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Response of Plant Growth to Different Salinization in Root Zone

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ABSTRACT

General objectives in this study were to investigate differences in movement of sodium (Na) and chloride (Cl) through a soil profile, to assess the effect and relationship of salt movement or concentration in soil on plant and root growth, and to evaluate the role of ion uptake based on the relation with osmotic adjustment. It was proposed that additional salt unexpectedly was not dispersed through the soil profile during the experiment. Hence, in salinized plants, the concentration of Na decreased corresponding to an increase in salt levels, while shoot dry weight increased in all species. We noted the positive correlation between Na concentration of root and Na concentration of surrounding soil of root. Zonal salinization of soil did not affect plant calcium (Ca) and magnesium (Mg) content. This suggested that adequate accumulation of potassium (K) in shoots and Na in roots might be corresponded to osmotic adjustments in salinized plants. Large interspecific differences in root responses to a salt layer were observed.

Keywords: glycinebetaine and amino acids, ions, plant growth, root-box, zonal salinization

INTRODUCTION

Among the environmental constraints that limit crop yield in many semi-arid and arid regions, salinity of soils and irrigation water is certainly one of the most critical (Hasegawa et al., 2000). Salt-affected areas are now estimated to be 10% of the world's cropland and increasing especially in semi-arid and arid regions. Therefore, it is important to understand and to find the means to minimize the injurious effects of salinity on crop growth. Undoubtedly, soil salinity

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is the most prevalent and widespread problem limiting crop production in irrigated agriculture. Through the past six to seven decades a considerable body of information has been accumulated, which has promoted the understanding of the principles involved and helped to develop the technology for coping with the problems (Shalheve, 1994). A common problem in irrigated agriculture is the gradual buildup of salts in the root zone. Periodic leaching with low-saline water can greatly reduce the concentration of soluble salts (Laura et al., 2002). The degree of growth reduction is dependent on a variety of factors such as plant species, growth stage (Francois et al., 1989), kind and concentration of the salt in the root zone (Lacuter and Munns, 1986), and duration of the exposure to injurious concentrations of salts (Rodriguez et al., 1997). Salinity results in the exposure of roots to high concentration of ions that after absorption are readily transported into root cells. The lowering of the pressure potential accounts for the inhabitation of root extension (Rodriguez et al., 1997). Osmotic adjustment helps cells of higher plants to withstand salt stress and water deficiency by maintaining sufficient turgor for growth to proceed. Glycinebetaine is one of the compatible solutes that accumulate in the chloroplasts of certain halotolerant plants when these plants are exposed to salt (Murata and Tasaka, 1997). Glycinbetaine, amino acid, and praline are organic solutes that may function as osmotically active (osmolytes) substances in *Gramineae spp* (Rhodes and Hanson, 1993). Numerous studies of organic solute accumulation in leaves have been reported, but much less is known concerning the role of organic solutes in salinized root tissues, and no data exists from experiments with organic solutes such as glycinbetaine, praline and amino acids. Recent studies show that the reduction in growth of primary roots of maize seedlings induced by salinization of the nutrient medium with 100 mM sodium chloride (NaCl) was accompanied by reductions in the length of root tip elongation zone (Zidan et al., 1990). The purpose of this study was: i) to investigate differences movement of sodium (Na) and chloride (Cl) through the soil profile; ii) to assess the effects and relationship of either salt movement or concentration in soil on plant and root growth; and iii) to evaluate the role of ions uptake based on the relation with osmotic adjustment.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

The experiment consisted of a randomized complete block design with four replications. Treatments consisted of factorial combinations of a variety of zonal salt levels, a no salt treatment, and three different grass species. Four replications of each treatment were tested, which gave rise to total of 48 experiments units (pots). Data were subjected to an analysis of variance (ANOVA) using Statistical

Analysis System (SAS) and followed by LSD multiple range tests. Terms were considered significant at $P < 0.05$.

Plant Materials

Seeds of tall wheatgrass (*Agropyron spp.*; TW) and perennial ryegrass (*Lolium perene*; PR) were collected from a hot Persian Gulf coastal area of Iran which is dominated by saline-sodic soils. African millet (*Eleusine coracana L.*; AM) was used as a commercial plant in this experiment.

