

Differential Response of Bean (*Phaseolus vulgaris* L.) Roots and Leaves to Salinity in Soil and Hydroponic Culture

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Abstract

The present study aimed to investigate the response of common bean (*Phaseolus vulgaris* L. Volare) roots and leaves to salinity in different growth mediums (soil and hydroponic culture) through physiologic and biochemical analyses. The relative water content (RWC) and total chlorophyll (CHL) content decreased with 300 mM NaCl treatment in both cultures but did not change with 150 mM treatment in soil culture. Similarly, the malondialdehyde (MDA) content did not change with 150 mM treatment in soil culture, whereas it increased in all other treatments. The highest increase in hydrogen peroxide (H₂O₂) content was observed with 300 mM treatment in hydroponic culture. The highest increase in superoxide dismutase (SOD) activity was observed in plant leaves in the hydroponic culture. Catalase (CAT) activity did not change with 150 mM treatment in soil culture but decreased with 300 mM treatment in both cultures. Ascorbate peroxidase (APX) activity decreased in all treatments, except in the roots in the hydroponic culture. The Na⁺ and Cl⁻ contents were higher in the hydroponic culture than in the soil culture. Salt stress induced more serious oxidative damage in the hydroponic culture compared to the soil culture.

Keywords: antioxidant enzymes, hydroponic, *Phaseolus vulgaris*, salt stress, soil

Introduction

Several environmental stress factors, such as air pollution, climate change, and drought, limit the crop yield and quality around the world. Salinity is also a significant stress factor that limits the plant growth and influences agricultural practices in various parts of the world (Afzal *et al.*, 2006). The ever-increasing salinity problem has already affected 20% of irrigated land resources worldwide (Kader *et al.*, 2008). Salt stress causes osmotic stress and ion toxicity in plants (Hasegawa *et al.*, 2000). Osmotic stress starts after a threshold salt concentration around the roots is reached, leading to inhibition of water uptake and cell expansion, whereas ion toxicity starts with salt accumulation in the leaves, resulting in an increase in chlorosis and necrosis. In addition, salinity affects the photosynthesis rate (Sudhir and Murthy, 2004) because of decreased chlorophyll pigment and closure of stomata (Soussi *et al.*, 1998; Bethke and Drew, 1992). The limitation of photosynthesis causes an over-reduction of photosynthetic electron chains and the production of reactive oxygen species (ROS), which are harmful to plant growth and development. These ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are highly reactive and can cause serious damage on membrane lipids, proteins, and nucleic acids. Salinity induces an oxidative stress in plant cells (Imlay, 2003). To protect the cells against oxidative damage, plants develop complex defense mechanisms, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase

(APX), and glutathione reductase (GR) (Noctor and Foyer, 1998). The role of antioxidant enzymes in stress conditions has been well documented for many plant species (Bowler *et al.*, 1992; Shalata *et al.*, 2001; Sairam *et al.*, 2005; Ashraf *et al.*, 2008).

The common bean (*Phaseolus vulgaris* L.) is one of the most important grains for human nutrition. It is also a valuable leguminous crop worldwide because of its ability to fixate atmospheric nitrogen into the root nodules in a symbiotic interaction with soil rhizobia. This plant is classified as relatively more salt-sensitive compared with the other members of the legume family; more than 50% yield losses are experienced at soil salinities of over 2 dS m⁻¹ (Gama *et al.*, 2007). Salinity also significantly reduces the growth of these species. Such salinity-induced impacts may result in changes in dry-matter allocation, ion relationships, water status, physiologic processes, biochemical reactions, or a combination of all these parameters (Khadri *et al.*, 2001). The salt tolerance of glycophytes is believed to be related to their ability to avoid accumulating excess monovalent cations in their leaves while taking up Cl⁻ ions (Lauter *et al.*, 1988). High Cl⁻ concentrations lead to nutritional imbalance and changes in water relations (Seeman *et al.*, 1985; Gama *et al.*, 2007).

