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# SALINITY INDUCED DIFFERENCES IN GROWTH AND NUTRIENT ACCUMULATION IN FIVE BARLEY CULTIVARS

Bengü TÜRKYILMAZ* Lale YILDIZ AKTAŞ Avni GÜVEN

*Ege University, Faculty of Science, Department of Biology, Turkey*
**Corresponding author: bengu35540@hotmail.com*

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## ABSTRACT

**Salinity-induced changes in growth, photosynthetic performance and nutrient accumulation were determined in five barley (*Hordeum vulgare* L.) cultivars, subjected to different NaCl concentrations (120 and 240 mM) under controlled conditions. According to germination test data, two of these cultivars (Suleyman Bey and Vamik Hoca) were evaluated as more tolerant while Anadolu 98, Efes 3 and Gem cultivars were assessed as less tolerant. In the early growth stage (one-week-old seedlings) salinity caused an increase of the root/coleoptile length ratio in the less tolerant cultivars while it was not changed in the more tolerant ones. In 4-week-old plants, PSII activity, chlorophyll *a* and chlorophyll *b* were not affected negatively by NaCl stress, the carotenoids being even increased. $Na^+$ and $Cl^-$ accumulation in all genotypes were raised by NaCl salinity and the shoots accumulated ions at least 4-fold more than the roots. The $K^+/Na^+$ and $Ca^{2+}/Na^+$ ratio and Mg accumulation in the shoots of more tolerant Suleyman Bey and Vamik Hoca cultivars remained almost constant with control level. The study established that evolved salt tolerance strategy among the barley cultivars was based on maintenance of more cationic nutrients in shoots (K, Ca and Mg) and protection of photosynthetic apparatus in saline conditions.**

**Key words: *Hordeum vulgare*, NaCl, ion uptake, salt tolerance, photosynthesis.**

## INTRODUCTION

Soil salinity is a world-wide problem affecting about 20% of the world's cultivated land and nearly half of all irrigated lands (Zhu, 2001). It is well established that excessive salts in the growth medium are toxic to various physiological and biochemical processes taking place in crop plants (Ashraf and Harris, 2004; Munns et al., 2006). According to the FAO/UNESCO Soil Map of the world, around 1.5 million hectares of land in Turkey have both salinity and sodicity problems (Anonymous, 2008). Salt stress disrupts in water potential and ion distribution by inducing inhibition in the uptake of nutrients like $K^+$, $Ca^{2+}$ and $NO_3^-$ and accumulation of $Na^+$ and $Cl^-$ to potentially toxic levels within cells **(**Marschner, 1995; Mengel and Kirkby, 2001; Zhu, 2001; Krouma, 2009). Following the primary stresses, NaCl causes metabolic modifications by inducing generation of reactive oxygen species (ROS) (Ashraf and Harris, 2004), reducing the activity of certain enzymes (Munns, 1993) and impaired photosynthesis (Soussi et al., 1998; Loreto et al., 2003; Tatar et al., 2010), nitrogen (Mansour, 2000; Santos et al., 2002; Krouma, 2009) and carbon (Balibera et al., 2003) metabolism. This might leads, inhibition of cell division and expansion directly thus cell death is accelerated (Zhu, 2001; Hasegawa et al., 2000).

Barley (*Hordeum vulgare* L.) displays a relatively high salt tolerance and can grow in the areas with elevated salt concentrations (Maas and Hoffman, 1977; Lawlor et al., 1981). *H. vulgare* is known as the most salt tolerant cereal compared with wheat and other cultivated *Triticeae*, but barley cultivars still experienced a decline in biomass in saline environments (Greenway, 1962). Biomass production is regarded as a good predictor of yield also under salinity and is used as a major index of salt tolerance (Munns, 2002). Based on this knowledge, biomass production, growth attributes in seedling stage and $K^+/Na^+$ ratio have been suggested as criteria for improvement of salt tolerance in barley (Chen et al., 2005; Leonova et al., 2005; Huang et al., 2006; Munns et al., 2006). Limited information, however, is available on other physiological characters as related to salt tolerance in this important cereal crop (Munns et al., 2006; Huang et al., 2006). The objectives of the present investigation were to study the effects of salinity stress on the germination, early growth stage, accumulation of nutrient, photosynthetic rate, mature growth stage in *Hordeum vulgare* L., in order to understand the adaptation mechanism of *Hordeum vulgare* L. to salinity.

## MATERIALS AND METHODS

### *Plant material and treatments*

Salt tolerant cultivars (Anadolu 98, Efes 3, Gem, Suleyman Bey and Vamik Hoca) of barley (*Hordeum vulgare* L.) were chosen according to preliminary experiments among 13 cultivars (Akhisar, Çumra 2001, Angora, Çatalhöyük, Akhisar, Bornova 92, Kaya, Şerife

Hanım). Preliminary screening was made for various concentrations (0, 120, 240 mM) of NaCl to obtain the optimum response in germination and the variations to salinity tolerance. The final germination percentage was recorded after a period of one-week and embryonic root and coleoptile lengths were determined in the early germination stage.

For the second group of experiments, seeds were sown in pots (20 x 30 cm, 50 seeds for each) filled with perlite and grown under controlled conditions (light/dark regime of 16/8 h at 19±1 °C, relative humidity of 60-70%, total irradiance 350 µmol $m^{-2}$ $s^{-1}$ at the leaf level). Seedlings were irrigated with distilled water every other day; and with 0, 120 and 240 mM NaCl and Hoagland solution at the end of the week for four weeks. After two days from the final application, plants were harvested and sampled for measurement of growth attributes, chlorophyll fluorescence (using a plant efficiency analyzer-Hansatech, UK), leaf pigment content (Linchtenthaler, 1987) and nutrient content (were conducted at the Ege University, Faculty of Agriculture, Department of Soil Science Izmir, Turkey).

