EFFECT OF SALINISATION OF SOIL ON GROWTH AND NUTRIENT ACCUMULATION IN CERTAIN DOMINANT TREE SPECIES AT AND AROUND JAMNAGAR

Thesis

For The Degree Of

DOCTOR OF PHILOSOPHY

In

BIOSCIENCES (BOTANY)

Submitted by

SEEMA ABHAY HARDIKAR

Registration No. 3536, Date: 31 July 2006

To

SAURASHTRA UNIVERSITY
RAJKOT

Department of Biosciences
Saurashtra University
Rajkot 365 005

November 2008

CERTIFICATE

I have pleasure in forwarding this thesis of **Mrs. Seema Abhay Hardikar** entitled **"EFFECT OF SALINISATION OF SOIL ON GROWTH AND NUTRIENT ACCUMULATION IN CERTAIN DOMINANT TREE SPECIES AT AND AROUND JAMNAGAR"** for acceptance for the degree of Ph. D. in Botany. The results embodied in this thesis are original and have not been submitted for the award of any degree of any University.

Mrs. Seema Abhay Hardikar has put in more than five terms of research work in this department under my supervision.

Forwarded through **(Prof. A. N. Pandey)**

Guide Teacher

(Professor and Head)

Department of Biosciences,

Saurashtra University,

Rajkot.

Dedicated to
the memory of my Father
Late Somnath Deshpande
&
My loving son
Anshul

ACKNOWLEDGEMENTS

I owe to all the people who made my work possible and brought me up to this level. From the bottom of my heart I am indebted to my calm and generous, mentor and guide Dr. Amar Nath Pandey, Department of Biosciences, Saurashtra University, Rajkot for confiding in me and guiding me to complete my work successfully. His positive approach and continuous encouragement helped me throughout my work. I have great respect for him which can not be expressed in words.

My sincere thanks are to Prof. S. P. Singh, Head of the Department, and all the faculty members of the Department of Biosciences for their support.

I am running short of words to express my gratitude towards some of my well wishers, Mr. Jayvin and Mrs. Bhavna Dave, Ms. Disha, Ms. Jasmita and Mr. Ashutosh Parihar for their whole hearted support. I am thankful to Shri Sheelu bhai, Shri Rathhod bhai, Forest Department, Jamnagar and Shri Vyas bhai, Ayurved University, Jamnagar for providing me information, regarding habitats of test plants.

My hanks are due to Dr. Ashish, Ms. Pranali, Ms. Pratiksha and Ms. Neha for their help in various ways. I specially thank Jeevanbhai, caretaker of the botanical garden for his timely help.

I am deeply grateful to my husband Abhay and mother Smita, whose unconditional support was instrumental in accomplishing this task. I am delighted to thank my father-in-law Mr. Jageshwar and mother-in-law Mrs. Suman for encouraging me to go ahead with my work.

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GENERAL INTRODUCTION

Introduction

High concentrations of sodium are toxic to most plant species, making soil salinity a major abiotic stress in plant productivity world- wide. Seven percent of the land's surface and five percent of cultivated lands are affected by salinity (Flowers *et al.* 1997). The world's surface area occupies about 13.2 billion ha, but no more than 7 billion ha are arable and 1.5 billion ha are cultivated (Massoud, 1981). Of the cultivated lands about 0.34 billion ha (23%) are saline (salt-affected) and another 0.56 billion ha (37%) are sodic (sodium-affected). Thus, saline and sodic soils cover about 10% of the total arable lands and exist in over 120 countries (Tanji, 2002). Another set of database (FAO, 1989) indicates that the world has about 227 million ha of irrigated lands of which 20% are salt affected. Salinisation leads to an excessive increase of water soluble salts in soil. The accumulated salts include, sodium, potassium, magnesium and calcium, chloride, sulphate, carbonate and bicarbonate (mainly sodium chloride and sodium sulphate). A distinction can be made between primary and secondary salinisation process. Primary salinisation involves salt accumulation through natural process due to a high salt content of the parent material or in groundwater. Secondary salinisation is caused by human interactions such as inappropriate irrigation practices, e.g., with salt rich irrigation water and/or insufficient drainage. The accumulation of salts, particularly sodium salts, is one of the main physiological threats to ecosystems. According to Taiz and Zeiger (2006) high salinity can impair plant functions, growth and developmental processes. In the extreme, it can reduce plant survival. Plants can be divided in to two groups based on response to high concentrations of salts. Halophytes are native to saline soils and complete their life cycles in that environment. Glycophytes (literally "sweet plants")

or non halophytes, are not able to tolerate salts to the same degree as halophytes. Three major injurious effects of salts to plants are recognized:

Osmotic effects

Dissolved solutes in the root zone cause a low (more negative) osmotic potential that reduces the soil water potential. The general water balance of plants is thus affected, because root hydraulic conductance and transport in to cells of leaves requires development of even lower water potential to maintain a "downhill" gradient of water potentials that facilitates water movement from the soil in to leaves. This osmotic effect of dissolved solutes is similar to that of a soil water deficit and initial plant responses to excessive level of soil salinity are the same as for water deficit (Taiz and Zeiger, 2006).

Osmotic stress can occur in the leaf apoplast and this mechanism of Na^+ toxicity was first proposed by Oertli (1968). High apoplastic Na^+ concentrations can induce a flux of water from the cells, causing a decrease in turgor and an increase in concentrations of intracellular solutes (Marschner, 1995).Older leaves commonly show such damage first, as they have been around for longer and have therefore had more time to accumulate Na^+ that enters the apoplast from the xylem stream and is left behind as the water evaporates. Such osmotically driven removal of water from cells puts a strain on membranes and macromolecules, disrupts normal cellular activities and could even cause death. As water generated turgor pressure is a driving force for cell expansion, the decrease in turgor pressure is a driving force for cell expansion, the decrease in turgor could also result in reduced rates of cell expansion. Further, osmotic stress may sometimes occur in the leaf vacuoles too as Na^+ may sometimes be unsuitable as an osmoticum due to its slightly disruptive effect on the lattice structure of water around proteins (Marschner, 1995). With high concentrations of

Na^+ in the leaf apoplast and sometimes the vacuole, plant cells may encounter difficulties in maintaining low cystosolic Na^+ and high K^+: Na^+ ratio. This may bring about a different form of stress that is known as ionic stress.

Ionic stress

In saline substrates Na^+ and Cl^- are usually the dominant ions. Despite the essentiality of chloride as a micronutrient for all higher plants and of sodium as mineral nutrient for many halophytes and some C4 species, the concentrations of both ions in saline substrates by far exceed this demand and lead to toxicity in non-salt-tolerant plants. In many herbaceous crop species, grapevine, and many fruit trees growth inhibition and injury of the foliage (marginal chlorosis and necrosis on mature leaves) occur even at low levels of NaCl salinisation (Sykes, 1992; Maas, 1993). Under such conditions water deficit is not a constraint(Greenway and Munns, 1980) and, atleast in *Citrus* species, high chloride sensitivity and thus chloride toxicity is the major constraint (Maas, 1993). Deciduous woody trees such as *Tilia* and *Aesculus*, as well as coniferous trees such as *Picea omorika* also suffer from chloride toxicity in the foliage when grown on substrates high in NaCl (Alt *et al*., 1982; Mekdaschi *et al.*, 1988).

Under nonsaline conditions, the cytosol of higher –plant cells contains about 10 mM Na^+, an ionic environment in which enzymes are operationally functional. An abnormally high ratio of Na^+ and K^+ and high concentrations of total ions inactivate enzymes and inhibit protein synthesis. Enzymes isolated from halophytes are just as sensitive to the presence of NaCl as are enzymes from glycophytes. Hence halophytes do not possess salt- tolerant metabolism (Taiz and Zeiger ,2006).

At a high concentration, Na^+ can displace Ca^{2+} from the plasma membrane, resulting in a change in plasma membrane permeability that can be detected as a leakage of K^+

from the cells (Cramer *et al*., 1985). Na^+ negatively disturbs ion homeostasis, affecting plant nutrient status in a variety of ways, such as by inhibiting acquisition of the essential element K^+ both by competition for sites on transport proteins and through intracellular processes yet to be fully deciphered. Photosynthesis is inhibited when high concentrations of Na^+ and / or Cl^- accumulate in chloroplasts. Since photosynthetic electron transport appears relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected.

Nutrient imbalance

Saline soils may be characterized by low nutrient ion activities and extreme ratios of Na^+/Ca^{2+}, Na^+ / K^+, Ca^{2+} / Mg^{2+}, and Cl^- / NO_3^- in the soil solution. When glycophytes, which encompass most cultivated crops are imposed to saline conditions, nutritional disorders may develop. These disorders vary in their intensity and can differ among species as well as among cultivars within a species. Nutrient imbalance may result from the effect of salinity on nutrient availability, uptake or partitioning within the plant or may be caused by physiological inactivation of given nutrient, resulting in an increase in plant's internal requirement for that essential element (Feigin, 1985; Grattan and Grieve, 1994).

Salinity causes reduction in N and P accumulation in plants, although this effect may not always be growth limiting (Bernstien *et al*., 1974; Champagnol, 1979; Munns and Termaat, 1986; Grattan and Grieve, 1994). The decreased N and P concentrations under salt stress have been attributed to increased Cl^- uptake (Feigin, 1985). Plants grow in N^- and P^- deficient environments respond positively to addition of these elements provided the plant is not experiencing severe salt stress (Bernstein *et al*., 1974; Feigin, 1985). Levitt (1980) has concluded that a reduced uptake of a nutrient is insufficient evidence, by itself that salt injury is due to a secondary nutrient

deficiency. It may, for instance result from and osmotically produced decrease in root growth or a reduced uptake of a nutrient will occur as a result of salt induced decrease in growth. Therefore, evidence of the reduced uptake must be followed by an elimination of the salt stress injury when the nutrient supply.

It is reported that K^+ concentration in plant tissues is reduced as Na^+ salinity or Na^+ / K^+ratio in the root medium is increased(Janzen and Chang, 1987; Subbarao *et al*., 1990).The effect of Na^+ on K^+ uptake is two fold. At low concentrations, Na^+ may actually increase K^+ uptake, though decreases it at higher concentrations (Levitt, 1980). There is evidence that Na^+ can partially substitute for K^+ in many glycophytic species without affecting growth, which, however, depends upon the crop species. Despite the overwhelming data that show reduced uptake and translocation of K^+ by plant growth in high Na^+ substrate, there are few data which show that addition of K^+ to sodium dominated soils improved plant growth or yield (Bernstein *et al*., 1974; Grattan and Grieve, 1994). Sodium induced Ca^{2+} deficiencies have growth distorting effects in developing leaves. The uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation and increases in the ionic strength that reduce the activity of $Ca^{2+.}$ These combined effects are mainly responsible for reduced yield under saline or sodic conditions (Bernstein, 1975; Janzen and Chang, 1987). Nutritional imbalances in salt stressed cereals under different molar ratios of Na^+ and Ca^{2+} have demonstrated striking intergeneric differences in corn, rice, wheat, sorghum and pearl millet species (Grattan and Grieve, 1994).

Besides macro- nutrients, salinity may also lead to deficiencies or disorders in the levels of micro- nutrients such as zinc, manganese, iron and copper (Hassan *et al*., 1970; Doering *et al*., 1984; Suhayda *et al*., 1992). In saline and sodic soils, the solubility of micro- nutrients is particularly low and plants grown in these soils often

exhibit deficiencies in these elements (Page *et al*., 1990). However, the micro-nutrient concentration in plant shoots may increase, decrease or have no effect, depending upon the type of plant, tissue, salinity, micro- nutrient concentration and environmental conditions. For instance salinity increased the concentrations of Zn, Mn and Fe in barley (Suhayda *et al*., 1992) but decrease in corn (Hassan *et al*., 1970). However, the available literature indicate that a mineral deficiency or toxicity may some time contribute to salt injury but is rarely the sole cause of the injury or inhibition (Levitt, 1980; Grattan and Grieve, 1994).

Secondary effects

The above are examples of primary deleterious effects of salinity on plants; however, secondary events also inhibit plant function. These arise from disruption of cell membrane integrity and metabolism, production of toxic molecules such as reactive oxygen species and death of cells. It is not known how these primary and secondary effects of salt stress perturb cell division and expansion but these processes are substantially modulated at sublethal salt concentrations (Taiz and Zeiger, 2006).

Mechanisms of salt tolerance

Mechanisms of salt tolerance take place at three levels of organization: whole plant, cellular and molecular.

Control at the whole plant level

Physiological mechanisms conferring exclusion that operate at the cellular and whole plant level have been described in reviews (Greenway and Munns, 1980; Lauchli, 1984; Munns *et al*., 1983; Pitman 1984 and Storey and Walker, 1990) and with particular reference to selectivity for K^+ over Na^+ (Jeschke, 1984; Jeschke and Hartung, 2000). Salt tolerance depends on the ability of the plant to control the transport of salt at five sites, as summarized by Munns *et al*., (2002):

1. Selectivity of uptake by root cells.

It is still unclear which cell types control the selectivity of ions from the soil solution. The initial uptake of Na^+ and Cl^- could occur at the epidermis, at the exodermis or if soil solution flows apoplastically across the root cortex, it would occur at the endodermis.

2. Loading of the xylem.

There is evidence for a preferential loading of K^+ rather than Na^+ by the cells of stele.

3. Removal of salt from the xylem in the upper part of the roots, the stems, petiole or leaf sheaths.

In many species Na^+ is retained in the upper part of the root system and in the lower part of the shoot, indicating an exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stem and petioles.

4. Loading of the phloem.

There is little retranslocation of Na^+ or Cl^- in the phloem, particularly in the more tolerant species. This ensures that salt is not exported to growing tissues of the shoot.

5. Excretion through salt glands or bladders.

Only halophytes have well-developed mechanisms to control the uptake, transport and excretion of salts. Glycophytes rely on the first three mechanisms and exhibit these mechanisms to various degrees. Genetic variation within a given species, or between closely related species, has in most cases been identified as due to different degrees of control of salt uptake by roots, or in loading of the xylem.

There are contributory features that function to maintain low rates of salt accumulation in leaves. High shoot: root ratios and high intrinsic growth rates (Pitman, 1984), and absence of an apolastic pathway in roots (Garcia *et al*., 1997), all

will serve to reduce the rate at which salt enters the transpiration stream and accumulates in the shoot.

Control at the organelle level: ion compartmentation

There is no evidence of adaptations in enzymes to the presence of salt (reviewed by Munns *et al*., 1983), so mechanisms for the salt tolerance at the cellular level involve keeping the salt out of the cytoplasm, and sequestering it in the vacuole of the cell. That this occurs in most of the species is indicated by the high concentrations found in leaves that are still functioning normally, concentrations well over 200 mM, while it is found that these same concentrations will completely repress enzymes activity *in vitro* (Munns *et al*., 1983. Generally Na^+ starts to inhibit most enzymes at concentrations above 100 mM.The concentration at which Cl^- become toxic is less well defined, but is probably in the same range as that for Na^+. If Na^+ and Cl^- are sequestered in the vacuole of the cell, K^+ and organic solutes should accumulate in the cytoplasm and organelles to balance the osmotic pressure of the ions in the vacuole. The organic solutes that accumulate most commonly under salinity are proline and glycine betaine, although other molecules can accumulate at the lesser degrees (Hassegawa et.al. 2000).

Control at the molecular level: ion transporters

The ion channels and transporters that regulate the net movement of salt across cell membranes have been recently reviewed (Amtmann and Sanders, 1999; Blumwald, 2000; Schatchtman and Liu, 1999; Tyreman and Skerrett, 1999). The mechanisms that control Na^+ transport were summarized by Munns *et al*. (2002). There is no specific Na^+ transporter, Na^+ entry being gained by competition with other cations, in particular K^+. Na^+ could enter the cell through high affinity carriers or through low affinity channels called non selective cation channels that are strongly influenced by

Ca^{2+}. These cation channels could allow entry of large amounts of Na^+ from a highly saline soil if not adequately regulated (Amtmann and Sanders, 1999). Na^+ can effluxed from the cytoplasm through Na^+/H^+ antiporters, driven by the pH gradient across the plasmalemma (Blumwald, 2000). These transport processes all work together to control the rate of net uptake of Na^+ by a cell. Intracellular compartmentation is by a vacuolar Na^+/H^+ antiporter, driven by a pH gradient across the tonoplast (Blumwald, 2000). The transporters that maintain low Na^+ concentrations in organelles such as chloroplasts and mitochondria are not known. In some species Cl^- transport is associated with salt tolerance. Mechanisms that control Cl^- movement across membranes have been comprehensively reviewed by White and Broadley (2001).

Salinity problems in western Gujarat

The western region of Gujarat state in India can be divided in to two zones: (i) the Kutch, a northern saline desert and (ii) the Saurashtra, to the south of the Kutch. The Saurashtra zone includes a peripheral coastal area along the shore of the Arabian Sea and the central area. Intensive agriculture is restricted to the central area, which is characterized by semi arid ecoclimate. In the coastal area and saline desert of the Kutch vegetation is sparse mainly because of soil salinity and aridity. Moreover, in coastal area, salt concentration is increasing due to ingress of Arabian Sea. In addition, a considerable increase in soil salinity has been recorded in many parts of the central area and also in several other parts of Gujarat state. Groundwater at many places in Gujarat contains excess amount of salt and is not fit for drinking. Evidently, soil salinity is one of the major ecological problems of Gujarat state.

Aim

Certain tree species namely *Acacia senegal*, *Tamarindus indica*, *Thespesia populnea*, *Salvadora oleoides*, *Sapindus emarginatus* and *Cassia fistula* successfully grow and are dominant in coastal area of Jamnagar district in Saurashtra. The aim of the present study was to understand salt avoidance or salt tolerance mechanisms of these tree species which allow them to grow and survive in saline coastal area.

Objectives

The objectives of the present study were to assess the following responses of the test plants to soil salinity in order to achieve the aim:

I. Effect of soil salinity on emergence of seedlings.

II. Effect of soil salinity on shoot and root elongation.

III. Effect of soil salinity on dry weight accumulation in seedlings.

IV. Effect of soil salinity on water content of plant tissues.

V. Effect of soil salinity on water potential of plant tissues.

VI. Effect of soil salinity on proline accumulation in plant tissues.

VII. Effect of soil salinity on accumulation of macro- and micro- nutrients in plant tissues.

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Map of Gujarat state in India showing the location of the study area.
The inset is a map of India.

EXPERIMENT – 1

PUBLISHED IN

Anales de Biologia

***Acacia Senegal* (L.) Willd. (Mimosaceae)**

Growth, water status and nutrient accumulation of seedlings of *Acacia senegal* (L.) Willd. in response to soil salinity

Seema Abhay Hardikar[1] **& Amar Nath Pandey**[2]

1 Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:abhay.hardikar@sgs.com

E-mail:seemahardikar@yahoo.com

2 Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:anpandey2001@gmail.com

Short title (to be used as running head): **Effect of salinisation of soil on *Acacia senegal*.**

Correspondence

A. N. Pandey

E-mail:anpandey2001@gmail.com

Tel (O): +91-281-2586419

Tel (M): +919427495989

Fax (O): +91-281-2577633

Abstract

Greenhouse experiments were conducted to assess the effects of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Acacia senegal* (L.) Willd.(Mimosaceae). NaCl was added to the soil and salinity was maintained at 0.2, 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1}. Salinity caused reduction in water content and water potential of tissues, which resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased with increase in soil salinity. Proline content in tissues increased with increase in soil salinity. There were no effective mechanisms to control net uptake of Na and its transport to shoot tissues. Nitrogen, phosphorus, potassium and calcium content significantly decreased in tissues in response to salinity. Changes in tissues and whole-plant accumulation patterns of other elements, as well as possible mechanisms to avoid Na toxicity in this tree species in response to salinity, are discussed.

Key words: Soil salinity, seedling growth, proline content, water potential, macro- and micro-nutrients, salt tolerance.

Introduction

Saline soils are abundant in semi arid and arid regions where the amount of rainfall is insufficient for substantial leaching (Marschner 1995). Salinity is a scourge for agriculture, forestry, pasture development and other similar practices. An understanding of responses of plants to salinity is of great practical significance. High concentrations of salts have detrimental effects on plant growth (Taiz & Zeiger 2006, Ramoliya et al. 2006) and excessive concentrations kill growing plants (Garg & Gupta, 1997). There occurs retardation of germination and growth of seedlings at high salinity (Bernstein 1962, Garg & Gupta 1997, Ramoliya et al. 2006). However, plant species differ in their sensitivity or tolerance to salts (Marschner 1995). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas & Hoffman 1977, Munns 2002, Ramoliya et al. 2006).However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt–induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g. Cramer et al.1989, Maas & Grieve 1987, Ramoliya et al. 2006, Patel & Pandey 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al. 2000). An understanding of growth and survival of plants under saline habitat conditions is

needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Acacia senegal (L.) Willd.(Mimosaceae), a small deciduous tree species grows abundantly in coastal forests of Saurashtra in Gujarat State of India. It also grows successfully on marginal-saline lands of Kutch (north-west saline desert) contiguous to Saurashtra. *A. senegal,* yields commercial gum arabic. Wood is a good fuel. Leaves and pods are eaten by herbivores. The present investigation was performed with the following objectives :(i) to understand the adaptive features of *A. Senegal* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

Material and Methods

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22^0 18' N Lat, $70^0$56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6 % sand, 20.3 % silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dSm^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%,

respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier (Patel & Pandey 2007). The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid – June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395mm at Bhuj ($23^0$15' N Lat, $69^0$49' E Long) in Kutch and about 554mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June – September) and winter (November – February). The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Salinisation of soil

Surface soil was collected, air dried and passed through a 2 mm. mesh screen. Six lots of soil of 100 kg each, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 390, 700, 1070, 1275 and 1530g was then thoroughly mixed with soil of five lots, respectively to give electrical conductivities of 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1}. There was no addition of NaCl to sixth lot of soil that served as control. The electrical conductivity of control soil was 0.2dSm^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to

stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 18 August 2006. Seeds of *A. Senegal* were collected from the coastal area of Arabian Sea in Jamnagar city of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. immediately after sowing soils were watered and thereafter watering was carried out on alternate days. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity, using the expression:

$Sin^{-1}\sqrt{p} = \beta_0 + \beta_1 X$

where, $Sin^{-1}\sqrt{p}$ 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 estimated using the model.

Seedling growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 3.9 and 6.2 dSm^{-1} salinity exhibited emergence of the second leaf after 15 days whereas the second leaf on seedlings grown in 8.1, 10.0 and 11.9 dsm^{-1} appeared after 23 days.

Emergence of the second leaf confirmed the establishment of seedlings. However, only 19.6% seed germination was recorded in soil at 11.9 dSm^{-1} salinity, and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialized with five grades of soil (0.2, 3.9, 6.2, 8.1, and 10.0dSm^{-1}) were prepared. This gave a total of 100 bags, which were arranged in 20 randomized blocks. Seedlings were watered (about 250 ml water was added to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on 18 February 2007. Five plants grown in soil at 10 dSm^{-1} salinity died during the course of experiment. Seedlings contained in 15 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of different components were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of water potential and proline content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by

Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

Mineral analyses of plant materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three sub samples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper 1944). Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3: H_2SO_4:$HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

Results

Effect of salinisation on seedling emergence

Seedlings began to emerge 3 days after sowing and 84.8% seed germination was obtained over a period of 20 days, under control (0.2 dSm^{-1} salinity) conditions

(Fig.1). Seedling emergence in saline soils was also recorded 3 days after sowing. Seedling emergence lasted for 18, 19, 18, and 18 and 12 days in soils with 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1} salinities, respectively and corresponding seed germination was 62.8%, 58.4%, 50.4%, 44% and 19.6%. There was a significant reduction in seed germination ($p<0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y= 68.654 - 3.134 X$, ($R^2_{adj}=0.937$, $p<0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinisation on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p<0.01$) stem and root elongation (Table1). Nevertheless, root length was remarkably greater than shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p<0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p<0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p<0.01$).

Effect of salinisation on dry weight

Dry weight significantly decreased($p<0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p<0.01$).

Percent relative weight of tissues of salinised plants compared to those of control plants were computed as: (salinised tissue dry weight / control dry weight) ×

100. Dry weight values of tissues given in Table 1 were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 87.2 to 54% for leaves, from 78.1 to 50.2 % for stems, 77.5 to 41.3 % for tap roots and from 76 to 39.7 % for lateral roots in response to increasing soil salinity from 3.9 to 10.0dSm^{-1}. As has been estimated using regression equations given in results, the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 10.8, 9.3, 7.9 and 7.7 for leaves, stems ,tap roots and lateral root tissues, respectively. Root/shoot dry weight ratio was 1.10 under control conditions, while it was 1.05, 0.93, 0.89 and 0.87 for seedlings grown in soils at 3.9, 6.2, 8.1 and 10.0 dSm^{-1} salinities, respectively. Root / shoot dry weight ratios significantly decreased ($p< 0.01$) as soil salinity increased. There was a negative relationship between root / shoot dry weight ratio and soil salinity ($r = -0.665$, $p<0.01$).

Effect of salinisation on water content of tissues

Water content in leaves, stems, taproots and lateral root tissues significantly decreased ($p<0.01$) with increasing concentration of salt in soil (Fig. 2A). There was maximum water content in lateral roots and minimum in tap roots. Tissues according to their water content can be arranged in following decreasing order: lateral roots >leaves > stems > tap roots. There was a negative relationship between water content in different tissues and salt concentration ($r = -0.744$, -0.770, -0.872 and -0.793, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on water potential of tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p<0.01$) as soil salinity increased (Fig. 2B). Tissues according to their water potential values (low to high negative) can be arranged in the following

order: leaves > lateral roots > tap roots > stems. There was a negative relationship between water potential of tissues and salt concentration (r = -0.961, -0.906, -0.933 and -0.980, $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively). A positive relationship was obtained between water content and water potential (negative value) (r = 0.997, 0.813, 0.980 and 0.972, $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on proline content of tissues

Proline content (μ mol/g FW material) significantly increased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues with increase in soil salinity (Fig. 2C) .Tissues according to their proline content can be arranged in following decreasing order: stems > tap roots > leaves > lateral roots. There was a positive relationship between salt concentration and proline content of tissues(r = 0.949, 0.953, 0.977 and 0.954, $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content (r = -0.954, -0.829, -0.919 and -0.963, $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content (r = -0.986, -0.956, -0.998 and -0.986, $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on mineral accumulation

Potassium and sodium content and K/Na ratio

Potassium content (as mg g^{-1} dry weight) significantly decreased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a negative relationship between potassium content in tissues and

increase in salt concentration in soil ($p<0.01$). Sodium content significantly increased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress ($p<0.01$). K/Na ratio significantly decreased ($p<0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K /Na ratio in tissues and increase in salt stress ($p<0.01$).

Nitrogen, phosphorus, calcium and magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen, phosphorus, potassium and calcium content significantly decreased in leaves, stems, tap roots and lateral root tissues ($p<0.01$), as the salinity increased (Table 2). A negative relationship was obtained in N, P, K and Ca content of tissues and salt concentration ($p<0.01$). Magnesium content exhibited a significant increase ($p<0.01$) in leaves, stems, taproots and lateral root tissues in response to increase in salt stress. There was a significant positive relationship between Mg content in tissues and salt concentration in soil ($p<0.01$).

Micro –elements

There was a significant decrease in the concentration of Zn, Cu, Mn and Fe ($p<0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress (Table2) . A negative relationship was obtained between soil salinity and Zn, Cu, Mn and Fe content in tissues ($p<0.01$).

Discussion

Earlier work (Ramoliya et al. 2004), indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dSm^{-1},but for *Acacia senegal* SG_{50} was obtained at 5.9 dSm^{-1}.That would suggest that this plant species is relatively salt tolerant at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 2.0 to 5.0 dSm^{-1}. Eventually, seeds of *A. senegal* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 10.0dSm^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentration of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Slater et al. 2003) and induces changes in the activities of many enzymes (Dubey & Rani 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz & Zeiger 2006). Moreover, tap root elongation for seedlings grown in control and saline soils both was greater than that of shoot. Result suggests that this species has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington 1987) and is

considered an adaptation to survive in dry habitats. Root / shoot dry weight ratio of *A. senegal* was 1.1 under control conditions and was greater than that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya et al. 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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 (Kramer 1983, Garg & Gupta 1997). Results for reduction of shoot growth and leaf area development of *A. senegal* with increasing salt concentration are in conformity with the 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. Also high concentration of salt tends to slow down or stop root elongation (Kramer 1983) and causes reduction in root production (Garg & Gupta 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of tap roots and lateral roots while lowest in leaves. Consequently, leaves were more resistant than roots to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: leaves>stems>tap roots=lateral roots. The greater reduction in root weight than in shoot weight resulted in decreased root/shoot dry weight ratio for seedlings with increase in salinity. In principle, salt tolerance can be achieved by

salt exclusion or salt inclusion (Marschner 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration. The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ (Marschner 1995). Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *A. senegal* survived up to the soil salinity of 10 dSm^{-1} and, therefore, this tree species is moderate salt tolerant. In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates (Greenway & Munns 1980). As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway & Munns 1980). Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al., 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (Stewart & Lee, 1974). In addition, the

primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar et al. 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis (Schachtman et al. 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow et al. 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox & Guerinot 1998). In the present study, significant decrease of K^+ content in all the tissues of seedlings with increasing soil salinity suggests that Na^+ inhibited K^+ uptake. The exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salts to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues of *A. senegal* suggests that this mechanism to block Na^+ transfer to growing tissues was not effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively low accumulation of K^+ than that of Na^+. As a consequence there were no effective mechanisms to control net uptake of Na^+ on root plasma membrane and subsequently its transport to shoot tissue. The pattern of accumulation of K^+ and Na^+ in *A. senegal* conforms to group C and / or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar (Watad et al.1991, Schroeder et al. 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}. These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated (Amtmann & Sanders 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Watad et al.1991, Schroeder et al. 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis & Sanders 1994, Niu et al. 1995). The K^+ and Na^+ profiles of *A. senegal* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al. 2001). As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant.

