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Research Article

Agrophysiological barley associated with flag leaf temperature and canopy light interception under salinity and zinc foliar application

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Abstract

To understand the agrophysiological barley associated with flag leaf temperature and canopy light interception under salinity and zinc foliar application (ZnFA), a field experiment was conducted in a strip-plot design with three replications in Isfahan, Iran. Saline irrigation water in three levels [2 (low), 10 (moderate) and 18 (high) dS m^{-1}] were applied as vertical factors. Three barley genotypes ['Morocco' (salt-sensitive), 'Nosrat' (semi-salt-tolerant) and 'Khatam' (salt-tolerant)] were arranged within the vertical factors. The horizontal factors included four ZnFA [Nano-ZnO, Zn-EDTA, simultaneous applications of (Nano-ZnO + Zn-EDTA), and water (control)]. With increasing salinity, light interception (LI), maximal efficiency of PSII (Fv/Fm), chlorophyll content (SPAD), relative water content (RWC), number of spike (NS), kernel number per spike (KNS), thousand-kernel weight (TKW), and grain yield (GY) decreased, whereas electrolyte leakage (EL), flag leaf temperature (FLT) and proline increased. Nano-ZnO had the highest EL and the lowest FLT, RWC, NS and KNS. Zn-EDTA application provided the highest LI, RWC, TKW and GY, and the lowest proline. Minus zinc application (check) had minimum LI, Fv/Fm, SPAD and GY. The tolerant genotype had maximum LI, proline, SPAD, RWC, KNS and GY, and minimum FLT, EL, NS and TKW. Overall, it was concluded that Zn-EDTA can be as a proper tool for increasing barley yield under salinity stress conditions. Likewise, this study has highlighted the close relationships existing between GY with, TKW (r= 0.89**), KNS (r= 0.46**), RWC (0.45**), NS (r= 0.36**), FLT (r= -0.32**), EL (r= -0.21**), and SPAD (r= 0.20**). These findings indicated that these physiological traits could be key factors, as well as tools for screening, and provide useful information about stress tolerance mechanisms, which could be useful to plant breeders for selecting and developing salt-tolerant genotypes.

Keywords: Canopy temperature, Electrolyte leakage, Light interception, Proline

Introduction

Salinity is a major stress limiting crop production around the world, affecting almost 80 million hectares of agricultural lands (Mahlooji, 2017). The use of recycled water, sea water and drainage water for crop production has been suggested as part of the solutions to such problems (Yordanov *et al.*, 2003). Droughts and application of reusable water cause salinity stress. Growth, productivity of plant species and photosynthetic processes are restricted by salt stress (Tabatabaei and Ehsanzadeh, 2016). Understanding salt-tolerant mechanisms is imperative for crop improvement in salt-affected areas. Screening techniques based on the grain yield for salt tolerance are expensive and time-consuming (Kiani-Pouya and Rasouli, 2014). Therefore, there is a need for introduction of reliable physiological markers for selection of salt-tolerant genotypes to be planted. Major agrophysiological responses, including light interception, flag leaf temperature, chlorophyll fluorescence, chlorophyll content, relative water content and electrolyte leakage can be used to monitor plant responses to salt stress (Izadi *et al.*, 2014; Florence *et al.*, 2019; Bingham *et al.*, 2019). Therefore, using these measurements to screen for salinity tolerance and reducing expenses are thought to be more reliable than selecting for salt tolerance based on the yield (Rahnama *et al.*, 2011).

Light (solar radiation) provides the energy to drive photosynthesis. As light passes through the canopy it is absorbed or reflected, and the remaining light is transmitted to the lower leaves. Therefore, at a particular moment the fraction of incident light radiation intercepted depends on the green area index and how the leaves are geometrically arranged in the canopy (Florence *et al.*, 2019). Many plant traits and environmental variables play roles in the energy balance of the plant canopy affecting its temperature. The surface temperature of the canopy is related to the amount of transpiration resulting in evaporative cooling. Genotypes with 'cooler' canopy temperatures can be used to indicate a better hydration status (Rebetzke *et al.*, 2012). Canopy temperature (CT) is the ideal physiological selection trait in many ways since measurement is quick, simple, accurate estimation of

