

Full Length Research Paper

Determination of physiological responses on hyacinth (*Hyacinthus orientalis*) plant exposed to different salt concentrations

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Plant growth is restricted by many environmental factors. Soil salinity is considered as an important agricultural problem for dry and semi-dry fields in many regions around the world. It is known that salinity is an important stress factor restricting water and nutrient intake of plants. In this study, the physiological responses of hyacinth (*Hyacinthus orientalis*) exposed to different salt concentrations (0, 50, 100, 200 and 400 mM) were investigated. The stomata status, osmotic potential, proline content, chlorophyll and caretonoid contents and protein variances were examined in the plants exposed to salt. The physiological responses of the hyacinth varied depending on the salt concentration. Stress was kept under control at concentrations of 50, 100 and 200 mM; however, bulbs were decayed and necrosis was formed on the leaves at concentration of 400 mM. This study on hyacinth will help us to learn about tolerance mechanisms raised by plants with bulb against salt stress. It was seen in this study that the stomata size decreased when the salt concentration was increased and the chlorophyll and caretonoid contents also decreased. A significant decrease was seen in proline content and it increased at 400 mM and some protein bands which existed in control group disappeared in electrophoresis study.

Key words: Salt, stress, hyacinth.

INTRODUCTION

High salinity soils have major ecological effects on plant growth and productivity (Kumar et al., 2003). Saline soils comprise 831 million hectares on earth and saline-alkaline soils occupy 434 million hectares (Jin et al., 2006). The main characteristics of these soil types include high levels of Na⁺ and Cl⁻, and CO₃⁻² is often a co-occurring anion (Wang et al., 2008).

Natural flora on soils with high salt concentration is called halophyte. The plants which may be harmed at concentrations lower than 200 mM, are non-halophytes. Some non-halophytes can continue to grow at 200 mM NaCl concentration. These are considered as plants tolerant to salt (Greenway and Munns, 1980). *Hyacinthus orientalis* L. is a perennial flowering plant, native to Southwestern Asia and Southern and Central Turkey. Ornamental potential, propagation and perfumery value

have been investigated in several studies.

Salinity stress is an environmental stress factor for cultural plants and it is among the chemical stress group. If cultivation media has problems relating to salt, many other negative effects are also seen. Plant physiologists and improvers have focused on variations of cultural plant species with respect to resistance against salinity in recent years and have made durable species against salinity. Plant physiologists are studying to explain molecular bases of this durability. They have reported that, plants follow two ways in resisting against salinity and similar stress factors. The first one is avoidance; for this purpose, plants conduct morphologic and chemical variations in their structure. The second resistance mechanism is tolerance. In other words, they work to reduce the effects of the stress factors. They make changes in their cells and tissues. They also strengthen cell walls, produce secondary metabolite and synthesize stress proteins like proline (Avcioglu et al., 2003).

Specific plant tolerances may vary depending on the

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climate, soil conditions, cultural practices and different species. Salinity tolerance varies between species. Salinity is not a problem if decrease in growth due to salinity does not exceed plant's tolerance. When soil salinity exceeds plant's tolerance, growth will decrease (Battal et al., 2006). Difficulties occur in water and it is known that, salt (NaCl) concentration in roots of plants causes chlorosis because Na^+ accumulates in leaves and chlorophyll molecules are replaced with Mg^{2+} and the structure of chlorophylls are disturbed. Under similar conditions and at the same Na^+ concentration, production and accumulation of proline, this is a stress protein in cells increase (Avcioglu et al 2003).

Although, studies examining free proline content did not report appreciable increases under NaCl conditions (Dix and Pierce, 1981; Jain et al., 1987), proline accumulation is still considered as one of the several plant adaptations to salinity and water deficits (Wang et al., 2008).

Chlorophyll b is one of the 8 different chlorophyll molecule groups existing in plant kingdom and it accounts for the largest chlorophyll molecule group existing in mesophyll tissue after chlorophyll a. The determined chlorophyll b content by analyzing its percentage is a good indicator for resistance against salinity (Demiroglu et al., 2001).