In determination of nutrients, the dried ground shoot and root material (1g) was digested with sulfuric acid and hydrogen peroxide according to the method of Wolf (1982). The digested material was filtered and used for the determination of cations. $K^+$, $Na^+$ and $Ca^{2+}$ were determined by a flame photometer (Jenway, UK) and $Mg^{2+}$ was determined by using a micro flame photometer (Varian, Austria). Chloride analysis was performed with a titrimetric method by using a flame photometer (Jenway, UK) (Johnson and Ulrich, 1959). Phosphorous (P) was analyzed with a spectrophotometer (Jenway, UK) using Barton’s reagent (Jackson, 1958). Barton’s reagent was prepared from ammonium molybdate, ammonium metavanadate, and concentrated $HNO_3$. Total nitrogen (N) in digests was determined by the Kjeldahl method.

Experimental data were analyzed with the SPSS statistical computer package (SPSS for WINDOWS, Standart version 11.0) and with the protected least significant difference (LSD) test at $p<0.05$ level.

## RESULTS

### *Growth analysis*

In our study germination percentage in all cultivars showed considerable decrease with increasing salinity. Responses to NaCl salinity in early germination stage of the barley cultivars were obtained by measuring lengths after one week germination in different concentrations of NaCl (120 and 240 mM). Increasing salt levels in the germination medium caused a marked inhibitory effect on coleoptile and embryonic root lengths of all barley cultivars in a week. The adverse effect of salt was more pronounced on coleoptile than embryonic root length of Anadolu 98, Efes 3 and Gem. In contrast, in the more tolerant Suleyman Bey and Vamik Hoca which have constitutively longer roots than the other genotypes, salinity effect on root and coleoptile length was not evidently different. Root/coleoptile ratio varied among cultivars. There was a sharp increase in Anadolu 98, Efes 3 and Gem about 3.8, 3.6 and 2.9-fold (240 mM NaCl) respectively; in the other cultivars (Suleyman Bey and Vamik Hoca), the ratios were almost constant in all NaCl concentrations ( Table 1).

**Table 1.** Germination percent and Root and Coleoptile lengths of one-week-old seedlings of *Hordeum vulgare* L. cultivars subjected to varying levels of NaCl salinity (n= 100, ± SE).

| Barley cultivars | NaCl (mM) | Germination (%) | Root length (cm) | Coleoptile length (cm) | Root/Coleoptile |
|---|---|---|---|---|---|
| Anadolu 98 | 0 | $100^a$ | $6.15±0.08^a$ | $6.12±1.11^a$ | 1.00 |
| | 120 | $100^a$ | $2.22±0.21^b$ | $0.76±0.22^b$ | 2.92 |
| | 240 | $66^b$ | $1.11±0.03^b$ | $0.29±0.01^b$ | 3.83 |
| Efes 3 | 0 | $100^a$ | $6.13±1.28^a$ | $7.14±1.59^a$ | 0.86 |
| | 120 | $96^b$ | $1.15±0.15^b$ | $0.57±0.01^b$ | 2.01 |
| | 240 | $80^c$ | $0.83±0.12^b$ | $0.27±0.01^b$ | 3.07 |
| Gem | 0 | $92^a$ | $4.98±0.86^a$ | $6.35±0.76^a$ | 0.78 |
| | 120 | $92^a$ | $2.37±0.57^b$ | $2.09±0.87^b$ | 1.13 |
| | 240 | $88^b$ | $0.73±0.13^c$ | $0.32±0.01^c$ | 2.28 |
| Suleyman Bey | 0 | $98^a$ | $8.40±1.81^a$ | $4.62±0.96^a$ | 1.82 |
| | 120 | $98^a$ | $4.04±0.88^b$ | $2.25±0.47^b$ | 1.80 |
| | 240 | $90^b$ | $1.09±0.20^c$ | $0.64±0.02^c$ | 1.70 |
| Vamik Hoca | 0 | $98^a$ | $9.14±2.33^a$ | $8.34±2.30^a$ | 1.10 |
| | 120 | $96^b$ | $1.93±0.05^b$ | $1.78±0.47^b$ | 1.08 |
| | 240 | $92^c$ | $0.44±0.08^c$ | $0.48±0.13^b$ | 0.92 |

Different letters indicate a significant difference at 0.05 level of probability as evaluated by ANOVA (LSD) test.

Although, there were no significant differences in root fresh weight and root dry weight, four-week-old barley seedlings were affected significantly by NaCl treatment in shoot length in Efes 3 and Suleyman Bey, in shoot fresh weight in Vamik Hoca, in shoot dry weight Anadolu 98, Efes 3, Suleyman Bey and Vamik Hoca, in root length in Anadolu 98 and Suleyman Bey (Table 2).

**Table 2.** Growth attributes of four-week-old seedlings of *Hordeum vulgare* L. cultivars subjected to varying levels of NaCl salinity (n=100, ± SE)

