

Effect of salinization of soil on emergence, growth and survival of *Albizzia lebbek* seedlings

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Abstract: Effect of soil salinization on emergence, growth and physiological attributes of seedlings of *Albizzia lebbek* (Linn.) Benth. (Mimosaceae) was studied. A mixture of chlorides and sulphates of Na, K, Ca and Mg was added to the soil and salinity was maintained at 4.1, 6.3, 8.2, 10.1, 12.2 and 14.1 dSm⁻¹. A negative relationship between percent seed germination and salt concentration was obtained. Seedlings did not emerge when soil salinity exceeded 12.2 dSm⁻¹. Results suggested that this tree species is salt tolerant at seed germination stage. Seedlings survived and grew up to soil salinity of 12.2 dSm⁻¹. Eventually, this species is salt tolerant at seedling stage too. Stem and root elongation were retarded by increasing salt stress. However, this species has a tendency for rapid root penetration and roots are able to extract water from very dry saline soil (6.8 % moisture). Young roots and stems were most tolerant to salt stress and were followed by leaves and old roots successively. Leaf tissues exhibited drastic reduction in dry mass production in response to increasing salt stress. However, production of young roots and death of old roots were found to be continuous and plants apparently use this process as an avoidance mechanism to remove excess ions and delay onset of ion accumulation in this tissue. This phenomenon, designated “old fine root turnover”, is of importance to the mechanisms of salt tolerance. The ability of this plant to thrive in dry regions is further conferred by the xeromorphic features of its leaves.

Resumen: Se estudió el efecto de la salinización del suelo sobre la emergencia, el crecimiento y los atributos fisiológicos de las plántulas de *Albizzia lebbek* (Linn.) Benth. (Mimosaceae). Se añadió al suelo una mezcla de cloruros y sulfatos de Na, K, Ca y Mg y se mantuvo la salinidad a 4.1, 6.3, 8.2, 10.1, 12.2 y 14.1 dSm⁻¹. Se obtuvo una relación negativa entre el porcentaje de germinación y la concentración de sal. Las plántulas no emergieron cuando la salinidad del suelo excedió de 12.2 dSm⁻¹. Los resultados sugieren que esta especie arbórea es tolerante a la sal en la etapa de germinación. Las plántulas sobrevivieron y crecieron en salinidades del suelo de hasta 12.2 dSm⁻¹. A la larga, esta especie también es tolerante a la sal en la etapa de plántula. La elongación del tallo y de las raíces se vio retardada conforme incrementó el estrés salino. Sin embargo, esta especie tiene una tendencia hacia una penetración rápida de las raíces y éstas son capaces de extraer agua de suelos salinos muy secos (6.8% humedad). Las raíces jóvenes y el tallo mostraron la mayor tolerancia al estrés por salinidad, seguidos sucesivamente por las hojas y las raíces viejas. Los tejidos foliares mostraron una reducción drástica en la producción de biomasa seca como respuesta a un incremento en el estrés por salinidad. Sin embargo, se encontró que la producción de raíces jóvenes y la muerte de raíces viejas son continuas y aparentemente las plantas usan este proceso como un mecanismo para evitar remover los iones en exceso y retrasar el inicio de acumulación de iones en este tejido. Este fenómeno, denominado “recambio de raíces finas viejas”, tiene importancia para los mecanismos de

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tolerancia a la sal. La habilidad de esta planta para prosperar en regiones secas está dada además por los rasgos xeromórficos de sus hojas.

