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Effect of Salinity on Growth and Nitrogen Fixation of Alfalfa (*Medicago sativa*)

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Abstract: To investigate the effect of salinity on the amount of nodulation and nitrogen fixation and protein in alfalfa, a factorial experiment arranged randomized complete blocks, with four replications and 4 treatments was conducted in the Faculty of Agriculture University Islamic Azad Mahabad branch in 2010. Treatments included a combination of salt stress in 3 levels (control, 50 mM, 100mM, 150 mM, mM) on alfalfa cultivars (Hamadani, Qara yonjeh, Renjer, Scuttelata annual medic) supplemented with locally Rhizobium and planted in pots that were filled by perlit. Hoagland solution, without nitrogen, was used to supply nutrients. At first, the pots were irrigated with tap water. After the emergence of main leaflet, irrigation with Hoagland solution was started. After 25 days, salinity treatment was followed with Hoagland solution and it was continued for 65 days. The results showed plant dry weight, the number of active nodules and nitrogen content, relative water content (RWC) and leaf content chlorophyll were affected under salt stress in all tested cultivars. Plant dry weight decreased by about 56% with the highest salt dosage (150 mM NaCl). Also the number of active nodules and Nitrogen content, dry weight, RWC and leaf chlorophyll content decreased significantly in the highest level of salinity. Because of the growth stage in which the salinity was applied, all the cultivars had a similar response to salinity treatments and none of them could show its ability and difference.

Key word: Nitrogen Fixation · Nodule · Salinity Stress · Chlorophyll Content · Dry Matter · Hoagland Solution

INTRODUCTION

Salinity in the arid and semi-arid regions of the world as well as in irrigated lands is a serious threat to agriculture, affecting plant growth and crop yields [10, 24]. Current estimates indicate that 10 - 35% of the world's agricultural land is now affected, with very significant areas becoming unusable each year. Soil salinization significantly limits crop production and consequently has negative effects on food security. The consequences are damaging in both socioeconomic and environmental terms.

Salinity is increasing as a problem on an irrigated farm, it may be necessary to select crop varieties that have a greater tolerance to salt.

An ecologically and agriculturally relevant aspect of legume biology is their specific ability to interact with soil bacteria to form root nodules which fix atmospheric nitrogen. The symbiotic bacteria, differentiated into bacteroids and surrounded by a peribacteroid membrane (that isolates them from the host cytoplasm), fix nitrogen inside the plant cells of this organ [7]. However, this symbiotic interaction is affected by salt stress. Legumes are usually more sensitive to salinity than rhizobia which can be tolerant up to 700 mM NaCl [1, 8, 21]. Different steps of the symbiotic interaction as well as nodule development and metabolism are affected by salt stress, leading to a reduction in nodule number and limited nitrogen fixation [21]. Salt stress notably affects rhizobial colonization of roots and early infection events [18, 22]. In addition, the nitrogen fixation process is very sensitive to salt stress, affecting peribacteroid membrane structure and bacteroid number [4].

In the present work, the response 4 cultivar of Medicago Sativa symbiosis to salt stress during the vegetative period when symbiosis is well established, has been studied. The aim is to ascertain whether the negative effect of salt stress on nitrogen fixation is due to a limitation on 4 important traits including biological nitrogen fixation and the number of active nodules,

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content chlorophyll and relative water content (RWC) in vegetative stage in *Medicago sativa* life.

MATERIALS AND METHODS

In a pot experiment, 4 cultivars of Alfalfa (*Medicago sativa* L.) (Hamadani, Ghrai Yonjeh, Ranjer and Annual Medic Scotellata) were exposed to NaCl salinity using a factorial design setup in CRB with four replicates. 3 levels of salinity were used (control, 50 milimolar, 100 milimolar, 150 milimolar). This study was conducted in Iran, at agriculture college greenhouse, Islamic Azad University Mahabad branch during the spring of 2010. The seeds were provided from saline regions, inoculated with domestic rhizobium (that from Lucerne fields in the area before the test was extracted) and sown in pots which were filled with perlit. During the10 days following sowing, the number of plants was reduced to 10 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 15°C respectively. From sowing to emergence of main leaflets the irrigation was done with tap water. At this time when seed nutrient storage is finished, the irrigation with Hoagland solution free from nitrate was initiated. The aim of using this solution was, being sure that the source of nitrogen content was only from biological nitrogen fixation and nodule activation.

Twenty five days after sowing when the nodule activity completed the salinity treatment was applied with Hoagland solution and it was continued for 50 days.