MATERIALS AND METHODS

Growth media for plants

Plant bulbs used in the study were supplied by removing them from their habitat in 2007 to 2008. The obtained hyacinth (*H. orientalis* Bieb.) bulbs with a perimeter of 8 to 10 cm were left in growth media of plastic pots (diameter: 12 cm) filled with soil. The plants were exposed to temperatures of 25°C during day and 18°C at nights and also humidity of 70% with 10-h photoperiod in the growth chamber. Salt was applied on them by watering. For this purpose, NaCl solutions were prepared at 50, 100, 200 and 400 mM, and 100 ml of solution was added into pots at each time, once in two days. Salt application was started when first leaves has grown. Five different groups were established including control group, which was watered with tap water. At the end of the eighth week, the application was terminated and the plants were harvested for physiological analyses. The samples were kept in a deep freezer (-80°C) after they have been extracted until use (-80°C).

Determination of chlorophyll and carotenoid content

Fresh leaves were homogenized with pestle and mortar. Chlorophyll and carotenoids were extracted from leaves of 15-day-old plants with 80% acetone and absorbance values were measured at 663 and 645 nm wavelengths for chlorophyll and 450 nm for carotenoids in a UV-160 Shimadzu spectrophotometer. The amount of chlorophyll a, b, total chlorophyll and carotenoids was calculated according to Witham et al. (1971).

Examination of stomata samples

When plants were harvested from the pots, leaves were preserved in formalin-acetic acid- ethanol (FAA; 1:1:9) for microscopic

examination and photographed with Canon microscope apparatus (Zwieniecki et al., 2004).

Determination of osmotic potential and dry weight

One gram of fresh sample was ground with pestle and mortar. Osmotic potential of 15-day-old plants were measured with a Wescor 5100 vapour pressure osmometer. Osmotic potential were expressed as mmhos/gram. After the osmotic potential was determined, the seedlings were oven-dried at 65°C for 3 days for dry weight determination. The relative dry weight was calculated as $\text{DW}/\text{FW} \times 100\%$.

Measurement of proline contents

Free proline accumulation was determined using the method of Bates et al. (1973). 0.5 g fresh weight was homogenized with 2 ml 40% methanol, the homogenate was treated with acetic acid and orthophosphoric acid, ninhydrin, boiled for 1 h and then absorbance at 520 nm was determined by UV-visible spectrophotometer (UV-160 Shimadzu). Contents of proline were expressed as mg/g^{-1} FW.

Preparation of whole-cell proteins and SDS-PAGE analysis

One gram of fresh sample was ground into powder in liquid nitrogen and transferred into an Eppendorf tube. After adding 25 μl of denaturing buffer containing 0.06 M Tris-HCl, 2.5% glycerol, 0.5 SDS and 1.25% β - mercaptoethanol (pH: 6.8), the cells were stirred and the proteins were denaturated in boiling water for 5 min. After centrifugation for 3 min at 10.000 g, supernatant was kept at -50°C until electrophoresis was carried out.

Solubilized proteins were subjected to SDS PAGE in gel slabs 1 mm thick (3.5 cm, 4% stacking and 15.5 cm, 12% resolving gels) as described by Laemmli (1970). Protein molecular marker was estimated by comparison standards (Prestained SDS-PAGE Standarts, BIO-RAD). The gel was stained for 6 h in 0.001 (w/v) Coomassie Brilliant Blue R-250. Afterwards, the gel was destained in a methanol, acetic acid and water (3:1:6) mixture until the protein bands became clearly visible.

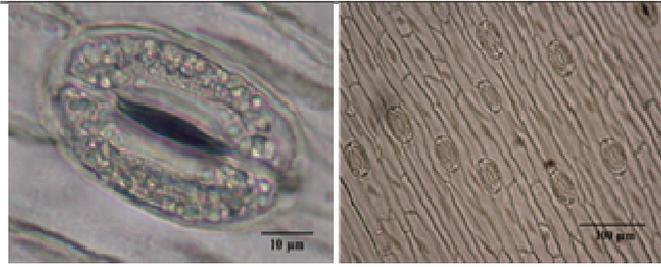
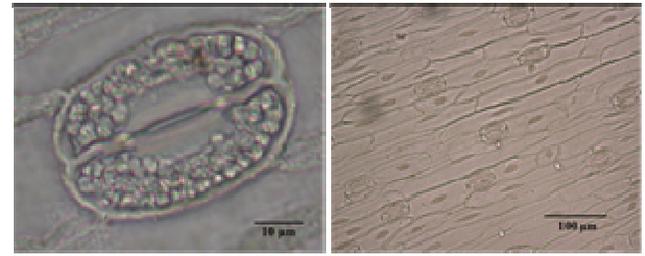
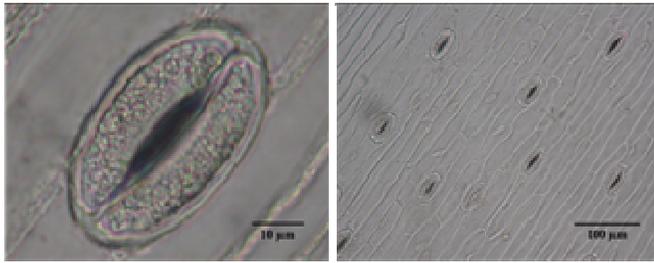
Soil analysis

