Effect of Salt Stress on Three Green Bean (Phaseolus vulgaris L.) Cultivars

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Abstract

Agriculture is increasingly forced to utilize marginal waters to meet its increasing demands, which in turn increases the risks of soil salinization and yield reduction in the arid and semi-arid areas of the Mediterranean basin. Given that the bean is an extremely salt sensitive species, the purpose of the present work was to study the effect of 0 and 75 mM sodium chloride (NaCl) on leaf characteristics, growth, pod yield and ion accumulation of three green bean (Phaseolus vulgaris L.) cultivars (‘Corallo Nano’, ‘Romano Bush Plaja’ and ‘Starazagorski’), widely used in Greece. Plants were grown in a greenhouse of Technological Educational Institute of Peloponnese in Messinia, Southern Greece, from April to June 2014, in hydroponics. The experimental design was the factorial completely randomized one with five replications; each replication consisted of the three plants grown on the same rockwool slab. The results of the majority of growth and yield parameters determined showed the superiority of ‘Corallo’ over ‘Romano’ whereas ‘Starazagorski’ tolerance was found to be intermediate. ‘Corallo’ tolerated NaCl salinity better due to its capacity for Na retention in the roots and maintaining appropriate K/Na and Ca/Na ratios, limiting the accumulation of toxic ions into actively growing shoots. The salt sensitivity of ‘Romano’ was related to its higher concentration of Na in the leaves and lower in the roots, to the greater decrease of the leaf number and leaf water content, as well as to the specific leaf area increase compared to the other two cultivars under saline conditions.

Keywords: growth, ion distribution, leaf morphology, pod yield, salt

Introduction

In regions affected by water scarcity such as the Mediterranean basin, water supplies are already degraded, or subjected to degradation processes, which worsen the shortage of water. In such regions, the competition for scarce water resources among users inevitably reduce the supplies of freshwater available for crop irrigation. As a consequence, agriculture is increasingly forced to utilize marginal waters to meet its increasing demands, which in turn increases the risks of soil salinization and yield reduction in the arid and semi-arid areas of the region (Paranychianakis and Chartzoulakis, 2005). Moreover, Greece is characterized by a severe water imbalance particularly in the summer months, due to low precipitation and, at the same time, increased demands for irrigation and water use due to tourism. Thus, the water quality of existing groundwater is deteriorating due to its overexploitation, which favors sea intrusion into aquifers (Chartzoulakis et al., 2001).

Common bean (Phaseolus vulgaris L.) is a popular crop worldwide as both grain legume and fresh vegetable (Nagesh Babu and Devaraj, 2008). As the consumption of the tender, fresh bean pods is top of the Greek consumers’ preferences all year round, the mean yield of the last decade in the country was 64,887 tones (FAOSTAT, 2013), relishing high market values. However, the bean, as an extremely salt sensitive species, suffers yield losses even at soil salinity of less than 2 dS m⁻¹ (Pessarakli, 1999). It has been repeatedly reported that salinity affects plant physiology through changes of water and ionic status in the cells because of ionic imbalance due to excessive accumulation of Na and Cl and reduced uptake of other mineral nutrients, such as K, Ca and Mg (Pessarakli, 1999; Hasegawa et al., 2000).

Although there are several green bean cultivars well adapted to the different environmental conditions of Greece that ensure high yield and income meeting the farmer’s and consumer’s requirements (Mavromatis et al., 2010), they have never been evaluated for their salt tolerance. The purpose of the present work was to study the effect of NaCl salt imposition on several plant growth and leaf parameters, fresh pod yield, and ion contents and distribution of three commercial green bean cultivars, widely cultivated in Greece.

