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# Some nutritional characteristics and mineral contents in barley (*Hordeum vulgare* L.) seeds cultivated under salt stress

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**RESEARCH ARTICLE**

## Abstract

Barley (*Hordeum vulgare* L.) is an important food crop grown worldwide and it is important to investigate the quality of its grain as affected by various environmental conditions. In this study, barley was grown under increasing salt stress (0, 40, 80, and 120 mmol/l of NaCl) and the nutritional quality of its seeds was evaluated for their proximate composition, phenolic compounds and micro- and macro-mineral contents. Phenolic compounds of barley seeds increased significantly from 1.75 (control) to 1.95 mg gallic acid equivalent/g dry weight of barley seed in 120 mmol/l NaCl. Cu, Mg, and Ni contents were decreased whereas Fe, Mo, and Zn contents were increased significantly by increasing salinity. Among the macro-minerals in barley seeds cultivated under salt stress only K contents increased whereas Ca, Mg, P, and S contents were decreased significantly.

**Keywords:** barley seed, macro minerals, micro minerals, phenolic compounds, salt stress

## 1. Introduction

Salinity is one of the major abiotic stresses that can reduce plant growth and crop productivity worldwide. Abiotic stresses resulting from excessive salinity led to reduction in photosynthesis, transpiration and other biochemical processes associated with plant growth, development and crop productivity (Tiwari *et al.*, 2010). Salt stress is a global issue in agriculture. The soil salinity is a common problem in arid and semi-arid regions. Soil salinity causes different problems in cultivation of crops, pasture development and other agronomic practices (Ramoliya *et al.*, 2004). It is essential to monitor the response of different plants grown under salt stress although it is well established that increased salt concentration in soil have potential negative effects on the growth and health of plants (Mer *et al.*, 2000). The excessively higher salt stress can even result in plant death (Ramoliya *et al.*, 2004). The ion homeostasis may be changed under salt stress due to excessive uptake of $Na^+$ and $Cl^-$. Competitions between these and further anions and cations are often reported in relation to their certain negative effects on plant growth and crop yield (De Pascale *et al.*, 2005). It is still unclear how the micronutrient contents of different plants are affected by salinity; however, it may depend on species or cultivar and plant organs (De Pascale *et al.*, 2005). It has been reported that salinity can affect physico-chemical attributes of strawberry fruit (Keutgen and Pawelzik, 2008) and romaine lettuce (Kim *et al.*, 2008), and phenolic compounds and biological properties of halophyte Cakile maritime leaves (Ksouri *et al.*, 2007). However, little or no information on the distribution of macro- and micronutrients and polyphenolic contents in barely (*Hordeum vulgare* L.) seed under NaCl salinity, is yet available. Thus, it is important to monitor the changes in nutritional characteristics of barley cultivated under salt stress (Keutgen and Pawelzik, 2008). It has also been reported that salinity can result in compositional changes in compounds containing N, proteins and free amino acids in particular (Keutgen and Pawelzik, 2008). It has been amply documented that different types of phenolic compounds from plant based foods can decrease or prevent different health problems due to their antiradical and antioxidant

activities (Surh, 2002). Cereals crops are very important source of carbohydrate and energy for masses around the globe. Different evidences are being obtained these days whereby researchers study the bioactive compounds potential of different cereal crops (Sun and Ho, 2005). Barley is a widely consumed cereal among the most ancient cereal crops. There have been reports that the seeds of barley can be considered as a source of phenolic compounds and there is need to find out the best suited method for their extraction (Liu and Yao, 2007).

The objective of carrying out this study was to evaluate barley seed, obtained after cultivation at varying salinity levels, for micro and macro minerals, phenolic compounds and proximate composition.

