## SOIL AND PLANT NUTRITION - Article

# **Improvement of saline soils with Vicia sativa L. from a semiarid region**

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**ABSTRACT:** In the Cienega de Chapala region of the state of Michoacan, Mexico, forage crops form a major pillar for traditional livestock systems. In this region, high soil salinity due to poor groundwater quality is a common problem. Thus, the objective of this research was to evaluate the potential of the forage species Vicia sativa L. for improved saline soil in a greenhouse and non-leaching conditions over the course of 90 days. In this experiment, three levels of NaCl salinity were tested: 5.3, 7.12 and 10.8 dS·m<sup>-1</sup>. Samples of soil were analyzed for electrical conductivity of the saturation paste extract (ECe), soluble and interchangeable cations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) content. Then, the forage species *Vicia sativa* L were divided into three categories (leaf, stem, and root) and their shoot biomass production and Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> content was determined. Analysis of variance was performed to examine the effects of salinity on each of the evaluated variables; Tukey's test was used to detect differences between the mean values of the variables per treatment. In a greenhouse experiment, the species *V. sativa* is confirmed to be tolerant to a moderately saline level. The high concentrations of Na+ found in both the stems and leaves of this plant confirm that it may be used for the improvement of moderately saline soils. The decrease in pH, ECe, SAR and ESP in the pots via non-leaching conditions confirmed their role in the improvement of the chemical characteristics of the soil.

**Key words:** Cienega de Chapala, glycophyte, non-leaching, phytodesalinization, salinity.

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## INTRODUCTION

In the Cienega de Chapala region of the state of Michoacán, Mexico, a semiarid region, forage crops represent a major pillar of traditional livestock systems. However, an increase in cultivated land area has resulted in the expansion of agriculture and less productive, marginal lands. A diverse set of problems has arisen due to water scarcity and increasing levels of salts and sodium in the soil, as a result of recurrent use of poor-quality groundwater (Silva-García et al. 2006). These environmental factors affect the establishment and development of agricultural crops and negatively impact the profitability of producers.

Salinity generally creates the following conditions for plants: a) ionic stress; b) osmotic stress; and c) disrupted homeostasis. In addition, a combination of different morphological, physiological and biochemical effects may occur (Munns and Tester 2008), negatively affecting nutrient acquisition, transpiration and the photosynthetic function of plants and resulting in stunted growth or potential plant loss. Such effects are reflected as economic losses for producers.

On the other hand, phytodesalination has shown great potential to remediate saline soil. This phytotechnology is based on the capacity of certain plant species to tolerate, absorb and accumulate sodium from the soil via its absorption and concentration in harvestable plant tissues (Rabhi et al. 2015). Studies on the phytodesalination of sodium chloride (NaCl) have analyzed the potential of several halophytic species (Hasanuzzaman et al. 2014); however, other studies, such as those of de Villa et al. (2006) on Capsicum annuum L., and Reves-Perez et al. (2013) on Ocimum basilicum L., have demonstrated the remediation potential of glycophytic species, although to a lesser extent. Such species were able to improve soils with moderate salinity  $(4.1-8.0 \text{ dS} \cdot \text{m}^{-1})$  to a sufficient extent, although this remediation was shown mainly in arid and semiarid regions with low levels of rainfall and inadequate irrigation systems.

Leidi and Pardo (2002) have highlighted the need to analyze the potential of a larger number of glycophytic species for the remediation of salinity-affected soils. The response capacity varies according to species and may vary within the same species, given its stage of growth or genetic variation, in addition to the level and type of salt in the soil.

Furthermore, the use of forage crops to improve salinesodic soils represents a cost-efficient and sustainable alternative to other means. In addition to reducing soil salinity, these crops are able to grow under conditions of residual humidity and may also be used as green fertilizers (Castro et al. 2017; Cerda et al. 2012). Moreover, they may be cultivated over a large land area and can decrease erosion (Abbasi et al. 2014). The present attempt is a step forward in this direction, testing the tolerance of *V. sativa* and its suitability to saline habitats, with the purpose to reach more meaningful conclusions (Parveen and Farrukh 2009; Orak and Ates 2005). Therefore, the objective of the present study was to evaluate the phytodesalination potential of the species *Vicia sativa* L. in greenhouse conditions, given soils with different levels of salinity.

