Rhizobium Symbiosis Contribution to Short-term Salt Stress Tolerance in Alfalfa (*Medicago sativa* L.)

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Introduction

Symbiotic nitrogen fixation (SNF) has profound agronomic, economic, and ecological impacts because availability of soil nitrogen most frequently limits agricultural production throughout the world. In SNF, plants provide carbon compounds and energy sources needed by rhizobia in exchange for fixed nitrogen. In addition to nitrogen fixation, rhizobia have been shown to benefit plants in many different ways, which imply that symbiosis may enhance the overall health status of plants and improve their tolerance to abiotic stresses, including salinity.

Aim

The objective of this study is to test our hypothesis that rhizobium symbiosis may improve alfalfa salt tolerance by affecting its physiological and biochemical processes such as enhancing antioxidant capacity during stress response. We studied the response of well-developed alfalfa plants with active nodules, inactive nodules and no nodules to salt stress by assessing survival rate and their ability to deal with oxidative and osmotic stress induced by salt shock.

Methods

Seeds of alfalfa (*Medicago sativa,* Ladak+) were surface-sterilized and germinated in Petri dishes in a growth chamber for 5 days. Seedlings were transferred to conical plastic pots containing sterilized quartz sand without added nutrients and cultured in the greenhouse. Seedlings with 10 cm shoot height were selected and randomly divided into 3 groups: (1) inoculated with *Rhizobium meliloti* and watered daily with 1/4 strength nitrogen-free Hoagland nutrient solution, which resulted in development of active nodules (**AN**); (2) inoculated and watered with 1/4 strength Hoagland nutrient solution daily, which led to development of inactive nodules (**IN**) due to inhibition of rhizobia by sufficient nitrogen in the nutrient solution; (3) not inoculated and watered with 1/4 strength Hoagland nutrient solution, which led to plants with no nodules (**NN**). Shoots were cut on the sixtieth day and ninetieth day after inoculation to promote root and/or nodule growth. After 120 days of growth, plants were subjected to salt stress for survival tests and physiological/biochemical analyses.

Results

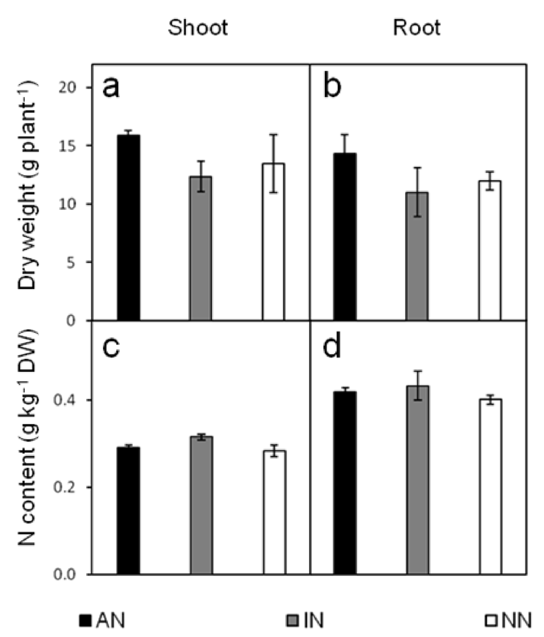

Fig. 1 Dry weight and nitrogen content in shoot and root of alfalfa prior to salt treatment (AN: alfalfa with active nodules; NI: alfalfa with inactive nodules; NN: alfalfa with no nodules. The data are means ± SE. Means are compared among the all groups. The significance is labeled with letters. The same as bellow except for specific notification.)

