SALINE IRRIGATION WATER AFFECTS ELEMENT UPTAKE BY BEAN PLANT (*VICIA FABA* L.)

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Use of saline water for agricultural irrigation is leading towards salt accumulation in the root zone and consequent damage to crop production and soil fertility. Furthermore, it is known that increased root zone salinity can potentially increase plant trace element uptake. In this context, crop salt tolerance and growth response assessment is useful tool in managing salinity stress. A greenhouse pot experiment was set up to study the effects of irrigation water salinity on growth and element uptake of faba bean (*Vicia faba* L.). Three weeks old faba bean seedlings were transplanted into pots and automatically fertigated with a modified Hoagland nutrient solution. Two weeks after transplanting, treatment with four NaCl salinity concentrations in nutrient solution was applied as follows: $NaCl_0$ – control (basic nutrient solution without added NaCl), $NaCl_{35}$ (control + 35 mM NaCl), $NaCl_{50}$ (control + 50 mM NaCl), $NaCl_{65}$ (control + 65 mM NaCl). Increasing root zone salinity significantly enhanced Na and Cl accumulation in faba bean leaves. A decrease in Mo and K leaf content occurred most significantly at $NaCl_{50}$ treatment, as well as an increase in Mn leaf content. NaCl treatments reduced P leaf content in regard to control but without significant difference amongst treatments. Results have shown that increased root zone salinity can affect certain faba bean leaf element accumulation, although trace element leaf content was not significantly altered. Hence, faba bean could be considered as rather salt tolerant horticultural crop.

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Introduction

An attempt to meet world food demands accompanied with decline in availability in fresh water has resulted in using water of poor quality for crop irrigation. It is known that horticultural production is dependent on soil and water quality. Use of saline water may alter soil's physical and chemical properties, which consequently may lead to decrease in crop yield.[1] Considering the need for increasing the crop yield, as well as the decline of good quality irrigation water, crop salt tolerance assessment can be a useful tool. It may provide information needed for deciding either to expose plants to moderate salt stress or to moderate water stress.[2] Furthermore, increased root zone salinity can affect plant element uptake. In the context of nutrient uptake, it reflects on fertilizers application. In addition, possible toxic element food chain intrusion is already recognized in a saline environment[3]. A site – specific approach that includes the specific crop salt stress response could be a potential solution in merging opposite agricultural demands. It will determine the economic threshold for growing horticultural crops in salt affected areas, as well as whether and when to irrigate crops with saline water.[2]

Using saline water for agricultural irrigation is prevalent in the Croatian Mediterranean coastal region, where seawater intrudes through porous media and salinizes both ground and surface waters. In addition, climatic conditions are increasing the demand for irrigation water, forcing farmers to utilize water of poor quality.[4]

Plant responses to salinity differ but main salinity effects on plant are osmotic and ionic stress. Ionic stress generates nutrient imbalances and affects their bioavailability, competitive uptake, transport or partitioning within the plant[4]. Common indicators of plant salt stress are increased tissue concentrations of sodium and chloride, accompanied with decreased potassium concentration.[3] Although, legume chickpea (*Cicer arietinum* L.) revealed no difference in shoot potassium content, during vegetative stage of development, under saline conditions.[5] Legumes are either sensitive or moderately tolerant to salinity but variability in salinity tolerance among legumes has also been reported. *Vicia faba* (L.) is moderately sensitive to salinity, registering 50% growth reduction at 6.7 dS m^{-1} salinity.[6]

Faba bean is one of the major cool season grain legume crops produced worldwide. It is mainly grown for its high protein content for food and feed. Faba bean popularity has increased recently as its high yield makes it attractive to producers while its high protein content makes it attractive to consumers.[7] Effect of salt stress on faba bean growth (plant height, number of leaves, leaf area, etc.) and yield has been studied.[8,9,10,11] Abdelhamid *et* al.[6] reported that salinity significantly decreased nitrogen, phosphorus, calcium, magnesium and potassium in faba bean leaves while significantly increasing sodium and chloride. Thus, investigations on faba bean element uptake in a saline environment have been limited.

The aim of this study was to elucidate *Vicia faba* (L.) salt stress response after exposing faba bean to rising irrigation water salinity.