## MATERIALS AND METHODS

The experiment was performed under greenhouse conditions. The soil used in the experiment was taken at a depth of 0 to 30 cm from a farm in the municipality of Jiquilpan (20° 0' 0" N and 102° 42' 30" W at an altitude of 1560 m above sea level, sampling was systematic, in zigzag, and compound samples were obtained. The soil texture was sandy clay loam (clay 40%, silt 22%, sand 38%); the pH was 7.4. The electrical conductivity of extracted saturated soil paste (ECe) was 1.4 dS·m<sup>-1</sup>; the sodium adsorption ratio (SAR) was 6.60 (mmol<sub>c</sub>·L<sup>-1</sup>)<sup>1/2</sup>. The soil had a water-retention capacity (WRC) of 91%, a cation-exchange capacity of 33 cmolc·kg<sup>-1</sup> and an exchangeable sodium percentage (ESP) of 7.28. In addition, the soil contained 0.77 % organic matter (% OM), 0.896 g·L<sup>-1</sup> salt (salt content, SC).

#### Seed material

The seeds of the forage crop, *Vicia sativa* L. X Mezquita, Correa Mexicana brand were obtained from a commercial seed and agrochemical company in the study region.

## Greenhouse experiment under non-leaching conditions

Approximately 580 kg of soil was collected, shade-dried, homogenized and transferred to black polyethylene 12-L pots. The pots were unperforated, and each was filled with 8 kg of soil. The experiment was carried out in March of 2016. Ten plants were sown in each pot and watered with tap water to 70% of the water-holding capacity of each container. On average, the temperature and relative humidity conditions in the greenhouse were 38/15 °C (day/night) and 60% (± 10%), respectively. The experiment was finalized 90 days after germination (DAG). The productivity of the glycophytes (PG) was estimated by crop in pots according to plant density, plant dry weight and the surface area of the pot.

## Physicochemical characteristics of irrigation water

The pots were irrigated manually and the physicochemical characteristics of the water are detailed as follows: The ECe was 0.5 dS·m<sup>-1</sup>, the pH was 8.81 and the sodium adsorption ratio (SAR) was 1.93. The water contained 155 mg.L<sup>-1</sup> of total dissolved solids (TDS), 0.42 mmol<sub>c</sub>·L<sup>-1</sup> Ca<sup>2+</sup>, 3.61 mmol<sub>c</sub>·L<sup>-1</sup> Mg<sup>2+</sup>, 2.75 mmol<sub>c</sub>·L<sup>-1</sup> Na<sup>+</sup>, 0.26 mmol<sub>c</sub>·L<sup>-1</sup> K<sup>+</sup>, 1.47 mmol<sub>c</sub>·L<sup>-1</sup> CO<sub>3</sub><sup>-2</sup>, 5.01 mmol<sub>c</sub>·L<sup>-1</sup> HCO<sub>3</sub><sup>-2</sup> and 0.153 mmol<sub>c</sub>·L<sup>-1</sup> SO<sub>4</sub><sup>-2</sup>.

#### Salinity treatments

Analytical-grade NaCl was used as the salinity source and applied to the soil in the pots 15 days prior to seed planting. Three levels of salinity were tested, in which different amounts of NaCl were added to achieve varying salt concentrations, beginning with 8.75 g·pot<sup>-1</sup> NaCl to increase the base conductivity from 1.4 to 5.3 dS·m<sup>-1</sup>, 12 g·pot<sup>-1</sup> NaCl to increase the conductivity from 1.4 to 7.12 dS·m<sup>-1</sup> and 16.25 g·pot<sup>-1</sup> NaCl to increase the conductivity from 1.4 to 10.8 dS·m<sup>-1</sup>. The three salinity levels tested in this study were based on the research by Parveen and Farrukh (2009), who suggested that *V. sativa* can tolerate moderate levels of salinity and might be cultivated on marginal saline habitat as a source of fodder and nutrients to the soil.