Materials and methods

Plant material and growth conditions

The plant material consisted of the three dwarf green bean (Phaseolus vulgaris, L.) cultivars: ‘Corallo Nano’ (‘Corallo’), ‘Romano Bush Plaja’ (‘Romano’) and ‘Starazagorski’. Their
seeds, after being surface sterilized, were placed in small pre-cut rockwool tubes and irrigated with the ½ Hoagland’s No 2 nutrient solution (Hewitt, 1966) for 15 days. Then, the seedlings, which were the most uniform in size, were placed on rockwool slabs (1.0 m length × 0.15 m width × 0.07 m height), three plants per slab, and grown in a greenhouse of Technological Educational Institute of Peloponnesse in Messina (longitude: 32° 1’ 43” E, latitude: 37° 3’ 22” N), Southern Greece, from April to June 2014. Before the onset of salt treatment, full Hoagland’s No 2 nutrient solution, the pH of which was 5.75 and the electrical conductivity (EC) 2.2 dS m⁻¹, was applied to plants for 20 days. Afterwards, two treatments (Tr) were applied to the plants, Tr0: Full nutrient solution + 0 mM NaCl and Tr75: Full nutrient solution + 75 mM NaCl. During the first days of salt imposition, the plants were irrigated with the NaCl solution in increments of 25 mM per day, in order to reach the desired NaCl concentration gradually, avoiding salt shock. After the onset of the full salt stress, the EC and pH of the nutrient solution applied, as well as those of the drainage solutions from the pots were recorded every week. The mean pH of the nutrient solution of both treatments was 6.0 whereas the mean EC of Tr0 was 2.2 and that of Tr75 8.5 dS m⁻¹. The mean pH of the drainage solutions from both treatments was 6.1 whereas the relevant EC ranged from 3.0 to 3.3 in Tr0 and from 9.6 to 9.9 dS m⁻¹ in Tr75. The duration of the salt imposition to plants was 32 days.

For controlling high temperatures inside the greenhouse during the experimental period, shade curtains were used continuously. Thus, the average daily air temperatures recorded were in April 18.9 °C (min 7.5, max 34.5), in May 21.8 °C (min 10.0, max 36.0) and in June 23.5 °C (min 16.0, max 35.5).

Growth parameters determination
During the experiment, four harvests of green pods were carried out in total; harvest 1, incipient 17 days after the full 75 mM NaCl imposition, harvest 2 20 days, harvest 3 26 days and harvest 4 32 days. At every harvest, the number, the fresh weight (FW) and the dry weight (DW) of the pods were recorded. At the end of the experiment, the plants were destructively harvested, separated into leaves, stems and roots and transferred to the laboratory where the relevant FW and DW were recorded. The root to shoot ratios (RT/SHT) and the leaf water content ((leaf FW-leaf DW)*100/leaf FW) were calculated. The leaf area was measured by the Area Meter ADC AM 300 of BioScientific Ltd. and the specific leaf area was calculated (leaf area/leaf DW, expressed as cm² g⁻¹).

Ion concentrations determination
Regarding the plant mineral nutrition, two samples per replication, the fully expanded leaves of comparable physiological age and the roots, were chemically analysed in order to determine the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Na, Cl, iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and boron (B). Specifically, fresh leaf and root samples were washed, dried to constant weight, ground to fine powder and dry-ashed in a furnace for 6 hours at 500 °C. The concentration of P was determined by vanado-molybdo-phosphate yellow color method, Cl by titration with 0.1 N silver nitrate, B by azomethin-H, K and Na by flame emission spectroscopy whereas Ca, Mg, Fe, Mn, Zn and Cu was determined by atomic absorption spectrometry (Varian SpectraAA, 240 FS) in the dry digest; N was determined by the indophenol-blue method in the wet digest (Allen, 1989; Karla, 1998).

Experimental design and statistical analysis
The experiment was conducted as a factorial completely randomized design with two NaCl treatments and three cultivars in five replications; each replication consisted of the three plants grown on the same rockwool slab.

The significant differences in mean values between treatments were evaluated by the ANOVA and determined by LSD test at $P=0.05$.