Daily rate of 100 ml of nutrient solution containing Sodium chloride was added to the pots. Weekly washing pots with distilled water to prevent salt accumulation around the roots looking washing.

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

Node counting**.** After 65 days from sowing, the plants were taken out pots and the number of active nodules were measured (the pink nodules) [5].

Dry weight: Then the plants(10 plant) were dried at 70°C for 48h and then weighted.

Nitrogen Fixation: For measuring nitrogen content, one gram was used from every sample. The nitrogen measurement was done with Tecator and Kejeldal method Then the nitrogen content was measured in consideration of dry matter [6].

Chlorophyll: TO measure of chlorophyll SPAD digital device was used(SPAD Unit).

Relative Water Content: The relative water content was determined according to Henson *et al.* (1981) using the following formula:

$$RWC = 100 \times (\text{fresh mass} - \text{dry mass}) / (\text{turgid mass} - \text{dry mass}).$$

Turgid mass was determined after saturation of leaf blades in distilled water in sealed lass tubes for 4 h at room temperature followed by over night storage at 5°C. The dry mass was determined after 48 h at 80°C [11].

Collected data were analyzed statistically using SPSS program and mean were compared by Duncan multiple range Test at $P < 0.05$.

RESULTS AND DISCUSSION

The growth response to salt stress of Medic cultivars is represented in Table 1. These data clearly indicate that all traits of plant were affected by 50 and 100 and 150 mM NaCl.

Table 1 shows that the salinity has a significant effect on dry matter. With increasing salinity the dry matter decreases from 2.75 gr in control level to 0. 76 in the highest level.

The ANOVA table shows that the salinity has a significant effect on root active nodules (Table 1). The mean comparison for number of active nodes on the root indicates that the control level is 13.2 and in 150 mM treatment the number decreased to 2.7 (Table. 2). With increasing salinity, the number of active nodes and nitrogen fixation percent were reduced (Fig. 1).

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

In several studies, the total number of active nodules in chick-pea and Vicia faba and Trifolium subterraneum decreased by salinity treatment.

Using salinity treatment nodules lost their pink color (leghemoglobin content) and the N2 fixation activity, made them white and inactive. The salt-induced distortions in nodule structure could also be a reasons for the decline in the N2 fixation rate by legumes subjected to salt stress. Likida, reported that the application of 13.7 mM NaCl decreased number of root hair and root curling in White Clover,also salinity reduced the number of bacteria attached to the root and nodules [16].

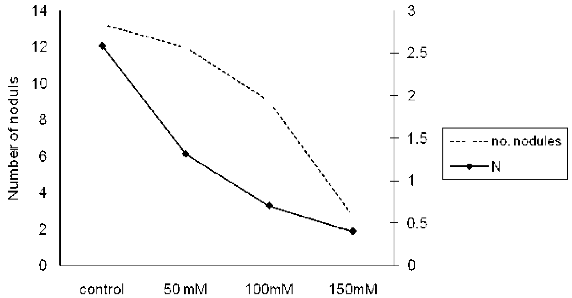

Fig. 1: Effect of different salinity on number of nodules and plant total N(%)

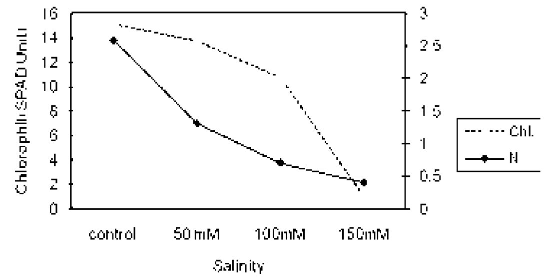

Fig. 3 : Effect of different salinity on leaf chloropyll content and plant total N(%)

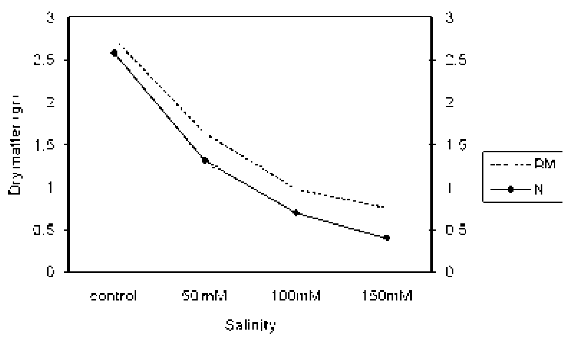

Fig. 2: Effect of different salinity on dry matter and plant total N(%)

Environmental factors not only affect symbiosism between legume and bacteria but also ensures their growth and reproduction. Increasing salinity has a significant effect on nitrogen content (Table. 1). Table 2 shows in control level the nitrogen content is 2.58 and at the highest level of salinity this decreases to 0.4. Intimate relationship between host plants and bacteria and their interactions increases plant tolerance to salinity. The varieties able to quickly form nodules have greater tolerance to salinity [24].