Resumo: O efeito da salinização do solo na emergência, crescimento e atributos fisiológicos das plântulas de *Albizzia lebbek* (Linn.) Benth. (Mimosaceae) foram estudados. Uma mistura de cloretos e sulfatos de Na, K, Ca e Mg foi adicionada ao solo e a salinidade mantida em 4.1, 6.3, 8.2, 10.1, 12.2 e 14.1 dSm⁻¹. Uma relação negativa entre a percentagem de semente germinada e a concentração salina foi obtida. As plântulas não emergiram quando a salinidade do solo excede os 123.2 dSm⁻¹. Os resultados sugerem que esta espécie é tolerante ao sal no estágio de germinação da semente. As plântulas sobreviveram e cresceram até a um nível de salinidade de 12.2 dSm⁻¹. Eventualmente, esta espécie será também tolerante ao sal no estágio de nascedio. O alongamento do caule e da raiz foram retardados pelo aumento do stress salino. Contudo, esta espécie tem a tendência para uma rápida penetração das raízes no solo e as raízes são capazes de extrair água de um solo salino muito seco (6,8 % de humidade). As raízes jovens e os caules são mais tolerantes ao stress salino sendo seguidos pelas folhas e raízes velhas, sucessivamente. Os tecidos das folhas exibiram uma redução drástica de produção de massa seca em resposta ao aumento do stress salino. A produção de raízes jovens e a morte de raízes velhas foi, contudo, contínua e as plantas aparentemente usam este processo como um mecanismo de prevenção para remover o excesso de iões e atrasar o início da acumulação destes iões nestes tecidos. Este fenómeno, designado “rotatividade das raízes finas velhas”, é importante para os mecanismos de tolerância ao sal. A aptidão desta planta para vegetar nas regiões secas é convergente com as características xeromórficas das suas folhas.

Key words: Adaptation, *Albizzia lebbek*, salt tolerance, seedling emergence, seedling growth.

Introduction

Salinization of soil is a world-wide problem. It is, however, more widespread and acute in arid and semi-arid regions than in humid regions. An understanding of responses of plants to salinity is of great practical significance as high concentrations of salts have detrimental effects on plant growth (Bernstein 1961; Garg & Gupta 1997; Kramer 1983; Mer *et al.* 2000) and excessive concentrations kill growing plants (Donahue *et al.* 1983). Many investigators have reported retardation of germination and growth of seedlings at high salinity (Ayers & Hayward 1948; Berstein 1962; Garg & Gupta 1997). However, plant species differ in their sensitivity or tolerance to salts (Brady & Weil 1996; Troech & Thompson 1993). There are many different types of salts and almost an equally diverse set of mechanisms of avoidance or tolerance. It is found that soil

salinity suppresses shoot growth more than the root growth (Garg & Gupta 1997; Ramoliya & Pandey 2003). However, fewer studies on the effect of soil salinity on root growth have been conducted (Garg & Gupta 1997). The effect of high soil salt concentrations on plant growth is primarily through soil solution. Therefore, it is expected that dry soil may affect plant growth more than wet soil. Also, occurrence of frequent droughts is almost a regular phenomenon in saline deserts. Eventually, responses of roots and shoots of plants to soil salinity need to be understood, especially, under wet and dry soil conditions. The knowledge acquired regarding the growth and survival of plants under natural conditions could be used for screening of plant species for the afforestation of saline deserts.

Albizzia lebbek (Linn.) Benth. (Mimosaceae) is one of the dominant tree species in the vast area of Kutch (north-west saline desert) of Gujarat in India. It also grows successfully in

alternate days. Emergence of seedlings was recorded every day, over a period of forty days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity using the expression: $\text{Sin}^{-1}\sqrt{P} = \beta_0 + \beta_1 X$, where, $\text{Sin}^{-1}\sqrt{P}$ is cumulative proportion of seed germination, X is soil salinity and β_0 and β_1 are constants. Salt concentration at which seed germination was reduced to 50% (SG_{50}) was determined using the model.