Construction of Root-Box Container System and Growth Conditions

Base on approach to the hypothesis, root-box container system was achieved with plexiglass ($H \times L \times W$: $63 \times 26 \times 14$ cm). Plants were grown in a greenhouse with 65/70% relative humidity, day / night temperatures of 28–30°C / 22–24°C and a photoperiod of 14 h at photosynthetic photon flux density of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ under high intensity incandescent light. Soil from the horizon (0–15 cm) of a loamy-sand soil belonging to the Research Station Fields, of the Agricultural Biotechnology Research of Institute, was utilized in this study. The soil was air-dried, ground, and passed through a 5-mm mesh screen and filled into containing 20 kg soil. Furthermore, the root-boxes were first filled with 10 kg up to 30 cm depth, the obtained soil moisture was at field capacity, and 1500 kg ha^{-1} calcium carbonate (CaCO_3), 200 kg ha^{-1} ammonium nitrate (NH_4NO_3), 150 kg ha^{-1} potassium phosphate (KH_2PO_4), and 80 kg ha^{-1} magnesium sulfate (MgSO_4) were added, and a pH 6.5. The rest of the root-box was filled with the same soil and 80 g of NaCl at several depths which were 5, 10, and 20 cm from the top soil as the salinity stress treatment. Six seeds of each species were sown in each root-box container system and covered with transparent plastic to prevent surface evaporation and to ensure gradual depletion of substrate moisture. The water loss was replaced by adding tap water if necessary during the experiment after daily weighing to replenish the soil to field capacity.

Soil Analysis, Plant Morphological, and Physiological Parameters

The soil column was sectioned into 5-cm portions and the roots in each section were sampled, washed, blotted, and weighed. The soil elector conductivity (EC) in root-box container system was measured with a saturation extract from the soil (70 mL: 200 g deionized water: dry soil) using an EC meter (TOA, Model CM-14P) after harvesting the plants.

Plants were grown in a plexiglass container (root-box container system) for four weeks at field capacity gravimetric soil water content. Throughout the experiment, water loss from the chambers was determined daily by weighing the container and it was replenished by adding tap water to the container to bring the soil water content to the original value of field capacity. Plants were harvested four weeks after sowing and shoot and root fresh weights were determined immediately after harvesting and the dry weight of the aboveground biomass and roots were determined after the plants were dried at 65°C for 48 h.

At the end of each experiment, harvested shoots and roots were oven-dried at 70°C for at least 48 h and dry masses determined. For elemental determination of shoot and root concentrations the dried samples were ground using a Wiley mill and the plant materials were digested with acid. Acid extraction of plant samples was provided from a mixture of 0.1 N nitric acid (HNO₃) and 1.3 N acetic acid. Therefore, sodium (Na) and potassium (K) were analyzed using a flame photometer. In addition, calcium (Ca) and magnesium (Mg) were determined using atomic absorption spectrophotometry. The nutrient content was calculated by multiplying the mineral concentrations by the dry masses of shoots and roots.

Determination of Glycinbetaine and Amino Acids

The glycinbetaine and amino acids contents of the plant tissues were extracted using the methanol extraction method of Rhodes and Rich (1988). Thinly cut plant samples were placed in a vial containing 10 mL methanol. After at least 48 h of storage at 4°C, chloroform and H₂O were added to give a final ratio of 10:5:6 (Methanol: chloroform: H₂O, v/v/v). The upper aqueous phase was then removed and concentrated to dryness under a stream of dry air. Each sample was dissolved in 2 mL H₂O and applied to a 1-cm column of Dowex-1-OH⁻¹ resin, and betaines were eluted with 6 mL H₂O. This aqueous eluent was then applied to a 1-cm column of Dowex-50-H⁺. The columns were washed with 6 mL H₂O and betaines eluted with 6 mL of 6 N ammonium hydroxide (NH₄OH). The latter fraction was then concentrated to dryness under a stream of dry air. Samples were dissolved in 1 mL H₂O and 10 μL injections were used for glycinbetaine determination by HPLC using a TSK-GEL Amide column (4.6 mm × 25 cm, Tosoh). Monitoring was done at 200 nm using UV-Vis detector (Shimadzu SPAD-10A).