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the temperatures of different organs and inexpensive (Jones *et al.*, 2009; Munns *et al.*, 2010; Cossani *et al.*, 2012). Many studies have confirmed that CT is associated with crop yield (Blum *et al.*, 1982; Reynolds *et al.*, 1994; Olivares-Villegas *et al.*, 2007) as well as a range of physiological traits including stomatal conductance (Amani *et al*., 1996), plant water status (Blum *et al.*, 1982; Balota *et al*., 2008; Elbashier *et al.*, 2012), and deep roots. Moreover, cooler canopies have been associated with high yield (Rebetzke *et al.*, 2012). In terms of plant characteristics that determine genotypic differences in CT the most important traits are: (i) the vascular system of leaves, shoots and roots which determines the capacity for transpiration, (ii) stomatal aperture which regulates transpiration rate and may be influenced by hormonal signals (Davies *et al.*, 2005), (iii) root depth which determines access to water (Lopes and Reynolds, 2010), (iv) metabolism which if constrained for any reason will cause feedback inhibition of CO_2 fixation and therefore influence stomatal aperture (Reynolds *et al.*, 2000), and (v) source–sink balance (Bingham *et al.*, 2019) since a strong demand for assimilates will result in increased CO_2 uptake associated with larger stomatal conductance (Reynolds *et al.*, 2005).

The chlorophyll content (SPAD) in flag leaves is an important physiological index representing the degree of photosynthesis in plants. Reduction in net photosynthesis under stress has been attributed to reduction in SPAD of plants (Ebrahimi *et al.*, 2014). Decrease in leaf water potential induces stomatal closure and thus inhibits photosynthetic metabolism with evident changes in the actual quantum efficiency of PSII (Azizpour *et al.*, 2010), while no or little changes and effects are recorded in Fv/Fm (Seckin *et al.*, 2010). Some researchers have demonstrated that salt stress inhibits PSII activity (Hichem *et al.*, 2009); whereas others have indicated that salt stress has no effect on PSII (Demiral and Turkan, 2006). However, some studies have shown changes in chlorophyll (Chl) fluorescence (Fv/Fm ratio after dark-adaptation of the leaf) as a result of salinity stress (Castillo *et al.*, 2005). Chl fluorescence methodology can be conveniently used, to screen in a short time, many samples for tolerance to abiotic stresses, and also provides useful information about stress tolerance mechanisms (Izadi *et al.*, 2014). Also, leaf electrolyte leakage (EL) is considered as a good physiological index reflecting the degree of plant injury caused by salt stress. Increasing membrane ion leakage under stress conditions has been reported by Roy *et al.* (2009). The relative water content (RWC) of a leaf is a measurement of its relative hydration status to maximum water holding capacity at full turgidity.

In calcareous soils, zinc precipitates in unavailable forms to plants (Morshedi and Farahbakhsh, 2012). By reducing the amount of soil moisture in saline soils, Zn and Fe in the soil solution are reduced in mobility. Application of Zn fertilizers is a common practice to compensate Zn deficiency. Zinc deficiency in plants grown in calcareous soils can be moderately corrected by the application of inorganic zinc salts. However, soil applications of Zn have not been very successful under furrow irrigation. Most Zn deficiencies can be corrected with foliar zinc application (Christensen and Peacock, 2000). Plant element deficiencies can be compensated for by spraying appropriate foliar solutions to compensate for the deficiency (Cakmak, 2008). Zn is an essential micronutrient, which is deficient in many regions worldwide, such as in calcareous and salt-affected soils of central Iran (Khoshgoftarmanesh *et al.*, 2004). Morshedi and Farahbakhsh (2012), Keshavarz and Saadat (2016) have reported that zinc applications increased yields and had a positive effect on salt tolerance of wheat and barley.

There is a lack of information on the use of agrophysiological responses as selection markers for barley genotypes under saline stress conditions. Therefore, the purpose of this study was to determine the agrophysiological responses of different barley genotypes to salinity stress and to investigate the role of zinc fertilizer application in reducing the effects of salinity stress.