Soil of experimental groups were analyzed for texture (Bouyoucoucous, 1951), soil reaction (Jackson, 1958), total salt content (Jackson, 1958), percentage lime content (Hizalan and Ünal, 1966) and percentage organic matter (Walkey, 1947).

RESULTS

Morphologic observations

Hyacinths in control group were grown normally and any yellowing or necrotic layers were not observed on leaves; however, yellowing and necrotic layers were seen on leaves at salt concentrations of 200 and 400 mM. It was found that the growth of the plants exposed to the concentrations of 50 and 100 mM was closer to that of the control group. The color of leaves converted to yellowish brown in the parts close to the root in the plants exposed to the concentrations of 200 to 400 mM died.

**A****B****C****D****E**

Salt accumulation on soil surface was observed at 200 and 400 mM.

Stomatal differantions

Stomata number in unit area and stomata openings were determined depending on the salt concentration. It was observed that the stomata were contracted and the stomata number in unit area decreased and stomata openings decreased depending on the increase in salt concentration (Figure 1).

Wet-dry weight ratios

It was seen that the dry matter content decreased due to

Table 1. *H. orientalis* dry weight ratio and soil humidity (water status).

Salt conc. (mM)	Dry weight (%)	Soil humidity
Control	85.86965±0.08	25.7406
50	82.35586±0.12	22.9104
100	82.99756±0.09	23.9377
200	81.78003±0.14	25.0582
400	78.59263±0.11	25.8995

increase in salt concentration in wet-dry weight ratios. It was observed that soil dried at the end of the watering in control group; however, soil humidity was higher in the group exposed to the salt concentration of 400 mM when compared with other groups. Table 1 shows dry weight ratio and humidity (water status) of the growth media.

Table 2. Some physical and chemical features of soil.

Salt conc. (mM)	pH	Salt (μ S)	Lime	Texture	OM (%)	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Control	8.75	627	6.02	Sandy loamy	1.04	0.052	5.63	782	7671	702	7.05	23.16	4.08	1.85
50	8.25	2575	6.02	Sandy loamy	1.11	0.055	4.89	245	7762	405	8.1	28.36	3.8	2.03
100	8.19	3755	6.42	Sandy loamy	1.08	0.054	4.89	229	7580	380	9.84	39.20	2.52	1.65
200	8.09	3990	6.02	Sandy loamy	0.99	0.049	4.32	209	6339	314	9.58	39.68	2.36	1.89
400	7.9	9666	5.21	Sandy loamy	1.18	0.059	5.32	245	7954	383	9.52	39.1	2.27	1.71

Soil analysis

According to soil analysis, mineral matter contents and salt contents varied depending on the concentration. Soil pH was strong alkali in control group, while it was moderate at 50, 100 and 200 mM and in 400 mM salt application, alkaline property was identified. Salinity was not seen in control group, while it was slight at 50 mM, moderate at 100 and 200 mM, and severe at 400 mM. Organic matter content, N, P, K, Ca, Mg, Fe, Mn, Zn and Cu contents were within sufficient limits. Some physical and chemical features and mineral matter contents occurred as a result of applications under controlled conditions which are shown in Table 2.

Osmotic potential

It was seen that osmotic potentials of plants were different from each other in all salt applications of the pot experiments. It was observed that osmotic potential was kept in balance despite increase in salt concentration. Figure 2 shows osmotic potentials of hyacinth in mmol/kg

Proline contents

Significant decrease in proline content was

observed at all salt concentrations with the exception of the control group. The lowest proline content occurred at 100 mM (Figure 3).

Chlorophyll and caretonoid

It was found that the salt exposure had negative effects on chlorophyll a and b and caretonoid contents and it decelerated the plant metabolism (Figure 4).

Protein profiles

Samples were collected during growth and harvest in order to compare protein profiles. Bands seen in the second week of growth decreased at harvest time and some of them disappeared (Figure 5).

