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Effects of Salinity and Nitrogen Supply on Nitrogen-Fixation Nodules and Nitrogen, Sodium and Potassium Concentration of Alfalfa Cultivars

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ABSTRACT

In order to determine the effect of salinity and nitrogen supply on the total numbers of nodes and active nodes of nitrogen - fixation and nitrogen concentration in roots and shoots of in two cultivars alfalfa (Bami and Gareh), the experiment was carried out in the form of completely randomized design with factorial layout, including two Iranian cultivars of alfalfa (Bami and Gareh), two levels of salinity (1.1 dS/m(S_0) and 12 dS/m(S_1)), three levels of nitrogen (seeds inoculation before planting by rhizobium meliloti bacteria (N_1), taking 400 mg/lit ammonium nitrate in food solution without seeds inoculation by bacteria (N_2), seeds inoculation before planting by rhizobium meliloti bacteria with taking 400 mg/lit ammonium nitrate in food solution (N_3) with three repetitions during 2010-2011 in greenhouse of Karaj Agricultural University in Iran. salinity stress reduced significantly the total numbers of nodes and active nodes of nitrogen- fixation. In the Way of nitrogen supply (ammonium nitrate), the total numbers of root nodes and numbers of root`s active nodes also reduced considerably. In both levels of salinity (S0, S1) and both Alfalfa cultivars, N1 treatment had the maximum nodes and active nodes in root. Salinity (Na concentration) dramatically increased in roots and shoots. The two figures of Alfalfa were varied in terms of sodium concentration in roots and shoots and sodium concentration in roots and shoots of Ghareh figure was markedly higher than the Bami figure. Salinity was considerably reduced K concentration in the roots and shoots of both varieties. The two varieties had significant differences in the concentration of potassium of roots and shoots that potassium concentration in roots and shoots of Ghareh figure was significantly higher than the Bami figure. Consequently, it seems that the Bami figure has a higher resistance to salinity than the Ghareh figure, and salinity has less effect on dry weight, nitrogen and potassium concentration in the Bami cultivar.

KEY-WORDS: Alfalfa, salinity stress, Nitrogen-fixation, rhizobium bacteria

INTRODUCTION

Increasing soil salinity is one of the destructive actors of natural that have detrimental effects on plant growth. Most crop species are glycophyte and their growth becomes reduced in the saline soil and water. With increasing salinity arable land increases need to salinity-resistant varieties (Szabolcs , 1994).

Due to the arid and semi-arid climate in Iran, the main problem of nearly 50% of areas under cultivation with various crops is being salty and alkaline (Meybodi and Garehyazy, 2001). In Iran, in 2002, the proportion of forage production (Corn, Alfalfa, Barley and Other forage plants) to the other total crops was15,078 tons(equal to 24.3 percent). As the average of alfalfa production between 1994 and 2000 were 4.314 tons which was a considerable amount of ratio to other forage plants and shows the importance of Alfalfa among other forage plants in Iran (Ministry of Agriculture, 2002). Alfalfa is compared with other forage plants of interest to farmers due to rapid growth after harvest, nutritive forage production and symbiotic nitrogen fixation. Alfalfa is a plant that is relatively resistant to Salinity (Nobel, 1984). Salinity has the effect on the soil microbial population and its relationship with the root in rhizosphere zone. For example, rhizobium bacteria (Rhizobium meliloti) and legume root of alfalfa, which have a symbiotic relationship, are resistant to salinity ratio to the host plant. But nevertheless salinity has the effect on exist of nitrogen- fixation nodules of legume root and activity by these nodules (Kordovyla et al 1997). AS they studied on (Viciafaba L) expressed that plant growth is more sensitive to salinity than symbiotic nitrogen fixation by rhizobium bacteria, although salinity also reduced markedly nitrogen fixation. The study of ammonium immobilization in root nodules has shown that the activity of glutamine synthetize is more resistant than glutamate synthetize enzyme to salinity salinity stress and reduction of immobilization of ammonium in effect of salinity is largely due to decreasing the activity of glutamate synthetize (Kordovyla et al, 1997). Eschie et al (2002) showed that the salinity of 12 dS/m caused a significant reduction in shoot growth of alfalfa. But the supply of mineral nitrogen to form of ammonium or nitrate reduces the negative effects of salinity on shoot growth. Also they suggested that due to the strong inhibition of denitrogenize enzyme in saline conditions, the addition of inorganic nitrogen partly canimprove the effects of salinity on the Alfalfa yield. The main purpose of this study was to investigate the effect of salinity and nitrogen supply on the

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total numbers of nodes and active nodes of nitrogen-fixation and concentration of nitrogen in roots and shoots of the two cultivars Alfalfa (Bami and Ghareh).