Results and discussions
Salt toxicity symptoms
The main symptoms observed consisted of local, non-chlorotic wilting spots in the leaves, which later turned to necrotic spots. Symptoms were more severe in ‘Romano’ and started to appear 15 days after the salt imposition. Similar symptoms were reported by Slabu et al. (2009) in Vicia faba plants grown under salinity.

Growth
The main effect of the cultivar on the majority of growth parameters determined was that ‘Romano’ presented significantly lower values compared to ‘Starazagorski’ whereas ‘Corallo’ showed intermediate ones (data commented the main effects are not shown). Regarding leaf area and leaf number per plant, ‘Corallo’ showed the lowest leaf number but the greatest leaf area.

The main effect of NaCl imposition on plant growth parameters was that plants under 75 mM NaCl presented significantly decreased values as compared to those of plants under Tr0. The leaf number of ‘Corallo’ under Tr75 was not significantly differentiated compared to Tr0, it remained almost unaffected whereas those of ‘Romano’ and ‘Starazagorski’ were significantly reduced (Table 1). The plant leaf FW was significantly reduced in every cultivar, however ‘Corallo’ followed by ‘Starazagorski’ presented lower decreases (27-30%) compared to ‘Romano’ (59%). Similarly to plant leaf FW, the upper plant part and the total plant FW were less reduced in ‘Corallo’ and ‘Starazagorski’ compared to ‘Romano’. The stem FW was reduced by 23-29% in all the three cultivars. Regarding root FW, ‘Romano’ under Tr75 presented greater decrease (by 67%) compared to ‘Corallo’ (36%) and ‘Starazagorski’ (44%). The RT/SHT of ‘Romano’ presented the greatest decrease as well, followed by ‘Starazagorski’. The fact that salt stress resulted in a considerable decrease of leaves, stems and root weights has been mentioned by other researchers (Nagesh Babu and Devaraj, 2008; Ziaf et al., 2009). However, salinity affected shoot growth less than root growth in the present work. These results corroborate the findings of Cordovilla et al. (1999) with Vicia faba plants but they contradict those of Wignarajah (1992), Bayudo-Jimenez et al. (2002) and Tejera et al. (2005) with Phaseolus. There is still an active debate related to the physiological significance of RT/SHT variations in salt stress adaptation (Moya et al., 1999). An increased RT/SHT is usually documented as a general response to salinity as cultivars with enhanced root growth would colonize a greater soil volume and find parts of the soil with a less salt concentration (Cuartero and Fernández-Munáoz, 1999). On the other hand, a reduced
RT/SHT may improve salinity tolerance by restricting the flux of toxic ions to the shoot, delaying the onset of the tolerance threshold.

As aforementioned, the leaf number of 'Corallo' under saline conditions was not significantly reduced whereas the relevant ones of 'Romano' and 'Starazagorski' were. The leaf area of both 'Corallo' and 'Romano' presented significant decreases, however that of 'Starazagorski' did not (Table 1). Leaf number and/or the leaf area reductions have been reported in several plants under saline conditions (Lycoskoufis et al., 2005; Rodriguez et al., 2005), as well as the highest reductions of many leaf characteristics have been recorded in the more salt sensitive cultivars as compared to the more tolerant ones (Ziad et al., 2009). Specific leaf area, an indicator of leaf thickness that has often been observed to be reduced under saline conditions, was significantly increased in 'Romano' but not significantly affected in 'Corallo' and 'Starazagorski'. It could be assumed that 'Romano' salt sensitivity was related not only to its greater leaf thickness increase of 'Romano' are associated with the cultivar lower water use efficiency under saline conditions (Rahimi et al., 2011).

Yield

The main effect of the cultivar on yield parameters (marketable pod number, marketable pod FW and DW) was that there were no significant differences among the three cultivars whereas the main effect of NaCl imposition was their significant decrease under Tt75.