| Barley cultivars | NaCl (mM) | Shoot length (cm) | Shoot fresh weight (g) | Shoot dry weight (g) | Root Length (cm) | Root fresh weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|---|---|
| Anadolu 98 | 0 | $20.8\pm3.6^{a}$ | $0.131\pm0.018^{a}$ | $0.011\pm0.001^{a}$ | $13.0\pm2.3^{a}$ | $0.064\pm0.007^{a}$ | $0.006\pm0.001^{a}$ |
| | 120 | $22.1\pm3.5^{a}$ | $0.162\pm0.028^{a}$ | $0.016\pm0.002^{b}$ | $10.8\pm1.5^{b}$ | $0.063\pm0.011^{a}$ | $0.006\pm0.001^{a}$ |
| | 240 | $18.4\pm2.5^{a}$ | $0.130\pm0.023^{a}$ | $0.015\pm0.001^{b}$ | $10.7\pm0.8^{b}$ | $0.071\pm0.013^{a}$ | $0.007\pm0.001^{a}$ |
| Efes 3 | 0 | $19.1\pm3.2^{a}$ | $0.129\pm0.023^{a}$ | $0.012\pm0.002^{a}$ | $11.7\pm1.4^{a}$ | $0.076\pm0.011^{a}$ | $0.006\pm0.001^{a}$ |
| | 120 | $25.1\pm2.5^{b}$ | $0.150\pm0.031^{a}$ | $0.016\pm0.002^{b}$ | $13.4\pm2.0^{a}$ | $0.064\pm0.010^{a}$ | $0.006\pm0.001^{a}$ |
| | 240 | $20.1\pm4.2^{ab}$ | $0.127\pm0.025^{a}$ | $0.015\pm0.002^{b}$ | $11.5\pm1.2^{a}$ | $0.076\pm0.013^{a}$ | $0.007\pm0.001^{a}$ |
| Gem | 0 | $20.4\pm4.4^{a}$ | $0.141\pm0.021^{a}$ | $0.013\pm0.002^{a}$ | $11.2\pm1.5^{a}$ | $0.068\pm0.013^{a}$ | $0.006\pm0.001^{a}$ |
| | 120 | $22.5\pm3.9^{a}$ | $0.164\pm0.027^{a}$ | $0.015\pm0.003^{a}$ | $10.6\pm1.3^{a}$ | $0.071\pm0.012^{a}$ | $0.006\pm0.001^{a}$ |
| | 240 | $20.2\pm5.6^{a}$ | $0.145\pm0.030^{a}$ | $0.015\pm0.002^{a}$ | $09.5\pm1.3^{a}$ | $0.081\pm0.014^{a}$ | $0.006\pm0.001^{a}$ |
| Suleyman Bey | 0 | $14.8\pm3.2^{a}$ | $0.110\pm0.023^{a}$ | $0.009\pm0.001^{a}$ | $12.8\pm0.8^{a}$ | $0.064\pm0.010^{a}$ | $0.005\pm0.001^{a}$ |
| | 120 | $21.5\pm2.8^{b}$ | $0.136\pm0.021^{a}$ | $0.012\pm0.002^{b}$ | $09.0\pm1.4^{b}$ | $0.066\pm0.013^{a}$ | $0.005\pm0.001^{a}$ |
| | 240 | $19.3\pm2.9^{ab}$ | $0.138\pm0.030^{a}$ | $0.012\pm0.001^{b}$ | $08.3\pm0.8^{b}$ | $0.072\pm0.014^{a}$ | $0.005\pm0.001^{a}$ |
| Vamik Hoca | 0 | $15.5\pm2.9^{a}$ | $0.120\pm0.020^{a}$ | $0.01\pm0.002^{a}$ | $10.2\pm0.9^{a}$ | $0.076\pm0.011^{a}$ | $0.007\pm0.001^{a}$ |
| | 120 | $19.8\pm3.8^{a}$ | $0.173\pm0.025^{b}$ | $0.014\pm0.001^{b}$ | $09.4\pm1.0^{a}$ | $0.083\pm0.018^{a}$ | $0.006\pm0.001^{a}$ |
| | 240 | $16.4\pm2.5^{a}$ | $0.133\pm0.019^{ab}$ | $0.011\pm0.001^{a}$ | $09.2\pm0.8^{a}$ | $0.080\pm0.015^{a}$ | $0.007\pm0.001^{a}$ |

Different letters indicate a significant difference at 0.05 level of probability as evaluated by ANOVA (LSD) test.

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### *PSII quantum yield and photosynthetic pigments*

The maximum PSII quantum yield ($F_V/F_M$) after full dark-adaptation varied among 4-week-old control plants; it ranged from 0.706 (Efes 3) to as much as 0.788 (Vamik Hoca). However, NaCl treatment did not cause significant differences in PSII activity of the cultivars (Table 3).

NaCl salinity did not affect photosynthetic pigment concentrations of Anadolu 98 and Vamik Hoca. It resulted in a significant increase in chl *a* concentration in Efes 3 and Gem in all levels of NaCl and Suleyman Bey cultivar only in 120 mM NaCl, while chl *b* concentration was not changed by NaCl treatment, except for Gem (240 mM NaCl). Total chlorophyll concentration and chl *a/b* ratios were not drastically affected by NaCl stress in any barley cultivar. Noticeably, the carotenoid concentration was increased by NaCl exposure, in Efes 3 at the higher concentrations and in Gem and Suleyman Bey at all concentrations up to 31% (Table 3).

### *Nutrient accumulation*

In 4-week-old seedlings of the control group there was no significant difference among varieties in roots $Na^+$ accumulation; however, shoots of Suleyman Bey and Vamik Hoca have accumulated at least 2.5-fold more $Na^+$ compared to other cultivars. Comparing between organs, $Na^+$ level in shoots was higher by 2.6, 1.9 and 3.3-fold in Anadolu 98, Efes 3 and Gem, respectively, compared to the roots. Suleyman Bey and Vamik Hoca showed a 5.1 and 6.5-fold higher $Na^+$ ion accumulation in shoots than in roots in non-saline conditions. $Cl^-$ accumulation in the roots was changed in a similar trend with $Na^+$ accumulation. In the control group, Suleyman Bey and Vamik Hoca had higher $Cl^-$ concentration in the roots and shoots than other cultivars. Accumulation of $Cl^-$ in the shoots was 21.4, 12.5, 10.7, 7.9 and 6.7-fold more than in roots of Gem, Efes 3, Vamik Hoca, Anadolu 98 and Suleyman Bey cultivars, respectively (Table 4).