Most of the studies previously conducted on salinity in crops were carried out in hydroponic systems, pots, or fields. Certainly, there are significant differences between the plants grown in each one of these systems. For instance, in soil experiments, the cation exchange mechanism affects soil

features by changing the cation and anion activities. However, in hydroponic systems, the plants have to overcome the osmotic stress of the solution. Thus, it is important to elucidate the salt tolerances or responses of plants in different mediums. Although many studies have revealed the effects of salt stress on bean plants at different stages (Bayuelo-Jimenez *et al.*, 2002; Esechie *et al.*, 2002), there is no report in the literature about a comparative analysis of the changes in physiologic (growth and water content) and biochemical (chlorophyll content, antioxidant enzymes, and ion concentration) characteristics of the same bean plants exposed to salinity in different mediums (hydroponic and soil culture). Therefore, the present study aimed to investigate the response of common bean (*Phaseolus vulgaris* L. Volare) roots and leaves to salinity in different growth mediums (soil and hydroponic culture) through physiologic and biochemical analyses, compare the salt tolerance mechanisms of the plants in different mediums to obtain more tolerant cultivars, and underline the degrees of salinity-induced damage in different mediums.

Materials and methods

Plant material and experimental design

Common bean (*Phaseolus vulgaris* L.) seeds were obtained from a commercial seed company (Tasaco) in Antalya, Turkey. The seeds were sterilized in 5% hypochloride solution for 10 minutes, rinsed three times with distilled sterile water, and then sown in plastic trays (10 x 14 cm) filled with soil. After germination, seedlings were taken into a growth chamber at 25 °C with 16/8 h day/night photoperiod and light intensity of 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The seedlings were watered with Hoagland solution (Hoagland and Arnon, 1950). After 14 days of growth in the chamber, some of the plants were transferred to hydroponic culture. A week later, the seedlings were exposed to saline solutions of 150 mM NaCl and 300 mM NaCl. To reach the desired concentrations, 50 mM was added to Hoagland solution once every 12 h for 48 h in both groups (hydroponic and soil culture). The plants were then harvested and stored at -20 °C until further analyses.

Relative water content

The relative water content (RWC) was calculated in accordance with Smart and Bingham (1974). The leaves were floated on deionized water for 5h in low irradiance; then the turgid tissue was quickly blotted to remove excess water, and the turgid weights (TW) were determined. DW was determined after seedlings were dried in the oven at 70 °C for 72 h.

Chlorophyll content

The chlorophyll contents of the leaves were measured in accordance with the method specified by Lichtenthaler and Wellburn (1983). The pigments from 0.1 g fresh leaves were extracted by using 80% acetone.

H₂O₂ content

The H₂O₂ content was determined according to Velikova *et al.* (2000). Fresh leaves (0.1 g) were homogenized in 5ml of 0.1% trichloroacetic acid (TCA)

and centrifuged at 12,000 rpm for 15 minutes. The supernatant (0.5 ml) was then mixed with 0.5 ml of buffer (10 mM potassium phosphate, pH 7) and 1ml of 1M KI. The absorbance reading was taken at 390 nm.

Na⁺ and Cl⁻ ion content

The Na⁺ content was determined by flame photometry according to Mathis (1956). The Cl⁻ concentration was obtained by wet oxidation of dried leaf tissue with nitric and perchloric acids in accordance with the method adapted by Johnson and Ulrich (1959). The digest was diluted with 0.1 N perchloric acid, and Cl⁻ concentrations were determined by atomic absorption spectrophotometry.

Malondialdehyde content

The level of lipid peroxidation in leaf samples was determined in terms of the malondialdehyde (MDA) content according to the method specified by Madhava Rao and Sresty (2000). The MDA content, an end product of lipid peroxidation, was determined by using the thiobarbituric acid reaction. The MDA concentration was calculated from the absorbance at 532 nm, and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. An extinction coefficient of 155 mM⁻¹ cm⁻¹ was used to determine the MDA concentration.

For protein and enzyme extractions, 0.5 g of fresh leaf samples were homogenized in 1.5 ml of 50 mM sodium phosphate buffer (pH 7.8) containing 1mM ethylenediaminetetraacetic acid (EDTA) Na₂ and 2% (w/v) polyvinylpyrrolidone (PVPP). All operations were done at 4 °C. Samples were centrifuged at 14 000×g for 30 min, and supernatants were used to determine the protein content and enzyme activities. All spectrophotometric analyses were done on a UV-visible spectrophotometer.

SOD activity

The superoxide dismutase (EC 1.15.1.1) activity was assayed by its ability to inhibit the photochemical reduction of nitrotriazolium blue chloride (NBT) at 560 nm (Beauchamp and Fridovich, 1973).

