Ecophysiology of seed dormancy and salt tolerance of
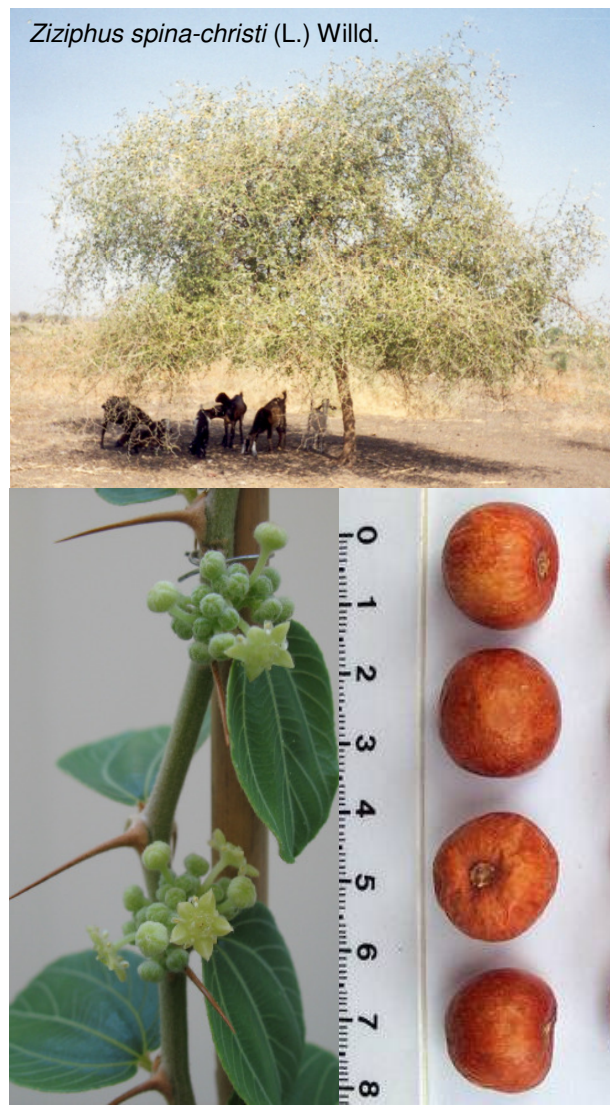# *Grewia tenax* (Forssk.) Fiori and *Ziziphus spina-christi* (L.) Willd.

Muhammad Sohail

Grewia tenax (Forssk.) Fiori

Ziziphus spina-christi (L.) Willd.

Die vorliegende Arbeit wurde vom Fachbereich Agrarwissenschaften der Universität Kassel als Dissertation zur Erlangung des akademischen Grades eines Doktors der Agrarwissenschaften (Dr. agr.) angenommen.

Tag der mündlichen Prüfung   15. Oktober 2009

Acknowledgements

Table of Contents

Summary

Ecophysiology of seed dormancy and salt tolerance of
Grewia tenax (Forssk.) Fiori and *Ziziphus spina-christi* (L.) Willd.

The overall forest cover of Pakistan is only 4.8% of the total land area. At the same time, Pakistan's deforestation rates are alarmingly high and threatening the lives of many people who are dependent upon them for their livelihood. More than 70% of the country area is exposed to arid and semi-arid tropical climate conditions including 11.5 million hectares of deserts which are characterized by extreme temperatures and erratic rainfall. These dry areas are suffering from recurrent droughts and their vegetation cover greatly depends on the amount of precipitation. Increasing aridity and soil salinity are considered important abiotic factors that threaten crop biodiversity and ultimately land degradation in these areas. Drought and salinity stress are interrelated ecophysiological phenomena and cause many problems for seed germination and plant growth inducing physiological and biochemical disorders in metabolic processes.

Utilization of salt tolerant plant species is considered a sustainable adaptation strategy to soil salinity in drylands, which is otherwise impossible to manage through agronomic or engineering practices. Trees and shrubs, which are adapted to local conditions, can play a vital role to combat dryland salinity and rehabilitate degraded lands. The potential role of indigenous multipurpose fruit trees has often been overlooked in this contest. Some of these species are very important to rural population to secure their livelihood.

Pakistan harbors many tropical fruit tree species. *Grewia tenax* (Forssk.) Fiori and *Ziziphus spina-christi* (L.) Willd. are two indigenous tropical fruit trees that

grow wild and are extensively used by the rural population. Their fruits are edible and consumed either fresh or dried and their leaves provide palatable fodder for livestock especially during the dry periods of the year. Despite their great ability to withstand drought and high temperature, wild stands of the species are sparse.