The Amino acids were eluted from the Dowex-1-OH⁻ resin column with 6 ml 2.5 N hydrochloric acid (HCl). The eluent was concentrated to dryness, redissolved in 2 mL H₂O, and applied to a 1-cm Dowex-50-H⁺ columns. The columns were washed with 8 mL H₂O, and amino acids were eluted with 6 mL of 6 M NH₄OH. The column was washed and the eluted was concentrated to dryness under pressure. The residue was then dissolved in 50 μL ethyl acetate: acetic anhydride (1:1, v/v). Routinely, 1 μL injection was utilized for the

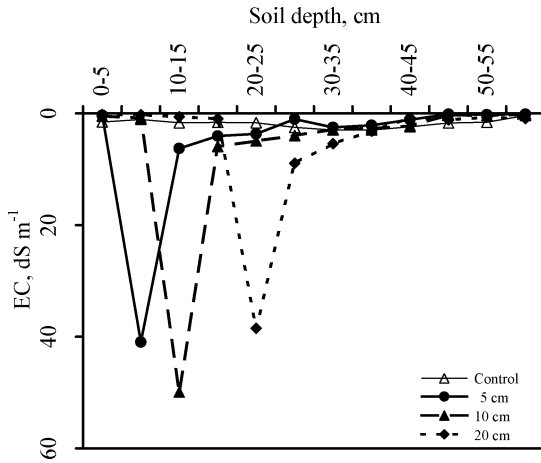


Figure 1. Salt distribution under different salt placements.

determination of the individual amino acids by gas chromatography (Shimadzu model GC-14A).

RESULTS

Salt Movement in the Soil Column System

The electrical conductivities (EC) of the different levels of soil were not significantly different through the experiment (Fig. 1). This observation indicated that placed salt was not distributed through. The soil EC was 41, 50, and 40 dS m⁻¹ at depths of 5, 10, and 20 cm of the soil column, respectively.

Effect of Zonal Salinization on Plant Morphology

Under different treatments of zonal salinization, no nutrient deficiencies were observed. But different treatments significantly affected the accumulation of dry matter in salinized plants under the same circumstances. Thereby, a positive correlation appeared between depth of salt placement and plant growth inhibition (Table 1). The growth of AM was slightly less than control plants, which showed about 3% at the 5 cm depth of salt placement in the experimental soil. Whereas, TW was the most tolerant to all of the treatments of zonal salinization. In this experiment, data demonstrated a big response of shoot growth to salt, which achieved about 51% reduction as compared to control plants.

Table 1

Dry weight of leaves and roots of grass species in relation to zonal salinization of the root system of three forage crops

Grass species	Salt placement depth (cm)	Shoot dry weight (g pot ⁻¹)	% of control	Root dry weight (g pot ⁻¹)	% of control
T. wheatgrass	5	0.86 ± 0.18	49	0.31 ± 0.02	84
	10	1.29 ± 0.18	74	0.50 ± 0.02	134
	20	1.67 ± 0.18	96	0.45 ± 0.02	121
	Control	1.74 ± 0.18	100	0.37 ± 0.02	100
P. ryegrass	5	0.08 ± 0.08	19	0.05 ± 0.04	36
	10	0.40 ± 0.08	93	0.24 ± 0.04	171
	20	0.80 ± 0.08	186	0.22 ± 0.04	154
	Control	0.43 ± 0.08	100	0.14 ± 0.04	100
A. millet	5	0.64 ± 0.63	3	0.11 ± 0.15	2
	10	9.02 ± 0.63	37	0.80 ± 0.15	18
	20	13.80 ± 0.63	56	2.24 ± 0.15	50
	Control	24.70 ± 0.63	100	4.50 ± 0.15	100

Each value is the mean of 2 replicates ± SE.

Hence, reduction in root growth in salinized plants showed a similar pattern of decreasing shoot dry weight as well.

A 50% reduction in root dry weight of AM at the 20-cm depth of salt placement was observed. However, an increase in the root dry weight was found in TW and PR between 21–54%.

In this study, root length extension of different species of grass showed better development in TW than AM and PR (Fig. 2). The results also indicated no significant effect of placed salt on the root development of TW. No remarkable root extension of PR in the horizontal layer of soil was noted.

Effect of Zonal Salinization Ions and Organic Solute Accumulation

The observation of ion accumulation in salinized plants is shown in Table 2. The results defined high shoot Na content and subsequent ion accumulation in salinized plants grown in treatments with a 5-cm salt placement. Likewise, a high content of K was accumulated in the same plants. There was no influence of root salinization on Ca²⁺ and Mg²⁺ accumulation in the three grass species in terms of no significantly different data.