#### Chemical analysis of soil and plants

Particle-size analysis of the original soil was performed according the method developed by Huluka and Miller (2014). ECe, SAR, and ESP were determined in the saturated paste extract. Therefore, soil samples were randomly taken, dried, ground, and separated with a 2-mm-meshed sieve before distilled water was added until saturation. The obtained saturated pastes were covered and left overnight at ambient temperature. The extraction was performed under vacuum. ECe and the concentrations of Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> were measured in the extracts. The plant material from each replicate and treatment (1.4, 5.3, 7.12 and 10.8 dS·m<sup>-1</sup>) was chemically analyzed 112 days after sowing. Plants were first washed with distilled water, and the roots, stems and leaves were then separated and quantified for their fresh and dry weights (g). Samples were oven-dried at 70 °C for 48 h before being ground. Ion extraction was carried out by incubating 20 mg of each sample in 30 ml HNO<sub>3</sub> (0.5%) solution for three days with agitation from time to time. Extracts were then paper-filtered and immediately analyzed for Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> concentrations.

The cations of both soil and plant samples was determined in HNO<sub>3</sub> extracts and in saturated paste extracts, respectively. Measurements of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> content in both the plants and soil samples was performed by atomic absorption spectroscopy (Nemati et al. 2011), using a SensAA spectrometer (AAS).

#### Calculations

Relative rate of phytodesalination (RRP)

To quantify the relative rates of phytodesalination by the species *V. sativa* L. at different levels of salinity were used the following Eq. 1 proposed by Rabhi et al. (2015):

$$RRP (kg Na^{+} t^{1} DW \cdot day^{-1}) = RRG \cdot (Na_{f}^{+} - Na_{i}^{+})/(DW_{f} - DW_{i})$$
(1)
$$RRG = (lnDW_{f} - lnDW_{f})/\Delta t$$

where RRP = the measure of a plant's ability to accumulate Na<sup>+</sup> ions, expressed as unit of biomass (dry weight, DW) per unit of time; RRG = the relative rate of growth; Na<sub>f</sub><sup>+</sup> – Na<sub>i</sub><sup>+</sup> = the difference between the Na<sup>+</sup> concentrations at the beginning and end of the experiment (kg Na<sup>+</sup>·t<sup>-1</sup> DW·day<sup>-1</sup>); and  $\Delta t$  = the duration of the experiment (days).

#### Data analysis

A completely randomized design was employed, with 18 experimental units per treatment; each experimental unit comprised 10 plants. An analysis of variance was performed to examine the effects of the studied factors on each of the evaluated variables. Tukey's test was then used, with a significance threshold of  $p \le 0.05$ , to determine whether the average values of each analyzed variable differed significantly among treatments. All analyses were performed using SAS software version 9.1 (SAS Institute 2004).

## RESULTS

Table 1 shows the content of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> in the roots (R), stems (T) and leaves (H) of *Vicia sativa* L. plants after undergoing saline treatments at different NaCl concentrations under non-leaching conditions for a period of 90 days.

#### Na<sup>+</sup> content

The plants of the evaluated forage species showed comprehensive and significant differences ( $p \le 0.05$ ) in their Na<sup>+</sup> contents when grown with different NaCl treatments and under controled conditions. The Na<sup>+</sup> content in root tissues increased from the control value by 29.78 and 35.05% for saline treatments at 5.30 and 7.12 dS·m<sup>-1</sup>, respectively; for the 10.8 dS·m<sup>-1</sup> treatment, an even higher increase of 38% was observed. In stems, the Na<sup>+</sup> content under the 5.30 and 7.12 dS·m<sup>-1</sup> treatments increased from the control value by 24.25 and 71.68%, respectively; for the 10.8 dS·m<sup>-1</sup> treatment increase of 87.5% was recorded. In leaves, the Na<sup>+</sup> content under 5.30 dS·m<sup>-1</sup> treatment under 5.30 dS·m<sup>-1</sup> treatment increased from the control value by 13.04%; for the 7.12 and 10.8 dS·m<sup>-1</sup> treatments, greater accumulation was observed, with increases of 41.13 and 67.15%, respectively.

#### K<sup>+</sup> content

In the evaluated forage plants, the  $K^+$  content also increased significantly (p  $\leq$  0.05). In root tissues, the  $K^+$  content

increased from the control value by 391.81 and 412.68% for the 5.30 and 7.12 dS·m<sup>-1</sup> treatments, respectively; for the 10.8 dS·m<sup>-1</sup> treatment, a 436.37% increase was observed. In stems, an increasing trend was also observed. For the 5.30 and 7.12 dS·m<sup>-1</sup> treatments, the K<sup>+</sup> content rose from the control value by 346.84 and 369.23%, respectively; for the 10.8 dS·m<sup>-1</sup> treatment, a 385.57% increase in K<sup>+</sup> accumulation was observed. In leaves, the K<sup>+</sup> content under the 10.8 dS·m<sup>-1</sup> treatment increased from the control value by 314.60%, while the 5.3 and 7.12 dS·m<sup>-1</sup> treatments corresponded to smaller increases of 209.33 and 231.87%, respectively.

