Morphological and physiological behaviour of sea barley (*Hordeum marinum ssp marinum*) genotypes originating from Soliman Sebkha under increasing salinity

Saoudi Warda[1*], Taamalli Wael[1,2], Abdelly Chedly[1] & Badri Mounawer[1]

[1] *Laboratory of Extremophile Plants, Center of Biotechnology of Borj-Cedria, B.P. 901, Hammam-Lif 2050, Tunisia,*
[2] *Laboratory of Olive Biotechnology, Center of Biotechnology of Borj-Cedria, B.P. 901, Hammam-Lif 2050, Tunisia*

Article info	Abstract

*Corresponding author
saoudiwarda1@gmail.com

Evaluation of germplasm in salt stress environment may help identifying salt-tolerant genotypes. In this study, we analyzed the genetic diversity of *Hordeum marinum*, genotypes originated from Soliman Sebkha in response to salinity (0, 250 and 400 mM) by measuring a set of parameters related to growth, water statue and photosynthetic activity. Results of ANOVA showed that variability of measured parameters was explained by the effects genotypes, treatment and their interaction, with treatment factor recorded the highest values. Salinity significantly reduced the photosynthetic rate and related parameters, including stomatal conductance (gs), intercellular CO_2 concentration (Ci), and transpiration (E). Several indices for salt tolerance indices comprising tolerance index (TOL), stress tolerance index (STI) and Salt Tolerance (ST) were calculated based on drought biomass (ADW) under non-stressed and under stress conditions. Results showed that lower values of TOL and higher values of ST and STI indicate the superiority of the genotypes having enough plasticity to respond to extreme conditions. Principal Component Analysis clustered genotypes into sensitive (21.43%), moderately tolerant (50%), and highly tolerant (28.57%) groups, depending on their relative potential to sustain good growth under high salinity. Results indicating sufficient genetic variability for salinity tolerance among the studied genotypes, hence tolerant genotypes can be good candidates in future breeding programs.

1. INTRODUCTION

Most cultivated crops are salt sensitive and therefore salinity is an ever-present threat to agriculture (Flowers and Flowers 2005). Thus, new sources of salinity tolerance are needed for crops grown on salt-affected land. This would be particularly effective in areas with subsoil salinity, which is extensive in many landscapes dominated by sodic soils. Crop wild relatives (CWR) are undomesticated species related to the cultivated crops that are considered to be a rich source of untapped genetic diversity that can be exploited to favor the climate adaptation of crops (Anada et al. 2020; Maxted et al. 2015). In fact, the "wild" nature of these species obliges them to survive without the support of farmers, (Coyne et al. 2020 ; Maxted et al. 2015). It is estimated that wild relatives (Nevo and Chen, 2010) contributed 30 –50% of the favorable alleles in modern breeding lines.

Moreover, crop wild relatives (CWRs) are a key resource in meeting this challenge as they are often found in a wide range of habitats, under variable environmental conditions. Improving the salt tolerance of crop and pasture species requires access to new genetic diversity (either natural or transgenic), and efficient techniques for identifying salt-tolerance (Munns and James, 2003). Although, both organic and inorganic solutes play crucial roles in osmoregulation of higher plants subjected to saline conditions, but their relative contribution varies among species, among genotypes and even between different compartments within the same plant (Siddiqui et al. 2009). Therefore, differential responses of plants toward salinity stress rely upon their

genetic make-up and the environment, thus to overcome the problem of salinity stress, it is important to screen varieties of crops for salinity tolerance and/or to enhance the salinity tolerance level of crops (Wu et al., 2015). Munns and James (2003) reported longer-term experiments have been necessary to detect genotypic differences in the effects of salinity on growth, and it is necessary to expose plants to salinity for at least two weeks, and sometimes several months.

Photosynthesis is crucial for the survival of all living biota, playing a key role in plant productivity by generating the carbon skeleton that is the primary component of all biomolecules. Salinity stress is a major threat to agricultural productivity and sustainability as it can cause irreversible damage to photosynthetic apparatus at any developmental stage (Zahraa et al. 2022). Hence, Ashraf and Harris (2013) state that the mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems, the electron transport system, and CO_2 reduction pathways. Any damage at any level caused by a stress factor may reduce the overall photosynthetic capacity of a green plant. Although the factors that limit photosynthesis in salt-stressed plants have been investigated in numerous species, the mechanism underlying inhibition is unclear (Steduto et al. 2000). Photosynthesis may decrease due to stomatal closure or through direct effects of salt in the photosynthetic apparatus. However, conflicting results on stomatal and non-stomatal limitation of photosynthesis have been reported. For instance, in bean (*Phaseolus vulgaris*) and cotton (*Gossypium hirsutum*), a reduction in assimilation was found to be mostly due to stomatal limitation, whereas other reports have attributed the reduction in photosynthesis to non-stomatal limitation (Steduto et al. 2000).