Seedling growth

Seedlings of *A. lebbek* were grown in Petri dishes from medium sized seeds collected at Naliya in Kutch. Soil of each concentration of salt was filled in forty open-bottomed PVC cylinders (10 cm diameter and 10 cm depth) and bulk density of soil was maintained at 1 g cm^{-3} . Thereafter, tap water was added to the soils up to field capacity (sufficient water to initiate drainage). A high mortality of seedlings was expected with increase of salinity, so single seedlings (with root length varying from 0.5-1.5 cm) on 24 June 2000 were planted on soil of 4.1 dSm^{-1} , two seedlings each on soils of 6.3 and 8.2 dSm^{-1} and three seedlings each on soils of 10.1 , 12.2 and 14.1 dSm^{-1} salinity filled in cylinders. The bottom of each cylinder was fixed with a wire-net so that roots can easily pass through. Cylinders were kept over Petri dishes to enable collection of leachate caused by watering which were returned to the soils. Cylinders with seedlings were kept inside the cage for 20 days to allow establishment of seedlings. During this period the cylinders were watered on alternate days. The mean maximum temperature of the cage during seedling establishment phase was about $32.1 \pm 0.2^\circ\text{C}$. About 96%, 82%, 68%, 48%, 38% and 26% seedlings survived at 4.1 , 6.3 , 8.2 , 10.1 , 12.2 and 14.1 dSm^{-1} salinity, respectively. Emergence of the second leaf occurred 6 days after the transplantation of seedlings on soils with 4.1 , 6.3 and 8.2 dSm^{-1} salinity. However, on soils with 10.1 , 12.2 and 14.1 dSm^{-1} salinity the second leaf emerged after 9 days. Growth experiment was not conducted on seedlings grown in soil at 14.1 dSm^{-1} conductivity because seedlings were remarkably weak and also seeds did not germinate in soil where salinity exceeded 12.2 dSm^{-1} . Seedlings were

thinned such that only one was allowed to grow after full emergence of the second leaf. Soil was last watered on 13 July 2000.

Thirty seedlings in each soil salinity treatment were further selected for two water treatments. Fifteen seedlings were grown in soil at field capacity (22.4% moisture content) and 15 in soil at 10% water content. On 14 July 2000 each cylinder was placed on top of an identical cylinder filled with soil at similar concentration of salt and maintained at either field capacity or at 10% water content. The junction of upper and lower cylinders was sealed with waterproof adhesive tape. The soil surface in the upper cylinder was covered with an aluminum foil to prevent evaporation loss and both the cylinders together were wrapped with polyethylene sheet. Fifteen replicates for each of the two water treatments, factorialized with 5 grades of soil (4.1 , 6.3 , 8.2 , 10.1 and 12.2 dSm^{-1}) were prepared, giving a total of 150 cylinders, which were arranged in 15 randomized blocks.

Forty-five days after the cessation of watering about 40-50% seedlings on 12.2 dSm^{-1} conductivity and under drier water treatment began to wilt and the experiment was terminated. Plants were washed to retrieve the root system from soil. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Dry weights of leaves, stems and roots in upper and lower cylinders were determined together with residual water content of soil. Data were analyzed by two-way ANOVA to assess the effect of water treatment and salinity on plant growth. Salt concentration at which dry weight of stem, upper root and lower root components of seedlings was reduced to 50% (DW_{50}) was determined by fitting a straight line relationship between the response and salt concentration.

Physiological attributes

Additional seedlings grown on soils with 4.1 and 12.2 dSm^{-1} conductivity and under field capacity treatment were used to determine certain physiological attributes. Fifteen days before the termination of the experiment water loss during 24 h through transpiration was

determined. For transpiration measurement, eight plants (4 plants grown in soil with 4.1 dSm⁻¹ and 4 in soil at 12.2 dSm⁻¹ conductivity) were washed to obtain intact root systems. A cotton plug was fitted to each plant around the stem, and the plant was then inserted into a conical flask filled with a measured volume of water so that root system remained deeply immersed, and the mouth of the flask was sealed. The conical flasks containing plants were covered with black cloth and placed inside the cage. After 24 h, the volume of water in the flask was measured. The difference in volume between the two measurements was used to determine water loss through transpiration. Details of the method are given by Ramoliya & Pandey (2003).

