Effect of irrigation with saline water on root distribution and forage yield by different root types of alfalfa (Medicago sativa) under different soil salinity conditions with zero leaching

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in

Genier G. (ed.), Prosperi J.M. (ed.).
The Genus Medicago in the Mediterranean region: Current situation and prospects in research

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 18

1996
pages 119-125

EFFECT OF IRRIGATION WITH SALINE WATER ON ROOT DISTRIBUTION AND FORAGE YIELD BY DIFFERENT ROOT TYPES OF ALFALFA (*MEDICAGO SATIVA*) UNDER DIFFERENT SOIL SALINITY CONDITIONS WITH ZERO LEACHING*

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ABSTRACT

Most of the cultivated lands in Egypt are irrigated lands. Thus, the problem of salt-affected soils are old, but their extent and their intensity are increasing quickly in recent decades due to large scale efforts to bring additional areas under irrigation utilizing poor-quality water. Therefore, research directed to evaluate the consequences of irrigation with saline water and its effects on crop plants and soils is needed. Considerable variation exists in root distribution, but a number of comparisons refer to inter-specific differences. Differences in the number and size of roots occur also between individual plants of a cultivar and between cultivars. It is likely that such differences have an important bearing on the adaptability of alfalfa cultivars to escape the adverse conditions, i.e., salinity. Transparent viewing planes provide a useful and popular means of observing and quantifying root growth.

This study was conducted to determined the limiting factors in utilization of saline irrigation water by alfalfa plants which have different root distributions under non-destructive conditions with zero leaching. Two alfalfa cultivars which were selected for high and low fibrous root systems were planted in containers. During a period of about four months, root growth by depth was monitored and root length was measured. Herbage yield was harvested five times. Both herbage yield and root length showed highly significant differences under the four salinity levels that have been used among and within cultivars. The interactions between cultivars and salinity levels were significant and non-significant for herbage yield and root length, respectively. It is indicated from the results that high possibility is likely to exist for breeding alfalfa plants which retain salinity tolerance.

Key words: *Medicago sativa*, alfalfa, salinity, rhizotron, stress.

INTRODUCTION

It is indicated that on a worldwide basis there are between 400 and 950 million hectares of salt-affected soils (Epstein *et al.,* 1980). Moreover, the problems of salt-affected soils are old, but their extent and their intensity are increasing quickly in recent decades due to large scale efforts to bring additional areas under irrigation utilizing saline water. Marginal or poor quality water is being used in several places in the world and its use requires careful management. Most of this wastewater is still usable and its utilization often reduces the total volume of wastewater that must ultimately be disposed. Research project is designed to evaluate the long-term consequences of irrigation with saline water and to develop strategy to prolong the productivity of saline-irrigated soils. The source of this water is the Electrical Power Plant at Huntington, Utah operated by Utah Power & Light (UP & L), U.S.A. Water in which naturally occurring minerals have been concentrated is used to irrigate a wide variety of crops, including alfalfa. This study is in its fifteenth year, and salinity has so far not reduced yields of several forage crops. The saline irrigation water contained about 10 times more total salt, 25 times more chloride and 50 times more boron than did the non-saline water. One of the primary objectives of the project is to find and monitor an irrigation schedule that produces near maximal crop yields without leaching (Dudley *et al.,* 1991). Eliminating leaching will result in salt accumulation in the soil and decrease crop yields. Eventually, alfalfa production will be adversely affected. Deposition of salts under saline irrigation occurs first at the bottom of the rootzone. Francois (1981) demonstrated that alfalfa yield was drastically reduced once salt build-up began in the upper portion of the rootzone and also indicated that alfalfa can tolerate high salinity in lower portions

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*The study has been conducted as Post Doctor research by the Author in the Dept. of Plants, Soils, & Biometerology, Utah State Univ., Logan,Utah,USA, in 1993.

