Faba Bean (*Vicia faba* L.) Salt Stress Response under Different Soil Organic Matter Content

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Summary

Use of saline water for crop irrigation leads to rhizosphere salinization, which affects plant element uptake, as well as trace elements (TEs) accumulation in plant tissue. Moreover, imbalance in crop element uptake may reflect on crop productivity. Soil organic matter (SOM) plays an important role in soil biogeochemical processes and especially affects trace element mobility and bioavailability. Therefore, it is an important factor for assessment of plant responses under varying ecological conditions, including salinity. A greenhouse pot experiment was set up to study the effects of saline irrigation and increased SOM on faba bean (*Vicia faba* L.) salt stress response. Soil from arable land of Croatian coastal region was used for the trial. One half of the bulk of soil provided for the experiment was mixed with commercial peat (4:1) and two trial variants, unmodified and increased SOM content, were investigated. Two weeks after transplanting faba bean seedlings into pots, treatment with two levels of NaCl salinity (50 and 100 mM NaCl, respectively) was applied in a nutrient solution. Control plants were included in the measurements as well. Saline irrigation as well as increased SOM affected certain element accumulation in bean plant (leaf, pod and/or seed), although no significant interaction between rhizosphere salinization and SOM was revealed.

Key words

faba bean, nutrient uptake, rhizosphere salinity, trace element

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Received: November 13, 2013 | Accepted: March 26, 2014

ACKNOWLEDGEMENTS
This research was supported by the Croatian Ministry of Science, Education and Sports, Project No. 178-1782221-0350

Introduction

Salinization is one of the major environmental constraints that limit crop productivity and quality. Although being more expressed in southern regions of the world, this process is increasingly affecting European agricultural land as well. It is estimated that at least 20% and up to 50% of the irrigated land is affected by salinization (Pitman and Laüchli, 2002) and the problem of soil salinity is expected to amplify in the future (Tejera et al, 2005). In Mediterranean coastal areas, where seawater intrudes into rivers and aquifers (Zalidis et al, 2002), surface and groundwater resources are used as irrigation water supply. Unfortunately, such irrigation water furnishes crops with salts dissolved in it. In addition, agricultural production in this area is unfeasible without irrigation (Romic et al, 2008b) and farmers are compelled to use water of poor quality for crop irrigation (Ondrasek et al, 2006). It is known that horticultural production is dependent on soil and water quality. Using of saline water may alter soil physical and chemical properties, which consequently may lead to decrease in crop yield (Romic et al, 2008a). Although high agricultural productivity is the aim of extensive crop irrigation, use of saline water for irrigation threatens the sustainability of crop production on the irrigated land.

When soluble salts build up in the rhizosphere and start affecting plant element uptake, plants undergo salt stress. Salt stress leads to morphological, physiological, biochemical and molecular changes, which deleteriously affects plant growth and productivity (Wang et al, 2003). Common indicators of plant salt stress are increased tissue concentrations of sodium and chloride, accompanied with decreased potassium concentration (Ondrasek et al, 2009). Plant salt stress is actually comprised of osmotic and ionic stress, often leading to oxidative stress. High soil salinity causes significant changes in soil water potentials, imposing plants to osmotic stress. Osmotic stress can disrupt normal cellular activities and even cause plant death (Xiong and Zhu, 2002). Ionic stress generates alteration in nutrients uptake and affects their bioavailability, competitive uptake, transport and partitioning within the plant (Ondrasek et al, 2006). Consequently, ionic stress can cause molecular damage, growth retardation and cell death (Wang et al., 2008). Generally, most abiotic and biotic stresses induce plant oxidative stress, characterized by increased production of reactive oxygen species that may lead to the unspecific oxidation of proteins and membrane lipids and cause DNA injury (Schützendübel and Polle, 2002).