In general, salinity reduces N accumulation in plants (Feigin 1985). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Torres & Bingham 1973, Garg & Gupta 1997). The Interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol 1979, Grattan & Grieve 1992). However it is known that P concentration is related to the rate of

photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach et al. 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e. g., in preserving membrane integrity (Rengel 1992), signaling in osmoregulation (Mansfield et al. 1990) and influencing K^+/Na^+ selectivity (Cramer et al. 1987). In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen & Chang 1987, Garg & Gupta 1997). Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates (Marschner & Cakmak 1989). In the present study increase of Mg^{2+} in tissues may be of importance for plant growth and survival in saline soils.

It is difficult to suggest mechanistic explanations of salinity influence on micro–element concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al. 2000). In the present study, it appears that salinity reduced Zn, Cu, Mn and Fe accumulation, at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox system, Mn for Photolysis of water in photosynthesis and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner 1995). In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the

subunits in the thylakoid membranes. The simultaneous decrease of Zn, Cu, Mn and Fe in leaves of *A. senegal* might limit the growth of plants. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al. 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater et al. 2003). Decrease in Cu, Zn, Mn and Fe content at the whole plant level might affect the survival of *A. senegal* in saline soil where salinity exceeded 10.0dSm^{-1}.

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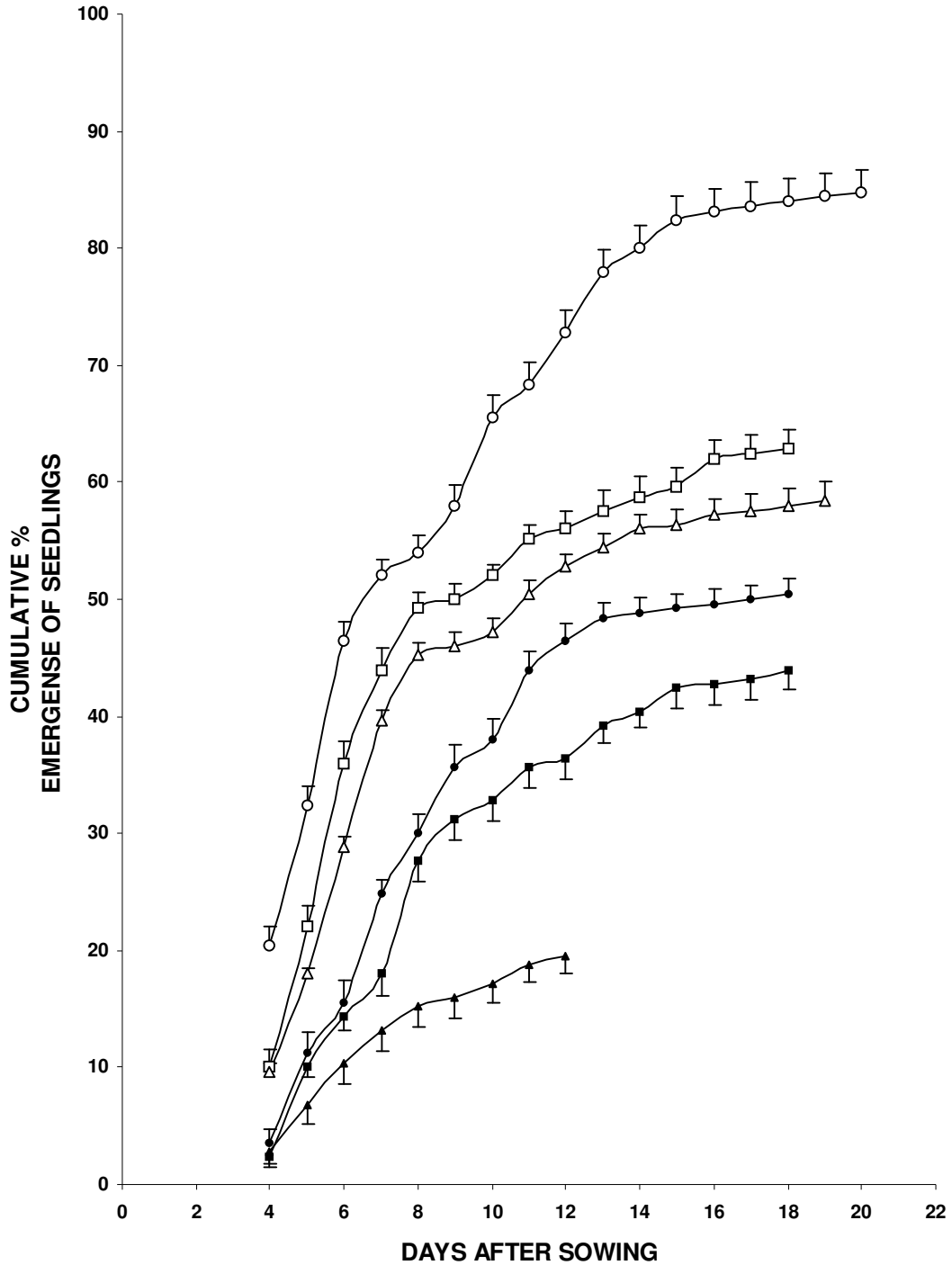

Fig.1. Cumulative emergence of seedlings of *Acacia senegal* in response to soil salinity 0.2 ds m^{-1} (○), 3.9 ds m^{-1} (□), 6.2 ds m^{-1} (Δ), 8.1 ds m^{-1} (●), 10.0 ds m^{-1} (▪) , and 11.9 ds m^{-1} (▲). Error bars represent SE.

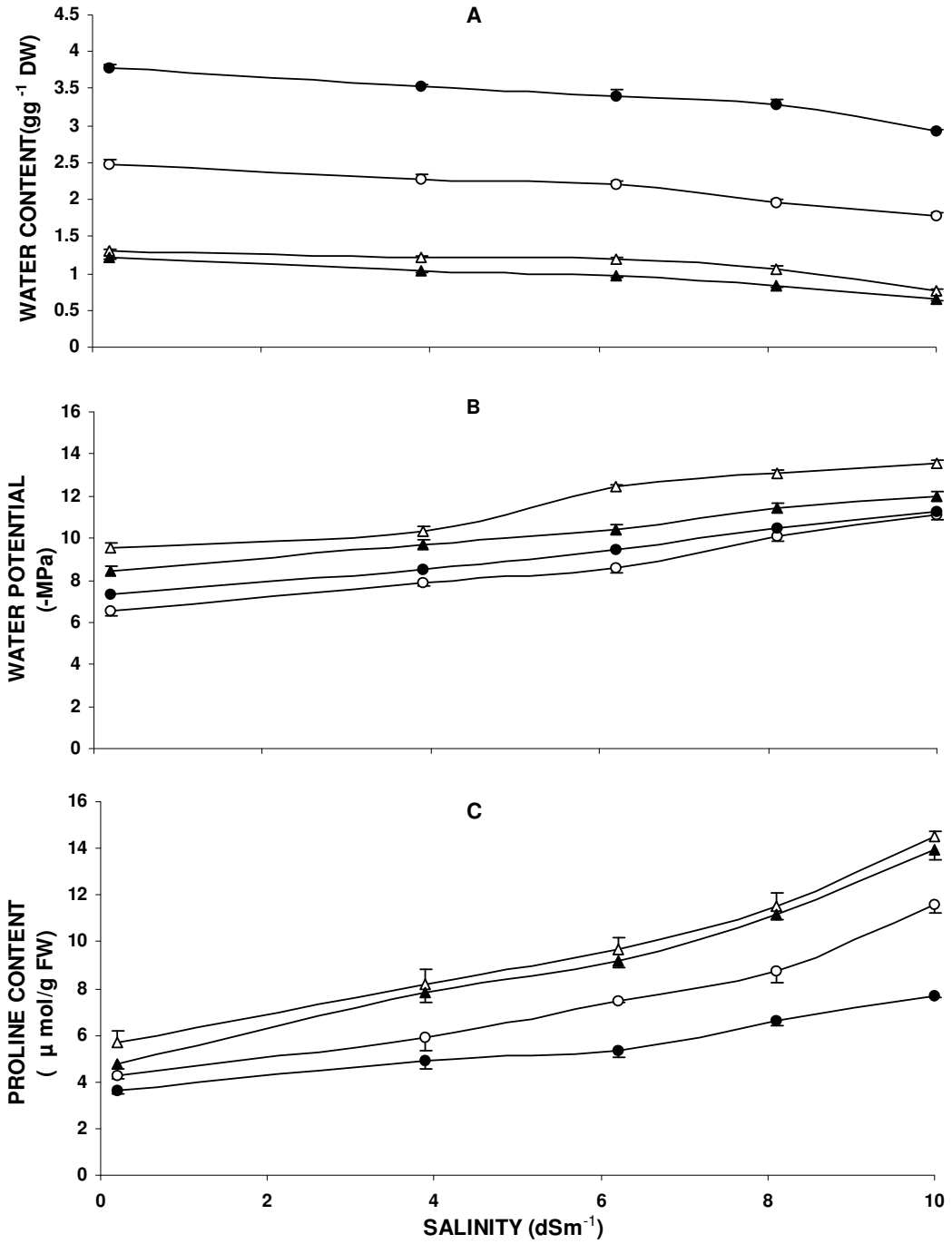

Fig .2 . Effect of soil salinity on water content (gg $^{-1}$DW) (A), water potential (-MPa) (B) and proline content (µ mol/g FW) (C) of leaves (○), stem (Δ), tap root (▲), and lateral roots (●) of *Acacia senegal* seedling. Error bars represent SE.

Table 1. Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Acacia senegal* as indicated by mean ± SEM and regression equation constants.

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area ($cm^2 plant^{-1}$)	Leaf weight (mg $plant^{-1}$)	Stem weight (mg $plant^{-1}$)	Shoot weight (leaf+stem) (mg $plant^{-1}$)	Tap root weight (mg $plant^{-1}$)	Lateral root weight (mg $plant^{-1}$)	Total root weight (mg $plant^{-1}$)
0.2	34.4±0.7	46.5±0.9	105.7±3.0	620.3±19.8	1880.6±19.1	2500.9±34.4	1674.5±42.8	1063.3±33.5	2737.8±57.9
3.9	29.3±0.7	38.5±0.8	85.2±2.8	540.8±17.5	1469.0±29.3	2009.8±30.8	1298.6±38.5	807.9±35.1	2106.5±50.6
6.2	25.6±0.8	30.5±0.7	66.3±3.0	455.7±11.9	1182.7±17.4	1638.4±25.3	930.3±33.6	592.5±27.3	1522.8±50.8
8.1	23.0±0.7	26.8±0.7	56.0±2.2	380.7±8.1	1031.7±18.2	1412.4±21.0	782.9±33.4	476.1±23.2	1259.0±42.7
10.0	19.3±0.4	24.5±0.9	46.8±2.5	334.7±8.9	944.6±14.4	1279.3±16.9	691.2±15.6	422.2±26.8	1113.4±34.9
α	34.97	46.77	107.17	638.57	1862.80	2501.40	1675.30	1062.30	2737.60
β	-1.52	-2.36	-6.18	-30.30	-98.78	-129.09	-105.60	-68.64	-174.25
r	-0.876	-0.924	-0.888	-0.885	-0.965	-0.941	-0.932	-0.896	-0.947
$LSD_{0.05}$	5.0	5.5	18.2	92.2	133.3	173.3	223.3	193.5	314.7

Relationship is significant at $p < 0.01$.

Table 2. (Part 1) Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Acacia senegal* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g\text{-}^{1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	27±1.0	2.4±0.1	10.5±0.4	3.8±0.3	14.9±0.1	5.9±0.1	2.8±0.2	114±4.1	38±2.3	103±3.5	796±5.0
	3.9	25±1.1	2.0±0.1	9.3±0.4	4.8±0.3	11.5±0.4	6.0±0.1	2.0±0.1	43±4.6	34±2.2	88±4.3	515±5.1
	6.2	24±1.1	1.6±0.1	9.2±0.1	7.2±0.3	10.2±0.3	6.1±0.0	1.3±0.0	38±2.6	28±2.6	86±2.6	419±4.3
	8.1	23±0.8	1.5±0.0	7.0±0.5	9.0±0.2	8.3±0.5	6.2±0.1	0.8±0.0	28±4.7	26±2.0	82±1.5	405±4.7
	10.0	20±0.6	1.2±0.2	6.3±0.4	10.8±0.2	8±0.5	6.8±0.1	0.6±0.0	27±3.6	24±1.3	59±2.5	353±4.0
	α	27.51	2.43	10.96	2.92	14.71	5.75	2.84	98.69	38.58	105.45	747.95
	β	-0.65	-0.12	-0.43	0.73	-0.72	0.07	-0.23	-8.57	-1.51	-3.84	-44.07
	r	-0.824	-0.913	-0.884	0.958	-0.958	0.733	-0.959	-0.883	-0.850	-0.777	-0.948
	$LSD_{0.05}$	2.9	0.4	0.4	0.9	1.3	0.4	0.5	11.8	6.3	9.0	13.7
Stem	0.2	22±1.1	2.0±0.1	6.6±0.2	2.1±0.1	10.9±0.3	3.1±0.2	3.2±0.2	138±3.2	35±2.0	50±1.5	780±3.5
	3.9	20±1.0	1.6±0.1	5.6±0.2	2.8±0.2	10.6±0.3	3.7±0.3	2.0±0.1	55±5.5	32±2.6	45±1.1	676±6.1
	6.2	18±1.5	1.2±0.1	4.8±0.2	4.5±0.3	9.4±0.4	4.0±0.3	1.1±0.1	46±3.6	28±2.5	38±1.7	556±3.6
	8.1	16±1.1	1.1±0.0	3.9±0.2	4.7±0.3	9.1±0.4	4.3±0.2	0.8±0.1	40±4.1	24±1.7	29±2.0	480±3.7
	10.0	15±0.6	0.8±0.2	3.8±0.3	5.2±0.2	7.1±0.5	5.6±0.5	0.7±0.1	35±4.3	23±0.7	24±2.6	361±5.5
	α	22.44	2.05	6.69	1.93	11.48	2.85	3.05	119.83	35.91	52.91	812.29
	β	-0.74	-0.12	-0.30	0.33	-0.36	0.22	-0.26	-10.04	-1.32	-2.76	-42.55
	r	-0.845	-0.919	-0.930	0.955	-0.829	0.795	-0.948	-0.884	-0.829	-0.939	-0.988
	$LSD_{0.05}$	3.3	0.4	0.8	0.4	1.3	1.0	0.4	12.4	6.0	5.6	13.6

Relationship is significant at $p < 0.01$.

Table 2. (Part 2) Effect of salinisation of soil on nutrient content of tissues (tap roots and lateral roots) of *Acacia senegal* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	18±1.0	1.6±0.1	5.0±0.1	2.0±0.2	8.8±0.3	2.8±0.2	2.6±0.3	131±4.1	32±1.5	68±2.5	3646±5.8
	3.9	16±0.6	1.2±0.1	4.5±0.2	2.3±0.1	6.8±0.5	3.3±0.3	2.0±0.2	59±4.9	22±1.1	55±1.5	3460±5.5
	6.2	15±0.7	1.0±0.1	3.0±0.2	3.7±0.2	4.9±0.4	4.1±0.2	0.8±0.1	36±3.0	20±1.7	41±3.6	3227±3.6
	8.1	14±0.5	0.7±0.1	2.8±0.3	4.4±0.3	4.8±0.4	4.3±0.3	0.7±0.1	34±3.7	17±2.0	29±1.1	3011±4.9
	10.0	12±0.7	0.6±0.1	2.5±0.2	5.4±0.1	3.6±0.5	5.1±0.4	0.5±0.0	31±4.3	15±0.5	25±1.7	2930±5.0
	α	18.29	1.61	5.15	1.51	8.77	2.61	2.63	115.79	30.81	70.14	3697.20
	β	-0.58	-0.10	-0.28	0.35	-0.52	0.23	-0.23	-10.14	-1.69	-4.67	-77.88
	r	-0.865	-0.893	-0.904	0.932	-0.928	0.850	-0.911	-0.904	-0.917	-0.971	-0.987
	$LSD_{0.05}$	2.3	0.4	0.7	0.6	1.3	0.9	0.6	12.0	4.4	6.7	14.8
Lateral roots	0.2	19±1.0	1.5±0.0	4.3±0.2	4.1±0.1	8.4±0.4	4.5±0.2	1.1±0.1	148±4.0	76±1.5	175±2.6	5225±4.5
	3.9	15±1.0	1.2±0.1	4.2±0.1	5.2±0.2	7.2±0.5	5.2±0.3	0.8±0.1	77±3.5	67±1.0	164±2.0	5016±5.7
	6.2	14±1.1	0.8±0.1	4.0±0.1	6.1±0.3	6.5±0.3	5.9±0.3	0.7±0.0	47±4.9	60±2.6	159±3.0	4743±5.1
	8.1	13±1.0	0.6±0.0	3.5±0.2	7.8±0.5	6.4±0.6	6.0±0.2	0.5±0.0	44±4.6	56±1.1	133±3.2	4572±5.5
	10.0	12±0.5	0.4±0.1	3.1±0.1	7.9±0.5	3.0±0.5	6.6±0.2	0.4±0.0	41±4.1	50±2.5	124±0.5	4543±4.5
	α	18.54	1.56	4.51	3.82	8.94	4.44	1.07	134.02	76.78	181.43	5251.30
	β	-0.69	-0.11	-0.12	0.42	-0.46	0.21	-0.06	-11.01	-2.63	-5.35	-75.96
	r	-0.848	-0.927	-0.808	0.917	-0.821	0.868	-0.934	-0.918	-0.957	-0.930	-0.982
	$LSD_{0.05}$	2.9	0.3	0.5	1.1	1.5	0.8	0.2	12.5	5.6	7.3	15.0

Relationship is significant at $p < 0.01$.

EXPERIMENT – 2

COMMUNICATED TO

Communications in Soil Science and Plant Analysis

***Tamarindus indica* Linn. (Caesalpiniaceae)**

Growth, Water Status and Nutrient Accumulation of Seedlings of *Tamarindus indica* Linn. in Response to Soil Salinity

Seema Abhay Hardikar[1] **and Amar Nath Pandey**[2*]

1 Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:abhay.hardikar@sgs.com

E-mail:seemahardikar@yahoo.com

2[*] **Correspondence**

A. N. Pandey

Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:anpandey2001@gmail.com

Tel (O): +91-281-2586419

Tel (M): +919427495989

Fax (O): +91-281-2577633

Short title (to be used as running head):

Effect of soil salinity on *Tamarindus indica*

ABSTRACT

Greenhouse experiments were conducted to assess the effect of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Tamarindus indica Linn.* (Caesalpiniaceae). NaCl was added to the soil and salinity was maintained at 0.2, 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. Salinity caused reduction in water content and water potential of tissues that resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased as salinity increased. Proline content in tissues increased as salinity increased. There were no effective mechanisms to control net uptake of Na^+ and subsequently its transport to shoot tissues. Nitrogen content significantly increased in tissues as salinity increased. Potassium and calcium content in tissues significantly decreased as salinity increased. Changes in tissues and whole-plant accumulation pattern of other nutrients, as well as possible mechanisms for avoidance of Na toxicity in this species in response to salinity, are discussed.

Key words: Soil salinity, seedling growth, proline content, water potential, macro- and micro-nutrients, salt tolerance.

INTRODUCTION

Saline soils are abundant in semi arid and arid regions where the amount of rainfall is insufficient for substantial leaching (Marschner, 1995). Salinity is a scourge for agriculture, forestry, pasture development and other similar practices. An understanding of responses of plants to salinity is of great practical significance. High concentrations of salts have detrimental effects on plant growth (Taiz and Zeiger, 2006; Ramoliya et al., 2006) and excessive concentrations kill growing plants (Garg and Gupta, 1997). There occurs retardation of germination and growth of seedlings at high salinity (Bernstein, 1962; Garg and Gupta, 1997; Ramoliya et al., 2006). However, plant species differ in their sensitivity or tolerance to salts (Marschner, 1995). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns, 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman, 1977; Munns, 2002; Ramoliya et al,. 2006). However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns, 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt–induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g. Cramer et al,. 1989; Maas and Grieve, 1987; Ramoliya et al., 2006; Patel and Pandey, 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al., 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the

afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Tamarindus indica Linn.(Caesalpiniaceae), a deciduous tree species grows abundantly in coastal area of Saurashtra in Gujarat State of India. It also grows successfully on marginal-saline lands of Kutch (north-west saline desert) contiguous to Saurashtra. Fruits with sticky brown pulp rich in tartaric- and citric acids and high amount of vitamin C and sugar, are used in Asian cookery. Wood is a good fuel. Leaves are eaten by herbivores. The present investigation was performed with the following objectives :(i) to understand the adaptive features of *T. indica* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

MATERIAL AND METHODS

Study Area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22^0 18' N Lat, $70^0$56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6 % sand, 20.3 % silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dS m^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical

properties of soil are given earlier (Patel and Pandey, 2007). The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid – June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj ($23^0$15' N Lat, $69^0$49' E Long) in Kutch and about 554 mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June – September) and winter (November – February).The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Salinisation of Soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil of 100 kg each, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 390, 700, 1070, 1275, 1530 and 1800 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.2 dS m^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling Emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on November 26, 2006. Seeds of *T. indica* were collected from the coastal area of Arabian Sea at Jamnagar district of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered and there after watering was carried out on alternate days. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity, using the expression:

$Sin^{-1}\sqrt{p} = \beta_0 + \beta_1 X$

where, $Sin^{-1}\sqrt{p}$ 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 estimated using the model.

Seedling Growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 3.9 and 6.2 dS m^{-1} salinity exhibited emergence of the second leaf after 26 days, whereas the second leaf on seedlings grown in 8.1, 10.0, and 11.9, 13.9 dS m^{-1} appeared after 31 and 34 days respectively. Emergence of the second leaf confirmed the establishment of seedlings. However, only 11.2 and 4% seed germination was recorded respectively in soils at 11.9 and 13.9 dS m^{-1} salinity, and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling

was further uprooted. Thus twenty replicates factorized with five grades of soil (0.2, 3.9, 6.2, 8.1, and 10.0 dS m^{-1}) were prepared. This gave a total of 100 bags, which were arranged in 20 randomized blocks. Seedlings were watered (about 250 ml water was added to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on May 26, 2007. Seedlings contained in 20 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of tissues were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of Water Potential and Proline Content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5 g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

Mineral Analyses of Plant Materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three sub samples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper, 1944). Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3:H_2SO_4:$HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

RESULTS AND DISCUSSION

Effect of Salinisation on Seedling Emergence

Seedlings began to emerge 16 days after sowing and 94.4% seed germination was obtained over a period of 39 days, under control (0.2 dS m^{-1} salinity) conditions (Figure 1). Seedling emergence in saline soils was recorded 17-27 days after sowing. Seedling emergence lasted for 39, 39, 39, 39, 37 and 36 days in soils with 3.9, 6.2, 8.1, 10.0,11.9 and 13.9 dS m^{-1} salinities, respectively and corresponding seed germination was 91.6%, 90.8%, 39.6%, 33.6%,11.2% and 4%. There was a significant reduction in seed germination ($p < 0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: Y= 90.913-

5.579X, (R^2_{adj} =0.885, $p < 0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of Salinisation on Stem and Root Elongation and Leaf Expansion

Increasing soil salinity significantly retarded ($p < 0.01$) stem and root elongation (Table 1). Shoot height was almost equal to root length for seedlings grown in control and saline soils. There was a negative relationship ($p < 0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p < 0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p < 0.01$).

Effect of Salinisation on Dry Weight

Dry weight significantly decreased ($p < 0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weight of tissues of salinised plants compared to those of control plants were computed as: (salinised tissue dry weight / control dry weight) × 100. Dry weight values of tissues given in Table 1 were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 82.4 to 49.4% for leaves, from 83.9 to 53.7 % for stems, 81.1 to 45.1 % for tap roots and from 82.8 to 47.9 % for lateral roots in response to increasing soil salinity from 3.9 to 10.0 dS m^{-1}. As has been estimated using regression equations given in results , the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 9.2, 10.6, 8.7 and 9.3 for leaves, stems, tap roots and lateral root tissues , respectively. Root/shoot dry weight ratio was 0.73 under control conditions

while it was 0.72, 0.71, 0.67 and 0.65 for seedlings grown in soils at 3.9, 6.2, 8.1 and 10.0 dS m^{-1}salinities, respectively. Root / shoot dry weight ratios significantly decreased ($p < 0.01$) as soil salinity increased. There was a negative relationship between root / shoot dry weight ratio and soil salinity ($r = -0.399$, $p < 0.01$).

Effect of Salinisation on Water Content of Tissues

Water content in leaves, stems, tap roots and lateral root tissues significantly decreased ($p < 0.01$) with increasing concentration of salt in soil (Figure 2A). There was maximum water content in leaves and minimum in tap roots. Tissues according to their water content can be arranged in following decreasing order: leaves > lateral roots > stems > tap roots. There was a negative relationship between water content in different tissues and salt concentration ($r= -0.881, -0.827, -0.833$ and -0.868, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of Salinisation on Water Potential of Tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p < 0.01$) as soil salinity increased (Figure 2B). Tissues according to their water potential values (low to high negative) can be arranged in the following order: leaves > lateral roots > tap roots > stems. There was a negative relationship between water potential of tissues and salt concentration ($r = -0.979, -0.966, -0.934$ and -0.938, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) . A positive relationship was obtained between water content and water potential (negative value) ($r = 0.980, 0.995, 0.945$ and $0.979, p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) .

Effect of Salinisation on Proline Content of Tissues

Proline content (μ mol/g FW material) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues, though amount of proline was low, with

increase in soil salinity (Figure 2C).Tissues according to their proline content can be arranged in following decreasing order: tap roots > stems > lateral roots > leaves. There was a positive relationship between salt concentration and proline content of tissues ($r = 0.925$, 0.976, 0.957 and 0.900, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content ($r = -0.919$, -0.918, -0.876 and -0.945, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content ($r = -0.997$, -0.980, -0.996 and -0.981, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of Salinisation on Mineral Accumulation

Potassium and Sodium Content and K/Na Ratio

Potassium content (as mg g^{-1} dry weight) significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a negative relationship between potassium content in tissues and increase in salt concentration in soil ($p < 0.01$). Sodium content significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress ($p < 0.01$). K/Na ratio significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K /Na ratio in tissues and increase in salt stress ($p < 0.01$).

Nitrogen, Phosphorus, Calcium and Magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen content exhibited a

significant increase ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress. There was a significant positive relationship between N content in tissues and salt concentration in soil ($p < 0.01$). Phosphorus, calcium and magnesium content significantly decreased in leaves, stems, tap roots and lateral root tissues ($p < 0.01$), as the salinity increased (Table 2). A negative relationship was obtained between P, K, Ca and Mg content of tissues and salt concentration ($p < 0.01$).

Micro –Elements

There was a significant increase in the concentration of Zn, Cu, Mn and Fe ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress (Table 2). A positive relationship was obtained between soil salinity and Zn, Cu, Mn and Fe content in tissues ($p < 0.01$).

Earlier work (Ramoliya et al,. 2004), indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dS m^{-1},but for *Tamarindus indica* SG_{50} was obtained at 7.3 dS m^{-1}.That would suggest that this plant species is relatively salt tolerant at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 2.0 to 5.0 dS m^{-1}. Eventually, seeds of *T. indica* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 10.0 dS m^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentrations of

salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Slater et al., 2003) and induces changes in the activities of many enzymes (Dubey and Rani, 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz and Zeiger, 2006). Root / shoot dry weight ratio of *T. indica* was 0.73 under control conditions and was greater than that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya et al., 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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(Kramer, 1983; Garg and Gupta, 1997).Results for reduction of shoot growth and leaf area development of *T. indica* with increasing salt concentration are in conformity with the finding of Curtis and 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 and 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. Also high concentration of salt tends to slow down or stop root elongation (Kramer, 1983) and causes reduction in root production (Garg and Gupta, 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of tap roots and lateral roots while lowest in stems. Consequently, stems and leaves were more resistant than roots to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: stems> leaves >lateral roots >tap roots. The greater reduction in root weight than in shoot weight resulted in decreased root/shoot dry weight ratio for seedlings with increase in salinity. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion (Marschner, 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration .The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ (Marschner, 1995).Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *T. indica* survived up to the soil salinity of 10 dS m^{-1} and, therefore, this tree species is moderate salt tolerant .In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates (Greenway and Munns, 1980). As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway and Munns, 1980). Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al., 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (Stewart and Lee, 1974).In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar et al., 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis (Schachtman et al., 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow et al., 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox and Guerinot, 1998). In the present study, significant decrease of K^+ content in all the tissues of seedlings with increasing soil salinity suggests that Na inhibited K^+ uptake. The exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salts to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues of *T. indica* suggests that this mechanism to block Na^+ transfer to growing tissues was not effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively low accumulation of K^+ than that of Na^+. As a consequence there were no effective mechanisms to control net uptake of Na on root plasma membrane and subsequently its transport to shoot tissue. The pattern of accumulation of K^+ and Na^+ in *T. indica* conforms to group C and / or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four

groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na^+ without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar (Watad et al. 1991; Schroeder et al., 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport system, which are necessary for K^+ acquisition .As a consequence ,Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}.These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated (Amtmann and Sanders, 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Watad et al,. 1991; Schroeder et al., 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis and Sanders, 1994; Niu et al,. 1995).The K^+ and Na^+ profiles of *T. indica* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al., 2001).As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant.