Materials and methods

This experiment was conducted in a strip-split-plot design with three replications in Isfahan Rodasht Drainage and Salinity Research Station (32º 30′ N, 52º 9′ E) during 2015-16. Three irrigation water salinity levels [control (S_1 = 2 dS m^{-1}), common salinity in the region (S_2 = 10 dS m^{-1}), and high salinity (S_3 = 18 dS m^{-1})] were evaluated as vertical strip factors. The horizontal factors were four zinc application levels, including nano zinc-oxide, Zn-EDTA, simultaneous applications (nano-ZnO and Zn-EDTA) and water (as a control). The application rates of nano-ZnO and Zn-EDTA included 100 and 1000 g ha^{-1}, respectively. Three barley genotypes [Morocco (salt-sensitive), Nosrat (semi-salt-tolerant) and Khatam (salt-tolerant)] were planted. 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^2 gr^{-1}. Due to the fact that nano-ZnO is not soluble in water, first, the nano-ZnO were suspended directly in deionized water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. The physical and chemical characteristics of the soil and irrigation water quality are shown in Table 1. The long term mean annual precipitation and temperature were 93.5 mm and 12°C, respectively. Seeds were sown with the density rate of 450 seeds m^{-2} on November 5 by a cereal row planting machine (Wintersteiger Plotman). Each subplot consisted of 6 rows, 6 m in length, with spacing of 20 cm apart. To irrigate the plots, water was delivered from the channel (S_1 = 2 dS m^{-1}), a local water well (S_2 = 10

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Table 1. Physico-chemical properties and water irrigation quality of the soil before sowing

Value	Soil characteristic	Water characteristics	Saline water		
			W_1=2	W_2=10	W_3=18
			dS/m	dS/m	dS/m
pH	7.7	pH	7.7	8.1	7.6
Electrical conductivity (dS m^{-1})	13	Electrical conductivity (dS/m)	1.4	9.7	17.8
Available K^+ (mg/kg)	340	So_4^{2-} (meq/lit)	0.8	26.9	172.3
Available Zn^{2+} (mg/kg)	0.72	HCo_3^- (meq/lit)	2.0	5.7	6.4
Available Fe^{2+} (mg/kg)	5.54	Cl^- (meq/lit)	1.4	60	111
Available Na^+ (meq/lit)	79.1	Na^+ (meq/lit)	1.5	47.8	99.3
Available Ca^{2+} +Mg^{2+} (meq/lit)	60	Ca^{2+}+Mg^{2+} (meq/lit)	2.6	44	72

dS m^{-1}), and mixed drainage water and local water well (S_3 = 18 dS m^{-1}).

At heading stage (between 10:00-14:00h), the quantum yield (Fv/Fm) was measured by the uppermost fully-expanded leaf using a fluorometer (chlorophyll fluorometer; Optic Science-OS-30, USA) (Pask *et al.*, 2012). For this purpose, the plants were adapted to darkness for 20 minutes using a special clamp and then the fluorescence amounts were measured in 1,000 (µM photon m^{-2} s^{-1}), and calculation was performed using the formula (Arnon, 1949):

$PSII = (F_m-F_0)/F_m= F_v/F_m$

PSII; quantum yield amount of photosystem II, F_m or maximum fluorescence after a saturated light pulse on plants adapted to darkness and F_0, the minimal fluorescence in the light adapted, which was determined by illumination with far-red light. Chlorophyll meter (SPAD Konica, Minolta, Japon) and infrared thermometer (AUTOPRO, Raytek, Ltd, USA) were used to measure chlorophyll content and flag leaf temperature (FLT) at heading stage, respectively. Taking light interception measurements with a hand-held ceptometer (Sun Scan Delta-T Devices, Ltd, England) at flowering stage at noon witl formula (Balota *et al.*, 2007; 2008).

Light interception (%) = ((A-B) - C) / (A-B) × 100

Where: A = above-canopy PAR; B = reflected PAR; and, C = below canopy PAR. The range that can be used by plants for photosynthesis are wavelengths between 400 nm (blue) and 700 nm (red), and is termed 'photosynthetic active radiation' (PAR).

Relative water contents of the flag leaves were measured as described by Pask *et al.* (2012), and the electrolyte leakage was measured using the methods of Ahmadizadeh *et al.* (2011). Fresh leaves samples at flowering stage were analyzed for proline contents (Bates *et al.*, 1973). Grain yield was measured in 0.4×4 m^2 plots. Analyses of variances were conducted on the data to determine differences among the treatments using the general linear model (GLM) in SAS 9.1 (SAS Institute, Cary, NC). Mean comparisons were conducted using Fisher's least significant differences (LSD) test at 0.05. Relationships between traits were examined using simple linear correlations performed using SAS.