Green pod yield results of the 1st harvest showed that 'Starazagorski' under salinity was the only cultivar that did not significantly differentiate the total pod FW compared to 'Corallo' and 'Romano' whose to total pod FW decreased by 41%-45%. The same cultivar under Tt75 presented neither a significantly reduced pod number nor the mean pod FW compared to Tr0 (Table 2). However, the results of the 2nd harvest showed that 'Starazagorski' under Tt75 significantly decreased both the total pod FW and the pod number whereas the mean pod FW remained unaffected. In the 3rd harvest, the cultivar that presented the lower total pod FW and pod number decreases under salinity was 'Corallo' compared to total pod FW and pod number decreases of both 'Romano' and 'Starazagorski'. The total pod DW varied similarly to the total pod FW of the three cultivars (decreased by 27% in 'Corallo' but by 47% in 'Romano' and 'Starazagorski') (data not shown). In the 4th harvest, 'Romano' presented the greatest percent decreases related either to total pod FW or to pod number. Therefore, in the first stages of salinity the salt imposition affected less the yield of 'Starazagorski' compared to 'Corallo' and 'Romano' whereas in the later stages 'Corallo' was the cultivar with the smaller percent yield decreases.

Regarding the results of the pod yield harvested during the whole experiment, the FW of the marketable pods produced by 'Corallo' decreased by 35% whereas that of the two other cultivars by 45-46%. The number of the marketable pods per plant as well as the total pod dry matter were not significantly differentiated because of salinity only in 'Corallo'. The mean pod FW was found to be significantly decreased in all the three cultivars but the decrease was lower in 'Corallo' and 'Starazagorski' (Table 2). The yield reduction of 'Corallo' under saline conditions was due to a greater extent to pod size reduction and less to pod number which agrees with relevant results in other plants (Caro et al., 1991; Van Ieperen, 1996).

| Cultivar | Treatment | Leaf number | Plant Leaf FW | Upper plant part/FW | Stem FW | Root FW | Total plant FW | RT/SHT FW | Leaf Water Content | Leaf Area | Specific Leaf Area |
|----------|-----------|-------------|---------------|----------------------|---------|---------|----------------|------------|-------------------|------------|-------------------|
| Corallo  | 0         | 632         | 50983d        | 7488c                | 217d    | 1300c   | 8490c          | 790c       | 0.39ab            | 809a      | 115c              |
| Corallo  | 75        | 662a        | 3526b         | 5163b                | 1657b   | 835ab   | 9996b          | 794b       | 0.37ab            | 869a      | 765b              |
| Romano   | 0         | 806b        | 4651ab        | 5994c                | 3190b   | 1402c   | 7896c          | 939b       | 0.25b             | 876a      | 798b              |
| Romano   | 75        | 866a        | 1953a         | 2875a                | 897a    | 466a    | 3414a          | 653a       | 0.16a             | 708b      | 588a              |
| Starazag. | 0         | 666b        | 525d          | 754c                 | 1229c   | 1921c   | 9215c          | 1161c      | 0.25b             | 874a      | 908b              |
| Starazag. | 75        | 672a        | 3823ac        | 540b                 | 1628b   | 1073c   | 6223b          | 805b       | 0.20ab            | 881a      | 708b              |

The values followed by different letters within a column are significantly different at P<0.05.

Table 1. Effects of 0 and 75 mM NaCl in the nutrient solution on growth and plant parameters of three green bean cultivars

