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A preliminary study on salt tolerance of some barley genotypes

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Abstract

In this study, salt (NaCl) tolerance of five barley genotypes (Avcı-2002, Aydanhanım, Bülbül-89, Orza-96, Tarm-92) was investigated. These genotypes were exposed to different salt concentrations (0 mM (control), 100 mM, 200 mM and 300 mM) and then some basic growth parameters (root length, shoot length total plant length, fresh weight, dry weight) were determined on the 18 d old plants. Tolerance indices for each genotype were calculated on the basis of root and shoot length. Salt stress significantly inhibited root and shoot growth in all genotypes, except for genotype Bülbül-89. Inhibition degree of root and shoot growth was found to be proportional to the salt concentrations applied. Maximum inhibitory effect of salinity on root and shoot growth was observed in genotype Avcı-2002 at maximum salinity level (300 mM), while Bülbül-89 was less affected by the same level of salinity. In addition, salt stress disturbed water relation in barley genotypes dependent on the organ type, as demonstrated by more severe inhibition in shoot fresh weight as compared to root fresh weight. These results may show that salt stress reduced translocation of water from roots to shoots rather than water uptake from growth medium in barley genotypes used in this study. Changes in dry weight of roots and shoots indicated that salt stress more severely reduced biomass accumulation in roots in barley genotypes. The calculated tolerance indices demonstrated that Bülbül-89 is the most tolerant barley genotype to salinity, while Avcı-2002 was the most susceptible one.

Keywords: Barley, *Hordeum vulgare*, salt tolerance, tolerance indices

1. INTRODUCTION

Salinity is one of the major problems restricting agricultural production, especially in arid and semi-arid areas [1]. The concept of soil salinity indicates the amount of soluble salts present in the soil in the unit volume [2]. Soluble salts are the natural components of the soil and many of them are nutrients necessary for plants. However, the accumulation of these salts in the soil prevents the cultivation of the crop plants and reduces agricultural yield. The reason for this is that high salt concentrations are toxic to plants and cause

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the physicochemical structure of the soil to deteriorate [3]. One of the most important causes of soil salinity is the release of various types of soluble salts such as Na^{+1}, Ca^{+2}, Mg^{+2}, sulfate and carbonate from the parent rocks by the influence of climatic factors and to participate in soil structure [4]. Another source of salt accumulation in agricultural land is the height of the groundwater. As the groundwater rises, the salts found in the lower layers of the soil come out of the upper layers and the soil fertility starts to decrease [5]. Sodium chloride (NaCl) is the most important salt compound that causes salinity in the soil. It is also the most toxic type of salt for plants because of its high solubility [5].

Salinity is one of the most important problems of the earth [7]. Approximately 1.5 million hectares of our country's land are also facing salinity problems [8]. According to the latest estimates, over 800 million hectares of agricultural land in the world are affected by salinity [9]. This amount corresponds to more than 6% of the total agricultural land on the earth. The danger of salinization is continuously increasing for our fertile soils that can still be cultivated.

Soluble salts in the soil can be easily taken up by plants. Salt stress could damage plants in different ways. High soil salinity, for example, may reduce the osmotic potential of the soil solution and cause physiological drought for plants. In addition, salt stress reduces the growth rate of plants by disrupting mineral nutrition. Finally, Na^{+} and Cl^{-} ions may represent specific toxic effects for plant growth and development [10, 11]. All of these factors lead to multiple negative effects on plant growth and development at the physiological, biochemical and molecular levels. For example, it has been reported that salinity reduces plant growth by slowing down mitosis in the meristematic cells [12, 13]. Hasanuzzaman et al. (2009) found that salt stress significantly reduced total plant height and leaf area in rice plants [14]. Guan et al. (2011), on the other hand, have demonstrated that salt stress significantly reduced total plant height, number of side branches as well as shoot thickness in *Suaeda salsa* [15]. Salt stress also affects fresh and dry weights of various organs in plants. For example, salt stress has been found to reduce the total plant dry weight in *Raphanus sativus* [16]. Leidi and Saiz (1997) observed that salt stress reduced dry weight in cotton plant [17]. Kurban et al. (1999) have reported that low salt concentrations (50 mM) increased total plant weight in *Alhagi pseudoalhagi* while higher salt concentrations (200 and 300 mM) caused lower plant weight [18]. However, cultivated plants exhibit a wide variation in susceptibility to salt stress. It has been well known that plants are more susceptible to salt stress at the early seedling stage [19].