In general, salinity reduces N accumulation in plants (Feigin, 1985), but in this plant nitrogen increased with increase in salinity. Dubey and Rani (1989) reported that protein level in several crops under salinisation increases due to the increased synthesis of pre-existing and certain new sets of proteins. The Interaction between

salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol, 1979; Grattan and Grieve, 1992). However it is known that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach et al., 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e. g., in preserving membrane integrity (Rengel, 1992), signalling in osmoregulation (Mansfield et al., 1990) and influencing K^+/Na^+ selectivity (Cramer et al., 1987). In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions , precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen and Chang, 1987; Garg and Gupta, 1997).Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates, which when impaired leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner and Cakmak, 1989).

It is difficult to suggest mechanistic explanations of salinity influence on micro–element concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al., 2000). In the present study, it appears that salinity enhanced Zn, Cu, Mn and Fe accumulation, at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox system, Mn for photolysis of water in photosynthesis and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner, 1995). In addition, high

concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner, 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al., 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater et al., 2003). Increase in Cu, Zn, Mn and Fe content at the whole plant level might be the requirement of this plant for survival and growth in response to salinity.

CONCLUSIONS

As the salt concentration increased during soil salinisation, seed germination decreased in a linear fashion. The SG_{50} concentration was estimated to be 7.3 dS m^{-1} suggesting that this plant species is comparatively salt tolerant at seed germination. Expansion of leaves and elongation of stems and roots also decreased, exhibiting a linear trend in response to increasing soil salinity. Furthermore, dry weight of leaves, stems, tap roots and lateral roots of seedlings decreased following a linear pattern, with increasing concentration of salt in soil. Dry- weight reduction was lowest for stems, while it was maximum for tap roots and lateral roots in response to increasing salinity. Water content and water potential of tissues of salt- stressed plants decreased and might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. The greatest reduction in root weight than in shoot weight resulted decrease in root/shoot dry weight ratio for seedlings as the salinity increased. Proline content in tissues increased, exhibiting a linear trend in response to increasing soil salinity.

Sodium content increased, while potassium content decreased in tissues in a linear fashion in response to increase in salinity. Results further suggested that Na inhibited K uptake and there were no effective mechanisms to block subsequent Na^+ transfer to shoot tissues. A significant decrease of calcium content in all the tissues with increase in salinity suggested that Na^+ induced Ca^{2+} deficiency in tissues. This result implicates that Ca fertilizers may mitigate Na^+
toxicity to plants. Concentration of N increased, while concentration of Mg decreased with increase in soil salinity. There was a significant increase in the concentration of Zn, Cu, Mn and Fe in tissues in response to increase in salt stress. Increase in concentration of these micro-elements at the whole- plant level might be an advantage to this plant for survival and growth in saline soils.

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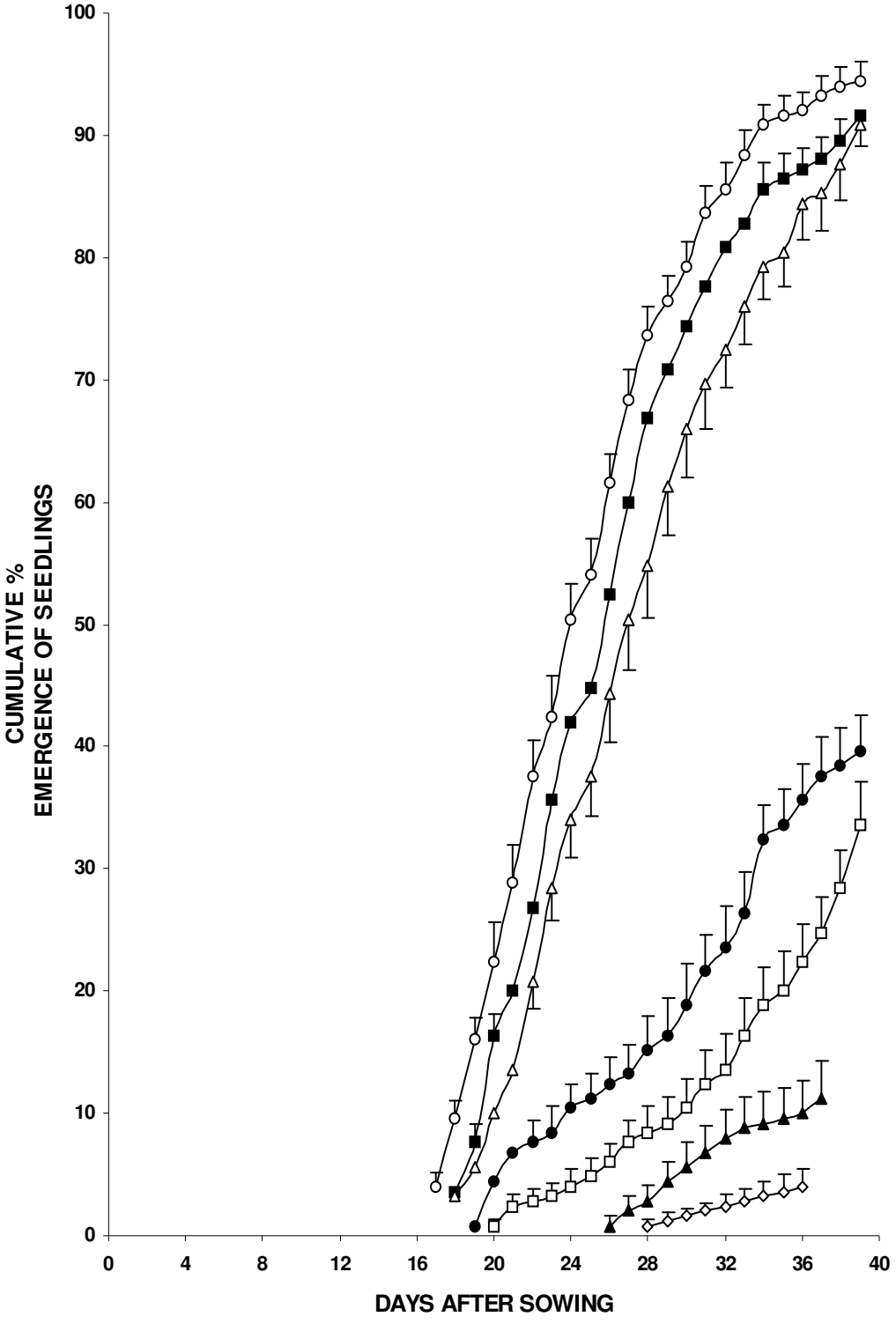

Figure 1. Cumulative emergence of seedlings of *Tamarindus indica* in response to soil salinity 0.2 dS m^{-1} (○), 3.9dS m^{-1} (■), 6.2dS m^{-1} (Δ), 8.1dS m^{-1} (●), 10.0dS m^{-1} (□) , 11.9dS m^{-1} (▲) and 13.9dS m^{-1} (◊). Error bars represent SE.

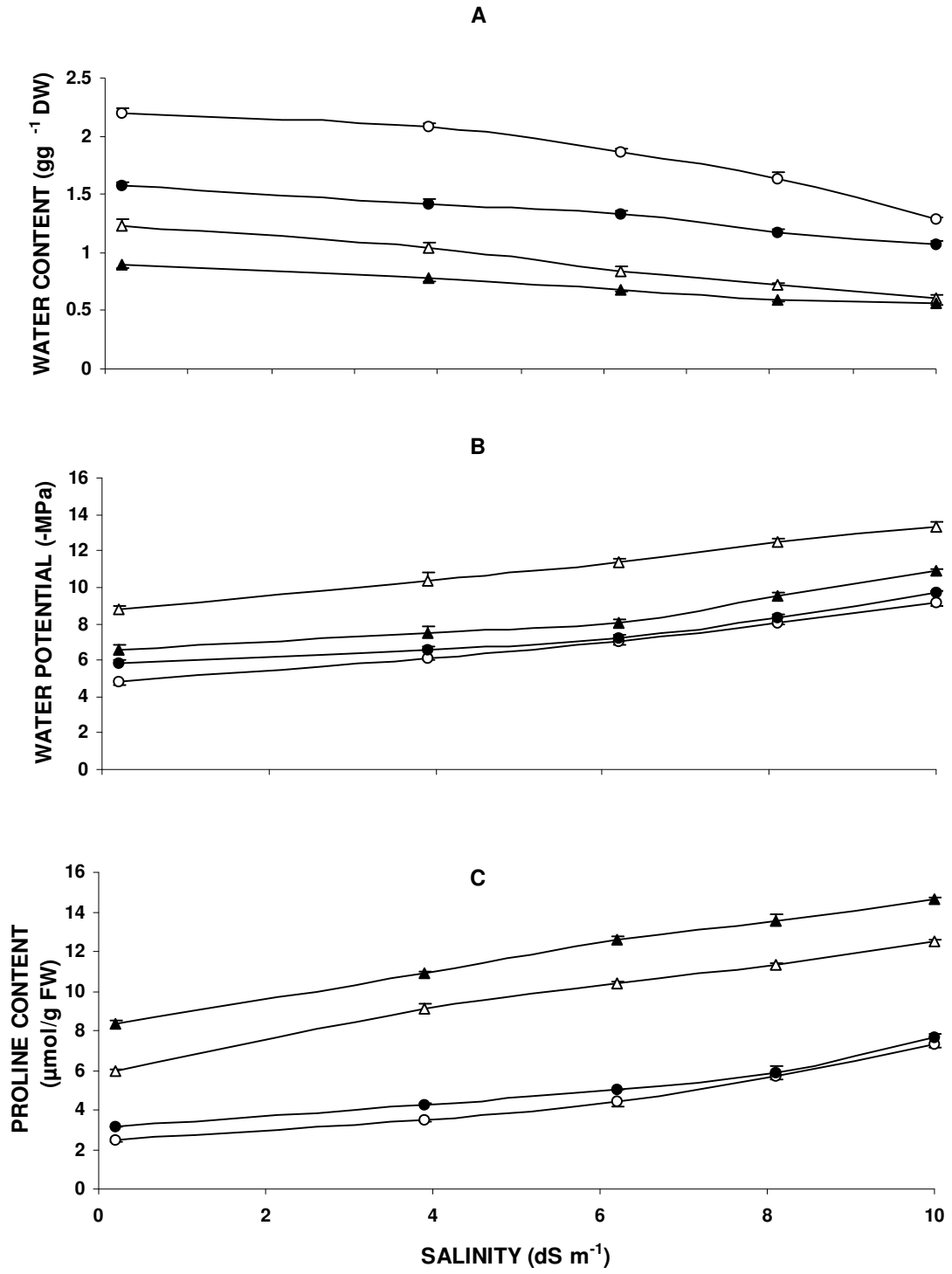

Figure 2. Effect of soil salinity on A. water content (gg $^{-1}$ DW), B. water potential (-Mpa) and C. proline content (µ mol/g FW) of leaves (○), stem (Δ), tap root (▲), and lateral roots (●) of *Tamarindus indica* seedlings. Error bars represent SE.

Table 1. Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Tamarindus indica* as indicated by mean ± SEM and regression equation constants.

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area ($cm^2 plant^{-1}$)	Leaf weight (mg $plant^{-1}$)	Stem weight (mg $plant^{-1}$)	Shoot weight (leaf+stem) (mg $plant^{-1}$)	Tap root weight (mg $plant^{-1}$)	Lateral root weight (mg $plant^{-1}$)	Total root weight (mg $plant^{-1}$)
0.2	35.4 ± 0.7	34.7±0.7	339.6±7.8	1781.7±26.2	1338.2±28.8	3119.9±44.2	1509.5±34.0	753.8±20.1	2263.3±36.5
3.9	29.0±0.8	27.9±0.8	257.2±7.8	1468.7±24.3	1123.6±25.5	2592.3±38.7	1225.3±22.6	624.7±15.3	1850.0±25.0
6.2	24.9±0.6	22.7±0.6	187.2±8.0	1140.2±26.6	1017.1±30.6	2157.3±46.6	1022.2±32.0	501.3±24.0	1523.5±45.9
8.1	21.3±0.5	20.4±0.6	151.1±7.5	934.3±21.2	775.1±14.9	1709.4±29.7	731.8±8.7	411.6±12.0	1143.4±18.8
10.0	19.0±0.7	17.6±0.7	135.3±4.4	880.5±20.3	719.1±20.2	1599.6±36.3	681.2±23.1	361.3±10.3	1042.5±27.0
α	35.6	34.6	338.5	1801.3	1369.0	3170.4	1544.1	768.07	2312.2
β	-1.70	-1.76	-21.91	-98.63	-65.91	-164.56	-89.81	-41.82	-131.63
r	-0.903	-0.905	-0.930	-0.955	-0.913	-0.959	-0.943	-0.908	-0.958
LSD	4.8	4.8	51.1	156.5	162.0	259.3	168.5	112.4	210.3

Relationship is significant at $p < 0.01$.

Table 2. (Part 1) Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Tamarindus indica* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	21.9±0.8	2.8±0.2	26.6±0.7	4.5±0.2	19.0±0.6	5.8±0.2	5.9±0.4	14±0.6	6±1.2	37±1.5	375±7.1
	3.9	24.4±0.9	2.4±0.2	22.2±0.8	5.2±0.2	16.8±0.3	5.6±0.2	4.3±0.3	25±0.9	9±0.8	56±2.5	402±7.4
	6.2	25.0±0.9	2.0±0.1	21.2±0.5	7.4±0.2	13.6±0.3	5.1±0.2	2.9±0.1	27±0.8	10±0.6	58±1.2	589±6.4
	8.1	27.5±0.9	1.8±0.1	19.0±0.6	9.8±0.1	11.2±0.3	4.9±0.2	1.9±0.1	31±0.8	22±2.3	69±2.6	686±7.6
	10.0	28.3±0.8	1.2±0.1	17.9±0.7	13.9±0.2	9.1±0.2	4.6±0.2	1.29±0.1	46±1.0	26±1.5	71±2.0	785±6.7
	α	21.66	2.92	26.37	2.90	19.87	5.92	6.04	12.28	2.66	38.47	314.00
	β	0.66	-0.15	-0.87	0.92	-1.04	-0.12	-0.49	2.87	2.10	3.47	44.61
	r	0.870	-0.928	-0.943	0.920	-0.976	-0.829	-0.979	0.937	0.879	0.949	0.954
	$LSD_{0.05}$	2.5	0.4	2.0	0.5	1.1	0.6	0.7	2.4	4.1	6.0	20.6
Stem	0.2	16.8±0.3	2.0±0.1	15.5±0.6	3.4±0.2	13.9±0.6	5.5±0.2	4.6±0.5	16±0.3	16±1.1	23±2.3	496±6.0
	3.9	17.9±0.2	1.8±0.1	13.9±0.4	5.3±0.1	10.3±0.5	5.1±0.3	2.6±0.1	18±0.4	21±1.3	28±1.7	835±8.5
	6.2	18.4±0.3	1.6±0.1	13.2±0.2	6.3±0.2	8.9±0.3	4.5±0.3	2.1±0.1	37±0.5	23±1.5	41±1.5	1091±8.3
	8.1	20.1±0.2	1.2±0.2	12.6±0.4	8.6±0.2	8.1±0.2	4.0±0.2	1.5±0.1	38±0.6	26±1.7	58±2.1	1207±5.8
	10.0	22±0.1	1.0±0.1	11.8±0.4	9.0±0.1	6.9±0.2	3.7±0.3	1.3±0.1	53±1.2	29±0.9	60±1.2	1277±7.2
	α	16.16	2.15	4.34	3.10	13.58	5.65	4.34	10.80	15.64	18.00	509.26
	β	0.50	-0.11	-0.33	0.60	-0.69	-0.19	-0.33	3.80	1.29	4.22	82.83
	r	0.931	-0.896	-0.936	0.976	-0.964	-0.864	-0.936	0.935	0.917	0.940	0.987
	$LSD_{0.05}$	0.6	0.3	1.2	0.5	1.1	0.8	0.7	1.9	3.9	5.3	21.2

Relationship is significant at $p < 0.01$.

Table 2. (Part 2) Effect of salinisation of soil on nutrient content of tissues (tap roots and lateral roots) of *Tamarindus indica* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	12.6±0.2	1.6±0.2	15.1±0.4	3.0±0.2	5.7±0.3	8.1±0.3	5.0±0.3	20±1.0	33±1.0	32±2.1	830±12.2
	3.9	14.7±0.3	1.2±0.1	14.8±0.3	4.5±0.3	4.9±0.2	6.8±0.5	3.3±0.2	27±0.7	35±1.4	44±1.2	1138±10.7
	6.2	16.7±0.1	1.0±0.1	14.1±0.4	5.6±0.2	4.2±0.1	5.4±0.4	2.5±0.1	38±0.6	45±1.5	63±1.5	2126±10.3
	8.1	18.6±0.3	0.9±0.1	13.6±0.4	6.4±0.1	3.7±0.1	4.3±0.3	2.1±0.1	53±1.0	54±1.1	84±1.0	2690±12.8
	10.0	19.6±0.2	0.8±0.1	12.9±0.3	8.8±0.3	3.2±0.2	3.3±0.4	1.5±0.1	89±1.2	111±2.6	121±1.7	2767±9.0
	α	12.21	1.56	15.38	2.52	5.80	8.42	4.92	8.68	17.35	19.32	637.36
	β	0.74	-0.08	-0.22	0.55	-0.25	-0.50	-0.35	6.46	6.73	8.71	224.08
	r	0.987	-0.862	-0.818	0.956	-0.952	-0.951	-0.963	0.897	0.796	0.939	0.961
	$LSD_{0.05}$	0.6	0.3	1.0	0.6	0.6	1.1	0.6	2.7	4.8	4.5	32.4
Lateral roots	0.2	11.0±0.1	1.2±0.1	20.8±0.9	9.7±0.1	8.0±0.3	9.0±0.2	2.1±0.1	32±0.4	47±1.2	58±1.2	5356±11.0
	3.9	12.8±0.1	1.0±0.1	18.5±0.8	10.6±0.2	6.6±0.3	8.8±0.3	1.7±0.1	37±1.7	62±1.5	78±2.1	6741±8.7
	6.2	14.2±0.2	0.9±0.1	15.6±0.9	12.1±0.2	5.8±0.2	8.7±0.2	1.3±0.1	39±1.6	82±1.7	118±1.7	6783±10.5
	8.1	15.6±0.2	0.8±0.1	14.9±0.8	13.5±0.3	5.1±0.2	8.5±0.1	1.1±0.1	54±1.2	99±0.6	126±1.2	6897±9.1
	10.0	16.1±0.1	0.7±0.1	13.3±0.4	14.6±0.3	4.1±0.3	7.6±0.2	0.9±0.0	111±2.1	126±2.1	149±2.5	7103±7.6
	α	10.85	1.20	21.05	9.16	8.12	9.21	2.18	16.47	38.27	51.61	5640.30
	β	0.54	-0.05	-0.78	0.51	-0.38	-0.12	-0.13	6.71	7.90	9.52	164.74
	r	0.989	-0.852	-0.916	0.965	-0.963	-0.733	-0.953	0.782	0.968	0.978	0.900
	$LSD_{0.05}$	0.4	0.2	2.3	0.6	0.7	0.6	0.3	4.4	4.4	5.3	27.7

Relationship is significant at $p < 0.01$.

EXPERIMENT – 3

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Thespesia populnea (L.) Sol. ex Correa (Malvaceae)

Growth, water status and nutrient accumulation of seedlings of *Thespesia populnea* (L.) Sol. ex Correa in response to soil salinity

Seema Abhay Hardikar[1] **and Amar Nath Pandey***[2]

[1] *Department of Biosciences, Saurashtra University, Rajkot – 360005 India*

E-mail:abhay.hardikar@sgs.com

E-mail:seemahardikar@yahoo.com

***[2]Correspondence**

A. N. Pandey

Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:anpandey2001@gmail.com

Tel (O): +91-281-2586419

Tel (M): +919427495989

Fax (O): +91-281-2577633

Short title (to be used as running head):

Effect of salinisation of soil on *Thespesia populnea* (L.) Sol. ex Correa

ABSTRACT

Greenhouse experiments were conducted to assess the effect of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Thespesia populnea* (L.) Sol. ex Correa (Malvaceae). Sodium chloride (NaCl) was added to the soil and salinity was maintained at 0.2, 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. A negative relationship between seedling emergence and salt concentration was obtained. Salinity caused reduction in water content and water potential of tissues (leaves, stems, tap roots and lateral roots) that resulted in internal water deficit to plants. Consequently, shoot and root elongation, leaf expansion and dry matter accumulation in leaves, stems, tap roots and lateral root tissues of seedlings significantly decreased in response to increasing concentration of salt. Proline content in tissues increased with increase in soil salinity. Evidently, nitrogen, phosphorus and calcium content significantly decreased as soil salinity increased. There were no effective mechanisms to control net uptake of Na^+ and subsequently its transport to shoot tissues. Sodium and potassium content significantly increased in tissues in response to salinity, but increase of Na^+ was relatively more rapid than that of K^+. Changes in tissues and whole-plant accumulation pattern of other elements tested, as well as possible mechanism for avoidance of Na toxicity in this tree species in response to salinity, are discussed.

Key words: macro- and micro-nutrients, proline content, salinisation of soil, salt tolerance, seedling growth, water potential.

INTRODUCTION

Salt affected soils represent a stress condition for plants. An understanding of responses of plants to salinity is of great practical significance. High concentrations of salts have detrimental effects on plant growth (Taiz and Zeiger, 2006; Ramoliya et al., 2006) and excessive concentrations kill growing plants (Garg and Gupta, 1997). There occurs retardation of germination and growth of seedlings at high salinity (Bernstein, 1962; Garg and Gupta, 1997; Ramoliya et al. 2006). However, plant species differ in their sensitivity or tolerance to salts (Marschner, 1995). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns, 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman, 1977; Munns, 2002; Ramoliya et al., 2006). However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns, 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt–induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g., Maas and Grieve, 1987; Cramer et al., 1989; Ramoliya et al., 2006; Patel and Pandey, 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al., 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Thespesia populnea (L.) Sol. ex Correa (Malvaceae), a deciduous and small tree species grows abundantly in coastal area of Saurashtra in Gujarat State of India. It also grows successfully on marginal-saline lands of Kutch (north-west saline desert) contiguous to Saurashtra. It has many uses including coastal protection, animal fodder, wind breaks and living fences. The most common use is probably as an ornamental tree, despite its valuable timber. When a bud or young fruit is cut transversally, a copious yellow and gummy fluid known as gossypol exudes from the surfaces. Gossypol is a substance that helps protect the plant against predators because in a large quantity it is toxic to mammals including humans. The present investigation was performed with the following objectives: (i) to understand the adaptive features of *T. populnea* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

MATERIAL AND METHODS

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22^0 18' N Lat, $70^0$56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil, which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6% sand, 20.3% silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dS m^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%,

respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier (Patel and Pandey, 2007). The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid – June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj ($23^0$15' N Lat, $69^0$49' E Long) in Kutch and about 554 mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June – September) and winter (November – February).The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Salinisation of soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil of 100 kg each were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 390, 700, 1070, 1275, 1530 and 1800 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.2 dS m^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 7 December 2006. Seeds of *T. populnea* were collected from the coastal area of Arabian Sea at Jamnagar district of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered and there after watering was carried out on alternate days. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity, using the expression:

$Sin^{-1}\sqrt{p} = \beta_0 + \beta_1 X$

where, $Sin^{-1}\sqrt{p}$ 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 estimated using the model.

Seedling growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 3.9, 6.2 and 8.1 dS m^{-1} salinity exhibited emergence of the second leaf after 24 days, whereas the second leaf on seedlings grown in 10.0, 11.9 and 13.9 dS m^{-1} appeared after 29 days. Emergence of the second leaf confirmed the establishment of seedlings. However, only 13.6 and 4.4% seed germination was recorded respectively in soils at 11.9 and 13.9 dS m^{-1} salinity, and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus

twenty replicates factorized with five grades of soil (0.2, 3.9, 6.2, 8.1, and 10.0 dS m^{-1}) were prepared. This gave a total of 100 bags, which were arranged in 20 randomized blocks. Seedlings were watered (about 250 ml water was added to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on 7 June 2007. Seedlings contained in 20 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of tissues were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of water potential and proline content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

Mineral analyses of plant materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three sub samples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper 1944). Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3: H_2SO_4: $HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined

RESULTS

Effect of salinisation on seedling emergence

Seedlings began to emerge 13 days after sowing and 63.8% seed germination was obtained over a period of 32 days, under control (0.2 dS m^{-1} salinity) conditions (Fig. 1). Seedling emergence in saline soils was recorded 13-19 days after sowing. Seedling emergence lasted for 31, 31, 31, 30, 29 and 26 days in soils with 3.9, 6.2, 8.1, 10.0,11.9 and 13.9 dS m^{-1} salinities ,respectively and corresponding seed germination was 58%, 55.2%, 48.8%, 34.8% ,13.6% and 4.4%. There was a significant reduction in seed germination ($p < 0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y= 61.609-3.033X$, ($R^2_{adj} =0.837$, $p <$

0.01), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinisation on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p < 0.01$) stem and root elongation (Table 1). Nevertheless, root length was remarkably greater than shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p < 0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p < 0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p < 0.01$).

Effect of salinisation on dry weight

Dry weight significantly decreased ($p < 0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weight of tissues of salinised plants compared to those of control plants were computed as: (salinised tissue dry weight / control dry weight) × 100. Dry weight values of tissues given in Table 1 were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 78.1 to 56.8% for leaves, from 75.3 to 52.3 % for stems, 75.3 to 43.4 % for tap roots and from 75.4 to 49.9 % for lateral roots in response to increasing soil salinity from 3.9 to 10 .0 dS m^{-1}. As has been estimated using regression equations given in results, the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 11.2, 10.1, 8.4 and 9.6 for leaves, stems, tap roots and lateral

root tissues, respectively. Root/shoot dry weight ratio was 0.46 under control conditions while it was 0.45, 0.41, 0.40 and 0.39 for seedlings grown in soils at 3.9, 6.2, 8.1 and 10.0 dS m^{-1}salinities, respectively. Root / shoot dry weight ratios significantly decreased ($p < 0.01$) as soil salinity increased. There was a negative relationship between root / shoot dry weight ratio and soil salinity ($r = -0.685$, $p < 0.01$).

Effect of salinisation on water content of tissues

Water content in leaves, stems, tap roots and lateral root tissues significantly decreased ($p < 0.01$) with increasing concentration of salt in soil (Fig. 2A). There was maximum water content in lateral roots and minimum in tap roots. Tissues according to their water content can be arranged in following decreasing order: lateral roots > leaves > stems > tap roots. There was a negative relationship between water content in different tissues and salt concentration (r= -0.796, -0.838, -0.826 and -0.844 , $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on water potential of tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p < 0.01$) as soil salinity increased (Fig. 2B). Tissues according to their water potential values (low to high negative) can be arranged in the following decreasing order: lateral roots > leaves> tap roots > stems. There was a negative relationship between water potential of tissues and salt concentration ($r = -0.948$, -0.902, -0.971 and -0.914, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). A positive relationship was obtained between water content and water potential (negative value) ($r = 0.987$, 0.938, 0.974 and 0.962, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on proline content of tissues

Proline content (μ mol/g FW material) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues, though amount of proline was low, with increase in soil salinity (Fig. 2C).Tissues according to their proline content can be arranged in following decreasing order: leaves> stems>tap roots> lateral roots. There was a positive relationship between salt concentration and proline content of tissues ($r = 0.980, 0.959, 0.929$ and 0.967, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content ($r = -0.889, -0.838, -0.896$ and -0.832, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content ($r = -0.957, -0.985, -0.972$ and -0.990, $p < 0.0$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on mineral accumulation

Potassium and sodium content and K/Na ratio

Concentration of potassium and sodium content significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a positive relationship for potassium and sodium content in tissues with salt concentration in soil ($p < 0.01$). K/Na ratio significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K /Na ratio in tissues and increase in salt stress ($p < 0.01$).