#### Ca<sup>2+</sup> and Mg<sup>2+</sup> content

The contents of  $Ca^{2+}$  and  $Mg^{2+}$  in the forage plants differed significantly (p  $\leq$  0.05). As the concentration of salt increased, the contents of  $Ca^{2+}$  and  $Mg^{2+}$  also increased.

The Ca<sup>2+</sup> content of the root tissues increased with respect to the control by 60.76, 483.86 and 565.98% under the 5.30, 7.12 and 10.8 dS·m<sup>-1</sup> treatments, respectively. Meanwhile, the Mg<sup>2+</sup> content of the root tissues increased with respect to the control by 60.27 and 76.48% under the 5.30 and 7.12 dS·m<sup>-1</sup> treatments, respectively; an increase of 93.71% was observed for the 10.8 dS·m<sup>-1</sup> treatment. The Ca<sup>2+</sup> content in the stems showed a marked increase relative to the control under the treatment conditions, increasing by 21.23 and 23.68% in the 5.30 and 7.12 dS·m<sup>-1</sup> treatments, respectively. The accumulation was even greater for the 10.8 dS·m<sup>-1</sup> treatment, for which an increase of 42.04% was

Table 1. Content of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> of the roots (R), stems (T) and leaves (H) of Vicia sativa L. at 90 DAG.

	Organ	Mg∙kg⁻¹DW					
Treatments (dS·m⁻¹)		Na⁺	K+	Ca <sup>2+</sup>	Mg <sup>2+</sup>		
Control	R	4270.30 ± 172.8 <sup>a</sup>	1476.3 ± 181.9 <sup>a</sup>	$33.9 \pm 11.8^{a}$	1750.32 ± 129.6°		
	Т	$14086.60 \pm 388.4^{\circ}$	1925.6 ± 205.2ª	295.78 ± 22.3ª	1495.36 ± 321.2ª		
	Н	$9474.23 \pm 441.5^{a}$	3055.5 ± 211.8°	338.41 ± 16.2ª	1904.76 ±155.2°		
5.3	R	5542.12 ± 120.7 <sup>b</sup>	7260.6 ± 132.7 <sup>b</sup>	21.50 ± 10.9°	2805.25 ± 117.4 <sup>b</sup>		
	Т	$17504.05 \pm 231.2^{b}$	8604.4 ± 230.3 <sup>b</sup>	$358.60 \pm 17.3^{\text{b}}$	2632.21 ± 210.2 <sup>b</sup>		
	Н	$10709.78 \pm 367.6^{\text{b}}$	9451.7 ± 361.2 <sup>b</sup>	375.36 ± 22.4 <sup>a</sup>	$3134.88 \pm 180.5^{b}$		
7.12	R	$5767.10 \pm 140.8^{\circ}$	7568.70±182.4 <sup>bc</sup>	$197.93 \pm 31.2^{b}$	3089.12 ± 142.7°		
	т	24184.83 ± 365.6°	$9035.55 \pm 245.2^{\circ}$	365.84 ± 32.8 <sup>b</sup>	3056.11 ± 163.2°		
	Н	13371.63 ± 627.1°	$10140.4 \pm 241.1^{\circ}$	$421.94 \pm 18.4^{\circ}$	3220.53 ± 121.3°		
10.8	R	$5920.41 \pm 218.2^{\rm bc}$	$7918.48 \pm 132.7^{\circ}$	225.77 ± 30.6 <sup>b</sup>	3390.61 ± 188.5 <sup>d</sup>		
	Т	$26413.69 \pm 395.6^{d}$	$9350.19 \pm 235.6^{\circ}$	$420.13 \pm 24.2^{bc}$	3212.23 ± 215.8°		
	Н	$15835.31 \pm 508.2^{d}$	$12668.2 \pm 393.2^{d}$	477.53 ± 25.5 <sup>b</sup>	3609.23 ± 134.9 <sup>d</sup>		

In each row, different letters represent significant differences according to Tukey's test ( $p \le 0.05$ ). Values are averages of 18 replicates ± standard error, (R: Roots; H: Leaves and T: Steam). DAG (days after germination).

observed. The Ca<sup>2+</sup> content of leaves under the 5.30 dS·m<sup>-1</sup> treatment was 10.91% greater than in the control; for the 7.12 and 10.8 dS·m<sup>-1</sup> treatments, the Ca<sup>2+</sup> content rose by 24.68 and 41.10%, respectively.