| Cultivar | Treatment | Total pod FW | Mean pod FW | Total Pod FW | Mean pod FW | Total pod FW | Mean pod FW | Total pod FW | Mean pod FW | Total pod FW | Mean pod FW | Total pod FW | Mean pod FW | Total pod FW | Mean pod FW | Total pod FW | Mean pod FW | Total pod FW | Mean pod FW |
|----------|-----------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|
| Corallo  | 0         | 422bc        | 71c         | 642c         | 71d         | 89ab        | 126c        | 134bc        | 75c         | 73b          | 169bc        | 165bc        | 771bc        | 646b        | 904bc        | 85c         |
| Corallo  | 75        | 349a         | 57a         | 359a         | 58c         | 67a         | 76ab        | 107bc        | 314bc       | 85a          | 137bc        | 153bc        | 591bc        | 545bc        | 778a         | 66b         |
| Romano   | 0         | 570d         | 61b         | 460c         | 50b         | 91ab        | 106c        | 150c         | 72c         | 79b          | 147bc        | 153b6d       | 723bc        | 596b         | 103bc        | 70b         |
| Romano   | 75        | 366b         | 47a         | 321a         | 42a         | 75ab        | 447a        | 86a          | 195a        | 38a          | 773a         | 105a         | 403a         | 415a         | 813a         | 49a         |
| Starazag. | 0         | 717d         | 61bc        | 59ab         | 57bc        | 97b         | 107c        | 153c         | 447b        | 50a          | 1711c        | 181bc        | 781bc        | 626b         | 116c         | 67b         |
| Starazag. | 75        | 800cd        | 58b         | 337a         | 52bc        | 68a         | 66b         | 82a          | 195a        | 28a          | 1097b        | 125ab        | 423bc        | 44b          | 772a         | 55a         |

The values followed by different letters within a column are significantly different at P<0.05.

Table 2. Effects of 0 and 75 mM NaCl in the nutrient solution on yield parameters of three green bean cultivars
Considering the total plant biomass (total plant+ pods FW) produced under salinity, ‘Romano’ presented the greater decrease (47%) compared to ‘Corallo’ and ‘Starazagorski’ (30% and 36%, respectively. Based on total plant biomass DW, the lowest dry matter decrease was recorded in ‘Corallo’ under Tr75 as compared to Tr0, indicating the superiority of ‘Corallo’ over ‘Romano’ and ‘Starazagorski’. Similar conclusions regarding salt tolerance evaluation of bean cultivars were reported by other researchers (Kaymakova and Stoeya, 2008; Gutierrez et al., 2009; Ndadkimi and Makoi, 2009) as well as in pepper and cotton cultivars by Ziaf et al. (2009) and Dai et al. (2014), respectively.

**Ion concentrations**

The main effect of NaCl imposition on leaf and root Na and Cl concentrations of plants under Tr75 was their significant increases as compared to control plants. The greatest Na concentrations under Tr75 were detected in the roots and the lowest ones in the leaves (Table 3). These results are in accordance with those of Loupassaki et al. (2002).

Among the three cultivars, ‘Corallo’ was the one which did not significantly increase leaf Na under Tr75 compared to Tr0 whereas ‘Romano’ under Tr75 concentrated the greatest leaf Na quantities (14 times higher than controls). With regard to root Na, ‘Starazagorski’ followed by ‘Corallo’ retained greater Na whereas ‘Romano’ lower (Table 3). The Na exclusion from the shoots of ‘Corallo’ and its retention by the roots may represent the tolerance mechanism of the cultivar, which has proved as the most salt tolerant one on the basis of growth, yield and leaf characteristics (Heimler et al., 1995; Aktas et al., 2006; Yasar et al., 2006; Gama et al., 2007). Adversely to Na, leaf and root Cl results did not reflect the salt tolerance of the cultivars tested. Comparing K concentrations in the leaves of plants under Tr75 to those under Tr0, significant increases were observed in every cultivar. Adversely to leaf K, root K under Tr75 was found to be significantly decreased compared to Tr0. Moreover, root K of every cultivar under Tr75 decreased by five times compared to Tr0 whereas leaf K increased by 28%, respectively (Table 3). The great decreases of K in the roots suggest that plants became able to concentrate higher K levels in the leaves maintaining K-Na selectivity (Kaya et al., 2001; Zhang and Flowers, 2010). However, the leaf or root K concentrations under Tr75 were not significantly differentiated among the three cultivars tested. Aktas et al. (2006) reported very similar shoot K and Ca concentrations between the sensitive and the tolerant pepper genotypes under salt treatment as well.