Relative water content (RWC) of leaves was determined following Barrs & Weatherley (1962). The leaves were detached from the plants at about 10 a.m. Twenty leaflets of bipinnately compound leaves were taken and their fresh weight was measured. Weighed leaflets were then placed in water for 4 hours at 4°C, after which these leaflets were carefully blotted to remove surface water and turgid weight of leaflets were taken to make calculation for water uptake. Dry weight of the leaflets was determined by drying the tissues at 80°C to constant weight. Fresh weight (FW), water uptake (turgid weight, TW) and dry weight (DW) data of leaflets were used for the

determination of relative water content (RWC):

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

For the stomatal study, a collodion solution was applied to the upper and lower leaf surfaces at mid-day and then the dry films were removed and the stomatal number and size of the stomatal aperture were determined under the microscope.

Results

Seedling emergence

Seedlings began to emerge 2 days after sowing and 90% seed germination was obtained over a period of 15 days under control (4.1 dSm⁻¹) (Fig. 1). Seedling emergence in saline soils was recorded 4-6 day after sowing. Emergence lasted for 15, 14, 12 and 11 days in soils with 6.3, 8.2, 10.1 and 12.2 dSm⁻¹ salinities, respectively, and corresponding seed germination was 80%, 64%, 44% and 22%. Seedlings did not emerge on soils with further increase in salinity. There was a significant reduction in seed germination ($P < 0.01$) with increasing salt stress. A negative relationship between percentage seed germination and concentration of salt was obtained according to the following expression: $Y = 98.8 - 5.7 X$, ($r^2 = 0.992$, $P < 0.01$), where, Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Stem and root elongation and leaf expansion

The drier treatment significantly reduced ($P < 0.01$) shoot height, leaf area and root length of seedlings (Fig. 2). Increasing concentration of salt in soil also retarded ($P < 0.01$) elongation of stem and root of seedlings. However, the effect of salt was more pronounced under drier treatment. There was a negative linear relationship between shoot height and increasing salt concentration ($r^2 = 0.501$ and 0.512 , $P < 0.01$, for moist and drier treatments, respectively). A negative linear relationship was also obtained between root length and salt concentration ($r^2 = 0.544$ and 0.565 , $P < 0.01$, for moist and drier treatments, respectively). Nevertheless, roots penetrated the 10 cm thick column of dry and saline subsoils (soils contained in lower cylinders) to their full depth

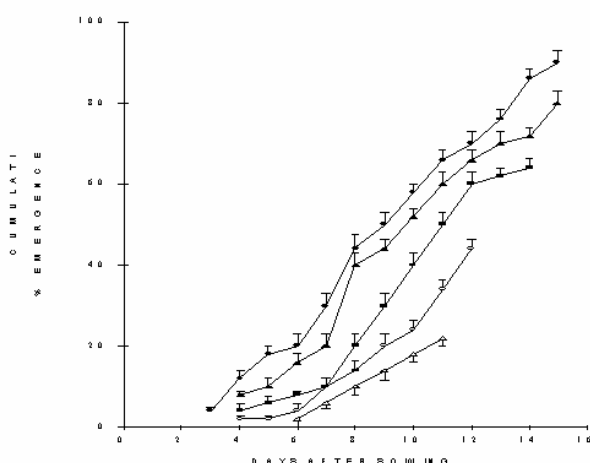


Fig. 1. Cumulative emergence of seedlings of *Albizzia lebbek* in response to soil salinity. 4.1 dS m⁻¹ (●), 6.3 dSm⁻¹ (▲), 8.2 dSm⁻¹ (■), 10.1 dSm⁻¹ (○), 12.2 dSm⁻¹ (△). Bars on symbols represent the SE.