## 2. Materials and methods

### Materials

The seeds of barley variety bajwar-200 were obtained from Ayub Research Institute (Faisalabad, Pakistan) and planting trials were carried out in a greenhouse farm. The average temperatures during day and night (± standard error; n =8) of the complete growth cycle of barely were 30±9 °C and 15±7 °C, respectively. The average relative humidity ranged from 41 to 69% and natural photoperiod from 10 to 12.5 h. The experimental trials were based on a complete random design consisting of 3 types of salt treatments and a control (0% NaCl). The salt stress conditions were created by using 40, 80, and 120 mmol/l of NaCl. The hardened clay pots (30 cm diameter) were lined with polyethylene. Each one was filled with 9.0 kg of pure sand obtained from a river and around 50 seeds were sown in each one. Plants were allowed to germinate for 15 days after which thinning was done to keep 6 plants in each pot. The plants were provided with 2 l/pot of full strength Hoagland's nutrient solution each week prior to the onset of the salt stress experiments. Once plants had grown for a total period of 67 days after the onset of germination, the NaCl dissolved in Hoagland's nutrient solution was given using aliquots of 40 mmol/l each day. A total of 2 l/pot desired treatment solution was given to plants every week. The reason for providing this much volume was to wash any salts pre-existing in soil. Distilled water (200 ml/pot) was added every week to maintain the moisture contents for plants. Seeds were obtained after harvesting the mature plants and ground to powder farm (0.5 mm sieve). Chemicals used in this study were of analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA).

### Proximate analyses

Moisture, crude lipid, protein and fibre were determined according to the standard AOAC (1990) method. Protein content was established according to Dupon method. Crude protein was calculated by using a nitrogen conversion factor of 6.25. Protein content was determined by the Dumas Nitrogen Analyzer (Velp NDA 70; Velp Scientifica, Usmate, Italy) using $O_2$ flow rate of 400 ml/min, He flow rate of 195 ml/min, combustion reactor temperature at 1,030 °C, reduction reactor temperature at 650 °C and pressure of 881 mbar.

### Total phenolics

The total phenolic compounds of barley seed powder (6.52% w/w moisture and passed through 0.5 mm sieve) were analysed by extraction using ethanol (50%). The extracts were incubated at room temperature for 24 h under dark conditions. The suspension was centrifuged at 10,000×*g* for 10 min at room temperature and the supernatant was collected. The determination of total phenolic compounds was carried out using Folin-Ciocalteu reagent (FCR) method. Once the reaction of extract with reagents (1 N FCR and 10% $Na_2Co_3$ solution) was completed, the absorbance was recorded at 765 nm using a UV-visible spectrophotometer (Ultrospec II 4050; LKB Biochrom, Cambridge, UK) according to the method of Ghafoor *et al.* (2012). Gallic acid was used for preparing a standard curve and the results were expressed as milligrams of gallic acid equivalent (mg GAE)/ g of dry weight (DW).

### Mineral analyses

Approximately 0.5 g barley samples were taken in burning cups with 15 ml of pure $NHO_3$ and 2 ml $H_2O_2$ (30%, w/v). Afterwards the samples were incinerated in a microwave oven (MARS 5, CEM Corp. Matthews, NC, USA) at 210 °C. Distilled deionised water and ultrahigh-purity commercial acids were used in preparation of reagents, standards, and samples. Samples were filtrated after digestion using Whatman no. 42 filter papers (Whatman, Göttingen, Germany). The filtrates were collected in 50 ml flasks and analysed by inductively coupled plasma atomic emission spectroscopy using a Varian Vista system from Varian Inc. (Palo Alto, CA, USA). Radio frequency power was 0.7-1.5 kW (1.2-1.3 kW for axial); plasma gas flow rate was 10.5-15 l/min; viewing height was 5-12 mm; copy/reading time was 1-5 s (max 60 s) and copy time was 3 s (max 100 s). The mineral contents of the samples were quantified against standard solutions of known concentrations which were analysed concurrently.

### Statistical analyses

All analytical measurements were accomplished in triplicates and results are presented as means ± standard deviation. Data analysis, for statistical significance, was carried out using analysis of variance and Duncan's multiple range test using the statistical analysis system (SAS, version

9.1; SAS Institute Inc., Cary, NC, USA). Significance was defined as $P<0.05$.

## 3. Results and discussion

### Effect of salt stress on proximate composition of barely seeds

The data showing proximate composition of barley seeds obtained after cultivating the plants under increasing salt stress in shown in Table 1. Moisture contents decreased significantly ($P<0.05$) from 7.05 to 6.81% with increasing salt stress up to 120 mmol/l. Crude oil contents also decreased significantly ($P<0.05$) from 2.27 to 1.87%.