MATERIALS AND METHODS

Experiment design and location

In this reserch, we were performed the effects of salinity in 2 levels (1.1 dS/m(S_0) and 12 dS/m(S_1)), the supply of nitrogen in 3 levels (inoculating seeds before planting by rhizobium meliloti bacteria (N_1), taking 400 mg/lit ammonium nitrate in food solution without inoculating seeds by bacteria (N_2), inoculating seeds before planting by rhizobium meliloti bacteria with taking 400 mg/lit ammonium nitrate in food solution (N3)) and two figure of Alfalfa (Bami, and Ghareh) with three replications. Experiments were conducted in a completely randomized design with factorial layout during 2010-2011 in the greenhouse of Karaj Agricultural University in Iran.

Plant nutrient and irrigation

The number of seeds planted in each pot was 10 that after the establishment of plants in pots, five plants were maintained per pot. Nutrients needed for plant growth were conduct by irrigating plants every three days with Hoagland solution (Table 1). In the treatment N1 was used N-free solution and in two other treatments were used Hoagland solution in addition to 400 mg/l of ammonium nitrate. After planting seeds in pots were irrigated with Hoagland Solution and 7 days after planting and early vegetative stage, treatment (S1) were irrigated with Hoagland solution containing sodium chloride. For maintaining the normal growth and prevention of plant sudden stress, plant were placed gradually exposed to salinity and thus the salinity concentration of 2 dS/m was started and within 10 days to 12 dS/m.

Table 1: Macro and micro element concentrations of Hoagland solution

Element	*Concentration(ppm)*
P	*31*
K	*234*
Ca	*200*
Mg	*48*
B	*1.5*
Cu	*0.05*
Fe	*5*
Mn	*0.5*
Mo	*0.01*
Zn	*0.05*

Plant sampling

90 days after planting and the beginning of the flowering, pot plants removed and washed with distilled water and the total numbers of nodules, number of active nodes were counted in the roots. Active nodes were identified based on the color reddish pink from passive nodes. In order to measure the dry weight of plants were harvested at the beginning of flowering stage. Plants were removed completely from the pot and divided into two parts, roots and shoots. Roots and shoots were dried in the oven for 48 hours and their dry weights were measured. After digestion, the nitrogen concentrations of roots and shoots were measured using kejeltec system.

Statistical analysis

Data from experiments were analyzed using SAS software (SAS, 1985). Averages of main and interactions effects of experimental factors were evaluated by Duncan test. For drawing diagrams was used EXCEL software.

RESULTS AND DISCUSSION

The total number of nitrogen-fixation nodes:

The effect of salinity stress: The effect of salinity was significant on the total number of nodes and salinity reduced this parameter markedly (Figure 1). There was a significant difference between the two figures (Ghareh and Bami) in terms of total number of nodes and number of root nodules in Ghareh figure was substantially higher than the Bami figure. The interaction effect of salinity and figure was not significant on the total number of nodes. Djilianov et al (2003) expressed that salinity reduces the number of nitrogen-fixation nodules in Alfalfa root, but this reduction in the amount of salt-resistant varieties are less than susceptible varieties to salinity. Etelvina (2005) reported that the effect of salinity stress on growth of pea , the salinity of 50 mM has no impact on the number of root nodes, but the time of nodes formation delays in the root. Cordovilla et al (1997) reported that in saline conditions, the activity of symbiotic nitrogen-fixation is related to susceptibility of the host plant and rhizobium bacteria. Lavchely (1984) placed the legume in the salt-sensitive plants and reported that limitations of the growth of legumes in saline environments are weak connection between host plant and symbiotic bacteria. Salinity reduced N concentration of root and shoot in both cultivars and reduction of nitrogen concentrations was higher in the Bami figure.

The effect of nitrogen supply (ammonium nitrate):
The effect of ammonium nitrate (NH_4 NO_3) was significant on the total number of root nodules and ammonium nitrate consumption reduced this parameter considerably(Figure 1). The interactive effects of salinity and nitrogen supply were significant on the total number of root nodules and ammonium nitrate supply under saline conditions compared to control (non-salin) reduced the total number of root nodules. Esechie et al (2002) expressed nitrate affect a wide range of processes that root inoculated by bacteria which including reduction in the formation of hairy roots, reducing the bacteria associated with the hairy roots and reduction in the number of successful insemination and thus reduces the formation of nitrogen-fixationof nodules on the roots of legumes.