APX activity

The ascorbate peroxidase (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). The assay depended on the decrease in absorbance at 290nm as ascorbate was oxidized. The reaction mixture contained 50 mM Na-phosphate buffer (pH 7.0), 50 mM ascorbate, 0.1 mM EDTA Na₂, 1.2 mM H₂O₂, and 0.1 ml of enzyme extract in a final assay volume of 1 ml. The concentration of oxidized ascorbate was calculated by using an extinction coefficient of 2.8 mM⁻¹ cm⁻¹. One unit of APX was defined as 1mmol ml⁻¹ ascorbate oxidized min⁻¹.

CAT activity

The catalase (EC 1.11.1.6) activity was assayed in a reaction mixture (2 ml) containing 50 mM K-phosphate buffer (pH 7.0). Then, 12.2 mM H₂O₂ was added, and the reaction was started by adding 100 μl supernatant. The activity was determined by monitoring the degradation of H₂O₂ at 240 nm over 2 min against a supernatant-free blank. Enzyme-specific activities were expressed as $\mu\text{mol of H}_2\text{O}_2$ oxidized min⁻¹mg⁻¹ protein (Bergmeyer, 1970).

Statistical analysis

All data were subjected to analysis of variance, and the mean differences were compared by least significant difference (LSD) testing at $p < 0.05$ level. Each data point represented the mean of six replicates

Results and discussion

Salt and water stress are well known to limit the availability of water in tissues and to reduce the relative water content (RWC) in *Phaseolus* plants (Bayuelo-Jimenez et al., 2002; Kabir et al., 2004; Lazcano-Ferrat Lovatt, 1999). In the present study, compared with the control group in soil culture, the RWC did not change significantly in leaves with 150 mM NaCl treatment but decreased by 42.61 % in hydroponic culture. In addition, 300 mM NaCl treatment inhibited the RWC by 31.30 and 58.47% in the soil and hydroponic cultures, respectively (Tab. 1). The decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process, as also reported by Katerji et al. (1997). Besides, high exogenous salt concentrations lead to ion toxicity and osmotic stress damage in plant cells (Cuartero et al., 2006). It could be suggested that the decreased water content was related to osmotic stress in the leaves of *Phaseolus vulgaris* because, in the hydroponic culture, osmotic stress affected the roots by decreasing the water content in the nutrient solution directly and more negatively than in the soil culture at the same NaCl concentration (300 mM NaCl).

Stepien and Klobus (2006) reported strong evidence of salt effects on photosynthetic enzymes, chlorophylls, and carotenoids of plants. This is related to the interference of salt ions with the de novo synthesis of proteins and the structural component, rather than the breakdown, of chlorophyll (Jaleel et al., 2007). In the present study, compared with the control treatment, the total chlorophyll content did not change with 150 mM NaCl treatment in both the soil and hydroponic cultures, whereas it decreased by 50 and 26.30%, respectively, with 300 mM NaCl treatment in the hydroponic and soil cultures (Tab. 1). Similarly, Gadallah (1999) showed that NaCl decreased the chlorophyll content in bean plants. However, Delfine et al. (1999) reported no changes in chlorophyll content over 20 days in salt-stressed spinach (*Spinacia oleracea* L.) plants.

Such findings agree with our current results. In the present study, the higher chlorophyll reduction rates in the hydroponic culture compared to the soil culture may be related to the higher Na^+ content in leaves with 300 mM NaCl treatment.

Hydrogen peroxide (H_2O_2) is a versatile molecule that may be involved in several cell processes in normal and stress conditions (Quan et al., 2008). In stress conditions, H_2O_2 is produced and accumulated, leading to oxidative stress in plants. Plants possess a battery of antioxidant mechanisms, both enzymatic and nonenzymatic, by which ROS are removed from the cells (Noctor and Foyer, 1998). In the present study, compared with the control treatment, the H_2O_2 content of roots and leaves increased by 5.7 and 5.4%, respectively, with 150 mM NaCl treatment. Moreover, 300 mM NaCl treatment increased the H_2O_2 content of roots and leaves by 39.12 and 48.93%, respectively. In the hydroponic culture, 150 mM NaCl treatment increased the H_2O_2 content by 35.69% in roots and 21.56% in leaves. Treatment with 300 mM NaCl increased the H_2O_2 content by 71.59% in roots and 46.03% in leaves (Tab. 2). These findings are in agreement with the results of Sairam and Srivastava (2002). It can be suggested that the higher H_2O_2 content in roots with 300 mM NaCl treatment in the hydroponic culture may have led to more oxidative damage