Nitrogen, phosphorus, calcium and magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen , phosphorus and Calcium content significantly decreased in leaves, stems, tap roots and lateral root tissues ($p <$

0.01), as the salinity increased. A negative relationship was obtained for N, P and Ca content of tissues with salt concentration ($p < 0.01$). Magnesium content exhibited a significant increase in leaves, stems, tap roots and lateral root tissues ($p < 0.01$) in response to increase in salt stress (Table 2). There was a positive relationship for Mg content in tissues with salt concentration in soil ($p < 0.01$).

Micro –elements

There was a significant increase in the concentration of Cu, Mn and Fe ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress. A positive relationship was obtained between soil salinity and Cu, Mn and Fe content in tissues ($p < 0.01$).However, concentration of Zn significantly decreased in leaves, stems, tap roots and lateral roots ($p < 0.01$) with increase in soil salinity (Table 2).There was a negative relationship between Zn content in tissues with salt concentration in soil ($p < 0.01$).

DISCUSSION

Earlier work (Ramoliya et al. 2004), indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dS m^{-1}, but for *Thespesia populnea* SG_{50} was obtained at 5.3 dS m^{-1}.That would suggest that this plant species is relatively salt tolerant at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 2.0 to 5.0dS m^{-1}. Eventually, seeds of *T. populnea* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 10.0 dS m^{-1} was detrimental to seed germination that can

be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentrations of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated. It has been reported that salinity reduces protein hydration (Slater et al., 2003) and induces changes in the activities of many enzymes (Dubey and Rani, 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz and Zeiger, 2006). Moreover, tap root elongation for seedlings grown in control and saline soils both was greater than that of shoot. Result suggests that this species has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington, 1987) and is considered an adaptation to survive in dry habitats. Root / shoot dry weight ratio of *T. populnea* was 0.46 under control conditions and was equal to that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya et al., 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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 (Kramer 1983, Garg and Gupta, 1997).Results for reduction of shoot growth and leaf area development of *T. populnea* with increasing salt concentration are in conformity with the finding of Curtis and 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 and 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. Also high concentration of salt tends to slow down or stop root elongation (Kramer, 1983) and causes reduction in root production (Garg and Gupta, 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of tap roots while lowest in leaves. Consequently, leaves were most resistant and tap roots were sensitive to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: leaves > stems > lateral roots > tap roots. The greater reduction in root weight than in shoot weight resulted in decreased root/shoot dry weight ratio for seedlings with increase in salinity. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion (Marschner, 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration .The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ (Marschner, 1995).Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *T. populnea* survived up to the soil salinity of 10 dS m^{-1} and, therefore, this tree species is moderate salt tolerant. In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much

reduced growth rates on saline substrates (Greenway and Munns, 1980). As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway and Munns, 1980). Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al*.,* 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (Stewart and Lee, 1974). In the present study osmotic adjustment was achieved by increase in quantity of proline and K^+ tissues when water content decreased with increase in salinity. In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar et al., 1994). In the present study, proline accumulation was greater in leaves and stems than that in tap roots and lateral roots as salinity increased. Result corroborates the conclusion of Munns (2002) that organic solutes are often lower in roots than shoots.

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis (Schachtman et al., 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow et al. 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox and Guerinot, 1998). In the present study, significant increase of K^+ content in all the tissues of seedlings with increasing soil salinity might be due to high selectivity of *T. populnea,* for K^+. Gorham (1990) reported that in wheat, salt tolerance is associated with low

rates of transport of Na^+ to shoots with high selectivity for K^+ over Na^+. Further, the exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salt to leaves or growing tissues. Moreover, the significant increase of Na^+ to all tissues of *T. populnea* suggests that this mechanism to block Na^+ transfer to growing tissues was not sufficiently effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively more rapid accumulation of Na^+ than that of K^+. As a result *T.populnea* lacks effective mechanisms to control net uptake of Na^+ on root plasma membrane and subsequently its transport to shoot tissue. The pattern of accumulation of K^+ and Na^+ in *T. populnea* conforms to group C and / or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na^+ without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar (Watad et al.,1991; Schroeder et al., 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}.These cation channels could allow entry of large amount of Na^+ from a highly

saline soil if not adequately regulated(Amtmann and Sanders, 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Watad et al., 1991; Schroeder et al., 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis and Sanders, 1994; Niu et al., 1995). The K^+ and Na^+ profiles of *T. populnea* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al., 2001). As a result, calcium fertilizers may mitigate Na toxicity to the plants.

In general, salinity reduces N accumulation in plants (Feigin, 1985). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Torres and Bingham, 1973; Garg and Gupta, 1997). The Interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol, 1979; Grattan and Grieve, 1992). However it is known that P concentration is related to the rate of photosynthesis, but it decreases the conversion of fixed carbon in to starch (Overlach et al., 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e. g., in preserving membrane integrity (Rengel, 1992), signalling in osmoregulation (Mansfield et al., 1990) and influencing K/Na selectivity (Cramer et al., 1987). In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen and Chang, 1987; Garg and Gupta, 1997). Besides the role of Mg in chlorophyll structure and as an enzyme cofactor,

another important role of Mg in plants is in the export of photosynthates. Therefore, increase of Mg content in all tissues suggests that it has an implication on growth of seedlings.

It is difficult to suggest mechanistic explanations of salinity influence on micro – element concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al., 2000). In the present study, it appears that salinity increased Cu, Mn and Fe accumulation, while reduced Zn accumulation at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox systems (Marschner, 1995), Mn for photosynthetic reaction as part of water splitting enzyme of photosystem II (Cheniae, 1970) and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner, 1995). In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner, 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al. 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater et al., 2003). Increase in Cu, Mn and Fe content at the whole plant level might be the requirement of this plant for survival and growth in response to salinity.

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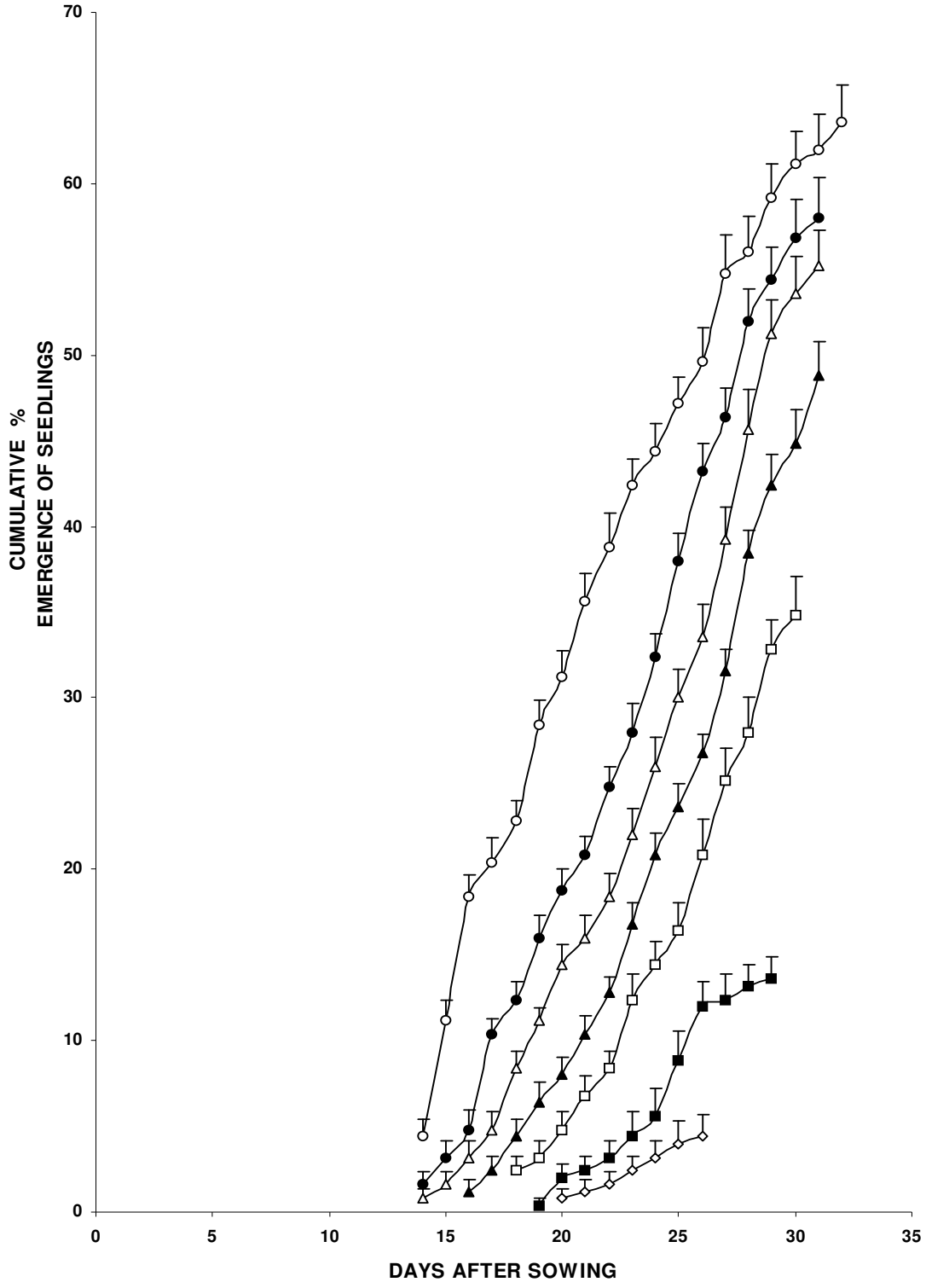

Fig.1. Cumulative emergence of seedlings of *Thespecia populnea* in response to salinity 0.2 dS m^{-1} (○), 3.9dS m^{-1}(●), 6.2dS m^{-1} (Δ), 8.1dS m^{-1} (▲), 10.0dS m^{-1} (□) , 11.9dS m^{-1} (■) and 13.9 dS m^{-1} (◊). Error bars represent SE.

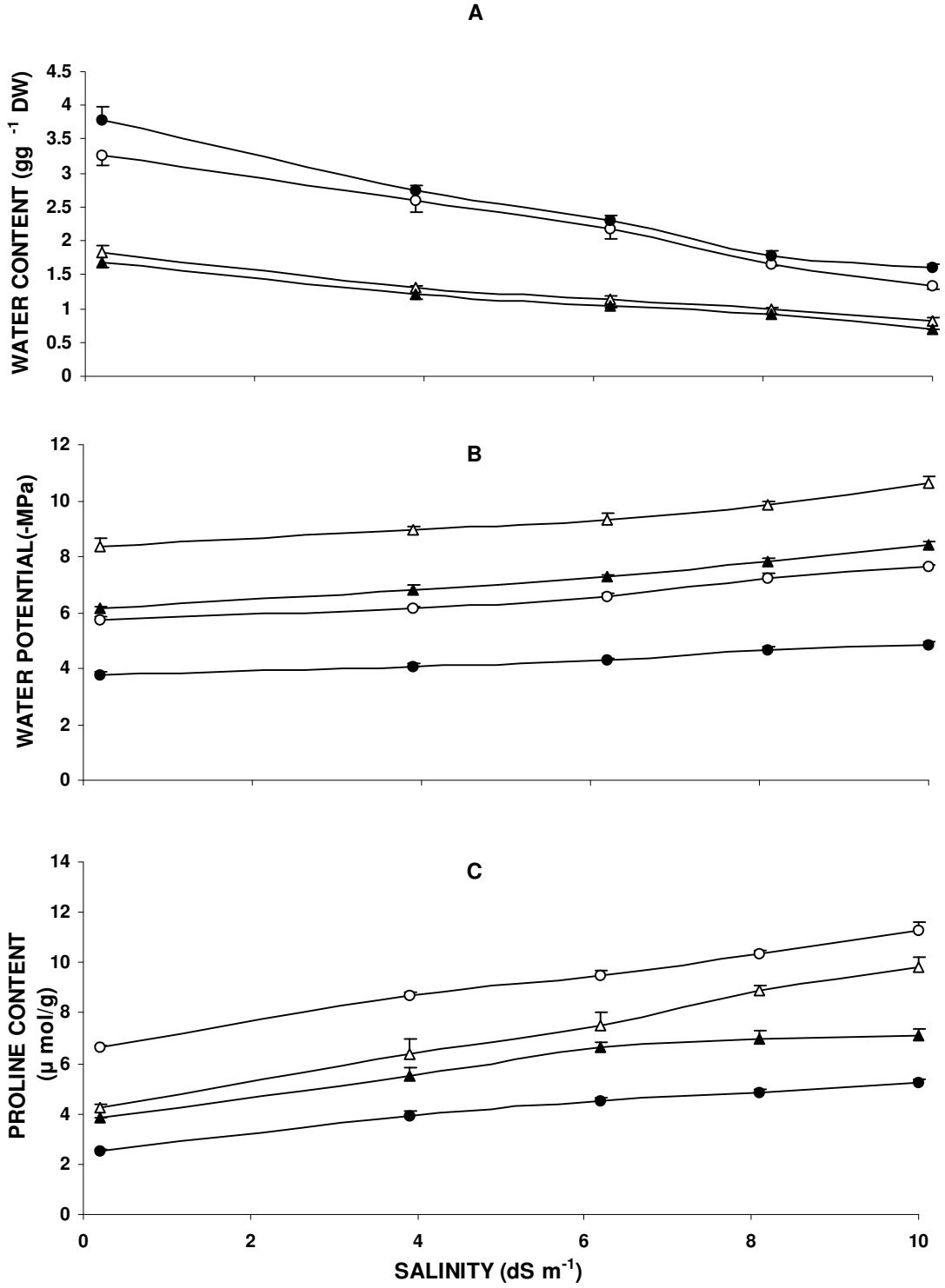

Fig.2. Effect of soil salinity on water potential (gg $^{-1}$ DW) (A),water potential (-Mpa) (B) and proline content (µ mole/g FW) (C) of leaves (○),stem(Δ),tap root(▲) and lateral roots(●) of *Thespesia populnea* seedlings. Error bars represent SE.

Table 1. Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Thespesia populnea* as indicated by mean ± SEM and regression equation constants.

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area (cm^2plant^{-1})	Leaf weight (mg $plant^{-1}$)	Stem weight (mg $plant^{-1}$)	Shoot weight (leaf+stem) (mg $plant^{-1}$)	Tap root weight (mg $plant^{-1}$)	Lateral root weight (mg $plant^{-1}$)	Total root weight (mg $plant^{-1}$)
0.2	37.2±0.7	42.6±0.7	276.3±4.5	1756.0±33.0	1593.2±30.9	3349.1±49.7	1019.0±18.5	530.9±13.5	1549.9±22.5
3.9	30.4±0.6	36.4±0.7	226.8±3.7	1372.1±26.4	1200.5±15.6	2572.6±34.1	767.3±20.4	400.4±7.0	1167.7±24.1
6.2	27.0±0.6	32.8±0.6	215.3±3.9	1262.3±15.3	1097.5±24.6	2359.8±33.4	630.6±8.4	342.5±9.3	973.1±14.8
8.1	23.7±0.4	25.4±0.8	182.2±2.2	1102.7±13.3	936.8±11.9	2039.5±19.2	503.8±8.5	318.0±11.4	821.8±13.8
10.0	21.4±0.4	22.5±0.7	176.7±1.7	997.9±14.8	834.1±16.4	1831.9±24.3	442.7±9.0	262.7±3.7	705.4±12.5
α	37.2	43.91	274.37	1732.00	1566.00	3298.00	1014.60	521.64	1536.20
β	-1.62	-2.10	-10.37	-76.38	-76.33	-152.72	-60.19	-26.54	-86.74
r	-0.910	-0.908	-0.910	-0.930	-0.933	-0.953	-0.954	-0.898	-0.961
$LSD_{0.05}$	4.3	5.3	25.7	166.2	159.0	255.4	106.0	72.7	137.5

Relationship is significant at $p < 0.01$.

Table 2. (Part 1) Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Thespesia populnea* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	26.4±0.5	2.9±0.1	13.5±0.2	4.5±0.1	24.6±0.6	4.0±0.3	3.0±0.1	96±1.2	8±0.6	58±1.5	311±4.2
	3.9	20.8±0.4	2.6±0.1	18.9±0.2	8.8±0.7	20.7±0.6	4.6±0.2	2.2±0.1	68±2.1	9±1.0	62 ±1.3	329±2.1
	6.2	18.7±0.4	2.4±0.1	20.6±0.9	11.1±0.5	20.2±0.3	4.9±0.2	1.9±0.1	54±2.3	10±1.1	66±1.8	370±1.7
	8.1	14.6±0.3	2.3±0.1	22.2±1.7	12.5±0.2	17.6±0.5	5.4±0.2	1.8±0.0	43±2.6	12±1.2	74±2.0	494±5.6
	10.0	14.4±0.2	2.1±0.2	24.1±1.0	13.9±0.2	10.7±0.6	5.8±0.1	1.7±0.0	36±1.5	15±1.4	94±1.2	495±4.5
	α	26.27	2.93	13.90	4.71	25.84	3.90	2.78	94.61	6.96	52.55	278.69
	β	-1.28	-0.08	1.04	0.96	1.24	0.18	-0.12	-6.19	0.67	3.23	21.32
	r	-0.977	-0.854	0.924	0.979	0.908	0.915	-0.892	-0.984	0.790	0.867	0.909
	$LSD_{0.05}$	1.1	1.1	2.9	1.2	1.6	0.5	0.3	5.9	3.2	4.7	11.5
Stem	0.2	21.5±0.5	1.9±0.1	11.8±0.0	3.4±0.2	13.9±0.5	3.7±0.0	3.5±0.2	60±1.5	11±1.5	19±1.4	211±1.7
	3.9	18.8±0.2	1.7±0.1	12.9±0.1	5.3±0.1	10.3±0.3	4.9±0.1	2.4±0.1	46±3.0	13±1.0	20±1.5	283±2.1
	6.2	15.1±0.6	1.5±0.1	14.1±0.1	6.3±0.1	8.9±0.2	5.1±0.1	2.2±0.0	43±1.7	15±1.2	22±2.1	313±1.5
	8.1	14.0±0.1	1.3±0.1	14.8±0.1	8.6±0.7	8.1±0.5	5.5±0.1	1.7±0.0	26±3.1	16±1.04	29±1.4	325±2.6
	10.0	12.8±0.6	1.1±0.1	15.1±0.2	9.2±0.7	6.9±0.6	5.7±0.2	1.6±0.0	23±2.3	18±0.3	39±1.1	402±1.0
	α	21.75	1.94	11.72	3.06	13.59	3.87	3.38	61.74	10.59	14.98	206.31
	β	-0.93	-0.08	0.35	0.61	0.69	0.19	-0.19	-3.89	0.70	1.90	17.69
	r	-0.967	-0.888	0.981	0.945	0.953	0.946	-0.959	-0.944	0.839	0.833	0.971
	$LSD_{0.05}$	1.3	0.3	0.3	1.3	1.3	0.3	0.3	7.1	3.2	4.5	5.5

Relationship is significant at $p < 0.01$.

Table 2. (Part 2) Effect of salinisation of soil on nutrient content of tissues (tap roots and lateral roots) of *Thespesia populnea* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	18.7±0.3	1.4±0.1	11.3±0.1	3.0±0.1	6.7±0.1	3.4±0.1	3.8±0.1	65±2.1	9±0.6	29±1.6	1218±5.3
	3.9	15.8±0.1	1.2±0.1	12.6±0.1	4.5±0.6	4.9±0.2	4.4±0.1	2.8±0.0	64±1.2	11±1.5	33±1.0	1554±6.5
	6.2	13.7±0.4	1.1±0.1	13.5±0.2	5.6±0.1	4.5±0.2	4.6±0.0	2.4±0.1	43±2.6	13±1.2	38±1.1	1630±5.8
	8.1	12.2±0.8	1.0±0.1	13.7±0.2	6.4±0.2	4.3±0.1	4.8±0.1	2.1±0.1	38±3.5	15±1.4	40±0.8	1738±6.2
	10.0	10.8±0.4	0.9±0.0	13.9±0.2	8.8±0.2	3.6±0.0	5.1±0.1	1.6±0.0	31±3.1	17±0.7	45±0.8	1974±4.0
	α	18.88	1.43	11.45	2.53	6.50	3.56	3.75	69.93	8.33	27.86	1217.20
	β	-0.81	-0.05	0.27	0.55	0.29	0.16	-0.21	-3.82	0.82	1.60	71.40
	r	-0.974	-0.875	0.944	0.948	0.949	0.937	-0.982	-0.909	0.858	0.951	0.983
	$LSD_{0.05}$	1.3	0.2	0.5	0.8	0.4	0.3	0.2	7.6	3.3	3.2	16.5
Lateral roots	0.2	19.8±0.2	1.6±0.1	13.3±0.2	6.7±0.2	8.0±0.2	7.7±0.1	2.0±0.0	85±2.1	13±1.2	93±1.5	5612±4.2
	3.9	16.7±0.3	1.4±0.1	17.0±0.6	10.6±0.5	6.6±0.2	8.5±0.1	1.6±0.0	73±0.6	15±1.0	101±1.2	5925±6.5
	6.2	15.1±0.3	1.3±0.1	18.1±0.4	12.1±0.6	5.8±0.1	8.7±0.2	1.5±0.0	60±1.7	17±0.8	108±2.0	6009±4.9
	8.1	14.1±0.2	1.1±0.1	18.9±0.2	13.9±0.2	4.8±0.2	8.8±0.2	1.4±0.0	49±2.6	18±1.0	135±1.8	6696±6.7
	10.0	11.5±0.2	1.0±0.1	20.8±0.3	15.6±0.2	4.1±0.1	8.9±0.2	1.3±0.0	43±1.2	20±1.0	147±2.0	6724±5.5
	α	20.02	1.60	13.52	6.72	8.13	7.84	1.93	87.48	12.59	84.62	5500.10
	β	-0.80	-0.05	0.72	0.89	0.40	0.11	-0.06	-4.48	0.70	5.66	122.03
	r	-0.985	-0.836	0.966	0.982	0.986	0.834	-0.964	-0.979	0.857	0.926	0.939
	$LSD_{0.05}$	0.6	0.3	1.1	1.1	0.4	0.5	0.1	5.2	2.9	5.1	16.5

Relationship is significant at $p < 0.01$.

EXPERIMENT – 4

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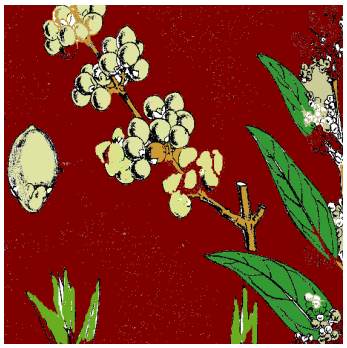

Salvadora oleoides **(Decne.) (Salvadoraceae)**

Growth, water status and nutrient accumulation of seedlings of *Salvadora oleoides* (Salvadoraceae) in response to soil salinity

Seema Abhay Hardikar[1] **and Amar Nath Pandey**[2*]

1 Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:abhay.hardikar@sgs.com

E-mail:seemahardikar@yahoo.com

2* **Correspondence**

A. N. Pandey

Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:anpandey2001@gmail.com

Tel (O): +91-281-2586419

Tel (M): +919427495989

Fax (O): +91-281-2577633

Short title (to be used as running head):

Effect of soil salinity on *Salvadora oleoides*.

Abstract

Greenhouse experiments were conducted to assess the effect of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Salvadora oleoides* (Decne.) (Salvadoraceae). NaCl was added to the soil and salinity was maintained at 0.2, 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. Salinity caused reduction in water content and water potential of tissues that resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased as salinity increased. Proline content in tissues increased as salinity increased. Potassium and sodium content significantly increased in tissues as salinity increased. Moreover, this species has high selectivity for K^+ and lacks effective mechanisms to control net uptake of Na^+ and its transport to shoot tissues. Changes in tissues and whole-plant accumulation patterns of other nutrients, as well as possible mechanisms for avoidance of Na toxicity in this species in response to salinity, are discussed.

Key words: Soil salinity, seedling growth, proline content, water potential, macro- and micro-nutrients, salt tolerance.

Introduction

Soil salinity has detrimental effects on seed germination and plant growth (Bernstein 1962; Taiz and Zeiger 2006; Ramoliya *et al.* 2006). However plant species differ in their sensitivity or tolerance to salts (Marschner 1995). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman 1977; Munns 2002; Ramoliya *et al.* 2006).However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt – induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g., Maas and Grieve 1987; Cramer *et al.* 1989; Ramoliya *et al.* 2006; Patel and Pandey 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu *et al.* 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanisms that plants use in the avoidance and /or tolerance of salt stress.

Salvadora oleoides (Decne.)(Salvadoraceae), a tree species is native to India and Pakistan grows naturally by seed germination and is one of the dominant tree species in the vast area of Kutch (north-west saline desert) in Gujarat State of India. It also grows successfully in the coastal area as well as in the non- saline and marginal semi-arid (closer to arid) central area of Saurashtra region, to the south of Kutch. This tree species is of multipurpose use because of its oil-yielding potential,

pharmaceutical application, fodder and fuel values and many others. However, the potential of this tree species to grow and survive in coastal area of Saurashtra and in saline desert of Kutch is not known. The present investigation was performed with the following objectives: (i) to understand the adaptive features of *S.oleoides* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

Materials and Methods

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22^0 18' N Lat, $70^0$56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil, which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6 % sand, 20.3 % silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dS m^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier (Patel and Pandey, 2007). The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid – June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj ($23^0$15' N Lat, $69^0$49' E Long) in Kutch and about 554 mm at Rajkot in the central Saurashtra which occurs

totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June – September) and winter (November – February).The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Salinisation of soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil of 100 kg each were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 390, 700, 1070, 1275, 1530 and 1800 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.2 dS m^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 17 July 2007. Seeds of *S. oleoides* were collected from the coastal area of Arabian Sea at Jamnagar district of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered (about 300 mL water was added to raise the soil moisture to field capacity) and thereafter similar amount of water was added to the soil on alternate days. Irrigation of soil with required amount of water was taken as a measure to

control the level of soil salinity. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity using the expression:

$Sin^{-1}\sqrt{p} = \beta_0 + \beta_1 X$

where, $Sin^{-1}\sqrt{p}$ 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 estimated using the model.

Seedling growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 3.9 and 6.2 dS m^{-1} salinity exhibited emergence of the second leaf after 21 days, whereas the second leaf on seedlings grown in soils at 8.1, 10.0, 11.9 and 13.9 dS m^{-1} salinity appeared after 27 days. Emergence of the second leaf confirmed the establishment of seedlings. Moreover, only 12% seed germination was recorded in soils at 13.9 dS m^{-1} salinity, and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialized with six grades of soil (0.2, 3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1}) were prepared. This gave a total of 120 bags, which were arranged in twenty randomized blocks. Seedlings were watered (to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. For each salinity level, about 50 g surface soil was taken out from 3 bags at the intervals of one month and salinity was measured. Soil salinity varied within a limited range with time. Experiment was terminated on 17 January 2008. The diurnal temperature inside the greenhouse was similar to that of ambient atmosphere. Seedlings contained in 20 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root

length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of tissues were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of water potential and proline content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates *et al.* (1973). Extract of 0.5 g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

Mineral analyses of plant materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three subsamples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper 1944). Concentrations of Ca, Mg, Na K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3:H_2SO_4:$HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear

regression equations between mineral content and salt concentrations were determined.

Results

Effect of salinisation on seedling emergence

Seedlings began to emerge 6 days after sowing and 73.6% seed germination was obtained over a period of 25 days, under control (0.2 dS m^{-1} salinity) conditions (Fig. 1). Seedling emergence in saline soils was recorded 6-11days after sowing. Seedling emergence lasted for 26, 26, 26, 25, 25 and 22 days in soils with 3.9, 6.2, 8.1, 10.0,11.9 and 13.9 dS m^{-1} salinities, respectively and corresponding seed germination was 62.8%, 52%, 49.6%, 43.2%, 35.6% and 12%. There was a significant reduction in seed germination ($p < 0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y= 62.846-2.521X$, ($R^2_{Adj}=0.895$, $p < 0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinisation on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p < 0.01$) stem and root elongation (Table 1). Nevertheless, root length was remarkably greater than shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p < 0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p < 0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p < 0.01$).

Effect of salinisation on dry weight

Dry weight significantly decreased ($p < 0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing

concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weight of tissues of salinised plants compared to those of control plants was computed as: (salinised tissue dry weight / control dry weight) × 100. Dry weight values of tissues given in Table 1 were used for the calculation of percent relative weight of tissues. Values of percent relative weight varied from 77.6 to 49.5% for leaves, from 78.1 to 43.5 % for stems, 74.6 to 39.6 % for tap roots and from 74.8 to 45.9 % for lateral roots in response to increasing soil salinity from 3.9 to 11.9 dS m^{-1}. As has been estimated using regression equations given in results , the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 10.8, 9.3, 8.9 and 9.8 for leaves, stems, tap roots and lateral root tissues , respectively. Root/shoot dry weight ratio was 0.60 under control conditions while it was 0.58, 0.58, 0.57, 0.55 and 0.53 for seedlings grown in soils at 3.9, 6.2, 8.1, 10.0 and 11.9dS m^{-1}salinities, respectively. Root / shoot dry weight ratios significantly decreased ($p < 0.05$) as soil salinity increased. There was a negative relationship between root / shoot dry weight ratio and soil salinity ($r = -0.300$, $p < 0.01$).