Legumes can support biological nitrogen fixation and, according to some authors, they offer more environmentally sound and sustainable source of nitrogen to cropping systems (Crews and Peoples, 2004). Furthermore, screening for legumes that can grow and provide economic yield under saline conditions has a dual ecological benefit: (i) less nitrogen fertilization needed; (ii) ability to grow in a saline environment would reduce the effects of the saline irrigation water. Faba bean is one of the major cool season grain legume crops produced worldwide, as its high yield makes it attractive to producers and its high protein content makes it attractive to consumers (Daur et al, 2010). Legumes are either sensitive or moderately tolerant to salinity. *Vicia faba* (L.) is moderately tolerant to salinity, with vegetative growth reduction at irrigation water electrical conductivity of 6 dS m^{-1} and more (Al–Tahir and Al–Abdulsalam, 1997). The fact that crop performance may be adversely affected by salinity–induced nutritional disorders fostered research on salinity–mineral nutrient relations in horticultural crops (Grattan and Grieve, 1999; Parida and Das, 2005). Research on faba bean nutrient removal (Daur et al, 2010) and salt stress effect on growth, water use efficiency and yield has been conducted (Al–Tahir and Al–Abdulsalam, 1997; Katerji et al, 2005; Qados et al, 2011; Katerji et al, 2011). Srinivasarao et al. (2004) observed considerable differences in faba bean nutrient acquisition when subjected to salinity. Abdelhamid et al. (2010) reported that salinity significantly decreased nitrogen, phosphorus, calcium, magnesium and potassium in faba bean leaves while significantly increasing sodium and chloride.

Low levels of SOM can favor the negative effects of salinization (Muhammad et al, 2008) and the addition of organic matter is one of the soil improving measures for overcoming the detrimental effect of salinity (Aydin et al, 2012). The remediation of salt–affected soil using organic matter is an effective, low cost and simple approach. The physical, chemical and biological properties of saline soils can be improved by the application of organic matter, consequently enhancing plant growth and development (Cha–um and Kirdmanee, 2011). Furthermore, SOM is an important factor in soil biogeochemical processes. It plays significant role in retaining TEs in soil, therefore affecting their mobility and bioavailability. In addition, increased rhizosphere NaCl salinity can affect plant trace element uptake. Excessive concentration of dissolved chloride may increase trace element solubility due to chloride ion complexes (Cl–ligands) and potentially increase trace element phyto-accumulation (Zovko et al, 2011).

The aim of this research was to examine faba bean (*Vicia faba* L.) salt stress response and element plant tissue concentrations, after exposing plants to rising irrigation water salinity under different SOM content.

Materials and methods

Growing conditions

The study was carried out from April, 2 – June, 15 2012, in a greenhouse at the experimental station of the Faculty of Agriculture University of Zagreb, Croatia. Faba bean (*Vicia faba* L. cv. Aguadulce) seeds were sown into the polystyrene cups containing a peat soil (Potgrond P, Klasmann). Three weeks old uniform faba bean seedlings were transplanted into pots (one plant per pot) containing (i) agricultural soil and (ii) agricultural soil with added commercial peat (4:1) to increase SOM content. From the beginning of the experiment until the end of study, the plants were irrigated using automatic drip irrigation system. During the first two weeks after transplanting, pots were fertigated daily with a basic nutrient solution (Poly–Feed Drip 20–20–20 with micronutrients: B, Cu, Fe, Mn, Mo and Zn; c = 2 g/l). Good drainage was ensured to provide aeration of soil and soil/peat mixture and prevent waterlogging. The fertigation rate and frequency was adjusted to the plant phenology and to the climatic conditions in the greenhouse, being the same for all the treatments.