Seed dormancy is a typical feature of dryland tropical species facilitating their survival under unfavorable climatic conditions. At the same time, increasing dryland soil salinity might be a possible threat to seedling survival. As emergence and early seedling growth are critical stages in the survival of most woody plants, seed germination and sensitivity of seedlings to soil salinity are important areas of research. This thesis comprises three experiments conducted under controlled climatic conditions to study how to overcome seed dormancy and to examine seedling's ability to tolerate NaCl salinity.

The results of the study about seed dormancy breaking of *G. tenax* show that incubation of seeds at 40°C for 4 weeks before sowing significantly increased total germination from 20% (control) to 70%. Heat incubation also significantly reduced days to first emergence and mean days to emergence as compared to the untreated control. The results indicate that seeds of *G. tenax* have a physiological dormancy which can be overcome by pre-sowing heat treatment of seeds (constant heat exposure) at 40°C for 4 weeks.

Other two studies determined the salt tolerance potential of *G. tenax* (chapter 4) and *Z. spina-christi* seedlings (chapter 5). Six-week old seedlings of both species were subjected to 0, 40, 80 and 160 mM NaCl salinity, corresponding to electrical conductivities (EC) of 1.8, 5.6, 8.6 and 15.2 dS m^{-1}, respectively in separate experiments. Initially seedlings of both species were given half strength of the proposed final salt levels for four weeks to avoid young plants collapsing due to a sudden salt shock, especially at high salinity.

NaCl salt treatment up to 160 mM had no effect on the survival of *G. tenax* seedlings; nevertheless, salt injury symptoms were noticed on the surface of

mature basal leaves at this salinity level after 6 weeks of salt application. At the same time, significant reduction in plant height was only observed at 160 mM NaCl salinity. The presence of NaCl in the growing medium significantly reduced total leaf area per plant at high salinity. At the same time, specific leaf area (SLA) increased significantly at the high (160 mM) salt level. A consistent decline in specific root length (SRL) was observed in plants at higher salinity as compared to the control plants. Addition of NaCl to the nutrient solution also led to an increase in relative water content of *G. tenax* seedlings. This effect was most distinct in leaf tissues. Remarkable increase in Na and Cl ions concentrations in all plant tissues was observed with increasing salinity which indicates *G. tenax* as ion accumulator. NaCl-induced salinity did not result critical deficiency of macronutrients in plant tissues. Despite excessive accumulation of salt ions, decrease in K content of leaf tissues was insignificant at all salt levels. Significant reduction in leaf chlorophyll was only observed in plants treated with the high salt level (160 mM NaCl) compared to control plants. Net photosynthesis (P_n) was only decreased significantly at 80 and 160 mM NaCl salinity in comparison to untreated plants. Stomatal conductance (G_s) and transpiration (E) were reduced significantly at all salt levels applied, nevertheless significant increase in internal CO_2 concentration (C_i) was only observed at 160 mM salinity.

As seedlings of *G. tenax* survived and continue to grow up to 160 mM NaCl salinity, our results have the indication that *G. tenax* is equipped with sufficient adaptive mechanisms to tolerate high salinity.

On other hand, seedlings of *Z. spina-christi* showed significant reduction in plant height, number of leaves per plant, total leaf area and dry matter accumulation with increasing salinity. Species showed significant increase in their leaf water contents. Remarkable increases in Na and Cl concentrations were also observed in leaf, stem and root tissues with increasing salinity. At later stages of the experiment, excessive accumulation of toxic salt ions in

growing tissues of *Z. spina-christi* seedlings caused severe foliar damage (chlorosis and necrosis). At 80 and 160 mM NaCl salinity seedlings showed severely stunted growth and 20% mortality. Nevertheless, seedlings of tolerated moderate salinity (40 mM NaCl) most likely through adequate root uptake efficiency for macronutrients (ionic balance) and increased leaf water content of stressed plant (salt dilution). Over all, our results allow to classify *Z. spina-christi* a moderate salt tolerant fruit tree species at the seedling stage.

During our studies we also observed considerable variation to salt tolerance within species that indicated genetic differences among seedlings of both species. This points to the potential of breeding for increased tolerance to salt stress.

