



## Relationship between salinity stress and ion uptake of hyacinth (*Hyacinthus orientalis*)

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### Abstract

Salinity is one of the most severe environmental factors limiting the agricultural productivity. Salt stress is also of rising importance in landscaping. Salinity is a reality in coastal area and in countries where de-icing salts are applied to roadways. Plants are affected in a different way by amount of salt and depending on their growth and development stage. Major saline ions can affect nutrient uptake which may cause reductions in plant growth. Hyacinth (*Hyacinthus orientalis* L.) is grown worldwide as a commercially important bulbous ornamental plant. In this study the effect of salt stress on ion uptake of hyacinth plants were investigated. The plants were irrigated with different NaCl concentration by the addition of 0, 25, 50, 75, 100, 200, 400 and 600 mM for 15 days with two days intervals in soil medium under controlled conditions. Salt treatments were imposed to plants at the beginning of flowering stage. According to the results Na<sup>+</sup> uptake were significantly increased by salt stress. The higher concentration of NaCl treatments (75 mM and upper) caused sharply reduction on K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios. This study showed that measuring of Na<sup>+</sup> concentrations and calculating the ratios of K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup>, in leaf tissue are reliable physiological parameters for predicting salt damage of hyacinth plant.

**Keywords:** Salinity, hyacinth, ion concentrations, bulbous plant

### Sümbül bitkisinde (*Hyacinthus orientalis*) tuzluluk stresi ve iyon alımı arasındaki ilişki

#### Özet

Tuzluluk tarımsal verimliliği sınırlayan en ciddi çevresel faktörlerden biridir. Tuz stresinin önemi peyzaj düzenlemelerinde de giderek artmaktadır. Tuzluluk, deniz kıyısındaki bölgelerin ve yol buzlanmalarına karşı tuz uygulamasının yapıldığı ülkelerin önemli bir gerçeğidir. Bitkiler tuz stresinden stresin şiddeti ve bitkinin büyüme ve gelişme aşamasına bağlı olarak farklı şekillerde etkilenmektedir. Tuzluluğa yol açan iyonları, bitki besin elementi alımını etkileyerek bitki büyümesinde azalmaya yol açabilmektedir. Sümbül (*Hyacinthus orientalis* L.) ticari olarak önemli bir soğanlı süs bitkisi olarak dünya çapında yetiştirilmektedir. Bu çalışmada sümbül bitkisinin iyon alımı üzerine tuz stresinin etkisi araştırılmıştır. Bitkiler, kontrollü koşullar altında, toprak ortamında 0, 25, 50, 75, 100, 200, 400 ve 600 mM tuz konsantrasyonu ile iki gün aralıklarla 15 gün süre boyunca sulanmıştır. Tuz uygulamaları bitkilere çiçeklenme döneminin başında uygulanmıştır. Sonuçlara göre, tuz stresinin artışı ile Na<sup>+</sup> alımı önemli ölçüde artmıştır. Yüksek NaCl uygulamaları (75 mM ve üstü) K<sup>+</sup>/Na<sup>+</sup> ve Ca<sup>2+</sup>/Na<sup>+</sup> oranlarında keskin bir düşüşe neden olmuştur. Bu çalışma, yaprak dokularında Na<sup>+</sup> konsantrasyonunun ölçülmesi ve K<sup>+</sup>/Na<sup>+</sup> ve Ca<sup>2+</sup>/Na<sup>+</sup> oranlarının hesaplamasının sümbül bitkisinde tuz zararının tahmin edilmesinde için güvenilir fizyolojik parametreler olduğunu göstermektedir.

**Anahtar Kelimeler:** Tuzluluk, sümbül, iyon konsantrasyonu, soğanlı bitki

## Introduction

Salinity is a problem for plant production worldwide, which can affect not only the yield of ornamental plants, but also their quality. Soil and water that contain salt naturally, causes problems in plant growth (Grattan and Grieve, 1999). Ornamental plants are used to improve the aesthetic quality of urban and rural landscapes, recreational areas and commercial sites. In relation to numerous ornamental plants species that can potentially be utilized in the landscape, the facility of choosing genotypes able to cope with salt stress is high. Sometimes in marginal conditions plant survival is the only aim of cultivation. However, to promote the use of salinity waters or soils while minimizing salt injury, the salt response of ornamentals needs to be determined (Niu and Rodriguez, 2006; Cassaniti et al., 2012).