Treatments applied and experimental design

Alluvial surface soil (5 – 25 cm) with surficial organic deposit removed (0 – 5 cm) from horticultural land in a Croatian coastal region was used for the experiment. Soil was initially purged from roots and other plant parts, manually fragmented and passed through a 1 cm mesh. Soil was then mixed manually in 4:1 rate (volume ratio) with commercial peat (Potgrond P, Klasmann) to obtain increased SOM content. Peat used for the trial was enriched with certain nutrients stated on its declaration as follows: 210 mg N L^{-1}, 240 mg P_2O_5 L^{-1}, 270 mg K_2O L^{-1} and 120 mg Mg L^{-1}. Furthermore, peat was digested in aqua regia (HRN ISO 11466 2004) with the microwave technique on a MARSXpress system (CEM) for the determination of TEs concentrations (Cu, Fe, Mn, Mo and Zn) by inductively coupled plasma optical emission spectroscopy (Vista MPX, Varian). Determined TEs concentrations were 20.76 ± 0.6 mg Cu kg^{-1}, 0.92 ± 0.04 g Fe kg^{-1}, 53.04 ± 3.7 mg Mn kg^{-1} and 15.25 ± 0.5 mg Zn kg^{-1}, respectively.

Six samples per treatment (SOM_0 and SOM_1) were randomly selected for organic carbon (C) analysis. Soil samples were air–dried, ground and passed through a 0.5 mm mesh. Soil organic carbon was determined by sulfochromic oxidation (HRN ISO 14235: 1998). Referent soil sample (WEPAL ISE) and blank were included in organic C determination. Mean soil organic C value for SOM_0 was 27.7 g kg^{-1} dried soil (105 °C) and for SOM_1 53.6 g kg^{-1} dried soil (105 °C). Two SOM trial variants, unmodified (SOM_0) and increased (SOM_1), were investigated.

Three weeks after transplanting plants, treatment with raising NaCl concentrations in nutrient solution was applied for five weeks as follows: $NaCl_0$ – control (basic nutrient solution without added NaCl), $NaCl_{50}$ (control + 50 mM NaCl), $NaCl_{100}$ (control + 100 mM NaCl).

Split–plot experimental design with three blocks was applied. In each block, the main plots were assigned to two SOM variants and the sub-plots were randomly assigned to three NaCl salinity treatments.

Data collecting and sampling

Per each SOM and salinity treatment, two samples of leafs, pods and seeds were collected from three plants, five weeks after salinity treatment started. Each leaf sample consisted of fully developed leaves located next to the pods. Pod sample consisted of all pods and seed sample consisted of all seeds collected from the pods.

Plant tissue analysis

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

Statistical analysis

Plant tissue analysis data were subjected to the analysis of variance by using MIXED procedure. The significance of differences between the means was determined using a Tukey–Kramer's test at $P<0.05$. Statistical analysis was done using the SAS statistical software package (SAS Institute, 2007).

Results

Plant tissue element concentrations

Enhanced SOM induced a decrease in faba bean leaf chloride concentration, as well as an increase of magnesium and molybdenum concentration (Table 1). Pod tissue phosphorus and molybdenum concentration increased, along with seed phosphorus, molybdenum and zinc concentration. Higher SOM also induced a decrease in pod copper concentration (Table 1).

Irrigation with saline water decreased faba bean leaf dry matter (DM) content (Table 1). However, pod DM percentage increased with raising irrigation water salinity, yet only in regard to control plants. Leaf, pod and seed sodium concentration rose proportionally with the NaCl treatments, as well as the chloride concentration. Irrigation water salinity significantly decreased potassium concentration in faba bean leaves only. Calcium concentration decreased in leaves, pods and seeds, although significant difference occurred only between the control plants in comparison with NaCl treated plants, and no significant difference was determined amongst treatments themselves. Magnesium leaf and pod tissue concentration was affected by NaCl treatments in a same way as plant tissue calcium concentration. The salinity treatments did not affect phosphorus plant tissue concentration. Saline irrigation water induced a slight decrease in sulfur seed concentration. Leaf trace element concentrations were not affected by increased root zone salinity. Different irrigation water salinity level affected pod molybdenum and copper concentration, as well as seed molybdenum concentration (Table 1).

No statistically significant interaction between SOM content and raising irrigation water (NaCl) salinity was revealed for DM content and element concentration in faba bean leaves, pods or seeds (Table 1).

