Research Journal of Agriculture and Biological Sciences, 4(5): 413-415, 2008

The Effect of Salinity on the Nitrogen Fixation in 4 Cultivars of *Medicago sativa* L. in the Seedling Emergence Stage

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Abstract: In order to study the effect of salinity on the nitrogen fixation in *Medicago sativa* L. seedling emergence stage, this experiment was conducted in research greenhouse of Karaj Agriculture college, in spring 2006 using a factorial as complete block design with four replications. Treatments included a combination of salt stress in 4 levels (control, 40 mM, 80mM, 120 mM, 160 mM) on alfalfa cultivars (Bami, Yazdi, Shoorkat, Yugoslavi) supplemented with MS26 Rhizobium and planted in pots that were filled by perlit. Hoagland solution, without nitrogen, was used to supply nutrition. At first, the pots were irrigated with tap water. After the emergence of main leaflet, irrigation with Hoagland solution was started.After 21 days, salinity treatment was followed with Hoagland solution and it was continued for 46 days. The results showed plant dry weight, the number of active nodules and nitrogen content were affected under salt stress in all tested cultivars. Plant dry weight decreased by about 82% with the highest salt dosage (160 mM NaCl). The number of active nodules and Nitrogen content decreased significantly in the highest level of salinity. Because of the growth stage in which the salinity was employed, all the cultivars have a similar response to salinity treatments and none of them could show its ability and difference.

Key word: Nitrogen Fixation, Nodule, Salinity Stress, Hoagland Solution.

INTRODUCTION

Salinity is a serious threat to agriculture in arid and semiarid regions[12]. Nearly 40% of the world's land surface can be categorized as having potential salinity problems[4]. Increasing salt concentration may have a detrimental effect on soil microbials as a result of direct toxity as well as through osmotic stress[15].

As with most cultivated crops, the salinity response of legumes varies greatly and depends on such factors as climatic conditions, soil properties and the stage of growth[5].

Alfalfa is the most important forage crop for the arid and semi-arid areas where increased salinity of irrigated fields is one of the major constraints that limit crop productivity[8].

Alfalfa may be affected by salinity throughout its growth. Three developmental stages have been studied, although these stages have often not been clearly defined. These three stages are[1] germination[2] seedling emergence and growth[3] mature plant[13].

Salinity may affect symbiotic N2 fixation in alfalfa in various ways. Included among these are effects on both the Rhizobium bacteria, which infects alfalfa (*Rhizobium meliloti* L.) andcarries out N2 fixation, as well as the alfalfa host plant itself, which provides photosynthates to fuel fixation[2]. Salinity retard initiation or growth of new nodules reduced the efficiencyof fully formed nodules which had developed earlier under nonsaline conditions[2] and decrease the proportion of those nodules that are initiated in saline conditions, that are able todifferentiate fully in to active N2-fixing nodules[17].

The purpose of this study was to investigate the effect of salinity on tow important traits including biological nitrogen fixation and the number of active nodules in a special stage in *Medicago sativa* life. Since finding the resistant cultivar was not the aim, the seeds were chosen from saline regions.

MATERIAL AND METHODS

In a pot experiment, 4 cultivars of Alfalfa *(Medicago sativa* L.) (Bami, Yazdi, Shoorkat,

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Yugoslavi) were exposed to NaCl salinity using a completely randomized block design with factoria treatments in four replication. 5 levels of salinity were used (control, 40 milimolar, 80 milimolar, 120 milimolar, 160 milimolar). This study was conducted in Iran, at Karaj agriculture college greenhouse, during the spring of 2006. The seeds were provided from saline regions, inoculated with MS 26 Rhizobium and sown in pots which were filled with perlit. During the 10 days following sowing, the number of plants was reduced to 5 per pot by thinning. Supplementary light was provided for 16h per day. The day time and night time air temperatures of the greenhouse were 28 and 12°c respectively. From sowing until emergence of main leaflets the irrigation was done with tap water. At this time with finishingseed storage, the irrigation with Hoagland solution free nitrate was started. The cause of using this solution was, being sure that the source of nitrogen content is only from biologicalnitrogen fixation and nodule activation.

After passing 21 days from sowing when the nodule activity was completed, the salinity treatment was followed with Hoagland solution and it was continued for 46 days.

In order to feel sure of using exact salinity the water EC under the pot was measured everyweek.

After passing 67 days from sowing, the plants were taken out pots and the number of active nodules were measured (the pink nodules).