of the rootzone (at 1.8 m) by increasing water uptake from higher rootzone regions that are lower in salinity. The concept that plants extract water from non-saline regions was also supported by Hanks *et al.*, (1977) who demonstrated that high salinity in the upper levels of the rootzone was associated with no reductions in yield with the application of saline irrigation water. Alfalfa is considered moderately sensitive to salinity; its yield is reduced approximately 7.3% for each EC unit (dS m^{-1}) above the threshold value of 2.0 (Maas,1990). Root weight has been the root character most frequently studied as a response to salinity, but root distribution parameters such as volume, length, diameter, depth of penetration, degree of branching and number of root hairs have also been the subject of comparative studies. Variation among root systems has also been found for characters (i.e. root length, root length density, root length index) other than those related to the quantity of roots (Bohm *et al.*,1977; Gerard,1978; Schenk and Barber,1979; McIntosh and Miller,1980; Waisel and Breckle,1987; Burt and Christians,1990; Cramer *et al.*,1990; Glinski *et al.*,1993). Root growth distribution, such as any character, may result from interaction of genetically and environmentally induced factors. Moreover, salinity in soils irrigated with saline water could vary with both the depth and the time based upon the salt concentration and the amount of the water applied. In the area of breeding alfalfa cultivars with high potential for nitrogen fixation, two alfalfa cultivars were developed using two cycles of bi-directional selection from Mn PL alfalfa gene pool which are characterized by high-and low-fibrous root systems (Viands *et al.*, 1981). These materials seem ideal to test our hypothesis. Two alfalfa cultivars (Mn 4311 and Mn 4661) which were characterized with high and low fibrous root systems, respectively; were kindly supplied by Dr. D. K. Barnes, USDA-ARS and Dept. Agron., Univ. of Minn. The objectives of this study were to determine the limiting factors in utilization of saline irrigation water by alfalfa plants having different root distributions under non-destructive conditions with zero leaching in the greenhouse.

MATERIALS AND METHODS

Forty polyvinylchloride (PVC) cylindrical tubes (10 cm-diam. by 120 cm long; 5-mm all thickness) with a 10-cm wide band sliced off extending to the length of the tube with electrical saw and covered with plexiglas glued with epoxy glue and sealed from outside with silicon were constructed. The PVC tubes were bottom-capped with PVC caps have four holes 3 mm diam. with a layer of wire screen as filter for aeration. The caps had been glued with epoxy glue and sealed with silicon to the bottom of the tubes. Ten separate batches of soil were obtained from the Ap horizon of Coarse-silty, mixed (calcareous), mesic soil (Typic Torriorthents) at Utah Power and Light Research Farm, Huntington, Utah, USA. Field-moist soils were screened (3-cm mesh) to remove plant residues and larger rocks. Soils were sampled separately for testing at USU Analytical Laboratories, Utah State Univ. Logan, Utah. Based upon simulated model developed to predict water and salt balance of chemically reactive species in soil profile after 20 years, 30 years and actual analysis after 13 years of using saline irrigation water for alfalfa irrigation (Dudley *et al.*, 1991), calculated amounts of salts were, added as solutions, mixed with soils to construct the simulated levels of salinity after 20 years, 30 years and actual soil profiles after 13 years of using saline irrigation water. Soils were resembled for testing at the same lab after were left to dry (Table 1). The salts were added to the soils to mimic the salt composition along the soil profile extended to 120 cm depth from soil irrigated with saline water for 13 years as well as simulated soil profiles for salt balance of chemically reactive species after 20 and 30 years (Table 2). From soil analysis it was clear that we increased the salinity level of the soils but we could not realize the average salt composition intended (Table 2). Soils were used to fill the tubes and construct four levels of salinity with various salt levels at different depths along the soil profile in the tubes (Table 2). One-kilogram increments of soils with salinity level and depth intended were successively packed into the tubes. Packing followed by tamping continued until the tubes filled. All 40 tubes were of approximately equal volume and received equal volumes of soil. Soil heights varied (+3 cm) and were adjusted to equivalent levels with leaving 8 cm on the top for watering the plants. The bulk density was approximately 1.3 Mg m^{-1}. After packing, tubes were saturated with tap water to promote soil settling and left for 24 h. The tubes were weighted to figure out how much water was used to reach the field capacity and were set on a plywood A-frame structure at 25^{o} from vertical so that roots would tend to grow and be visible along plexiglas surface. To exclude light from the roots, each tube was wrapped in two layers of 0.1-mm black polyethylene plastic. Tubes were weighted and randomly rotated on the racks to monitor ET and eliminate any variation could result from differences due to environmental conditions, if any, respectively. Twenty plants from each of two alfalfa cultivars (MN 4311 and MN 4661) which were selected for high- and low-fibrous (secondary) root systems, respectively, (Viands *et al.*, 1981) were established in the 40 tubes on 26 Jan., 1993 in the greenhouse. Seeds from each of the two alfalfa cultivars were treated with inoculant of alfalfa- clover, Urban Laboratories, St. Joseph, MO., and fungicide Apron with rate 0.0025g Apron (12.5%) /g alfalfa seed and soaked for 6h in tap water. Then, five seeds were sown in each tube based upon the design, covered with 0.5 cm soil and sprayed with tap water. Spraying with the tap water continued till 25 Feb., 1993. Then saline water (Table 3) was used in irrigation of the plants. Germination percentage was very good in all the tubes and seedling were thinned to one seedling per tube after 15 d of sowing. Plants were grown in a greenhouse with supplementary lighting provided by high pressure sodium bulbs (900-1000 u mol photons m^{-2} s^{-1}) on a 14/10-h day/night cycle. Throughout daytime of the growing period (26 Jan. - 20 June 1993), greenhouse temperature was maintained at 25°C+4°C. Plants were treated with timik based upon the rate recommended on 25 Feb., 1993. Since it was reported that salinity could affect some Rhizobium strains for nitrogen fixation by alfalfa plants (Mohammad *et al.*, 1989), a mixture of four salt-tolerant Rhizobium strains (No. 1027,1029,1030, and 1031) which were kindly supplied by Dr. W.F. Campell, Professor, Plant Sci. Dept. Utah State Univ., Utah. The four strains were mixed and diluted to 40ml, and 1ml was injected in each tube, 4 cm deep in the soil, near the root (crown) after taking the second harvest on 23 March, 1993. Soil analysis indicated that soil pH was 7.7 and potassium and calcium were adequate. Phosphorous was only below Utah Extension Service recommendations and increased to 90.7 kg P_2O_5 ha^{-1} (0.649g P_2O_5 per tube) after 20 days of sowing. Nitrogen was applied as a solution 56.7 kg N ha^{-1} (0.809g 30-10-10 per tube) on 29 March, 1993, since some of the tubes had no nodules on the roots during tracing the roots before the second harvest. Root tracing began day before taking the first harvest on 3 March. Clear Strapless Transparency sheets (Xerox Corporation Rochester, New York) were used to trace the roots. Roots within a 8-cm-wide band extending the length of the tube were traced onto the sheets on the plexiglas side of each tube. Tracings were made once the day before each harvest, using Stadler Lumicolor permanent marking pens (Stadler, Nurnberg, Germany). Different colored markers distinguished harvests. Root length was measured using Super Calc 4 (Computer Associates, 1986). Herbage yield was harvested about 10-cm above the soil surface when in partial bloom on 5 March, 20 March, 6 April, 4 May, and 4 June, 1993. Plant height and number of stems per plant were recorded before cutting each harvest. Fresh herbage weight was recorded in g/plant and stored in a freezer in Z-L bags for dry weight and chemical analysis determinations. Before cutting the third harvest plants were heat-stressed due to some problems in the cooling system for 24h. Fourteen plants, most of them had low yielding in the first two harvests and belong to the low fibrous root system cultivar and high salinity level treatments, were affected by high temperature and died after the third cut. Data recording, continued on the rest of the plants (26 plants).