The exposure of barley to NaCl salinity caused a marked rise in both shoot and root $Na^+$ and $Cl^-$ accumulation to varying levels among cultivars (Table 4). Genotype-specific differences in the $Na^+$ and $Cl^-$ concentrations in roots and shoots of the cultivars also appeared as a higher accumulation of the ions in Suleyman Bey and Vamik Hoca cultivars than in other cultivars. Noticeably, Gem (2.6-fold) and Efes 3 (2.3-fold) genotypes appeared to have larger increase of $Na^+$ accumulation in shoots than the other

**Table 3.** Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), Chlorophyll *a/b* (Chl *a/b*), Total Chlorophyll, Carotenoids (Car) (mg $g^{-1}$ fresh weight) concentrations and PSII quantum yield in leaves of four-week-old seedlings of *Hordeum vulgare* L. cultivars subjected to varying levels of NaCl salinity (n=4, ± SE).

| Barley cultivars | NaCl (mM) | Chl a | Chl b | Chl a/b | Total Chl | Fv/Fm | Car |
|---|---|---|---|---|---|---|---|
| Anadolu 98 | 0 | $0.68\pm0.06^{a}$ | $0.25\pm0.02^{a}$ | $2.77\pm0.02^{a}$ | $0.93\pm0.05^{a}$ | $0.778\pm0.003^{a}$ | $4.9\pm0.4^{a}$ |
| | 120 | $0.73\pm0.02^{a}$ | $0.24\pm0.01^{a}$ | $3.02\pm0.01^{b}$ | $0.97\pm0.01^{a}$ | $0.732\pm0.006^{b}$ | $5.5\pm0.1^{a}$ |
| | 240 | $0.67\pm0.03^{a}$ | $0.22\pm0.01^{a}$ | $2.99\pm0.04^{b}$ | $0.89\pm0.02^{a}$ | $0.721\pm0.004^{c}$ | $4.9\pm0.2^{a}$ |
| Efes 3 | 0 | $0.73\pm0.08^{a}$ | $0.23\pm0.03^{a}$ | $3.14\pm0.06^{a}$ | $0.89\pm0.07^{a}$ | $0.706\pm0.008^{a}$ | $4.7\pm0.6^{a}$ |
| | 120 | $0.87\pm0.09^{b}$ | $0.23\pm0.01^{a}$ | $3.18\pm0.08^{ab}$ | $0.93\pm0.02^{a}$ | $0.717\pm0.003^{b}$ | $5.3\pm0.2^{ab}$ |
| | 240 | $0.84\pm0.09^{ab}$ | $0.27\pm0.03^{a}$ | $3.08\pm0.02^{ac}$ | $1.12\pm0.07^{b}$ | $0.734\pm0.005^{c}$ | $6.1\pm0.6^{b}$ |
| Gem | 0 | $0.69\pm0.08^{a}$ | $0.25\pm0.03^{a}$ | $2.82\pm0.02^{a}$ | $0.94\pm0.06^{a}$ | $0.773\pm0.003^{a}$ | $5.0\pm0.5^{a}$ |
| | 120 | $0.82\pm0.06^{b}$ | $0.27\pm0.02^{a}$ | $3.00\pm0.04^{b}$ | $1.10\pm0.04^{b}$ | $0.779\pm0.002^{a}$ | $6.0\pm0.4^{b}$ |
| | 240 | $0.93\pm0.10^{b}$ | $0.31\pm0.03^{a}$ | $3.05\pm0.02^{b}$ | $1.24\pm0.08^{c}$ | $0.719\pm0.007^{b}$ | $6.6\pm0.7^{b}$ |
| Suleyman Bey | 0 | $0.73\pm0.06^{a}$ | $0.27\pm0.02^{a}$ | $2.74\pm0.01^{a}$ | $1.00\pm0.05^{a}$ | $0.713\pm0.004^{a}$ | $5.2\pm0.4^{a}$ |
| | 120 | $0.86\pm0.01^{b}$ | $0.29\pm0.01^{a}$ | $2.98\pm0.01^{b}$ | $1.15\pm0.01^{b}$ | $0.721\pm0.007^{a}$ | $6.4\pm0.1^{b}$ |
| | 240 | $0.80\pm0.11^{ab}$ | $0.27\pm0.04^{a}$ | $2.96\pm0.02^{b}$ | $1.07\pm0.09^{a}$ | $0.717\pm0.001^{a}$ | $6.1\pm0.9^{b}$ |
| Vamik Hoca | 0 | $0.66\pm0.04^{a}$ | $0.23\pm0.01^{a}$ | $2.83\pm0.04^{a}$ | $0.89\pm0.03^{a}$ | $0.788\pm0.002^{a}$ | $4.8\pm0.2^{a}$ |
| | 120 | $0.69\pm0.05^{a}$ | $0.23\pm0.12^{a}$ | $3.06\pm0.08^{b}$ | $0.92\pm0.04^{a}$ | $0.800\pm0.001^{b}$ | $5.2\pm0.4^{a}$ |
| | 240 | $0.67\pm0.09^{a}$ | $0.22\pm0.02^{a}$ | $3.02\pm0.02^{b}$ | $0.89\pm0.07^{a}$ | $0.804\pm0.010^{b}$ | $4.9\pm0.7^{a}$ |

Different letters indicate a significant difference at 0.05 level of probability as evaluated by ANOVA (LSD) test.

genotypes. Comparison between the organs in respect of $Na^+$ and $Cl^-$ accumulation, NaCl treatments resulted in significantly higher shoot $Na^+$ and $Cl^-$ levels than the roots of barley cultivars 4 weeks following the treatment initiation.

Slight decreases were found in the concentrations of $K^+$ and $Ca^{2+}$ in both roots and shoots of barley cultivars subjected to NaCl except for Suleyman Bey and Vamik Hoca in which both ions increased or remained same at all salt levels applied (Table 4).