Results and discussion

The results of the analysis of variance of the data indicated that the effects of saline irrigation water were significant on light interception (LI), flag leaf temperature (FLT), proline content, chlorophyll content (SPAD), relative water content (RWC), number of spike (NS), kernel number per spike (KNS), as well as thousand-kernel weight (TKW) and grain yield (GY). As analyses indicated, zinc applications had significant on LI, SPAD and electrolyte leakage. The genotype had significant effects on all of the agrophysiological responses (LI, FLT, Proline, Fv/Fm, SPAD, RWC, EL, NS, KNS, TKW, and GY) (Table 2).

Light interception (LI): Data analysis showed that LI was significantly affected by salinity of water irrigation, barley genotypes and zinc fertilizer applications. As shown by the results, LI was decreased by increasing salinity (Table 2). Salinity of water irrigation S_1 improved in the LI from 98.87% to 99.47% relative to S_3. The LI was the greatest in both F2 and F3 treatments and the least in the treatment F4. Similarly, in the zinc fertilizer applications F_2 (Zn-EDTA: from 99.09% to 99.29%), and simultaneous applications F_3 (Nano-ZnO + Zn-EDTA: from 99.09% to 99.29%) enhanced LI compared to F_4 (check), respectively. Also, results revealed that the LI was significantly ($P < 0.01$) influenced by genotype. Similarly, the application of salt-tolerant genotype (Khatam) and semi-salt-tolerant (Nosrat) had raised the light interception compared to salt-sensitive (Morocco). Therefore, the Khatam genotype had maximum LI between genotypes. Our results appeared positive correlation grain yield versus LI ($r = 0.44^{**}$) (Table 3). On the other hand, some researchers have affirmed that grain yield was positively related to radiation use efficiency in winter wheat Yang *et al.* (2017); Li *et al.* (2008). The salinity of water reduced the solar radiation interception (due to a decrease in the number and size of leaves, rolling up the leaves and the total leaf area), if the water salinity was prolonged (Mahlooji *et al.*, 2015, 2017, 2018; Jafaraghaei and jalali, 2019).

Flag leaf temperature (FLT): With increasing salinity, FLT was high. Khatam genotype had minimum temperature (cooler) between genotypes. Mixing (nano and EDTA) of zinc treatment had the highest FLT (Table 2). Seem to have, less FLT, more tolerant to salinity stress. Likewise, significant negative correlation between FLT ($r = -0.32^{**}$) and yield stress condition confirms this result (Table 3). On the other hand,

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Table 2. Effects of water quality and fertilizer application on photosynthetic parameters of barley genotypes

Treatments	Light interception (%)	Flag leaf temperature (°c)	Proline (µgr/gr)	Fv/Fm	SPAD value	Relative water content (RWC%)	Electrolyte leakage (EL%)	Number of spike (NS)	Kernel number per spike (KNS)	Thousand-kernel weight (gr) (TKW)	Grain yield (GY) (kg ha^{-1})
Quality(dS m^{-1})											
S_1=2	99.47^{a}	30.16^{b}	204.40^{c}	0.799^{a}	44.97^{b}	87.27^{a}	35.42^{b}	517.92^{a}	32.57^{a}	37.86^{a}	6006.30^{a}
S_2=10	99.25^{b}	30.71^{a}	214.15^{b}	0.795^{a}	47.36^{a}	83.99^{ab}	37.80^{ab}	457.81^{b}	29.30^{b}	34.98^{b}	4592.20^{b}
S_3=18	98.87^{c}	30.90^{a}	219.26^{a}	0.792^{a}	43.83^{c}	81.34^{b}	38.98^{a}	389.19^{c}	25.50^{c}	26.94^{c}	2054.40^{c}
LSD 5%	0.053	0.344	4.747	0.015	0.98	4.22	3.14	30.36	1.65	1.28	361.04
Fertilizer											
F_1=Nano-ZnO	99.11^{b}	30.27^{b}	218.74^{b}	0.795^{ab}	44.06^{b}	83.06 ^{a}	41.38^{a}	454.63^{a}	28.09^{b}	32.90^{a}	4163.30^{a}
F_2=Zn-EDTA	99.29^{a}	30.43^{b}	184.44^{d}	0.794^{ab}	45.48^{b}	84.92 ^{a}	34.09^{b}	457.07^{a}	29.06^{ab}	33.98^{a}	4365.10^{a}
F_3=Mix	99.29^{a}	31.00^{a}	195.83^{c}	0.801^{a}	48.15^{a}	84.89 ^{a}	39.53^{a}	448.41^{a}	29.58^{ab}	33.81^{a}	4209.80^{a}
F_4=Check	99.09^{b}	30.65^{b}	251.39^{a}	0.790^{b}	43.86^{b}	83.93 ^{a}	34.60^{b}	459.78^{a}	29.77^{a}	32.35^{a}	4132.40^{a}
LSD 5%	0.172	0.571	6.249	0.015	1.18	3.80	1.95	28.01	1.52	1.85	386.9
Genotype											
G_1=Morocco	98.86^{c}	31.17^{a}	200.13^{c}	0.795^{ab}	42.57^{c}	79.34^{c}	39.67^{a}	567.56^{a}	18.02^{c}	34.43^{a}	3843.59^{b}
G_2=Nosrat	98.97^{b}	30.63^{b}	209.08^{b}	0.789^{b}	43.89^{b}	83.68^{b}	38.06^{a}	427.03^{b}	33.27^{b}	31.96^{c}	4402.67^{a}
G_3=Khatam	99.76^{a}	29.97^{c}	228.59^{a}	0.801^{a}	49.70^{a}	89.59^{a}	34.46^{b}	370.32^{c}	36.08^{a}	33.39^{b}	4406.68^{a}
LSD 5%	0.101	0.278	3.854	0.015	2.03	1.98	1.92	15.14	1.52	0.82	176.41
S (Quality)	**	**	**	ns	**	*	ns	**	**	**	**
F (Zn-fertilizer)	ns	ns	ns	ns	**	ns	**	ns	ns	ns	ns
S*F	**	**	**	**	**	ns	*	ns	ns	**	*
G (genotype)	**	**	**	*	**	**	**	**	**	**	**
G*S	**	ns	**	ns	**	ns	ns	**	**	**	**
G*F	**	ns	**	**	**	ns	*	**	*	*	ns
G*S*F	**	ns	**	ns	ns	ns	**	**	ns	**	*
CV%	2.15	1.92	3.82	2.23	5.09	4.97	10.81	7.02	10.97	5.20	8.82