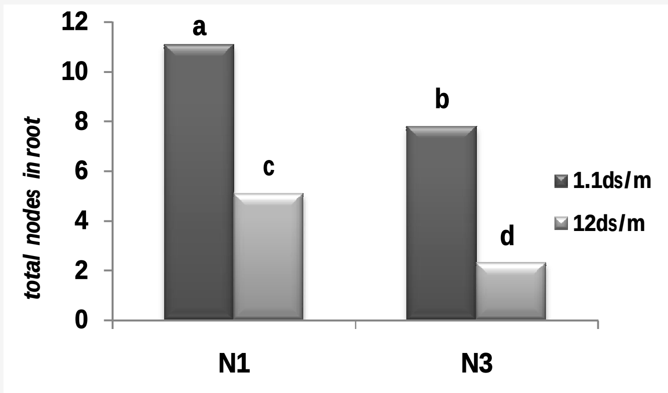

Figure 1: Effects of salinity and nitrogen supply on the total number of root nodes

The active number of nitrogen-fixation nodes:
The effect of salinity stress: Salinity effect was significant on the active nodes number of root and salinity substantially reduced this parameter. There were no considerable differences between figures in terms of active nodes number. The interaction effect of salinity and figure was significant on the active nodes number of root. In the control conditions the active nodes number in the Ghareh figure was markedly higher than the Bami figure. But in the Salinity stress, the active nodes numbers were not significant in both figures (Figure 2). Lopez et al (2008) expressed that one of the reasons for the inactivation of nitrogen-fixation nodes in the saline conditions was decreasing the leg hemoglobin content. In this relationship, Fernandez (1996) has been noted the reduction of the leg hemoglobin content in the saline conditions. Gordon (1987) said that nitrogen-fixation in root nodules is dependent on sucrose intake from host plants and this sucrose is hydrolyzed by sucrose synthetize and alkaline invertase enzyme in the root nodes. In this case, Gordon (1993) pointed that the activity of nitrogenase enzyme along with reduction the activity of sucrose synthetize enzymein saline condition reduced in root nodules of soybean. In keeping with these results, Lopez (2008) also suggested that reducing photosynthesis of host plant in effect of salinity resulting in decreased production ofsucrose and its transfer to the root nodules and thusreduced nitrogen-fixation in these nodes. Lopez et al (2008) and Gordon (1987) showed that Symbiotic nitrogen-fixation is dependent on metabolic energy and expressed that one of the reasons reduced symbiotic nitrogen-fixation under saline stress is reduced metabolic energy required for the reduction of molecular nitrogen.

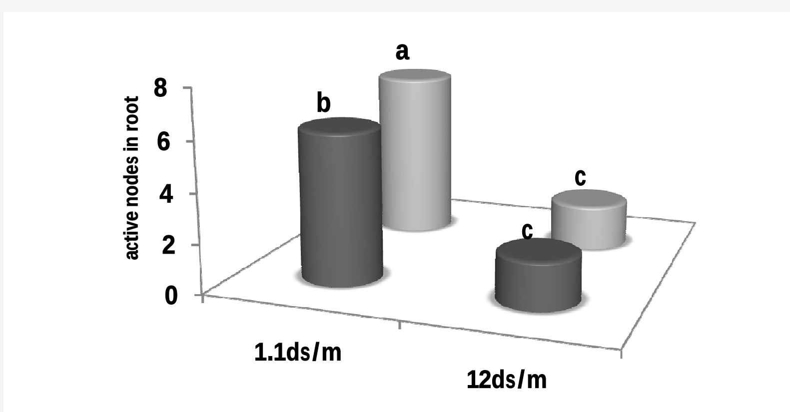

Figure 2: The effect of salinity on the number of active root nodes in two Alf alfa cultivars

The effect of nitrogen supply (ammonium nitrate):

The effect of ammonium nitrate (NH_4 NO_3) was significant on the number of active root nodules and ammonium nitrate consumption reduced this parameter substantially. The interactive effect of salinity and nitrogen supply was considerable on the number of active root nodules and ammonium nitrate supply in saline conditions compared with control reduced the number of active root nodules (Figure 3). Kennedy et al (1975) and Pagan et al (1977) reported that the inhibitory effect of nitrate on symbiotic nitrogen-fixation activity has been attributed to the presence of nitrate reductase in the root nodules. As they suggested the reduction of nitrate to nitrite is adjusted by nitrate reductase and nitrate by creating complex with leg hemoglobin prevent leg hemoglobin combines with oxygen and thus inhibiting of nitrogen fixation in the root nodes. In the new report, inhibition of symbiotic nitrogen-fixation in the presence of inorganic nitrogen in the soil is attributed to carbohydrate deficiency for metabolic processes (nitrate assimilation) (Waterer and Vessy, 1984).