The ratio of $K^+/Na^+$ greatly varied with genotypes and salt levels (Fig. 1). In roots of barley cultivars the $K^+/Na^+$ ratio was significantly reduced by NaCl salinity except for Suleyman Bey where this ratio was not significantly changed. Barley cultivars maintained a considerably higher $K^+/Na^+$ ratio in the shoots than those in the roots. In the shoots of Suleyman Bey and Vamik Hoca, $K^+/Na^+$ ratio remained relatively constant after NaCl exposure. In contrast, the $K^+/Na^+$ ratio was considerably reduced in the shoots of the other genotypes by NaCl salinity (Fig. 1).

In roots $Ca^{2+}/Na^+$ ratios were reduced significantly by NaCl treatment except Suleyman Bey in which the effect of NaCl on $Ca^{2+}/Na^+$ ratios was very slight and independent from salt levels (Fig. 2). Among other cultivars, Vamik Hoca and Gem were less affected by NaCl treatment compared with Anadolu 98 and Efes 3. The $Ca^{2+}/Na^+$ ratio of Suleyman Bey and Vamik Hoca remained almost constant after NaCl treatment, but in the other cultivars particularly in Efes 3, NaCl treatment caused sharp decreases in the ratio (Fig. 2).

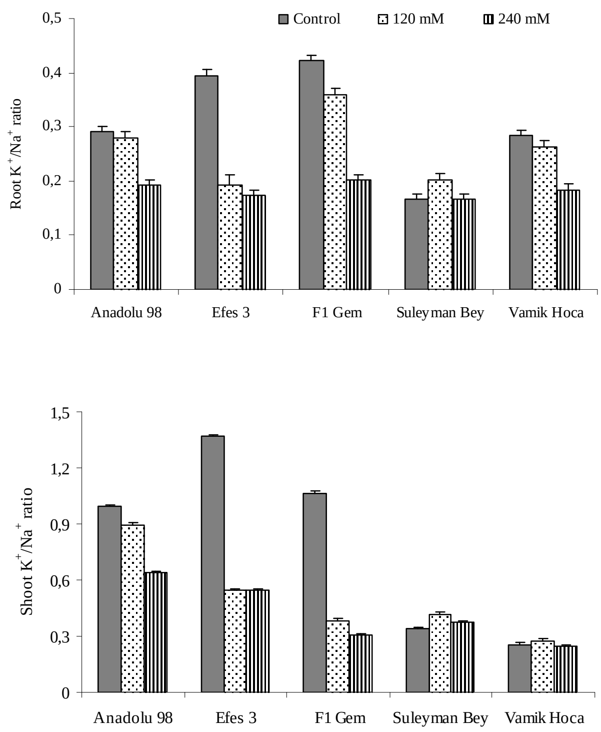


**Figure 1.** $K^+/Na^+$ ratios in roots and shoots of four-week-old seedlings of *Hordeum vulgare* L. cultivars subjected to varying levels of NaCl salinity.

**Table 4.** Concentration of Na (mmol $g^{-1}$ dry weight), K (mg $g^{-1}$ dry weight), Ca (mg $g^{-1}$ dry weight), Cl (mg $g^{-1}$ dry weight), N (% dry weight), P and Mg (μg $g^{-1}$ dry weight) in roots and shoots of four-week-old seedlings of *Hordeum vulgare* L. cultivars subjected to varying levels of NaCl salinity (n=4, ± SE).