Salinity is of rising importance in ornamentals. Because of the increase of green areas in the urban environment the demand for water supply is increasing (Navarro et al., 2008; McCammon et al., 2009; Cassaniti et al., 2012). The shortage of good quality water in many regions has become a concern as the competition among agricultural, urban, industrial, environmental, and recreational groups continues to increase (Valdez-Aguilar et al., 2009a). Scarcity of water has led to the use of alternative water sources for irrigation. Alternative water sources might be recycled water, treated municipal effluent and brackish ground water, all of which generally have higher levels of salts (Niu et al., 2007). Salinity is also a reality to plants that grow on coastal gardens and landscapes (Parida et al., 2002; Ferrante et al., 2011). It is likewise a great problem of countries where large amounts of de-icing salts are applied (Townsend and Kwolek, 1987; Cassaniti et al., 2012).

Salinity is of concern because of its detrimental effect on plant growth, nutritional balance, and plant and flower marketable quality, including visual injury, flower distortion, and reduced stem length. Plant growth is affected by salinity as a result of the disruption of certain physiological processes that lead to reductions in yield and/or quality (Valdez-Aguilar et al., 2009a; Aydınşakir et al., 2010). The deleterious effects of salinity on plant growth are associated with low osmotic potential of the soil solution (secondary drought), nutritional imbalances (nutrient disorders), specific ion effects (sodium or chlorine toxicity) or a combination of all those factors (Grattan and Grieve, 1999; Parida and Das, 2005). Most horticultural crops are glycophytes and range from salt-sensitive to moderately salt-tolerant (Greenway and Munns, 1980). Floral and nursery

growers of ornamental species are unwilling to utilize poor quality water supply for irrigation because they assume floricultural species to be highly salt sensitive. However, some studies have denoted that moderately saline waters can be used to irrigate certain ornamental species without compromising economic value (Carter and Grieve, 2008; Friedman et al., 2007; Grieve et al., 2005; Shillo et al., 2002). Salt tolerance does, however, vary considerably among the different species of ornamentals which are used in landscaping (Cassaniti et al., 2012). On the other hand, little information is available to growers of ornamentals, however, on the limits salinity places on the growth, yield, and quality of many ornamental species. In the same way, landscape designers and gardeners have few guidelines for selection of plant species suitable for sites where soils are saline and/or irrigation waters are high in salinity (Valdez-Aguilar et al., 2009a).

It is known that hyacinth plant is sold commercially at the beginning of flowering. At this stage, hyacinth can be considered as an outdoor plant or an indoor plant in pots. Therefore, stress factors are considerably important to hyacinth plants on flowering stage. In this study the effect of salt stress on ion uptake of hyacinth (*Hyacinthus orientalis* L.) plants on flowering stage was investigated. For this propose, we have focused mainly on total plant fresh weight, relative fresh weight rate of plants, and  $K^+$ ,  $Ca^+$  and  $Na^+$  concentrations of leaves tissue as morphological and physiological parameters.

## Materials and Methods

This study was conducted in greenhouses at the Department of Horticulture, Cukurova University in Adana/Turkey. In this study *Hyacinthus orientalis* L. cv. 'Jan Boss' bulbs are used as a plant material. Salt treatments started a time close to the plants to blossom. The plants were irrigated with different NaCl concentration by the addition of 0, 25, 50, 75, 100, 200, 400 and 600 mM for 15 days with two days intervals in soil medium under controlled conditions. Soil medium were preferred for representing use in landscaping.

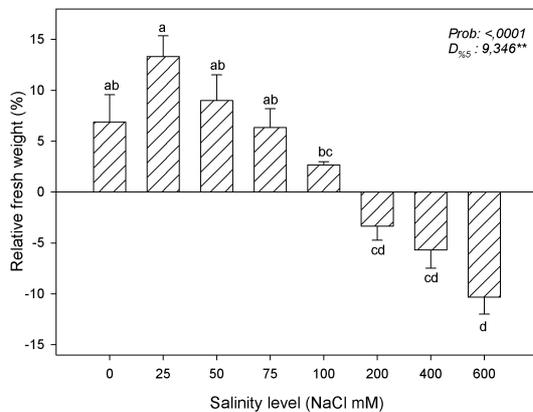
At the end of the experiment, after the harvesting, plants were uprooted carefully and washed thoroughly in a running tap water to remove soil particles. After rinsing with deionized water the fresh samples were weighed using a digital top loading weighing balance (Mettler AE 100) to determine the total fresh weight. Relative fresh weight rate was calculated from the change in fresh weight of plants at the beginning and at the end of the salt treatments. Fresh weight

rate to control was calculated from weights changing on plants which are salt stressed. Samples were oven dried for four days at 70°C. The dry leaves materials were ground. In this study, K<sup>+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup> were determined by Atomic Emission Spectrometer (AES) (Alkan-Torun et al., 2013). After determining ion concentrations, K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios were also calculated.