In comparison to other wild cereals, wild barley (from which belongs *H. marinum*) has a wide natural distribution area to which it is well adapted. *Hordeum marinum* is an annual, autogamous and facultative halophyte species that considered as an appropriate model to study mechanisms of salinity in cereals. In our previously research's (Saoudi et al. 2019a) reported the effect of salinity levels in populations of *Hordeum marinum* using morpho-physiological parameters for long term under a moderate salinity (200mM NaCl).It was found higher genetic diversity among and within population, with latter being the most important.

Multivariate analysis (Hierarchical classification) showed an overlap among lines form population in each group. For that reason, in the current research we tried to focus on genetic variation among lines originated from Soliman Sebkha at several salinity levels on growth, photosynthetic activity and tissue water content. Various stress indices are in widespread use for selecting genotypes based on their performance under a stressed environment (Singh et al. 2015; Morton et al. 2019) were calculated. We hypothesized that gradual effects of salinity level could be efficient for identifying contrasting genotypes that could be used for breeding programs.

2. MATERIAL AND METHODS

2.1. Plant material and growth conditions

For evaluation of salinity tolerance, 14 genotypes of *Hordeum marinum ssp marinum* originated from Soliman population, which were previously characterized by morphological, physiological and molecular (RAPD) markers (Saoudi et al. 2017, 2019a, 2019b), were used. Seeds of all genotypes were surface sterilized with 1% hypochlorite, rinsed thoroughly with distilled water and were germinated in pots full with moist sand. The experiment was conducted in a greenhouse at the Center of Biotechnology of Borj Cedria, with a completely randomized design. When seedlings grew the third leaf (about 15 days), they were irrigated gradually with Hewitt's nutrient solution (1966), under controlled conditions (16 h photoperiod, 350 μmol m^{-2} s^{-1} PAR, thermo-period 25/20_C day/night, 65±5% Relative Humidity). After 30 days of pretreatment period, plants (five replicates /genotype/ treatment) were subjected to a range of salt concentrations: 0, 250 and 400 mM NaCl gradually for 40 days. Gas exchange parameters was measured and immediately plants were harvested. Fresh shoot and roots were immediately weighted then oven dried at 70°C, until a constant weight was reached, for dry weight determination.

2.2. Morpho-physiological measured traits

Sixteen morpho-physiological traits related to growth, water content and photosynthetic activity were measured for the 14 genotypes of *H. marinum* at tillering stage. The morphological traits related to shoot height (SH), root length (RL), areal fresh weight (AFW), root fresh weight (RFW), areal dry weight (DWA), root dry weight (DWR) and the whole dry weight (HDW) were recorded. Shoot water content was estimated as the following equation: SWC (ml H_2Og^{-1} Dry

Weight) = (Fresh weight –Dry weigt)/ dry weight.

For photosynthesis measurements, Gas exchange measurements (net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (gs), and intracellular CO_2 concentration (Ci) were determined using a portable LiCor gas analyser (ADC BioScientific Ltd. Hoddesdon, United Kingdom). Measurements were carried between 09:30 am and 13:00 am in fully expanded leaves with five replicate/ genotype/ treatment. The photosynthetically active radiation during measurement under sunlight conditions was about 1200 µmol m^{-2} s^{-1}. During gas exchange measurements, the leaf temperature, the relative humidity, and the ambient CO_2 concentration were 25± 2 °C, 65± 5 %, and 380 µmol mol^{-1}, respectively. Water-use efficiency (WUE) as the ratio A/E, intrinsic water use efficiency (IWUE) as the ratio A/gs were estimated. Besides instantaneous carboxylation as A/Ci was calculated according to Frazão et al. (2020).

Different stress indices were calculated based on the phenotypic analysis for each genotype, using the following formulae:

Tolerance Index (TOL) = Yp- Ys (Rosielle and Hamblin, 1981)

Stress Tolerance Index (STI) = (Yp x Ys)/ (Xp)^2 (Fernandez, 1992)

Salt Tolerance (ST) = Y $_{salt}$ at T2/Y $_{control}$ at T2 Genc et al. (2007)

Here,

Yp = measured trait for each genotype under control condition.

Ys = measured trait for each genotype under stress condition.

Xp=Average of the observed trait for all genotypes under control condition

2.3. Statistical analyses

Analysis of the effects genotype (G), treatment (T) and genotype (G) x treatment (T) interaction on measured parameters was performed using using Proc GLM in SPSS version 20 (2011 ; IBM, SPSS, Chicago,IL, USA). The Means were compared using Turkey's multiple range test at 5%. Phenotypic correlations between measured parameters and salt stress indices under the different salt treatment were estimated by computing the Pearson Correlations (r) using the program XSTAT 2019. Measured traits of the 14 genotypes of Soliman Sebkha as well as the salt response indices were subjected to Principal component analysis (PCA) using R package R.0.3.5 (R core team. 2020).