Effect of salinisation on water content of tissues

Water content in leaves, stems, tap roots and lateral root tissues significantly decreased ($p < 0.01$) with increasing concentration of salt in soil (Fig. 2*A*). There was maximum water content in leaves and minimum in tap roots. Tissues according to their water content can be arranged in the following decreasing order: leaves > lateral roots > stems > tap roots. There was a negative relationship between water content in different tissues and salt concentration ($r= -0.709$, -0.582, -0.717 and -0.514, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on water potential of tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p < 0.01$) as soil salinity increased (Fig. 2*B*). Tissues according to their water potential values (low to high negative) can be arranged in the following decreasing order: lateral roots > leaves > tap roots > stems. There was a negative relationship between water potential of tissues and salt concentration ($r = -0.832$, -0.841, -0.855 and -0.889, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) . A positive relationship was obtained between water content and water potential (negative value) ($r = 0.898$, 0.944, 0.969 and 0.900, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) .

Effect of salinisation on proline content of tissues

Proline content (µ mol/g FW material) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues, with increase in soil salinity (Fig. 2*C*).Tissues according to their proline content can be arranged in the following decreasing order: stems > tap roots > leaves> lateral roots. There was a positive relationship between salt concentration and proline content of tissues ($r = 0.953$, 0.811, 0.908 and 0.948, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content ($r = -0.887$, -0.794, -0.826 and -0.860, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content ($r = -0.980$, -0.975, -0.965 and -0.960, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on mineral accumulation

Potassium and sodium content and K/Na ratio

Potassium and sodium content (as mg g^{-1} dry weight) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a positive relationship for K and Na content in leaves, stems, taproots and lateral roots with increase in salt concentration in soil ($p < 0.01$).

The K/Na ratio significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A positive relationship was obtained between K /Na ratio in tissues and increase in salt stress ($p < 0.01$).

Nitrogen, phosphorus, calcium and magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen content exhibited a significant increase ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress. There was a significant positive relationship between N content in tissues and salt concentration in soil ($p < 0.01$). Phosphorus, calcium and Magnesium content significantly decreased in leaves , stems , tap roots and lateral root tissues ($p < 0.01$), as the salinity increased (Table 2). A negative relationship was obtained between P, Ca and Mg content of tissues and salt concentration ($p < 0.01$).

Micro –elements

There was a significant increase in the concentration of Cu, Mn and Fe ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress (Table 2). A positive relationship was obtained between soil salinity and Cu, Mn and Fe content in tissues ($p < 0.01$).However, concentration of Zn significantly decreased in leaves, stems, tap roots and lateral roots ($p < 0.01$) with increase in soil salinity (Table 2).There was a negative relationship between Zn content in tissues and salt concentration in soil ($p < 0.01$).

Discussion

Earlier work (Ramoliya *et al.* 2004) indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dS m^{-1},but for *Salvadora oleoides* SG_{50} was obtained at 6.9 dS m^{-1}.That would suggest that this plant species is relatively salt tolerant at seed germination. Under

field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 2.0 to 5.0 dS m^{-1}. Eventually, seeds of *S. oleoides* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 11.9 dS m^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentrations of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Slater *et al.* 2003) and induces changes in the activities of many enzymes (Dubey and Rani 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz and Zeiger, 2006). Root / shoot dry weight ratio of *S. oleoides* was 0.60 under control conditions and was greater than that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya *et al.* 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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(Kramer 1983; Garg and Gupta 1997).Results for reduction of shoot growth and leaf area development of *S. oleoides* with increasing salt concentration are

in conformity with the finding of Curtis and 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 and 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. Also high concentration of salt tends to slow down or stop root elongation (Kramer 1983) and causes reduction in root production (Garg and Gupta 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of tap roots while the lowest in leaves. Consequently, leaves were most resistant and tap roots were sensitive to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: leaves >lateral roots> stems >tap roots. The greater reduction in root weight than in shoot weight resulted in decreased root/shoot dry weight ratio for seedlings with increase in salinity. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion (Marschner 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration .The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ (Marschner 1995).Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *S. oleoides* survived up to the soil salinity of 11.9 dS m^{-1} and, therefore, this tree species is moderate salt tolerant .In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates (Greenway and Munns 1980). As a

result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway and Munns 1980). Considering selectivity of ions by root cells it is still unclear which cell types control the selectivity of ions from the soil solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa *et al.* 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (Stewart and Lee 1974).In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar *et al.* 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis (Schachtman *et al.* 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow *et al.* 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox and Guerinot 1998). In the present study, significant increase of K^+ content in all the tissues of seedlings with increasing soil salinity might be due to high selectivity of *S. oleoides* for K^+. Gorham (1990) reported that in wheat, salt tolerance is associated with low rates of transport of Na^+ to shoots with high selectivity for K^+ over Na^+. Further, the exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salt to leaves or growing tissues. Moreover, the significant increase of Na^+ to all tissues of *S. oleoides* suggests that this mechanism to block Na^+ transfer to growing tissues was not sufficiently effective at high salt concentration. Significant increase in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively more rapid accumulation of K^+ than that of Na^+. At the same time, results also suggest that there

were no effective mechanisms to control net uptake of Na on root plasma membrane and subsequently its transport to shoot tissues. The pattern of accumulation of K^+ and Na^+ in *S. oleoides* conforms to group C and / or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na^+ without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar (Watad *et al.* 1991; Schroeder *et al.* 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}.These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated (Amtmann and Sanders 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Watad *et al.* 1991; Schroeder *et al.* 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis and Sanders 1994; Niu *et al.* 1995).The K^+ and Na^+ profiles of *S. oleoides* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus *et al.* 2001).As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant. Results further suggest that sodium accumulation was greater in stem tissues than in leaves. It can be attributed to cell types in stems that are better able to retain Na^+. Considering that stem tissues will be reinforced by growth with

time, it can be predicted that after seedling stage Na tolerance of plants may increase above 11.9 dS m^{-1} salinity which is maximum salt concentration in this experiment.

In general, salinity reduces N accumulation in plants (Feigin 1985), but in this plant nitrogen increased with increase in salinity. Dubey and Rani (1989) reported that protein level in several crops under salinisation increases due to the increased synthesis of pre-existing and certain new sets of proteins. The interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol 1979; Grattan and Grieve 1992). However it is known that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach *et al.* 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e. g., in preserving membrane integrity (Rengel 1992), signalling in osmoregulation (Mansfield *et al.* 1990) and influencing K^+/Na^+ selectivity (Cramer *et al.* 1987). In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions , precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen and Chang 1987; Garg and Gupta 1997).Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates, which when impaired leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner and Cakmak, 1989).

It is difficult to suggest mechanistic explanations of salinity influence on micro–element concentration due to relatively smaller differences between control and salinised tissues (Tozlu *et al.* 2000). In the present study, it appears that salinity

increased Cu, Mn and Fe accumulation, while reduced Zn accumulation at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox systems (Marschner 1995), Mn for photosynthetic reaction as part of water splitting enzyme of photosystem II (Cheniae 1970) and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner 1995). In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani *et al.* 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater *et al.* 2003). Increase in Cu, Mn and Fe content at the whole plant level might be the requirement of this plant for survival and growth in response to salinity.

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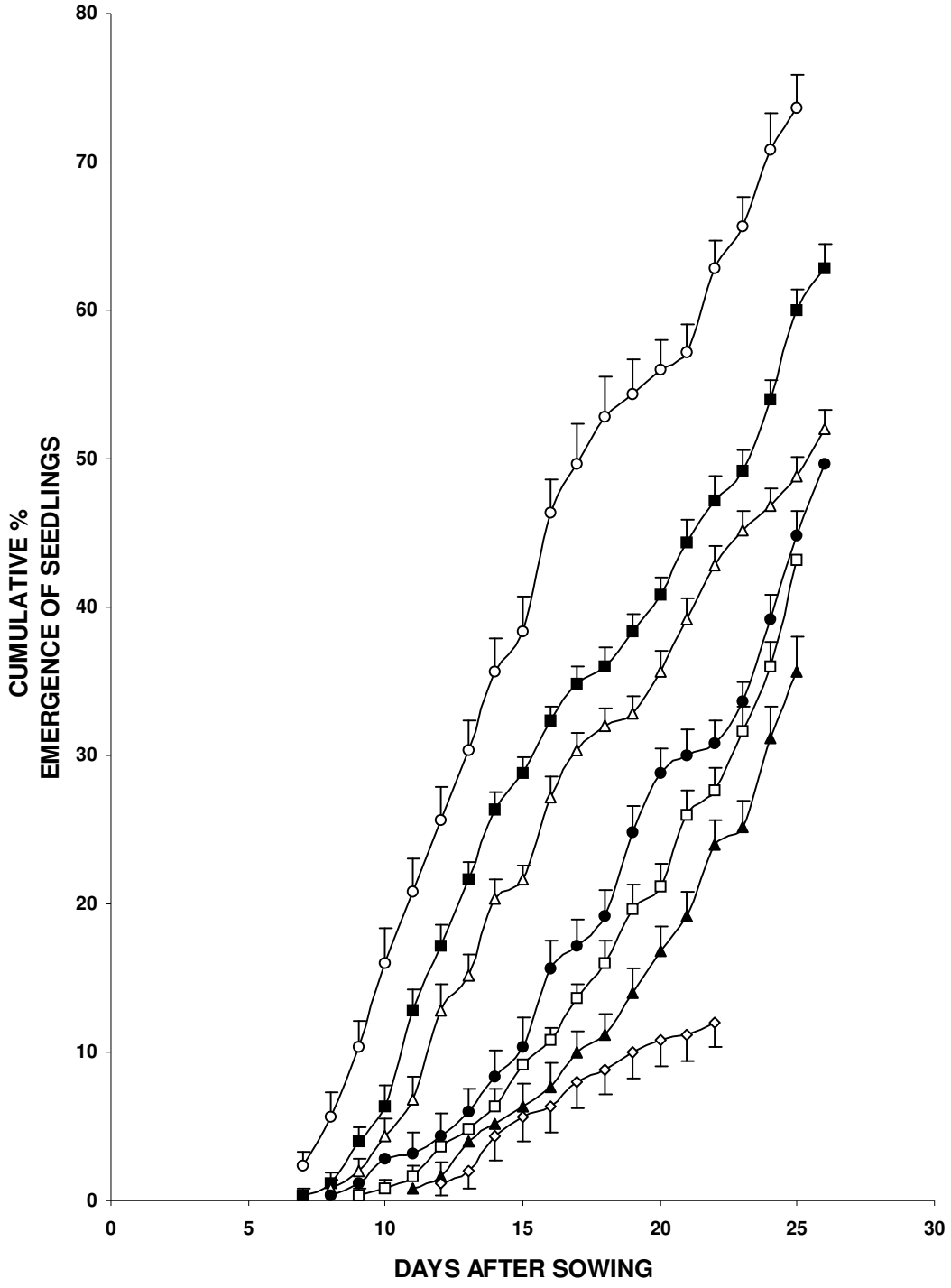

Figure 1. Cumulative emergence of seedlings of *Salvadora oleoides* in response to soil salinity 0.2 dS m^{-1} (○), 3.9dS m^{-1} (■), 6.2dS m^{-1} (Δ), 8.1dS m^{-1} (●), 10.0dS m^{-1} (□) , 11.9dS m^{-1} (▲) and 13.9dS m^{-1} (◊). Error bars represent SE.

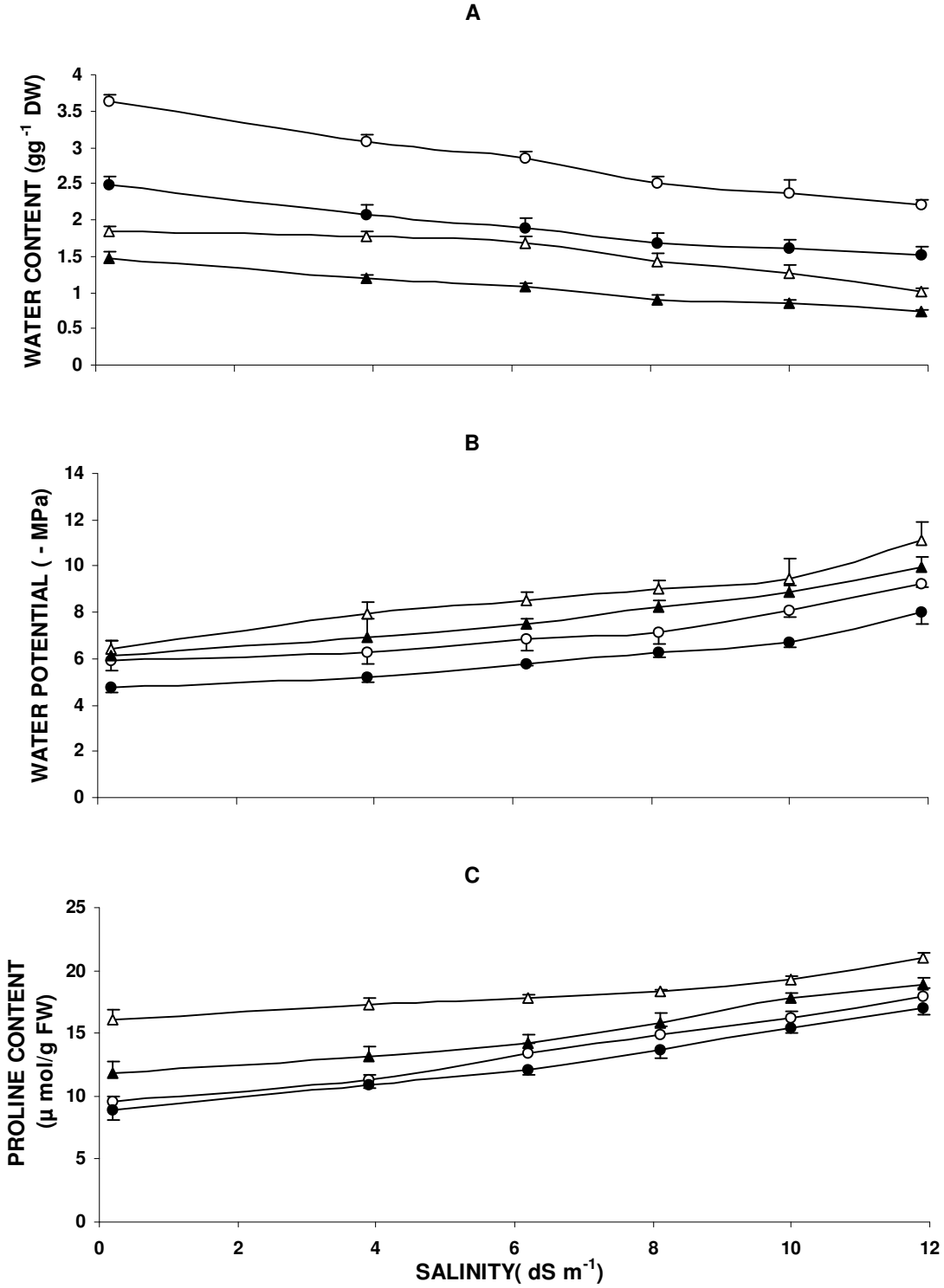

Fig.2. Effect of soil salinity on A. water content (gg $^{-1}$ DW), B. water potential (-Mpa) and C. proline content (µ mol/g FW) of leaves (○), stem (Δ), tap root (▲), and lateral roots (●) of *Salvadora oleoides* seedlings. Error bars represent SE.

Table 1. Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Salvadora oleoides* as indicated by mean ± SEM and regression equation constants.

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area ($cm^2 plant^{-1}$)	Leaf weight (mg $plant^{-1}$)	Stem weight (mg $plant^{-1}$)	Shoot weight (leaf+stem) (mg $plant^{-1}$)	Tap root weight (mg $plant^{-1}$)	Lateral root weight (mg $plant^{-1}$)	Total root weight (mg $plant^{-1}$)
0.2	27.1 ± 0.9	35.5±0.7	59.5±1.9	340.7±8.0	390.1±9.4	730.8±13.7	330.3±6.0	104.5±2.9	434.8±6.6
3.9	25.1±0.7	31.9±0.7	54.2±2.0	264.6±8.8	304.6±10.7	569.1±17.8	246.6±3.9	78.2±3.5	324.8±6.8
6.2	22.8±0.7	27.1±0.8	45.6±1.3	230.3±8.6	221.4±8.8	451.7±11.2	192.3±4.6	67.4±1.7	259.5±5.2
8.1	20.7±0.7	24.1±0.7	40.0±1.6	194.6±7.2	198.5±7.5	393.1±9.4	166.4±4.0	56.9±2.7	223.2±5.4
10.0	18.2±0.7	22.2±0.6	32.9±1.7	184.9±7.8	183.8±5.9	368.7±8.3	152.2±4.5	50.9±1.9	203.1±5.7
11.9	16±0.6	20.3±0.7	28.8±1.1	168.7±5.82	169.9±3.6	338.6±8.2	130.7±2.3	48±1.2	178.7±2.8
α	28.2	36.07	62.22	329.9	375.41	705.31	317.63	100.48	418.08
β	-0.97	-1.37	-2.79	-14.78	-19.46	-34.24	-17.06	-4.89	-21.95
r	-0.75	-0.865	-0.827	-0.849	-0.881	-0.910	-0.942	-0.855	-0.943
$LSD_{0.05}$	5.7	5.2	12.4	58.6	60.4	90.3	33.1	18.7	42.2

Relationship is significant at $p < 0.01$.

Table 2. (Part 1)Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Salvadora oleoides* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g\text{-}^{1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	17.6±0.2	2.4±0.2	22.3±0.6	6.8±0.1	34.6±0.4	8.5±0.3	3.3±0.1	53±1.2	49±0.6	44±0.1	251±3.5
	3.9	19.6±0.2	2.3±0.1	26.5±0.3	7.1±0.1	26.4±0.1	7.4±0.2	3.7±0.1	46±0.5	58±1.0	49±2.1	269±0.6
	6.2	20.2±0.1	1.9±0.2	31.1±0.6	7.9±0.1	24.6±0.6	6.7±0.2	3.9±0.1	38±0.3	66±0.8	53±0.2	331±2.3
	8.1	21.1±0.0	1.4±0.1	34.6±0.8	8.6±0.2	20.8±0.1	4.2±0.1	4.0±0.1	30±0.1	73±0.3	61±0.9	335±2.6
	10.0	21.7±0.1	1.2±0.1	39.8±0.1	9.7±0.2	18.4±0.2	3.1±0.1	4.1±0.1	27±0.3	81±1.4	68±2.1	389±1.2
	11.9	22.6±0.1	1.1±0.1	42.9±0.2	10.3±0.1	16.2±0.5	3.0±0.1	4.2±0.0	23±0.3	88±0.6	71±1.2	460±1.5
	α	17.69	2.55	20.6	6.24	33.91	9.06	3.38	54.28	46.44	40.83	223.00
	β	0.41	-0.12	1.82	0.32	-1.54	-0.53	0.07	-2.69	3.42	2.49	17.29
	r	0.989	-0.906	0.985	0.952	-0.986	-0.956	0.878	-0.988	0.991	0.961	0.949
	$LSD_{0.05}$	0.4	0.4	1.5	0.4	1.1	0.5	0.3	1.7	2.5	3.9	6.4
Stem	0.2	10.5±0.3	1.7±0.1	31.9±0.3	10.8±0.2	36.3±0.1	7.6±0.2	3.0±0.0	68±0.7	29±0.9	37±0.9	186±2.1
	3.9	11.2±0.4	1.2±0.1	37.5±0.6	12.2±0.2	30.0±0.6	6.2±0.2	3.1±0.1	65±1.1	34±1.0	38±0.9	197±0.6
	6.2	12.2±0.2	1.1±0.1	40.4±0.5	13.0±0.1	28.9±0.3	5.0±0.2	3.1±0.0	59±0.5	42±0.6	39±0.1	255±1.7
	8.1	13.2±0.1	0.9±0.2	43.9±0.5	13.6±0.2	26.8±0.9	3.3±0.1	3.2±0.1	52±1.9	56±1.0	41±1.5	277±2.3
	10.0	14.3±0.2	0.7±0.2	46.5±0.3	14.0±0.1	25.8±0.6	2.9±0.1	3.3±0.0	48±1.1	63±0.3	43±0.3	286±1.0
	11.9	15.1±0.1	0.4±0.0	54.2±0.3	16.1±0.4	18.8±0.1	2.8±0.3	3.4±0.1	46±1.0	66±1.1	44±0.3	298±0.7
	α	9.97	1.17	30.53	10.54	36.46	7.68	2.93	70.57	24.51	35.78	177.78
	β	0.41	-0.10	1.76	0.41	-1.29	-0.45	0.04	-2.14	3.54	0.66	10.73
	r	0.958	-0.898	0.973	0.952	-0.945	-0.966	0.823	-0.961	0.966	0.885	0.959
	$LSD_{0.05}$	0.8	0.4	1.3	0.6	1.8	1.7	0.2	3.4	2.6	2.4	4.6

Relationship is significant at $p < 0.01$

Table 2. (Part 2) Effect of salinisation of soil on nutrient content of tissues (tap root and lateral root) of *Salvadora oleoides* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	11.6±0.3	1.9±0.1	28.6±0.3	6.6±0.1	26.4±0.3	5.7±0.2	4.3±0.1	47±0.6	42±1.5	36±0.6	199±1.3
	3.9	12.3±0.4	1.7±0.1	37.7±0.3	8.5±0.1	24.7±0.5	4.7±0.3	4.4±0.1	44±0.5	52±1.0	37±1.2	219±3.8
	6.2	13.2±0.6	1.3±0.1	42.8±0.8	9.3±0.1	20.8±0.1	4.1±0.2	4.6±0.0	35±0.5	61±1.8	39±0.3	264±1.0
	8.1	14.0±0.6	1.1±0.1	46.6±0.5	9.8±0.2	19.4±0.2	3.3±0.1	4.8±0.1	32±0.4	67±1.7	40±0.1	289±1.5
	10.0	14.8±0.6	0.9±0.1	49.8±0.8	10.0±0.1	17.7±0.3	3.0±0.6	5.0±0.1	30±0.2	72±1.1	42±0.5	320±1.6
	11.9	15.8±0.3	0.7±0.0	52.6±0.5	10.2±0.3	14.3±0.4	2.2±0.0	5.2±0.2	22±0.9	83±1.9	43±0.4	333±1.9
	α	11.20	2.01	29.18	7.04	27.51	5.81	4.21	49.27	39.93	35.43	186.53
	β	0.36	-0.11	2.06	0.30	-1.03	-0.29	0.07	-2.12	3.41	0.64	12.52
	r	0.884	-0.923	0.990	0.935	-0.976	-0.941	0.844	-0.972	0.980	0.954	0.983
	$LSD_{0.05}$	1.4	0.3	1.7	0.4	1.0	0.8	0.3	1.6	4.5	1.3	6.0
Lateral roots	0.2	16.4±0.3	2.8±0.1	25.9±1.1	10.4±0.5	20.7±0.2	7.8±0.2	2.5±0.2	59±0.4	23±0.3	57±0.3	829±1.2
	3.9	17.0±0.3	2.7±0.1	28.7±0.2	10.8±0.2	16.5±0.5	6.5±0.5	2.7±0.2	52±0.4	34±1.0	60±1.5	869±1.7
	6.2	17.2±0.2	2.3±0.1	32.4±0.0	11.2±0.1	15.6±0.2	6.4±0.4	2.9±0.0	45±1.6	42±1.5	66±1.0	908±1.5
	8.1	18.4±0.2	2.1±0.1	37.8±0.4	12.4±0.2	13.8±0.2	4.9±0.3	3.0±0.0	34±1.0	46±0.3	77±1.7	934±2.6
	10.0	19.3±1.0	1.9±0.1	42.2±0.4	13.5±0.3	12±0.5	3.9±0.0	3.1±0.1	33±0.4	53±1.1	106±2.1	978±1.3
	11.9	21.2±0.3	1.7±0.1	45.3±0.1	14.2±0.0	10.2±0.3	2.9±0.5	3.2±0.1	32±0.1	57±0.4	114±1.7	1049±0.6
	α	15.67	2.95	23.54	9.76	20.63	8.21	2.47	59.88	22.71	44.67	807.14
	β	0.38	-0.10	1.76	0.34	-0.87	-0.42	0.06	-2.59	2.94	5.22	17.97
	r	0.854	-0.921	0.972	0.924	-0.984	-0.931	0.890	-0.960	0.992	0.906	0.971
	$LSD_{0.05}$	1.4	0.3	1.5	0.7	1.0	1.1	0.2	2.4	2.6	3.8	4.7

Relationship is significant at $p < 0.01$

EXPERIMENT – 5

COMMUNICATED TO

South African Journal of Botany

***Sapindus emarginatus* Vahl. (Sapindaceae)**

Growth, water status and nutrient accumulation of seedlings of *Sapindus emarginatus* Vahl. in response to soil salinity

Seema Abhay Hardikar[1] **and Amar Nath Pandey**[2*]

1 Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:abhay.hardikar@sgs.com

E-mail:seema.a.hardikar@gmail.com

2[*] **Correspondence**

A. N. Pandey

Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:anpandey2001@gmail.com

Tel (O): +91-281-2586419

Tel (M): +919427495989

Fax (O): +91-281-2577633

Short title (to be used as running head):

Effect of soil salinity on *Sapindus emarginatus*.

Abstract

Greenhouse experiments were conducted to assess the effect of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Sapindus emarginatus* Vahl.(Sapindaceae). NaCl was added to the soil and salinity was maintained at 0.2, 2.1, 3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1}. Salinity caused reduction in water content and water potential of tissues that resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased as salinity increased. Proline content in tissues increased as salinity increased. There were no effective mechanisms to control net uptake of Na^+ and subsequently its transport to shoot tissues. Sodium content significantly increased in tissues as salinity increased. Nitrogen, potassium and calcium content in tissues significantly decreased as salinity increased. Changes in tissues and whole-plant accumulation pattern of other nutrients, as well as possible mechanisms for avoidance of Na toxicity in this species in response to salinity, are discussed.

Key words: Soil salinity, seedling growth, proline content, water potential, macro- and micro-nutrients, salt tolerance.

1. Introduction

Salinisation of soil is common in arid and semi arid regions where evapotranspiration is greater than rainfall. High concentrations of salts have detrimental effects on seed germination and plant growth (Bernstein, 1962; Taiz and Zeiger, 2006; Ramoliya et al., 2006) and excessive concentrations kill growing plants (Garg and Gupta, 1997). However, plant species differ in their sensitivity or tolerance to salts (Marschner, 1995). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns, 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman, 1977; Munns, 2002; Ramoliya et al., 2006).However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns, 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt – induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g., Maas and Grieve, 1987; Cramer et al., 1989; Ramoliya et al., 2006; Patel and Pandey, 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al., 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Sapindus emarginatus Vahl. (Sapindaceae), a deciduous tree species, is native to south India and grows abundantly in coastal area of Saurashtra in Gujarat State of

India. It also grows successfully on marginal-saline lands of Kutch (north-west saline desert), north to Saurashtra. The nut shell contains saponin, which acts like soap as soon as it gets in contact with water. The skin of the fruit is highly valued by the rural folks as a naturally produced shampoo for washing hair. Leaves are palatable to domestic animals. The present investigation was performed with the following objectives: (i) to understand the adaptive features of *S. emarginatus* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

2. Material and methods

2.1. Study Area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22^0 18' N Lat, $70^0$56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6 % sand, 20.3 % silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dS m^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier (Patel and Pandey, 2007). The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-

western monsoon which causes the onset of wet season in mid – June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj ($23^0$15' N Lat, $69^0$49' E Long) in Kutch and about 554 mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June – September) and winter (November – February).The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

2.2. Salinisation of Soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil of 100 kg each, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 210, 390, 700, 1070, 1275 and 1530 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 2.1, 3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1}. There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.2 dS m^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

2.3. Seedling Emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 31 August 2007. Seeds of *S. emarginatus* were collected from the coastal

area of Arabian Sea at Jamnagar district of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered (about 300 mL water was added to raise the soil moisture to field capacity) and thereafter similar amount of water was added to the soil on alternate days. Irrigation of soil with required amount of water was taken as a measure to control the level of soil salinity. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity, using the expression:

$$Sin^{-1}\sqrt{p} = \beta_0 + \beta_1 X$$

where, $Sin^{-1}\sqrt{p}$ 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 estimated using the model.