Table 1. The average composition of the soil solution and EC of 10 samples of 10 soil batches after adding salts tested in USU Analytical Laboratories

ID	SP	EC_e	Cl	Na	Ca	MG	S
	%	mmhos/cm^{-1}	meq/L	mg/L			
1	58.3	6.5	28.1	467.6	655.0	177.6	711.1
2	71.7	5.6	19.4	304.3	625.8	142.9	475.6
3	66.6	7.3	33.0	513.9	734.7	165.1	631.6
4	56.6	4.5	5.2	189.9	570.5	155.0	601.7
5	54.2	6.7	23.2	468.3	626.9	233.5	826.7
6	56.7	9.6	46.4	819.5	694.3	229.9	865.5
7	59.1	8.1	35.5	635.0	665.0	207.1	806.2
8	53.3	16.0	62.8	1397.6	647.1	523.5	1269.9
9	52.8	18.0	71.5	1656.7	630.8	608.9	1546.1
10	60.8	1.4	1.5	42.9	185.3	47.6	68.8

STATISTICAL ANALYSIS

For root distribution screening of two alfalfa cultivars under different levels of salinity, a split-plot experiment was arranged in a randomized complete block design with level of salinity (four levels) as the main plot factor and cultivars (two cultivars) as the sub-plot treatment in five replications (Snedecor and Cochran, 1980).