Means with the same letters in each column are not significantly different (LSD 5%). ns, * and **: show no significant, significant at 5% and 1% level of probability, respectively.

Table 3. Coefficient correlations between traits of three barley genotypes grown under different salinity

Traits	Grain yield (GY)	Light interception (LI)	Flag leaf temperature (FLT)	Proline	Fv/Fm	SPAD value	Relative water content (RWC)	Electrolyte leakage (EL)	Number of spike (NS)	Kernel number per spike (KNS)	Thousand-kernel weight (TKW)
GY	1										
LI	0.44**	1									
FLT	-0.32**	-0.27**	1								
Proline	-0.08	0.12	-0.02	1							
Fv/Fm	0.04	-0.04	-0.05	-0.39**	1						
SPAD	0.20*	0.40**	-0.24*	-0.04	0.05	1					
RWC	0.45**	-0.25**	0.06	0.13	0.16	0.51**	1				
EL	-0.21*	-0.25**	0.06	0.03	-0.06	-0.24*	-0.26**	1			
NS	0.36**	-0.10	0.16	-0.28**	-0.03	-0.36**	-0.32**	0.07	1		
KNS	0.46**	0.48**	-0.42**	0.12	0.02	0.45**	0.62**	-0.31	0.55**	1	
TKW	0.89**	0.31**	-0.25**	-0.14	0.06	0.20*	0.32**	-0.11	0.45**	0.16	1

*** and **, Correlation coefficient significant at the 0.01 and 0.05 levels of probability, respectively**

numerous researchers affirmed that with high leaf area, transpiration, photosynthesis, fixing carbon dioxide, dry matter, produced more yield (Balota *et al.*, 2007; Rebetzke *et al.*, 2012; M'hamed *et al.*, 2015). As shown by the results, genotypes with cooler FLT under salinity conditions were able to gain higher grain yield. According to Yang *et al.* (2017), increased flag leaf area and decreased leaf temperature led to increased grain yield of wheat. Thus, FLT temperature can also be considered as one of the effective traits in stress resistance, these results correspond with other researchers (Pinter *et al.*, 1990).