Accumulation of ions in roots of salinized plants is shown in Table 3. Consequently, it indicated that salinized root response to accumulate K and subsequent ions in AM was kept constant with the increasing salt depth treatments. Whereas, K accumulation in PR was somewhat increased from the 5 to 10 cm salt placement depth (107.8 to 110.1, respectively) and then decreasing

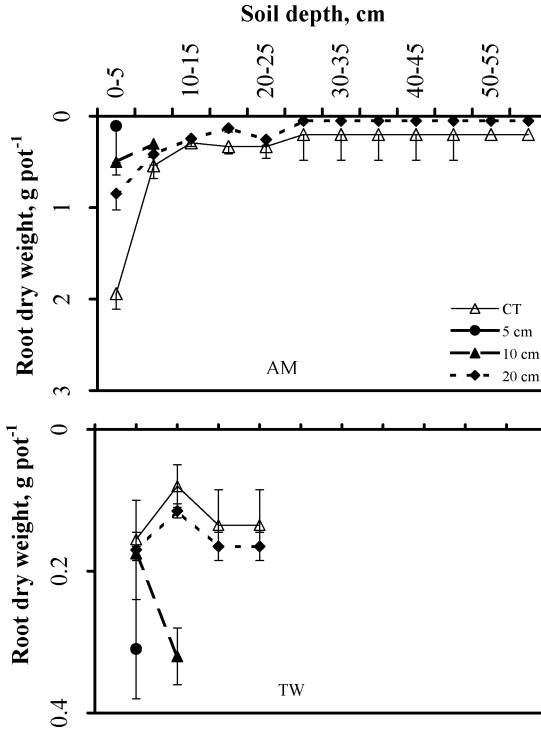


Figure 2. Root dry weight of tall wheatgrass (TW) and African millet (AM) along 5-cm sections of the root system in relation to zonal salinization. The \pm SD is shown by the vertical bars ($n = 2$).

at the 20 cm depth (27.5), and K accumulation in TW significantly decreased from 107.0 (5 cm) to 74.0 (10 cm), respectively.

With regard to concentration of Na in root, higher accumulation of K was determined in the roots of all salinized plants. Consequently, in salinized plants no significant difference was observed in ions such as Ca and Mg in shoot when compared to control plants.

A view of Na, K, and glycinebetaine distribution in roots of salinized plants in Fig. 3 indicated a negative correlation between accumulation of Na and glycinebetaine in root and salt depth placement. However, K concentration in root was kept constant under the same circumstances.

In this experiment, arising of osmoticum (total osmotic concentration) based on synthesis of either organic (glycinebetaine and amino acids) or inorganic (Na, K, Ca and Mg) solutes confirmed the assumption of their activities and uniform distribution throughout all tissue compartments. Potassium is one of the major contributors to osmoticum in shoots of salinized grass. Furthermore, Na, Ca, Mg, and organic solutes all contribute to the osmotic adjustment, along with K.

Table 2
 Inorganic and organic solute concentrations in leaves of grass species as affected by salinization of the root system of three forage crops

Species	Salt placement depth (cm)	Inorganic solute conc.					Organic solute conc.		
		Na	K	Ca	Mg	Amino acids	Glycinebetaine		
T. wheatgrass	5	126.4 ± 2.0	239.0 ± 8.5	55.7 ± 2.5	7.0 ± 0.9	7.9 ± 1.1	24.8 ± 0.5		
	10	30.9 ± 2.0	353.6 ± 8.5	62.6 ± 2.5	12.0 ± 0.9	9.2 ± 1.1	30.9 ± 0.5		
	20	13.9 ± 2.0	354.0 ± 8.5	48.5 ± 2.5	13.2 ± 0.9	9.7 ± 1.1	13.9 ± 0.5		
Control		14.9 ± 2.0	354.7 ± 8.5	43.2 ± 2.5	15.2 ± 0.9	12.3 ± 1.1	9.2 ± 0.5		
P. ryegrass	5	69.7 ± 3.3	194.7 ± 4.3	89.3 ± 1.9	13.8 ± 0.5	5.1 ± 1.4	3.4 ± 0.2		
	10	118.6 ± 3.3	260.7 ± 4.3	77.3 ± 1.9	25.4 ± 0.5	12.4 ± 1.4	3.4 ± 0.2		
	20	29.3 ± 3.3	481.0 ± 4.3	54.2 ± 1.9	24.2 ± 0.5	12.6 ± 1.4	0.9 ± 0.2		
Control		26.8 ± 3.3	258.0 ± 4.3	60.0 ± 1.9	25.4 ± 0.5	19.4 ± 1.4	0.9 ± 0.2		
A. millet	5	138.9 ± 1.5	225.0 ± 2.6	86.7 ± 0.9	32.5 ± 0.8	24.7 ± 0.7	9.6 ± 0.9		
	10	24.0 ± 1.5	244.0 ± 2.6	75.3 ± 0.9	31.4 ± 0.8	13.6 ± 0.7	7.6 ± 0.9		
	20	38.4 ± 1.5	337.0 ± 2.6	88.7 ± 0.9	46.5 ± 0.8	31.6 ± 0.7	8.4 ± 0.9		
Control		4.0 ± 1.5	187.9 ± 2.6	59.1 ± 0.9	43.1 ± 0.8	19.7 ± 0.7	0.3 ± 0.9		