Tab. 1. Changes in relative water content and total chlorophyll in leaves of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture

Hydroponic	Leaves	
	Relative water content (%)	Total chlorophyll (mg/ g FW)
C	56.88±3.25 ^a	6.5±2.86 ^a
150	32.51±2.67 ^b	6.1±2.14 ^a
300	23.62±3.87 ^c	4.7±2.78 ^b
Soil		
C	84.27±2.98 ^a	7.8±3.14 ^a
150	82.13±3.89 ^a	6.9±2.18 ^a
300	57.89±21.98 ^b	3.9±1.67 ^b

The different letters indicate a significant difference ($p < 0.05$) (n=6). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

Tab. 2. Changes in malondialdehyde content and hydrogen peroxide in roots and leaves of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture

Hydroponic	Roots		Leaves	
	Malondialdehyde (nmol gr FW ⁻¹)	Hydrogen peroxide (µM/gr)	Malondialdehyde (nmol gr FW ⁻¹)	Hydrogen peroxide(µM/gr)
C	24.38±5.46 ^a	70.34±2.76 ^a	23.67±4.20 ^a	82.63±2.13 ^a
150	32.78±4.98 ^b	95.45±1.68 ^b	36.12±3.29 ^c	100.45±1.19 ^b
300	39.17±3.67 ^c	120.7±2.89 ^c	27.24±5.12 ^b	120.67±2.56 ^c
Soil				
C	20.27±5.83 ^a	72.36±2.56 ^a	21.41±3.29 ^a	80.35±2.78 ^a
150	22.38±4.12 ^a	76.49±1.68 ^b	23.16±2.87 ^a	84.69±2.67 ^b
300	30.12±3.87 ^b	100.67±1.56 ^c	29.35±4.69 ^b	119.67±1.67 ^c

The different letters indicate a significant difference ($p < 0.05$) (n=6). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

compared to the soil culture. Our results are also in good agreement with the findings of Mittova *et al.* (2004), who reported a decrease in hydrogen peroxide content in the roots of tomatoes.

Plants develop antioxidant enzymes, such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase, to repair the damage to cellular components caused by reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) (Noctor and Foyer, 1998). SOD plays a significant role in the removal of superoxide radicals from the compartments where they are formed (Alscher *et al.*, 2002). In the present study, the SOD activity in the roots and leaves increased by 33.33 and 34.94%, respectively, with 150 mM NaCl treatment in the soil culture. However, with 300 mM NaCl treatment, the SOD activity increased by 36.38% in roots and 36.11% in leaves. In the hydroponic culture, the SOD activity in the roots and leaves increased by 34.12 and 38.23%, respectively, with 150 mM NaCl treatment, whereas with 300 mM NaCl treatment, the activity increased by 71.93% in leaves and 56.45% in roots (Fig. 1a and b). Parallel to the current findings, previous studies also reported increased SOD activities for cotton and tomato plants under salt stress

(Meloni *et al.*, 2003; Mittova *et al.*, 2001). It could be suggested that the oxidative damage was greater in the roots than in the leaves in terms of the unchanged SOD activity in both cultures with 300 mM NaCl treatment. However, there was a decrease in SOD activity in the hydroponic culture, which might have led to an induction of MDA content in roots and leaves in the soil culture.

In the present study, the CAT activity in the roots and leaves did not change with 150 mM NaCl treatment in the soil culture. However, it decreased by 39.21 and 27.69%, respectively, in the leaves and roots with 300 mM NaCl treatment. These results are in agreement with the findings on the hydrogen peroxide content of the leaves and roots with 150 mM NaCl treatment. However, 300 mM NaCl treatment clearly increased the hydrogen peroxide content in the roots and leaves by 25.30 and 11.02%, respectively, and decreased CAT activity (Fig. 2a and b). Similar decreases in CAT activity (Cavalcanti *et al.*, 2004) and unchanged CAT activity (Azevedo Neto, 2005) under salt stress have been reported. Similarly to the soil culture, 150 mM NaCl treatment increased the CAT activity by 21.56 and 56%, respectively, in the leaves and roots in the hydroponic culture. Treatment with 300 mM NaCl decreased the CAT activity in the roots and leaves by 23.12

