INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 14, No. 2, p. 123-130, 2019
http://www.innspub.net

RESEARCH PAPER | OPEN ACCESS

Salt tolerant strains of *Sinorhizobium meliloti* improved the forage yield and nutrient contents of *Medicago sativa* under salt toxicity of soil

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Article published on February 28, 2019

Key words: Salinity, Alfalfa, Nutrients, *Sinorhizobium meliloti*, Forage yield.

Abstract

Salt stress extremely disrupts the growth and developments of plants, whereas nutrients play a vital role in regulating plant responses to salinity stress. Bacteria is attracting increasing attention in recent years as a potential soil amendment under stress condition. This study addressed the use of salt tolerant strains (three strain) of *Sinorhizobium meliloti* to mitigate salt-stressed soil (7.34 dS m^{-1}) and evaluated the levels of some nutrients in alfalfa (*Medicago sativa* L.) ecotypes (Kara yonje, Hamedani and Malek kandi). All of the *Sinorhizobium meliloti* strains under the salt stress, improved the forage yield in ecotype of Hamedani. In ecotype of Malek kandi, inoculation of seeds by strains 1 and 3 increased the dry weight of forage in compared to the non-inoculated crops, strain 2 did not show a tangible effect on forage yield. The most beneficial effect of *Sinorhizobium meliloti* on rising fresh and dry weights of alfalfa ecotypes occurred in ecotype of Hamedani with strain 2. Inoculation of alfalfa ecotypes by *Sinorhizobium meliloti* exposed various results depends to ecotypes, but in general in all of the ecotypes, *Sinorhizobium meliloti* improved the manganese content in plant tissues. This nutrient showed a significant correlation with alfalfa growth under salt stress.

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Introduction

Salt toxicity of soil is a main factor inhibiting global agricultural productivity. About 20% of the world's irrigated land were affected by high soil salinity, which has been a major limitation to agricultural productivity (Munns and Tester, 2008; Gupta and Huang, 2014; Farhangi-Abriz and Torabian, 2017). Natural activities or crop irrigation with saline water can cause salt toxicity of soil in different semi-arid and arid areas of the world (Parida and Das, 2005; Rivero *et al.,* 2014). Salinity causes several destructive effects on crop growth and productivity, which are due to a low hydraulic potential of soil in the rhizosphere (osmotic stress), oxidative stress and ion toxicity (Sheldon *et al.,* 2017; Farhangi-Abriz and Ghassemi-Golezani, 2018). In legumes, salinity can inhibit nodulation and nitrogen metabolism in different parts of plants such as root and leaf (Farhangi-Abriz and Torabian, 2018 a). Salt ions such as sodium and chlorine can reduce, absorb of mineral nutrient through competitive interaction or altering membrane selectivity. For example, a high level of sodium toxicity reduced calcium, magnesium and potassium absorption in bean plants (Farhangi-Abriz and Torabian, 2018 b; Ghassemi-Golezani and Farhangi-Abriz, 2018).

Alfalfa (*Medicago sativa*) is an important forage crop from legume families with great agronomical importance, due to the high nutritional value, low production cost and its quality, nitrogen fixing capability and perennial growth (Hanson, 2015; Warnke and Ruhland, 2016). This forage crop was cultivated all over the world for silage making, hay and pasture. About 30 Mha of alfalfa is grown in worldwide. Most of commonly available alfalfa cultivars and ecotypes are deficient in salt tolerance (Bao *et al.,* 2016; Putnam *et al.,* 2017). The productivity of this forage crop decrease by the salt stress, and also its capacity of nitrogen metabolism and nodulation inhibit by salt stress. Several tactics such as inoculation of plants with plant growth-promoting rhizobacteria have been recognized to decrease the toxic effects of salt ions in plants (Egamberdieva and Lugtenberg, 2014; Shrivastava and Kumar, 2015; Habib *et al.,* 2016). *Sinorhizobium meliloti* is important bacteria which can help to increasing forage yield and growth of alfalfa under salt toxicity of soil.