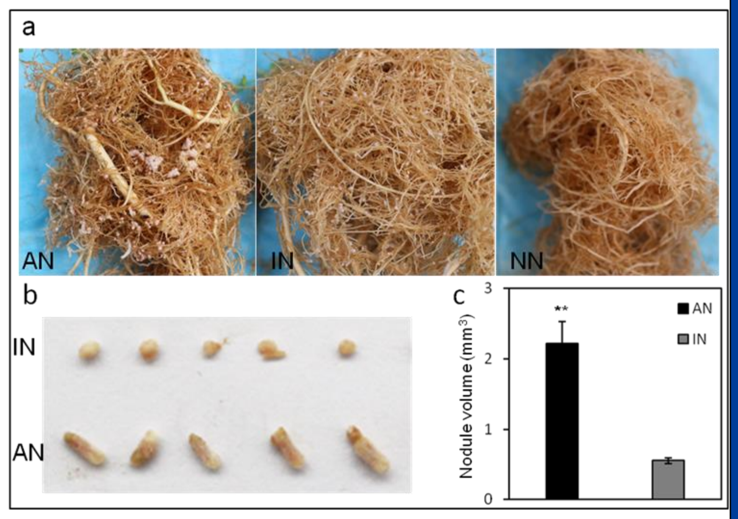

Fig. 2 Alfalfa root and nodule color, nodule size, and volume (Data are means ± SE of ten experiments. Different letters indicate significant difference (p<0.05) amount AN and IN plants.)

Table 1 Variance analysis of survival rate, fresh weight of regenerated shoots, activities of SOD, POD, CAT and APX in the leaf and root of alfalfa under different treatments of nodulation and salt shock

Sources (factors)	DF	Survival F value	Survival P value	Fresh weight F value	Fresh weight P value	SOD F value	SOD P value	POD F value	POD P value	CAT F value	CAT P value	APX F value	APX P value
Leaf													
N	2	5.33	0.022	8.05	0.001	2.19	0.122	39.79	<0.001	49.79	<0.001	37.96	<0.001
S	5	133.67	<0.001	105.78	<0.001	9.08	<0.001	47.51	<0.001	23.39	<0.001	24.16	<0.001
N*S	10	5.33	0.007	4.08	0.001	7.50	<0.001	21.97	<0.001	11.84	<0.001	7.84	<0.001
Root													
N	2					15.64	<0.001	53.41	<0.001	56.22	<0.001	3.57	0.035
S	5					15.1	<0.001	87.34	<0.001	5.48	<0.001	3.08	0.016
N*S	10					6.12	<0.001	21.22	<0.001	2.94	0.005	4.33	<0.001

N and S are the abbreviation of nodulation and salt shock, respectively.

Table 2 Variance analysis of contents of $O_2^{-}\cdot$, MDA, GSH, proline, soluble sugar and soluble protein in the leaf and root of alfalfa under different treatments of nodulation and salt shock

Sources (factors)	DF	$O_2^{-}\cdot$ F value	$O_2^{-}\cdot$ P value	MDA F value	MDA P value	GSH F value	GSH P value	Proline F value	Proline P value	Soluble sugar F value	Soluble sugar P value	Soluble protein F value	Soluble protein P value
Leaf													
N	2	44.79	<0.001	4.11	0.022	8.64	0.001	6.02	0.004	0.82	0.446	3.11	0.053
S	5	2.93	0.021	14.16	<0.001	12.76	<0.001	10.85	<0.001	6.44	<0.001	5.71	<0.001
N*S	10	22.97	<0.001	20.27	<0.001	7.94	<0.001	2.20	0.031	5.60	<0.001	13.03	<0.001
Root													
N	2	21.08	<0.001	22.14	<0.001	1.84	0.168	0.04	0.962	26.17	<0.001	3.75	0.030
S	5	6.29	<0.001	5.27	0.001	8.57	<0.001	7.70	<0.001	3.58	0.007	3.54	0.008
N*S	10	4.99	<0.001	6.71	<0.001	6.78	<0.001	5.77	<0.001	3.94	<0.001	3.54	0.001

N and S are the abbreviation of nodulation and salt shock, respectively.