3. RESULTS AND DISCUSSION

3.1. Analysis of variance of salinity-tolerant traits

In the current research, we tried to evaluate the genotypic variability of sea barley at increasing levels of salinity. The relative decrease in growth related traits are strong indicators of salt stress response in plants (Negrão et al. 2017). Therefore, we have selected some traits for the screening of sea barley genotypes under different salinity level. Fourteen genotypes of *Hordeum marinum* originated from Soliman sebkha, previously characterized, in our previous work (Saoudi et al. 2019a), by morpho-physiological traits under moderate salinity level (200mM NaCl), were screened under 0. 250 and 400mM NaCl. Results from ANOVA showed that variation of measured parameters was explained by the effects genotype (G), treatment (T) and genotype (G) x treatment (T) interaction (Table 1). These parameters were mostly influenced by the treatment factor. It was found that all the measured characters were significantly explained by genotype x treatment interaction. These results indicate that genotypes had higher value of these analyzed parameters in normal conditions are not necessarily having similarly under salt stress and vice versa.

In this investigation, we found significant decrease of the main measured traits related to growth by increasing salinity. Otherwise as showed in Fig. 1, except the parameter Root length (RL), the parameters shoot height (SH), areal drought weight (ADW) and root drought showed significant decrease and being more affected at 400Mm. this results was similar to that previously reported by Hafsi et al. (2007). However, Yousfi et al. (2010) showed less reduction for growth parameters even under 300mM, this discrepancy could explained by short-term growth experiment (only 60h under salt stress). Munns and James (2003) proclaimed that short-term growth experiment revealed little difference between genotypes that differ in long-term biomass production or yield. Root elongation is selection criteria in response to salt stress (Munns and James, 2003), in the current research, even though the root drought weight (RDW) showed a significant reduction under salinity stress (about 50% under 400mM NaCl), the root length remained unaffected. Presumably, the tolerance of sea barley under high salinity was

Table 1. ANOVA results of measured traits for salinity stress tolerance in the genotypes of *H. marinum* after treatment with 250 and 400 mM NaCl

	Genotype (G)		Treatment (T)		GXT	
	F	*P*	*F*	*P*	*F*	*P*
SH	13.456	0.000	250.518	0.000	8.106	0.000
NT	7.301	0.000	140.569	0.000	3.886	0.000
RL	6.56	0.000	1.772	0.173	2.188	0.002
FWR	6.373	0.000	48.817	0.000	3.947	0.000
FWA	6.698	0.000	60.539	0.000	4.895	0.000
DWR	3.483	0.000	50.826	0.000	2.819	0.000
DWA	29.761	0.000	142.439	0.000	8.874	0.000
HDW	26.867	0.000	156.485	0.000	8.649	0.000
SWC	9.306	0.000	3.008	0.052	2.208	0.001
Ci	1.66	0.074	7.725	0.001	2.595	0.000
E	9.283	0.000	113.764	0.000	16.676	0.000
Gs	18.855	0.000	143.048	0.000	25.511	0.000
A	14.184	0.000	14.647	0.000	28.631	0.000
WUE(A/E)	13.231	0.000	91.653	0.000	27.046	0.000
IWUE(A/gs)	12.058	0.000	54.073	0.000	27.71	0.000
ICE (A/Ci)	*4.587*	*0.000*	*3.635*	*0.029*	*8.16*	*0.000*

F: Coefficient of Snedecor-Fisher, not significant ($P>0.05$), significant ($P\leq0.05$).
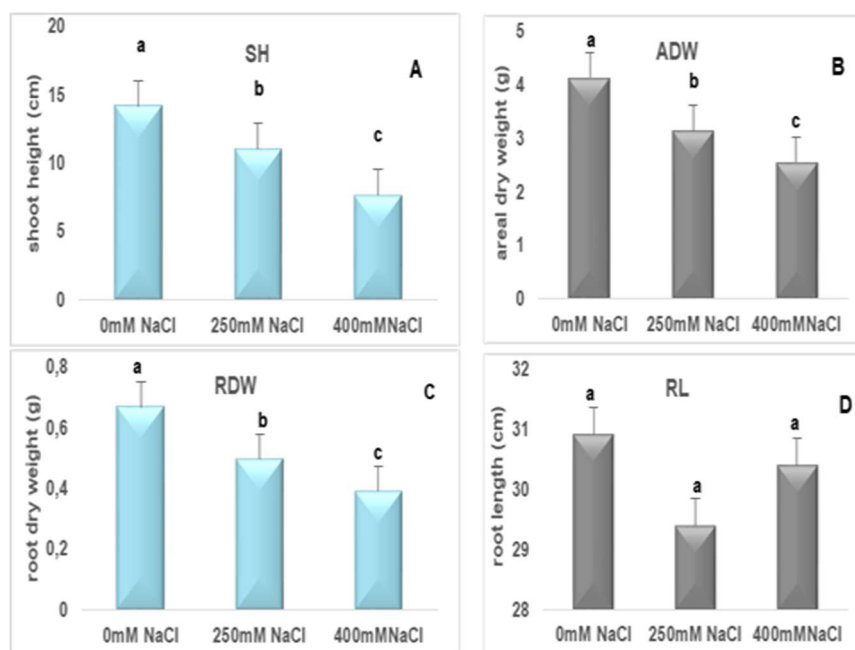