DISCUSSION

This study aimed to examine the performance of ornamental plant (hyacinth) bulbs under saline water irrigation. The effect of saline irrigation on ornamental plants has been investigated to a much lesser extent because ornamentals are normally irrigated with high quality water. Increasing awareness of the need for environmental

protection and the necessity for water saving have led producers to explore salinity tolerance research of ornamental plants (Ruth et al., 2002).

Symptoms determined in the form of chlorosis and necrosis on the leaves when salt concentration increases in watering water in our study appear under many stress conditions (Ahmad et al., 2005). Deficiency of K^+ leads to chlorosis followed by necrosis of leaves. Deficiency of Ca^{2+} leads to chlorosis and results in necrotic spots on the leaf surface. It was also found that decrease in chlorophyll a and b and caretonoid contents is correlated with chlorosis and necrosis as determined in our study (Oncel and Keleş, 2002). The fact that osmotic potential of plants increases depending on salt concentration is an expected result.

Plants reduce respiration while they withhold water inside their tissues as a response against salt stress. The plant might have adapted itself to this event by closing its stomata and reducing stomata number in unit area at the increased salt concentrations to protect itself because a similar respiration occasion would increase salt concentration in the test groups when compared with the control group (Omami et al., 2006).

It is known that proline content increases under salt stress. However, imbalance in proline content may indicate that the plant produced a different adaptation mechanism in its response against salt stress (Yakit and Tuna, 2006). Many researchers

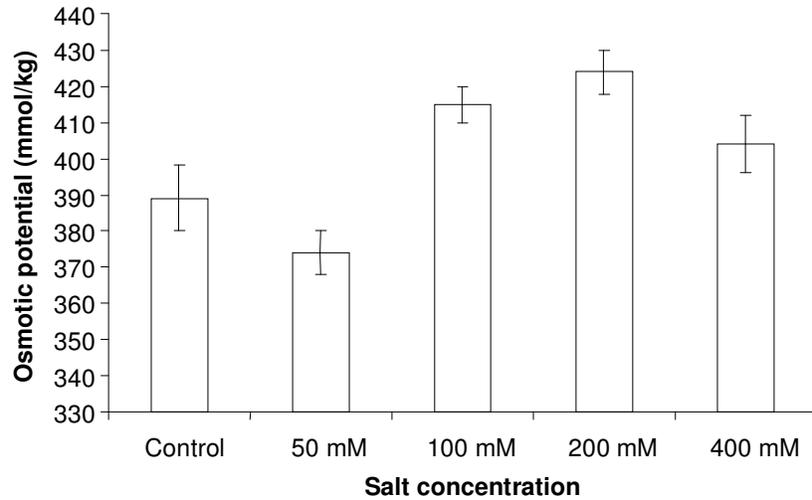


Figure 2. Osmotic potentials of hyacinth in mmol/kg

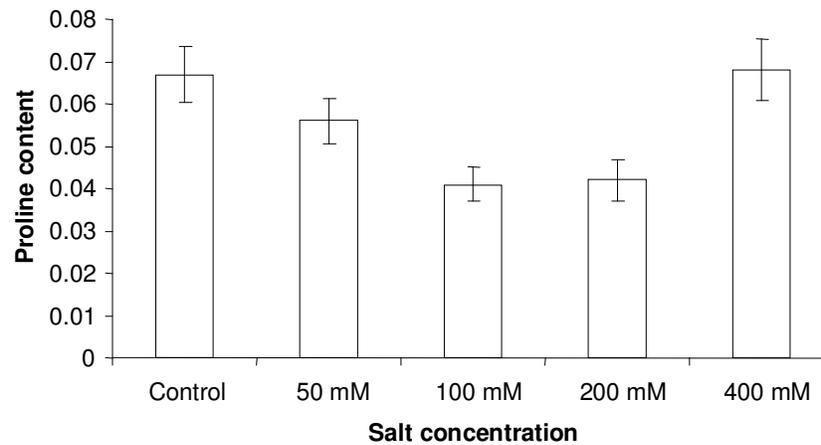


Figure 3. Effects of different salt concentrations on proline content of hyacinth.