The Mg<sup>2+</sup> content in stems under the  $5.30 \text{ dS} \cdot \text{m}^{-1}$  treatment was 76.02 % higher than the control; for the 7.12 and 10.8 dS·m<sup>-1</sup> treatments, the Mg<sup>2+</sup> content rose by 104.37 and 114.81%, respectively. Likewise, the Mg<sup>2+</sup> content in the leaves under the  $5.30 \text{ dS} \cdot \text{m}^{-1}$  treatment was 64.58% greater than in the control; for the 7.12 and 10.8 dS·m<sup>-1</sup> treatments, the increases were 69.07 and 89.48%, respectively.

Overall, increased concentrations of NaCl in the soil led to increased Na<sup>+</sup> content in the plant tissues of *V. sativa* while decreasing the rate of Ca<sup>2+</sup> and Mg<sup>2+</sup> accumulation, demonstrating a clear antagonistic relationship among Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> (Läuchli and Grattan 2007).

As shown in Table 2, the final dry weights of the roots decreased relative to the control by 13.85, 21.42 and 25.32% under the 5.30, 7.12 and 10.8 dS·m<sup>-1</sup> treatments, respectively. Furthermore, the final dry weights of the shoots relative to the control increased by 47.76, 193.36 and 304.46% under the 5.30, 7.12 and 10.8 dS·m<sup>-1</sup> treatments, respectively. The final dry weights of the whole plants increased with increasing NaCl concentration. Compared to the control (1.4 dS·m<sup>-1</sup>), the final dry weight of plants subjected to 5.30 dS·m<sup>-1</sup> treatment increased by 33.41%; the corresponding increases under the 7.12 dS·m<sup>-1</sup> and 10.8 dS·m<sup>-1</sup> treatments were 143.34% and 227.67%, respectively

Table 3 shows the electrical conductivity (ECe), pH, SAR and ESP of the soil and lists the relative rate of phytodesalination (RRP) of *V. sativa* L. after being subjected to different NaCl concentrations under non-leaching conditions for 90 days.

At the end of the experiment, the results also revealed reductions in the concentrations of soluble sodium in the soil.

The evaluated species has a high capacity to eliminate this cation, as reductions of 27.7, 30.02 and 46.6% were observed for the 5.30, 7.12 and 10.8 dS·m<sup>-1</sup> treatments, respectively. The elimination of sodium was also reflected by decreases in the SAR of 6.18, 10.01 and 15.07% for the 5.30, 7.12 and 10.8 dS·m<sup>-1</sup> treatments, respectively.

Analysis of soil samples, taken after the final harvest of the plant species, indicates that the desodication succeeded in all the treatments. The reduction in the SAR of the *V. sativa* plant is notable, given an ECe of 10.8 dS·m<sup>-1</sup>, as such a reduction is substantial for a glycophyte.

In addition, the exchangeable sodium percentage (ESP) decreased by 7.1, 10 and 14.5% in the 5.30, 7.12 and  $10.8 \,\mathrm{dS} \cdot \mathrm{m}^{-1}$  treatments, respectively, while the control showed an increase of 14.7% by the end of the experiment.

## DISCUSSION

Overall, the results demonstrated that increasing concentrations of soil NaCl led to increasing Na<sup>+</sup> accumulation in plants, which mainly occurred in the stem and leaves. These results agree with those of Munns (2002), who showed that for the majority of plants, the principal site of Na<sup>+</sup> toxicity is the leaf lamina. Due to transpiration, Na<sup>+</sup> is deposited and accumulates in leaves rather than in roots. In perennial species, Na<sup>+</sup> accumulation in leaves mainly results from their longer lifespan and thus longer duration of transpiration.