or to a considerable depth. Seedlings began to

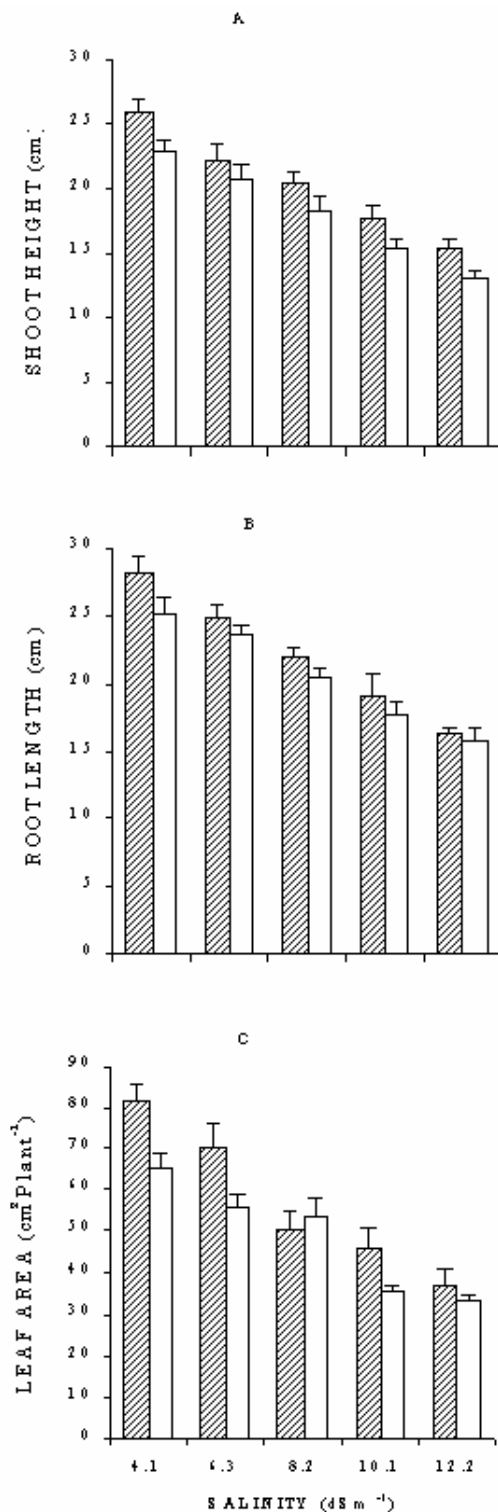


Fig. 2. Effect of salinization of soil under field capacity (■) and drier (□) (10% soil moisture) water treatments on elongation of A. shoot; B. root and C. expansion of leaf of *Albizzia lebbek* seedlings. Line bars on histogram bars represent the S.E.

wilt when soil with 12.2 dSm⁻¹ conductivity in the upper cylinders above the dry subsoil dried to 6.8%, below the permanent wilting percentage (values for residual soil moisture are not shown). As a result, it appears that *A. lebbek* extracts water from highly dry saline soil. Leaf emergence was delayed by increasing salt stress. Further, leaf expansion was significantly reduced ($P < 0.01$) by increasing concentration of salt under both moist and drier treatments, but effect was more pronounced with drier soil. A negative relationship was obtained between leaf area and salt concentration ($r^2 = 0.412$ and 0.467 , $P < 0.01$, under moist and drier treatments, respectively).

Dry weight

Dry weight of leaf, stem, shoot, upper root, lower root and total root of seedlings significantly decreased ($P < 0.01$) in response to drier treatment and increasing concentration of salt (Fig. 3). A negative relationship was obtained between dry weight of different tissues and salt concentration ($r^2 = 0.261$, 0.321 , 0.521 , 0.503 and 0.542 , $P < 0.01$, for leaf, stem, shoot, upper root and total root, respectively) under moist treatment and ($r^2 = 0.504$, 0.363 , 0.654 , 0.522 and 0.555 , $P < 0.01$, for leaf, stem, shoot, upper root and total root, respect-

ively) under drier soil. However, root dry weight in lower cylinders did not exhibit a significant negative relationship with increasing concentration of salt under both water treatments.

