Impact of Salinity on Biological Nitrogen Fixation in Alfalfa (*Medicago sativa*) and the Response to Applied Mineral Nitrogen

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Introduction

- Due to water scarcity, saline waters deemed unsuitable for irrigation are now considered as valuable resources to supplement fresh water supplies. Saline water resources include agricultural drainage waters, ground waters and wastewaters from animal production and food processing.
- Salinity in soil or irrigation water negatively impacts plant growth by reducing the soil water potential, making it more difficult for roots to extract water, and by causing foliar injury due to sodium or chloride toxicity.
- In California, attention is also focused on nitrogen management due to concerns for nitrate contamination of groundwater. Alfalfa is one of the few crops that can fix atmospheric nitrogen (N_2) and it therefore has a lower requirement for N fertilizer.
- Adverse effects of salinity on N_2-fixing plants could include reductions in root nodulation, the N_2 fixation capacity of the Rhizobia and ultimately, the amount of N fertilizer required by the alfalfa.

Objectives

- Evaluate the impacts of irrigation water salinity on biological nitrogen fixation (BNF)
- Determine the extent to which salinity-induced reductions in BNF can be offset by N-fertilizer additions.

Materials & Methods

- Variety: CUF 101 (grown in sand medium)
- Inoculant: Nitragin Gold consisting of *Sinorhizobium meliloti* strain of bacteria.
- 2 salinity levels : Low salinity(LS) = 1.3 dS/m EC,
  High salinity (HS) = 8.5 dS/m EC*
  **for emergence phase, the HS salinity level was 6.6 dS/m*
- 3 Nitrogen levels: N1 = 0 ppm (without nitrogen)
  N2 = 18.7 ppm (1/12 Hoagland's solution)
  N3 = 112 ppm (1/2 Hoagland's solution)
- Inoculated treatment (60 pots) + non- inoculated treatment (40 pots) = 100 pots (depth of pots - 40cm). Prior to seeding the sand medium was sterilized in a propane boiler.

Acknowledgment: The authors thank the California State University Agricultural Research Institute (ARI) for funding and Novozymes Inc. for the inoculants and general assistance.

Experimental setup

Emergence Percentage: Final emergence was counted for 50 seeds per pot 30 days after sowing.

Mature Plant Response: Plants were thinned to 2 per pot after emergence count. Shoots were harvested after every 3-4 weeks for determination of dry matter and total N, Na, K and Cl. Excavated plants were measured for nodule count, nodule weight and root biomass.

Biological nitrogen fixation will be measured by Total Nitrogen Difference method using the non- inoculated ones as reference plants.

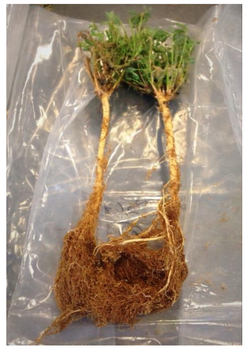
Greenhouse pots with irrigation emitters

Table 1: Irrigation water salinity and nitrogen concentrations.
LS = low salinity and HS = high salinity. The pH was 7.3 to 7.5.

	Inoculated		Non-Inoculated	
Treatment	EC (dS/m)	N (mg/L)	EC (dS/m)	N (mg/L)
LS-N1	1.5	1.9		
LS-N2	1.5	15.7	1.5	16.9
LS-N3	1.4	107.9	1.5	113.2
HS-N1	7.5	2.3		
HS-N2	7.8	15	7.8	15.7
HS-N3	7.8	110.7	7.8	113.8

Irrigation tanks outside greenhouse (monitored for pH, EC, nitrate)

Seedlings from low and high salinity treatments



Plants under high (left) and low (right) salinity treatments with nodulation

Results and Discussion

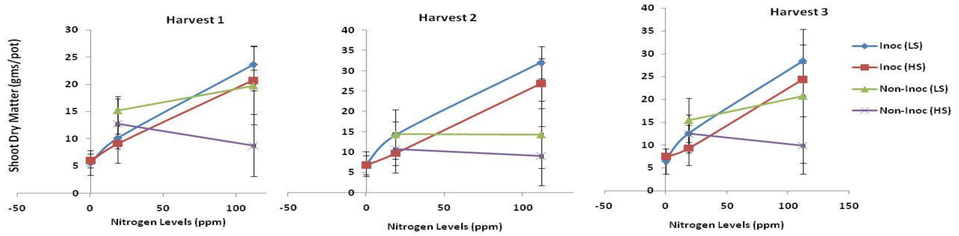
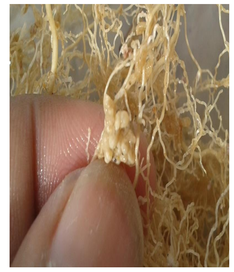


Fig 1. Effect of N-Levels in irrigation water on shoot dry matter yield of 3 harvests (7/28/14, 8/23/14 and 9/29/14)

For all three harvests,

- Salinity significantly reduced DM yield in both inoculated and non-inoculated plants.
- N concentration had a significant effect on DM yield in inoculated plants for Harvest 2 & 3.
  -- non-inoculated plants exposed to high salinity had a negative response to increasing N, possibly due to a combined effect of salinity, high N and temperature affecting nodulation.



Photos above: Root nodules from inoculated treatment

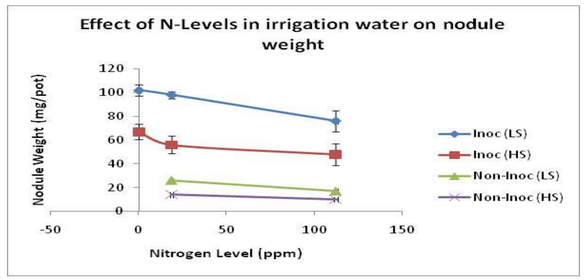


Fig4. Effect of N-Levels in irrigation water on nodule weight

- There was a significant difference in nodule weight between the N1 and N3 treatments.
- High salinity did not significantly affect nodule weight in any of the treatments.
- For both inoculated and non-inoculated treatments, the most active nodules (based on color) were present in the LS-N1 treatment and the N3 level treatments had the fewest nodules.

Conclusions

- High greenhouse temperatures in July (42 - 45°C) and irrigation fluctuations likely reduced the activity of the N_2-fixing bacteria resulting in a greater response to applied N in the inoculated treatments.
- For non-inoculated treatments, low soil moisture may explain the lack of response to applied N.
- Total N data will be used to assess the effect of salinity on alfalfa growth, N_2 fixation and N fertilizer requirement for maximal growth.

Future Work

The next experiment will include the following changes:

- greenhouse temperatures maintained below 35 C,
- N2 level increased to 28 ppm and salinity to 10 dS/m.
- Zero N treatment included for non-inoculated plants.

Methods are being investigated to better assess the activity of the N_2-fixing bacteria under saline conditions and to better isolate the non-inoculated plants so that nodules do not form.