We observed that reduced levels of  $K^+$  correspond with increased concentrations of Na<sup>+</sup>. According to Conn and Gilliham (2010), higher levels of Na<sup>+</sup> interfere with the accumulation of  $K^+$ , as these ions compete for the same subcellular sites.

Treatments (dS·m <sup>-1</sup> )	Organ	Initial dry weight (g·plant-1)	Final dry weight (g∙plant⁻¹)	Dry weight (g·pot⁻¹)	
Control	Whole plant	$0.0569 \pm 0.0041^{\rm a}$	$0.1984 \pm 0.0242^{\circ}$	$1.9912 \pm 0.0215^{a}$	
Control	Ratio (Root/Shoot)	$0.2902 \pm 0.0112^{\rm a}$	0.3035 ± 0.0614°		
5.3	Whole plant	$0.0533 \pm 0.0031^{\circ}$	$0.2647 \pm 0.0317^{\rm b}$	$2.6518 \pm 0.0327^{\text{b}}$	
	Ratio (Root/Shoot)	$0.3808 \pm 0.0317^{\text{b}}$	$0.1769 \pm 0.0123^{\text{b}}$		
7.12	Whole plant	$0.0435 \pm 0.0049^{\circ}$	$0.4828 \pm 0.0334^{\circ}$	$4.8308 \pm 0.0026^{\rm c}$	
	Ratio (Root/Shoot)	$0.4403 \pm 0.0359^{\circ}$	$0.0812 \pm 0.0214^{\circ}$		
10.8	Whole plant	$0.037 \pm 0.0058^{\rm ab}$	$0.6501 \pm 0.0187^{d}$	$6.5112 \pm 0.0243^{d}$	
	Ratio (Root/Shoot)	$1.384 \pm 0.1172^{d}$	$0.0560 \pm 0.0421^{d}$		

Table 2. Dry weight of V. sativa plant organs during the first phase (21 DAG) and at the end of the experiment (90 DAG).

In each row, different letters represent significant differences according to Tukey's test (p ≤ 0.05). Values are averages of 18 replicates ± standard error. (R: Roots; H: Leaves and T: Steam). DAG (days after germination).

 Table 3. Electrical conductivity (ECe), pH, SAR and ESP of the soil and relative rate of phytodesalination (RRP) of V. sativa L. after being subjected to different NaCl concentrations under non-leaching conditions for 90 days.

	Treatments (dS·m <sup>-1</sup> )							
Parameters	Control		5.3		7.12		10.8	
	I	F		F	I	F		F
ECe (dS·m <sup>-1</sup> )	1.40ª	1.62ª	5.30ª	4.63 <sup>b</sup>	7.12ª	5.62 <sup>b</sup>	10.8ª	7.24 <sup>b</sup>
рН	<b>7.40</b> ª	7.51ª	7.92ª	7.81ª	8.15ª	7.98 <sup>b</sup>	8.48ª	8.23 <sup>b</sup>
Na <sup>+</sup> (mmol <sub>c</sub> ·L <sup>-1</sup> )	23.7ª	15.21 <sup>b</sup>	33.47ª	24.19 <sup>b</sup>	45.65ª	31.95 <sup>b</sup>	75.28ª	40.58 <sup>b</sup>
Ca <sup>2+</sup> (mmol <sub>c</sub> ·L <sup>-1</sup> )	13.3ª	5.78 <sup>b</sup>	20.58ª	12.67 <sup>b</sup>	27.61ª	17.65 <sup>b</sup>	47.27ª	21.74 <sup>b</sup>
Mg <sup>2+</sup> (mmol <sub>c</sub> ·L <sup>−1</sup> )	12.5ª	3.55 <sup>b</sup>	16.25ª	9.2 <sup>⊾</sup>	21.34ª	12.18 <sup>b</sup>	49.22ª	15.86 <sup>b</sup>
Na⁺ (cmol <sub>c</sub> ·kg¹)	4.15ª	3.61 <sup>b</sup>	6.27ª	4.11 <sup>b</sup>	7.40ª	4.93 <sup>b</sup>	9.96ª	7.05 <sup>b</sup>
K⁺ (cmol <sub>c</sub> ·kg¹)	2.01ª	1.85 <sup>b</sup>	2.45ª	2.13 <sup>b</sup>	2.52ª	2.20 <sup>b</sup>	2.57ª	4.09 <sup>b</sup>
Ca <sup>2+</sup> (cmol <sub>c</sub> ·kg <sup>-1</sup> )	18.32ª	38.34 <sup>b</sup>	29.78ª	41.92 <sup>b</sup>	32.69ª	45.9 <sup>b</sup>	33.33ª	51.89 <sup>b</sup>
Mg <sup>2+</sup> (cmol <sub>c</sub> ⋅kg <sup>-1</sup> )	8.72ª	12.07 <sup>b</sup>	9.34ª	11.24 <sup>b</sup>	9.64ª	9.32 <sup>b</sup>	9.78ª	8.77 <sup>b</sup>
SAR (mmol <sub>c</sub> ·L <sup>-1</sup> ) <sup>1/2</sup>	6.60ª	7.04 <sup>b</sup>	7.80ª	7.31 <sup>b</sup>	9.19ª	8.27 <sup>b</sup>	11.01ª	9.35 <sup>b</sup>
ESP	7.28 ª	8.35 <sup>b</sup>	9.29ª	8.63 <sup>b</sup>	10.96ª	9.85 <sup>b</sup>	13.03ª	11.14 <sup>b</sup>
RRP (kg·Na⁺·t¹ DW·day¹) 0.0261 <sup>a</sup>		0.0297 <sup>b</sup>		0.03940°		0.0439 <sup>d</sup>		