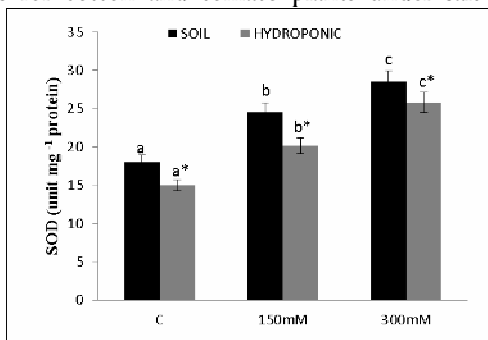


Fig. 1a. Changes in SOD activities in leaves of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture. The different letters indicate a significant difference ($p < 0.05$). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

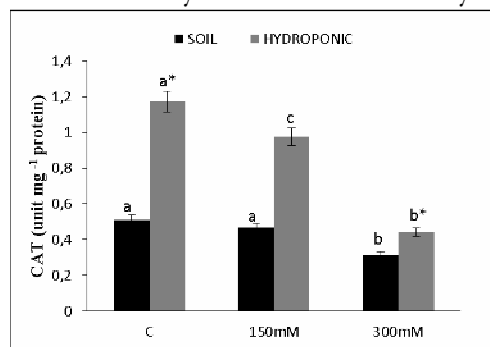


Fig. 2a. Changes in CAT activities in leaves of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture. The different letters indicate a significant difference ($p < 0.05$). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

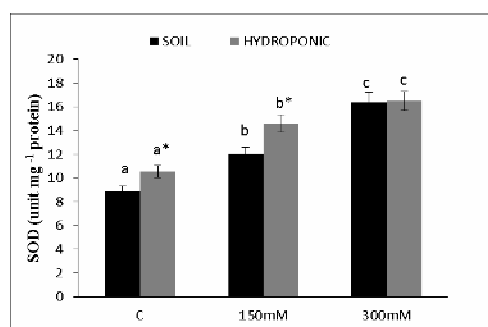


Fig. 1b. Changes in SOD activities in roots of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture. The different letters indicate a significant difference ($p < 0.05$). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

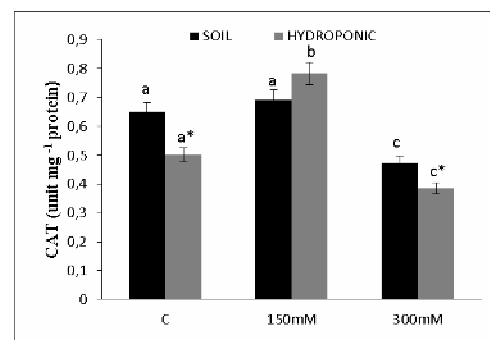


Fig. 2b. Changes in CAT activities in roots of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture. The different letters indicate a significant difference ($p < 0.05$). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

and 62.39%, respectively. Parallel to the current findings, Vaidyarathan *et al.* (2003) and Shalata *et al.* (2001) also reported increased CAT activities in rice and tomato plants under salt stress.

The predominant peroxidase enzyme is ascorbate peroxidase (APX; EC 1.11.1.11), which catalyzes the oxidation of ascorbate (AsA) by H₂O₂, generating dehydroascorbate radicals (Hideg, 1999). In the present study, compared with the control treatment, the APX activity in the leaves and roots increased by 53.60 and 76.92%, respectively, with 150 mM NaCl treatment in the soil culture. The APX activity in the roots did not change with 300 mM NaCl treatment, but it increased by 45.36% in the leaves. In the hydroponic culture, 150 mM NaCl treatment did not change the APX activity in the leaves but

increased that in the roots by 44.11%. In addition, 300 mM NaCl treatment increased the APX activity in the roots and leaves by 36.76 and 33.33%, respectively (Fig. 3a and b). Parallel to the current findings, the APX activity in peas and tomatoes reportedly increased under salt stress (Hernandez *et al.*, 2000; Mittova *et al.*, 2004), whereas it did not change in citrus plants under salinity (Arbona *et al.*, 2003).