Fig. 1. Effects of salinity (0, 250 and 400mM NaCl) on (A) Shoot height (SH), (B)Areal drought weight (ADW), (C) root drought weight (RDW) and (D) root length (RL) in lines of *H. marinum*Soliman Sebkha . Means of 14 lines with five replicates per line. Means followed by the same or common letters are not significantly different among treatment according to Tukey' test at 5% .

related to maintain high length of root for soil nutrient uptake. These results were in corcondance to that reported in seaside barley by Saoudi et al. (2019a) and Isayenkov et al. (2020), who annaounced that while more roots were maintained in their primordial state under salt stress, the total surface area and the lenght of the stressed roots was higher. They explained these results due to the production of a larger

number of thinner roots that improved root-rhizosphere interaction. Consequently, Isayenkov et al. (2020), suggested that roots appaer to paly the central role in salinity adaptation for *H. marinum*, ensured by the development of thinner and likely lignified roots, as well as fine-tuning of membrane transport for effective management of restriction of ion entry and sequestration, accumulation of osmolytes,

and minimization of energy costs. As observed in the parameter RL, *H. marinum* maintained higher shoot water content (SWC) by increasing salinity (Table 2), which remained marginal even at the highest salinity level (400mMNaCl). It is noteworthy that even under severe salinity, sea barley plants maintained shoot hydratation. Similar findings were reported by Hafsi et al (2007), who found that tissue water content remained high in shoots even at 300Mm NaCl. Besides Isayenkov et al. (2020) showed that sea barley exhibited a greater aability to retain water under conditions of high salinity and reported that *H. marinum* exhibited stronger resistance salinity stress as reflected in enhenced water retention, preserved shoot growth and , possibly, sustained biosynthesis activity. In previous study, Saoudi et al. (2019a) revealed that osmotic adjustement in sea barley

plants remained effective even under long-term salt stress (more than three months).

The effect of salinity on assimilation (A), stomatal conductance (gs), intercellular CO2 concentration (Ci) and transpiration (E) of *H. maritimum* leaves are shown in Fig. 2. By comparison to their respective control, stomatal conductance (gs), showed a significant declined with increasing salt concentration, being more pronounced at high salinity level (400Mm NaCl). The parameters intercellular CO2 concentration (Ci) and transpiration (E) were declined significantly in plants subjected only to high salinity level. In spite of the previous measured photosynthetic parameters, assimilation (A) was unaffected by salinity. For the ratio (Table 2), Water use efficiency (WUE) estimated by A / E, and Intrinsic water use efficiency (IWUE) as the ratio between A/gs were not affected by

Table 2. Variation of water use efficiency (WUE, $\mu mol\ CO_2 mmol\ H_2O$), intrinsic water use efficiency (IWUE, $\mu mol\ CO_2\ mol^{-1}\ H_2O$), instantaneous carboxylation efficiency (ICE, $mol\ air\ m^{-2}s^{-1}$) and Shoot water content (TWC, $H_2O\ (ml.g^{-1}\ DW)$) in genotypes of *Hordeum marinum* from Soliman Sebkha. Means of 14 lines with five replicates per line. Means followed by the same or common letters are not significantly different among treatment according to Tukey' test at 5% .

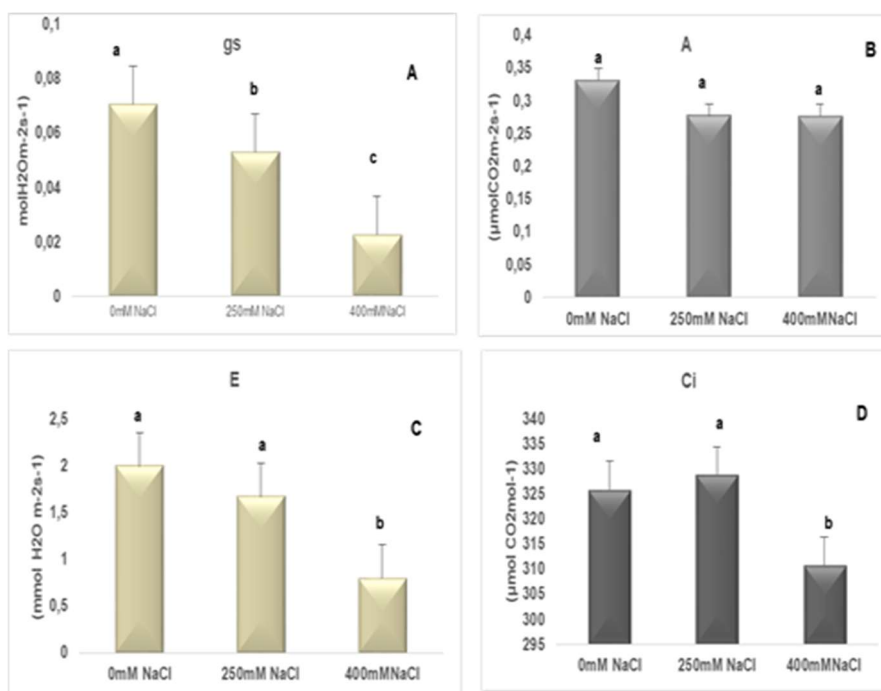| Salt treatment	0 mM NaCl	250 mM NaCl	400 mM NaCl
WUE(A/E) ($\mu mol\ CO_2 mmol\ H_2O$)	0,266a	0,226a	0,53b
IWUE(A/gs) ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)	10,257a	7,764a	16,843b
ICE (A/Ci) ($mol\ air\ m^{-2}s^{-1}$)	0,001a	0,0009a	0,001a
SWC H_2O ($ml.g^{-1}\ DW$)	2.545a	2.996a	2.762a

