



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

Anna ASSIMAKOPOULOU*, Ioannis SALMAS, Kallimachos NIFAKOS, Panagiotis KALOGEROPOULOS

Technological Educational Institute of Peloponnese, Department of Agricultural Technology, 24100 Antikalamos, Messinia, Greece; a.assimakopoulou@teikal.gr (*corresponding author); isalmas@teikal.gr; kallimachos@teikal.gr; pkalog@teikal.gr

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

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seeds, after been 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 x 0.15 m width x 0.07 m height), three plants per slab, and grown in a greenhouse of Technological Éducational Institute of Peloponnese in Messinia (longitude: 22° 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 33.5).

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 cm²g¹).

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 6h 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 SpectrAA, 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 discusions

Salt toxicity symptoms

The main symptoms observed consisted of local, nonchlorotic 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), Bayuelo-Jiménez 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 lesser salt concentration (Cuartero and Fernândez-Munäoz, 1999). On the other hand, a reduced

Cultivar	Treat- ment	Leaf number	Plant Leaf FW	Upper plantpartFW	Stem FW	RootFW	Totalplant FW	Total plant DW	RT/SHT FW	Leaf Water Content	Leaf Area	Specific Leaf Area
	mМ				~					04	cm ²	cm²g¹leaf
	NaCl				g					70	un	DW
Corallo	0	63.2 a	500.8cd	7180c	2172c	130.0c	848.0c	97.0c	0.19ab	88.9a	119.1c	597.4ab
Corallo	75	562a	352.6b	5163b	163.7b	83.5 ab	599.8b	79.4ab	0.17ab	869a	76.5b	631.8ab
Romano	0	88.0b	460.4 bcd	599.4bc	139.0ab	140.2c	739.6bc	93.9b	0.25b	87.6a	79.8b	545.2a
Romano	75	668a	1903a	297.5a	1072a	46.6a	344.1 a	63.5a	0.16a	78.8b	53.8a	650.1b
Starazagorski	0	86.6b	525.5d	755.4c	229.9c	192.1 d	921.5c	1168c	0.25b	87.4a	90.8b	547.2a
Starazagorski	75	672a	382.2bc	545.0b	162.8b	107.3bc	6523b	80.5ab	020ab	88.1 a	70.0ab	569.6ab

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

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

Cultivar	Treat- ment	Total pod H1	Mean pod H1	Total Pod H2	Mean pod H2	Pod number H2	Total pod H3	Pod number H3	Total pod H4	Pod number H4	Total biomass	Total biomass	Total marketable pod	Total marketable pod	Total marketable pod number	Mean pod
	mM NaCl	gF	W	gF	W		gF	W	gFW		gFW	gDW	gFW	gDW		gFW
Corallo	0	42.2bc	7.1c	61.2c	7.1d	8.9ab	1267c	13.4bc	75.5c	7.6b	1635.4c	161.5cd	771.2b	646b	90.0ab	85c
Corallo	75	249a	5.7b	39.5 ab	5.8c	6.7a	75.0b	10.7ab	30.4ab	45a	1137.3b	133.4abc	5043a	540ab	77.8a	6.6b
Romano	0	57.0cd	6.1bc	46.6abc	5.0b	9.1 ab	1065c	15.0c	72.9c	7.9b	1471.4c	153.6bcd	723.7b	59.8b	103.8bc	7.0Ь
Romano	75	31.6ab	4.7a	32.1 a	42a	7.5ab	44.7a	8.6a	19.5a	3.8a	773.1a	105.4a	400.3a	41.9a	81.3a	49a
Starazagorski	0	71.7d	6.1bc	55.2bc	5.7bc	9.7b	107.5c	15.3c	44.7b	5.0a	1711.1c	181.6d	781.2b	62.0b	1160c	67b
Starazagorski	75	60.0cd	5.8b	33.7a	5.2bc	68a	45.0a	8.2a	15.9a	2.8a	1099.7b	125.3ab	423.2a	448a	77.2a	5.5a

^{*}The values followed by different letters within a column are significantly different at P>0.05.