Each value is the mean of 2 replicates ± SE.

Table 3
Inorganic and organic solute concentrations of root of the grass species as affected by salinization of the root system of three forage crops

Species	Salt placement depth (cm)	Inorganic solute conc.				Organic solute conc.		
		Na	K	Ca	Mg	Amino acids	Glycinebetaine	Glycinebetaine
T. wheatgrass	5	396.0 ± 3.1	107.0 ± 1.9	83.4 ± 0.7	22.1 ± 0.6	8.3 ± 0.6	1.50 ± 0.5	
	10	83.1 ± 3.1	74.0 ± 1.9	32.5 ± 0.7	14.0 ± 0.6	8.0 ± 0.6	4.22 ± 0.5	
	20	34.2 ± 3.1	52.2 ± 1.9	42.0 ± 0.7	8.7 ± 0.6	4.6 ± 0.6	4.10 ± 0.5	
	Control	16.0 ± 3.1	47.1 ± 1.9	25.0 ± 0.7	7.2 ± 0.6	2.9 ± 0.6	0.83 ± 0.5	
P. ryegrass	5	490.7 ± 10.1	107.8 ± 2.7	24.5 ± 2.3	9.8 ± 0.8	ND	ND	
	10	168.2 ± 10.1	110.1 ± 2.7	38.5 ± 2.3	10.5 ± 0.8	6.4 ± 0.1	ND	
	20	19.6 ± 10.1	27.5 ± 2.7	32.4 ± 2.3	8.2 ± 0.8	2.8 ± 0.1	ND	
	Control	21.4 ± 10.1	45.3 ± 2.7	38.4 ± 2.3	6.7 ± 0.8	2.5 ± 0.1	ND	
A. millet	5	96.0 ± 1.7	38.4 ± 1.0	31.9 ± 1.4	14.3 ± 0.6	4.0 ± 0.5	0.20 ± 0.1	
	10	65.2 ± 1.7	35.4 ± 1.0	22.5 ± 1.4	17.6 ± 0.6	4.0 ± 0.5	0.80 ± 0.1	
	20	44.7 ± 1.7	25.0 ± 1.0	24.9 ± 1.4	11.4 ± 0.6	4.3 ± 0.5	0.72 ± 0.1	
	Control	20.7 ± 1.7	40.5 ± 1.0	25.9 ± 1.4	11.5 ± 0.6	6.5 ± 0.5	0.33 ± 0.1	

Each value is the mean of 2 replicates ± SE. ND; not determined.