In each row, different letters represent significant differences according to Tukey's test ( $p \le 0.05$ ). Values are averages of 18 replicates  $\pm$  standard error. (I: Initial Stage before the seed sowing; F: Final Stage after the seed sowing).

Moreover, the greatest accumulation of  $K^+$  was found in leaves, followed by stems and roots. This distribution of ions in the different organs of *V. sativa* agrees with the report of González (2013), who found that in soy plants, the greatest  $K^+$  accumulation occurred in leaves, followed by stems and roots, while the greatest Na<sup>+</sup> accumulation was found in stems, followed by roots and leaves.

This pattern suggests that potassium uptake constitutes the principal mechanism for combating saline stress in this species. Potassium maintains cell turgor, regulates osmosis and participates in cell expansion (Pares and Basso 2013). This idea is further evidenced by the growth of the leaves and stems over time (reflected in the dry weight), as discussed below. With respect to this final point, recent studies have confirmed that K<sup>+</sup> is one of the main cations in plants and contributes more than 6% of dry weight, due to its positive influence on energy metabolism and carbohydrates and especially on protein synthesis during plant growth (Africano Perez and Pinzon Sandoval 2015).

The greatest levels of Ca<sup>2+</sup> were found in the leaves, followed by the stem and roots; Ca<sup>2+</sup> is necessary for regulating cellular metabolism and for protecting membranes from potential saline stress-induced damage (Casierra et al. 2006).

In addition, the increase in  $Mg^{2+}$  in plants over the control levels may be attributed to the function of  $Mg^{2+}$  in photosynthesis and in the biosynthesis of proteins and

chlorophyll. Thus, elevated Mg<sup>2+</sup> uptake is largely necessary for plants growing in high salinity, as this ion is necessary to maintain a high growth rate in roots and young shoots (Maathuis 2009).

The increases in the dry biomasses of plants exposed to moderate and relatively high salt concentrations may be due to an increase in the synthesis of organic solutes (e.g., sugars, proteins and amino acids). Such solutes serve to counter the osmotic effects of salinity during the growth stage (Khan et al. 2000) and may be associated with mechanisms of salinity tolerance in the *V. sativa* L. cultivar.

According to Balibrea et al. (2003), for plants grown under saline conditions to osmotically adjust and to increase their internal osmotic potential, they must use photosynthetic products at a higher rate, as confirmed by the high levels of  $K^+$  and  $Mg^{2+}$  in the foliar area.

Notably, saline tolerance is evaluated according to the degree of dry matter reduction (Niu and Cabrera 2010). In this respect, *V. sativa* may be considered saline tolerant, as the rate of water adsorption in the evaluated plants was not reduced as the salinity levels of the treatments increased. On the contrary, plants would expend a greater amount of energy, and carbon capture and photosynthesis per foliar unit area would be reduced (Moradi and Ismail 2007). The saline tolerance of *V. sativa* was also confirmed by a reduction in the root-to-shoot ratio as the NaCl concentration increased;

this reduction is evidence of an equilibrium process and demonstrates that the salinity levels did not affect the growth of leaves or lead to a relative increase in the dry weight of the root area. Hence, roots grew at a slower rate to reduce, by dilution, the concentration of toxic ions in their tissues.