Leaf Ca and Mg under Tr75 were found to be significantly increased compared to Tr0 whereas the relevant ones in the root were significantly decreased; there were no significant differences among the three cultivars under Tr75 in most cases (Table 3). The increased leaf K, Ca and Mg under salinity may have been a consequence of a concentration effect because of the limited leaf growth. In accordance with our results are Melgar et al. (2008) and Rousses et al. (2011) whereas Semiz et al. (2014) found not significantly affected K and Mg concentrations in pepper leaves under salinity. However, the increase of leaf K, Ca and Mg under Tr75 is not very common, since the majority of the literature reports quite drastic decreases of their concentration as high NaCl uptake competes with their uptake, especially with regard to K (Alfocea et al., 1993; Cuartero and Fernandez-Munoz, 1999; Khan et al., 1999; Kaya et al., 2001; Rus et al., 2004; Weisany et al., 2014). Nevertheless, when the leaf and root contents of K, Ca and Mg were calculated (as g of nutrient per upper plant part and per root DW), then the results were fully reversed, as in all the cases the content of these three nutrients was significantly decreased under salinity (data not shown). This is mainly the result of the lower total upper plant part and root DW and the strong competition between Na and K, Mg and Ca as well as among K-Mg-Ca. Although the leaf K concentrations under Tr75 were not significantly differentiated among the three cultivars, the leaf K/Na and Ca/Na ratios were found to be significantly highest in ‘Corallo’ and lowest in ‘Romano’ (Table 3). It has been pointed out that low values of K/Na and Ca/Na ratios of several crops appeared as better indicators of salt stress than the Na concentration alone (Rengel, 1992; Alfocea et al., 1993; Gadallah, 1999; Ruhfio-Casal et al., 2003).

The main effect of NaCl imposition on leaf Na and P concentrations was their significant decreases. However,
'Corallo' changed neither leaf N nor P under Tr75 (Table 3). Decreased leaf N under salinity was reported by Grattan and Grieve (1999) whereas decreased P by Sharpley et al. (1992). The root reduction observed under Tr75 could also be attributed to the lower root P determined, as P is a major element for root growth and development (Shibli et al., 2001). However, the results of other studies in various crops indicated that salinity either increased or had no effect on P uptake (Grattan and Grieve, 1999).

The main effect of the cultivar on leaf micronutrient concentrations was that Fe, Mn and B were significantly lower in 'Corallo', Zn was significantly lower in 'Romano' whereas Cu was not significantly differentiated among the three cultivars. The main effect of salt imposition on leaf micronutrient concentrations were the significant decreases of only leaf Fe and B. Among the three cultivars, leaf Fe under Tr75 was significantly lower in 'Corallo' and 'Starazagorski' and leaf B in 'Romano' (Table 4). With regard to availability of micronutrients to plants growing on salt affected soils, Grattan and Grieves (1999) pointed out that they may increase, decrease, or have no effect whereas Shibli et al. (2001) and Dai et al. (2014) noted that tissue Fe, B, Zn, Mn and Cu were generally decreased with elevated salinity.

Conclusions

The conducted study confirmed the genetic variability in salt tolerance among three green bean cultivars which are widely used in Greece. 'Corallo', followed by 'Starazagorski', was found to be the most tolerant cultivar based on the majority of growth parameters assessed. On the contrary, the most salt sensitive one was 'Romano', as it presented their greatest suppression. Moreover, the salt sensitivity of 'Romano' was related to its lower root reduction observed under Tr75 could also be attributed to the lower root P determined, as P is a major element for root growth and development (Shibli et al., 2001). However, the results of other studies in various crops indicated that salinity either increased or had no effect on P uptake (Grattan and Grieve, 1999).

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