Fig. 2. Effects of salinity (0, 250 and 400mM NaCl) on (A) stomatal conductance (gs), (B) assimilation rate (A), (C) transpiration rate (E), (D) intercellular CO2 concnetration (Ci) in lines of *H. marinum* Soliman Sebkha. Means of 14 lines with five replicates per line. Means followed by the same or common letters are not significantly different among treatment according to Tukey' test at 5% .

moderate salinity level, while significantly increased at 400Mm treated-plants compared to control. Nonetheless, instantaneous carboxylation efficiency (ICE = A/Ci) was not affected by all applied treatment.

Salinity stress drastically reduced the photosynthetic rate and other photosynthetic responses including transpiration and stomatal conductance. The decline in productivity observed in many plant species subjected to excess salinity is often associated with a reduction in photosynthetic capacity. In the current research, stomatal limitation of photosynthesis, which arises from decreased stomatal conductance (gs) in response to salt stress, was more dramatic at high salinity level. Munns and James (2003) reported that stomatol conductance could provide a positive screen for salinity tolerance, and supposed that the reduced stomatal conductance was due to osmotic stress, generated via root signals, as it occurred before NaCl started to build up to high levels in the leaf. Similar results were previously found in *H. marinum*, under either low level (Talbi et al. 2012) or high level of salinity Yousfi et al. (2010). The reduction in transpiration during mid-day hours indicates stomatal closure to some degree. Therefore, it is assumed that salinity reduced the transpiration rates mainly by stomatal closure in this study.

Water use efficiency (WUE), calculated by dividing A by E values and represents the ratio of water used in photosynthesis to water lost by the plant through transpiration, Debez et al. (2008) reported that increase in WUE is considered an important adaptive trait for plant survival under high salinity. In the current study, an increase in WUE appears to be due to a higher photosynthetic rate as compared to transpiration rate. Similar findings has previously reported by Yousfi et al. (2010) when studying short-term effects of salinity on *H. marinum* at different levels of salinity. Likewise, similar behavior was detected for the ratio IWUE (A/gs), which increased significantly in plants subjected to the high level of salinity, which may be owing to the larger decrease in stomatol conductance compared to CO2 assimilation rate (A). Stomatal conductance is one of the main factors affecting photosynthesis. It appears that plants use efficient mechanisms to limit the loss of water before the inhibition photosynthesis. Consequently, water status (SWC) remained unchanged under high salinity associated to higher WUE and IWUE, this results suggest that

osmotic adjustment is very efficient in *H. marinum* even under 400 m MNaCl.