In this experiment dry weight was reduced Photosynthesis in plants facing to salinity have

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

MS						
Source of Variations	df	*P.D.W (gr)*	N.A.N	L.Ch.C (SPAD Unit)	P.N.C(mgr/gr dry mater	RWC (%)
Cultivar (A)	3	1.07**	42.913**	0.251*	14.25*	926.4**
Salinity (B)	3	2.99**	178.68**	2.43**	56.82**	153^{ns}
Salt x Cultivar	9	0.134*	1.682**	0.118*	0.642*	10.4 ^{ns}
Error	48	0.006	0.069	0.002	0.068	11. ^{ns} 81

ns: non-significant

*: significant at $p \leq 0.05$.

**: significant at $p \leq 0.01$.

P.D.W= Plant dry weight, N.A.N= Number of active nodules

P.N.C=Plant nitrogen fixation, L.Ch.C=Leaf chlorophyll content, R.W.C= Relative water content

Table 2: Effects of four levels of Nacl on growth and nitrogen fixation parameters

MS					
Salinity levels (mM NaCl)	*PDW(gr)*	N.A.N	P.N.content (%)	L.Ch.content (SPAD Unit)	(RWC)(%)
Control	2.75a	13.23a	2.58a	15.11a	91a
50 mM	1.64b	11.94b	1.31b	13.73b	80b
1oo mM	0.98c	9.016c	0.7c	10.7c	44c
150 mM	0.76	2.7d	0.4d	9.0d	35d

Means with the same letter in each column are not significantly different at the probability level of (Duncan 5%).

Table 3: Effects of four levels of Nacl on growth and nitrogen fixation parameters of four alfalfa cultivars

MS					
Cultivars	*PDW(gr)*	N.A.N	P.N.content (%)	L.Ch.content (SPAD Unit)	(RWC) (%)
Hamadani	1.14^{b}	12.34^{a}	2.23^{b}	12.8^{c}	55^{bc}
Garh Yonjeh	1.05^{c}	9.78^{b}	2.09^{c}	11.61^{d}	53^{c}
Ranjer	1.06^{c}	9.12^{c}	2.27^{a}	13.75^{a}	56^{b}
Scutellata	1.59^{a}	8.67^{d}	2^{d}	13.4^{b}	50^{d}

Means with the same letter in each column are not significantly different at the probability level of (Duncan 5%).

decreases. With decreases in dry mater,nitrogen fixation percent reduces (Fig. 2). Ranjer varity with the value %2.27 N per total dry weight was highest level (Table 3).

The analysis of variance shows that the salinity has a significant effects on amount chlorophyll (Table 1). With increasing salinity the amount of chlorophyll decreases from 15.11 in control level to 9.00 in the highest level (Table. 2). Chlorophyll decrease in salinity has been linked to more activity of chlorophylase [9], also chlorophyll reduction can be due to changes in nitrogen metabolism to make compounds such as proline, which is used in the osmotic adjustment [17]. Atlassi *et al.* (2009) reported that salinity decreases amount of chlorophyll in tolerant Brassica variety [3]. Fig.3 shows with the reducing of amount of chlorophyll in the leaves, the percentage of nitrogen in the plant will be fixed, which may be due to decreased photosynthesis.

Salt stress decreased the RWC of all four cultivars and it increased with salinity level. Table (2) shows greater reduction in the RWC is related to 150 mM salinity. Our results are in accordance with those of Sairam *et al.* who report a greater reduction in the RWC of salt sensitive wheat cultivar as compared with tolerant one under salt stress [19].

Ability to maintain higher RWC at any water potential of the stability of the cell wall which is indicative of damage caused by stress [14]. Reduction of water in the environment of roots decreases RWC and reduced total water potential of the plant [15].

Kapulnik, observed reduction in total N produced through N2 fixation, with increasing salinity (0, 100 and 200 Mm NaCl) in 45-day-old seedlings and in mature plants [12]. Khan *et al.* reported that the number of active nodes and nitrate activity in alfalfa varieties significantly decreased with increasing salinity showed [13].

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

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