It has been reported that the salts found in the soil could be removed by washing after the drainage system has been provided [20]. However, this method is impractical because of being expensive. The second and most economical method for reclaiming these areas is to select and cultivate salt tolerant plant species and genotypes [21]. In environments containing high concentrations of various soluble salts, the ability of plants to grow and complete their life cycle is known as salt tolerance [22]. It has been determined that salt tolerance of barley varies depending on genotypes.

Accordingly, the aim of this study is to examine the effects of salt (NaCl) stress in some growth parameters of five different barley genotypes [Avcı-2002 (six-row feed barley), Aydanhanım (two-row malting barley), Bülbül-89 (two-row feed barley), Orza-96 (two-row feed barley) and Tarm-92 (two-row feed barley)] which are commonly grown in Turkey. In addition, tolerance indices were calculated in order to determine the variation between barley genotypes in terms of salt tolerance.

2. MATERIAL AND METHODS

2.1. Plant Material, Growth Conditions and Experimental Design

Seeds of barley cultivars (*Hordeum vulgare* L. cvs. Avcı-2002, Aydanhanım, Bülbül-89, Orza-96 and Tarm-92) were obtained from Field Crops Central Research Institute, Ankara, Turkey. All seed samples were surface sterilized in 5% sodium hypochlorite solution for 10 minutes before sowing. After washing in distilled water, three seeds of each cultivar were sown in plastic pots (14 cm in diameters) containing perlite. The experiment was performed in a controlled growth chamber (25/20 °C day-night temperature) under a PPFD of 200 µmole m^{-2} s^{-1} light intensity, with a photoperiod of 16 h and 50±5 relative humidity. The perlite moisture was maintained at field capacity for 12 days (d), after which some of the pots from each cultivar were exposed to salt stress (100, 200 and 300 mM NaCl) for 6 d. The control plants were watered for additional 6 d with Hoagland nutrient solution. At the 18th d of the experiment, plants were harvested and morphological measurements were performed immediately.

2.2. Determination of root and shoot length

Measurements of root and shoot length were done with a millimetric ruler on 7 plants from each treatment and 3 independent replicates (n=21). The longest root was taken into consideration for measurement. Root length, shoot length and total plant length were expressed as cm $plant^{-1}$.

2.3. Determination of fresh and dry weight

After harvesting, fresh weights (FW) of roots and shoots were determined separately (7 plants from each treatment and 3 independent replicates; n=21). Dry weight (DW) of roots and shoots was measured after drying in hot-air oven at 80 °C for 2 d. The fresh and dry weight of roots and shoots were expressed as g $plant^{-1}$.

2.4. Calculation of tolerance indices

Tolerance indices (TI) were calculated on the basis of root length (RL) and shoot length (SL) according to the following formula [23]:

$$TI\ (\%) = (RL\ or\ SL_{NaCl} / RL\ or\ SL_{Control}) x100$$

According to the calculated tolerance indices for each salt concentration applied, five different barley genotypes were scored between one and five. One score was given to the genotype with minimum tolerance indices while five score was given to the genotype with maximum tolerance indices. Then, the scores obtained for each salt concentration for each genotype were added to obtain the total score [24].

2.5. Statistical analysis

The experimental design was a complete randomised block with three independent replicates. The significance of difference between controls and applications (mean values) was determined by one-way ANOVA at 95% confidence level by using SPSS 11.0 statistical program for Windows. Means and s.e. values represent seven replicates (n=7) for all measurements.