Table 2. The average composition of accumulated salt and EC after 13 years (actual analysis) and simulated after 20 and 30 years in soil profile (120 cm depth)

Depth cm	Trt.4 years	Ca	Na	SO_4^{2-}	Mg	Cl	EC
		mmol/L					mmhos/cm^{-1}
0-30	13 field[1]	17.4	23.6	31.5		15.5	28.8
	GH[2]	16.4	20.3	22.2	7.3	28.1	6.5
	20 simu[3]	13.4	13.7	29.1	15.9	8.1	6.7
	GH	14.3	8.3	18.8	6.4	5.2	4.5
	30 simu.	13.4	13.7	29.0	15.9	8.7	6.7
	GH	14.3	8.3	18.8	6.4	5.2	4.5
	0.0 GH	4.6	1.9	2.2	2.0	1.5	1.4
30-60	13 field[1]	14.4	23.6	31.5		15.5	28.8
	GH[2]	16.4	20.3	22.2	7.3	28.1	6.5
	20 simu.	12.7	29.8	45.5	33.4	28.1	11.3
	GH	15.7	20.4	25.8	9.6	23.2	6.7
	30 simu.	12.7	29.8	45.5	33.4	28.1	11.3
	GH	15.7	20.4	25.8	9.6	23.2	6.7
	0.0 GH	4.6	1.9	2.2	2.0	1.5	1.4
60-.90	13 field[1]	14.2	24.7	31.4		14.6	31.0
	GH[2]	15.7	13.2	14.9	5.9	19.4	5.6
	20 simu.	11.6	56.7	74.1	62.0	49.0	18.1
	GH	17.4	35.6	27.1	9.5	46.4	9.6
	30 simu.	10.8	69.4	87.8	67.7	63.0	21.1
	GH	16.6	27.6	25.2	8.5	35.5	8.1
	0.0 GH	4.6	1.9	2.2	20.	1.5	1.4
90-120	13 field[1]	14.1	40.8	36.9		24.7	41.1
	GH[2]	18.4	22.3	19.7	6.8	33.0	7.3
	20 simu.	11.4	101.9	87.1	68.6	76.8	23.2
	GH	16.2	60.8	39.7	21.5	62.8	16.0
	30 simu.	9.1	103.0	140.6	98.1	89.1	30.6
	GH	15.8	72.0	48.3	25.1	71.5	18.0
	0.0 GH	4.6	1.9	2.2	2.0	1.5	1.4

1: Actual analysis of soil profile of alfalfa field irrigated with saline water; 2: Soil profile constructed in the greenhouse (GH) with soil mixed with salt; 3: Simulated deposited salt after 20 and 30 years, 4 Treatments (four); 13- Actual analysis of soil profile after 13 years, 20- Simulate profile after 20 years, 30- Simulated profile after 30, and 0.0- No salts added to the profile.

Table 3. The average composition of the saline irrigation water applied to alfalfa plants in the greenhouse