| Cultivars | Organ | NaCl | Na | K | Ca | Cl | Mg | N | P |
|---|---|---|---|---|---|---|---|---|---|
| Anadolu 98 | Roots | 0 | 118±02$^{a}$ | 34±1$^{a}$ | 23±1$^{a}$ | 2540±0019$^{a}$ | 22±1$^{a}$ | 12320±10$^{a}$ | 470±1$^{a}$ |
| | | 120 | 121±02$^{a}$ | 34±1$^{a}$ | 26±1$^{b}$ | 2320±0330$^{a}$ | 21±1$^{a}$ | 10920±10$^{b}$ | 435±2$^{b}$ |
| | | 240 | 167±03$^{b}$ | 32±1$^{b}$ | 19±1$^{c}$ | 5740±0270$^{b}$ | 18±3$^{a}$ | 10080±9$^{c}$ | 411±1$^{c}$ |
| | Shoots | 0 | 309±03$^{a}$ | 31±1 | 45±1$^{a}$ | 20073±0964$^{a}$ | 55±1$^{a}$ | 40880±10$^{a}$ | 101±1$^{a}$ |
| | | 120 | 343±01$^{b}$ | 31±1 | 46±1$^{a}$ | 24150±0004$^{b}$ | 46±1$^{b}$ | 43960±15$^{b}$ | 86±1$^{b}$ |
| | | 240 | 479±03$^{c}$ | 39±1$^{b}$ | 45±1$^{a}$ | 32749±0026$^{c}$ | 42±3$^{b}$ | 39480±10$^{c}$ | 84±1$^{b}$ |
| Efes 3 | Roots | 0 | 109±01$^{a}$ | 43±1$^{a}$ | 29±1$^{a}$ | 2583±0005$^{a}$ | 24±3$^{a}$ | 12880±10$^{a}$ | 425±1 $^{a}$ |
| | | 120 | 190±01$^{b}$ | 35±1$^{b}$ | 22±1$^{b}$ | 6543±0033$^{b}$ | 20±2$^{a}$ | 11480±10$^{b}$ | 624±4$^{b}$ |
| | | 240 | 116±03$^{a}$ | 27±1$^{c}$ | 10±1$^{c}$ | 4175±0350$^{a}$ | 20±1$^{a}$ | 12320±10$^{c}$ | 557±1$^{c}$ |
| | Shoots | 0 | 205±03$^{a}$ | 28±1$^{a}$ | 54±1$^{a}$ | 32368±1485$^{a}$ | 42±1$^{a}$ | 46200±10$^{a}$ | 84±1$^{a}$ |
| | | 120 | 469±03$^{b}$ | 25±1$^{b}$ | 46±1$^{b}$ | 34746±1059$^{b}$ | 42±4$^{a}$ | 49840±10$^{b}$ | 91±1$^{b}$ |
| | | 240 | 477±21b | 26±1$^{b}$ | 49±1$^{c}$ | 35094±1652$^{b}$ | 53±1$^{b}$ | 53480±10$^{c}$ | 101±1$^{c}$ |
| Gem | Roots | 0 | 85±03$^{a}$ | 36±1$^{a}$ | 22±1$^{a}$ | 1785±0034$^{a}$ | 18±1$^{a}$ | 11200±10$^{a}$ | 641±4$^{a}$ |
| | | 120 | 99±01$^{b}$ | 36±1$^{a}$ | 24±1$^{b}$ | 3996±0256$^{a}$ | 23±1$^{a}$ | 10920±10$^{b}$ | 625±2$^{b}$ |
| | | 240 | 140±01$^{c}$ | 28±1$^{b}$ | 23±1$^{a}$ | 7181±0001$^{b}$ | 19±2$^{a}$ | 08680±10$^{c}$ | 397±2$^{c}$ |
| | Shoots | 0 | 279±14$^{a}$ | 30±1$^{a}$ | 54±1$^{a}$ | 38322±1000$^{a}$ | 50±1 $^{a}$ | 43120±10$^{a}$ | 105±1$^{a}$ |
| | | 120 | 703±03$^{b}$ | 27±1$^{b}$ | 53±1$^{a}$ | 50838±0960$^{b}$ | 48±8$^{a}$ | 41440±10$^{b}$ | 94±1$^{b}$ |
| | | 240 | 723±15$^{c}$ | 22±1$^{c}$ | 36±1$^{b}$ | 35498±0288$^{c}$ | 39±1$^{b}$ | 27160±10$^{c}$ | 82±1$^{c}$ |
| Suleyman Bey | Roots | 0 | 143±03$^{a}$ | 24±1$^{a}$ | 18±1$^{a}$ | 8524±0337$^{a}$ | 15±1$^{a}$ | 8960±10$^{a}$ | 354±3$^{a}$ |
| | | 120 | 158±02$^{b}$ | 32±1$^{b}$ | 19±1$^{a}$ | 8842±0232$^{a}$ | 16±2$^{a}$ | 10780±10$^{b}$ | 370±2$^{b}$ |
| | | 240 | 196±01$^{c}$ | 33±1$^{b}$ | 22±1$^{b}$ | 10117±0150$^{a}$ | 18±1$^{a}$ | 10920±10$^{c}$ | 642±1$^{c}$ |
| | Shoots | 0 | 730±12$^{a}$ | 25±1$^{a}$ | 34±1$^{a}$ | 56778±4144$^{a}$ | 33±1$^{a}$ | 32760±10$^{a}$ | 73±1$^{a}$ |
| | | 120 | 882±12$^{b}$ | 29±1$^{b}$ | 35±1$^{a}$ | 58757±2023$^{a}$ | 34±1$^{a}$ | 37720±10$^{b}$ | 74±1$^{a}$ |
| | | 240 | 930±12$^{c}$ | 35±1$^{c}$ | 33±1$^{a}$ | 68932±0474$^{b}$ | 33±1$^{a}$ | 38360±10$^{c}$ | 94±1$^{b}$ |
| Vamik Hoca | Roots | 0 | 126±03$^{a}$ | 36±1$^{a}$ | 21±1$^{a}$ | 6418±0811$^{a}$ | 15±2$^{a}$ | 11760±10$^{a}$ | 522±3$^{a}$ |
| | | 120 | 131±02$^{a}$ | 34±1$^{b}$ | 19±1$^{b}$ | 7089±0243$^{a}$ | 16±1$^{a}$ | 10920±10$^{b}$ | 542±1$^{b}$ |
| | | 240 | 213±15$^{b}$ | 39±1$^{c}$ | 19±1$^{b}$ | 11042±0010$^{b}$ | 15±1$^{b}$ | 8400±10$^{c}$ | 540±6$^{b}$ |
| | Shoots | 0 | 823±11$^{a}$ | 21±1$^{a}$ | 29±1$^{a}$ | 68593±2169$^{a}$ | 29±1$^{a}$ | 42280±10$^{a}$ | 81±1$^{a}$ |
| | | 120 | 970±03$^{b}$ | 27±1$^{b}$ | 34±1$^{b}$ | 50009±1993$^{b}$ | 37±1$^{b}$ | 36960±10$^{b}$ | 107±1$^{b}$ |
| | | 240 | 1093±03$^{c}$ | 27±1$^{b}$ | 32±1$^{c}$ | 67744±4602$^{a}$ | 33±1$^{ab}$ | 34440±10$^{c}$ | 99±1$^{c}$ |

Different letters indicate a significant difference at 0.05 level of probability as evaluated by ANOVA (LSD) test.

Mg content showed variability among cultivars and organs (Table 4). Upon salt exposure, Mg content of Anadolu 98 showed slight decreases in both root and shoot. Although Mg contents of Efes 3 did not change in roots, the mineral accumulated in shoots as much as 26% in the highest NaCl concentration. Mg content in roots of Gem rose up to 28% in 120 mM NaCl concentration whereas the nutrient in shoots decreased in 240 mM NaCl treatment compared to control. Suleyman Bey also showed a different response to NaCl salinity in respect of Mg content; while the nutrient concentration of roots increased in the highest NaCl concentration, shoots were not influenced by the stress. NaCl salinity did not lead to any significant change in Mg concentration of both roots and shoots of Vamik Hoca cultivar.