3.2. Salinity-tolerant response of sea barley genotypes

The genotypic variability for salinity tolerance was assessed in the current study, based on different calculated salt tolerance indices such as Tolerance Index (TOL), the Stress Tolerance Index (STI) and Salt Tolerance (ST) which was proposed for yield-related traits and can be adapted for many growth-related traits (Fernandez, 1992). It accounts for both the overall performance of the plant population (e.g. all accessions) under control conditions as well as the ability to maintain yield (or other growth parameters) under stress conditions. A higher Stress Tolerance Index for a genotype indicates a higher stress tolerance potential and a higher yield/growth potential **(**Negaro et al. 2017**).** For analysis of genotype x salinity level treatment combinations, phenotypic characters such as areal drought weight were recorded at 40 days after salt treatment. The relative decrease in shoot growth, fresh weight or total biomass are strong indicators of salt stress response (Negrao et al. 2017), thus we have selected drought biomass for the selection of sea barley genotypes. A large variation in salt tolerance levels were detected among various sea barley genotypes (Fig. 3), which exhibited a significant reduction of stress indices from 15 to 62% in areal drought weight after treatment with 400 mM NaCl. Studied genotypes exhibited differences for various stress tolerance indices (TOL, St and ST). The highest ST values were obtained for SL3, SL1, SL6 and SL10, revealing that these genotypes showed a lesser reduction on growth and higher tolerance for the imposed salt stress, whereas the lowest ST values were registered for SL14, SL11 and SL8 (reduction of biomass exceeded 50%). The remaining genotypes showed moderate reduction varied from 29 to 45%. Similar results were obtained for STI. The STI and ST are commonly used indices that have been reported previously to select the tolerant genotypes (Singh et al. 2015; Jha et al. 2022). On the other hand, SL1, SL3 and SL6 showed the lowest TOL values and considered as the salt tolerant genotypes, in contrast to salt-sensitive genotypes SL14, SL13, SL11 and SL8 having highest TOL values. The TOL index measures the differences in biomass production or yield under stressed and control conditions (Rosielle and Hamblin 1981). In the current research, the lower values of TOL and
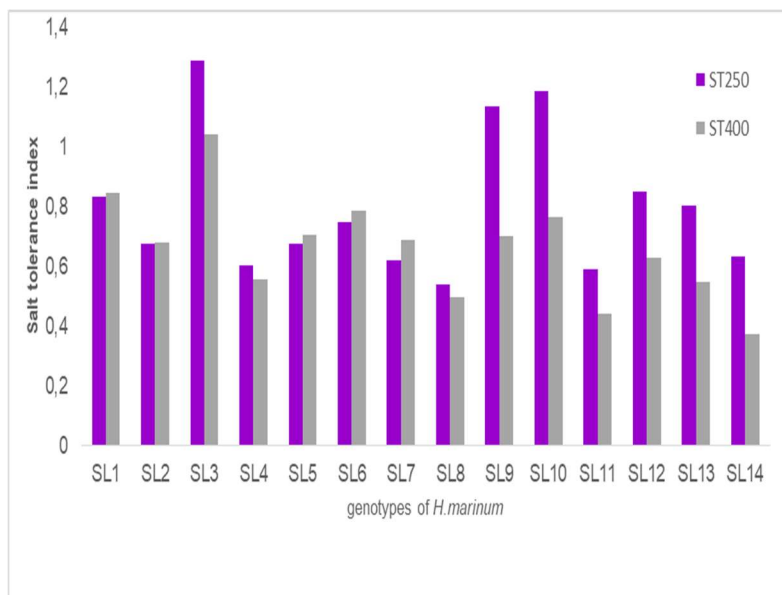
Fig. 3. Effects of salinity (0, 250 and 400mM NaCl) on salt tolerance index (STI) in lines of *Hordeum marinum* Soliman Sebkha. Means of 14 lines with five replicates per line. Means followed by the same or common letters are not significantly different among treatment according to Tukey' test at 5% .

higher values of STI and ST indicate the superiority of the genotypes having enough plasticity to respond to extreme conditions. These observations are in accordance with the earlier studies in wheat, pearl millet, sea barley and many other crops (Singh et al. 2015; Saoudi et al. 2019a; Jha et al. 2022).

3.3. Correlation analysis

To demonstrate the association among various stress indices and studied parameters, Pearson correlations (r) were calculated under different studied salt treatments, here we reported the correlations registered under high salinity level, as the main reduction was registered under 400 mM NaCl (Table 3). Otherwise, Negrão et al. (2017) reported that the correlation among various stress indices and the observed traits is a valuable parameter for the identification of superior genotypes. The current study demonstrated significant correlation between traits related to biomass where the highest one were registered between DWA and HDW (r= 0.985, p<0.0001), between FWR and DWR (r= 0.826, p<0.0001). Furthermore, higher significant and positive correlations were obtained among the traits related photosynthetic parameters including E and gs (r= 0.913, p<0.0001), between WUE and IWUE (r= 0.851, p<0.0001), ICE (r= 0.545, p<0.0001) and A (r= 0.589, p<0.0001), and between A and IWUE (r= 0.684, p<0.0001) and ICE (r= 0.692, p<0.0001). Otherwise, moderate and significant correlations were registered between E and SWC (r= 0.412, p<0.0001), ST (r= 0.415, p<0.0001) and STI (r=

0.415, p<0.0001), which suggesting that tolerant-genotypes with higher STI and ST maintained higher water statue suitable for photosynthetic activity.

In this study, photosynthesis-related parameters, such as stomatal conductance (gs), transpiration (E), and intracellular CO_2 concentration (Ci) were affected by salinity stress. Kwon et al (2019) reported that transpiration (E) is usually strongly correlated with stomatal conductance (gs). As the case in our study, Similar behavior was observed and higher correlation was registered between transpiration (E) and stomatal conductance (gs), (r= 0.913, p<0.0001). This result supported previously ascertainment and it is assumed that salinity reduced the transpiration rates mainly by stomatal closure in this study

Conversely, a significant negative association was found between TOL and STI (r= -0.906, p<0.0001) and between TOL et ST (r= -0.906, p<0.0001), these results indicate that selection based on low values of TOL are associated with salt tolerance under a stressed environment. The STI has shown a higher significant positive correlation with ST (r= 1, p<0.0001), while negatively correlated with TOL. These results indicated that these stress indices can distinct between sensitive and tolerant genotypes, and could be used as selection criteria for tolerant genotypes of seaside barley under slat stress. Similar findings were reported by Singh et al (2015) in wheat and Jha et al (2022) in pearl millet.