3. RESULTS AND DISCUSSION

Decreased growth rate in plant species has been considered as one of the most obvious effects of salt stress [25]. Growth ability of plants under salt stress has been used as a common screening criterion to determine salt tolerance degree of plant genotypes [22]. In addition, identification of plant species and genotypes that are tolerant to high soil salinity is very important for reclamation of salinized areas for agricultural purposes [26]. In our study, it was observed that salt stress decreased root growth of all barley genotypes significantly, except for genotype Bülbül-89, as compared to controls (Figure 1A) ($P<0.05$). Also, our results showed that inhibition degree of growth in roots was proportional to the salt concentrations applied. The inhibitory effect of salt stress on root growth was observed in all barley genotypes used in this study. However, root growth in Avcı-2002 was found to be the most susceptible to salt concentrations, while Bülbül-89 was the most tolerant genotype because of insignificant decrease in root growth as a result of salt applications. Root growth in Avcı-2002 and Bülbül-89 was inhibited by 33% and 15% at 300 mM salt application, as compared to controls, respectively. Similarly, salt stress decreased shoot growth rate in barley genotypes in a concentration dependent manner as well (Figure 1B). Furthermore, our results showed that roots and shoots of barley genotypes used in this study represented considerable variation with respect to salinity tolerance and sensitivity. In Aydanhanım, for example, root growth was found to be more sensitive to salt stress while shoot growth was more tolerant. In Bülbül-89 and Orza-96, on the other hand, shoot growth was sensitive and root growth was tolerant to salinity. These results are in accordance with the findings of Zaimoğlu and Doğru [27], who reported that sensitivity and/or tolerance degree of different maize genotypes may show great variation under saline conditions. Similarly, root and shoot growth was decreased more severely as the salt concentration increased in our study, as indicated by Pessarakli et al. [28] in *Distichlis spicata* plants. It has been reported that salt stress could reduce mitotic activity in the meristematic cells in plants, depending on the plant species, genotype, salt concentration and exposure time [29]. Accordingly, we may conclude that mitotic activity in the salt-stressed barley genotypes used in this study showed considerable variation in an organ dependent manner. Total plant length, on the other hand, was found to be significantly lower than respective controls in all salt-stressed barley genotypes, possibly due to cumulative effect of decreased root and shoot growth (Figure 1C).

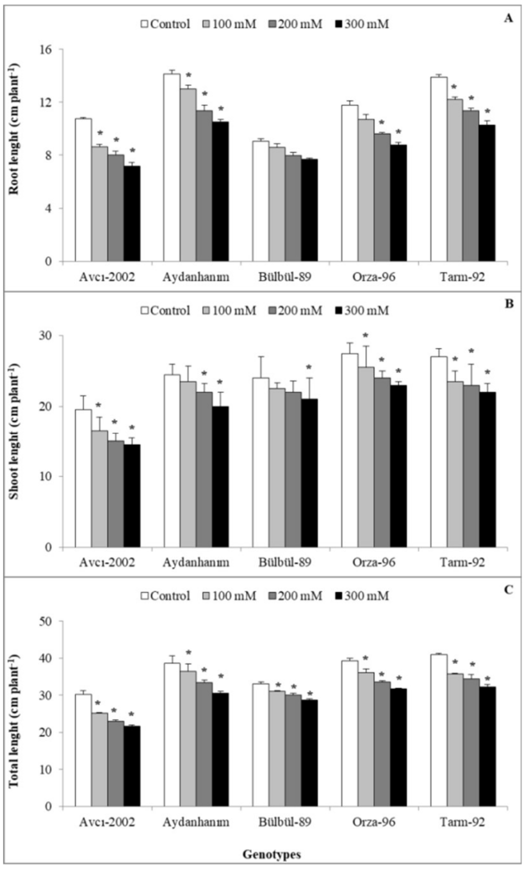

Figure 1. The effect of salt stress on (A) root length, (B) shoot length and (C) total length in barley genotypes (Significant differences from controls ($P<0.05$) are marked with an asterisk. Abbreviations and statistical evaluations are the same for the following figures and table(s)).