Taarit *et al.* (2010) observed that cultivation of sage under NaCl resulted in significant ($P<0.05$) reduction of certain fatty acid contents. Talha and Osman (1974) observed that cultivation under water stress resulted in a decrease of oil contents (from 31.9 to 24.7%) in sunflower seed. Similarly, Jose and Mark (2009) observed that stress cultivation of soybean caused 35% reduction in its seed oil contents. Hence it becomes evident that oil contents can be reduced in crops cultivated under environmental stress. In contrary to oil contents the crude protein contents remained almost unchanged instead there was a slight increase from 20.13 to 20.93% with increasing salinity. Similarly to crude oil and moisture, crude fibre also decreased from 6.37 to 5.90%. The salt stress can affect photosynthetic process in plants that can in return reduce crop productivity. It may also variably affect the composition of plant produce such as it was previously observed that a total carbohydrates and crude fibre in fennel were adversely affected due to salinity (Abd El-Wahab, 2006). Such adverse effects can be attributed to the nutritional imbalance and specific toxic effects of salinity.

### Effect of salt stress on total phenolics contents of barley seeds

The data showing total phenolic contents are presented in Figure 1. The phenolic contents rose from 1.75 (control)

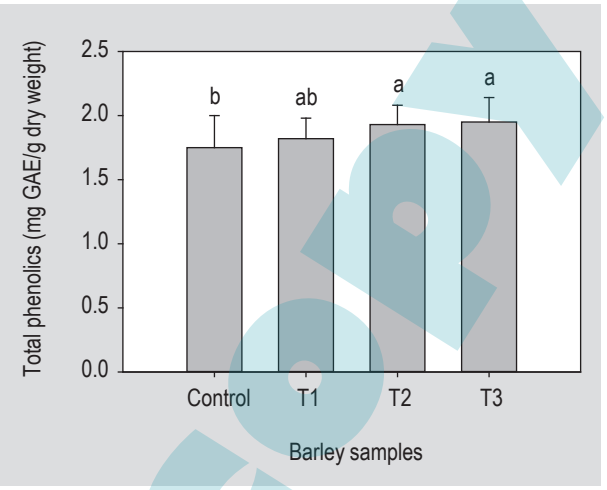


**Figure 1. Total phenolic compounds in barley grown under salt stress. Bars represent standard error of means (n=3) and means with different letters are significantly different ($P<0.05$). Control = no salt stress; T1 = 40 mmol/l NaCl; T2 = 80 mmol/l NaCl; T3 = 120 mmol/l NaCl; GAE = gallic acid equivalent.**

to 1.95 mg GAE/g DW of barley seed in T3 (120 mmol/l NaCl). T1 and T2 also showed higher phenolic contents (1.82 and 1.93 mg GAE/g DW, respectively) than control. In another study (Yuan *et al.*, 2010), the total phenolic contents of 5-7 day old radish sprouts grown under 100 mmol/l NaCl stress were significantly increased ($P<0.05$). In general, the synthesis of phenolic compounds in plants follows the phenylpropanoid pathway. These compounds can also be synthesised as a result of plant's response to environmental stresses and elicitor (Giorgi *et al.*, 2009). The phenolic compounds can be affected by increasing salt stress; however this mostly depends on the sensitivity of a plant towards salts stress (Kim *et al.*, 2008). For instance, an increase in phenolic compounds in sprouts treated with 100 mM of NaCl and similar effects on total phenolics of *Cakile maritima* and red pepper were reported by Ksouri *et al.* (2007) and Navarro *et al.* (2006), respectively. Strawberry fruit grown using 40 and 80 mmol/l NaCl also showed a significant increase in phenolic compounds in two types of cultivars namely Elsanta and Korona and both of these