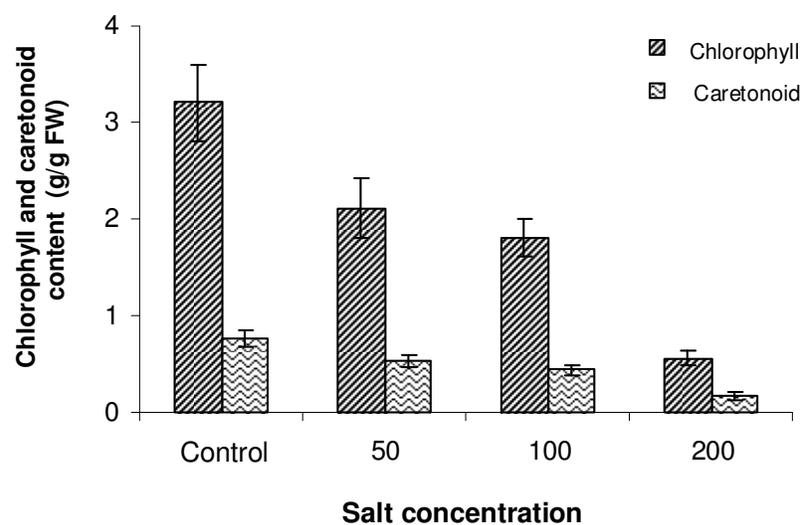


Figure 4. Chlorophyll a and b and caretonoid contents of hyacinths exposed to different salt concentrations.

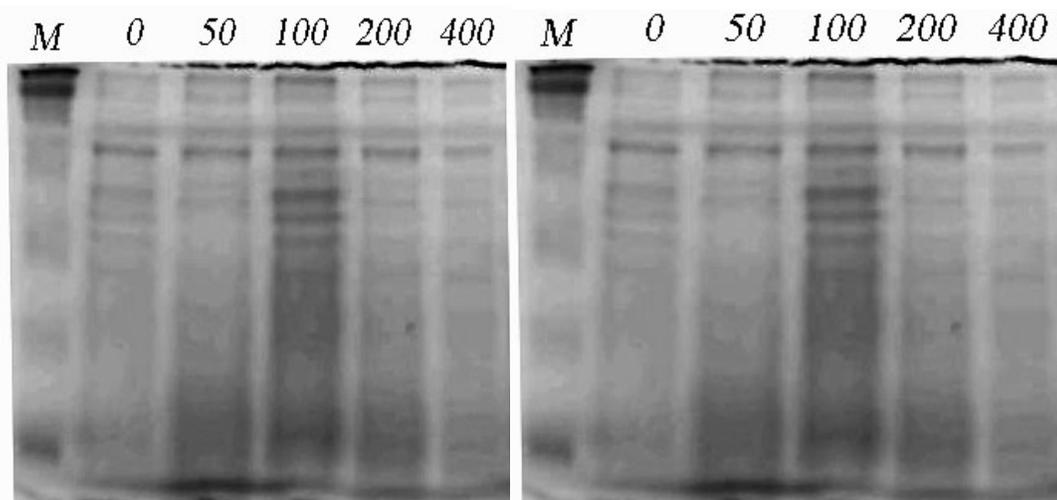


Figure 5. Protein bands in the salt concentrations of 0,50,100,200 and 400Mm in the second week of growth. M: Marker of protein ; 20, 40, 60, 80, 100, 120 KDa).

reported that decrease in chlorophyll a and b and caretonoid contents occurs as a result of general metabolic disturbance (Gadallah, 1999). Variation in photosynthesis rate in unit area on leaf de-pends on environmental factors significantly. Availability of water is a direct restrictive factor for photosynthesis because the closed stomata and the decreased photosynthesis rate in rainless fields are highly effective on dry-wet weights of plants (Ahmad et al., 2005).

Senna plants soluble protein content declined at each state with all NaCl treatments (Arshi et al., 2002). Under salinity, a low water potential enhances the synthesis of ABA which plays an essential role in plant water relations by affecting the solute and water movement in the tissue. ABA also reduces protein synthesis and accelerates protein degradation. Similarly, both osmotic and water stress enhance protein degradation and alter the pattern of protein synthesis. Similar to these data, the plant tolerated salinity until 100 mM concentration and produced structural proteins and different protein bands in protein profiles during growth and on harvest time; however, decrease in structural protein bands was seen at 200 and 400 mM concentrations. However, structural proteins were clear only in control group and at 50 mM concentration, while structural proteins were degraded at higher concentrations depending on the increase in salt concentration and different protein bands were not produced. Proline and chlorophyll ratios correspond with these findings.

Adaptation was understood and it was seen that plant responses against salt stress vary. Although, the concentrations of 0 to 200 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. Thus, more comprehensive studies are required to understand these adaptation mechanisms.

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