Data were subjected to ANOVA and means were separated by using the Tukey test at  $P \leq 0.05$ . All the statistical analyses were performed by using JMP software packages.

## Results

In general, relative fresh weight rate hyacinth plants varied following salinity treatments (Figure 1). According to the beginning of the salinity stress period, the rates of relative fresh weight of plants under salinity level up to 100 mM NaCl showed an increasing trend. However, the higher salinity treatments (200 mM and upper) had negative effects on the relative fresh weight rate. During salinity treatment period, the highest relative fresh weight rate was determined in the lowest salinity treatment (25 mM). On the other hand, the lowest relative fresh weight rate was found at 600 mM which is the highest NaCl stress condition.



\*Values are means from three replications and bars indicate standard errors. Values indicated by the different letters are statistically different at  $P \leq 0.05$  (Tukey test).

**Figure 1.** Relative fresh weight rate of hyacinth in terms of plant weight change between at the beginning and at the end of the experiment.

At the end of the experiment, total fresh weight (FW-g) and fresh weight rate to control (%) of hyacinth plants under salinity stress (NaCl) were given in Table 1. There was a significant variation in total plant fresh weight in response to salinity stress. The highest fresh weight was obtained at 25 mM NaCl level at the rate of  $\sim 113.33$  g.plant<sup>-1</sup>

while the lowest one was determined at 600 mM NaCl level, at the rate of  $\sim 89.67$  g.plant<sup>-1</sup>. Even though the total plant fresh weights were increased by salinity treatments up to 100 mM NaCl levels, higher concentrations of NaCl (200, 400 and 600 mM) caused reduction in total plant fresh weight in comparison to control (0 mM NaCl).

**Table 1.** Fresh weights (FW-g) and fresh weight rate to control (%) of hyacinth plants under salinity stress (NaCl).

NaCl (mM)	FW (g)	FW rate to control (%)
0	99.33±1.45 <sup>bcd y</sup>	0.00
25	113.33±2.03 <sup>a</sup>	12.86
50	109.00±2.52 <sup>ab</sup>	8.72
75	106.33±1.86 <sup>ab</sup>	5.82
100	102.67±0.33 <sup>abc</sup>	3.24
200	98.00±3.06 <sup>bcd</sup>	-3.36
400	95.00±3.51 <sup>cd</sup>	-6.38
600	89.67±1.67 <sup>d</sup>	-9.17
Prob>f	<.0001	--
D <sub>55</sub>	11.024**	

±Values are means from three replications and standard errors

∧Values in columns followed by the different letters are statistically different at  $P \leq 0.05$  (Tukey test). \*:  $P \leq 0.05$ , \*\*:  $P \leq 0.01$ .

Ion concentrations (K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>) of leaf tissues of *Hyacinthus orientalis* L. cv. 'Jan Boss' plants grown under NaCl stress were shown in Table 2. As shown in results Ca<sup>2+</sup> and Na<sup>+</sup> concentrations of hyacinth leaves significantly affected by salinity treatments. However, significant alteration on K<sup>+</sup> concentration of leaf tissues was not detected. The results showed that Ca<sup>2+</sup> concentration was the highest at 100 mM NaCl level and the lowest at 25 mM NaCl level. Sodium concentrations increased with increase in salinity. The maximum Na<sup>+</sup> concentration (approximately 0.75%) was found at 600 mM NaCl level, which is the highest NaCl stress condition, followed by 75, 100, 200 and 400 mM. Sodium concentrations of leaf tissues of hyacinth plants grown under lower NaCl concentrations (25 and 50 mM) were at the same level with control plants. Ratio of K<sup>+</sup>/Na<sup>+</sup> of hyacinth leaves significantly affected by salinity (Figure 2). and decreased dramatically with NaCl treatment. Ratios of K<sup>+</sup>/Na<sup>+</sup> were significantly lower in high levels of salt treatments (75, 100, 200, 400 and 600) compared to those in control and low levels of salt treatments (25 and 50 mM).