**Table 1. Proximate composition (%) of barley grown under salt stress conditions.[1,2]**

| Samples[3] | NaCl conc. (mmol/l) | Moisture | Crude oil | Crude protein | Crude fibre |
|---|---|---|---|---|---|
| control | 0 | 7.05±0.89$^{a}$ | 2.27±0.12$^{a}$ | 20.13±1.16$^{b}$ | 6.37±0.11$^{a}$ |
| T1 | 40 | 6.92±1.27$^{ab}$ | 2.04±0.43$^{b}$ | 20.85±1.67$^{a}$ | 6.06±0.43$^{b}$ |
| T2 | 80 | 6.86±1.32$^{b}$ | 1.80±0.35$^{c}$ | 20.92±0.54$^{a}$ | 6.11±0.38$^{b}$ |
| T3 | 120 | 6.81±0.91$^{c}$ | 1.87±0.56$^{bc}$ | 20.93±0.29$^{a}$ | 5.90±0.89$^{c}$ |

[1] Data are presented as means ± standard deviation (n=3).
[2] Means with different superscript letters within a column are significantly different ($P<0.05$).
[3] Control = no salt stress; T1 = 40 mmol/l NaCl; T2 = 80 mmol/l NaCl; T3 = 120 mmol/l NaCl.

also had improved colour due to increased anthocyanins. It was further reported that the taste of these strawberry fruits was also improved due to increased sugars, the reason may also be higher sugars to moisture ratio (Keutgen and Pawelzik, 2008). One of the reasons of increased phenolic contents is that plants gown under salt stress exhibit a protection mechanism against an increase in free radicals generated under stress by maintaining a sufficient quantity of phenolic and other antioxidant compounds. Therefore, the contents and composition of plant phenolics and their biological properties may also depend on environmental factors such as salt stress (Falleh *et al.*, 2012). Hence, it is evident that environmental stresses can decrease crop yield but on the other hand they may favour development of certain quality characteristics in agricultural produce.

### Effect of salt stress on some micro- and macro-nutrients in barley seeds

The results showing the effects of salt stress on micro-mineral contents of barely seeds are given in Table 2. There was a significant ($P$<0.05) decrease in concentration of majority micro-minerals (Cu, Fe, Mo, Mn, and Zn) except Ni, the quantity of which remained significantly ($P$<0.05) higher in normally grow barely seed. In case of Fe and Mo there were non-significant effects of increasing salinity level however Zn contents (81.6 mg/k) in T3 (120 mmol/l NaCl) were significantly ($P$<0.05) higher than rest of the treatments showing that increase in salinity resulted in accumulation of more zinc in barley seeds. In case of Cu (9.7 mg/kg) and Mn (18.0 mg/kg), T1 obtained using 40 mmol/l NaCl showed higher contents then the rest of the treatments. It is difficult to suggest mechanistic reasons for variations in micro-minerals due to their smaller proportions. Only Ni and Cu were decreased with increasing salinity whereas rest of the micro-minerals remained un-changed or their contents increased. All these minerals have high nutritional importance and their deficiencies can lead to certain health disorders in humans (Onabanjo and Oguntona, 2003).

The effect of increasing salinity stress on the macro minerals (Ca, Mg, K, P, and S) is shown in Table 3. The concentration of Ca, Mg, P, and S was significantly ($P$<0.05) reduced due to salt stress whereas that of K was significantly ($P$<0.05) higher in salt treated seeds and was the highest in T3 treated with 120 mmol/l of salt. In particular, there was marked decrease in Ca contents in salt treated barley seed. The increase in K contents in barley seed was similar to its increase in leaves and stems of *Salvadora persica* grown under increasing salinity in a study carried out by Ramoliya *et al.* (2004).

The cation K plays an important role during cell expansion, osmo-regulation and cellular and whole-plant homeostasis

**Table 2. Trace or micro minerals concentration (mg/kg, dry matter) in barley grown under salt stress.[1,2]**

| Samples[3] | Cu | Fe | Mn | Mo | Ni | Zn |
|---|---|---|---|---|---|---|
| Control | 9.2±1.2$^{b}$ | 82.9±4.6$^{b}$ | 12.3±0.8$^{d}$ | 4.066±0.51$^{b}$ | 0.431±0.012$^{a}$ | 69.1±2.1$^{b}$ |
| T1 | 9.7±1.6$^{a}$ | 90.6±1.1$^{a}$ | 18.0±0.9$^{a}$ | 5.317±0.25$^{a}$ | 0.370±0.017$^{b}$ | 68.7±2.9$^{b}$ |
| T2 | 8.7±0.6$^{c}$ | 92.2±2.8$^{a}$ | 14.1±0.5$^{c}$ | 5.385±0.26$^{a}$ | 0.365±0.019$^{b}$ | 71.5±4.6$^{b}$ |
| T3 | 8.6±0.5$^{c}$ | 93.6±7.1$^{a}$ | 16.5±0.3$^{b}$ | 5.379±0.37$^{a}$ | 0.324±0.011$^{c}$ | 81.6±0.8$^{a}$ |