Discussion

Soil degradation caused by salinity affects the amount of plant tissue input and the rate of SOM decomposition, consequently affecting SOM turnover (Setia et al, 2011). Furthermore, SOM plays an important role in TEs biogeochemical processes by retaining TEs in soil, thus affecting their mobility and bioavailability. For any factor that affects the solubility of soil TEs (e.g., SOM) it is generally assumed having an impact on TEs uptake by plants (Tack, 2010). In the present study, significant effect of SOM addition to experimental soil on faba bean (*Vicia faba* L.) P, Mg, Mo, Zn and Cu concentration has been revealed (Table 1). Increase in Mo (leaf, pod and seed), P (pod, seed), Mg (leaf) and Zn (seed) concentration with the SOM addition could simply be the result of the improvement of water-air relations in pots.

Table 1. Effect of saline irrigation water (0, 50 and 100 mM NaCl), soil organic matter (unmodified – SOM_0 and increased – SOM1) and their interaction on dry matter content and element concentrations in faba bean (*Vicia faba* L.) leaves, pods and seeds.

Treatment	DM	Na	Cl	K	Ca	Mg	P	S	Mo	Cu	Fe	Zn	Mn
	%	g kg^{-1} DM							mg kg^{-1} DM				
					Leaf tissue element concentration								
SOM_0	12.7a	34.7a	54.3a	19.1a	34.9a	3.3b	2.4a	2.7a	0.6b	8.5a	662.2a	14.3a	88a
SOM_1	12.7a	31.4a	46.4b	17.7a	37.6a	4.2a	2.5a	2.6a	5.1a	8a	674.9a	14.1a	84.8a
$NaCl_0$	15.2a	2.3c	9.4c	33.7a	42.7a	4.8a	2.7a	2.8a	2.6a	6.5a	297.7a	14.5a	92.6a
$NaCl_{50}$	12b	38.8b	56.1b	13.3b	32.9b	3.1b	2.5a	2.6a	2.7a	9.1a	803.2a	14.3a	86.9a
$NaCl_{100}$	10.9c	57.9a	85.6a	8.1c	33b	3.4b	2.2a	2.6a	3.3a	9.2a	904.8a	13.7a	79.7a
SOM*NaCl interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
					Pod tissue element concentration								
SOM_0	14.3a	4.7a	6.2a	18.5a	2a	1.2a	3.1b	1a	3.6b	5.5a	41.9a	14.6a	12.5a
SOM_1	13.9a	4.4a	6.2a	18.6a	2.1a	1.3a	3.4a	1a	12a	4.3b	40.3a	16.6a	12.9a
$NaCl_0$	13b	0.3c	1.4c	18.3a	2.5a	1.5a	3.2a	1a	6.4c	4.4b	50.9a	14.6a	12.3a
$NaCl_{50}$	14.7a	5.1b	6.2b	18.6a	1.9b	1.2b	3.3a	1a	7.8b	5.7a	31.8a	16.3a	13a
$NaCl_{100}$	14.6a	8.3a	10.9a	18.8a	1.8b	1.2b	3.2a	0.9a	9.2a	4.7ba	40.7a	16a	12.7a
SOM*NaCl interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
					Seed tissue element concentration								
SOM_0	20.4a	0.36a	1.2a	17.8a	0.9a	1.2a	6b	1.6a	8b	10.9a	47.7a	27.2b	9.3a
SOM_1	21a	0.34a	1.1a	17.8a	0.9a	1.3a	6.5a	1.6a	21.6a	11.4a	55a	29.9a	8.7a
$NaCl_0$	20.5a	0.05c	0.8b	17.8a	1a	1.2a	6.5a	1.8a	13.5b	13.4a	63.1a	29.8a	8.9a
$NaCl_{50}$	19.8a	0.42b	1.3a	18a	0.8b	1.2a	6.2a	1.5b	14.7ba	10.3a	47.3a	28.8a	9.3a
$NaCl_{100}$	21.8a	0.57a	1.4a	17.7a	0.8b	1.2a	6a	1.5b	16.2a	9.8a	43.7a	27.1a	8.8a
SOM*NaCl interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Means with the same letter are not significantly different at $P<0.05$; n.s.: non–significant interaction.