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 (Ziaf 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 number and leaf area decrease but also to the inability of the cultivar to avoid leaf thinness; thicker leaves usually have a higher density of chlorophyll and proteins per unit leaf area and, hence, have a greater photosynthetic capacity than thinner leaves by increasing salinity (Giuffrida et al., 2001). Regarding the leaf water content, the only cultivar that significantly decreased this parameter was 'Romano' under Tr75. Reduced water uptake is the common response of plants subjected to salt stress (Munns, 2002; Habtamu, 2013). The lower leaf water content in combination with the leaf thinness increase of 'Romano' are related to 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 Tr75.

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 Tr75 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 Tr75 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).

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Table 3. Effects of 0 and 75 mM NaCl in the nutrient solution on leaf and root Na, Cl, K, Ca, Mg, N and P concentrations and leaf K/Na, Ca/Na ratios of three green bean cultivars

Treat-	Leaf	Root	Leaf	Root	Leaf	DantV	Leaf	Root	Leaf	Root	Leaf	Leaf	Root	Leaf	Leaf
ment	Na	Na	Cl	Cl	Κ	NOUL	Ca	Ca	Mg	Mg	Ν	Р	Р	K/Na	Ca/Na
mMNaCl						g/kgDV	W								
0	03a	3.0a	1.7b	12bc	41.5a	26.0b	302a	23.1c	40ab	53c	33.7ab	2.6a	8.0bc	-	-
75	12ab	25.6c	22c	1.5 cd	48.6b	5.5a	34.1 bcd	15.9ab	4.7c	33a	32.2a	2.8ab	5.9a	39.7 a	28.8a
0	1.1 ab	3.4a	12a	0.7a	38.6a	29.7bc	33.6abc	17.0ab	3.6a	5.8c	35.4bc	3.1 ab	9.5d	-	-
75	17.5c	19.6b	25c	1.7d	50.8b	45a	37.6d	15.1 a	43bc	42ab	32.2a	3.2.ab	5.6a	3.0b	2.2b
0	0.9ab	2.3a	13ab	1.0ab	39.2a	32.7c	31.2.ab	19.1 abc	3.7a	44b	35.7c	33b	83ad	-	-
75	2.6b	28.8d	25c	2.4e	52.7b	72a	36.1 cd	19.5bc	43bc	44b	33.9b	4.0c	67ab	28.1ab	184ab
	Treat- ment 0 75 0 75 0 75 0 75	Treat- ment Leat mMNaCl 0 0 0.3a 75 1.2ab 0 1.1ab 75 17.5c 0 0.9ab 75 2.6b	Trate Laf Root ment Na Na 0 03a' 30a 75 12ab 256c 0 11ab 34a 75 175c 196b 0 09ab 23a 75 26b 288d	Treat- Leaf Root Leaf ment Na Na Cl mMNaCI	Treat Leaf Root Leaf Root ment Na Na Cl Cl mMNaCI	Treat- Leaf Root Leaf Root Leaf ment Na Na Cl Cl K mMNaCI	Treat- ment Leaf Na Root Cl Leaf Root Cl Root Root Na Leaf Root Na Root Na Root Na Root	Treat- ment Leaf Na Root Cl Leaf Kalon Root Ca Leaf Ca MMAGI	Treat- ment Leaf Root Leaf Root Leaf Root ment Na Na Cl Cl K RootK Ca Ca mMNaCI State State State State State State State State State Ca Ca Ca Ca Ca State State State State State Ca Ca Ca Ca State State State Ca Ca Ca Ca Ca Ca State State	Treat- ment Leat Na Root Cl Leat Ca Root Ca Leat Root Leat Leat Ca Root Mag Leat Root Leat Leat Ca Root Mag Leat Mag Root Leat Leat Ca Root Leat Leat Root Leat Mag Root Leat Leat Root Leat Leat Root Leat Roo	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $

The values followed by different letters within a column are significantly different at *P*>0.05

Table 4. Effects of 0 and 75 mM NaCl in the nutrient solution on leaf and root Fe, Mn, Zn, Cu and B concentrations of three green bean cultivars