Then the plants were dried at 70°C for 48h and then weighted. For measuring nitrogen content, one gram was used from every sample. The nitrogen measurement was done with Tecator and Kejeldal method[3]. Then the nitrogen content was measured in consideration of dry matter. The data were analyzed and the mean comparisons were made following least significant different at P=0.05.

RESULTS AND DISCUSSION

The variance analysis of the obtained data shows that the traits in this experiment were significantly affected by salinity (Table 1).

Table 1 shows that the salinity has a significant effect on dry matter. With increasing salinity the dry matter decreases from 3.16 gr in control level to 0.57 in the highest level.

The table 1 shows that there is no different between cultivars in dry matter trait, because the time of salinity application was in the emergence of seedling stage. In this stage the plants are weak and can not show their abilities in the stress condition.

Salinity reportedly reduces shoot and root weights in several legumes such as faba bean[17, 18], soybean (Glycine max)[9], and bean (Phaseolus vulgaris)[16].

Table 1: Analysis of variance for the traits investigated in 4 alfalfa cultivars in response to salinity stress

		Mean square		
Source of variation	d.f.	Plant dry weight	The no. of active nodules	Plant nitrogen content
Replication	3	0.08^{ns}	68.21^{**}	0.06^{ns}
Cultivar	3	0.10^{ns}	5.74^{ns}	0.05^{ns}
Salinity	4	17.50^{**}	6554.67* *	2.99^{**}
Sal. * Cult.	12	0.08^{ns}	12.39^{ns}	0.01^{ns}
Error	57	0.09	13.67	0.03

ns : nonsignificant
* : significant at 0.05 probability levels
** : significant at 0.01 probability levels

Table 2: The effect of NaCl treatment on plant dry weight, the number of active nodules and plant nitrogen content of 4 alfalfa cultivars

	Mean		
Salinity levels (mM NaCl)	Plant dry weight	The no. of active nodules	Plant nitrogen content
Control	3.1 6a	51.25a	1.34a
40 mM	1.97b	29.37b	0.93b
80 mM	1.30c	16.94c	0.62c
120 mM	0.79d	7.00d	0.39d
160 mM	0.57e	0e	0.28d

Within column means followed by the same letter (a ...) are not significantly different at the 0.05 level, according to L.S.D. test

Most examinations of Alfalfa seedling response to osmotic-salinity stress have focused on forage yield under saline conditions. Significant declines (=45%) in seedling shoot weight were observed in 56-day-old greenhouse-grown plants irrigated with 80mM NaCl (=0.26 MPa) compared to plants irrigated with no saline water[10].

Salinity has a significant effect on root active nodules (Table 1). The means in the table 2 shows that in control level the number of active nodules is 51.25 and in 160mM NaCl, this amount decreases to 0.

The reduction of N2- fixing activity by salt stress is usually attributed to a reduction in respiration of the nodules and a reduction in cytosolic protein production, specifically leghemoglobin, by nodules[7].

In a study[6], the total number of active nodules in Vicia faba decrease with salinity treatment. Also it was reported that salinity significantly decreased total N in both shoots androots of V. faba ($p<0.05$). With using salinity treatment nodules lost their pink color (leghemoglobin content) and by interference N2 fixation activity, became white and inactive. The salt-induced distortions in nodule structure could also be reasons for the declinein the N2 fixation rate by

legumes subject to salt stress[14]. A favorable Rhizosphere environment is highly important to the interaction between root hairs and Rhizobium, as it not only encourages the growth and multiplication of rhizobia but also ensures the healthy development of root hairs. Any environmental stress that affects these processes is also likelyto influence infection and nodulation[1]. Increasing salinity has a significant effect on nitrogen content (Table 1). Table 2 shows in control level the nitrogen content is 1.34 and at highest level of salinity this decreases to 0.28. In this experiment, the active nodules are the only source of producing nitrogen so decrease in number of active nodules decreases nitrogen content.

The depressive effect of salt stress on N2 fixation by legumes is directly related to the salt-induced decline in dry weight and N content in the shoot[5].

Kapulnik (1989), observed reduced total N produced through N2 fixation with in increasing salinity (0, 100 and 200 Mm NaCl) in 45-day-old seedlings and in mature plants.

Overall, the result of plant dry weight, nodule number and nitrogen content indicate that NaCl concentrations can cause severe damage to alfalfa plants in the seedling emergence stage.

It must be noticed, in the application of salinity, the stage of growth is a very important factor. Since the time of salinity application in this experiment is at the seedling emergence stage, the cultivars couldn't show their abilities and differences. So using of salinity in different stages of growth can be suggested.

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