An increase in the level of MDA, produced during the peroxidation of membrane lipids, is often used as an indicator of oxidative damage. Many studies have reported that the lipid peroxidation levels (MDA) in plants under stress conditions are decreased by an effective antioxidant enzyme system. In the current results, compared with the control treatment, the MDA content in the leaves and roots did not change with 150 mM NaCl treatment in the soil culture but increased by 41.42 and 48.59 %, respectively, with 300 mM NaCl treatment. In the hydroponic culture, 150 mM NaCl treatment increased the MDA content by 24.41% in leaves and by 34.35% in roots. In comparison, the MDA content in the leaves and roots increased by 57.32 and 60.66%, respectively, with 300 mM NaCl treatment. The highest increase was observed in the roots and leaves with 300 mM NaCl treatment in the hydroponic culture. The MDA content in mulberries and *Phaseolus vulgaris* has also been reported to increase under saline conditions (Sudhakar and Giridarakumar, 2001; Babu and Devaraj, 2008).

In the soil culture with 150 mM NaCl treatment, the increased APX activity was not efficient in terms of maintaining the hydrogen peroxide content (slight increase) in the leaves and roots because of the unchanged CAT activity. Besides, it could be suggested that the unchanged MDA content was related to the smaller increase in hydrogen peroxide caused by the increased SOD activity. The increase in APX activity with 300 mM NaCl treatment was also inefficient because the decrease in CAT activity might have led to an increase in hydrogen peroxide.

In the hydroponic culture, the increase in hydrogen peroxide with 150 mM NaCl treatment could be related to the decreased CAT activity. Similarly to the soil culture, the increase in APX activity was not sufficient to reduce hydrogen peroxide in the roots. However, at the same concentration, the unchanged CAT and APX activities clearly led to an increase in the hydrogen peroxide content in the leaves. The fact that 300 mM NaCl treatment increased the hydrogen peroxide content in leaves could be due to a decrease in CAT activity; similarly, the induction of APX activity was not enough to protect cells from oxidative damage.

In the present study, compared with the control treatment, the Na⁺ content in the leaves increased 4.5- and 13.75-fold with 150 mM and 300 mM NaCl treatment, respectively. However, in the roots, the Na⁺ content increased by 98.02% with 150 mM NaCl and 2.62-fold with 300 mM NaCl treatment (Tab. 3). Glycophytic plants adapt to high salt concentrations by lowering their tissue osmotic potentials through an increase in inorganic ions from an external solution (Cacchorro *et al.*, 1995). In *Phaseolus* species, the inorganic ions in leaves, especially Cl⁻, Na⁺, and K⁺, increase as the salt level increases. In the present study, the cultivated samples (*Phaseolus vulgaris* L. Volare) accumulated Na⁺ ions in their leaves, as also found

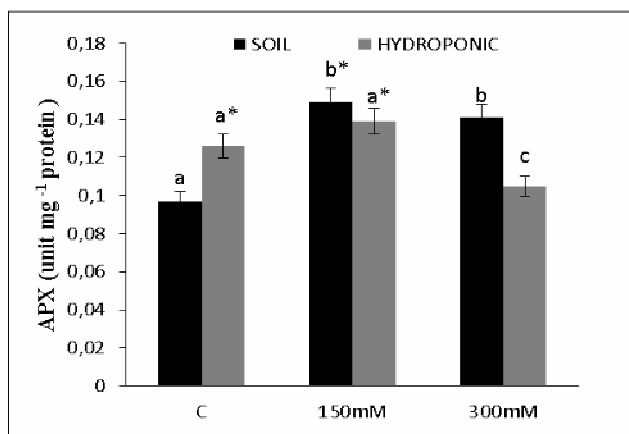


Fig. 3a. Changes in APX activities in leaves of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture. The different letters indicate a significant difference ($p < 0.05$). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

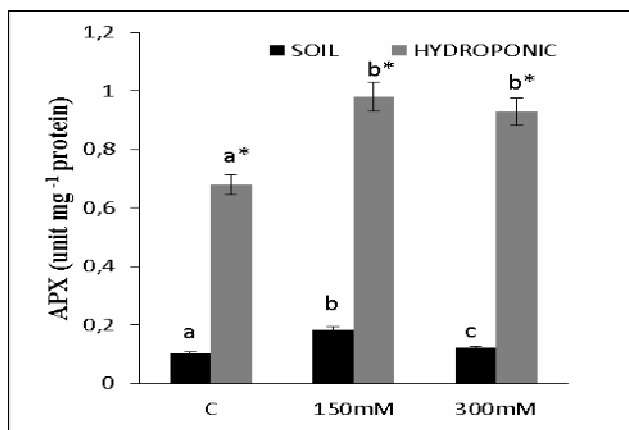


Fig. 3b. Changes in APX activities in roots of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture. The different letters indicate a significant difference ($p < 0.05$). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