0.1:	Treat-	Leaf	Root	Leaf	Root	1 (7	Root	Leaf	Root	Leaf	Root
Cultivar	ment	Fe	Fe	Mn	Mn	Leaf Zn	Zn	Cu	Cu	В	В
	mM NaCl					mg kg ⁻¹ D	W				
Corallo	0	142.9 c [*]	1269.9 Ь	151.6 a	217.2 с	69.6 b	200.9 d	11.8 ab	39.9 c	57.8 a	36.9 bcd
Corallo	75	106.6 a	1028.4 a	154.5 a	150.0 a	73.9 b	165.5 b	10.0 a	29.3 b	52.2 a	35.0 bcd
Romano	0	152.9 c	1229.8 ab	174.3 ab	203.7 abc	51.8 a	132.5 a	10.1 a	19.0 a	63.9 b	33.1 bc
Romano	75	136.8 bc	1168.4 ab	188.1 b	159.8 ab	68.5 b	171.2 bc	12.6 b	40.7 c	57.6 a	31.4 a
Starazagorski	0	172.8 d	1122.7 ab	202.9 b	187.0 abc	72.7 b	176.6 bcd	11.3 ab	35.1 bc	69.8 b	37.6 cd
Starazagorski	75	121.7 ab	1203.9 ab	203.6 b	211.4 bc	71.4 b	197.1 cd	10.6 ab	31.7 bc	64.1b	39.5 d

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

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 (Kaymakanova and Stoeva, 2008; Gutierrez *et al.*, 2009; Ndakidemi 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 Roussos 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 Fernândez-Munäoz, 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; Rubio-Casal et al., 2003).

The main effect of NaCl imposition on leaf N 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 greater decrease of the leaf number and leaf water content as well as to the increased specific leaf area. The effect of salt imposition on the green pod yield and pod number was that 'Corallo' suffered the least decrease whereas in the case of the mean pod FW, it was less decreased in both 'Corallo' and 'Starazagorski'. With regard to ion contents, 'Corallo' accumulated less Na and Cl in the leaves by retaining higher Na in the roots. On the contrary, the salt sensitive 'Romano' concentrated higher Na in the leaves and lower Na in the root. Moreover, 'Corallo' was able to maintain higher leaf K/Na and Ca/Na compared to the most salt sensitive 'Romano'. In conclusion, 'Corallo' tolerated better NaCl salinity due to its capacity of ion retention of Na in the roots, maintaining appropriate K/Na and Ca/Na ratios, limiting the accumulation of these ions into actively growing shoots.

References

- Aktas H, Abak K, Cakmak, I (2006). Genotypic variation in the response of pepper to salinity. Scientia Horticulturae 110:260-266.
- Alfocea FP, Estan MT, Caro M, Bolar MC (1993). Response of tomato cultivars to salinity. Plant and Soil 150:203-211.
- Allen SE (1989). Chemical Analysis of Ecological Materials. 2nd Edition. Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Melbourne.
- Bayuelo-Jiménes JS, Debouck DG, Lynch JP (2002). Salinity tolerance of *Phaseolus* species during early vegetative growth. Crop Science 42:2184-2192.