Sinorhizobium meliloti is a Gram-negative nitrogen-fixing bacterium (Rhizobium). It forms a symbiotic relationship with legumes from the genera Medicago, Melilotus, and Trigonella, including the model legume Medicago truncatula (Palma *et al.,* 2014; López-Gómez *et al.,* 2017). Several reports have been dedicated to the formation of symbioses between root-nodule bacteria and legume plants under salt stress (Song *et al.,* 2017; Farhangi-Abriz and Torabian, 2018 a). Under the salt toxicity of soil, the survival of root-nodule bacteria in soil and rhizosphere decrease and the cell ultrastructure disrupts (Roumiantseva and Muntyan, 2015; Sabagh *et al.,* 2017). However, it is still unidentified whether root-nodule bacteria can affect the tolerance of nitrogen-fixing symbioses with respect to salt toxicity of soil. High activity of root nodules can supply the mineral nutrients and increase plant growth rate under salt stress (Benidire *et al.,* 2017; Tedersoo and Brundrett, 2017). Thus, the objective of this work was to assess the effect of symbiotic relationship between *Sinorhizobium meliloti* bacteria and alfalfa on forage yield and nutrient contents under salt toxicity of soil.

Materials and methods

Experimental conditions

A field experiment with three replications was conducted during the two growing seasons in an experimental farm (latitude 38.15° N, longitude 46.45° E, altitude 1349 m) with a split plot arrangement (Bacterias in the main plot and alfalfa ecotypes in sub plot) based on randomized complete block design. The average yearly precipitation, which occurs mostly during the autumn and winter months, is 300 mm for the research farm. The annual mean temperature is 12.4°C. The main properties of the experimental soil with loam, clay sand structure appear in Table 1. Three salt tolerant strains of *Sinorhizobium meliloti* were chosen according to the pretests (Strain 1, Strain 2, and Strain 3), then three ecotypes of alfalfa (Kara yonje, Hamedani and Malek kandi) were inoculated with these bacterias (Seeds of these echo tips were inoculated with 10^8 bacterias perml). Before the sowing, 20 kg urea per hectare was added to the farm (Starter fertilizer for legumes) and inoculated seeds were sown. The first year was dedicated to the establishment of the crop, and the measurements began in the second year.

Table 1. Some chemical characteristics of the experimental soil.

Property	pH	EC	C	N	P	K	Cu	Mn	Fe	Zn
		dS m^{-1}	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	7.9	7.34	0.35	44	3.94	235	0.9	3.5	1.7	0.6

Harvesting method

The plants at $1m^2$ of the middle part of each plot were separately harvested at three stages (Cut off 5cm from the soil surface at the 25% of flowering stage) than fresh weight and number of branches were determined. The dry weight of each sample was determined after oven drying at 80°C for 48h.

Nutrient contents

About 1 gram of dried samples was powdered and used for determination of nitrogen content in plant tissues with kjeldahl (Nelson and Sommers, 1973). Phosphorus (P) was measured by yellow method, in which vanadate–molybdate (Tandon *et al.,* 1968) is used as an indicator. P content was determined at 430nm using a spectrophotometer (Shimadzu UV3100, Japan). To determine the Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn) content in plant tissues of alfalfa, plant samples were dry-ashed at 500°C for 7h and then, 500mg of dried samples was digested in 5ml HNO_3. Tubes were filled up to volume (50ml) with double-distilled water and analyzed for ion content (mg g^{-1} dry weight) with atomic absorption spectrophotometry (Shimadzu model: AA-7000, Kyoto, Japan).

Statistical analysis

All the data were analyzed on the basis of experimental design, using SPSS 16 software. Excel software was used to draw figs.

Results

Forage yield

The ecotype of Malek kandi showed the highest weight of forage in comparison with other ecotypes (Fig. 1). Inoculation of alfalfa seeds by different strains of *Sinorhizobium meliloti* exposed various results. Inoculation of Kara yonje seeds by Strain 3 increased the forage yield (Dry weight) under salt stress, however, other strains did not show the beneficial effect on this trait. All of the *Sinorhizobium meliloti* strains under the salt stress, improved the forage yield in ecotype of Hamedani, but the strain 2 had a better effect than other strains on this improvement. In ecotype of Malek kandi inoculation of seeds by strains 1 and 3 increased the dry weight of forage in compared to the non-inoculated crops, strain 2 did not show a tangible effect on forage yield. The most beneficial effect of *Sinorhizobium meliloti* on rising fresh and dry weights of alfalfa ecotypes occurred in ecotype of Hamedani with strain 2.