Proline content: Salinity increased flag leaf proline content in the plant. The results showed that salt-

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tolerant genotype (Khatam) had the most proline content following by Nosrat, although salt-sensitive genotype (Morocco) had the least proline content (Table 2). Proline amino acid is an organic molecule, protects membranes, participating in osmotic regulation and can play a role in salinity stress. Proline is a positive factor for adaptation under salinity stress (Peng *et al.*, 1996; Girousse *et al.*, 1996; Mansour, 1998; Hong *et al.*, 2000; Barzegari *et al.*, 2019). It seems that under high salinity conditions of irrigation water, salt-tolerant genotype had the most proline content as well as grain yield and therefore, it is recommended. According to Mahlooji (2017) flag leaf leaf proline content was higher in salinity tolerant and semi-tolerant cultivars compared to semi-salinity cultivar. These barley cultivars with high Fv/Fm ratio and maximum potential in producing proline under stress conditions were able to have less yield reduction. Tolerant genotype had higher stomatal conductance, mesophyll conductance, stomatal mesophyll, water use efficiency, photosynthetic rate and grain yield (Mahlooji *et al.*, 2015; Mamnoe *et al.*, 2010).

Chlorophyll fluorescence: The maximal quantum yield (F_v/F_m), which characterizes maximum efficiency of PSII photochemistry, can be used as a good estimatior for photosynthetic performance. The effects of salinity levels and zinc applications on Fv/Fm were not significant, whereas genotypic differences in Fv/Fm were significant (Table 2). In comparison with S_1, Fv/Fm had a decline about 0.5% and 1% in S_2 and S_3, respectively. It has been reported that mild-salinity levels do not induce sustained photodamage to PSII as revealed by unvaried Fv/Fm ratio in plants (Naumann *et al.*, 2007) even in reduction of leaf gas exchanges. Salt-tolerant genotype (Khatam) had higher Fv/Fm than the salt-sensitive genotype (Morocco). The results showed that the leaf Fv/Fm gradually decreased with increasing salinity in barley (Table 2). This could result from damaged leaf cell membranes, reducing leaf area and irreversible photoinhibition resulting from stress. These results are in agreement with those reported by James *et al.* (2002); Movahhedi Dehnavi and Jalil Sheshbahre (2017) and Asadi and Eshghizadeh (2020).

Chlorophyll content (SPAD): SPAD was reduced due to high salinity stress. With increasing salinity up to 10 dS m^{-1} (S_2), SPAD increased approximately 5%, but in S_3 (18 dS m^{-1}) SPAD reduced about 2.6%, in comparison with S_1 (2 dS m^{-1}). Maximum SPAD was at a medium level of salinity and the greatest reduction was observed in S_3 treated with high saline water. However, under lower salt stress (S_2), the SPAD was higher compared with the control (S_1). Conditions of medium salinity (S_2) may stimulate photosynthesis due to tolerance mechanisms such as leaf area reduction and leaf thickness increasing the concentration of chlorophyll in the leaf surface (Mahlooji, 2017). According to Mohammadkhani and Heidari (2007), SPAD increased at moderate stress level. Increasing the salinity up to (18 dS m^{-1}) lowered SPAD values. Reduction of the SPAD could be due to destroying the chloroplasts, increasing the stomatal resistance, decreasing the stomatal conductance as well as a reduction in the amount of chlorophyll. Similar results have been reported by Azizpour *et al.* (2010); Mahlooji *et al.* (2015).

The effects of zinc application treatments in SPAD were significant ($P \leq 0.01$). Simultaneous application treatments (F_3=nano-ZnO and Zn-EDTA) in SPAD increased about 10%, in comparison with F_4 (check=only water). SPAD of F_4 was the lowest and the differences between F_1 (nano-ZnO), F_2 (Zn-EDTA) and F_4 were not statistically significant (Table 2). SPAD increased by zinc applications (F_1, F_2 and F_3). Higher chlorophyll accumulation may be due to complementary effects of nutrients like zinc. The positive effects of zinc applications under salt stress included: Protecting chlorophyll against free radicals, removing the reactive oxygen species, preventing the degradation of chlorophyll, increasing potassium concentration in the leaves, reducing sodium in the plasma membrane and maintaining cell integrity (Pask *et al.*, 2012). This is in consistent with our study and Cakmak (2008) in which zinc applications improved the SPAD.