NaCl salinity resulted in slight differences in N concentration of roots and shoots of the barley cultivars (Table 4). In roots, N concentration was decreased in Anadolu 98 at all NaCl concentrations, in Efes 3 at 120 and 180 mM NaCl treatment and in Gem at 240 mM NaCl treatment. The highest decrease rate in roots was observed at 240 mM NaCl in Vamik Hoca. However, N concentration slightly increased up to 22% in Suleyman Bey and 16% in Efes 3 compared to the non-saline control. In case of shoots

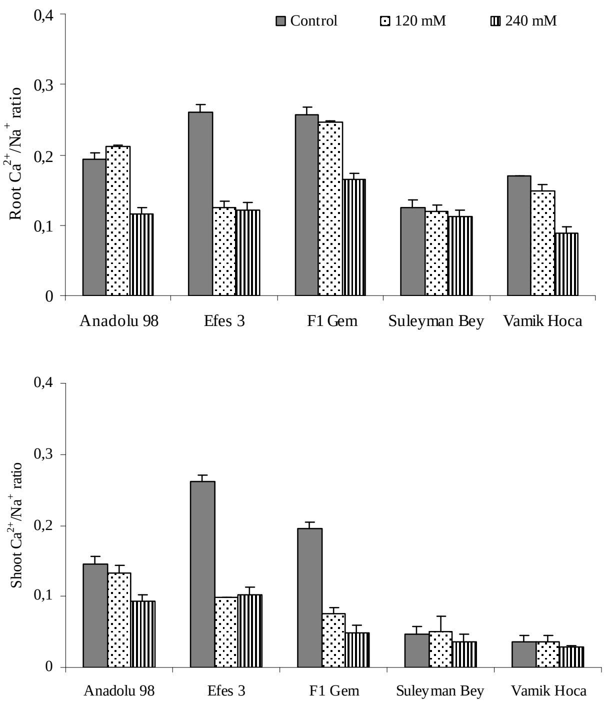


**Figure 2.** $Ca^{2+}/Na^+$ ratios in roots and shoots of four-week-old seedlings of *Hordeum vulgare* L. cultivars subjected to varying levels of NaCl salinity

of the cultivars, N concentration decreased slightly at all NaCl levels in Vamik Hoca and Gem, at 240 mM NaCl in Anadolu 98 and at 120 mM NaCl in Suleyman Bey. However it rose at all NaCl levels in Efes 3, at 120 mM NaCl in Anadolu 98 and at higher NaCl treatments in Suleyman Bey compared to their non-saline controls.

While P concentration in roots of Anadolu 98 and Vamik Hoca was not significantly changed, it was sharply increased in Efes 3 at all NaCl concentrations and in Suleyman Bey at 240 mM NaCl (Table 4). In case of shoots, salinity treatment caused a slight rise in P concentration of Suleyman Bey at 240 mM NaCl, and at all salt concentrations in Efes 3 and Vamik Hoca, the highest rate in the latter being 32% over the control.

## DISCUSSION

In our study, increasing levels of NaCl caused a marked reduction in % germination, coleoptile and embryonic root length of one week old seedlings of barley cultivars (Table 1). These data demonstrate that seedling length is a very sensitive trait related to salinity, which would be adequate for recognizing tolerance of barley cultivars to NaCl salinity in early growth stage. Our results are compatible with data of Leonova et al. (2005), Iqbal et al. (2006) who found lesser reduction in plant length due to salinity as a step toward adjustment. Also, inhibition of germination due to salinity has been reported by Megdiche et al. (2007), Abd El-Monem and Sharaf (2008), Hussain et al. (2009), Afkari (2010), Khalid et al. (2010)**,** Li et al. (2010) and Mustafa et al. (2010).

Root/coleoptile ratio in the less tolerant barley genotypes (Anadolu 98, Efes 3 and Gem) increased in response to NaCl (Table 1) which is in line with the data of Mozafar and Goodin (1986), Cicerali (2004) showing that it is a behavior characteristic to tolerance in wheat. However, root/coleoptile ratio is maintained constant in saline conditions in the more tolerant genotypes (Suleyman Bey and Vamik Hoca) (Table 1), this pointing to its possible use as a criterion for salt tolerance in the early growth stage of barley.

Growth attributes in four-week-old barley seedlings were not influenced significantly by NaCl salinity (Table 2). These results indicate that early growth stage in barley is more responsive to salt stress particularly in seedling length, which is in accordance with Kingsbury and Epstein (1984) pointing out that tolerance ability to salt stress can enhance with the age of wheat plants.

Photosynthesis is an important parameter used to monitor plant response to abiotic stress. A close association was found between growth and photosynthetic rate in sunflower (Ashraf, 1999) and wheat genotypes (El-Hendaway et al., 2005) differing in salt tolerance. Decrease of chlorophyll content of rice leaves under salinity were reported by Lutts et al. (1996) and Mitsuya et al. (2003). In contrast, Singh and Dubey (1995) and Asch et al. (1997, 2000) reported that the chlorophyll concentration of young and photosynthetically active rice leaves did not decrease but rather increased with increasing $Na^+$ or $Cl^-$ concentrations. Our data showed that the barley cultivars maintained PSII activity in leaf tissues following exposure to different NaCl concentrations. This corroborate results indicating that the rate of photosynthesis in leaves of tolerant barley genotypes was not directly affected by high $Na^+$ or $Cl^-$ concentrations (Rawson et al., 1988) and thus the cultivars maintained growth potential in saline conditions (Munns et al., 2006). In our study NaCl exposure did not cause drastic changes in chlorophyll *a* and *b* pigments, this being parallel to the results of Huang et al. (2006) on salt tolerant barley cultivars. Noticeably we established for the first time increases in carotenoid concentration in barley subjected to salt stress (Table 3). Carotenoids concentration was significantly increased in most tolerant and local Sorghum genotype under salinity treatments (Ashraf et al., 2009). This can be a common characteristic of tolerance associated with high antioxidant capacity and better protection of photosynthetic apparatus.