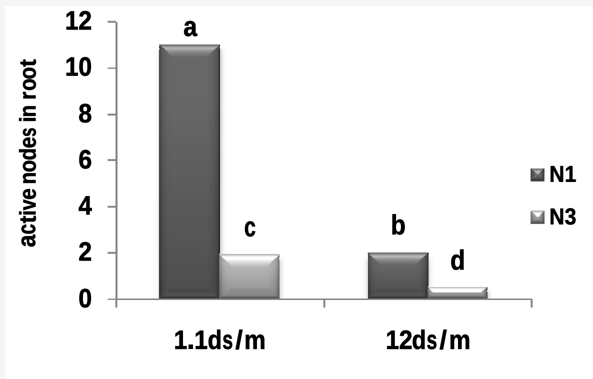

Figure3: Effects of salinity and nitrogen supply on thethe number of active root nodes

Nitrogen concentration of roots and shoots:

Statistical analysis of data related to the nitrogen concentration of roots and shoots are summarized in Table 2.

The effect of salinity stress:

In the control conditions (non-saline), N concentration of roots and shoots in the Ghareh figure was higher than the Bami figure. But under saline conditions (12 dS/m), root and shoot N concentration reduced in both cultivars and decreasing the nitrogen concentration were higher in shoots and roots of Bami figure. Considering the positive correlation between root and shoot nitrogen concentration and dry matter production in these organs, probably to maintain higher levels of nitrogen in resistant varieties to salinity can be a key factor for increasing salt tolerance in Alfalfa cultivars. Pessarakli (1994)and Khan et al (1998) also expressed that in saline conditions the nitrogen concentration reduction will be less in resistant cultivars to salinity in compared with the susceptible cultivars.

The effect of nitrogen supply:

The highest nitrogen concentration in roots and shoots was treatments N2 and N3. Nitrogen concentration of roots in all three forms of nitrogen supply in Ghareh figure was higher than Bami figure. But nitrogen concentration of shoots was not significantly in the two varieties. Using ammonium nitrate in the N_3 treatment seems to have the inhibitory effect on symbiotic nitrogen-fixation and prevent symbiotic nitrogen-fixation in plant and adjusted nitrogen content. Kennedy (1975) and Pagan (1977) pointed that the inhibitory effect of mineral nitrogen on symbiotic nitrogen-fixation due to the presence of nitrate reductase enzyme inhibitor that causes the reduction of nitrate to nitrite in the root nodes.

Sodium concentration in roots and shoots

The effect of salinity stress:

Salinity had a significant effect on Na concentration in roots and shoots and sodium ion concentrations increased substantially in roots and shoots. The two figures of Alfalfa were different in terms of sodium concentration in the roots and shoots and sodium concentration in roots and shoots of Ghareh figure was significantly higher than the Bami figure. Interactive effect of variety and salinity was considerable on root and shoot Na concentration indicated that the figures in these parameters had not a similar reaction.

Means comparisons showed that under non_saline conditions, the sodium concentration was not statistically significant in shoots and roots of both cultivars. However in the saline conditions, Sodium concentration in roots and shoots of Ghareh figure was significantly higher than the Bami figure. Accordingly, we can say that the Bami figure avoid from sodiumentrance and transport to their organs, in particular shoots.

The effect of nitrogen supply:

The effect of nitrogen supply on root and shoot sodium concentration was not significant. As the interaction effect of nitrogen supply and cultivar was not considerable in the root and shoot sodium concentration. Therefore response of both figures was similar to the forms of nitrogen supply. The interactions of cultivar × salinity × nitrogen supply was not substantial on root and shoot sodium concentration. In this regard, Mans (2002) reported that the effects of osmotic, oxidative and toxicity of sodium ions that causes reduced plant growth. Therefore it can be concluded that the likely mechanism of ion desorption is used for salt tolerance. Lynch and Laochely (1984) pointed to the ability of Alfalfa in disposal of sodium ion from the shoots. Flowers and Hajbagheri (2001) have been reported the ability of resistant cultivars to salt stress in the desorption of sodium ion from shoots under salt stress.