Tab. 3. Changes in Na⁺ and Cl⁻ ion contents in roots and leaves of *Phaseolus vulgaris* L. under salt stress for 48 hours in hydroponic and soil culture

Hydroponic	Roots		Leaves	
	Na ⁺ (%)	Cl ⁻ (%)	Na ⁺ (%)	Cl ⁻ (%)
C	0.33±4.21 ^a	0.67±4.22 ^a	0.04±5.12 ^a	0.66±4.99 ^a
50	4.92±4.12 ^b	3.95±4.09 ^b	5.93±5.03 ^b	7.01±4.32 ^b
300	11.76±3.97 ^c	11.46±4.32 ^c	7.4±5.12 ^c	8.7±4.81 ^c
Soil				
C	0.53±3.64 ^a	1.01±4.35 ^{a*}	0.04±5.39 ^a	1.76±3.56 ^{a*}
150	1.05±3.12 ^{b*}	0.95±4.23 ^a	0.18±5.02 ^a	1.54±3.12 ^a
300	1.39±3.65 ^b	0.96±4.12 ^a	0.55±4.99 ^b	2.45±3.49 ^b

The different letters indicate a significant difference ($p < 0.05$ ($n=6$)). C: control; 150 mM NaCl; 300 mM NaCl. *Indicates significant differences between the two mediums at the same concentrations

by Bayuelo-Jimenez *et al.* (2002). In the hydroponic culture, compared with the control treatment, the Na⁺ content in the leaves increased 14.82-fold with 150 mM NaCl and 18.5-fold with 300 mM NaCl treatment. In the roots, compared with the control treatment, the Na⁺ content increased 14.90- and 35.63-fold with 150 mM and 300 mM NaCl treatment, respectively. As a result, there were differences in ion accumulation between the hydroponic culture and the soil culture, as also found by Tavakkoli *et al.* (2010). The higher Na⁺ and Cl⁻ contents in the hydroponic culture, compared to the soil culture, may be related to the roots taking up minerals directly from the external solution more rapidly over 48 h and transmitting them to the leaves in response to salinity. In the soil culture, the soil matrix may have led to a decrease in the Na⁺ content by ion exchange mechanisms. It can be suggested that the higher MDA content in the hydroponic culture compared to the soil culture is related to the toxic effects of accumulated Na⁺ ions.

In the soil culture, the Cl⁻ content in the leaves increased by 12.3% with 150 mM NaCl treatment by 39.20% with 300 mM NaCl treatment. However, the Cl⁻ content in the roots did not change with 150 mM NaCl and 300 mM NaCl treatment. In the hydroponic culture, the Cl⁻ content in the leaves increased 10.62-fold with 150 mM NaCl and 13.18-fold with 300 mM NaCl treatment. In the roots, the Cl⁻ content increased 5.89- and 17.10-fold with 150 mM NaCl and 300 mM NaCl treatment, respectively (Tab. 3). The Cl⁻ ions clearly increased to a greater extent in the hydroponic culture than in the soil culture. Dasgan and Koc (2009) and Tavakkoli *et al.* (2012) also reported that Cl⁻ ions increased in bean plants under salinity. Because both Na⁺ and Cl⁻ are toxic to plants, and Na⁺ in particular causes the deterioration of the physical structure of soil, these ions are considered the most important (Dubey, 1997; Hasegawa *et al.*, 2000). Unchanged Cl⁻ ions in the roots can be related to the soil matrix, which leads to the cation exchange mechanism. The increase in Cl⁻ was higher than that in Na⁺ in all species, as also found by Bayuelo-Jimenez *et al.* (2002).

Conclusions

Based on the results of this study, it can be suggested that salt stress causes more oxidative damage in hydroponic culture than in soil culture. The changes in antioxidant enzyme responses in different mediums could be explained

by a disturbance in the redox balance through the accumulation of toxic ions and osmotic stress, which was more rapid in the hydroponic culture.

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