Percentage relative weight of tissues of salinized plants compared to control plants were computed as: (salinized tissues dry weight/control dry weight) \times 100. Dry weight values shown in Fig. 3 were used for the calculation of percentage relative weight of tissues. Values of percentage relative weight varied from 95 to 72.5 for young (lower) roots, from 92.9 to 67.2 for stem, from 89.2 to 64.7 for leaf and from 88.5 to 56.2 for old (upper) roots under moist treatment, whereas these values varied from 91.5 to 63.7 for young (lower) roots, from 86.9 to 58.6 for stem, from 83.0 to 55.0 for leaf and from 80.4 to 50.1 for old (upper) roots under drier treatment in response to increasing soil salinity from 6.3 to 12.2 dSm⁻¹. The salt

concentrations in moist soil at which dry weights will be reduced to 50% of those of control plants (DW_{50}) were 15, 16, and 13 dSm^{-1} for leaf, stem and upper root tissues, respectively. Root/shoot dry weight ratio was 0.7 under control conditions and moist treatment. It did not change in response to increasing salt stress under moist and drier water treatments.

Physiological attributes

Plants on soils with 4.1 and 12.2 dSm^{-1} salinity and at field capacity moisture exhibited significant decrease ($P < 0.05$) in specific leaf area in response to increased salinity when t-test was applied (Table 2). However, stomatal number, size of stomatal aperture, transpirational loss of water and relative water content of leaf did not differ in response to increased salinity. The size of stomatal aperture was small, transpirational loss of water was low and relative water content of leaf was high.

Our earlier work (Mer *et al.* 2000) indicated that seed germination of salt tolerant barley (*Hordeum vulgare*) crop was reduced to 50% (SG_{50}) in soil with salinity at 4 dSm^{-1} , but for *A. lebbek* SG_{50} was obtained at 9.5 dSm^{-1} , which is a remarkably high salinity level. It indicates that the species is salt tolerant at seed germination phase. However, salt concentration exceeding 12.2 dSm^{-1} was detrimental to seed germination and this can be attributed to decreasing osmotic potential of the soil solution with increasing concentration of salt. It was observed that seeds began to shrink within a few days in the soil with high salt concentration and later became nonviable. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it is reported that salinity reduces protein hydration (Kramer 1983) and induces changes in the activities of many enzymes (Dubey & Rani 1990; Garg *et al.* 1993) in germinating seeds.