SPAD was significantly affected by genotype ($P \leq 0.01$). SPAD of the tolerant genotype (G_3=Khatam) was the highest. SPAD decreased in Morocco (G_1=sensitive) and Nosrat (G_2=semi tolerant) around 14 and 12%, respectively as compared to G_3 (Table 2). This was because of less degradation of chlorophyll and having the higher Chl content in the salt tolerant barley genotype (Khatam). Other studies have shown a similar effect (Kumar Parida and Bandhu Das, 2005). SPAD was positively correlated ($r= 0.21^{**}$) with salinity tolerance of genotypes (Table 3). The higher SPAD in Khatam (tolerant genotype) may be related to its ability to repair injury or to use an efficient mechanism for the uptake of necessary elements for Chl under saline soil. These results were generally consistent with findings of Mahlooji *et al.* (2018) in barley genotypes under zinc applicaction.

Relative water content (RWC): With increasing salinity levels, RWC declined significantly ($P \leq 0.05$). There was a reduction in RWC about 4 and 7% in S_2 and S_3, respectively in comparison with S_1. The RWC differences were small and not significant between S_1 and S_2 or S_2 and S_3, whereas were significant in S_1 and S_3 (Table 2). High sodium ion absorption under saline conditions may have impaired water absorption and reduced RWC. Reduction in RWC may be due to reduced water, high concentrations of sodium and chloride ion, and reduced leaf area (Munns and Tester, 2008; Ebrahimian and Bybordi, 2011; Farhoudi and Khodarahmpour, 2015).

RWC was not affected significantly ($P>0.05$) by zinc applications (Table 2). However, application of Zn-EDTA slightly increased RWC, but nano-ZnO application reduced it, as compared to F_4. Although zinc is considered to protect vital cell components under

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stress, it is not known to increase the water absorption potential of plants or affect RWC. Consequently, lack of a significant effect of zinc applications on RWC in the present experiment may be acceptable. These results are in accordance to those reported by (Cakmak, 2008).

RWC was significantly ($P \leq 0.01$) affected by genotypes. Tolerant (G_3) and sensitive (G_1) genotypes had maximum and minimum RWC, respectively. There was a reduction in RWC about 12 and 7% in G_1 and G_2, respectively, in comparison to G_3 (Table 2). Although G_2 was more saline tolerant than genotype G_1, the significant decreases in RWC due to salinity stress implies that G_2 is also slightly sensitive to saline water stress. The results demonstrated that the tolerant genotype (Khatam) rather than the sensitive genotype (Morocco) showed a higher RWC under salinity stress. It has been shown that the ability to attract and maintain osmotic potential for higher RWC in saline soil water is an effective mechanism in salt-tolerant genotypes. This is in accordace with the results of Ganji Arjenaki *et al.* (2012); Noroozi *et al.* (2013).

It has also been found that higher RWC indicates a better plant water status. Thus, it can be assumed that increase in RWC has increased the chlorophyll content and Fv/Fm (Table 2). The ability of plants to maintain their RWC under stress conditions has been suggested as a tolerance mechanism (Kadkhodaei *et al.*, 2014; Maghsoodi and Razmjoo, 2014). The RWC was positively correlated ($r= 0.45^{**}$) with grain yield (Table 3). Similar results have also been reported by Kadkhodaei *et al*. (2014).

Electrolyte leakage (EL): The effects of salinity levels on EL were not significant. Despite the lack of significant effects of salinity levels on EL, there was an increase of about 7% and 10% in S2 and S3, respectively, in comparison to S1. However, at the 18 dS m-1 salinity level (S3), EL was significantly higher than at S1. This implies that the high levels of salinity exerted more EL effects (Bilal *et al.*, 2015). The results showed that the EL gradually increased with increasing salinity. This has been attributed to leaf cell membranes being damaged by salt stress (Kaya *et al.*, 2001; Kashani *et al*., 2018).

The effects of zinc application treatments on EL of the flag leaf were statistically significant ($P \leq 0.01$). Nano-ZnO (F_1) and simultaneous applications of (F_3: nano-ZnO + Zn-EDTA) increased the EL. The highest EL was in treatments with nano particle contents (F_1 and F_3). Under environmental stresses, plant membranes are subjected to changes often associated with the increases in permeability and loss of integrity (Bilal *et al.*, 2015).

Varietal differences in EL were significant ($P \leq 0.01$). There were increases in EL of about 10 and 15% in G_2 and G_1, respectively, in comparison with G_3. No significant differences between G_1 and G_2 were obtained, but G_2 had a lower EL than G_1. However, the results showed that EL in G_3 (salt tolerant) and G_2 (semi-salt tolerant) were lower in comparison with the G_1 (salt sensitive). In line with the experiment results, Roy *et al.* (2009) and Mahlooji *et al.* (2018) reported that EL increased under salt conditions, and the varietal differences between the genotypes may offer partial explanations for the differential tolerance to salinity stress.