2.4. Seedling Growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 2.1, and 3.9 dS m^{-1} salinity exhibited emergence of the second leaf after 14 days. Emergence of the second leaf confirmed the establishment of seedlings. However, only 11.2 and 0.8 % seed germination was recorded respectively in soils at 6.2 and 8.1 dS m^{-1} salinity, and further experiments were not conducted on those seedlings. Seeds did not germinate when soil salinity exceeded 8.1 dS m^{-1}. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorized with three grades of soil (0.2, 2.1 and 3.9 dS m^{-1}) were prepared. This gave a total of 60 bags, which were arranged in 20 randomized blocks. Seedlings were watered (to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on 28 February 2008. Seedlings contained in

20 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of tissues were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

2.5. Determination of Water Potential and Proline Content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5 g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

2.6. Mineral Analyses of Plant Materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three sub samples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper, 1944). Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn

and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3:H_2SO_4:$HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

3. Results

3.1. Effect of Salinisation on Seedling Emergence

Seedlings began to emerge 3 days after sowing and 62.4% seed germination was obtained over a period of 18 days, under control (0.2 dS m^{-1} salinity) conditions (Fig. 1). Seedling emergence in saline soils was recorded 5-13days after sowing. Seedling emergence lasted for 18, 19, 16,and 15 days in soils with 2.1,3.9, 6.2 and 8.1 dS m^{-1} salinities, respectively and corresponding seed germination was 51.2%, 46%, 11.2% and 0.8%. There was a significant reduction in seed germination ($p < 0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y= 58.655 – 6.171X$, ($R^2_{adj} =0.934$, $p < 0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

3.2. Effect of Salinisation on Stem and Root Elongation and Leaf Expansion

Increasing soil salinity significantly retarded ($p < 0.01$) stem and root elongation (Table 1). Nevertheless, root length was nearly double of shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p < 0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p < 0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p < 0.01$).

3.3. Effect of Salinisation on Dry Weight

Dry weight significantly decreased ($p < 0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weight of tissues of salinised plants compared to those of control plants was computed as: (salinised tissue dry weight / control dry weight) × 100. Dry weight values of tissues given in Table 1 were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 51.6 to 27.5% for leaves, from 77.2 to 34.6 % for stems, 61.0 to 28.4 % for tap roots and from 85.3 to 54 % for lateral roots in response to increasing soil salinity from 2.1 to 3.9 dS m^{-1}. As has been estimated using regression equations given in results , the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 2.6, 3.2, 2.7 and 4.5 for leaves, stems, tap roots and lateral root tissues , respectively. Root/shoot dry weight ratio was 1.2 under control conditions while it was, 1.2 and 1.3 for seedlings grown in soils at 2.1 and 3.9 dS m^{-1}salinities, respectively. Root / shoot dry weight ratio did not change as soil salinity increased.

3.4. Effect of Salinisation on Water Content of Tissues

Water content in leaves, stems, tap roots($p < 0.01$) and lateral root tissues significantly decreased ($p < 0.05$) with increasing concentration of salt in soil (Fig. 2A). There was maximum water content in lateral roots and minimum in stem. Tissues according to their water content can be arranged in following decreasing order: lateral roots>leaves > tap roots > stems. There was a negative relationship between water content in different tissues and salt concentration ($r= -0.370,-0.578, -0.437$, $p < 0.01$ for leaves, stems, tap roots and -0.309, $p < 0.05$, for lateral roots, respectively).

3.5. Effect of Salinisation on Water Potential of Tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p < 0.01$) as soil salinity increased (Fig. 2B). Tissues according to their water potential values (low to high negative) can be arranged in the following order: lateral roots > leaves > stems > tap roots. There was a negative relationship between water potential of tissues and salt concentration ($r = -0.840, -0.935, -0.871$ and -0.884, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) . A positive relationship was obtained between water content and water potential (negative value) ($r = 0.958, 0.999, 0.975$ and 0.958, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) .

3.6. Effect of Salinisation on Proline Content of Tissues

Proline content (μ mol/g FW material) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues with increase in soil salinity (Fig. 2C).Tissues according to their proline content can be arranged in following decreasing order: leaves > tap roots > stems > lateral roots. There was a positive relationship between salt concentration and proline content of tissues ($r = 0.925, 0.901, 0.958$ and 0.883, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content ($r = -0.711, p < 0.05$, for leaves and $-0.766, -0.788 -0.848$, $p < 0.01$, for stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content ($r = -0.981, -0.996, -0.999$ and -0.996, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

3.6. Effect of Salinisation on Mineral Accumulation

3.6.1. Potassium and Sodium Content and K/Na Ratio

Potassium content (as mg g^{-1} dry weight) significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a negative relationship between potassium content in tissues and increase in salt concentration in soil ($p < 0.01$). Sodium content significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress ($p < 0.01$). K/Na ratio significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K /Na ratio in tissues and increase in salt stress ($p < 0.01$).

3.6.2. Nitrogen, Phosphorus, Calcium and Magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen, phosphorus, calcium and Magnesium content significantly decreased in leaves, stems, tap roots and lateral root tissues ($p < 0.01$), as the salinity increased (Table 2). A negative relationship was obtained between N, P, Ca and Mg content of tissues and salt concentration ($p < 0.01$).

3.6.3. Micro –Elements

There was a significant increase in the concentration of Zn, Cu, Mn and Fe ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress (Table 2). A positive relationship was obtained between soil salinity and Zn, Cu, Mn and Fe content in tissues ($p < 0.01$).

4. Discussion

Earlier work (Ramoliya et al,. 2004), indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dS m^{-1},but for *Sapindus emarginatus* SG_{50} was obtained at 2.2 dS m^{-1}.That would suggest that this plant species is relatively salt sensitive at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 2.0 to 5.0 dS m^{-1}. Eventually, seeds of *S. emarginatus* can germinate on low saline lands and achieve establishment during the rainy season. However, salt concentration exceeding 3.9 dS m^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentrations of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Slater et al., 2003) and induces changes in the activities of many enzymes (Dubey and Rani, 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz and Zeiger, 2006). Moreover, tap root elongation for seedlings grown in control and saline soils both was greater than that of shoot. Result suggests that this species has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington 1987) and is considered an adaptation to survive in dry habitats. Root / shoot dry weight ratio of *S. emarginatus* was 1.2 under control conditions and was two and a half time greater

than that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya et al., 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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(Kramer, 1983; Garg and Gupta, 1997).Results for reduction of shoot growth and leaf area development of *S. emarginatus* with increasing salt concentration are in conformity with the finding of Curtis and 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 and 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. Also high concentration of salt tends to slow down or stop root elongation (Kramer, 1983) and causes reduction in root production (Garg and Gupta, 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of leaves and tap roots while lowest in lateral roots. Consequently, lateral roots were most resistant and leaves were sensitive to salt stress. Tissues can be arranged in decreasing order of salt tolerance as: lateral roots > stems > tap roots > leaves. Root/shoot dry weight ratio did not change with increase in salinity due to the concurrent and differential reduction in dry weight of tissues. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion (Marschner, 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion

requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration .The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ (Marschner, 1995).Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *S. emarginatus* survived only up to the soil salinity of 3.9 dS m^{-1} and, therefore, this tree species has low tolerance to salt. In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates (Greenway and Munns, 1980). As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway and Munns, 1980). Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al., 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (Stewart and Lee, 1974).In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar et al., 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis (Schachtman et al., 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow et al., 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox and Guerinot,

1998). In the present study, significant decrease of K^+ content in all the tissues of seedlings with increasing soil salinity suggests that Na inhibited K^+ uptake. The exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salts to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues of *S.emarginatus* suggests that this mechanism to block Na^+ transfer to growing shoot tissues was not effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively low accumulation of K^+ than that of Na^+. As a consequence there were no effective mechanisms to control net uptake of Na on root plasma membrane and subsequently its transport to shoot tissue. The pattern of accumulation of K^+ and Na^+ in *S.emarginatus* conforms to group C and / or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na^+ without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar (Watad et al. 1991; Schroeder et al., 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}.These cation channels could allow entry of large amount of Na^+ from a highly

saline soil if not adequately regulated (Amtmann and Sanders, 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Watad et al,. 1991; Schroeder et al., 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis and Sanders, 1994; Niu et al,. 1995).The K^+ and Na^+ profiles of *S. Emarginatus* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al., 2001).As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant.

In general, salinity reduces N accumulation in plants (Feigin 1985). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Torres & Bingham 1973, Garg & Gupta 1997). The Interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol, 1979; Grattan and Grieve, 1992). However it is known that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach et al., 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e. g., in preserving membrane integrity (Rengel, 1992), signalling in osmoregulation (Mansfield et al., 1990) and influencing K^+/Na^+ selectivity (Cramer et al., 1987). In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions , precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen and Chang, 1987; Garg and Gupta, 1997).Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates, which

when impaired leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner and Cakmak, 1989).

It is difficult to suggest mechanistic explanations of salinity influence on micro–element concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al., 2000). In the present study, it appears that salinity enhanced Zn, Cu Mn and Fe accumulation, accumulation at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox system, Mn for photolysis of water in photosynthesis and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner, 1995). In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner, 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al., 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater et al., 2003). Increase in Cu, Zn, Mn and Fe content at the whole plant level might be the requirement of this plant for survival and growth in response to salinity.

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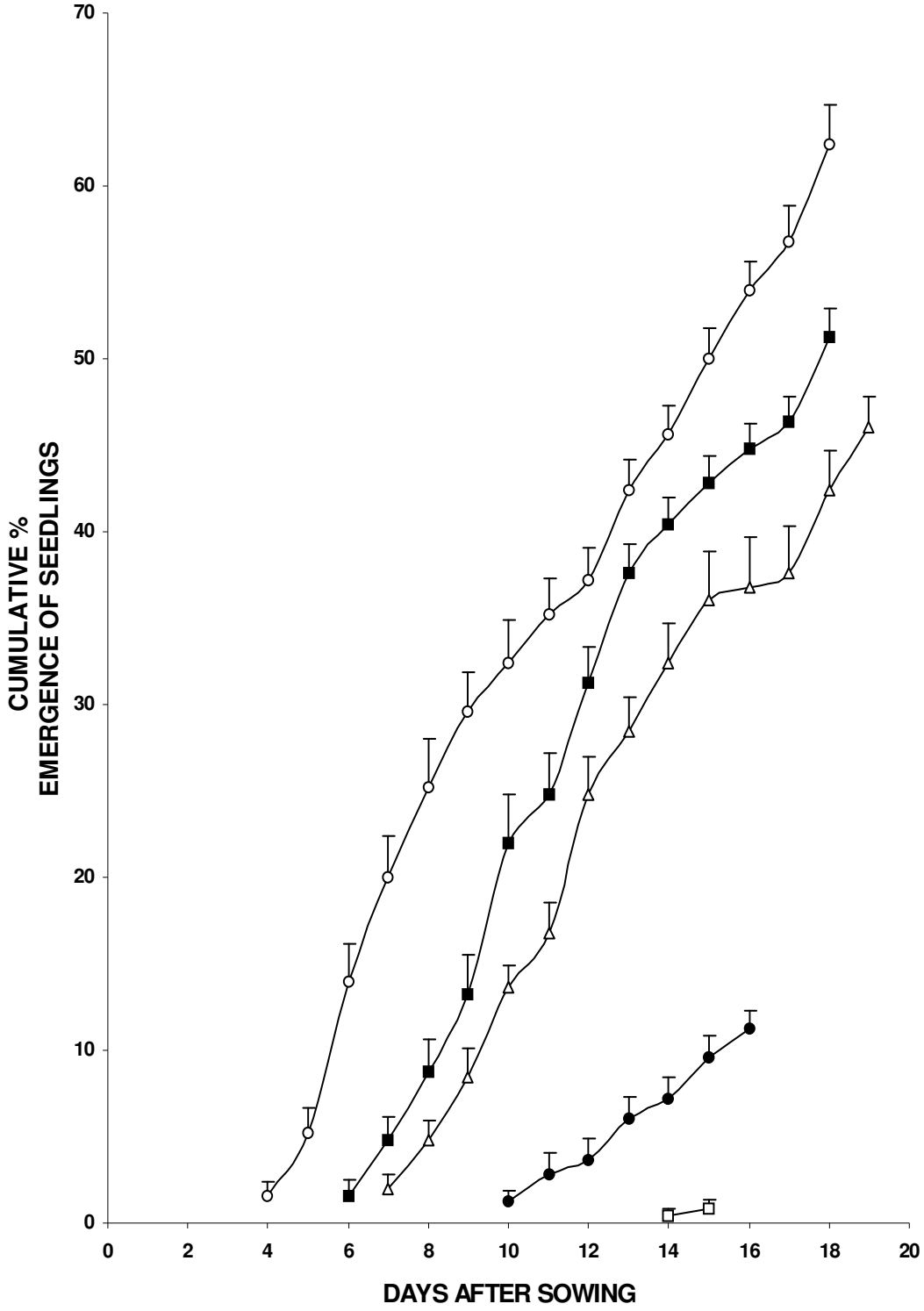

Fig.1.Cumulative emergence of seedlings of *Sapindus emarginatus* in response to soil salinity 0.2 dS m^{-1} (○), 2.1dS m^{-1} (■), 3.9dS m^{-1} (Δ), 6.2dS m^{-1} (●), and 8.1 dS m^{-1} (□) . Error bars represent SE.

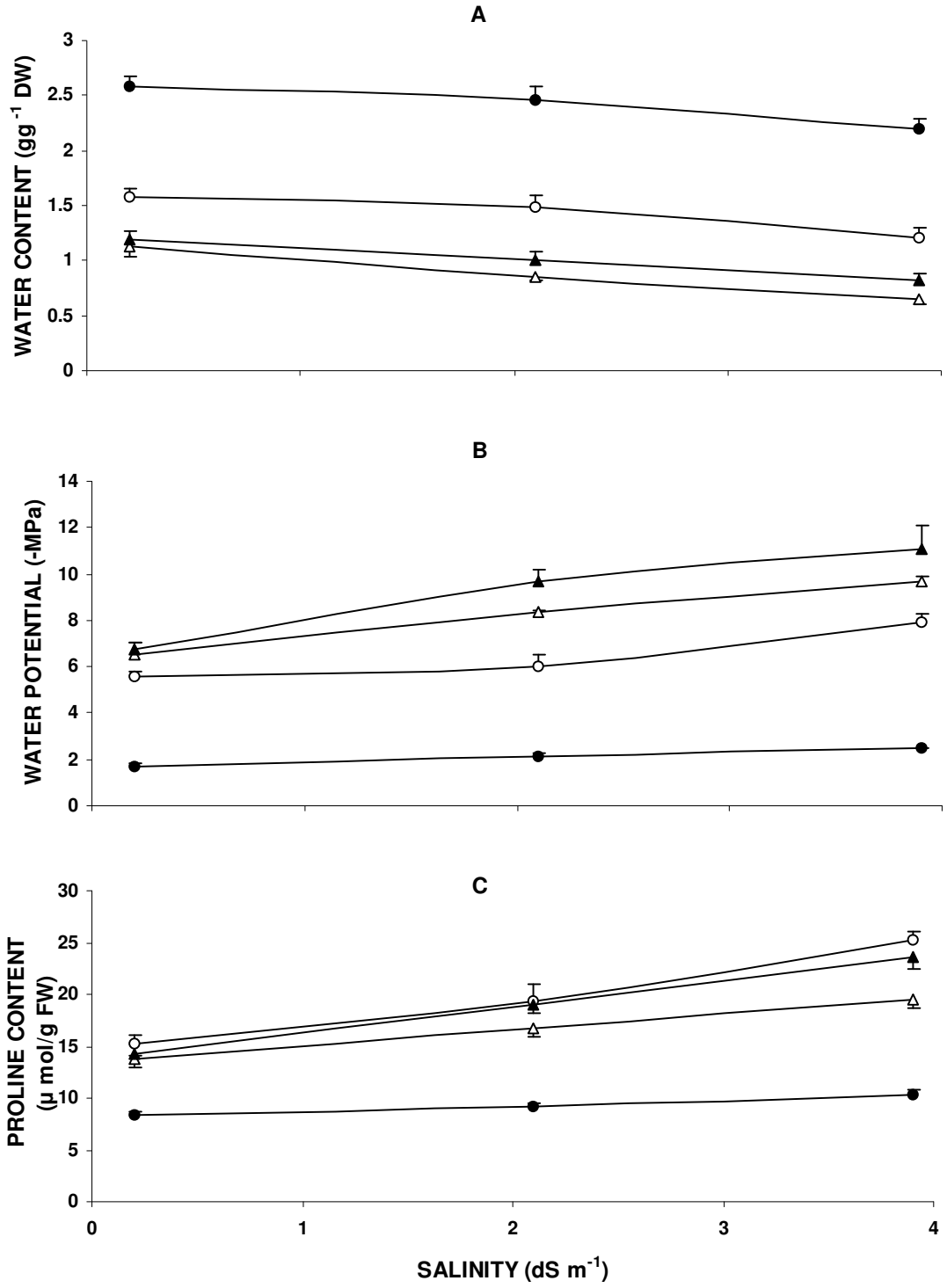

Fig. 2.Effect of soil salinity on A. water content (gg^{-1} DW), B. water potential (-Mpa) and C. proline content (µ mol/g FW) of leaves (○), stem (Δ), tap root (▲), and lateral roots (●) of *Sapindus emarginatus* seedlings. Error bars represent SE.

Table1. Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Sapindus emarginatus* as indicated by mean ± SEM and regression equation constants.

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area (cm^2plant^{-1})	Leaf weight (mg $plant^{-1}$)	Stem weight (mg $plant^{-1}$)	Shoot weight (leaf+stem) (mg $plant^{-1}$)	Tap root weight (mg $plant^{-1}$)	Lateral root weight (mg $plant^{-1}$)	Total root weight (mg $plant^{-1}$)
0.2	19.7 ± 0.6	44.7±1.9	106.1±5.6	395.3±9.6	374.1±7.9	769.4±14.4	686.1±13.0	196.6±8.6	882.7±18.2
2.1	14.2±0.6	27.7±2.2	48.6±2.6	204.1±9.2	288.8±6.5	462.9±7.8	418.8±16.2	167.7±7	556.5±18.1
3.9	12.7±0.8	21.6±1.9	33.2±1.5	108.8±9.8	129.4±6.4	238.1±13.2	195.1±15.1	106.1±5.3	301.2±15.3
α	19.48	44.27	103.5	396.53	400.38	782.22	707.75	207.11	905.16
β	-1.9	-6.25	-19.8	-77.66	-65.95	-143.76	-132.79	-24.36	-157.3
r	-0.700	-0.870	-0.970	-0.929	-0.941	-0.969	-0.951	-0.754	-0.952
$LSD_{0.05}$	4.8	8.2	17.5	72.1	52.7	91.9	112.1	53.7	130.3

Relationship is significant at $p < 0.01$.

Table 2. (Part 1) Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Sapindus emarginatus* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	28.3±0.2	1.9±0.1	28.0±0.5	4.3±0.1	24±0.1	7.5±0.2	6.5±0.2	82±0.6	53±0.4	69±3.0	486±9.6
	2.1	21.8±0.3	1.6±0.1	26.4±0.3	5.6±0.3	21±0.2	6.9±0.1	4.8±0.4	85±0.2	66±0.5	151±3.4	521±7.8
	3.9	20.9±0.6	1.4±0.1	24.8±0.3	7.8±0.2	19±0.5	5.7±0.4	3.2±0.0	97±0.5	76±0.3	173±5.4	659±9.5
	α	27.83	1.87	28.18	3.95	23.69	7.69	6.67	79.69	52.15	72.62	459.24
	β	-2.01	-0.14	-0.86	0.93	-0.82	-0.47	-0.89	4.02	6.22	28.25	46.49
	r	-0.911	-0.888	-0.924	0.963	-0.856	-0.887	-0.970	0.933	0.995	0.945	0.927
	$LSD_{0.05}$	1.1	0.2	1.1	0.7	0.9	0.7	0.7	1.4	1.3	12.0	26.4
Stem	0.2	19.3±0.5	1.5±0.0	24.8±0.2	4.9±0.2	26±0.1	4.6±0.2	5.1±0.2	47±0.3	49±0.6	49±1.8	273±0.4
	2.1	16.4±0.2	1.3±0.1	23.0±0.3	5.7±0.2	24±0.6	3.8±0.1	4.1±0.2	57±0.5	73±0.8	143±1.3	279±3.5
	3.9	10.2±0.3	1.2±0.1	21.4±0.4	9.2±0.1	22±0.6	2.5±0.1	2.3±0.1	63±0.2	84±1.1	152±1.7	289±0.6
	α	20.36	1.46	24.95	4.19	26.22	4.8	5.35	46.75	49.07	56.72	271.26
	β	-2.45	-0.07	-0.92	1.15	-1.07	-0.56	-0.74	4.33	9.49	28.04	4.39
	r	-0.967	-0.896	-0.952	0.923	-0.919	-0.969	-0.961	0.988	0.977	0.908	0.908
	$LSD_{0.05}$	1.0	0.2	0.9	0.5	1.4	0.4	0.5	1.1	2.6	4.8	6.1

Relationship is significant at $p < 0.01$

Table 2. (Part 2) Effect of salinisation of soil on nutrient content of tissues (tap roots and lateral roots) of *Sapindus emarginatus* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	17.2±0.3	1.4±0.1	24.0±0.1	3.3±0.1	25±0.6	2.9±0.0	7.2±0.2	52±0.6	33±0.6	45±3.5	316±6.2
	2.1	14.9±0.1	1.2±0.0	22.3±0.3	4.4±0.2	18±0.2	1.9±0.0	5.0±0.1	58±0.1	36±0.1	135±1.2	415±4.9
	3.9	9.9±0.9	1.1±0.1	21.0±0.4	5.1±0.1	16±0.3	1.5±0.1	4.1±0.1	66±1.0	38±0.1	164±1.8	588±5.3
	α	18.07	1.35	24.08	3.3	24.7	2.34	7.18	50.86	32.87	47.87	288.15
	β	-1.97	-0.06	-0.79	0.47	-2.43	-0.36	-0.83	3.77	1.35	32.31	73.31
	r	-0.97	-0.875	-0.942	0.964	-0.945	-0.969	-0.964	0.980	0.964	0.957	0.982
	$LSD_{0.05}$	0.6	0.1	0.9	0.4	1.1	0.1	0.4	2.1	1.1	11.6	16.1
Lateral roots	0.2	17.0±0.3	1.3±0.0	37.0±0.4	7.6±0.1	22±0.4	5.1±0.1	4.9±0.1	77±0.3	32±0.9	91±1.2	927±1.7
	2.1	14.1±0.2	1.1±0.0	32.3±0.2	8.1±0.1	17±0.0	4.7±0.3	4.0±0.0	79±0.6	42±0.6	165±1.5	982±1.2
	3.9	12.8±0.3	1.0±0.0	21.2±0.4	8.8±0.1	15±0.6	3.7±0.1	2.4±0.1	81±0.2	46±0.7	177±1.0	1042±2.1
	α	17.00	1.31	38.92	7.49	21.93	5.29	5.12	77.03	32.15	96.01	919.47
	β	-1.14	-0.09	-4.23	0.33	-1.89	-0.38	-0.66	0.99	3.80	23.38	31.06
	r	-0.956	-0.915	-0.967	0.966	-0.958	-0.909	-0.980	0.930	0.959	0.928	0.997
	$LSD_{0.05}$	0.8	0.1	1.0	0.2	1.2	0.5	0.2	1.2	2.2	3.5	5.0

Relationship is significant at $p < 0.01$

EXPERIMENT – 6

COMMUNICATED TO

Plant Physiology and Biochemistry

***Cassia fistula* L. (Fabaceae)**

Growth, water status and nutrient accumulation of seedlings of *Cassia fistula* L. in response to soil salinity

Seema Abhay Hardikar[1] **and Amar Nath Pandey**[2*]

1 Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:abhay.hardikar@sgs.com

E-mail:seema.a.hardikar@gmail.com

2[*] **Correspondence**

A. N. Pandey

Department of Biosciences, Saurashtra University, Rajkot – 360005 India

E-mail:anpandey2001@gmail.com

Tel (O): +91-281-2586419

Tel (M): +919427495989

Fax (O): +91-281-2577633

Short title (to be used as running head):

Effect of soil salinity on *Cassia fistula*.

Abstract

Greenhouse experiments were conducted to assess the effect of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Cassia fistula* L. (Fabaceae). NaCl was added to the soil and salinity was maintained at 0.2, 2.1, 3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1}. Salinity caused reduction in water content and water potential of tissues that resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased as salinity increased. Proline content in tissues increased as salinity increased. There were no effective mechanisms to control net uptake of Na^+ and subsequently its transport to shoot tissues. Sodium content significantly increased in tissues as salinity increased. Nitrogen, potassium and calcium content in tissues significantly decreased as salinity increased. Changes in tissues and whole-plant accumulation pattern of other nutrients, as well as possible mechanisms for avoidance of Na toxicity in this species in response to salinity, are discussed.

Key words: Macro- and micro-nutrients, proline content, salt tolerance, seedling growth, soil salinity, water potential.

1. Introduction

Salinisation of soil is common in arid and semi arid regions where the amount of rainfall is insufficient for substantial leaching. High concentrations of salts have detrimental effects on plant growth [3, 38, 31]. However, plant species differ in their sensitivity or tolerance to salts [22]. There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions [23]. It is reported that soil salinity suppresses shoot growth more than the root growth [19, 24, 31]. However, fewer studies on the effect of soil salinity on root growth have been conducted [24]. The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt – induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied [18, 7, 31, 27], but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood [40]. An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Cassia fistula L. (Fabaceae), is an ornamental small tree and is native to tropical Asia. This tree species is found abundantly in marginal-saline area of Kutch (north-west saline desert) in Gujarat State of India. It also grows successfully in coastal area as well as in non saline and semi-arid central area of Saurashtra region, to the north of Kutch. This tree is considered as a firewood source. The reddish wood, strong and durable, is suited for farm implements. The drug “Cassia fistula”, a mild laxative, is obtained from the sweetish pulp around the seed. In addition medicines are

extracted from fruits for the treatment of abdominal pain, fever, heart disease and leprosy. However, the potential of this tree species to grow and survive in coastal area of Saurashtra and in saline desert of Kutch is not known. The present investigation was performed with the following objectives: (i) to understand the adaptive features of *C. fistula* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

2. Results

2.1. Effect of Salinisation on Seedling Emergence

Seedlings began to emerge 6 days after sowing and 72.8% seed germination was obtained over a period of 30 days, under control (0.2 dS m^{-1} salinity) conditions (*Figure 1*). Seedling emergence in saline soils was recorded 6-12days after sowing. Seedling emergence lasted for 29, 29, 28, 25, 23 and 21 days in soils with 2.1,3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1} salinities, respectively and corresponding seed germination was 67.6%, 61.6%, 50%, 27.6%, 16.4% and 10%. There was a significant reduction in seed germination ($p < 0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y= 66.328 - 3.225X$, ($R^2_{adj} = 0.918$, $p < 0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

2.2. Effect of Salinisation on Stem and Root Elongation and Leaf Expansion

Increasing soil salinity significantly retarded ($p < 0.01$) stem and root elongation (*Table I*). Root length was almost equal to shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p < 0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p < 0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p < 0.01$).

2.3. Effect of Salinisation on Dry Weight

Dry weight significantly decreased ($p < 0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (*Table I*). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weight of tissues of salinised plants compared to those of control plants was computed as: (salinised tissue dry weight / control dry weight) × 100. Dry weight values of tissues given in *Table I* were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 61.8 to 35.7% for leaves, from 63.2 to 42.1 % for stems, 64.4 to 38.1 % for tap roots and from 64.1 to 47.9 % for lateral roots in response to increasing soil salinity from 2.1 to 6.2 dS m^{-1}. As has been estimated using regression equations given in results , the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 4.3, 4.6, 4.5 and 5.2 for leaves, stems, tap roots and lateral root tissues , respectively. Root/shoot dry weight ratio was 0.59 under control conditions and did not change as soil salinity increased.

2.4. Effect of Salinisation on Water Content of Tissues

Water content in leaves, stems, tap roots and lateral root tissues significantly decreased ($p < 0.01$) with increasing concentration of salt in soil (*Figure 2A*). There was maximum water content in leaves and minimum in tap roots. Tissues according to their water content can be arranged in following decreasing order: leaves > stems> lateral roots > tap roots. There was a negative relationship between water content in different tissues and salt concentration (r= -0.520, -0.565, -0.374 and -0.612, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

2.5. Effect of Salinisation on Water Potential of Tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p < 0.01$) as soil salinity increased (*Figure 2B*). Tissues according to their water potential values (low to high negative) can be arranged in the following order: lateral roots > leaves > stems > tap roots. There was a negative relationship between water potential of tissues and salt concentration (r = -0.895, -0.826, -0.958 and -0.967, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) . A positive relationship was obtained between water content and water potential (negative value) (r = 0.921, 0.923, 0.992 and 0.979, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively) .

2.6. Effect of Salinisation on Proline Content of Tissues

Proline content (μ mol/g FW material) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues, with increase in soil salinity (*Figure 2C*).Tissues according to their proline content can be arranged in following decreasing order: tap roots > stems > leaves> lateral roots. There was a positive relationship between salt concentration and proline content of tissues (r = 0.882, 0.833, 0.938 and 0.803, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content (r = -0.933, -0.772, -0.940 and -0.736, $p < 0.01$, for leaves, stems, tap roots and lateral roots,

respectively). Similarly, a negative relationship was obtained between water content and proline content ($r = -0.921, -0.954, -0.994$ and -0.977, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

2.7. Effect of Salinisation on Mineral Accumulation

2.7.1. Potassium and Sodium Content and K/Na Ratio

Potassium content (as mg g^{-1} dry weight) significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (*Table II*). There was a negative relationship between potassium content in tissues and increase in salt concentration in soil ($p < 0.01$). Sodium content significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress ($p < 0.01$). K/Na ratio significantly decreased ($p < 0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K /Na ratio in tissues and increase in salt stress ($p < 0.01$).

