



Proceeding Paper

# Assessment of Morphological and Physiological Traits of Moroccan Barley (*Hordeum vulgare* L.) Varieties Submitted to Severe Salt Stress <sup>†</sup>

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**Abstract:** The maintenance of Moroccan barley (*Hordeum vulgare* L.) cultivation under rising saline conditions requires investigations to be performed. In the present work, we aimed to test the effect of salt stress on four Moroccan barley varieties (ADRAR, AMIRA, LAANACEUR, and MASSINE). Salt stress was applied by increasing NaCl concentration gradually in nutrient solution to 300 mM (Severe stress). Our results showed that salt stress induces significant decreases in RDW, SDW, and Chl content. In addition, significant increases of EL% and proline content were recorded. Analysis of variance showed a significant intraspecific variability between varieties and a significant effect of treatment and combination of the varieties factor and treatment factor. Principal component analysis (PCA) showed that under NaCl treatment, MASSINE is the genotype that kept significantly high values of SDW and RDW.

**Keywords:** *Hordeum vulgare*; salinity; Moroccan barley



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## 1. Introduction

In the Mediterranean area, salinity is a serious problem reducing plants growth and crop productivity [1] markedly affecting agricultural land. For instance, more than 500,000 ha of the saline area are considered as damaged soils [2]. Salt stress affects morphological, physiological, and biochemical parameters as well as crop production in plants including cereals [3]. In Morocco, barley (*Hordeum vulgare* L.) is the second cereal used by Moroccan population in their alimentation after wheat [4]. The degree of salt tolerance is not the same at all barley varieties [5], which makes the selection of Moroccan tolerant varieties very important to ensure nutrition security for the next decades. Here, we aimed to screen the effect of severe salt stress on some morphological and physiological traits at four barley Moroccan varieties.

## 2. Material and Methods

Experiments were conducted in a greenhouse at the experimental station. Six days after sowing, seedlings of four barley varieties were subsequently transferred to a hydroponic culture system (10 L per pot) containing a complete and continuously aerated Hoagland nutrient solution. Two treatments were applied, control without NaCl and stressed with 300 mM of NaCl in the nutrient solution. Plants were grown at 21 °C under natural light supplemented with artificial light with PAR of 300  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Fresh leaves and root samples used for analysis were harvested 10 days after the application of the treatment.

For morphological parameters, the dry weights of shoots and roots were determined after being dried at 70 °C for 72 h. The electrolyte leakage (EL) was assayed by the technique of [6]. Total chlorophyll (Chl) was determined according to the method of Burnison, B. K. (1980) [7]. The proline accumulation was assessed as described in Bates et al. (1973) [8]. For statistical analyses, we submitted data to a variance analysis (ANOVA) to test the effects of salt stress, genotype, and their interactions on the morphological, physiological and biochemical traits of the Barley. Mean comparisons were made using test (Fisher’s comparison) at  $p \leq 0.05$ . Different statistical approaches were performed using XLSTAT software (XLSTAT Version 2016.02.28451).

### 3. Results and Discussion

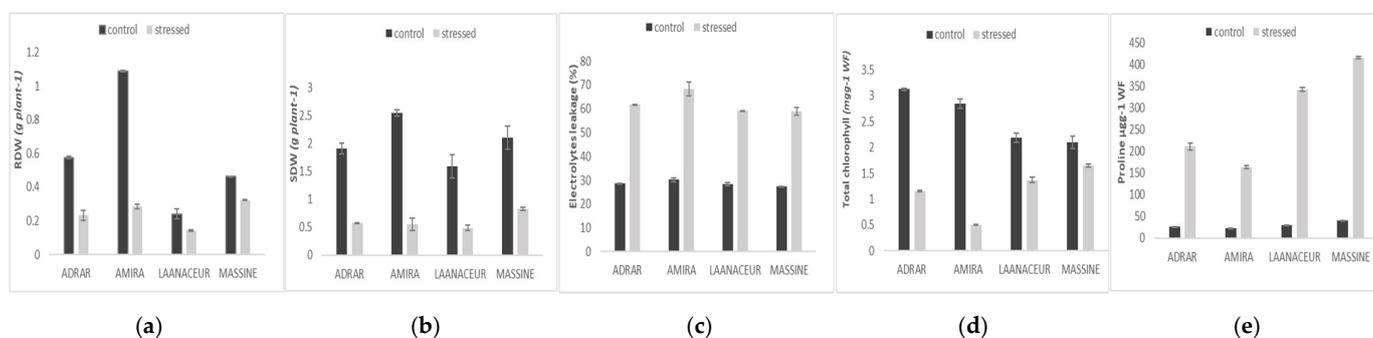
#### 3.1. Effect of Salt Stress on Barley Genotypes

Combined analysis of variance (Table 1), was used for biochemical and physiological traits of the four barley varieties measured after two-week under severe salt stress conditions. ANOVA also showed that salt stress significantly affected the biochemical traits as well as physiological traits. Moreover, highly significant differences were noticed among the genotypes for all the parameters. The ANOVA also disclosed a significant interaction between varieties and treatment for the biochemical and physiological traits. The comparisons of means (Figure 1) showed a significant reduction in root and shoot dry weight as well as Chl content in response to salt stress, AMIRA was found to have the most important reduction of RDW and SDW (79% and 82.2%, respectively) and total chlorophyll content (70%) when submitted to salinity. MASSINE displayed the lowest reduction percentage of RDW and SDW (59% and 71%, respectively), this variety showed also the highest score of proline accumulation (96%). The electrolyte leakage and proline content were significantly higher under salt stress compared to the control. The highest increase of the electrolyte leakage percentage was noted at AMIRA (55%).

