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# Responses of Physiological Related Traits in Mature Grains from Two Barley Cultivars (Acsad 1230 and Arig 8) Evaluated under Saline Stress

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**Abstract:** Two six row barley cultivars (*Hordeum vulgare* L.): Acsad 1230 and Arig 8, were grown under three salt treatments: Control, 70 and 140 mM of NaCl. Physiological related traits were assessed using mature kernels including 1000 kernal weight (TKW), soluble and total proteins, soluble and bounded phenols and $Na^+$ and $Cl^-$ content per kernel. Responses to salt tolerance were different for both cultivars. Results suggested that Acsad 1230 was more tolerant to salt treatment than Arig 8. Greater TKW and increased accumulation of soluble and total proteins as well as soluble and bound phenols were noted in kernels of Acsad 1230 along with high ions concentration of $Na^+$ and $Cl^-$. The amount of total protein and phenol components were found stable in Acsad 1230 whereas these components decreased significantly in Arig 8. Soluble proteins increased significantly in Acsad 1230 and were reduced in Arig 8. The higher level of soluble proteins in Acsad 1230 was associated with increased ions concentration of $Na^+$ and $Cl^-$. This investigation suggested that total and soluble proteins as well as phenol components along with $Na^+$ and $Cl^-$ concentrations may have a different control on the expression of TKW and indicates a different salt tolerance path expressed in both barley cultivars.

**Key words:** Barley, salinity stress, physiological parameters, mature kernels

## INTRODUCTION

Salt stress is considered a major limiting factor of cereal production worldwide because of increased sodic soils and greater use of irrigation with salty water. It has been reported that 25% of irrigated soils are largely affected by salt problems (Levigneron *et al*., 1995). More than 15 millions hectares in the Maghreb and in the Near East are affected by the salinity. These soils representing 15% of the total cultivated land are sequeletic and eroded (Le Houérou, 1986). Nowadays, several investigations were focused on plant species adapted to sodic soils or tolerant to salt stress in irrigated cropping systems. In the Mediterranean regions, several crop species are confronted to increased temperatures and water deficit during dry season and particularly in drought prone growing conditions. Under these growing conditions, salt could be accumulated on the surface because of the excessive evapo-transpiration and the insufficient ionic infiltration which is attributed to limited rainfall (Ben Naceur *et al*., 2001). The increased soil salinity was found to represent an irreversible growth for several plant species (Lachaal, 1998). These studies concluded that important salt responses diversity and various tolerance mechanisms to salt tolerance exist among the plant species and cultivated crops.

Currently salt tolerance of plant species is considered as a complex trait that involves responses to osmotic and ionic constraints and their secondary consequences as oxidative and nutritional stresses. The complexity and the polygenetic control of salt sensibility or salt tolerance were among the limiting factors that reduced the efficiency to develop salt tolerant cultivars. Breeding efforts were confronted to a lack of limited knowledge of salt tolerance mechanisms, absence of comprehensive screening tests that could be efficiently implemented in both the field and laboratory and finally because of the limited identified molecular and physiological markers Molecular markers (Zhu *et al*., 1997) have contributed to identify genes that are involved in the salt tolerance. However, these results have had a limited success because it described individual gene action

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instead of integrated salt tolerance mechanisms. Several other studies attributed protein changes to a metabolic adjustment as response to salt stress (Bohnert *et al*., 1995). However, these proteins could only depict changes on the expression of genes that have to be identified (Moons *et al*., 1997). Other investigations reported that several loci implicated within salt tolerance were determined in Arabidopsis thaliana (Zhu, 2000). These results were not fully exploited in breeding for salt tolerance.

Breeding for salt tolerance would consider along the morphological and grain yield selection criteria, the physiological related traits that would provide information on the level of salt tolerance such as total and soluble proteins, phenols and thousand kernel weight and $Na^+$ and $Cl^-$ concentration in mature kernels.

## MATERIALS AND METHODS

Two six row barley cultivars are used in this investigation: Arig 8 and Acsad 1230 which are originated from Morocco and Libya respectively. Both cultivars were grown in pots using 0; 70 mM and 140 mM of NaCl in the green house of the Agronomy National Institute of Tunis during 2003 cropping season. Irrigation was achieved every 5 to 6 days with tap water enriched with NaCl. Alternated leaching irrigation is carried out every other salt treatment.