- Caro M, Cruz V, Cuartero J, Estan MT, Bolarin MC (1991). Salinity tolerance of normal and cherry tomato cultivars. Plant and Soil 136:249-255.
- Chartzoulakis KS, Paranychianakis NV, Angelakis AN (2001). Water resources management in the island of Crete, Greece with emphasis on the agricultural use. Water Policy 3:193-205.
- Cordovilla MD, Ligero F, Lluch C (1999). Effect of salinity on growth, nodulation and nitrogen assimilation in nodules of faba bean (*Vicia faba* L.). Applied Soil Ecology 1:1-7.
- Cuartero J, Fernândez-Munäoz R (1999). Tomato and salinity. Scientia Horticulturae 78:83-125.
- Dai JL, Duan LS, Dong HZ (2014). Improved nutrient uptake enhances cotton growth and salinity tolerance in saline media. Journal of Plant Nutrition 37:1269-1286.
- Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT database) (2013). Production of green beans in Greece from 2004 to 2013. Available at http://faostat3.fao.org/browse/Q/QC/E.
- Gadallah MAA (1999). Effects of proline and glycinebetaine on Vicia faba response to salt stress. Biologia Plantarum 42:249-257.
- Gama PBS, Inanaga S, Tanaka K, Nakazawa R (2007). Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. African Journal of Biotechnology 6:79-88.
- Giuffrida F, Leonardi C, Noto G (2001). Response of soilless grown strawberry to different salinity levels in the nutrient solution. Acta Horticulturae 559:675-680.
- Grattan SR, Grieve CM (1999). Salinity-mineral nutrient relations in horticulture crops. Scientia Horticulturae 78:127-157.
- Gutierrez M, Escalante-Estrada JA, Rodriguez-Gonzalez MT (2009). Differences in salt tolerance between *Phaseolus vulgaris* and *Phaseolus coccineus* cultivars. International Journal of Agricultural Research 4:270-278.
- Habtamu Ashagre Asmare (2013). Impact of salinity on tolerance, vigor, and seedling relative water content of haricot bean (*Phaseolus vulgaris* L.) cultivars. Journal of Plant Sciences 1:22-27.
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology 51:463-499.
- Heimler D, Tattini M, Ticci S, Coradeschi MA, Traversi ML (1995). Growth, ion accumulation, and lipid composition of two olive genotypes under salinity. Journal of Plant Nutrition 18:1723-1734.
- Hewitt EJ (1966). Sand and Water Culture Methods Used in the study of Plant Nutrition. Commonwealth Bureau of Horticulture and Plantation Crops. East Malling, Maldstone, Kent, England.
- Karla Y (1998). Handbook of Reference Methods for Plant Analysis. CRC Press. N.Y.
- Kaya C, Kirnak H, Higgs D (2001). Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorous in tomato cultivars grown at high (NaCl) salinity. Journal of Plant Nutrition 24:357-367.
- Kaymakanova M, Stoeva N (2008). Physiological reaction of bean plants (*Phaseolus vulgaris*, L.) to salt stress. Genenal and Applied Plant Physiology 34:177-188.

- Khan MA, Ungar IA, Showalter AM (1999). Effects of salinity on growth, ion content, and osmotic relations in *Halopyrum mocoronatum* (L.) Stapf. Journal of Plant Nutrition 22:191-204.
- Loupassaki MH, Chartzoulakis KS, Digalaki NB, Androulakis II (2002). Effects of salt stress on concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium in leaves, shoots, and roots of six olive cultivars. Journal of Plant Nutrition 25:2457-2482.
- Lycoskoufis LH, Savvas D, Mavrogianopoulos G (2005). Growth, gas exchange and nutrient status in pepper (*Capsicum annum* L.) grown in re-circulating nutrient solution as affected by salinity imposed to half of the root system. Scientia Horticulturae 106:147-161.
- Mavromatis AG, Arvanitoyannis IS, Korkovelos AE, Giakountis A, Chatzitheodorou VA, Goulas CK (2010). Genetic diversity among common bean (*Phaseolus vulgaris* L.) Greek landraces and commercial cultivars: nutritional components, RAPD and morphological markers. Spanish Journal of Agricultural Research 8:986-994.
- Melgar JC, Syvertsen JP, Martínez V, García-Sánchez F (2008). Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. Biologia Plantarum 52:385-390.
- Moya JL, Primo-Millo E, Talon M (1999). Morphological factors determining salt tolerance in citrus seedlings: the shoot to root ratio modulates passive root uptake of chloride ions and their accumulation in leaves. Plant, Cell and Environment 22:1425-1433.
- Munns R (2002). Comparative physiology of salt and water stress. Plant, Cell and Environment 25:239-250.
- Nagesh Babu R., Devaraj VR (2008). High temperature and salt stress response in French bean (*Phaseolus vulgaris*). Australian Journal of Crop Science 2:40-48.
- Ndakidemi PA, Makoi JHHR (2009). Effect of NaCl on the productivity of four selected common bean cultivars (*Phaseolus vulgaris*). Science Research Essays 4:1066-1072.
- Paranychianakis NV, Chartzoulakis KS (2005). Irrigation of Mediterranean crops with saline water, from physiology to management practices. Agriculture, Ecosystems and Environment 106:171-187.
- Pessarakli M (1999). Handbook of Plant and Crop Stress. Second edition, Marcel Dekker, New York, USA.
- Rahimi A, Biglarifard A, Mirdehghan H, Borghei SF (2011). Influence of NaCl salinity on growth analysis of strawberry cv. Camarosa. Journal of Stress Physiology and Biochemistry 7:145-156.
- Rengel Z (1992). The role of calcium in salt toxicity. Plant, Cell and Environment 15:625-632.
- Rodriguez P, Torrecillas A, Morales MA, Ortuno MF, Sanchez-Blanco MJ (2005). Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. Environmental and Experimental Botany 53:113-123.