*Vicia sativa* showed a good capacity to extract sodium from the soil. The electrical conductivity (ECe) of the treatments at 5.30, 7.12 and 10.8 dS·m<sup>-1</sup> decreased by 7.71, 30.0 and 46.09%, respectively; in contrast, the control experiment showed an increase of 15.71% in the ECe at the end of the experiment. This result agrees with those reported by Cerda et al. (2010), who found that the decreasing trend in soil ECe is due to a greater uptake of Na<sup>+</sup> and Ca<sup>2+</sup> cations from the soil. Accordingly, the use of *V. sativa* in agricultural environments has the potential to improve crop output by decreasing osmotic effects and toxicity due to the presence of dissolved salts in the soil (Mogollón et al. 2015).

On the other hand, decrease of SAR in the *V. sativa* could be due both to the release of H- ions from the roots by an active electrogenic transport mechanism followed by Na<sup>+</sup> H exchange at the surface (Qadir et al. 1996), as well as the Ca<sup>2+</sup> and Mg<sup>2+</sup> provided by water during irrigation, since the dilution of the soil solution favors the adsorption of divalent at the expense of monovalent cations such as Na<sup>+</sup> (Jalali et al. 2008). In fact, the SAR reduction has also been found in *Sesbania aculeata* and *Leptochloa fusca* L., two glycophyte forages subjected to moderate salinity conditions such as this, which showed a capacity to reduce SAR by 42.9% and 37.5%, respectively, during a period of 150 days (Qadir et al. 2007, 1996).

Furthermore, *V. sativa* exhibited a higher rate of sodium accumulation (RRP) in their shoots under the 7.12 dS·m<sup>-1</sup> and 10.8 dS·m<sup>-1</sup> treatments, with corresponding values of 0.03940 and 0.0439 kg Na<sup>+</sup>·t<sup>-1</sup> DW·day<sup>-1</sup> respectively, when compared to the 5.3 dS·m<sup>-1</sup> treatment and the control. However, these values are minimal when compared to those of the halophytes species as *Suaeda* fruticosa, which has an RRP value of 3.5, or *Sesuvium portulacastrum*, which has an RRP value of 3.2 kg Na<sup>+</sup>·t<sup>-1</sup> DW·day<sup>-1</sup> (Rabhi et al. 2015).

This is due to the range of highly effective and complementary morphological, physiological and anatomical characteristics of halophytes to combat and even benefit from the saline environment (Shabala and Mackay 2011), that is their ability to use inorganic ions such as Na<sup>+</sup> and Cl<sup>-</sup> take it passively along the electrochemical gradient (ATP) to perform the osmotic adjustment in their tissues when they grow under saline conditions, a feature conferred by the constitutive expression of vacuolar-bound Na<sup>+</sup>/H<sup>+</sup> NHX (Apse and Blumwald 2007) supplemented by the efficient control of slow and rapid vacuolar ion channels (Bonales-Alatorre et al. 2013) to prevent Na<sup>+</sup> from re-penetrating the cytosol. This is contrary to what happens with the glycophytes, which have a limited capacity to use Na<sup>+</sup> in the osmotic adjustment (Flowers and Colmer 2008).

### CONCLUSION

The species *V. sativa* is confirmed to be tolerant to a salinity level corresponding to an ECe value of 10.8 dS·m<sup>-1</sup>, as plants did not exhibit reductions in water adsorption in the shoots when exposed to this level of salinity. The high concentrations of Na<sup>+</sup> found in both the stems and leaves of this plant confirm that it may be used for the phytodesalination of moderately saline soils. The decrease in pH, ECe, SAR and ESP in the pots after being subjected to different NaCl concentrations under non-leaching conditions confirmed their role in the improvement of chemical characteristics of the soil. In addition, the optimal rate of sodium accumulation of *V. sativa* was observed at saline concentrations corresponding to 7.12 and 10.8 dS·m<sup>-1</sup>.

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