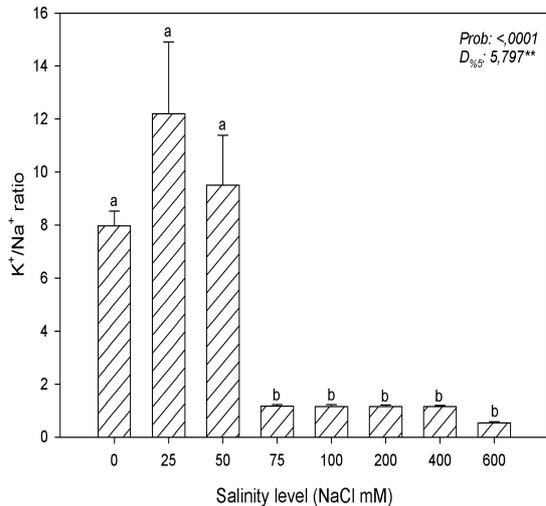
**Table 2.** Ion concentrations of hyacinth leaves under salinity stress (NaCl)

NaCl (mM)	$K^+$	$Ca^+$	$Na^+$
		(%)	
0	0.41±0.02	0.47±0.018 <sup>aby</sup>	0.05±0.00 <sup>c</sup>
25	0.40±0.03	0.44±0.019 <sup>b</sup>	0.04±0.01 <sup>c</sup>
50	0.39±0.01	0.55±0.060 <sup>ab</sup>	0.05±0.01 <sup>c</sup>
75	0.41±0.01	0.52±0.020 <sup>ab</sup>	0.36±0.01 <sup>b</sup>
100	0.39±0.00	0.59±0.026 <sup>a</sup>	0.34±0.02 <sup>b</sup>
200	0.40±0.02	0.55±0.008 <sup>ab</sup>	0.35±0.00 <sup>b</sup>
400	0.41±0.00	0.49±0.024 <sup>ab</sup>	0.36±0.01 <sup>b</sup>
600	0.40±0.01	0.53±0.001 <sup>ab</sup>	0.75±0.04 <sup>a</sup>
<i>Prob&gt;f</i>	0.9364	0.0257	<.0001
<i>D<sub>5%</sub></i>	<i>ns</i> <sup>2</sup>	0.135*	0.087**

±Values are means from three replications and standard errors

<sup>y</sup>Values in columns followed by the different letters are statistically different at  $P \leq 0.05$  (Tukey test).

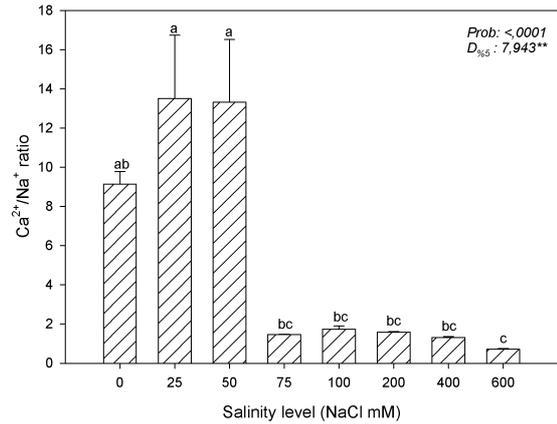
<sup>2</sup>ns: not significant, \*:  $P \leq 0.05$ , \*\*:  $P \leq 0.01$ .



Values are means from three replications and bars indicate standard errors. Values indicated by the different letters are statistically different at  $P \leq 0.05$  (Tukey test).

**Figure 2.**  $K^+/Na^+$  ratios of leaves of hyacinth plants under salinity stress (NaCl).

Salt treatments had significant effect on  $Ca^{2+}/Na^+$  ratios of hyacinth leaves (Figure 3). Even though  $Ca^{2+}/Na^+$  ratios evaluated in the study were significantly higher at 0, 25 and 50 mM NaCl in comparison with all NaCl treatments, the highest  $Ca^{2+}/Na^+$  ratios were found at 25 and 50 mM NaCl level. The higher concentration of NaCl treatments (75 mM and upper) caused a sharp reduction in  $Ca^{2+}/Na^+$  ratios. The lowest  $Ca^{2+}/Na^+$  ratio (~0.71) was determined at 600 mM NaCl level which is the highest NaCl stress condition.



Values are means from three replications and bars indicate standard errors. Values indicated by the different letters are statistically different at  $P \leq 0.05$  (Tukey test).

**Figure 3.**  $Ca^+/Na^+$  ratios of leaves of hyacinth plants under salinity stress (NaCl).

### Discussion

Several authors claimed that NaCl reduced plant growth (Aydınsakir et al. 2010; Niu, and Cabrera, 2010; Veatch-Blohm et al., 2013) and similarly in this study, relative fresh weight rate and total plant fresh weight were significantly reduced in plants under high salinity treatment (>100 mM NaCl). Reduced in plant growth of *H. orientalis* L. cv. 'Jan Boss' with salinity stress (>75 mM NaCl) was determined by Köksal and Külahlioglu (2013). On the other hand, Türkoglu et al., (2011) reported that salinity stress on hyacinth plants was kept under control at 50, 100 and 200 mM NaCl.