However, it could also be an indication of a more direct impact of SOM on plant mineral accumulation by retention and gradual release of nutrients, preventing them from leaching. Wichard et al. (2009) concluded that the binding of Mo to (natural) organic matter prevents leaching of Mo. Increase in plant P with SOM increase may be explained by microbial breakdown of SOM, which causes phosphate release into the soil solution, but also incurred organic molecules to compete with phosphate for adsorption sites, also affecting phosphate equilibrium (Mengel and Kirkby, 1979). Furthermore, addition of organic matter induced an increase of Mg (leaf) and Zn (seed) concentration, whereas Cu (pod) concentration decreased (Table 1). This somewhat inconsistent effect of SOM on mentioned cations still may be explained by the same mechanism. Charged sites enable SOM to retain cations in non-leachable but still exchangeable forms available to plants (Bohn et al., 2001). However, even though all cations may be sorbed on the surface sites of SOM, Cu forms stronger organic complexes than other divalent transition metals (Romic, 2012), e.g. Zn, which could cause its retention by organic matter, yet making it less available for plant uptake. Furthermore, Cu forms inner-sphere complexes with SOM (Boudescque et al., 2007), which are a stronger bond than outer-sphere complexes formed by alkaline earth metals, such as Ca and Mg (Tessier et al., 1996). Nevertheless, these results suggest that SOM application could induce a short–termed plant nutritional status improvement, although this particular effect could hardly be associated to saline soils improvement by increasing SOM per se.

Salinity treatments reduced faba bean leaf DM content (Table 1), which is a known feature of salt stressed plants (Ondrasek et al, 2009). As expected, increasing rhizosphere NaCl concentration significantly enhanced Na and Cl accumulation in faba bean leaves, pods and seeds (Table 1), proportionally to the salinity treatments, though considerably higher Na and Cl concentrations were observed in faba bean leaves comparing to pods or seeds. Even with the highest NaCl treatment, seeds Na and Cl concentrations (0.57 g kg^{-1} and 1.4 g kg^{-1}) were actually low comparing to leaves (57.9 g kg^{-1} and 85.6 g kg^{-1}, respectively) (Table 1). Furthermore, it is known that increased salinity may affect faba bean element uptake and accumulation (Matijevic et al, 2012). In this research, leaf K concentration significantly decreased with rising irrigation water salinity (Table 1). It is known that high rhizosphere Na concentration may interfere with K uptake by the roots, causing a K decrease in leaf tissue. Along with increased Na and Cl leaf tissue concentrations, decreased K leaf tissue concentration is considered to be a common plant salt stress symptom (Ondrasek et al, 2009). However, in a contrast to leaf K concentration, pod and seed K concentrations were not significantly affected by increased salinity (Table 1). Salinity tolerance is actually the ability of a plant to complete its life cycle under salt stress conditions (Yadav et al, 2011), which all plants in this trial were able to do. Mentioned, along with the fact that pod and seed tissue Na and Cl concentrations remained quite low (compared to leaf tissue), as well as K concentration undisturbed by increased root zone salinity, suggests that bean plants have activated a certain salt stress tolerance mechanism, reflected by protection of pod and seed from Na and Cl accumulation. A novel approaches for reduction of rhizosphere salt content are breeding and cultivating salt tolerant plants as prior (primer plants) or simultaneously (companion plants) with salt sensitive crops (Plaut et al, 2013). The primer or companion plants would take up the salts from soil and create a less saline environment for a salt sensitive crop. In this context, the fact that faba bean was able to complete its life cycle despite of high rhizosphere salinity and the amounts of Na and Cl accumulated in faba bean leaves at the highest salinity treatment (57.9 g kg^{-1} and 85.6 g kg^{-1}, respectively), suggest that faba bean may be used as primer and/

or companion crop in a cropping system. Nevertheless, further research on the issue is needed.