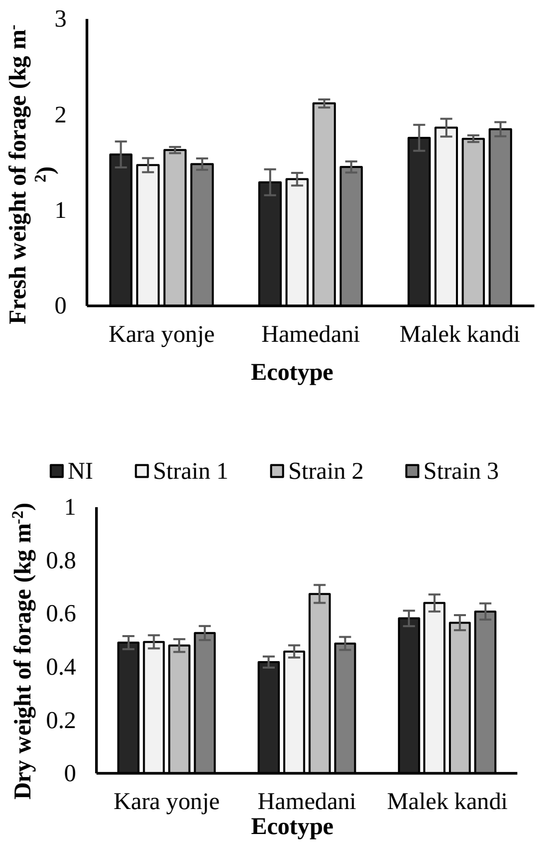


Fig 1. The effects of *Sinorhizobium meliloti* strains on fresh and dry weights of forage in alfalfa ecotypes under salt stress.

Number of branches

The ecotype of Malek kandi showed the highest number of branches between the ecotypes (Fig. 2). Inoculation of alfalfa by strains 1 and 3 improved the number of branches in Kara yonje ecotype, but in ecotype of Hamedani, only the strain 2 increased the number of branches in square meter and other strains did not showed the beneficial effect on the rising number of branches in this ecotype. None of the *Sinorhizobium meliloti* strains did not increase the number of branches in Malek kandi ecotype, also strain 2 decreased the number of branches in this ecotype.

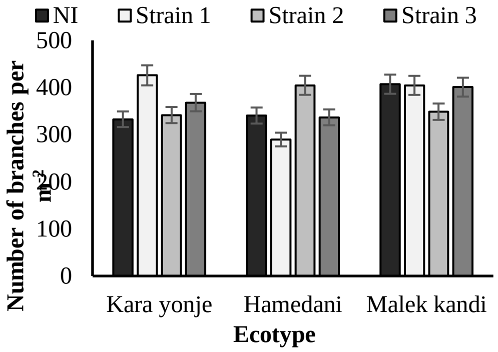
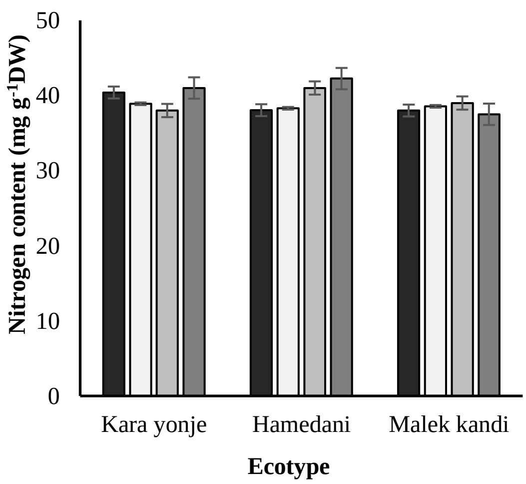
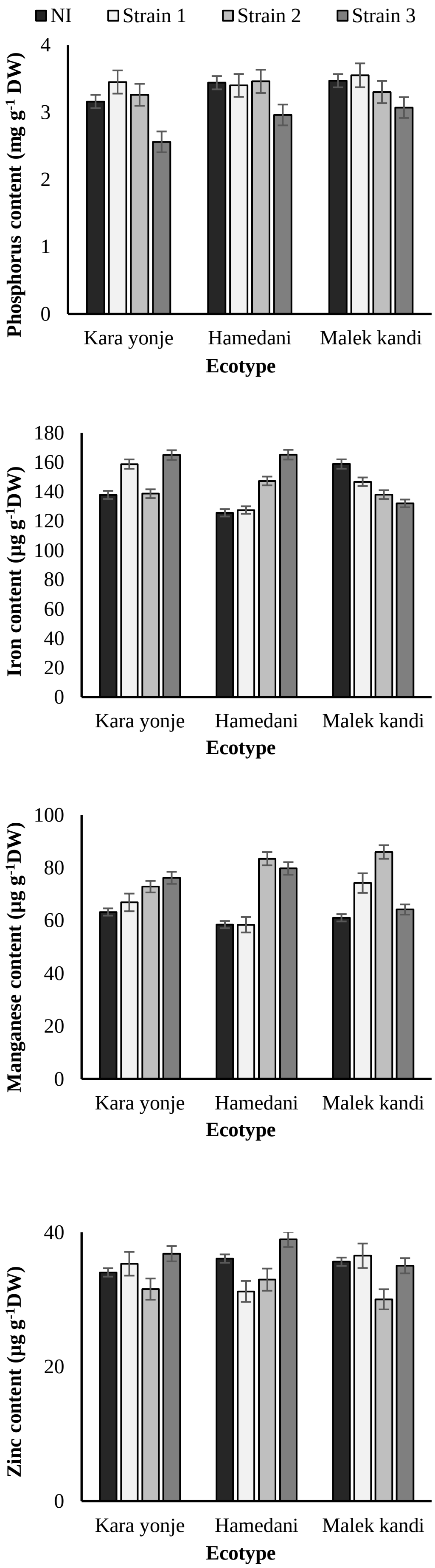