Discussion

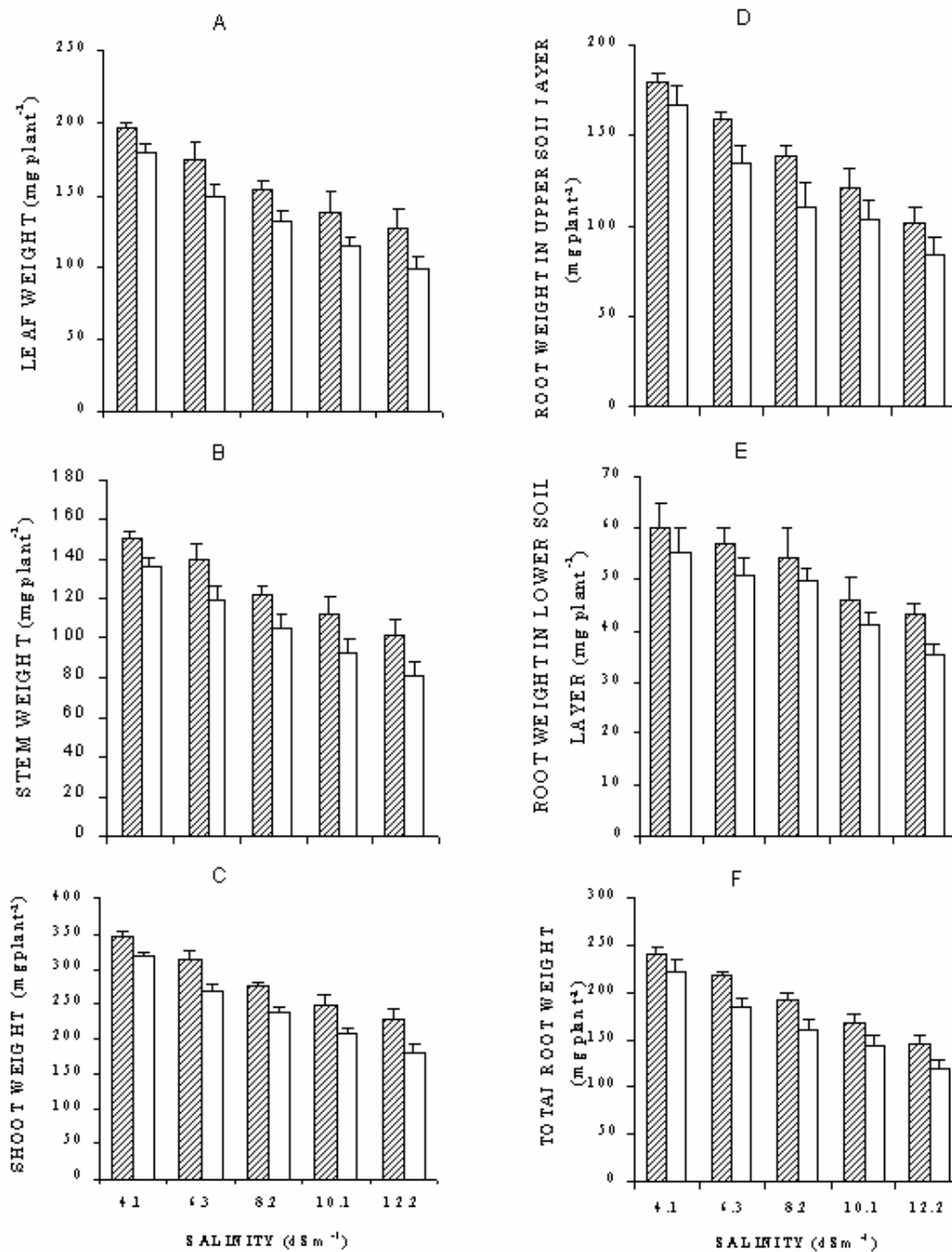


Fig. 3. Effect of salinization of soil under field capacity (■) and drier (□) (10% soil moisture) water treatments on dry weight (mg) of leaf (A), stem (B), shoot (C), root in upper soil level (D), root in lower soil level (E) and total root weight (F) of *Albizzia lebbek* seedlings. Symbol I represents LSD. Line bars on histogram bars represent the S.E.

Reduction in growth of shoot components of *A. lebbek* under drier treatment can be attributed to water stress. Kramer (1983) reported that plants subjected to water stress show a general reduction in size and dry matter

production. However, root penetration was not restricted by dry soil in the lower cylinders. In addition, greater decrease of moisture in soils contained in upper cylinders under drier treatment than under moist treatment suggests

Table 2. Effect of salinization of soil under field capacity moisture on selected traits of seedlings of *Albizia lebbek* (± 1 SE) in Kutch.

Leaf Traits	Salinity (dSm ⁻¹)	
	4.1	12.2
Specific leaf area (mm ² mg ⁻¹)	42.0 \pm 1.7	35.2 \pm 1.8
Number of stomata at upper surface of leaf (number mm ⁻²)	91.2 \pm 2.8	84.0 \pm 1.7
Number of stomata at lower surface of leaf (number mm ⁻²)	111.5 \pm 3.0	102.0 \pm 2.3
Length of stomatal aperture (μ m)	1.6 \pm 0.0	1.6 \pm 0.0
Width of stomatal aperture (μ m)	0.7 \pm 0	0.7 \pm 0.0
Water loss per unit leaf area (g dm ⁻² day ⁻¹)	25.7 \pm 0.7	24.2 \pm 0.6
Relative water content of leaves (%)	84.0 \pm 1.0	81.4 \pm 0.6

that root extension into the dry subsoil depended on the moisture content in the upper cylinders (values for soil moisture are not shown). Rapid root extension ensures the existence of plants in dry habitats (Etherington 1987; Pandey & Thakarakar 1997; Sydes & Grime 1984) and is an adaptation to survive in dry habitats. These results suggest that in dry regions where available rainfall can wet the surface soil, *A. lebbek* seedlings can utilize this moisture for the extension and proliferation of roots into the deeper layers of soil to achieve establishment during the rainy season. Our results corroborate the findings of Pandey *et al.* (1994) for the elongation and proliferation of roots of *Prosopis chilensis* seedlings in dry habitats. Root growth (upper and lower cylinders' root weight) was related with the growth of shoot and consequently root/shoot dry weight ratio was similar under the water treatments. Root/shoot dry weight ratio for *A. lebbek* (0.70) was higher than that for aridity and salt tolerant seedlings of *Prosopis chilensis* (0.40) when grown on dry soil (Pandey *et al.* 1994).