C h a p t e r 1

General Introduction

Pakistan is located between 24° and 37° N latitude and 61°and 75° E longitude covering an area of 88.2 million hectares (Roohi 2007). Because of its topographic diversity, Pakistan has diverse climatic zones from humid regions in the north to an arid tropical climate in southern and western parts of the country. More than 70% of Pakistan is classified as arid to semi-arid with less than 300 mm of mean annual rainfall (Siddiqui et al. 1999; Muhammad 2002; Roohi 2007). It also includes dry arid deserts of Cholistan, Thar, Thal and Kharan covering a total area of 11.5 million ha with an annual rainfall of less than 120 mm (FAO 2006; Ashraf 2006). The continental type of climate of these areas is characterized by maximum temperatures exceeding 50 °C.

Climate change due to gradual increase in atmospheric greenhouse gasses has increased the earth's surface temperature by 0.74 °C in the past century and will continue to do so (IPCC 2007) combined with significant changes in rainfall patterns (Houghton et al. 2001). At the same time mean annual surface temperature in Pakistan has risen since the beginning of the 20th century (Chaudhury 1994). Lal et al. (1995) reported that the surface air temperature over the Indian subcontinent is likely to rise from 1.0 °C (during the monsoon) to 2.0 °C (during the winter) by the middle of the 21st century. Moreover, the largest precipitations reductions (>1 mm day^{-1}; 60% decline in soil moisture) are expected for the arid regions of northwest India and Pakistan.

Ongoing global warming has led to increased aridity because of a rise in potential evapotranspiration (Pinol et al. 1998; Penuelas et al. 2005). Increasing soil salinity is becoming a more serious problem in arid zones where about 50% of the cropland is salt affected (FAO 2005; Flower and Yeo 1995). Excessive salt concentration transforms productive soil to barren land, destroy habitats for plants and animals and leads to a loss of biodiversity (Ghassemi et al. 1995).

Drought and soil salinity are prime agents for deforestation and ultimately land degradation. Soil salinity causes many problems for plant growth and

development through osmotic stress, ion toxicity, mineral deficiencies and induced physiological and biochemical disorders in metabolic processes (Yeo 1998; Hasegawa et al. 2000; Munns 2002). Increased salt concentrations in the root zone reduce the ability of plants to take up water and nutrients (osmotic stress). Water and nutritional deficiencies condition lead to slow, stunted or no growth. At this stage, abnormalities of cellular and metabolic processes are similar to those in plants under drought stress (Munns 2005). Longer exposure to salinity can cause excessive accumulation of salt ions in plant tissues through the transpiration stream leading to ionic toxicity. This can cause leaf senescence and further growth reductions in salt tolerant and growth termination in salt sensitive species (Munns 2005).

The natural vegetation in arid zones of Pakistan is predominated by hard-wooded tropical thorn forests in the plain areas and subtropical evergreen forests in the mountainous northern and western parts of the country (Champion et al. 1965; Michael 1997).

Wild growing indigenous fruit trees play an important role for the livelihoods of rural people providing food and feed (Saied et al. 2008), especially for those living in dry areas (Von Maydell 1990), where crop failure often results in poor nutrition of the local population (Maxwell 1991). These wild fruit tree species are often referred to as neglected or underutilized but their genetic resources contribute the biodiversity of agroecosystem (Hammer et al. 2001; Grivetti and Ogle 2000). *Ziziphus spina-christi* and *Grewia tenax* are two important fruit tree species growing wild in arid regions of Pakistan. Both species are of importance among rural communities given their multipurpose use.

As increasing aridity and soil salinity are becoming the major problems confronting biodiversity in arid and semi-arid regions of Pakistan; *Z. spina-christi* and *G. tenax* are part of the threatened biological assets of the rural poor. Although these species are adapted to adverse arid tropical climatic conditions, their wild stands become increasingly threatened. Poor germination and seedling mortality might be the most plausible reasons for

the propagation of both woody species. The studies summarized in this thesis were therefore conducted to contribute to an improved understanding of seed dormancy and seedling growth response of these species to salinity. It is hoped that the results obtained will contribute to the promotion of *G. tenax* and *Z. spina-christi* stands in the drylands of the tropics and subtropics.

References

Ashraf M (2006) Tolerance of some potential forage grasses from arid regions of Pakistan to salinity and drought. In: Öztürk, M.; Waisel, Y.; Khan, M.A.; Görk, G. (Eds.): Biosaline Agriculture and Salinity Tolerance in Plants. Birkhäuser Verlag/Switzerland, 15-27.