Experimental

Growing conditions

The study was carried out during autumn (9 September – 14 November 2011) in a polyethylene greenhouse at experimental station of the Faculty of Agriculture, University of Zagreb, Croatia. Faba bean (*Vicia faba* L. cv. Aguadulce) seeds were sown into polystyrene cups containing a peat soil (Klasmann, Potground P). Three weeks old uniform faba bean seedlings were transplanted into pots (3 litres) containing agricultural soil that was added to commercial substrate (1:1) to increase soil organic matter content. During the first two weeks after transplanting, the seedlings were irrigated daily, using automatic drip irrigation system with water–soluble NPK fertilizer (Poly–Feed Drip 20–20–20 with micronutrients: B, Cu, Fe, Mn, Mo and Zn; c = 2 g/l). In order to ensure soil/substrate mixture aeration and prevent waterlogging, good drainage conditions were ensured. The fertigation rate and frequency was the same for all treatments and was adjusted to the plant phenology and to the climatic conditions in the greenhouse.

Treatments applied and experimental design

Two weeks after transplanting (one plant per pot), treatment with four NaCl salinity concentrations in nutrient solution was applied as follows: $NaCl_0$ – control (basic nutrient solution without added NaCl), $NaCl_{35}$ (control + 35 mM NaCl), $NaCl_{50}$ (control + 50 mM NaCl), $NaCl_{65}$ (control + 65 mM NaCl).

The experimental design used was randomized block design with four replicates, including five plants per replicate, total of twenty plants per treatment.

Data collecting and sampling

Leaf samples were collected four weeks after salinity treatment started. One sample consisted of fully developed mature leaves from plants subjected to the same salinity treatment. Soil/substrate mixture samples from the pots were also collected at the same time. Soil/substrate mixture from pots subjected to the same salinity treatment was merged, mixed thoroughly and representative samples of each treatment were taken.

Plant and soil/substrate mixture analysis

Leaf samples were dried (24h at 60°C) and ground using an inox grinder (Zepter). Dried plant material was dissolved by multiwave–assisted digestion in concentrated HNO_3 : H_2O_2 (10:1, v/v) mixture. P, Fe, Mo, Mn, Cu and Zn concentrations were determined using inductively coupled plasma–optical emission spectrometry (ICP–OES Vista MPX, Varian). Na and K concentrations were measured by atomic emission spectrometry (Atomic Absorption Spectrometer 3110, Perkin–Elmer). Chloride content was measured in a plant water extract colorimetrically (470 nm) using continuous flow auto–analyzer (San++ Continuous Flow Analyzer, Skalar). Certified plant reference material (WEPAL) and blanks were included in digestion and mineral detection.

Soil/substrate mixture samples were air–dried and passed through a 2 mm mesh. All analyses were conducted in a saturated soil/substrate water extract. pH and electrical conductivity was measured. Na and K content was determined by atomic emission spectrometry (Atomic Absorption Spectrometer 3110, Perkin–Elmer). Cl was measured using continuous flow auto–analyzer (San++ Continuous Flow Analyzer, Skalar). Ca and Mg were determined titrimetrically.

Statistical analysis

Data on leaf and soil/substrate mixture element accumulation were subjected to the analysis of variance (ANOVA) using the SAS statistical software package (SAS Institute, 2007). The significance of differences between means was determined with Tukey's HSD test at $P \leq 0.05$.

Results

Soil/substrate mixture element content

Irrigation with saline water ($NaCl_{35}$, $NaCl_{50}$, $NaCl_{65}$) affected the ionic composition of saturated soil/substrate water extract (Table 1). The salinity treatments did not influence the pH of saturated soil/substrate water extracts. Electrical conductivity (dS m^{-1}) increased significantly, proportionally to the treatments, as well as the contents of sodium and chloride (Table 1). Salinity treatments significantly increased calcium, magnesium and potassium content in saturated soil/substrate water extract (Table 1).

Table 1. Effect of different irrigation water salinity levels on electrical conductivity (dS m^{-1}), sodium, chloride, potassium, magnesium and calcium content (mg L^{-1}) in a saturated soil/substrate mixture water extract.

		$NaCl_0$	$NaCl_{35}$	$NaCl_{50}$	$NaCl_{65}$
dS m^{-1}	**pH**	7.7	7.7	7.7	7.8
	E.C.	2.6 $_D$	4.7 $_C$	7.5 $_B$	8.6 $_A$
mg / l	Na^+	54.1 $_D$	335.8 $_C$	646.3 $_B$	841 $_A$
	Cl^-	160.1 $_D$	1193.9 $_C$	2185.3 $_B$	2735.3 $_A$
	K^+	31.1 $_{BC}$	30.7 $_C$	41 $_{BA}$	43.3 $_A$
	Mg^2+	95.3 $_B$	126.4 $_B$	166.7 $_A$	179.9 $_A$
	Ca^{2+}	396.8 $_B$	510.6 $_B$	747.1 $_A$	799.2 $_A$

Means with different superscripts in the same row are significantly different at $P \leq 0.05$.