It is crucial for salt tolerant plants to restrict $Na^+$ and $Cl^-$ flux into meristematic and actively growing and photosynthesizing cells (Hasegawa et al., 2000). Salt tolerance in *Hordeum vulgare* L. has previously been suggested to be related to the ability to selectively partition $Na^+$ into old leaves and sheaths and $K^+$ into growing tissues (Greenway, 1962; Boursier et al., 1987). The more tolerant Suleyman Bey and Vamik Hoca showed more $Na^+$ and $Cl^-$ accumulation into shoots independent from NaCl concentrations when compared to the other less tolerant barley cultivars (Table 4). Although our data are in contrast to references that suggest an association of salt tolerance to lower Na accumulation in the shoots of barley (Forster et al., 1994; Pakniyat et al., 1997; Wei et al., 2003), they coincide with data of Leonova et al. (2005) who found that salt tolerant barley cultivars accumulated much more sodium in their roots than susceptible cultivars. This case can result in a possibility of $Na^+$ and $Cl^-$ compartmentalization in vacuoles (Rausch et al., 1996) and different activities of ion transporters, such as $Na^+/H^+$-antiporters and proton pumps in the plasmalemma and tonoplast (Ershov et al., 2005). Similarly, at high leaf $Na^+$ concentrations (200-300 mM) Munns et al. (2006) reported on the efficient cellular and subcellular partitioning of both $Na^+$ and $K^+$ in barley.

The increase of $Na^+$ in plants is generally associated with a decrease in $K^+$. Thus, maintenance of low ratios of $K^+/Na^+$ will be suitable for the metabolic processes occurring within the plants and essential for the plants to survive salt stress (Ashraf and Khanum, 1997) and $K^+/Na^+$ may be used as a possible criterion for selecting salt tolerant genotypes (Salam et al., 1999; Chen et al., 2005). The preservation of the favorable $K^+/Na^+$ ratio in the cytoplasm under salt stress may be due to an effective partitioning of both ions (Munns et al., 2006). Our results are in support of the above data, as well as of the findings of Leonova et al. (2005), Carden et al. (2003), Eker et al. (2006), Chen et al. (2007), showing a significantly higher $K^+/Na^+$ ratio in shoots of Suleyman Bey and Vamik Hoca under all salinity treatments (Fig. 1). This character is compatible with the preserved root/coleoptile length ratio in these cultivars under NaCl stress, thus pointing to their higher salt tolerance as compared to the other genotypes studied.

$Ca^{2+}$ and $Mg^{2+}$ are shown to play an important role in the salt tolerance of plants by maintaining the structural integrity and functions of membranes and cell walls (Marschner, 1995). Recent studies showed that increase $Ca^+$ concentration in gourd and melon plants challenged with salinity stress could ameliorate the inhibitory effects of salinity stress on plant growth (Navarro et al., 2003; Kaya et al., 2003; Yetişir and Uygur, 2009). We observed a higher accumulation of shoot $Ca^{2+}$ and $Mg^{2+}$ and preserved $Ca^{2+}/Na^+$ ratio in the shoots under saline environment in the more tolerant Suleyman Bey and Vamik Hoca as compared to the decreasing trend observed in the other less tolerant cultivars (Fig. 2). This finding was reported for the first time in barley exposed to salt stress and could be proposed as a criterion for salt tolerance in this crop.

Plant N concentration was reported to decrease with increasing salinity in different species and culture systems (Bolarin et al., 1993; Hunt and Layzell, 1993; Sadiki and Rabih, 2001; Garg and Singla, 2004; Abdelly et al., 2005; Tejera et al., 2006; Krouma, 2009). In our study, the nitrogen percentage of most of the cultivars was decreased as salinity elevated in the medium. This result would be in agreement with the well-known $Cl^-$ antagonism with nitrate uptake (Knight et al., 1992). However, Klein et al. (1994) and Iqbal et al. (2006) reported an increase in shoot N concentration of salt tolerant Manzanillo olive cultivar and of three tolerant wheat cultivars with increased NaCl salinity in the growth medium. Our data confirmed these reports showing for the first time that in barley an increase in the shoot and root nitrogen percentage under saline conditions occurs in Suleyman Bey, thus pointing to its higher salt tolerance (Table 4).

NaCl treatments increased P concentrations in shoot and root (Yahya, 1998; Taban and Katkat, 2000). According to Roberts et al. (1984) such increase of P level in maize is the result of enhanced rates of uptake by the roots and of translocation to the shoots and not a concentration effect due to growth depression. Our results on the increased P accumulation in Suleyman Bey and Vamik Hoca are in agreement with these data (Table 4). On the other hand, reduction in the P concentration of the tissue with elevated salinity was reported in other plant species (Award et al., 1990; Al Karaki, 1997; Shibli et al., 2003). Similarly to these findings, shoot P concentration in Anadolu 98 and Gem declined in response to salt stress. These results indicate that in regard to salinity-induced P uptake distinct differences can exist between cultivars of the same species as also shown by Grattan and Maas (1984) for soybean. Thus, we may claim that higher P accumulation points to the differential salinity tolerance of the barley genotypes studied, and may be suggested as a tolerance-related criterion.

In conclusion, the photosynthetic traits and biomass production ability measured under NaCl treatments indicate that all barley cultivars studied can be regarded as salt tolerant. However, the better maintaining of $K^+/Na^+$ and $Ca^+/Na^+$ ratios and higher accumulation of $Mg^{2+}$ and P in the shoots, as well as the preserved root/coleoptile length ratio at the early germination stage in the most tolerant barley cultivars may be considered as additional salt tolerance criteria, allowing differentiation between barley genotypes.

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