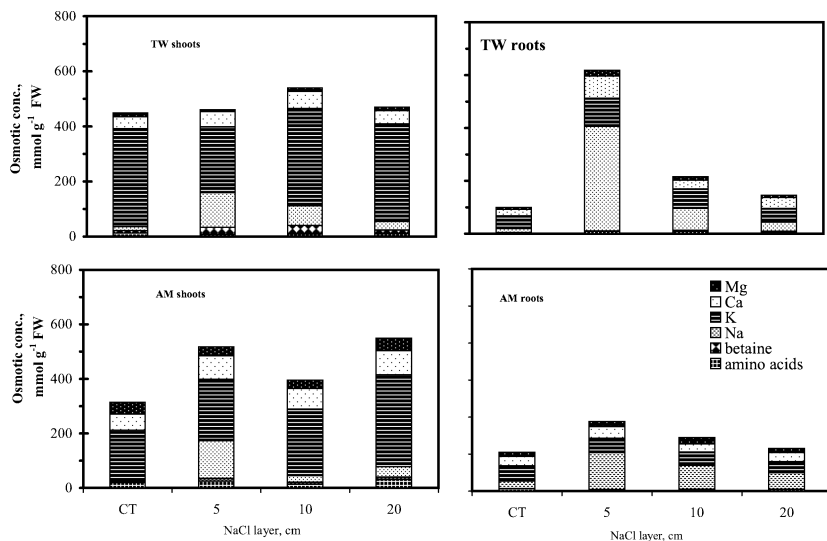


Figure 3. Contribution of inorganic and organic solutes to osmotic adjustment in relation to salinization of root system of African millet (AM) and tall wheatgrass (TW).

Finding results of osmolarity solutes showed that either Na⁺ or both glycine-betaine and amino acids contribute to osmotic adjustment in roots of TW with about 77% and 6% of osmoticum, respectively.

DISCUSSION

A common problem in irrigated agriculture is the gradual buildup of salts in the root zone. Periodic leaching with low-saline water can greatly reduce the concentration of soluble salts, but may have undesirable consequences for users of downstream drainage water. One strategy for reducing downstream impacts of low quality water is to forego leaching and store salt in the lower portion of the root zone. A root zone of heterogeneous salinity develops under irrigation without leaching, and yield reductions occur as salt accumulates first in the lower then the upper root zone, which is particularly salt sensitive (van Schilfgaarde et al., 1974; Jame et al., 1984; Smith, 1993).

In this experiment, salt placed at 5-cm depth caused about 50–97% reduction in shoot growth of all the three grass species, suggesting that salt distribution in soil profiles is one of the important factors affecting crop growth in saline areas. Also, the top portion of the system was considerably more salt sensitive than either the 10 or 20-cm profiles. The 5-cm horizon is the initial root zone that young roots encounter even though they are not yet able to withstand salt stress. As the salt placement depth increased, the roots had more salt-free

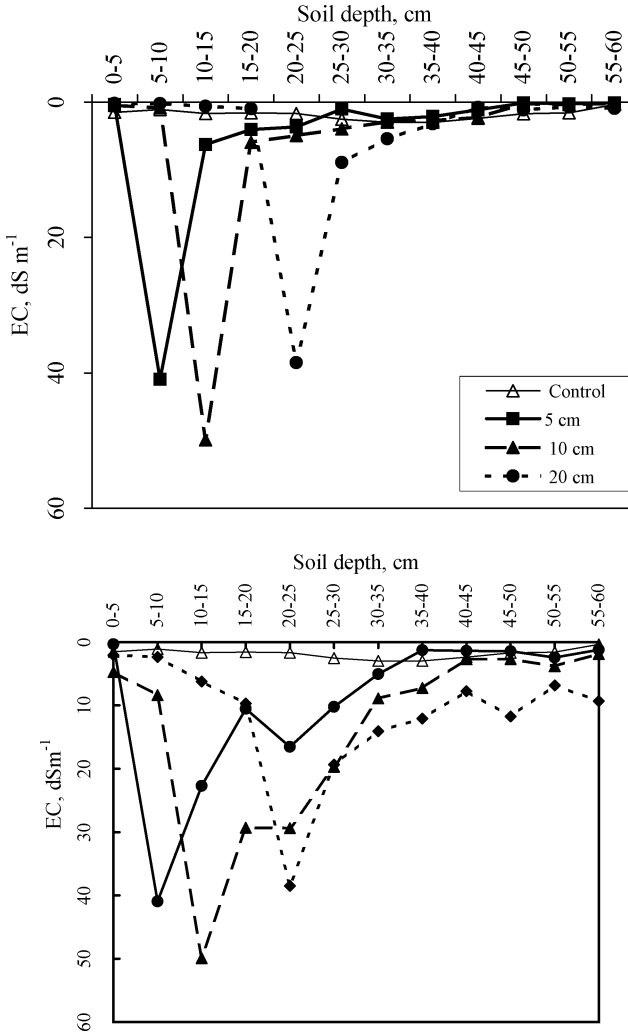


Figure 4. Salt distribution under different salt placements.

medium for initial growth and development with little damage. The observed growth differences might be due to the salinization treatment or the inherent differences in root activity along the root system depending upon the horizon under attention. It could be also the result of salt toxicity or nutritional imbalance due to accumulated ions (Pessaraki et al., 1991). The difference between these factors may be little; however, more observations are needed to provide a firm basis for the effect of root activity. Increasing root growth under salinity has been reported for Bermudagrass (Ackerson and Younger, 1975; Marcum

and Murdoch, 1990). The top portion of the root systems of alfalfa was found to be most sensitive to salt (Bernstein and Francois, 1973). These may be adaptations to salinity, leading to more efficient water and nutrient uptake by the high root production (Gorham et al., 1985).