At physiological maturity of grain three random samples of 1000 kernel were selected from each experimental unit and thousand kernel weights is determined. Grain dry weight is determined after drying thousand of kernel using 80°C during 72 h.

Whole grain samples (100 g) were ground with a cyclone mill. Flour samples were added with 5 mL acide nitro-perchlorique. The matras is covered with funnels and mineralized up till complete discoloration. $Na^+$ cation is estimated using spectrophotometry (photometer Eppendorf) and $Cl^-$ ion content is determined using Coulometry with Chloridometer (Buchler-Cotlove). Analysis of phenolic compounds is carried out using 50 mg of grinded kernel in cool temperature added with a solution of 1.5 mL of MeOH (80%) (El Hadrami *et al*., 1997). The supernatant was then agitated during 5 min at 4°C. The mixture is then centrifugated at 7000 g × 3 min and the supernatant is recovered and conserved at -20°C.

The residue is supplemented with 2 mL of NaOH and placed at 100°C × 2 h to extract non soluble phenolic compounds. After incubation the extracts were acidified to pH=2 with HCl 2 N. Ethyl acetate is then used to extract phenolic compounds and evaporated under dry.

The residues were than redissolved in MeoH and stored at-20°C. Soluble phenolic compounds evaluation was determined using Folin Ciocalteu's reactif (El Hadrami *et al*., 1997). Optic density recorded at 760 nm and phenolic compounds rates are expressed as mg of chlorogenic acid per g DM. Soluble proteins are determined according to Bradford (1976); whereas total proteins were determined according to Kjeldahl's methods.

The experiment was analyzed as completely randomized design of two barley cultivars, three salt levels and three replicates. Analysis of variance (ANOVA) was performed using proc anova of SAS with LSD (0.05) option for mean separation.

## RESULTS

**Thousand kernel weight (TKW):** Grain filling and assimilates translocation could be assessed using thousand kernel weight. Under control salt treatment, TKW in Arig 8 was 44.7 g which is greater than that observed for Acsad 1230 (37.9 g). Results suggested that TKW was not affected by salt treatment for Acsad 1230 while it has been reduced significantly 17% with 140 mM salt applications in Arig8 (Table 1) (Fig. 1).

**Amount of $Na^+$ and $Cl^-$:** The concentration of $Na^+$ per kernel was comparable for both barley cultivars grown under salt control treatment. These concentrations were 0.82 and 1.82 μeq $g^{-1}$ for Acsad 1230 and Arig 8, respectively. Increased concentrations of $Na^+$ were noted with greater salt treatment for both cultivars, with a more pronounced effect for Acsad 1230. When 70 mM NaCl was applied an increase of NaCl concentrations of 316% were obtained in Acsad 1230. Using 140 mM NaCl, an increase of $Na^+$ concentration of 683 and 403% were observed, respectively in kernel of Acsad 1230 and Arig 8 (Table 1) (Fig. 2).

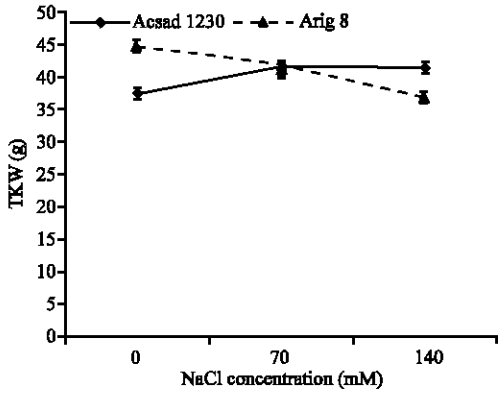


Fig. 1: Salt effect on TKW of two barley cultivars (Acsad 1230 and Arig 8).