Table 3. Correlation matrix (Pearson) between various stress indices and measured parameters for *Hordeum marinum* genotypes under 400mM NaCl. Values in bold are different from 0 with a significance level alpha=5E-4

Variables	FWR	DWR	RL	SH	DWA	HDW	FWA	NT	TWC	E	gs	TOL	ST	STI	WUE	IWUE	Ci	ICE	A
FWR	**1**																		
DWR	**0.826**	**1**																	
RL	**0.459**	**0.515**	**1**																
SH	-0.180	-0.296	-0.334	**1**															
DWA	0.221	0.298	0.025	-0.293	**1**														
HDW	0.353	**0.457**	0.115	-0.326	**0.985**	**1**													
FWA	**0.533**	**0.443**	0.115	0.022	**0.423**	**0.474**	**1**												
NT	0.268	0.213	0.009	0.001	-0.077	-0.034	**0.565**	**1**											
SWC	0.284	0.121	0.081	0.280	**-0.499**	**-0.444**	**0.524**	**0.544**	**1**										
E	-0.111	-0.072	-0.098	0.320	-0.205	-0.204	0.224	**0.422**	**0.412**	**1**									
gs	-0.117	-0.099	-0.225	0.304	-0.208	-0.212	0.152	**0.435**	0.349	**0.913**	**1**								
TOL	0.050	0.070	-0.032	-0.087	0.059	0.068	-0.004	-0.402	-0.111	-0.343	-0.330	**1**							
ST	-0.013	0.062	0.071	0.000	0.187	0.186	0.125	0.400	-0.030	**0.415**	0.374	**-0.906**	**1**						
STI	-0.013	0.062	0.071	0.000	0.187	0.186	0.125	0.400	-0.030	**0.415**	0.374	**-0.906**	**1.000**	**1**					
WUE	-0.109	-0.067	-0.069	0.077	-0.105	-0.110	-0.317	-0.341	-0.253	**-0.586**	**-0.512**	0.326	-0.380	-0.380	**1**				
IWUE	-0.133	-0.083	-0.029	-0.020	-0.044	-0.056	-0.180	-0.264	-0.182	**-0.477**	**-0.515**	0.390	**-0.424**	**-0.424**	**0.851**	**1**			
Ci	0.117	0.127	0.050	0.065	0.117	0.132	0.176	0.227	0.100	0.335	0.308	-0.102	0.166	0.166	**-0.493**	-0.453	**1**		
ICE	-0.118	-0.111	-0.139	0.028	-0.057	-0.072	-0.128	-0.168	-0.114	-0.153	-0.100	0.117	-0.158	-0.158	**0.545**	**0.538**	-0.754	**1**	
A	-0.026	-0.012	-0.105	0.116	-0.092	-0.088	-0.046	-0.004	0.006	-0.015	0.004	0.196	-0.243	-0.243	**0.589**	**0.684**	-0.236	**0.692**	**1**

The use of multivariate statistical algorithms is an important strategy for classification of germplasm and analysis of genetic relationships among breeding material (Sigh et al. 2015). In order to maintain, evaluate and utilize germplasm effectively, it is important to investigate the extent of genetic diversity available. Principal Component Analysis (PCA) based on measured parameters and various stress indices was performed to detect the most salt-tolerant genotypes among all sea barley genotypes under study (Fig. 4). The first three factors of the PCA account for about 58% of the