Fig 2. Number of branch per square of meter in alfalfa cultivars under saline condition and inoculation by *Sinorhizobium meliloti* strains.

Nutrient content

The content of nitrogen in ecotypes of Kara yonje and Hamedani was increased by inoculation of strain 3, however, this strain did not show a tangible effect on nitrogen content in Malek kandi ecotype (Fig 3). Strain 2 improved the content of nitrogen in the ecotypes of Hamedani and Malek kandi. Strain 1 did not alter the content of nitrogen in all of the ecotypes of alfalfa. Inoculation of alfalfa ecotypes by strain 1 enhanced the content of phosphorous in Kara yonje and Malek kandi ecotypes, but this strain of *Sinorhizobium meliloti* did not show a tangible effect on phosphorus content in ecotype of Hamedani (Fig 3). In ecotype of Hamedani inoculation by strain 2 did not alter the content of phosphorous, but this strain increased and decreased the content of phosphorous in Kara yonje and Malek kandi ecotypes respectively. Inoculation of alfalfa ecotypes by strain 3, decreased the content of phosphorous in all ecotypes. According to the Fig. 3, the content of iron in Kara yonje was increased by strain 1, but this strain reduced the iron content in ecotype of Malek kandi. Inoculation of Hamedani ecotype by strain 1 did not modify the iron content. Strain 2 of *Sinorhizobium meliloti* enriched the ecotype of Hamedani by iron, but this strain did not affect the content of iron in Kara yonje. Inoculation of Kara yonje and Hamedani by strain 3 increased the iron content in plant tissues, however, this strain reduced the iron content in Malek kandi ecotype. All of the *Sinorhizobium meliloti* strains improved the content of manganese in ecotypes of Malek kandi, Kara yonje and Hamedani, nonetheless in ecotype of Hamedani, inoculation of this ecotype by strain 1 did not show a superior effect on rising manganese content in this ecotype (Fig. 3). Inoculation of Kara yonje with strain 1 and 3 increased the content of zinc in this ecotype, however the strain 2 decreased this content. In ecotype of Hamedani, inoculation of this ecotype by strain 3 enhanced the zinc content, but other strains decreased this nutrient in plant tissues. Strain 1 increased and strains 2 and 3 decreed the zinc content in Malek kandi ecotype. The content of copper in ecotype of Kara yonje was enhanced by inoculation of this ecotype with strains 1 and 2 (Fig. 3). The strain 3 did not show a tangible effect on the copper content in Kara yonje. Inoculation of ecotype of Hamedani with strain 2 and 3 improved the content of copper, however the strain 1 did not show a significant effect on the copper content in this ecotype. None of the strains did not show a superior effect on rising copper content in Malek kandi ecotype.





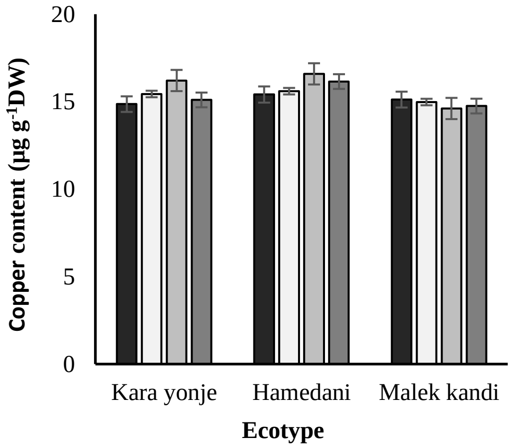


Fig 3. The content of nutrients in alfalfa ecotypes under salt stress and inoculation of *Sinorhizobium meliloti* strains.