[1] Data are presented as means ± standard deviation (n=3).
[2] Means with different superscript letters within a column are significantly different ($P$<0.05).
[3] Control = no salt stress; T1 = 40 mmol/l NaCl; T2 = 80 mmol/l NaCl; T3 = 120 mmol/l NaCl.

**Table 3. Macro minerals concentration (mg/kg, dry matter) in barley grown under salt stres.[1,2]**

| Samples[3] | Ca | Mg | K | P | S |
|---|---|---|---|---|---|
| Control | 1,160±82$^{a}$ | 1,810±46$^{a}$ | 1,822±218$^{c}$ | 5,254±114$^{a}$ | 1,734±94$^{a}$ |
| T1 | 721±50$^{b}$ | 1,699±38$^{c}$ | 7,796±362$^{b}$ | 4,609±100$^{b}$ | 1,457±54$^{c}$ |
| T2 | 473±37$^{bc}$ | 1,730±67$^{b}$ | 8,204±181$^{ab}$ | 4,583±243$^{c}$ | 1,523±79$^{bc}$ |
| T3 | 363±24$^{c}$ | 1,750±97$^{b}$ | 8,458±213$^{a}$ | 4,573±222$^{c}$ | 1,589±63$^{b}$ |

[1] Data are presented as means ± standard deviation (n=3).
[2] Means with different superscript letters within a column are significantly different ($P$<0.05).
[3] Control = no salt stress; T1 = 40 mmol/l NaCl; T2 = 80 mmol/l NaCl; T3 = 120 mmol/l NaCl.

(Schachtman *et al.*, 1997). There might have been great deal of K transfer from root and other parts of barley plant to its flower and subsequently to the seed. There is no clear explanation of the mechanism of a possibly complex interaction between salinity and P. Therefore it is not predictable whether P will increase, decrease or remain unchanged in response to salinity (Grattan and Grieve, 1992). It is understood that the concentration of P is co-related with the photosynthesis rate; however it reduces the fixed carbon conversion into starch (Overlach *et al.*, 1993). Calcium is important during salt stress, e.g. in preserving membrane integrity (Rengel, 1992), signalling in osmo-regulation (Mansfield *et al.*, 1990) and influencing K/Na selectivity (Cramer *et al.*, 1987). Due to this reason more Ca might have been transferred to leaves. In addition to the role of Mg in the structure of chlorophyll and as a cofactor for enzymes, an another function associated with Mg in plants is in the export of photosynthates, which is impaired and leads to enhanced degradation of chlorophyll in Mg deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner and Cakmak, 1989). These factors might have contributed in changing the composition of macro-elements in seeds of barley grown under varying stress that affected physiological processes of plants and hence accumulation of the macro-elements in seeds.

## 4. Conclusions

Barley was grown under increasing salt stress conditions and the nutritional quality of its grain was evaluated in terms of proximate composition, phenolic compounds and micro- and macro-mineral contents. Interesting results were observed as the crude protein contents remained unchanged whereas moisture, crude oil and crude fibre contents decreased with increasing salinity. Phenolic compounds of barley seeds which have important health benefits were increased under salinity. Among micro minerals Cu, Mg, and Ni contents were decreased whereas Fe, Mo, and Zn contents were increased with increasing salinity. Macro-minerals in barley seeds cultivated under salt stress were also evaluated and it was observed that only K contents increased whereas Ca, Mg, P, and S contents were decreased. Different physiological changes and/or adaptations by barley plant during growth under salinity might have affected nutritional quality of barely seed either in a negative or a positive way. Plant breeding techniques can also be used to ensure best quality crop, in both qualitative and quantitative terms, while grown under various environmental stresses.

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