Ca	Mg	Na	Cm	SO_4^{2-}	EC
mmol/L					mmhos/cm-1
8.0	4.0	5.0	5.0	12.0	4.0

RESULTS AND DISCUSSIONS

Data of herbage yield and root length traits as affected by different salinity levels and different root systems; high and low fibrous are presented. Average herbage yield (g/plant) as affected by four different salinity levels; (a) actual analysis of soil profile of alfalfa field irrigated with saline water for 13 years, (b) and (d) simulated deposited salts after irrigation with the saline water for 20 and 30 years; respectively, and (c) where no salts added to the soil profile averaged over two alfalfa cultivars characterized with high and low fibrous root systems differed significantly (Table 4). Control treatment (c) where no salts were added to soil profile gave the highest forage yield in the first three harvests. It is indicated from that salinity has detrimental effects on forage yield especially in the first two harvests (Table 4). This result is in agreement with what was demonstrated by Francois (1981) who stated that alfalfa yield drastically reduced once salt build-up began in the upper portion of the rootzone. On the other hand, the detrimental effects of salts on herbage yield were decreased in comparison with the control, especially in the fourth and the fifth harvests. An increase in herbage yield with about 70% and 30% for salinity levels (b) and (d) in the fourth harvest and 50% and 21% for salinity levels (b) and (a) in the fifth harvest in comparison with the control (Table 4). It is clear from the results that alfalfa genotypes which can tolerate high salinity levels in lower portions of the rootzone are existing. The concept that plants extract water from non-saline regions was supported by Hanks *et al.*, (1977) who demonstrated that high salinity in the upper levels of the rootzone was associated with no reductions in yield with the application of saline irrigation water. Approximately, the detrimental effects of salinity on root distribution (root length) averaged over the two alfalfa cultivars have the same trained which indicated with herbage yield trait (Table 5). The control has the highest root length in the first three harvests. However, high salinity levels ,i.e., (b) in the fourth harvest and (a) and (b) in the fifth harvest have root length greater than those of the control. Regarding the effects of different root systems; high and low fibrous on herbage yield (Table 6) and root length (Table 7) averaged over four salinity levels, significant differences were detected. It could be stated that differences in root system have clear effects for salinity tolerance. Root growth distribution such as any character, may result from interaction of genetically and environmentally induced factors. Mean squares of the analysis of variance of both herbage yield and root length at the first three harvests of the two alfalfa cultivars under four salinity levels are presented in Table 8. Highly significant differences were detected for both salinity and cultivar effects for all harvests regarding herbage yield and root length. While differences due to the interaction effects were significant only for herbage yield in the first and third harvests. Alfalfa is considered moderately sensitive to salinity; its yield is reduced approximately 7.3% for EC unit (dSm^{-1}) above the threshold value of 2.0 (Maas,1990). However, it is suggested that alfalfa can tolerate high salinity in lower portions of the rootzone (at 1.8 m) by increasing water uptake from higher rootzone regions that are lower in salinity (Francois, 1981). It is likely that differences detected in our study have an important bearing on the adaptability of alfalfa cultivars with respect to the capacity of seedling and other plants to escape the salinity detrimental effects. McIntosh and Miller (1980) indicated that homogenous correlation between the branching-root habit and the other agronomic traits among alfalfa cultivars were found. Several workers observed that winter-hardy alfalfa tend to have a greater degree of root branching than non-hardy cultivars (Pederson *et al.*,1984). More efforts should be directed towards investigating various mechanisms affect root distribution to tolerate salinity.

Table 4. Average herbage yield (g/plant) of four salinity levels averaged over two alfalfa cultivars

Salinity levels	Harvest no.				
	1	2	3	4	5
a	1.87	3.22	3.34	6.68	18.56
b	3.37	3.88	3.49	13.53	22.96
c	6.37	6.27	5.85	7.96	15.35
d	2.48	4.19	4.53	10.38	13.68
LSD.05	1.05	1.22	2.44	2.55	2.60
LSD.01	1.48	1.70	3.42	3.54	3.70

(a) Actual analysis of soil profile of alfalfa field irrigated with saline water for 13 years, (b) and (d) simulated deposited salts after irrigation with the saline water for 20 and 30 years; respectively, and (c) no salts added to the soil profile.

Table 5. Average root length (cm/plant) of four salinity levels averaged over two alfalfa cultivars

Salinity levels*	Harvest no.				
	1	2	3	4	5
a	114	380	380	872	1177
b	172	315	470	1017	1220
c	252	471	643	878	1092
d	143	277	401	605	720
LSD.05	94	195	290	300	350
LSD.01	132	273	406	450	460

*The same as Table 1.

Table 6. Average herbage yield (g/plant) of two alfalfa cultivars averaged over four salinity levels

Cultivar	Harvest no.				
	1	2	3	4	5
High fibrous	4.05	4.66	4.58	9.81	17.62
Low fibrous	2.99	4.12	4.03	9.15	15.95
LSD 0.05	0.79	0.93	1.87	1.00	1.05
LSD 0.01	1.09	1.29	2.58	2.00	2.09

Table 7. Average root length (cm/plant) of two alfalfa cultivars averaged over four salinity levels

Cultivar	Harvest no.				
	1	2	3	4	5
High fibrous	201	420	544	935	1161
Low fibrous	139	301	403	662	825
LSD 0.05	47	82	111	115	150
LSD 0.01	66	113	153	170	200

Table 8. Mean squares of herbage yield (HY) and root length (RL) at three harvests of two alfalfa cultivars under four salinity levels

S.O.V.	df	Harvest no.					
		1		2		3	
		HY	RL	HY	RL	HY	RL
Salinity(S)	3	39.8**	35145**	17.4**	72394*	13.4*	142425*
Error(a)	12	1.17	9309	1.56	40057	6.28	88447
Cultivar(C)	1	11.3**	38495**	2.92*	142205**	3.0	198781*
SxC	3	1.8*	2772	1.4	6512	9.5*	11352
Error	16	1.3	5074	1.9	14913	7.7	27481

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