Table 1: Mean squares of physiological traits: Thousand Kernel weight; sodium content ($Na^+$); chlorine ($Cl^-$); soluble and total proteins; soluble and bounded phenols content evaluated at mature grains stage for two barley cultivars assessed under salt treatments

| Source of Variation | df | Thousand Kernel weight | $Na^+$ (µeq/gDM) | $Cl^-$ (µeq/gDM) | soluble proteins (mg/gDM) |
|---|---|---|---|---|---|
| Cultivars (C) | 1 | 4.718 ns | 2.002 ns | 2.406 ns | 0.000008 ns |
| Salt (S) | 2 | 12.530 ns | 34.421** | 13.441** | 0.00004** |
| S* C | 2 | 47.056** | 0.916 ns | 7.534* | 0.00016** |
| Error | 12 | 5.952 | 0.826 | 1.125 | 0.000003 |
| C.V | | 5.949 | 28.560 | 14.097 | 4.966 |
| $R^2$ | | 0.634 | 0.879 | 0.766 | 0.956 |

| Source of Variation | df | Total proteins (mg/gDM) | Soluble phenols (µéq/gDM) | Bounded phenols (µéq/gDM) |
|---|---|---|---|---|
| Cultivars (C) | 1 | 0.0001 ns | 2023.907* | 119.451 ns |
| Salt (S) | 2 | 0.044 ** | 115.471 ns | 53.537 ns |
| S* C | 2 | 0.091** | 1155.557* | 200.352* |
| Error | 12 | 0.005 | 199.037 | 32.038 |
| C.V | | 10.979 | 14.681 | 37.137 |
| $R^2$ | | 0.821 | 0.792 | 0.619 |

*;** Significant at p<0.05 and p<0.01 probability levels respectively. ns no significant difference at p=0.05, DM: DRY Matter

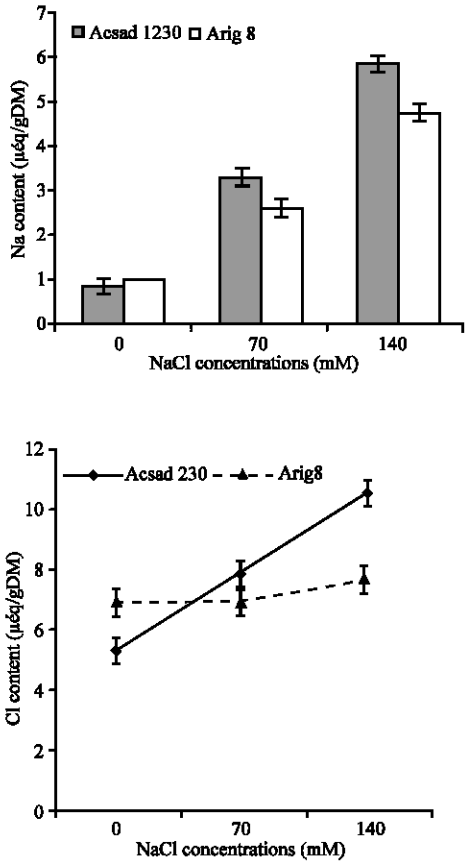


Fig. 2: Salt effect on $Na^+$ and $Cl^-$ content per kernel for two barley cultivars (Acsad 1230 and Arig 8)

This trend of $Na^+$ concentrations variations is also found for $Cl^-$. Hence under unsalted growing condition, both barley cultivars tended to accumulate comparable rates of $Cl^-$ per kernel of 5.31 µeq $g^{-1}$ for Acsad 1230 and 6.9 µeq $g^{-1}$ for Arig 8. However, greater and variable concentrations of $Cl^-$ were found to be associated with increased salt applications for Acsad 1230 (48 and 98% $Cl^-$ increase for 70 and 140 mM, respectively).

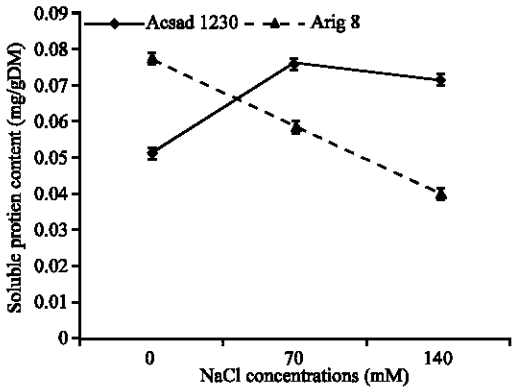


Fig. 3: Salt effect on soluble proteins content of two barley cultivars Acsad 1230 and Arig 8

$Cl^-$ concentrations were not affected by the level of salt application changes for Arig 8 (Table 1) (Fig. 2).