Faba bean Ca concentration (leaf, pod and seed) significantly decreased with rising irrigation water salinity comparing to control plants, though without significant difference amongst treatments (Table 1). Increased root zone salinity can affect Ca^{2+} plant uptake (Grattan and Grieve, 1999). Ca plays an important role in processes important for maintaining structural and functional integrity of plant membranes, cell wall structures stabilization, ion transport regulation and selectivity, control of ion–exchange processes and cell wall enzyme activities (Rengel, 1992). Results of this study indicate that excessive rhizosphere Na and Cl concentrations may have a detrimental effect on faba bean cell integrity. Furthermore, NaCl treatments induced a decrease in faba bean Mg leaf and pod concentration (Table 1), even though a decrease was significant only comparing control plants and NaCl treated plants, without significant difference amongst treatments. However, this is in accordance with other research reporting that rising rhizosphere NaCl salinity causes a decrease in plant Mg concentration (Yadav et al, 2011). Research on NaCl salinity effect on plant S uptake is generally scarce, as well as on Mo and Cu (Grattan and Grieve, 1999). In our experiment, with rising irrigation water salinity a slight decrease of seed S concentration, as well as an increase of pod and seed Mo concentration was recorded. Plants take up S as SO_4^{2-} which is an anion similar in size to molybdate (MoO_4^{2-}), the common form of Mo in soils (Mengel and Kirkby, 1979), and studies on the subject propose that SO_4^{2-} transport systems across the root plasma membrane are capable of MoO_4^{2-} transport as well (Kaiser et al., 2005), suggesting their antagonism for plant uptake. Furthermore, Cu pod concentration was also affected by saline irrigation water, although not corresponding linear to NaCl rising salinity (Table 1). Even though an increase in pod Cu concentration was recorded at first salinity treatment, suggesting increased Cu solubility with increased soil salinity, there was no statistically significant difference between Cu concentration in control plants and plants treated with the highest salinity treatment. Despite the fact that salinity effect was significant for faba bean pod Cu concentration, SOM has the major impact on Cu bioavailability in soil (Liao, 2000; Romic, 2012), and further research on the subject is needed to elucidate the behavior of Cu in a saline environment.

No statistically significant interaction between SOM and raising irrigation water salinity on faba bean element accumulation was revealed in this experiment (Table 1). Even though is known that salinity affects nutrient uptake, accumulation and partitioning within the plant (Romic et al, 2008a), as well as that SOM can retain TEs in soil, therefore affecting TEs uptake by plants (Tack, 2010), interaction between raising rhizosphere salinity and SOM content did not affect faba bean (leaf, pod or seed) element accumulation.

Conclusion

SOM addition may affect plant mineral accumulation by retention and gradual release of certain nutrients, preventing them from leaching. In this context, SOM application may induce a short–termed plant nutritional status improvement, although this particular effect could hardly be associated to saline soils improvement by increasing SOM per se. Moreover, this trial revealed no significant interaction between increasing rhizosphere salinity and SOM content on faba bean (leaf, pod or seed) element accumulation.

As expected, increasing rhizosphere NaCl concentration significantly enhanced Na and Cl accumulation in faba bean leaves, pods and seeds. However, pod and seed tissue Na and Cl concentrations remained quite low (compared to leaf tissue), as well as K concentration undisturbed by increased root zone salinity. This revealed the possibility that bean plants have activated a certain tissue specific (e.g. seed, pod) salt stress protection mechanism. Considering that leaves sampled for this trial was in fact located next to the pods, the pod and seed salt stress protection mechanism is indicated by Na and Cl accumulation in the leaves next to the pods, instead in pods or seeds, respectively. Furthermore, a possibility for using faba bean as primer and/or companion plant in a cropping system is suggested.

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