EL was used to assess membrane permeability. In our study, EL decreased in genotypes under salt stress. The leaf EL is considered as a good physiological marker reflecting the amount of plant membrane damage caused by salt stress (Kaya *et al.*, 2001). EL was negatively correlated ($r = -0.21^{**}$) with the salinity tolerance of the genotypes (Table 3) in agreement with the results of Peng *et al.* (2008).

Yield and yield components, yield components: The effects of salinity levels and genotypes were highly significant ($P \leq 0.01$), but there were no significant effects of fertilizer applications on yield components. Number of spike (NS) about 12% and 25%, kernel number per spike (KNS) about 11% and 22% and thousand-kernel weight (TKW) about 8% and 29% were reduced in S_2 and S_3, respectively, in comparison with S_1. Genotypic differences in NS, KNS, and TKW were significant. Khatam (G_3=tolerant) had the highest KNS and lowest NS, but Morocco (G_1=sensitive) had the lowest KNS and the highest NS and TKW (Table 2).

Grain yield (GY): GY was significantly affected by irrigation water quality and genotype ($P \leq 0.01$). GY reduced with increasing salinity levels. GY was reduced in S_2 and S_3 by 24% and 66%, respectively, in comparison with S_1. GY was not affected by zinc application treatments. No significant differences in GY were found among the zinc applications (Table 2). Although zinc fertilizer applications on GY were not significant, F_2 and F_3 increased to up around 6% and 2%, respectively, in comparison with F_4. The highest GY was produced in Zn-EDTA application treatments (Table 2).

GY was significantly ($P \leq 0.01$) affected by genotype. GY of the tolerant genotype (G_3=Khatam) was the highest and reduced the most in Morocco (G_1). Despite of non-significant effect of genotypes (G_2 and G_3) on grain yield (GY), there was about 14% increase in GY in comparison with G_1. GY was reduced by both salinity treatments, whereas genotypic differences were markedly significant at high salinity level (S_3). At low (S_1) to moderate (S_2) salinity levels, osmotic stress affects growth and ionic stress (Na^+-specific effect) at high salinity level (S_3) negatively influences reproductive growth and grain yield (Munns and Tester, 2008). High concentrations of Na^+, which accumulate in the chloroplasts under salinity stress, are known to damage thylakoid membranes and inactivate electron transport and photophosphorylation of isolated thylakoid membranes causing a reduction in photosynthetic capacity (Ashraf and Harris, 2013). Moreover, reduction in yield could be due to decrease in water absorption by plant tissues along with reduction in cellular growth and development as well as the decrease

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in growth of the plants under salt stress as suggested by Pirasteh-Anosheh *et al.* (2016). In line with the experiment results, Ashrafi *et al.* (2014) found that salinity reduced plants' dry weights.

Zn-EDTA application and the Khatam salt tolerant genotype provided higher grain yield. The Khatam (salt-tolerant) and Nosrat (semi salt-tolerant) are comparatively higher in KNS, RWC, SPAD, proline and LI than the salt sensitive genotype Morocco. The decrease in GY of salt-tolerant genotypes (Khatam and Nosrat) was mainly attributed to a decline in NS, but in the salt-sensitive genotype (Morocco) it was due to a reduction in KNS. Decrease in KNS of Morocco could be due to the lack of availability of photoassimilates accumulation before anthesis that may have reduced the KNS per plant. On the other hand, in (Khatam and Nosrat), there may have been no limitation in photoassimilates accumulation before anthesis resulting in more KNS. Also, significant positive correlations exist along with a rather high coefficient rates between GY and KNS ($r = 0.46^{**}$), TKW ($r = 0.89^{**}$) and NS ($r = 0.36^{**}$). These findings show that these agrophysiological traits could be the key factors involved in salt tolerance. They could also be used to screen many genotypes in a short time and provide useful information about stress tolerance mechanisms.

Conclusion

In the present investigation it can be concluded that high salinity decreased agrophysiological parameters (light interception, chlorophyll fluorescence, chlorophyll content, relative water content, number of spike, kernel number per spike, thousand-kernel weight, grain yield), but increased flag leaf temperature, proline and leaf electrolyte leakage of the flag leaf. A significant and positive correlation was found between grain yield and 1000- kernel weight, whereas a significant and negative correlation was noted between grain yield and flag leaf temperature.

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