**Effect on soluble proteins:** Soluble proteins content per kernel was comparable for both barley cultivars grown under salt control treatment. These concentrations were 0.05 and 0.07 mg $g^{-1}$ for Acsad 1230 and Arig 8, respectively. However it was noted a significant increase (49%) of soluble proteins for Acsad 1230 when treated with 70 and 140 mM; whereas reduced concentrations of 25 and 49% were observed for Arig8 treated with 70 and 140 mM, respectively (Table 1) (Fig. 3).

**Effect of salt on total proteins:** Superior total proteins content is noted for Arig 8 (0.83 mg g) than Acsad 1230 (0.58 mg $g^{-1}$) grown in unsalted conditions. Results suggested that total proteins content was not affected by salt treatment for Acsad 1230, while it has been reduced significantly with increased salt applications in Arig 8. These reductions were 20 and 49% respectively for 70 and 140 mM (Table 1) (Fig. 4).

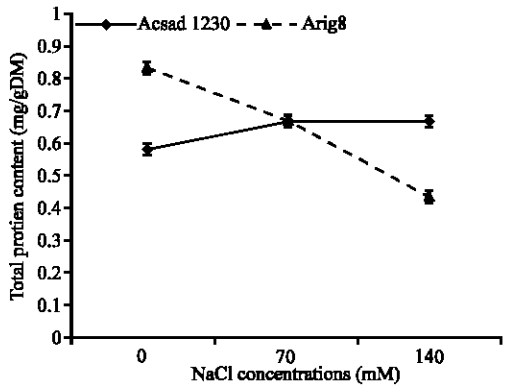


Fig. 4: Salt effect on total protein content for Acsad 1230 and Arig 8 barley cultivars

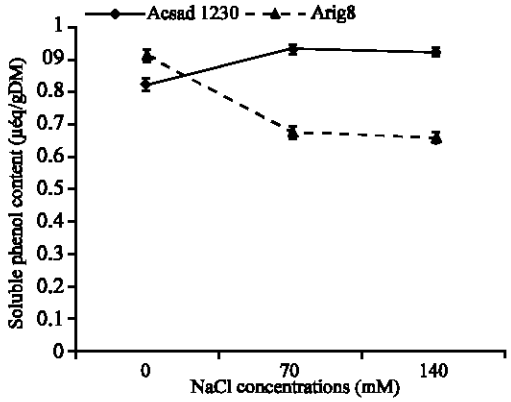


Fig. 5: Salt effect on soluble phenols content for Acsad 1230 and Arig 8 barley cultivars

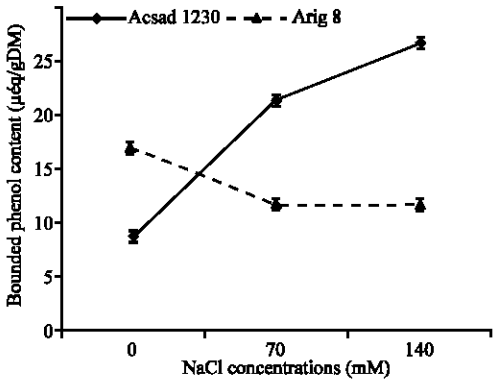


Fig. 6: Salt effect on bounded phenols content for Acsad 1230 and Arig 8 barley cultivars

**Effect of NaCl on soluble phenols:** Phenol accumulation in the grain was not significantly different for both barley cultivars grown under normal growing conditions. The average accumulation of phenols was 106 and 93 µeq $g^{-1}$ for Arig8 and Acsad 1230, respectively. Results suggested that soluble phenols content was not affected by salt treatment for both barley cultivars (Table 1) (Fig. 5).

**Effect of NaCl on bounded phenol:** The accumulation of bounded phenols of 16 µeq $g^{-1}$ in Arig 8 and only 8.17 µeq $g^{-1}$ for Acsad 1230 grown in unsalted conditions were obtained; however these differences are not significantly different. An increase of 146 and 207% of bounded phenols is found for 70 NaCl and 140 mM NaCl applications, respectively as compared to the control for Acsad 1230. A slight but no significant reduction of bounded phenols content is observed for Arig 8 for similar growing conditions (Table 1) (Fig. 6).