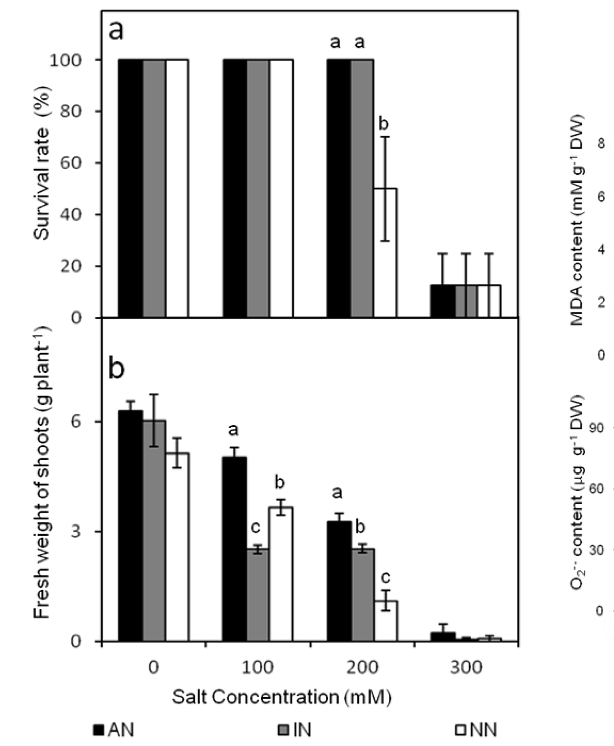

Fig. 3 Effect of nodulation on survival rate and shoot regrowth of alfalfa under salt treatment (Data are means ± SE of four experiments with two replications. Different letters indicate significant difference (p<0.05) among AN, IN and NN plants at the same salinity level.)

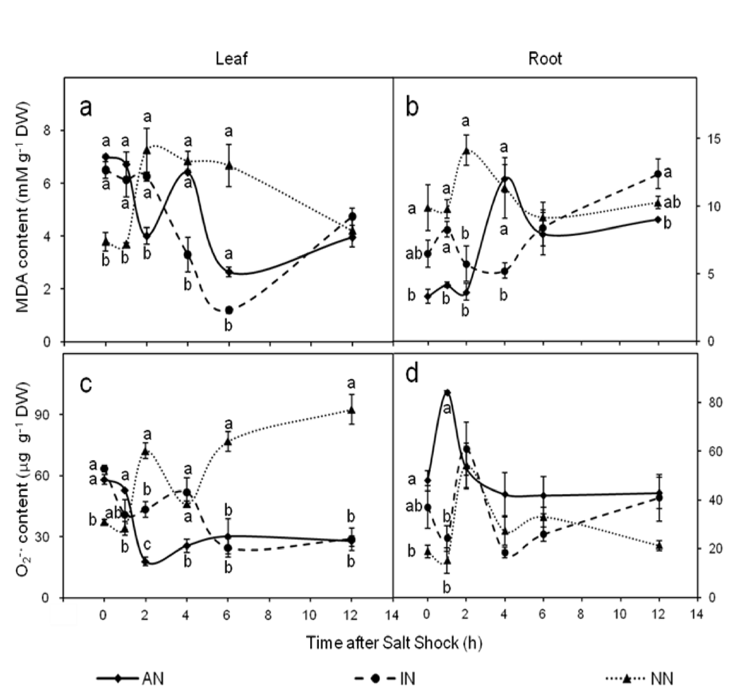

Fig. 4 Effect of nodulation on $O_2^{-}\cdot$ and MDA contents in alfalfa leaf and root under salt shock (Data are means ± SE of four experiments. Different letters indicate significant difference (p<0.05) amount AN, IN and NN plants at the same time point.)