2.7.2. Nitrogen, Phosphorus, Calcium and Magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen, phosphorus, calcium and Magnesium content significantly decreased in leaves, stems, tap roots and lateral root tissues ($p < 0.01$), as the salinity increased (*Table II*). A negative relationship was obtained between N, P, Ca and Mg content of tissues and salt concentration ($p < 0.01$).

2.7.3. Micro –Elements

There was a significant increase in the concentration of Zn, Cu and Mn ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt

stress. A positive relationship was obtained between soil salinity and Zn, Cu and Mn content in tissues ($p < 0.01$). However, concentration of Fe significantly decreased in leaves, stems, tap roots and lateral roots ($p < 0.01$) with increase in soil salinity (*Table II*).There was a negative relationship between Fe content in tissues with salt concentration in soil ($p < 0.01$).

3. Discussion

Earlier work [31], indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dS m^{-1},but for *Cassia fistula* SG_{50} was obtained at 4.9 dS m^{-1}.That would suggest that this plant species is relatively salt tolerant at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 2.0 to 5.0 dS m^{-1}. Eventually, seeds of *C. fistula* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 8.1 dS m^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentrations of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration [36] and induces changes in the activities of many enzymes [9] in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter

production [38]. Root / shoot dry weight ratio of *C. fistula* was 0.59 under control conditions and was greater than that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch [31].

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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 [17, 12].Results for reduction of shoot growth and leaf area development of *C. fistula* with increasing salt concentration are in conformity with the finding of Curtis and Lauchli [8], 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 and Gupta [12] reported that salinity causes reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield. Also high concentration of salt tends to slow down or stop root elongation [17] and causes reduction in root production [12].

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of leaves and tap roots while lowest in lateral roots. Consequently, lateral roots were most resistant and leaves were sensitive to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: lateral roots > stems > tap roots > leaves. Root/shoot dry weight ratio did not change with increase in salinity due to the concurrent and differential reduction in dry weight of tissues. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion [22]. The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion

requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration .The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ [22].Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *C. fistula* survived up to the soil salinity of 6.2 dS m^{-1} and, therefore, this tree species is moderate salt tolerant. In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates [14]. As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes [14]. Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species [15]. Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities [37]. In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants [30].

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis [34]. High stomatal K^+ requirement is reported for photosynthesis [5]. The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake [11]. In the present study, significant decrease of K^+

content in all the tissues of seedlings with increasing soil salinity suggests that Na inhibited K^+ uptake. The exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salts to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues of *C. fistula* suggests that this mechanism to block Na^+ transfer to growing shoot tissues was not effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively lower accumulation of K^+ than that of Na^+. As a consequence there were no effective mechanisms to control net uptake of Na on root plasma membrane and subsequently its transport to shoot tissues. The pattern of accumulation of K^+ and Na^+ in *C. fistula* conforms to group C and / or group D plants in Marschner's [22] classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na^+ without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar [35]. Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}.These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated [1]. Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted

[35]. Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways [25].The K^+ and Na^+ profiles of *C. fistula* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake [33].As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant.

In general, salinity reduces N accumulation in plants [10]. This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration [39, 12]. The Interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species [13]. However it is known that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch [26] and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e. g., in preserving membrane integrity [32], signalling in osmoregulation [20] and influencing K^+/Na^+ selectivity [6]. In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions , precipitation and increase in ionic strength that reduce the activity of Ca^{2+} [16, 12].Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates, which when impaired leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase [21].

It is difficult to suggest mechanistic explanations of salinity influence on micro–element concentration due to relatively smaller differences between control and salinised tissues [40]. In the present study, it appears that salinity enhanced Zn,

Cu and Mn accumulation, while reduced Fe accumulation at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox system, Mn for photolysis of water in photosynthesis and Zn for DNA replication, regulation of gene expression and integrity of biomembranes [22]. In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll [22]. Pushnik and Miller [29] reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism [4]. Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components [36]. Increase in Cu, Zn and Mn content at the whole plant level might be the requirement of this plant for survival and growth in response to salinity.

4. Methods

4.1. Study Area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22^0 18' N Lat, $70^0$56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6 % sand, 20.3 % silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dS m^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier [27]. The Kutch and Saurashtra regions are tropical

monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid – June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj ($23^0$15' N Lat, $69^0$49' E Long) in Kutch and about 554 mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June – September) and winter (November – February).The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

4.2. Salinisation of Soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil of 100 kg each, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 210, 390, 700, 1070, 1275 and 1530 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 2.1, 3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1}. There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.2 dS m^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

4.3. Seedling Emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were

sown on 7 February 2008. Seeds of *C. Fistula* were collected from the coastal area of Arabian Sea at Jamnagar district of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered (about 300 mL water was added to raise the soil moisture to field capacity) and thereafter similar amount of water was added to the soil on alternate days. Irrigation of soil with required amount of water was taken as a measure to control the level of soil salinity. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity, using the expression:

$$\mathrm{Sin}^{-1}\sqrt{p} = \beta_0 + \beta_1 X$$

where, $\mathrm{Sin}^{-1}\sqrt{p}$ 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 estimated using the model.

4.4. Seedling Growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 2.1, 3.9 and 6.2 dS m^{-1} salinity exhibited emergence of the second leaf after 19 days, whereas the second leaf on seedlings grown in soils at 8.1, 10.0, and 11.9 dS m^{-1} began to emerge after 26 days. Emergence of the second leaf indicated the probable establishment of seedlings. However, only 16.4 and 10% seed germination was recorded respectively in soils at 10.0 and 11.9 dS m^{-1} salinity, and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Plants grown in soil at 8.1 dS m^{-1} salinity died during the course of experiment. Thus twenty replicates factorized with four grades of soil (0.2, 2.1, 3.9 and 6.2 dS m^{-1}) were prepared. This gave a total of 80 bags, which were arranged in

20 randomized blocks. Seedlings were watered (to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on 7 August 2008. Seedlings contained in 20 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of tissues were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

4.5. Determination of Water Potential and Proline Content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. [2]. Extract of 0.5 g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

4.6. Mineral Analyses of Plant Materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three sub samples of

plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid [28]. Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3:H_2SO_4:$HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

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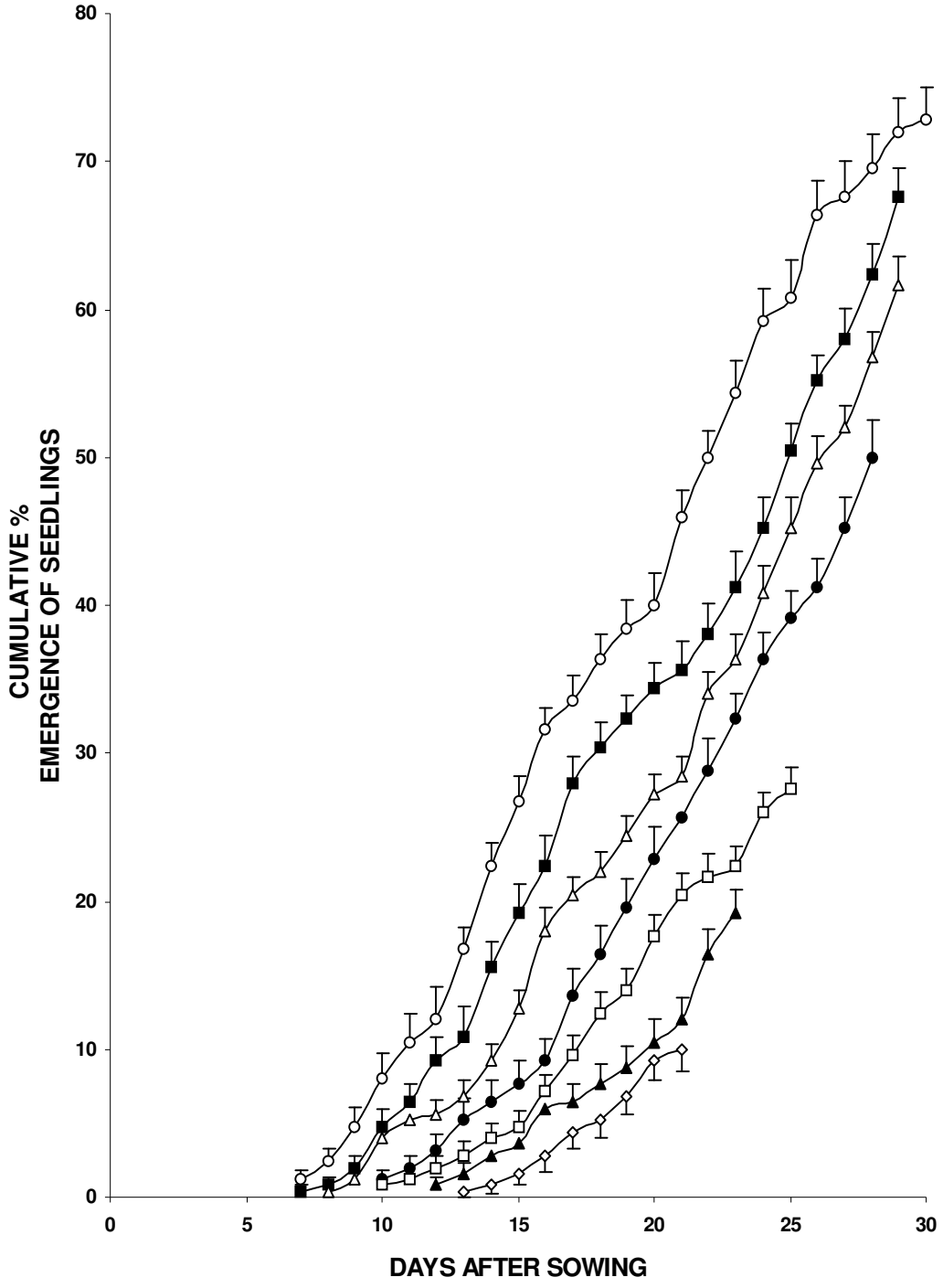

Figure 1. Cumulative emergence of seedlings of *Cassia fistula* in response to soil salinity 0.2 dS m^{-1} (○), 2.1 dS m^{-1} (■), 3.9dS m^{-1} (Δ) , 6.2dS m^{-1} (●) , 8.1dS m^{-1} (□) , 10.0dS m^{-1} (▲) and 11.9dS m^{-1} (◊) . Error bars represent SE.

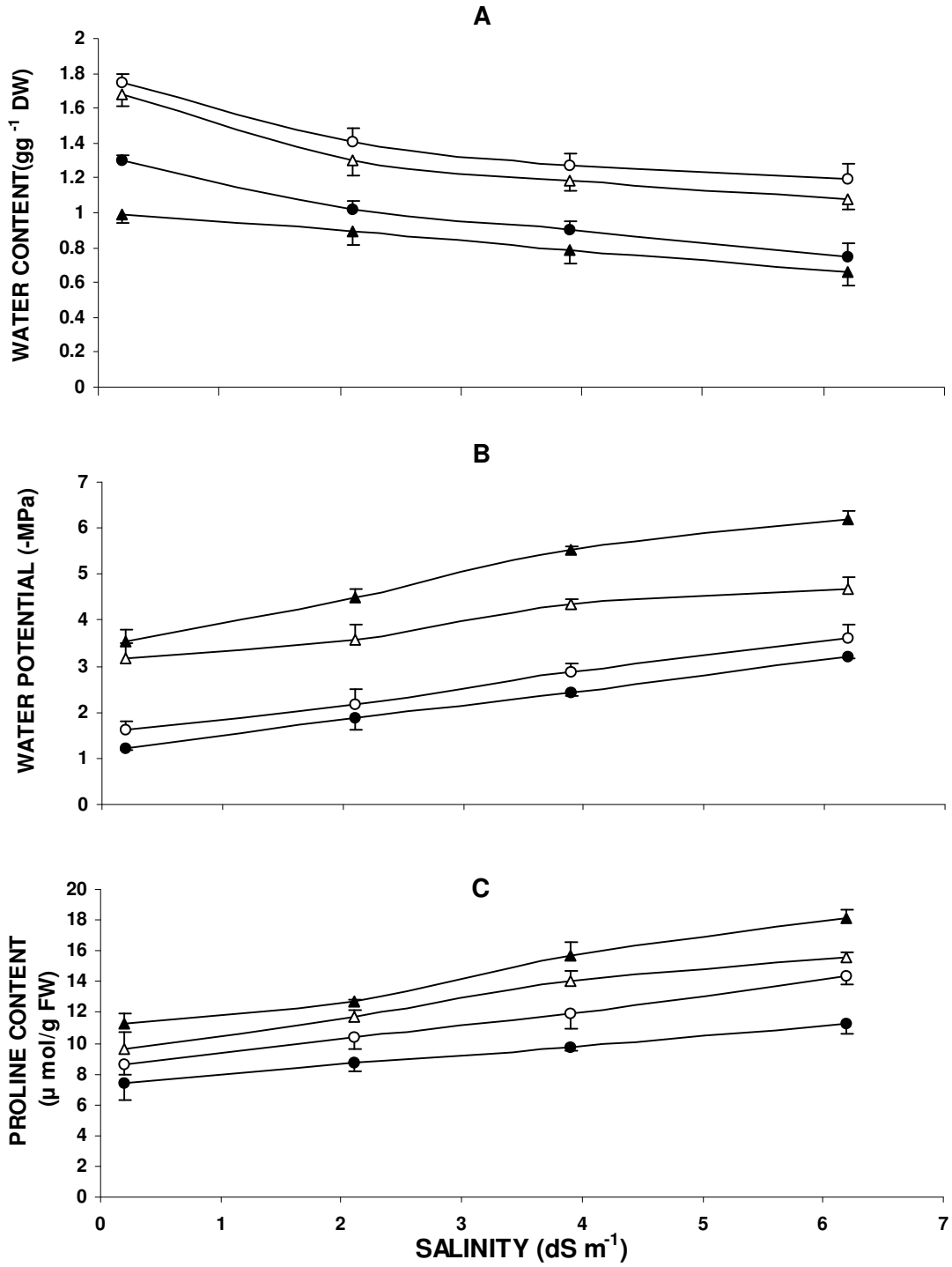

Figure 2. Effect of soil salinity on A. water content (gg^{-1} DW), B. water potential (-Mpa) and C. proline content (µ mol/g FW) of leaves (○), stem (Δ), tap root (▲), and lateral roots (●) of *Cassia fistula* seedlings. Error bars represent SE.

Table I. **Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Cassia fistula* as indicated by mean ± SEM and regression equation constants.**

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area ($cm^2 plant^{-1}$)	Leaf weight (mg $plant^{-1}$)	Stem weight (mg $plant^{-1}$)	Shoot weight (leaf+stem) (mg $plant^{-1}$)	Tap root weight (mg $plant^{-1}$)	Lateral root weight (mg $plant^{-1}$)	Total root weight (mg $plant^{-1}$)
0.2	32.3 ± 0.7	30.0±0.6	185.8±4.0	578.4±11.1	478.1±13.4	1056.4±19.5	418.5±9.0	197.2±2.2	615.7±9.0
2.1	26.9±0.7	25.7±0.7	107.7±3.1	357.7±10.9	302.1±10.1	659.8±17.6	269.5±7.2	126.4±3.4	395.9±6.2
3.9	21.7±0.7	18.0±0.7	82.0±0.8	295.3±6.3	246.8±5.3	543.0±7.0	222.2±8.2	109.7±1.9	331.8±8.2
6.2	18.1±0.6	15.6±0.6	67.0±1.1	206.2±6.5	201.2±4.0	407.3±6.9	159.4±5.3	94.4±2.8	253.7±6.4
α	32.16	30.16	169.5	542.2	443.92	986.12	395.29	182.03	577.32
β	-2.39	-2.53	-18.99	-58.98	-44.08	-103.07	-41.27	-16.18	-57.44
r	-0.865	-0.970	-0.892	-0.914	-0.872	-0.914	-0.905	-0.874	-0.918
$LSD_{0.05}$	5.2	5.0	19.8	68.1	68.3	106.0	57.0	19.9	57.0

Relationship is significant at $p < 0.01$.

***Table II*. (Part 1) Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Cassia fistula* as indicated by mean ± SEM and regression equation constants.**

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g\text{-}^{1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	29.3±0.2	1.7±0.1	36±0.3	2.0±0.1	32.3±0.6	2.7±0.1	18±0.4	45±0.2	10±0.6	54±0.2	420±2.3
	2.1	26.2±0.5	1.6±0.1	33.6±1.1	2.7±0.2	28.0±0.0	2.2±0.1	12.5±0.3	46±0.6	12±0.0	59±2.1	326±2.6
	3.9	24.4±0.1	1.3±0.1	28.8±0.3	4.3±0.2	26.6±0.6	2.0±0.0	6.8±0.3	49±0.5	19±0.3	67±0.5	239±0.6
	6.2	19.1±0.1	1.2±0.1	26.7±0.1	4.6±0.1	24.1±0.1	1.8±0.1	5.8±0.2	50±0.1	24±0.8	72±0.6	196±1.5
	α	29.87	1.72	36.37	1.94	31.78	2.62	17.29	45.71	8.60	53.35	412.75
	β	-1.65	-0.88	-1.64	0.46	-1.30	-0.15	-2.10	0.90	2.47	3.11	-37.9
	r	-0.983	-0.971	-0.958	0.942	-0.953	-0.902	-0.946	0.934	0.973	0.966	-0.977
	$LSD_{0.05}$	0.8	0.2	1.7	0.4	1.3	0.3	0.9	1.2	1.5	3.3	5.7
Stem	0.2	21.2±0.3	1.4±0.0	33.8±0.2	3.1±0.1	33.0±0.5	2.2±0.1	10.9±0.2	47±0.3	8.0±0.1	44±1.0	297±0.6
	2.1	16.3±0.3	1.2±0.1	28.6±0.2	3.9±0.4	29±0.6	1.8±0.1	7.3±0.1	49±0.3	9.0±0.3	45±0.6	184±1.5
	3.9	13.9±0.1	1.1±0.1	24.8±0.4	4.6±0.1	27±0.0	1.5±0.1	5.4±0.2	55±0.4	10±0.6	55±0.4	146±1.2
	6.2	11.9±0.	1.0±0.1	22.4±0.5	6.7±0.2	24.8±0.4	1.4±0.1	3.3±0.0	56±0.5	13±1.0	61±1.5	127±2.3
	α	20.52	1.36	33.3	2.76	32.59	2.14	10.56	46.66	7.46	41.71	272.85
	β	-1.54	-0.06	-1.90	0.58	-1.33	-0.14	-1.23	1.64	0.81	3.07	-27.2
	r	-0.963	-0.872	-0.970	0.966	-0.959	-0.889	-0.978	0.940	0.879	0.945	0.914
	$LSD_{0.05}$	0.6	0.2	1.1	0.4	1.3	0.3	0.5	1.1	1.8	2.9	4.5

Relationship is significant at $p < 0.01$

***Table II*. (Part 2) Effect of salinisation of soil on nutrient content of tissues (tap root and lateral root) of *Cassia fistula* as indicated by mean ± SEM and regression equation constants.**

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	18.0±0.1	1.0±0.1	19.0±0.1	3.7±0.3	21.5±0.3	1.8±0.1	5.2±0.4	35±0.5	9±0.2	54±0.2	527±2.0
	2.1	14.0±0.1	0.9±0.0	17.1±0.1	5.3±0.3	20.8±0.1	1.5±0.1	3.2±0.1	37±0.3	11±0.5	59±2.1	402±3.8
	3.9	12.0±0.2	0.8±0.1	15.7±0.9	6.3±0.1	20.2±0.1	1.3±0.1	2.5±0.1	40±0.1	13±0.1	67±0.5	316±2.5
	6.2	11.0±0.1	0.7±0.0	14.6±0.5	8.7±0.1	19.0±0.3	1.2±0.1	1.7±0.1	45±0.2	15±0.6	72±0.6	308±1.5
	α	17.27	1.03	18.86	3.48	21.68	1.77	4.84	34.3	8.88	53.35	502.21
	β	-1.14	-0.06	-0.72	0.81	-0.41	-0.10	-0.56	1.65	1.01	3.11	-36.76
	r	-0.944	-0.897	-0.899	0.981	-0.941	-0.852	-0.938	0.986	0.966	0.966	-0.923
	$LSD_{0.05}$	1.4	0.1	1.5	0.6	0.6	0.3	0.6	0.9	1.2	2.1	7.6
Lateral roots	0.2	17.0±0.3	1.2±0.0	20.6±0.5	6.5±0.1	30±0.2	5.4±0.2	3.2±0.1	52±0.6	11±0.5	44±1.0	1074±2.3
	2.1	15.2±0.1	1.1±0.0	17.0±0.1	6.6±0.1	27.0±0.0	4.9±0.3	2.6±0.0	55±0.3	17±0.5	45±0.6	1022±1.5
	3.9	13.5±0.1	1.0±0.0	16.0±0.2	7.1±0.1	26.0±0.6	4.7±0.3	2.3±0.0	64±1.0	22±0.4	55±0.4	1014±1.2
	6.2	10.7±0.2	0.9±0.0	15.0±0.6	10.1±0.3	23.7±0.0	3.9±0.1	1.5±0.0	73±0.2	28±0.5	61±1.5	986±2.1
	α	17.36	1.19	19.88	5.75	29.81	5.46	3.21	49.69	10.75	41.71	1066.6
	β	-1.04	-0.05	-0.88	0.58	-1.01	-0.24	-0.27	3.64	2.82	3.07	-13.72
	r	-0.991	-0.834	-0.899	0.87	-0.968	-0.858	-0.984	0.978	0.993	0.945	-0.949
	$LSD_{0.05}$	1.6	0.1	1.2	0.5	0.9	0.6	0.2	1.8	1.4	1.8	5.4

Relationship is significant at $p < 0.01$

GENERAL DISCUSSION

Seed germination

Seed germination was retarded for all the tree species with increasing salt concentration in soil. The maximum salt concentration above which seeds did not germinate differed for different tree species. A summary of the response of different tree species at seed germination stage is given in Table 1. The detrimental effect of salt to seed germination can be attributed to decreasing osmotic potential of the soil solution with increasing concentration of salt. Although the effect of high salt content on metabolic processes are yet to be fully elucidated, it is reported that salinity reduces protein hydration (Slater *et al*. 2003, Kramer, 1983), and includes changes in the activities of many enzymes (Dubey and Rani, 1990; Garg *et al*. 1993) in germinating seeds. Values of SG_{50} **(**salt concentration at which seed germination was reduced to 50%) for the test plant species ranged from 2.2 to 7.3 dS m^{-1} salinity which indicate that all tree species are salt tolerant at seed germination stage except for Sapindus emarginatus that is relatively salt sensitive. Moreover, tolerance limit varied among the plant species.

Growth of seedlings

Seedlings of *Acacia senegal*, *Tamarindus indica*, *Thespesia populnea*, *Salvadora oleoides*, *Sapindus emarginatus* and *Cassia fistula* survived up to 10.0, 10.0, 10.0, 11.9, 3.9 and 6.2 dS m^{-1} salinity, respectively (Table 1). Results indicate that tree species are moderate to high salt tolerant at seedling stage too. The salt concentration in soil at which dry weights of different plant tissues were reduced to 50% of those of control plants (DW_{50}) (as has been estimated using regression equations given in results) differed for leaves, stems, tap roots and lateral root tissues of seedlings for each species. The greater value of DW_{50} indicates greater tolerance to salt stress.

Percentage relative weights of tissues of salinised plants compared to those of control plants were also computed. Results suggest that tissues of seedlings of the test plants are not equally tolerant to salt stress. Tissues of different tree species can be arranged in the following decreasing order of salt tolerance:

Acacia senegal : Leaves > Stems > Tap roots = Lateral roots

Tamarindus indica : Stems > Leaves > Lateral roots > Tap roots

Thespesia populnea : Leaves > Stems > Lateral roots > Tap roots

Salvadora oleoides : Leaves > Lateral roots > Stems > Tap roots

Sapindus emarginatus : Lateral roots > Stems > Tap roots > Leaves

Cassia fistula : Lateral roots > Stems > Tap roots > Leaves

The differences among the plant tissues in salt tolerance are, perhaps, prerequisite for evolving salt avoidance mechanisms.

Mechanism of salt avoidance

Salt resistance includes both avoidance and tolerance mechanisms (Greenway and Munns, 1980; Wyn Jones, 1981; Gorham *et al*. 1985). Salt avoidance may operate through; exclusion and dilution of ions. An unavoidable consequence of growth of plants on a saline soil/solution containing high salt concentrations is the development of osmotic stress, which is followed by a loss of turgor. The tolerance or sensitivity of a given plant is indicated by the point or range in the continuum of the stress where the plant shows signs of being adversely affected. However, tolerance to osmotic stress may operate either through dehydration tolerance, which permits the cells to survive without growing when the turgor decreases, or by avoiding dehydration through osmoregulation. Among the studied tree species following two types of salt avoidance mechanisms were inferred:

1. Salt Exclusion

Cell membrane has low permeability for electrolyte ions such as Na^+, Cl^- and others. Consequently, roots prevent upward transport of ions from soil solution to shoot. This type of adaptation was found in *Acacia senegal*, *Tamarindus indica*, *Thespesia populnea*, *Salvadora oleoides*, *Sapindus emarginatus* and *Cassia fistula* to survive in saline and desert conditions. This type of adaptation is found normally in moderate salt tolerant plant species (Garg and Gupta, 1997).

2. Compartmentalization

Hallophytes and glycophytes alike accumulate ions intracellularly during salt adaptation and use these for osmotic adjustment necessary for cell expansion (Hasegawa *et al*. 2000). Cytosolic enzymes of plant cells can be severely inhibited by high concentration of ions (Na^+ and Cl^-). The accumulation of ions during osmotic adjustment appears to be restricted to the vacuoles where the ions are kept out of contact with enzymes in the cytosol or sub cellular organelles. Because of this compartmentalization of ions, other solutes must accumulate in the cytoplasm to maintain water potential equilibrium within the cell (Taiz and Zeiger, 2006).

These other solutes, called compatible solutes (or compatible osmolytes) are organic compounds that do not interfere with enzyme functions. Commonly accumulated compatible solutes include the amino acid proline, sugar alcohols (e.g., sorbitol and mannitol), and a quaternary amine called glycine betaine. Synthesis of compatible solutes helps plants adjust to increased soil salinity. Halophytes are efficient in compartmentalization of ions and have evolved this type of adaptation for surviving in saline habitats. The glycophytes or non-halophytes are unable to cause the sharp asymmetrical intracellular compartmentation of inorganic and organic solutes. All the

studied plants are glycophytes. This finding suggests that glycophytes can also grow successfully in saline desert provided they have evolved salt avoidance mechanisms.

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Table 1. Responses of different tree species to salt stress at seed germination and seedling growth stages and their salt tolerance mechanisms.

S. No.	Name of Tree species	Halophyte or glycophyte	Maximum soil salinity above which seeds did not germinate (dS m^{-1})	SG 50 (dS m^{-1})	Maximum soil salinity tolerated by seedlings (dS m^{-1})	Range of leaf water potential from control to maximum soil salinity (-Mpa)	Range of leaf proline content from control to maximum soil salinity (µ mol/gFW)	Range of leaf K/Na ratio from control to maximum soil salinity	Salt tolerance mechanism
1.	*Acacia senegal* (L.) Willd.	Glycophyte	11.9 dS m^{-1}	5.9	10.0 dS m^{-1}	6.6 ─ 11.1	4.2 ─ 11.6	2.8 ─ 0.6	Salt exclusion
2.	*Tamarindus indica* Linn.	Glycophyte	13.9 dS m^{-1}	7.3	10.0 dS m^{-1}	4.9 ─ 9.2	2.5 ─ 7.3	5.9 ─1.3	Salt exclusion
3.	*Thespesia populnea* (L.) Sol. ex Correa	Glycophyte	13.9 dS m^{-1}	5.3	10.0 dS m^{-1}	5.8 ─ 7.6	6.6 ─ 11.3	3.0─1.7	Salt exclusion
4.	*Salvadora oleoides* (Decne.)	Glycophyte	13.9 dS m^{-1}	6.9	11.9 dS m^{-1}	6.3 ─ 9.3	9.6 ─ 18.0	3.3 ─ 4.2	Salt exclusion
5.	*Sapindus emarginatus* Vahl.	Glycophyte	8.1 dS m^{-1}	2.2	3.9 dS m^{-1}	5.6 ─ 7.9	15.2 ─ 25.3	6.5 ─ 3.2	Salt exclusion
6.	*Cassia fistula* L.	Glycophyte	11.9 dS m^{-1}	4.9	6.2 dS m^{-1}	1.6 ─ 3.6	8.7 ─ 14.3	18 ─ 5.8	Salt exclusion

SUMMARY

Summary

Greenhouse experiments were conducted to assess the effects of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Acacia senegal* (L.) Willd., *Tamarindus indica* Linn., *Thespesia populnea* (L.) Sol. ex. Correa, *Salvadora oleoides* (Decne.), *Sapindus emarginatus* Vahl. and *Cassia fistula* L. Sodium chloride (NaCl) was added to the soil and different levels of salinity were maintained. The major findings of the experiments are summarized below:

1. *Acacia senegal* (L.) Willd. (Mimosaceae) is a glycophyte. This tree species is salt tolerant at seed germination stage. Seedlings exhibited salt tolerance up to 10 dS m^{-1}.