Discussion

Between the most communal forms of abiotic stress worldwide, salt toxicity can cause various antagonistic effects on growth, development and symbiotic activities in plants (Gupta and Huang, 2014). Particularly, reduced symbiotic activities and forage yield of alfalfa under salt stress (Latrach *et al.,* 2014) stem from the adverse effects of salinity on soil pH and rhizosphere biota, while salt toxicity reduces symbiotic activities in legumes. In general, salt toxicity of the soil decreases the growth of plants (Wang *et al.*, 2016), enhances the oxidative damage in plant tissues (Farhangi-Abriz and Torabian, 2017) and, in alfalfa, compromises shoot growth.

Our results described here, obviously show that salt stress has a various effect on different kind of alfalfa ecotypes. The ecotype of Malek kandi showed the highest dry weight of forage between the alfalfa ecotypes, this may be resulted from the high manganese content in these ecotypes under the salt toxicity, because according to the table 2, the content of manganese and dry weight of forage has a positive and significant correlation with each other.

Moreover, the ecotype of Malek kandi has the highest number of branches per square meter. The dry weight of forage in alfalfa ecotypes has a positive correlation with the number of branches, which means that dry weight of forage increases by the increasing number of branches in alfalfa ecotypes (Table 2).

Numerous parameters related to soil quality, including calcium level, pH, nitrogen availability and form of nitrogen, can affect the symbiotic activities in legume plants (Paul, 2014). Moreover, the strain of bacteria has an important role in successful symbiosis between the bacteria and host plant, for example, in this study different strains of *Sinorhizobium meliloti* showed the various results in different ecotypes of alfalfa. Strain 1 has a beneficial effect on rising dry weight of forage in ecotype of Malek kandi, while in ecotypes of Hamedani and Kara yonje, strains 2 and 3 exposed the superior effect on rising forage yield respectively (Fig 1).

This increment in dry weight of forage by inoculation with *Sinorhizobium meliloti* strains can be attributed to the rising manganese content in plant tissues (Table 2). Manganese is an important nutrient in plant cells, which has different roles in the plant life cycle (Fageria, 2016; Carrasco-Gil *et al.,* 2016): this element resembles iron in its function within the plant cells. Manganese is indirectly related to the formation of chlorophyll, (2) manganese is considered to be a constituent of some respiratory enzymes, (3) it is involved in the evolution of O_2 in photosynthesis (4) it involved in iron metabolism and nitrate assimilation and (5) it plays an important role in N_2 fixation in legumes. Improved plant performance in terms of increased nutrient content and the dry weights of organs play a vital role in the successful growth of alfalfa under salt stress.

Previous research has indicated that under salt stress, bacteria alters the oxidative stress in plant tissues and thereby improves plant growth (Kang *et al.,* 2014; Habib *et al.,* 2016). Inoculation of plants with *Sinorhizobium meliloti* improved plant performance under salt stress by providing more nutrients for rhizobium microorganisms in root zones. However, future research should further explore all reported benefits of *Sinorhizobium meliloti* on alfalfa cultivars.

Table 2. Correlation between the different parameters of Medicago sativa in this study.

	FW	DW	NB	N_2	P	Fe	Mn	Zn	Cu
FW	1.000								
DW	0.945^{**}	1.000							
NB	0.607^{**}	0.672^{**}	1.000						
N_2	0.007^{ns}	0.051^{ns}	-0.110^{ns}	1.000					
P	0.240^{ns}	0.164^{ns}	0.210^{ns}	-0.514^{**}	1.000				
Fe	0.083^{ns}	0.214^{ns}	0.451^{*}	0.592^{**}	-0.377^{*}	1.000			
Mn	0.484^{*}	0.475^{*}	0.161^{ns}	0.570^{**}	-0.235^{ns}	0.408^{*}	1.000		
Zn	-0.187^{ns}	-0.027^{ns}	0.320^{*}	0.366^{*}	-0.328^{*}	0.579^{**}	-0.097^{ns}	1.000	
Cu	0.044^{ns}	-0.066^{ns}	-0.098^{ns}	0.372^{*}	0.115^{ns}	0.172^{ns}	0.249^{ns}	0.005^{ns}	1.000

ns, * and ** non-significant, significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

FW: Fresh weight; DW: Dry weight; NB: Number of branch

Conclusions

In conclusion, our results showed that inoculation of *Sinorhizobium meliloti* improved the forage yield and nutrient contents of *Medicago sativa* under salt toxicity of soil. These inoculation treatments exposed various results in different ecotypes, but in general inoculation of *Sinorhizobium meliloti* increased manganese content in different ecotypes of alfalfa.

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