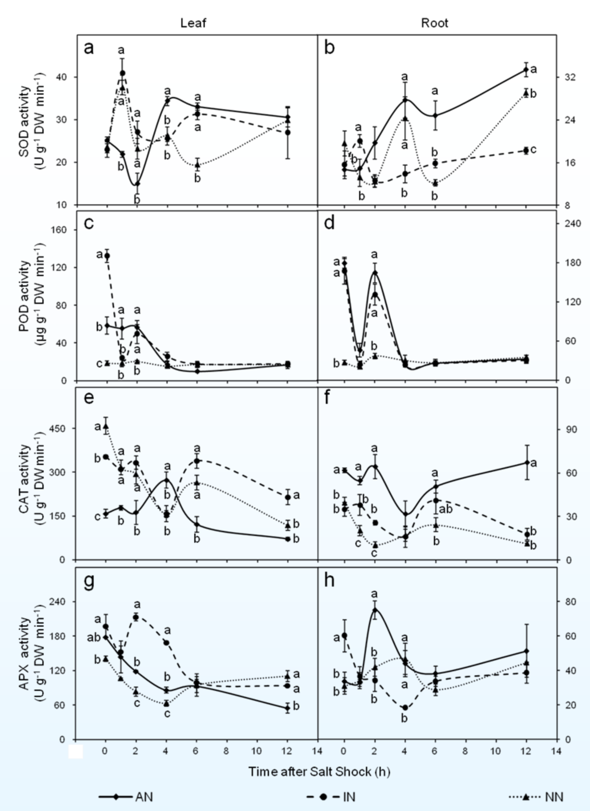

Fig. 5 Effect of nodulation on activities of SOD, POD, CAT and APX in alfalfa leaf and root under salt shock (Data are means ± SE of four experiments. Different letters indicate significant difference (p<0.05) amount AN, IN and NN plants at the same time point.)

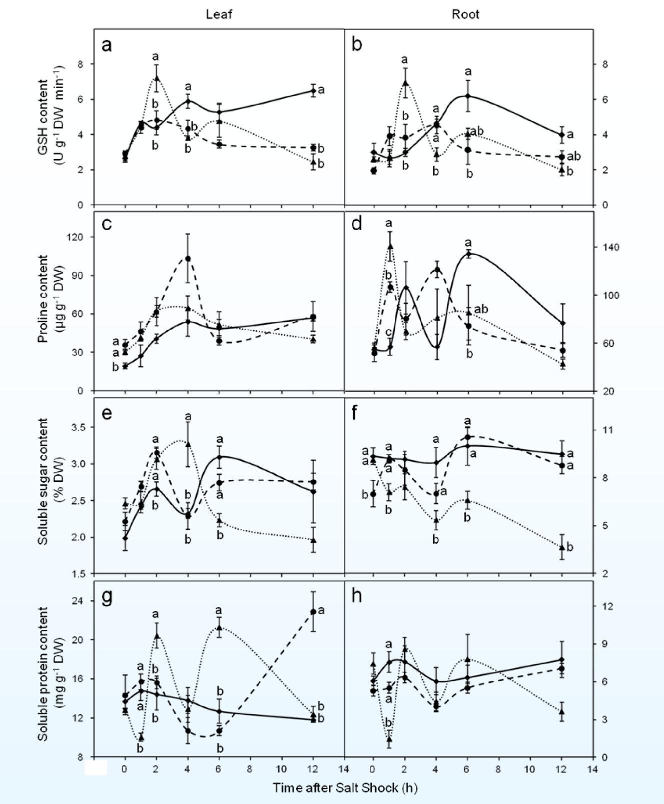

Fig. 6 Effect of nodulation on contents of GSH, proline, soluble protein and soluble sugar in alfalfa leaf and root under salt shock (Data are means ± SE of four experiments. Different letters indicate significant difference (p<0.05) amount AN, IN and NN plants at the same time point.)

Conclusion

Alfalfa with active nodules showed higher survival rate. Higher survival rate was associated with reduced lipid peroxidation, higher activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) as well as higher concentrations of reduced glutathione (GSH), and soluble sugar, especially in roots under salt stress.

Variance analysis indicated nodulation affected the activities of SOD, CAT, POD and APX along with concentrations of GSH, soluble sugar and soluble protein. Inoculation also resulted in higher basal levels of superoxide anion radical ($O_2^{-}\cdot$) without salt stress.

Rhizobium symbiosis had a positive effect on alfalfa salt tolerance by improving the activity of antioxidant enzymes and osmotic adjustment capacity.

Acknowledgements

This work was supported by the Project of National Natural Science Foundation of China (31372357, 31272490), the National Key Technology R&D Program in the 12th Five-Year Plan of China (2011BAD17B05), the major project for Tibetan forage industry (Z2014C02N02) and China Agriculture Research System (CARS-35). The authors thank Drs. Yajun Wu and Roger N Gates from South Dakota State University for their advice on statistical analysis and constructive comments to this project.

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