- Roussos PA, Dimou A, Assimakopoulou A, Gasparatos D, Kostelenos G (2011). Growth and nutrient status of five Greek olive (*Olea europaea* L.) varieties subjected to sodium chloride stress. OliveBioteq 2011. International Conference for olive tree and olive products. Chania, Greece, October 31-4 November, 2011. Proceedings pp. 367-372.
- Rubio-Casal AE, Castillo JM, Luque CJ, Figueroa ME (2003). Influence of salinity on germination and seeds viability of two primary colonizers of Mediterranean salt pans. Journal of Arid Environment 53:145-154.
- Rus A, Lee, B-H, Munoz-Mayor A, Sharkhuu A, Miura K, Zhu J-K, Bressan RA, Hasegawa PM (2004): AtHKT1 facilitates Na⁺ homeostasis and K⁺ nutrition in planta. Plant Physiology 136:2500-2511.
- Semiz GD, Suarez DL, Ulnukara A, Yurtseven E (2014). Interactive effects of salinity and N on pepper (*Capsicum annuum* L.) yield, water use efficiency and root zone and drainage salinity. Journal of Plant Nutrition 37:595-610.
- Sharpley AN, Smith SJ, Jones OR, Berg WA, Coleman GA (1992). The transport of bioavailable phosphorus in agricultural runoff. Journal of Environmental Quality 21:30-35.
- Shibli RA, Sawwan J, Swaidat I, Tahat M (2001). Increased phosphorus mitigates the adverse effects of salinity in tissue culture. Communications in Soil Science and Plant Analysis 32:429-440.
- Slabu C, Zörb C, Steffens D, Schubert S (2009). Is salt stress of faba bean (*Vicia faba*) caused by Na+ or Cl– toxicity? Journal of Plant Nutrition and Soil Science 172:644-650.
- Van Ieperen W (1996). Effects of different day and night salinity levels on vegetative growth, yield and quality of tomato. Journal of Horticultural Science 71:99-111.
- Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Badakhshan H (2014). Effects of zinc application on growth, absorption and distribution of mineral nutrients under salinity stress in soybean (*Ghycine max* L.). Journal of Plant Nutrition 37:2255-2269.
- Wignarajah K (1992). Growth response of *Phaseolus vulgaris* to varying salinity regimes. Environmental and Experimental Botany 2:141-147.
- Yasar F, Uzal O, Tufenkci S, Yildiz K (2006). Ion accumulation in different organs of green bean genotypes grown under salt stress. Plant, Soil and Environment 52:476-480.
- Zhang JL, Flowers TJ (2010). Mechanisms of sodium uptake by roots of higher plants. Plant and Soil 326:45-60.
- Ziaf K, Amjad M, Pervez MA, Iqbal Q, Rajwana IA, Ayyub M (2009). Evaluation of different growth and physiological traits as indices of salt tolerance in hot pepper (*Capsicum annuum* L.). Pakistan Journal of Botany 41:1797-1809.

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