**Table 1.** Mean squares of parameters studied in leaves and roots samples of four barley Moroccan varieties.

Source	Df	RDW	SDW	EL	Chl T	Proline
Variety	3	0.248947 ***	0.3165 ***	45.72 ***	0.2398 ***	22.872 ***
Treatment	2	0.7168 ***	12.2108 ***	6734.57 ***	11.7309 ***	386.290 ***
replicate	2	0.000165	0.0205	3.03	0.0082	0
Variety*trt	6	0.157225 ***	0.2287 ***	15.38 ***	1.2233 ***	17.974 ***
Residual	22	0.000272	0.0136	1.27	0.0039	16
Total	35					

Df: Degree of freedom. \*\*\*: significant at 0.001.



**Figure 1.** Effect of 300 mM NaCl treatment on RDW (a) SDW (b) EL% (c) chlorophyll content (d) and proline content (e) in four barley Moroccan varieties. Values indicate the means of three replicates. Treatment was applied during ten days.

It is widely spread in literature that salt stress induces a great reduction of plant growth and crop yield [5,9,10]. The decrease of RDW and SDW under osmotic stresses indicates that plants maintain resources by reducing their vegetative growth [11], which is well documented in many papers [12,13]. AMIRA in our results was the variety that showed the important decrease of SDW and RDW under salt stress, which indicates that this variety seems to be sensitive against this stress.

Photosynthetic pigments content is considered as good criterion for evaluating the tolerance of plants against abiotic stresses [14]. The reduction of total Chl content is considered as an indicator of abiotic stresses sensitivity [3], that was also recorded in many other studies [13], and could be due to occurrence of increase Chl degradation and pigment photo-oxidation [15]. The most important decrease of Chl content in our case was observed in AMIRA, which makes this variety, based on this parameter changes, more sensitive against salt stress. The increase of EL% under stress is always linked with sensitivity of plant into abiotic stress [16]. As in our case, many studies describe the increase of EL% under salt stress, which indicates cell membrane deterioration.

Under stressful conditions, as in our case, many other studies show a significant increase in leaves proline content [5]. This amino acid is considered as an osmoregulator for membrane stability, also it is known by its ability of buffering cellular redox potential, and for scavenging free radicals [17]. In our results, MASSINE and LAANACEUR are the varieties that accumulated more proline under salt stress, which make them more tolerant based on this criteria.

### 3.2. Correlations Matrix among Parameters Studied

Table 2 shows the correlations matrix among parameters studied, positive and negative correlations were recorded in our results. In control, a significant positive correlation of RDW with SDW, EL, and Chl content, and a significant negative correlation with proline content. SDW shows a significant positive correlation with EL. For EL, significant positive and negative correlations were recorded with Chl and proline contents, respectively. Furthermore, Chl content shows a significant negative correlation with proline. Under salt stress, RDW was in significant positive correlation with SDW. EL% correlates significantly and negatively with Chl and proline contents. A significant positive correlation between Chl and proline contents was recorded.

**Table 2.** Correlation matrix (Pearson) Somme parameters studied under control conditions and under salt stress.

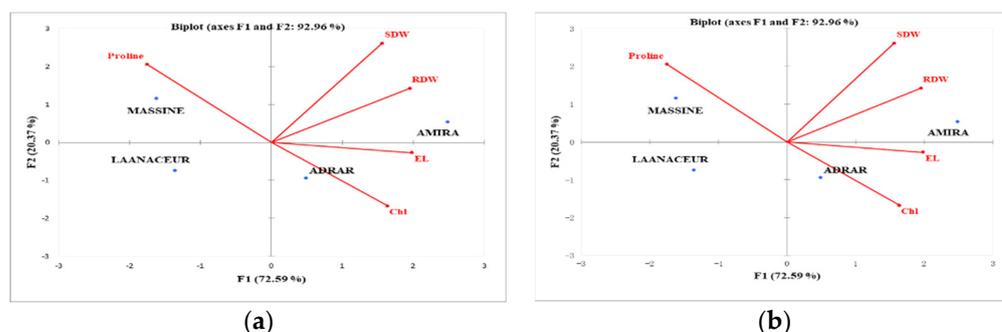
Variables	RDW	SDW	EL	Chl T	Proline
<b>Control</b>					
RDW		0.881 ***	0.779 ***	0.588 *	−0.578 *
SDW			0.591 *	0.356	−0.248
EL				0.615 *	−0.821 ***
Chl					−0.764 ***
<b>Salt Stress</b>					
RDW		0.703 *	0.298	−0.091	0.013
SDW			−0.366	0.498	0.540
EL				−0.916 ***	−0.799 ***
Chl					0.915 ***

\*: Significant at 0.05 probability level; \*\*\*: significant at 0.001 probability level.

### 3.3. Principal Component Analysis (PCA)

Principal component analysis (PCA) was used as a preferment multivariate statistical tool to discriminate between treatments and varieties based on two top first factors (F1 and F2). In control, F1 and F2 accounted, respectively, 72.59% and 20.37%, our biplot (Figure 2a) discriminates AMIRA in the positive side of F1 with high values of SDW, RDW, and EL%. The negative side of F1 shows MASSINE with high value of proline content. On the other

hand, F1 and F2 accounted, respectively, 63.38% and 34.23% under salt stress, the biplot created in this case (Figure 2b) discriminates MASSINE with high levels of proline and Chl T contents, and SDW, and RDW in the positive side of F1 and F2. The negative side of F1 shows AMIRA with high score of EL%. Under salt stress, PCA shows MASSINE variety with high values of tolerance indexes (SDW, RDW, and Chl and Proline contents). However, AMIRA is linked with sensitivity marker (EL%).



**Figure 2.** The loading biplot of principal component analysis for physiological responses in four barley Moroccan varieties under control conditions (a) and under severe salt stress (b).

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