In many studies, it is well demonstrated that increasing of NaCl stress level induces  $Na^+$  content of plants (Grattan and Grieve, 1999; Parida and Das, 2005; Nui et al., 2007). Similar results with previous papers were obtained in this study. Increasing of  $Na^+$  contents of hyacinth leaves under salinity stress (>75 mM) were significantly important in our study.

It is known that high NaCl induces calcium and potassium deficiencies in many plants (Valdez-Aguilar et al., 2009b; Parida and Das, 2005) However, in our study, we did not detect marked calcium and potassium deficiencies on hyacinth plants grown under NaCl stress. Besides, Veatch-Blohm and Morningstar (2011) determined that  $Ca^{2+}$  concentrations of calla lily shoots were increased by 25 and 50 mM NaCl despite high  $Na^+$  concentrations. They hypothesized that the maintenance of visual quality may in part be the result of increased  $Ca^{2+}$  concentration (up to four times the amount in the control) in the shoots of the salt-stressed plants. Similarly, in our study  $Ca^{2+}$  concentrations of hyacinth leaves were maintained

on a large scale to cope with salinity stress. On the other hand, the concentrations of ions in bulbous plants might be related to many factors such as plant species and cultivars, plant growth cycle, seasonal changes or partition among plant tissue. Seasonal changes in the concentration and content of macro- and micronutrients in roots, tubers, blades, petioles and flowers of *Zantedeschia elliottiana* are described by Clark and Boldingh (1991). They stressed that between 20 and 56% of the nutrient stored in tubers was removed to support new season's growth in the 5–7 weeks following planting. By contrast, there was minimal remobilisation of Ca, Mn or Na from tubers. Ramírez-Martínez et al. (2009) indicated that Ca partitioning was higher in leaves and stems, while K<sup>+</sup> was more abundant in bulbs on tulip. In our study, we were not detected significantly change on K<sup>+</sup> concentrations of hyacinth leaves with salinity treatments. This result can be due to blockage of the K<sup>+</sup> transport from the bulbs tissues to leaves. Similarly in our results, Veatch-Blohm et al. (2013) found that potassium concentrations in the shoots of four narcissus cultivars were mostly stable in response to salinity. They emphasized that the ability to maintain potassium content within the plant may help daffodils tolerate the negative effects of sodium.

There was irregular changing in Ca<sup>2+</sup> concentration and any significant alteration of the endogenous level of K<sup>+</sup> in leaves under salinity stress, in our study. On the other hand, results of K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> well indicate effects of salinity stress on hyacinth plants. It is well known that salt-stressed plants have lower K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios (Villora et al., 2000; Tuna et al., 2007; Navarro et al., 2008). This can result in low ratios that reduce plant growth and eventually become toxic. Our results are consistent with previous studies.

### Conclusion

Salinity creates irreversible harms in the growth and development of plants and restricts production. Salinity stress biology and plant responses to high salinity have been discussed in many studies up to now. Salt tolerance does, however, vary considerably among the different genotypes of ornamentals used in landscaping where soils are saline and/or irrigation waters are high in salinity as coastal areas. Adaptation of bulbous ornamental plants was poorly understood and it was seen that plant responses against salt stress vary.

This study showed that the differences in growth, Na<sup>+</sup> uptake, K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios of leaf tissues in hyacinth plant (*Hyacinthus orientalis*

L.) could be ascribed to the difference in mechanisms underlying ion uptake and subsequent tolerance to salinity. However, K<sup>+</sup> concentration of leaf was not found to be an effective determinant of salt tolerance in hyacinth. Reductions in K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratio of leaf under salt stress will greatly contribute to salt tolerance in hyacinth.

In conclusion, although, the concentrations of up to 75 mM are usually used for studies related to salt stress, we preferred higher salt concentrations to determine tolerance limits of hyacinth plant in this study. Measurement of Na<sup>+</sup> concentrations and calculation of K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios in leaf are reliable physiological parameters for ranking hyacinth cultivars and genotypes for their tolerance to salt toxicity based on the severity of damage and decreases in production under salt stress. Thus, more comprehensive studies are required to understand the effects of salinity on hyacinth of other cultivars and varieties.

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