Leaf damage symptoms

During the experiment, plants exposed to increased NaCl salinity developed salt burning symptoms at the leaf edges, causing marginal chlorosis on the basal, actually the oldest leaves. With time, chlorosis spread over the complete basal leaf area and gradually progressed to necrosis (data not shown).

Leaf tissue element content

Increased root zone salinity significantly increased sodium and chloride content (g kg^{-1}) in faba bean leaves (Figure 1).

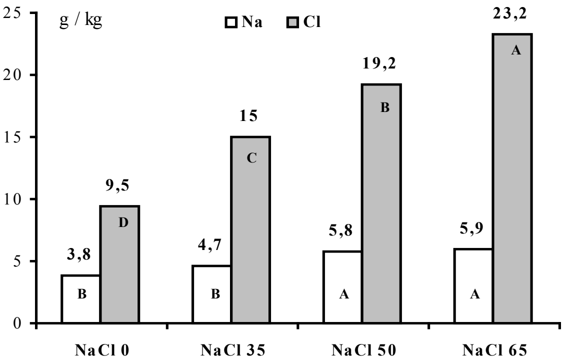

Figure 1. Effect of different irrigation water salinity levels on sodium and chloride content (g/kg) in faba bean. Means with different superscripts are significantly different at $P \leq 0.05$.

Irrigation water salinity significantly affected phosphorus and potassium content (g/kg) in faba bean leaves (Table 2). Phosphorus leaf content of all treated plants decreased regarding to control, but without significant difference amongst salinity treatments. Potassium leaf content most significantly decreased at $NaCl_{50}$ treatment regarding to control plants.

Table 2. Effect of different irrigation water salinity levels on potassium (g/kg), phosphorus (g/kg), molybdenum (mg/kg), manganese (mg/kg), iron (mg/kg), copper (mg/kg) and zinc (mg/kg) content in faba bean leaf tissue.

		NaCl $_{0}$	**NaCl** $_{35}$	**NaCl** $_{50}$	**NaCl** $_{65}$
g/kg	**K**	19.4_{A}	18.8_{BA}	15.4_{B}	17.7_{BA}
	P	4.4_{A}	3.4_{B}	3.2_{B}	3.5_{B}
mg/kg	**Mo**	8.9_{A}	7.6_{BA}	4.5_{B}	7.3_{BA}
	Mn	90_{B}	104.3_{BA}	113.2_{A}	95.3_{BA}
	Fe	99.2_{A}	122.1_{A}	122.7_{A}	106_{A}
	Cu	3.2_{A}	3.8_{A}	3.3_{A}	3.4_{A}
	Zn	27.1_{A}	31.6_{A}	26.8_{A}	25.2_{A}

Means with different superscripts in the same row are significantly different at $P \leq 0.05$.

Differences between control plants and salinity treated plants were determined for molybdenum and manganese leaf content (Table 2). At $NaCl_{50}$ treatment, molybdenum leaf content significantly decreased and manganese leaf content significantly increased, compared to control.

Compared to control plants, iron, copper and zinc faba bean leaf content was not significantly altered with the use of saline water.

Discussion

Salinity treatments significantly increased sodium, chloride, calcium, magnesium and potassium content in saturated soil/substrate water extract (Table 1). Increased potassium, magnesium and calcium content indicate that the process of ion exchange took place in the soil/substrate mixture[12]. If a soil contained equal amounts of the Na, K, Mg and Ca, their distribution would be Ca > Mg > K > Na on the soil adsorption complex[13]. Results suggest that excessive Na content added by saline irrigation water, induced release of K, Mg and Ca ions from soil/substrate adsorption complex into soil/substrate solution.

Increasing root zone salinity significantly increased Na and Cl accumulation in faba bean leaves. High sodium concentration in the rhizosphere may disrupt the integrity and selectivity of root membranes. As a result, imbalance in the availability of different ions may occur, affecting mineral uptake by roots[14,15]. In addition, high soil Na content may interfere with K uptake by the roots. K in plants plays an important role in metabolic processes, in regulation of ion transport and osmotic adjustment. Under salt stress, elevated tissue K levels are required for shoot growth and for the maintenance of full photosynthetic capacity[15]. Results shown in Table 2 suggest that irrigation water salinity caused a decrease in K faba bean leaf content, although not being linear in relation to treatments. Significant decrease in K leaf content occurred at $NaCl_{50}$ treatment, in reference to control plants. Results indicate that decreased K^{+} activity in soil and/or competitive mechanisms with Na^{+} at the root surface, took place under certain salinity levels. In the review given by Grattan and Grieve,[15] the decline in K concentration in plant tissue occurred with increased Na–salinity of the root media. However, some of the studies show K level increase in bean leaves caused by increasing salinity[16,17]. In our experiment, the critical level of salinity was $NaCl_{50}$, at which the decrease of K uptake was detected (Table 2). However, it seems that in further higher levels of salinity the plant of faba bean activates the salt stress tolerance mechanisms.