A large interspecific difference in plant response to zonal salinization was observed. Tall wheatgrass was able to withstand high salinity throughout the soil profile. Root growth even increased in the 10 and 20 cm salt placement depths, but African millet was very sensitive to salinization of the root zone. The roots of perennial ryegrass did not proliferate throughout the horizons, but it showed a relatively high root dry weight at 10 and 20-cm salt placements depths. Tall wheatgrass and presumably other plants are able to withstand substantial salinization of the root zone provided a portion of the root zone is free from excessive salts. This suggests the possibility of mechanism to overcome the injurious effects of salts at early seedling stage (Khosh Kholgh Sima et al., 1997). The response all the plants to zonal salinization is linked to maintenance of high Na and K content in the root. It is interesting to note the high accumulation of Na in the root parts exposed to NaCl in all the three species, suggesting the exclusion of Na from the other parts of the roots. By concentrating Na and K ions in the exposed parts of the root system, osmotic adjustment and maintenance of shoot osmoticum became possible, leading to better growth and development. Jeschke and Wolf (1988) reported that root apical zones accumulate high levels of cations via the phloem. Na and K were found to have accumulated in the same zone (Rodriguez et al., 1997).

The results of this study clearly show that glycinebetaine and amino acids frequently do not accumulate at high levels enough to be acting as true osmolytes. Osmotic adjustment was achieved rather by accumulation of K and Na. Tall wheatgrass and African millet were able to respond to zonal salinization by accumulating high amounts of Na and K. Together they accounted for 29–52% of osmoticum in the shoots of tall wheatgrass at 5-cm salt depth while, Na accounted for 64% in the root. Selection for K in shoot was much greater than in root. The selection for K might have prevented the entry of Na into leaves (Abbas et al., 1991).

The present studies were carried out under glasshouse conditions, yet they provided several interesting observations that are relevant to field conditions. For example, provided a substantial portion of the root system can grow and proliferate in relatively salt-free zone, a crop with extensive rooting habit may tolerate high salinity levels that will otherwise inhibit normal plant growth. Higher tolerance to salinity as characterized by the rapid and extensive root growth is an important factor that should be considered when selecting plant genotypes for establishment on saline areas. Likewise, under conditions favoring capillary rise and accumulation of salt in the different horizons, the salt distribution in the soil profiles can be one of the most influential factors affecting the crop growth in salt-affected areas. Downward movement of salts deeper than 20 cm may be an effective way to reduce injuries to many plants caused by

higher salinity stress. Additional studies are needed with other plant species to establish the general response of plants to variable salinity throughout the root zone. The observation in this study also showed that contribution of organic osmolytes was less than inorganic osmolytes in adjustment of osmolarity in salinized grass. The results allow authors to give an opinion that K, Ca, and Mg as inorganic elements and amino acid and glycinebetaine for inorganic substance can be classified for the most pioneer parameters to osmotic adjustments of salinized plants.

However, finally our suggestion in this evaluated study is to concerning on three strategies which are involved to minimize yield reduction under saline conditions: i) control of zone salinity; ii) reduced damage to the crop; and iii) reduced damage to individual plants.

CONCLUSIONS

The present studies were carried out under glasshouse conditions, yet they provided several interesting observations that are relevant to field conditions. For example, provided a substantial portion of the root system can growth and proliferate in relatively salt-free zone, a crop with extensive rooting habit may tolerate high salinity levels that will otherwise inhibit normal plant growth. Higher tolerance to salinity as characterized by the rapid and extensive root growth is an important factor that should be considered when selecting plant genotypes for establishment on saline areas. Likewise, under conditions favoring capillary rise and accumulation of salt in the different horizons, the salt distribution in the soil profiles can be one of the most influential factors affecting the crop growth in salt-affected areas. Downward movement of salts deeper than 20 cm may be an effective way to reduce injuries to many plants caused by higher salinity stress. Additional studies are needed with other plant species to establish the general response of plants to variable salinity throughout the root zone.

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