have the most GY and all yield components. As a result of consuming zinc element the total amount of carbohydrates, starches, and proteins produced by the plantincreases, and with increasing carbohydrates, the speed and duration of the grain filling period increase, resulting in increased grain yield. Similar results were observed by Mahmood (2011); Hagh Bahari and Seyed Sharifi (2013) reported that applying zinc in various ways, especially by spraying, increases the yield compared to the control.

In line with our results, Ashrafi et al. (2014) and Pirasteh et al. (2016) reported that the grain yield and yield components of barley genotypes were reduced under salt stress, but the reduction was greater in the salt-sensitive cultivar compared to the salt-tolerant one. They concluded that this was due to the reduction in water absorption by plant tissues, cellular growth and development, and physiological and biochemical traits. Consistent with these results, many researchers have shown that the growth of plants declined under saline conditions, but the degree of reduction depended on environmental conditions, salt content, water consumption, type of genotype (Abdallah et al., 2017), and stage of plant growth (Mahmood, 2011; Shafaqat et al., 2012).

4. Discussion

The world population is constantly increasing and is estimated to reach 9 billion by 2050. Therefore, there is a need to cultivate salinized soils to improve food production globally. In addition, salinized soils require irrigation water, which ultimately can result in increased soil salinity. Because arable land has varying degrees of salinity, the use of saline water in salinity soils seems to be necessary due to limited freshwater resources and the degree of sensitivity of different genotypes, so managing water use and selecting the right genotype are important. Various factors, such as plant species, ambient temperature, plant growth stage, soil or water composition, environmental variables, and plant resistance, affect salinity. In addition, the tolerance of plants to such elevated salinity levels varies from species to species. One solution to the salinity problem is using salt - tolerant species and cultivation of resistant cultivars within species. Barley can be salted for cultivation due to its high genetic diversity. Cultivation of this plant is also limited due to the need to use special genotypes that are highly adaptable to the conditions of the region and also due to the presence of very saline water drains (Mahlooji, 2017; Naeem et al., 2017).

Understanding salt-tolerant mechanisms is imperative for crop improvement in salt-affected areas. Tolerance to salinity often depends on the physiological and structural complexity of the plant. Traditional screening techniques for salt tolerance are usually based on the grain yield and are expensive and time-consuming. In recent years, the focus of screening has shifted towards examining germination characteristics and specific physiological traits involved in salt tolerance. The germination and seedling growth process depend on saline water quality and osmotic potential. however, little is known about the germination responses of genotypes to salinity. Therefore, there is a need for the introduction of reliable germination and physiological markers for the selection of salt-tolerant genotypes to be planted directly or used in breeding programs. Salt stress decreased the germination characteristics of the genotypes. In addition, tolerant cultivars had germination indexes unchanged or less affected by salinity. Many studies report that elevated salinity levels become a limiting factor for seed germination and seedling development. However, salinity levels showed an inhibitory effect on germination with an increase in salt concentration (Ahmed et al., 2017; Louf et al., 2018; Naeem et al., 2017; Sonia et al., 2019).

Among different abiotic stresses, salinity stress crucially reduces plant growth and also causes nutrient imbalances. Depending on the conditions, the use of field methods such as optimal levels of chemical fertilizers can also help to increase the growth and yield of crops to some extent. One easy, low-cost, and cost-effective solution is to use spraying to improve salinity tolerance and increase crop production in saline conditions. Under intolerable salinity, plant growth and yield are not raised by increasing nutrient concentration in soils because, in a saline environment, the micronutrient uptake is terribly variable. In calcareous soils, zinc precipitates in unavailable forms to plants. Soil salinity is also associated with zinc efficiency in alkaline. The high pH and $CaCO_3$ content of these soils are usually considered the reasons for the low availability of Zn. Micronutrient foliar application plays an important role in alleviating and increasing the nutrient uptake under salinity. Zn is used for improving seed germination, seedling development in barley, seedling vigor, field establishment, protein synthesis, membrane function, cell elongation, and tolerance to environmental stresses. The Zn foliar application method is a very economical alternative to more expensive broadcast Zn fertilizer applications and soil application. As a quick solution to the Zn deficiency problem in human populations, fertilizer strategies should be applied nationwide (Alvarez and Gonzalez, 2006; Cakmak, 2000; Gonzalez et al., 2007; Prasad and Sinha, 1981; Mahlooji, 2017).

It is clearly seen from our results that treated barley seeds with water followed by chelated ZnO with a concentration of 0.5 ppm have shown a significant increment in germination, shoot length, root length and seedling length vigor index over other concentrations of the same material and varying concentrations of another material (Nano ZnO) tested. The results showed that different levels of salinity had significant effects on germination percent and these longitudinal traits of seed. However, the performance of the zinc material is less than the control. both plant growth and yield decreased at higher concentrations of nanoscale ZnO, and these results were in accordance with the reports by Prasad et al. (2012). Such inhibitory effects of nanoparticles were also reported by Lin and Xing (2007). Hence, it may be concluded that the effects of different salt concentrations on physiological traits (grain filling rate, maximum grain weight, saturation water deficit, and proline content) and the grain yield of barley cultivars with contrasting salt tolerance (tolerant, intermediate, and sensitive) and foliar applications under field conditions are more operational. Salinity increased flag leaf proline content, saturation water deficit in the plant and declined the rate of grain filling. It was also found that a lower SWD indicates a better plant water status. In line with our results, Ganji Arjenaki et al. (2012) showed similar results in wheat and reported that tolerant genotypes maintained lower SWD under stress than sensitive ones. Compared to control, the proline content and SWD of flag leaf decreased with Zn-EDTA. Proline amino acid is an organic molecule and one of the most commonly used solutions that protect membranes in addition to participating in osmotic regulation and can play a role in salinity stress, though proline is a positive factor for adaptation under salinity stress (Hong et al., 2005; Peng et al., 1996; Mansour, 1998). The supply of nutrients allows for a longer period of grain filling. Although the plant's need for micronutrients is low, if not enough of these elements are available, the plants will be affected by the physiological stresses caused by the dysfunction of various enzymatic systems and other zinc-related metabolic functions. Consistent with these results, many researchers have shown that the use of zinc fertilizer has increased the rate of grain filling and the maximum weight of the grain, and the reason may be the increase in assimilation and, ultimately, the increase in material transfer to the grain (Yamaguchi et al., 1995; Seyed Sharifi and Nazarli, 2016).

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