Genotype Avcı-2002 had the lowest tolerance indices in root and shoot among all barley cultivars used in this study. This showed that meristematic cells located in the root and shoot tips were highly sensitive to salinity (Table 1). Bülbül-89, on the other hand, represented the highest tolerance indices in roots and shoots and was found to be the most salt tolerant barley genotype (Table 1). Total score, calculated on the basis of root and shoot length, confirmed our results as well. It was 6 for Avcı-2002 and displayed the highest salt-induced damage, while Bülbül-89 was less affected by salinity and maintained root and shoot growth, with a 29 of total score (Table 1). Shannon and Grieve [30] reported that one of the most important physiological growth parameters used to determine differences in salt tolerance between different plant species and genotypes is the growth rate of root and shoot. Anjum [31] has exposed two different *Citrus* rootstocks (Cleopatra and Troyer) to 40 and 80 mM salt stress and concluded that Cleopatra is more tolerant to salinity because of maintaining root and shoot growth under saline conditions. Several studies have demonstrated that salt stress decreased plant growth by causing hormonal imbalance [18], physiological drought [19], ion toxicity [4], enzyme inactivation [20], inhibition of protein synthesis [21] and oxidative stress [22]. However, these probabilities remained to be investigated in our study.

Our results showed that salt stress did not significantly affect fresh weight of roots in Avcı-2002, Aydanhanın and Orza-96 (Figure 2A) ($P>0.05$). However, in Bülbül-89 and Tarm-92, especially higher salt concentrations (200 and 300 mM) led to the relatively and significantly lower fresh weight in roots as compared to controls ($P<0.05$). The lower level of root fresh weight indicates severe impairment of water uptake from growth medium in Bülbül-89 and Tarm-92. Shoot fresh weight, however, was more severely and significantly affected by salt stress in barley cultivars except for Aydanhanım (Figure 2B) ($P<0.05$) when compared to respective controls and root fresh weights.

Table 1. Tolerance indices and total scores of barley cultivars under salt stress

	Root			Shoot			
	Tolerance indices (%)			Tolerance indices (%)			
Genotypes	100 mM	200 mM	300 mM	100 mM	200 mM	300 mM	Total score
Avcı-2002	80,4 (1)*	74,5 (1)	66,7 (1)	84,6 (1)	76,9 (1)	74,4 (1)	**6****
Aydanhanım	92,1 (4)	80,6 (2)	74,6 (3)	95,9 (5)	89,8 (4)	81,6 (3)	21
Bülbül-89	94,8 (5)	88,3 (5)	85,1 (5)	93,8 (4)	91,7 (5)	87,5 (5)	**29****
Orza-96	90,6 (3)	81,7 (3)	74,3 (2)	92,7 (3)	87,3 (3)	83,6 (4)	18
Tarm-92	87,8 (2)	81,8 (4)	73,8 (4)	87,0 (2)	85,2 (2)	81,5 (2)	16

* Values in the parenthesis indicate the scores given according to tolerance indices

** The highest score indicates the lowest damage and the lowest score indicates the highest damage

Therefore, changes in root and shoot fresh weights clearly indicated that salt stress affected water relations between barley plants and environment, depending on the salt concentration, organ type and genotype.