Reduction in the growth of seedlings was also recorded in response to increasing salt stress. Salinity can reduce the plant growth or damage the plants through: (i) osmotic effect (causes water deficit), (ii) toxic effects of ions and (iii) imbalance of the uptake of essential nutrients. These modes of action may operate on the cellular as well as on higher organizational levels and influence all the aspects of plant metabolism (Garg & Gupta 1997; Kramer 1983). Our results for reduction of shoot growth and leaf area development of *A. lebbek* with increasing salt concentration are in

conformity with finding of Curtis & Lauchli (1986), who reported that growth in Kenaf (*Hibiscus cannabinus*) under moderate salt stress was affected primarily through a reduction in elongation of stem and leaf area development. Garg & Gupta (1997) reported that salinity causes reduction in leaf area as well as in rate of photosynthesis which together result in reduced crop growth and yield. According to Kriedemann (1986), the early formative stages of shoots and leaves are governed by cell division, however, development of these tissues is limited by supply of assimilates. Also, high concentration of salt tends to slow down or stop root elongation (Kramer 1983) and causes reduction in root production (Garg & Gupta 1997). As a result, water stress and salt stress both reduce the plant growth and their effects are additive.

Results for dry weight and relative weight of tissues in response to increasing salinity under moist and drier treatments suggest that dry weight reduction was lowest for young (lower) roots. Salt resistance of tissues can be arranged in the following decreasing order: young root > stem > leaf > old root. The concurrent and differential reduction in dry weight of leaf, stem, old root and young root tissues resulted in constant root/shoot dry weight ratio. The rapid rate of reduction for old roots and constant root/shoot ratio suggest that production of young roots continued and production of sensitive leaf tissue was seriously reduced by increasing salt stress. The constant root/shoot dry weight ratio, rapid reduction of dry weight of old roots, and continuous production of young roots in response to increasing salinity suggest that *A. lebbek* shows old root turnover

(loss of old roots followed by subsequent production of new ones) to delay onset of salt stress by indirectly eliminating excess ions through the death of ion saturated old roots. However, in the present study dead and live roots were not quantified, the few dry and frail lateral roots were observed when the lateral roots were spread radially to measure extension of tap root. Tozlu *et al.* (2000) noted death of fine roots of *Poncirus trifoliata* in response to increasing concentration of NaCl and designated this mechanism as "fine root turnover". Moreover, since young roots and stem tissues are salt-resistant, it appears that *A. lebbek* may sequester the salts in roots and stems, thus minimizing the exposure of leaf cells and hence the photosynthetic apparatus to salt. This is a vital aspect of salt tolerance in the "integration in the whole plant" for glycophytes (Garg & Gupta 1997).

Lower specific leaf area of plants at salinity of 12.2 dSm⁻¹ compared to that of control plants indicates increase in leaf thickness in response to salt stress. Increase in leaf thickness is considered a mechanism of plants for salt tolerance (Garg & Gupta 1997). Low transpiration rate was related to small size of stomatal aperture. These characteristics confer xeromorphic features to this species. High relative water content of leaf is considered an adaptation to xeric conditions. Davidson & Reid (1989) studied the response of three *Eucalyptus* species to severe drought in summer of 1982/83 at Snug Plains, south-eastern Tasmania, Australia and reported that *E. pulchella* maintained higher relative water content of leaf and was relatively drought resistant species. Pandey *et al.* (1994) found that *Prosopis chilensis* contains high relative water content in leaf and grows successfully in dry regions of western India. Our results suggest that *A. lebbek* growing in dry habitats has experienced selection favouring water-conservative or drought-surviving adaptations.

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