Champion HG, Seth SK and Khattak GM (1965) Forest types of Pakistan. Pakistan Forest institute Peshawar.

Chaudhury QZ (1994) Pakistan's summer monsoon rainfall associated with global and regional circulation features and its seasonal prediction', in Proceedings of the International Conference on Monsoon Variability and Prediction, Trieste, Italy, 9–13.

FAO (2005) Global network on irrigated soil management for sustainable use of salt effected soils. FAO land and plant nutrition service, Rome, Italy

FAO (2006) Chief, Electronic Publishing Policy and Support Branch, Information Division FAO, Viale delle Terme di Caracalla 00100 Rome, Italy

Flower TJ and Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? Australian Journal of Plant Physiology 22, 875-884.

Ghassemi F, Jackman AJ and Nix AH (1995) Salinization of Land and water resources. CAB International, Wallingford, England.

Grivetti LE and Ogle BM (2000) Value of traditional food in meeting macro and micronutrients needs: the wild plant connection. Nutrtional Research Reviews 13, 31–46.

Hammer K, Heller J and Engels J (2001) Monographs on underutilized and neglected crops. Genetic Resource and Crop Evolution 48, 3–5.

Hasegawa PM, Bressen RA, Zhu JK and Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annual Review of Plant Biology 51, 463-499.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K and Johnson CA (2001) IPCC Third assessment report: climate change 2001. Cambridge University Press.

IPCC (2007) Climate Change 2007: the physical science basis. Contribution of Working Group I. In: Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M,

Lal M, Cubasch U, Voss R and Waszkewitz J (1995) Effect of transient increase in greenhouse gases and sulphate aerosols on monsoon climate. Current Science 69(9), 752–763.

Maxwell S (1991) To cure all hunger: food policy and food security in Sudan. Short Run Press, London

Michael RD (1997) the shift of tree cover from forest to farmers in Pakistan: along and broad view. In: Michael Arnold JE and Dewees A (ed.): Farms, trees and farmers: Response to agricultural intensification, Earthscan, 65-84.

Muhammad D (1998) Grassland and pasture crops: Country pasture/forage profiles,Pakistan. Food and Agriculture Organization of the United Nations.

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell and Environment 25, 239-250.

Munns R (2005) Genes and salt tolerance bringing them together. New Phytologist 167, 645-663

Munns R (2007) Utilizing genetic resources to enhance productivity of salt-prone land. Published as part of a theme on Salt-Prone Land Resources.

CAB Reviews: erspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2, No. 009.

Penuelas J, Filella I, Sabate S and Gracia C (2005) Natural systems: terrestrial ecosystems. In: Report on Climate Change in Catalonia (ed. Llebot JE), pp. 517–553. Institut d'Estudis Catalans, Barcelona, Spain.

Pinol J, Terradas J and Lloret F (1998) Climate warming, wildfire hazard, and wildfire occurrence in coastal eastern Spain. Climate Change 38, 347–357.

Roohi R (2007) Research on global changes in Pakistan. In: Baudo, R; Tartari, G; Vuillermoz (Eds): Mountains Witnesses of Global Changes. Elsevier B.V., 329

Saied AS, Gebauer J, Hammer K and Buerkert A (2008) *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. Genetic Resource and Crop Evolution 55, 929–937.

Siddiqui KM, Iqbal M and Ayaz M (1999) Forest ecosystem climate change impact assessment and adaptation strategies for Pakistan. Climate Research 12, 195–203.

Sivakumar MVK, Das HP and Brunini O (2005) Impacts of present and future climate variability and change on agriculture and forestry in the arid and semi-arid tropics. Climatic Change, 70, 31–72.

Somerville C and Briscoe J (2001) Genetic engineering and water. Science 292, 2217.

Von Maydell HJ (1990) Criteria for the selection of food producing trees and shrubs in semi-arid regions. In: Wickens GE, Haq N, Day P (eds) New crops for food and industry. Chapman and Hall, London, pp 66–75.

Walsh KJE and Ryan BF (2000) Tropical cyclone intensity increase near Australia as a result of climate change. Journal of Climate 13, 3029–3036.

Wery J, Silim SN, Knights EJ, Malhotra, RS and Cousin R (1994) Screening techniques and sources and tolerance to extremes of moisture and air temperature in cool season food legumes, Euphytica 73, 73–83.