2. *Tamarindus indica* Linn. (Caesalpiniaceae) is a glycophyte. This tree species is salt tolerant at seed germination stage. Seedlings survived and grew up to 10 dS m^{-1} salinity.

3. *Thespesia populnea* (L.) Sol. ex Correa (Malvaceae) is a glycophyte. This tree species is salt tolerant at seed germination stage. Seedlings did not survive when soil salinity exceeded 10 dS m^{-1}.

4. *Salvadora oleoides* (Decne.) (Salvadoraceae) is a glycophyte. This tree species is salt tolerant at seed germination stage. Salt tolerance of seedlings was obtained up to 12 dS m^{-1}.

5. *Sapindus emarginatus* Vahl. (Sapindaceae) is a glycophyte. This tree species is relatively salt sensitive at seed germination stage. Seedlings did not survive when soil salinity exceeded 3.9 dS m^{-1}.

6. *Cassia fistula* L. (Fabaceae) is a glycophyte. This tree species is salt tolerant at seed germination stage. Seedlings survived and grew up to 6.2 dS m^{-1} soil salinity.

PAPER

PUBLISHED

Anales de Biología 30: 17-28, 2008

Growth, water status and nutrient accumulation of seedlings of *Acacia senegal* (L.) Willd. in response to soil salinity

Seema Abhay Hardikar[1] & Amar Nath Pandey[2]

1 Department of Biosciences, Saurashtra University, Rajkot, 360005 India, e-mail:abhay.hardikar@sgs.com; seemahardikar@yahoo.com

2 Department of Biosciences, Saurashtra University, Rajkot - 360005 India, e-mail:anpandey2001@gmail.com

Correspondence
A. N. Pandey
Email:
anpandey2001@gmail.com
Tel (O): +91-281-2586419
Tel (M): +919427495989
Fax (O): +91-281-2577633
Received: 2 February 2008. **Accepted:** 30 July 2008.

Resumen

Crecimiento, estado hídrico, y acumulación de nutrientes en plántulas de Acacia senegal *(L.) Wild. en respuesta a la salinidad del suelo.*

Se llevaron a cabo una serie de experimentos en invernadero con el fin de evaluar los efectos de la salinidad del suelo sobre la emergencia, crecimiento, estado hídrico, contenido de prolina y acumulación de minerales de plántulas de *Acacia senegal* (L.) Willd.(Mimosaceae). Se añadió NaCl al suelo y se mantuvo la salinidad a 0.2, 3.9, 6.2, 8.1, 10.0 y 11.9 dSm^{-1}. La salinidad causó reducción del contenido de agua y del potencial hídrico de los tejidos, lo que resultó en un déficit interno de la planta. Consecuentemente, el crecimiento de las plántulas disminuyó significativamente, mientras que el contenido de prolina de los tejidos aumentó. No aparecieron mecanismos efectivos para controlar la absorción de Na y su transporte a los tejidos de los brotes. El contenido de N, P, K y Ca disminuyó significativamente en los tejidos como respuesta a la salinidad. Se discute sobre los cambios en los tejidos y el patrón global de acumulación de otros elementos, así como posibles mecanismos para evitar la toxicidad del Na en esta especie arbórea.

Palabras clave: Salinidad del suelo, Crecimiento de plántula, Contenido en prolina, Potencial hídrico, Macro/micronutrientes, Tolerancia a la sal.

Abstract

Greenhouse experiments were conducted to assess the effects of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Acacia senegal* (L.) Willd. (Mimosaceae). NaCl was added to the soil and salinity was maintained at 0.2, 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1}. Salinity caused reduction in water content and water potential of tissues, which resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased with increase in soil salinity. Proline content in tissues increased with increase in soil salinity. There were no effective mechanisms to control net uptake of Na and its transport to shoot tissues. N, P, K and Ca content significantly decreased in tissues in response to salinity. Changes in tissues and whole-plant accumulation patterns of other elements, as well as possible mechanisms to avoid Na toxicity in this tree species in response to salinity, are discussed.

Key words: Soil salinity, Seedling growth, Proline content, Water potential, Macro- and micro-nutrients, Salt tolerance.

Introduction

Saline soils are abundant in semi arid and arid regions where the amount of rainfall is insufficient for substantial leaching (Marschner 1995). Salinity is a scourge for agriculture, forestry, pasture development and other similar practices. An understanding of responses of plants to salinity is of great practical significance. High concentrations of salts have detrimental effects on plant growth (Taiz & Zeiger 2006, Ramoliya et al. 2006) and excessive concentrations kill growing plants (Garg & Gupta 1997). There occurs retardation of germination and growth of seedlings at high salinity (Bernstein 1962, Garg & Gupta 1997, Ramoliya et al. 2006). However, plant species differ in their sensitivity or tolerance to salts (Marschner 1995). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas & Hoffman 1977, Munns 2002, Ramoliya et al. 2006).However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt-induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt-stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g. Cramer et al. 1989, Maas & Grieve 1987, Ramoliya et al. 2006, Patel & Pandey 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al. 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Acacia senegal (L.) Willd. (Mimosaceae), a small deciduous tree species grows abundantly in coastal forests of Saurashtra in Gujarat State of India. It also grows successfully on marginal-saline lands of Kutch (north-west saline desert) contiguous to Saurashtra. *A. senegal*, yields commercial gum arabic. Wood is a good fuel. Leaves and pods are eaten by herbivores. The present investigation was performed with the following objectives: (i) to understand the adaptive features of *A. senegal* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro- and micro-nutrient accumulation within the tissues of this tree species in response to salt stress.

Material and Methods

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22º 18' N Lat, 70º56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6% sand, 20.3% silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.2dSm^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier (Patel & Pandey 2007). The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid - June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395mm at Bhuj (23º15' N Lat, 69º49' E Long) in Kutch and about 554mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April - mid June), monsoon (mid June-September) and winter (November-February).

The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Salinisation of soil

Surface soil was collected, air dried and passed through a 2 mm. mesh screen. Six lots of soil of 100 kg each, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 390, 700, 1070, 1275 and 1530g was then thoroughly mixed with soil of five lots, respectively to give electrical conductivities of 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1}. There was no addition of NaCl to sixth lot of soil that served as control. The electrical conductivity of control soil was 0.2dSm^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 18 August 2006. Seeds of *A. senegal* were collected from the coastal area of Arabian sea in Jamnagar city of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered and thereafter watering was carried out on alternate days. Emergence of seedlings was recorded daily over a period of 40 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 estimated using the model.

Seedling growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Seedlings grown in soils at 0.2, 3.9 and 6.2 dSm^{-1} salinity exhibited emergence of the second leaf after 15 days whereas the second leaf on seedlings grown in 8.1, 10.0 and 11.9 dSm^{-1} appeared after 23 days. Emergence of the second leaf confirmed the establishment of seedlings. However, only 19.6% seed germination was recorded in soil at 11.9 dSm^{-1} salinity, and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialized with five grades of soil (0.2, 3.9, 6.2, 8.1, and 10.0dSm^{-1}) were prepared. This gave a total of 100 bags, which were arranged in 20 randomized blocks. Seedlings were watered (about 250 ml water was added to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on 18 February 2007. Five plants grown in soil at 10 dSm^{-1} salinity died during the course of experiment. Seedlings contained in 15 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Sum of leaf and stem weight was considered as shoot weight. Water content (gg^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of different components were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of water potential and proline content

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and proline estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520 nm. Water potential and proline content of tissues were estimated in triplicate. Data were analyzed by one way ANOVA.

Mineral analyses of plant materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately. Plant samples were ground using mortar and pestle. Three sub samples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper 1944). Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3: H_2SO_4:$HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

Results

Effect of salinisation on seedling emergence

Seedlings began to emerge 3 days after sowing and 84.8% seed germination was obtained over a period of 20 days, under control (0.2 dSm^{-1} salinity) conditions (Fig.1). Seedling emergence in saline soils was also recorded 3 days after sowing. Seedling emergence lasted for 18, 19, 18, and 18 and 12 days in soils with 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1} salinities, respectively and corresponding seed germination was 62.8%, 58.4%, 50.4%, 44% and 19.6%. There was a significant reduction in seed germination ($p<0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression:

$Y= 68.654 - 3.134\ X$, ($R^{2adj}=0.937$, $p<0.01$)

where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.Increasing soil salinity significantly retarded ($p<0.01$) stem and root elongation (Fig. 1). Nevertheless, root length was remarkably greater than shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p<0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p<0.01$) by increasing concentration of salt in soil. A negative relationhip was obtained between leaf area and salt concentration ($p<0.01$).

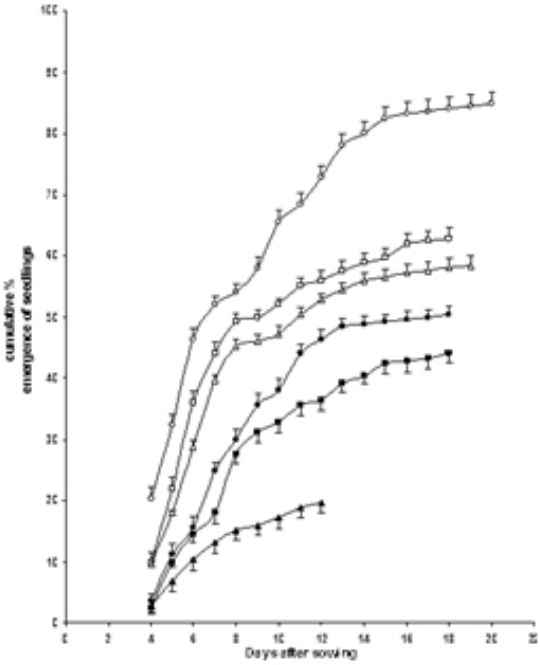

Figura 1. Emergencia acumulada de plántulas de *Acacia senegal* en respuesta a la salinidad del suelo. 0.2dSm-1(○), 3.9dSm-1(□), 6.2dSm-1(Δ), 8.1dSm-1(●), 10.0dSm-1(■) y 11.9dSm-1(▲). Las barras de error representan el SE.

Figure 1. Cumulative emergence of seedlings of *Acacia senegal* in response to soil salinity. 0.2dSm-1(○), 3.9dSm-1(□), 6.2dSm-1(Δ), 8.1dSm-1(●), 10.0dSm-1(■) and 11.9dSm-1(▲). Error bars represent SE.

Effect of salinisation on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p<0.01$) stem and root elongation (Fig. 1). Nevertheless, root length was remarkably greater than shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p<0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p<0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p<0.01$).

Effect of salinisation on dry weight

Dry weight significantly decreased ($p<0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap

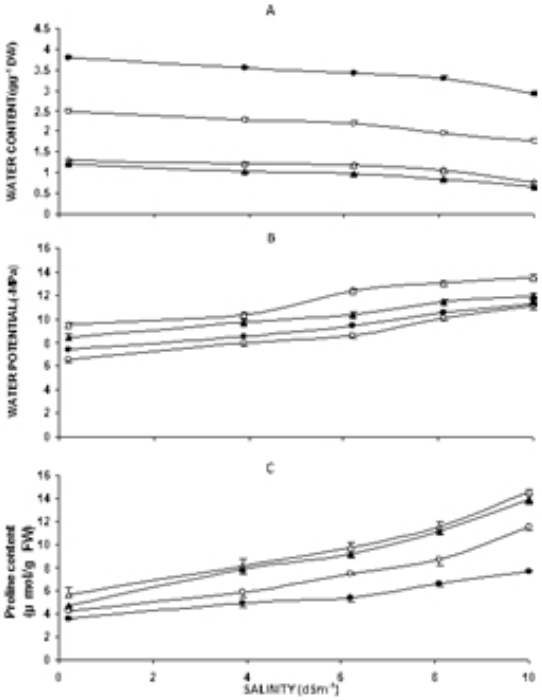

Figura 2. Efecto de la salinidad del suelo sobre **A:** contenido hídrico (gg^{-1} DW); **B:** agua potencial (-Mpa); **C:** contenido de prolina (µ mol/g FW) de hojas(○), tallo(Δ), raíz principal (▲) y raíces laterales (●) de plántulas de *Acacia senegal*. Las barras de error representan el SE.

Figure 2. Effect of soil salinity on **A:** water content (gg-1 DW); **B:** water potential (-Mpa); **C:** proline content (µ mol/g FW) of leaves (○), stem (Δ), tap root (▲) and lateral roots (●) of *Acacia senegal* seddling.

roots, lateral roots and total roots) and salt concentration ($p<0.01$).

Effect of salinisation on dry weight

Dry weight significantly decreased ($p<0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p<0.01$). Percent relative weight of tissues of salinised plants compared to those of control plants were computed as: (salinised tissue dry weight / control dry weight) × 100. Dry weight values of tissues given in Table 1 were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 87.2 to 54% for leaves, from 78.1 to 50.2% for stems, 77.5 to 41.3% for tap roots and from 76 to 39.7% for lateral roots in response to increasing soil salinity from 3.9 to 10.0dSm^{-1}. As has been estimated using regression equations given in results, the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 10.8, 9.3, 7.9 and 7.7 for leaves, stems ,tap roots and lateral root tissues, respectively. Root/shoot dry weight ratio was 1.10 under control conditions, while it was 1.05, 0.93, 0.89 and 0.87 for seedlings grown in soils at 3.9, 6.2, 8.1 and 10.0 dSm^{-1} salinities, respectively. Root/shoot dry weight ratios significantly decreased ($p<0.01$) as soil salinity increased. There was a negative relationship between root/shoot dry weight ratio and soil salinity ($r=-0.665$, $p<0.01$).

Effect of salinisation on water content of tissues

Water content in leaves, stems, taproots and lateral root tissues significantly decreased ($p<0.01$) with increasing concentration of salt in soil (Fig. 2A). There was maximum water content in lateral roots and minimum in tap roots. Tissues according to their water content can be arranged in following decreasing order: lateral roots >leaves > stems > tap roots. There was a negative relationship between water content in different tissues and salt concentration ($r=-0.744$, -0.770, -0.872 and -0.793, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on water potential of tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($p<0.01$) as soil salinity increased (Fig. 2B). Tissues according to their water potential values (low to high negative) can be arranged in the following order: leaves > lateral roots > tap roots > stems. There was a negative relationship between water potential of tissues and salt concentration ($r=-0.961$, -0.906, -0.933 and -0.980, $p<0.01$, for for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on proline content of tissues

Proline content (µ mol/g FW material) significantly increased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues with increase in soil salinity (Fig. 2C) .Tissues according to their pro-

Salinity (dSm-1)	Shoot height (cm)	Root length (cm)	Leaf area ($cm^2 plant^{-1}$)	Leaf weight ($mg\ plant^{-1}$)	Stem weight ($mg\ plant^{-1}$)	Shoot weight (leaf+stem) ($mg\ plant^{-1}$)	Tap root weight ($mg\ plant^{-1}$)	Lateral root weight ($mg\ plant^{-1}$)	Total root weight ($mg\ plant^{-1}$)
0.2	34.4±0.7	46.5±0.9	105.7±3.0	620.3±19.8	1880.6±19.1	2500.9±34.4	1674.5±42.8	1063.3±33.5	2737.8±57.9
3.9	29.3±0.7	38.5±0.8	85.2±2.8	540.8±17.5	1469.0±29.3	2009.8±30.8	1298.6±38.5	807.9±35.1	2106.5±50.6
6.2	25.6±0.8	30.5±0.7	66.3±3.0	455.7±11.9	1182.7±17.4	1638.4±25.3	930.3±33.6	592.5±27.3	1522.8±50.8
8.1	23.0±0.7	26.8±0.7	56.0±2.2	380.7±8.1	1031.7±18.2	1412.4±21.0	782.9±33.4	476.1±23.2	1259.0±42.7
10.0	19.3±0.4	24.5±0.9	46.8±2.5	334.7±8.9	944.6±14.4	1279.3±16.9	691.2±15.6	422.2±26.8	1113.4±34.9
α	34.97	46.77	107.17	638.57	1862.80	2501.40	1675.30	1062.30	2737.60
β	-1.52	-2.36	-6.18	-30.30	-98.78	-129.09	-105.60	-68.64	-174.25
r	-0.876	-0.924	-0.888	-0.885	-0.965	-0.941	-0.932	-0.896	-0.947
$LSD_{0.05}$	5.0	5.5	18.2	92.2	133.3	173.3	223.3	193.5	314.7

Relationship is significant at p < 0.01.

Tabla 1. Efecto de salinización del suelo en las características de hoja, tallo, brote y raíz de *Acacia senegal* indicado por la media ± DS y las constantes de la ecuación de regresión.

Table 1. Effect of salinisation of soil on leaf, stem, shoot and root characteristics of *Acacia senegal* as indicated by mean ± SEM and regression equation constants.

line content can be arranged in following decreasing order: stems > tap roots > leaves > lateral roots. There was a positive relationship between salt concentration and proline content of tissues(r=0.949, 0.953, 0.977 and 0.954, p<0.01, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content (r=-0.954, -0.829, -0.919 and -0.963, p<0.01, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content (r=-0.986, -0.956, -0.998 and -0.986, p<0.01, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on mineral accumulation

Potassium and sodium content and K/Na ratio

Potassium content (as mg g^{-1} dry weight) significantly decreased (p<0.01) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a negative relationship between potassium content in tissues and increase in salt concentration in soil (p<0.01). Sodium content significantly increased (p<0.01) in leaves, stems, tap roots and lateral root tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress (p<0.01). K/Na ratio significantly decreased (p<0.01) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K /Na ratio in tissues and increase in salt stress (p<0.01).

Nitrogen, phosphorus, calcium and magnesium

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen, phosphorus, potassium and calcium content significantly decreased in leaves, stems, tap roots and lateral root tissues (p<0.01), as the salinity increased (Table 2). A negative relationship was obtained in N, P, K and Ca content of tissues and salt concentration (p<0.01). Magnesium content exhibited a significant increase (p<0.01) in leaves, stems, taproots and lateral root tissues in response to increase in salt stress. There was a significant positive relationship between Mg content in tissues and salt concentration in soil (p<0.01).

Micro-elements

There was a significant decrease in the concentration of Zn, Cu, Mn and Fe (p<0.01) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress (Table 2) . A negative relationship was obtained between soil salinity and Zn, Cu, Mn and Fe content in tissues (p<0.01).

Discussion

Earlier work (Ramoliya et al. 2004), indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dSm^{-1}, but for *Acacia senegal* SG_{50} was obtained at 5.9 dSm^{-1}. That would suggest that this plant species is relatively salt toler-

ant at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0-15 cm depth) varies from 2.0 to 5.0 dSm^{-1}. Eventually, seeds of *A. senegal* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 10.0dSm^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil with high concentration of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Slater et al. 2003) and induces changes in the activities of many enzymes (Dubey & Rani 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz & Zeiger 2006). Moreover, tap root elongation for seedlings grown in control and saline soils both was greater than that of shoot. Result suggests that this species has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington 1987) and is considered an adaptation to survive in dry habitats. Root/shoot dry weight ratio of *A. senegal* was 1.1 under control conditions and was greater than that for aridity and salt tolerant seedlings of *A. catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya et al. 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect 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 (Kramer 1983, Garg & Gupta 1997). Results for reduction of shoot growth and leaf area development of *A. senegal* with increasing salt concentration are in conformity with the 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. Also high concentration of salt tends to slow down or stop root elongation (Kramer 1983) and causes reduction in root production (Garg & Gupta 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of tap roots and lateral roots while lowest in leaves. Consequently, leaves were more resistant than roots to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: leaves>stems>tap roots=lateral roots. The greater reduction in root weight than in shoot weight resulted in decreased root/shoot dry weight ratio for seedlings with increase in salinity. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion (Marschner 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration. The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgor maintenance or for the replacement of potassium ions in various metabolic functions by Na^+ (Marschner 1995). Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *A. senegal* survived up to the soil salinity of 10 dSm^{-1} and, therefore, this tree species is moderate salt tolerant. In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates (Greenway & Munns 1980). As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway & Munns 1980). Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	27±1.0	2.4±0.1	10.5±0.4	3.8±0.3	14.9±0.1	5.9±0.1	2.8±0.2	114±4.1	38±2.3	103±3.5	796±5.0
	3.9	25±1.1	2.0±0.1	9.3±0.4	4.8±0.3	11.5±0.4	6.0±0.1	2.0±0.1	43±4.6	34±2.2	88±4.3	515±5.1
	6.2	24±1.1	1.6±0.1	9.2±0.1	7.2±0.3	10.2±0.3	6.1±0.0	1.3±0.0	38±2.6	28±2.6	86±2.6	419±4.3
	8.1	23±0.8	1.5±0.0	7.0±0.5	9.0±0.2	8.3±0.5	6.2±0.1	0.8±0.0	28±4.7	26±2.0	82±1.5	405±4.7
	10.0	20±0.6	1.2±0.2	6.3±0.4	10.8±0.2	8±0.5	6.8±0.1	0.6±0.0	27±3.6	24±1.3	59±2.5	353±4.0
	α	27.51	2.43	10.96	2.92	14.71	5.75	2.84	98.69	38.58	105.45	747.95
	β	-0.65	-0.12	-0.43	0.73	-0.72	0.07	-0.23	-8.57	-1.51	-3.84	-44.07
	r	-0.824	-0.913	-0.884	0.958	-0.958	0.733	-0.959	-0.883	-0.850	-0.777	-0.948
	$LSD_{0.05}$	2.9	0.4	0.4	0.9	1.3	0.4	0.5	11.8	6.3	9.0	13.7
Stem	0.2	22±1.1	2.0±0.1	6.6±0.2	2.1±0.1	10.9±0.3	3.1±0.2	3.2±0.2	138±3.2	35±2.0	50±1.5	780±3.5
	3.9	20±1.0	1.6±0.1	5.6±0.2	2.8±0.2	10.6±0.3	3.7±0.3	2.0±0.1	55±5.5	32±2.6	45±1.1	676±6.1
	6.2	18±1.5	1.2±0.1	4.8±0.2	4.5±0.3	9.4±0.4	4.0±0.3	1.1±0.1	46±3.6	28±2.5	38±1.7	556±3.6
	8.1	16±1.1	1.1±0.0	3.9±0.2	4.7±0.3	9.1±0.4	4.3±0.2	0.8±0.1	40±4.1	24±1.7	29±2.0	480±3.7
	10.0	15±0.6	0.8±0.2	3.8±0.3	5.2±0.2	7.1±0.5	5.6±0.5	0.7±0.1	35±4.3	23±0.7	24±2.6	361±5.5
	α	22.44	2.05	6.69	1.93	11.48	2.85	3.05	119.83	35.91	52.91	812.29
	β	-0.74	-0.12	-0.30	0.33	-0.36	0.22	-0.26	-10.04	-1.32	-2.76	-42.55
	r	-0.845	-0.919	-0.930	0.955	-0.829	0.795	-0.948	-0.884	-0.829	-0.939	-0.988
	$LSD_{0.05}$	3.3	0.4	0.8	0.4	1.3	1.0	0.4	12.4	6.0	5.6	13.6

Relationship is significant at $p < 0.01$

Tabla 1-Parte 1ª (Hoja & tallo). Efecto de la salinización del suelo sobre el contenido de nutrientes de los tejidos (hoja, tallo, raíz primaria y raíces laterales) de *Acacia Senegal* indicado indicado por la media ± DS y las constantes de la ecuación de regresión.

Table 2-Part 1 (Leaf & stem). Effect of salinisation of soil on nutrient content of tissues (leaf, stem, tap root and lateral root) of *Acacia Senegal* as indicated by mean ± SEM and regression equation constants.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g\text{-}^{1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Tap roots	0.2	18±1.0	1.6±0.1	5.0±0.1	2.0±0.2	8.8±0.3	2.8±0.2	2.6±0.3	131±4.1	32±1.5	68±2.5	3646±5.8
	3.9	16±0.6	1.2±0.1	4.5±0.2	2.3±0.1	6.8±0.5	3.3±0.3	2.0±0.2	59±4.9	22±1.1	55±1.5	3460±5.5
	6.2	15±0.7	1.0±0.1	3.0±0.2	3.7±0.2	4.9±0.4	4.1±0.2	0.8±0.1	36±3.0	20±1.7	41±3.6	3227±3.6
	8.1	14±0.5	0.7±0.1	2.8±0.3	4.4±0.3	4.8±0.4	4.3±0.3	0.7±0.1	34±3.7	17±2.0	29±1.1	3011±4.9
	10.0	12±0.7	0.6±0.1	2.5±0.2	5.4±0.1	3.6±0.5	5.1±0.4	0.5±0.0	31±4.3	15±0.5	25±1.7	2930±5.0
	α	18.29	1.61	5.15	1.51	8.77	2.61	2.63	115.79	30.81	70.14	3697.20
	β	-0.58	-0.10	-0.28	0.35	-0.52	0.23	-0.23	-10.14	-1.69	-4.67	-77.88
	r	-0.865	-0.893	-0.904	0.932	-0.928	0.850	-0.911	-0.904	-0.917	-0.971	-0.987
	LSD $_{0.05}$	2.3	0.4	0.7	0.6	1.3	0.9	0.6	12.0	4.4	6.7	14.8
Lateral roots	0.2	19±1.0	1.5±0.0	4.3±0.2	4.1±0.1	8.4±0.4	4.5±0.2	1.1±0.1	148±4.0	76±1.5	175±2.6	5225±4.5
	3.9	15±1.0	1.2±0.1	4.2±0.1	5.2±0.2	7.2±0.5	5.2±0.3	0.8±0.1	77±3.5	67±1.0	164±2.0	5016±5.7
	6.2	14±1.1	0.8±0.1	4.0±0.1	6.1±0.3	6.5±0.3	5.9±0.3	0.7±0.0	47±4.9	60±2.6	159±3.0	4743±5.1
	8.1	13±1.0	0.6±0.0	3.5±0.2	7.8±0.5	6.4±0.6	6.0±0.2	0.5±0.0	44±4.6	56±1.1	133±3.2	4572±5.5
	10.0	12±0.5	0.4±0.1	3.1±0.1	7.9±0.5	3.0±0.5	6.6±0.2	0.4±0.0	41±4.1	50±2.5	124±0.5	4543±4.5
	α	18.54	1.56	4.51	3.82	8.94	4.44	1.07	134.02	76.78	181.43	5251.30
	β	-0.69	-0.11	-0.12	0.42	-0.46	0.21	-0.06	-11.01	-2.63	-5.35	-75.96
	r	-0.848	-0.927	-0.808	0.917	-0.821	0.868	-0.934	-0.918	-0.957	-0.930	-0.982
	LSD $_{0.05}$	2.9	0.3	0.5	1.1	1.5	0.8	0.2	12.5	5.6	7.3	15.0

Relationship is significant at $p < 0.01$

Tabla 1-Parte 2ª (Raíces). Efecto de la salinización del suelo sobre el contenido de nutrientes de los tejidos (hoja, tallo, raíz primaria y raíces laterales) de *Acacia Senegal* indicado indicado por la media ± DS y las constantes de la ecuación de regresión.

Table 2-Part 2 (Roots). Effect of salinisation of soil on nutrient content of tissues (leaf, stem, tap root and lateral root) of *Acacia Senegal* as indicated by mean ± SEM and regression equation constants.

solution.

In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al. 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (Stewart & Lee 1974). In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar et al. 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole-plant homeostasis (Schachtman et al. 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow et al. 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox & Guerinot 1998). In the present study, significant decrease of K_+ content in all the tissues of seedlings with increasing soil salinity suggests that Na^+ inhibited K_+ uptake.

The exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control to transport of salts to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues of *A. senegal* suggests that this mechanism to block Na+ transfer to growing tissues was not effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively low accumulation of K^+ than that of Na^+. As a consequence there were no effective mechanisms to control net uptake of Na+ on root plasma membrane and subsequently its transport to shoot tissue. The pattern of accumulation of K^+ and Na^+ in *A. senegal* conforms to group C and/or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K_+ and Na^+ are similar (Watad et al. 1991, Schroeder et al. 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high-affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca_{2+}. These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated (Amtmann & Sanders 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Watad et al. 1991, Schroeder et al. 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis & Sanders 1994, Niu et al. 1995). The K^+ and Na^+ profiles of *A. senegal* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al. 2001). As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant.

In general, salinity reduces N accumulation in plants (Feigin 1985). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Torres & Bingham 1973, Garg & Gupta 1997). The Interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol 1979, Grattan & Grieve 1992). However it is known that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach et al. 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e.g., in

take of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen & Chang 1987, Garg & Gupta 1997). Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates (Marschner & Cakmak 1989). In the present study increase of Mg^{2+} in tissues may be of importance for plant growth and survival in saline soils.

It is difficult to suggest mechanistic explanations of salinity influence on micro-element concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al. 2000). In the present study, it appears that salinity reduced Zn, Cu, Mn and Fe accumulation, at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox system, Mn for photolysis of water in photosynthesis and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner 1995). In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. The simultaneous decrease of Zn, Cu, Mn and Fe in leaves of *A. senegal* might limit the growth of plants. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al. 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater et al. 2003). Decrease in Cu, Zn, Mn and Fe content at the whole plant level might affect the survival of *A. senegal* in saline soil where salinity exceeded $10.0 dSm^{-1}$.

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