Potassium concentration in roots and shoots

The effect of salinity stress:

Salinity effect was significant on K concentration of roots and shoots and salinity was substantially reduced K concentration in the root and shoot. The two varieties had significant difference in potassium concentration of roots and shoots that potassium concentration in the roots and shoots of Ghareh figure was markedly higher than the Bami figure. The interactions of salinity and cultivars on roots potassium concentration were not considerable, but potassium concentration was significant in shoots. Mean comparisons showed that in non-saline conditions, shoot potassium concentration in Ghareh figure was significantly higher than the Bami figure, but in the saline conditions, the two varieties were not substantially different in this parameter (Table 2). There was a significant negative correlation between potassium and sodium concentrations in roots and shoots. In this regard, Kramer and et al (1993) reported that the main reason for reducing potassium concentration in the saline conditions is expressed instead of sodium replacing with calcium in the plasma membrane of root cells and potassium out of the membrane, due to the membrane permeability. Inthis case, Jsky (1979) stated that potassium transportation into the tissues growing reduced in the salinity effect and consequently decreased availability of potassium to the plant.

The effect of nitrogen supply:

The effect of nitrogen supply was significant on root and shoot K concentration. The highest K concentration in roots and shoots related to N2 and N3 treatments. The interactionsof nitrogen supply and cultivars were not significant on potassium concentrations of roots and shoots, indicating that the figures have shown a similar reaction to nitrogen forms. As the interactions of cultivar × salinity × nitrogen forms were not significant on root and shoot K concentration. K concentration in the roots and shoots under non-saline conditions in the plants that were fed with ammonium nitrate was higher than plants which received nitrogen in the form of symbiotic fixation in the root nodes. This has also been mentioned in report Atlovyna et al (2005).

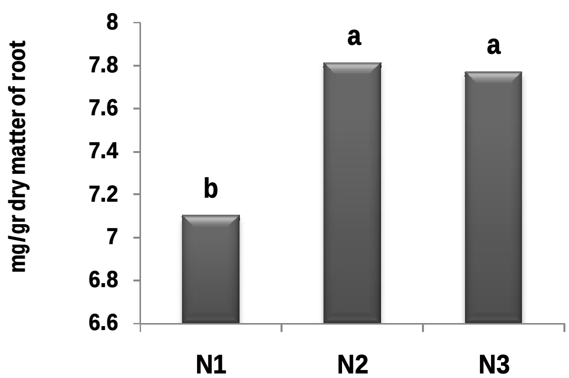

Figure 4: The effect of nitrogen supply on root potassium concentration

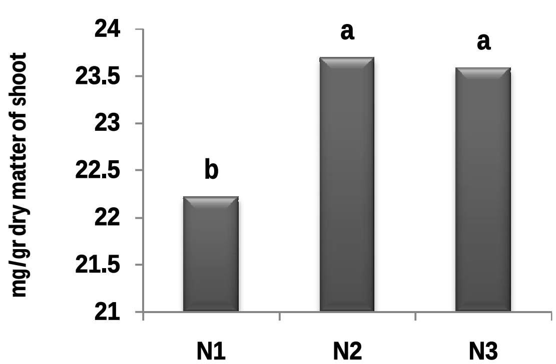

Figure 5: The effect of nitrogen supply on shoot potassium concentration

Conclusions

Consequently, salinity reduced significantly the total number of nodes, the number of active nodes, the root and shoot N concentration, Na and K concentration.The effect of ammonium nitrate (NH_4NO_3) was significant in the total number of nodes and the number of active nodes and decreased these parameters. But the consumption of ammonium nitrate increased Nitrogen and potassium concentration in the roots and shoots of both cultivars. According to the all parameters, it seems that the Bami figure is higher resistant to salinity than the Ghareh figure and salinity have lower effect on dry weight, concentration of nitrogen and potassium in the Bami cultivar. But in both cultivars there was no significant difference in the total number of nodes and the number of active nodes of nitrogen fixation.

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Table 2 : Analysis of effects of salinity and nitrogen supply on the concentration and dry weight of Alfalfa cultivars (Bami and Ghareh)

Nitrogen concentration		Dry matter				
Shoot	Root	Shrub	Shoot	Root	df	S.O.V
*	*	*	*	ns	2	Replication (R)
ns	ns	*	*	*	1	Cultivar (C)
**	**	**	**	**	1	Salinity (S)
**	**	**	**	**	2	Nitrogen supply (N)
**	**	**	**	**	1	C*S
*	ns	ns	*	ns	2	C*N
**	**	**	**	**	2	S*N
ns	ns	ns	ns	ns	2	C*N*S

** and * indicate significant difference at 1% and 5% probability level, respectively and ns is not significant

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