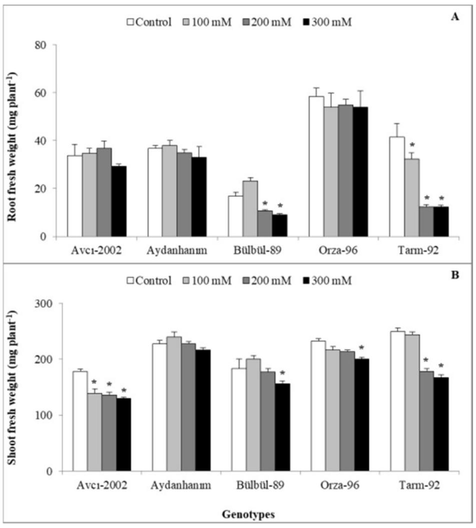

Figure 2. The effect of salt stress on (A) root fresh weight and (B) shoot fresh weight in barley genotypes

It has been reported that adjustment of water balance in the tissues of plants under biotic and abiotic stresses is a very important factor in terms of stress tolerance [32]. Several studies have indicated that decreases in the osmotic potential and water content in salt-sensitive plant species and genotypes are more prominent than tolerant species and genotypes [33]. In addition, it has been well known that fresh weight and water content in leaves is the most reliable suggestive indicator about physiological status in the salt-stressed plants [34]. Consequently, we may conclude that Avcı-2002, represented the lowest shoot fresh weight, is the most salt susceptible genotypes used in our study while Bülbül-89 was the most tolerant barley genotypes to salinity among others, with the highest shoot fresh weight. Similar to fresh weight, dry weights of barley genotypes were also affected by salt stress. Root dry weight was significantly lowered in Avcı-2002, Aydanhanım and Tarm-92 as compared to controls (Figure 3A) ($P<0.05$). In Bülbül-89 and Orza-96, however, it was not changed significantly as a result of salt applications ($P>0.05$). Shoot dry weight was found to be less affected in barley genotypes and only higher salt concentrations (200 and 300 mM) reduced shoot dry weight in Tarm-92 significantly (Figure 3B) ($P<0.05$). Obviously, our results showed that salt stress inhibited root metabolism more severely than leaves in barley cultivars used in this study, causing reduced biomass accumulation. The decrease in biomass accumulation in plants under salt stress has mostly been attributed to ion imbalance [12]. Davenport et al. [35] and Munns and Tester [36], for example, have stated that salt stress decreased photosynthetic activity and biomass in plants by reducing activities of some enzymes which are responsible for carbon fixation and pigment synthesis as a result of Na^+ accumulation in plant tissues.

In summary, our preliminary study showed that there is a broad variation between barley cultivars in terms of salt tolerance. We found that salt stress reduced root and shoot growth rate more severely than other physiological growth parameters evaluated in this study. Tolerance indices, calculated on the basis of root and shoot length, indicated that Bülbül-89 is the most salt-tolerant barley genotype and Avcı-2002 is the most susceptible one.

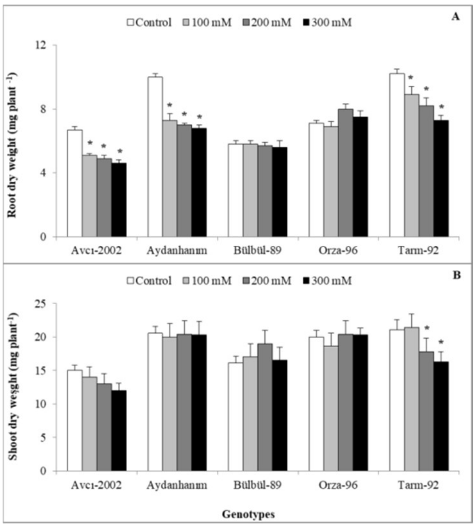

Figure 3. The effect of salt stress on (A) root dry weight and (B) shoot dry weight in barley genotypes

Aydanhanim, Orza-96 and Tarm-92, on the other hand, were found to be moderately tolerant to salinity. Fresh and dry weight of roots and shoots showed that salt stress affected water relation and biomass accumulation in barley plants depending on the genotype, salt concentration and organ type. However, we believe that changes in root and shoot growth rate in barley genotypes under salt stress may be used as a more reliable criterion for salt tolerance selection. In this study we have only identified the most salt-susceptible and salt-tolerant barley genotypes. Our future aim is to investigate the physiological and biochemical basis of salt responses of these two genotypes and our detailed experiments have been still continuing.

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