## DISCUSSION

Morphological modifications during vegetative growth stage could affect translocation efficiency and therefore grain filling rate reducing TKW (Hunt and Nicholls, 1986; Kuiper *et al*., 1988). Results from this investigation indicated that Acsad 1230 and Arig 8 have different responses to NaCl effects as depicted by TKW. Hence, TKW did not vary with increased levels of NaCl for Acsad 1230; whereas a significant reduction of TKW was noted for Arig 8 suggesting that the former cultivar is more tolerant to salt stress than the latter one. These results are comparable to those reported by Khatun (1995) who concluded that salt tolerance in rice cultivars was genotype dependant and closely associated with the level of expression of salt tolerance. The inhibition effects of NaCl on grain yield production in cereals were also described by other authors (Aljuburi, 1993; Hassan and El-Sammoudi, 1993; Ben Hamida, 2000). The total carbohydrates weight accumulation is often conditioned by the photosynthetic activity and translocation efficiency of vegetative tissues. Hence kernel number of both cultivars is also largely affected by salt treatment and was more reduced for Arig 8 and less reduced for Acsad 1230. The TKW was found to stable in Acsad 1230 with increased level of NaCl treatment is attributed to accumulation rate of dry matter within kernel rather than an accumulation of salt derived products within the various organs and grain filling rate as depicted by the difference between the total dry matter and major mineral ions present within kernel. It is however important to note that organic matter accumulation has a similar trend of accumulation as the total dry matter accumulation. These results would suggest that NaCl effect could be perceived as growth stimulatory effect rather than simple increase of biomass attributed to the uptake of ions from the substrates. The osmotic

adjustment would imply that organic dry matter would be used for compartimentation of excess of $Na^+$ and $Cl^-$. Excess accumulation rates of $Na^+$ and $Cl^-$ may inhibit other cellular metabolic activities such as protein synthesis (Xiong and Zhu, 2000) in sensitive cultivar causing a late reserve mobilization (Gomez and Sodek, 1988). In Acsad 1230, the amount of proteins per g of dry matter on per kernel basis was found to increase with increased NaCl levels but an opposite trend was noted for Arig 8. It is however observed that greater protein levels positively affect the germination rate and plant vigor. Since the kernel of plant specie represents a source of important molecules that control the quality of derived and products with regard to the nutritional and organoleptic values, it is important to assess the various components of proteins and their biosynthesis (Bénétrix and Autran, 1997). Dry kernels are quiescent and have an average 13% of water content which is bounded water so that storage will be easily carried out (Multon, 1982). During the maturation process of kernels, several mechanisms are induced to support greater drying tolerance among which the synthesis of hydrophilic proteins along the mRNA that are specifically induced by drought stress. These proteins could play a major stabilizing role of cell membranes (Jbir, 2002). Hence, under salt stress, the total proteins were found to stable in Acsad 1230 whereas these proteins decreased significantly in Arig 8. Previous studies (Bénétrix and Autran, 1997) demonstrated that the greater nitrogen levels in the kernel is particularly attributed to a higher nitrogen translocation efficiency from vegetative plant parts (amino-acids derived from hydrolyzed proteins) and/or a better nitrogen use efficiency. The nitrogen remobilization from leaves to kernels could be continued even in late growth stages and differences noted for protein contents is associated with enzymatic activity that is involved in protein degradation of vegetative tissues.

Phenolic compounds were higher in leaf tissues than in kernel for both barley cultivars. Similar results were reported by Wamska *et al.* (1988) in sorghum. The reduced amount of phenolic compounds in the kernels could be partly explained by the use of a proportion of these compounds to lignin compounds during the physiological maturation period particularly when salt stress is occurring. Results of this investigation showed that bounded phenolics compounds discriminate between cultivars under salt stress. Hence Acsad 1230 is characterized by a better accumulation of bounded phenolic compounds in kernel than in Arig 8. Therefore the greater accumulation of phenolic compounds in the former cultivars could be perceived as response to increased NaCl level present in the solution so that Acsad 1230 would be able to reinforce the cellular membranes through a process of lignifications to increase its resistance to decreased turgescence and water retention in the internal tissues (Cruz *et al.*, 1992).

Salt effect on the reproductive ability (TKW) could be used to discriminate between both cultivars and enable to conclude that:

- Thousand Kernel weight produced for Acsad 1230 under salt treatment is attributed to plant vigor during vegetative growth stage.
- The amount of proteins in Acsad 1230 and phenols content appeared to retard the critical accumulation of $Na^+$ and $CL^-$.

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