The effect of salinity on phosphorus (P) content of plant tissue depends on the level of salinity, plant available P content, plant species (cultivar) and developmental stage of plant.[15] Salt stress caused decrease in P concentration of the plant tissue[18], which is consistent with the results obtained in present study (Table 2). Use of saline irrigation water caused a decrease in phosphorus leaf content, but without significant difference amongst different salinity levels. The ionic strength effects that reduce the activity of phosphate, sorption processes that control phosphate concentrations in soil solution and low solubility of Ca–P minerals are the usual explanations for salinity–induced reduction in P availability.[15]

Studies on Mo uptake by crops in a saline environment are generally scarce. Grattan and Grieve[15] suggest that type of growing media (soil or solution) used in experiment plays role in molybdenum behavior in physiological processes. In a maize study carried out on soil, authors found that salinity increased Mo content[19]. Thus, other authors found salinity had no effect on plant Mo uptake from solution[20]. Results of this study (Table 2) show a slight decrease in Mo accumulation under saline conditions. Decrease was significant at $NaCl_{50}$ treatment, comparing to control plants. Mo plant availability depends on soil pH[21]. During this experiment, salinity treatments did not affect pH of saturated soil water extracts, thus indicating that the application of saline irrigation water could interfere with plant Mo uptake.

The majority of studies with crops grown under saline conditions, indicate that salinity decreases Mn shoot content[19,20]. Salt stress induced Mn deficiency in shoot has been reported for barley[22]. Authors have confirmed their hypothesis that increase in leaf Mn content, alleviate salt stress symptoms (increase in relative growth rate, net assimilation rate and net photosynthetic rate of salt stressed plants). In this experiment, treatments with saline irrigation water resulted with slight increase in Mn faba bean leaf content (Table 2). In reference to control plants, increase in Mn leaf content was significant at $NaCl_{50}$ treatment. Considering analyzed plant tissue, salinity composition and growing conditions have been constant for all the treatments during the experiment, variation in results of Mn leaf content can be assigned to different irrigation water salinity levels. In addition, Mn uptake is metabolically mediated[21] and faba bean leaves accumulate Mn from the soil solution[23]. Mn in soil solution participates in cation competition and magnesium particularly depresses Mn uptake[21]. Results at $NaCl_{50}$ treatment also show a significant increase in Mg content of a saturated soil/substrate water extract. Nevertheless, faba bean was able to increase Mn uptake from a soil/substrate mixture with high Mg content. Thus, salinity induced increase in Mn leaf content suggests presence of faba bean salt stress adaptation mechanism.

Mn in plants functions as an active center of superoxide dismutase (Mn–SOD) and participates in plant antioxidant defense. Such stress is produced by high levels of activated forms of oxygen and free radicals (ROS – reactive oxygen species), which are deleterious to plants. Mn–SOD is considered to play an important role in the adaptive plant responses and tolerance improvement under salt stress[24]. Transgenic *Arabidopsis* plants over–expressed Mn–SOD, which played a major role in preventing accumulation of ROS caused by salt stress, thus enhancing their salt stress tolerance[25]. Transgenic tomato plants also have improved NaCl–stress tolerance by Mn–SOD overexpression[26]. Thus, increased Mn level in plant tissue under salt stress suggests possibility that Mn is used for SOD activation, as an adaptive response to increased salinity.

At $NaCl_{50}$ treatment (7,5 dS/m), the most significant differences in faba bean leaf occurred in element accumulation under saline conditions (K and Mo most significantly decreased, Mn most significantly increased), as compared to control plants. Results of $NaCl_{65}$ treatment, actually the highest one, are also consistent with a general trend in salinity effect on element accumulation, in reference to control plants, but slightly diminishing that difference. These results suggest that at electrical conductivity of the root zone >7,5 dS/m, faba bean activates certain salt stress adaptation mechanism, which may be a subject of further studies.

Conclusions

Results of this study show that the use of saline irrigation water increases root zone salinity and affects certain faba bean leaf element accumulation. However, trace element leaf content was significantly altered only for Mo and Mn at certain salinity level. In addition, this research implies existence of Mn–associated faba bean salt stress adaptation and tolerance improvement mechanism. If so, this mechanism occurred as a natural faba bean salt stress response. Results provide foundation for further research to identify faba bean mechanisms of adaptive responses under salt stress. It can also be used as a basis for determination of salt tolerance traits in horticultural crops.

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