total salt response variability in the studied genotypes. The biplot diagram showed that the first principal component (PC1) accounted for maximum variability in the dataset (29.9%), and had a strong positive correlation with the salt stress indices (ST and STI), with parameters related to biomass (HDW, DWA, NT) and with photosynthetic parameters (E, gs and to a lesser degree Ci). Results indicated that these indices could identify the tolerant genotypes that executes well under salinity-stressed conditions. It was previously mentioned that E, gs and Ci showed significant decrease under high salinity
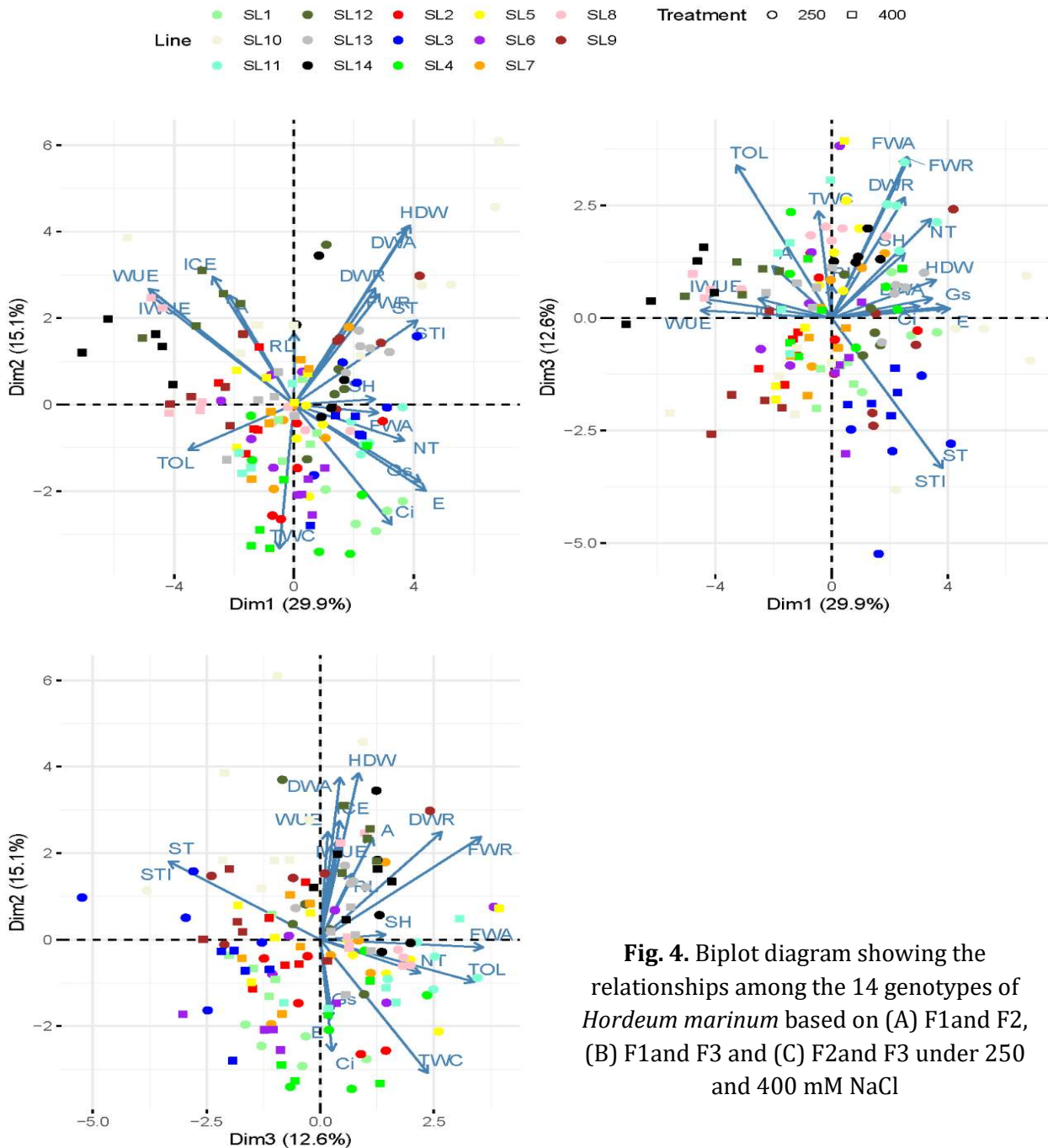

Fig. 4. Biplot diagram showing the relationships among the 14 genotypes of *Hordeum marinum* based on (A) F1 and F2, (B) F1 and F3 and (C) F2 and F3 under 250 and 400 mM NaCl

level, presumably this decrease mainly due to the closure of stomata and fewer leaves and /or leaf area as part of a strategy adopted by plants to reduce their transpiration surface.

Contrarily, first PCA was negatively correlated with TOL, WUE and IWUE. knowing that genotypes showing higher TOL index were the most sensitive, this result indicate that even the most sensitive sea barley genotypes tend to increase their water use efficiency (WUE) and), intrinsic water use efficiency (IWUE) when they are subjected salinity stress. The second component (PCA2) accounted for 15.1% of the total genetic variation between studied genotypes while the most contributing parameters are the photosynthetic ratios WUE, IWUE, and ICE. The third component (PCA3) explained 12.6% of the total variation among the14 genotypes while the parameters FWA, FRW and the TOL salt index being the most contributing characters. It can be concluded that the parameters, which contributed more to the first PCA, were the best indicator of salinity tolerance in sea barley genotypes in the current research

4. CONCLUSION

According to results, substantial genetic diversity among the 14 genotypes of sea barley for salinity stress tolerance was observed. Our observations was reported by extensive statistical analysis of morpho-physiological parameters such as correlation and PCA and the use of multiple stress indices to determine the salt tolerance potential of *H. marinum* genotypes. The tolerance of genotypes to salinity stress seams to depend in particular high biomass (HDW and ADW) and high salt stress indices such as ST and STI. Among 14 *H. marinum* genotypes, four highly tolerant genotypes (SL1, SL3 and SL6 and SL10), seven moderately tolerant and three (SL8, SL11 and SL14) salt sensitive genotypes. These contrasting genotypes of sea barley could be used for mining novel candidate genes imparting salt tolerance, aiming toward crop improvement through genomics and molecular breeding approaches. Despite our findings, further studies are required to better understand the additional mechanisms that contribute to the reduced photosynthetic rate under salinity stress in sea barley.

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