Yeo AR (1998) Molecular biology of salt tolerance in the context of whole-plant physiology. Journal of experimental Botany 49, 915-929.

C h a p t e r 2

Significance of *Grewia tenax* (Forssk.) Fiori and *Ziziphus spina-christi* (L.) Willd.: two wild growing fruit tree species of Pakistan

2.1 *Grewia tenax* (Forssk.) Fiori

Grewia tenax belonging to the Tiliaceae family is distributed throughout the western and eastern Sahelian zones, northern and southern Africa, the Arabian Peninsula and is also reported to grow from Iran to India (von Maydell 1990) on a wide array of soils (Bredenkamp 2000). It is a typical tropical plant species which can tolerate seasonal drought and withstand temperatures of more than 50°C (Gebauer et al. 2007b). Moreover, *Grewia tenax* is also known as dune fixing species because of its dense fast growing root system (Anonymous 2006). It is a deciduous fruit-producing shrub or small tree that may attain a height of 1 to 3 m.

Figure 1 *Grewia tenax* shrub growing at the upper edge of Wadi Muaydin, Oman (Photo: J. Gebauer)

Leaves are alternate and ovate to sub-orbicular with sharply toothed edges on stalks up to 1 cm long. The size of the leaves is up to 4.5 cm in width and 5.5 cm in length. Flowers are small, white, nectiferous, solitary or rarely paired with long stamens and even longer pistils. It bears globose, glabrous, fleshy, edible fruits arranged in 2-4 pairs. The fruit's color turns green to shiny orange or bright red when ripe containing one to two seeds in each fruit (Gebauer et al. 2007b).

Figure 2 Flowering branch of *Grewia tenax* with scale in cm (Photo: J. Gebauer).

Fruits and other parts of *G. tenax* contribute significantly to the food and energy needs of rural populations in multiple ways (El-Siddig et al. 2003; Vogt 1995). The fruit is eaten fresh but has commercial potential for consumption from beverages to ice cream, yogurt, porridge and confectionery. The juice made from its fruit is used as refreshing drink during the hot summer season.

Because of its high iron contents, fruits of *G. tenax* are often used in special diets for pregnant women and anemic children. Its leaves and twigs are palatable fodder for livestock (Gebauer et al. 2007b). Leaves and twigs of *G. tenax* are also an important component of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a poultice to treat swelling (El Ghazali et al. 1994; El Ghazali et al. 1997).

Figure 3 Ripened fruits of *Grewia tenax* with scale in cm (Photo: J. Gebauer).

2.2   *Ziziphus spina-christi* (L.) Willd.

Z. spina-christi belonging to the Rhamnaceae family is a medium-sized, thorny shrub or tree and is distributed throughout the tropical and subtropical regions of the world (Johnston 1963) including arid to semi-arid tropical forests of Pakistan where it is extensively used by the rural population (Sohail et al. 2008). The species can resist heat and drought (National Academy of Sciences 1980) and grows in desert areas with an annual rainfall of 50-300 mm (Jawanda and Bal 1978; Maydell 1986). It is evergreen but can drop some of its leaves during hot dry seasons (Maydell 1986). Leaves are simple, alternate, narrowly ovate, varying from 1 to 9 cm in length and 1 to 3.5 cm in width (Maydell 1986; Arbonnier 2004). The

Figure 4 *Z. spina-christi* near El Obeid, Central Sudan (Photo: J. Gebauer).

flowers are small, greenish yellow, sub-sessile with five minute sepals 2 mm long and five petals 1.5 mm long found in dense clusters in the axils of the leaves (El Amin 1990). The fruit is a globose drupe (about 1 to 1.5 cm in

diameter) and red-brown in color with a hard stone surrounded by a sweet edible pulp (Saied et al. 2008).

Fruits of *Z. spina-christi* are consumed either fresh or dried. The sweet pulp of the fruit is dried to produce fine flour which is either eaten raw or cooked in water, milk or buttermilk (Miller and Morris 1988; Saied et al. 2008). The leaves provide a palatable fodder for goats and sheep (Saied et al. 2008), under open grazing conditions (Verinumbe 1993), especially during fodder scarcity in dry seasons (Bunderson et al. 1990; Miehe 1986). The wood is used as a source of fuel, or for charcoal, furniture and construction material (Sudhersan and Hussain 2003). The timber is used for furniture, tool handles, fence posts, walk sticks, bent wood chairs, roofing beams, doors, windows and turned items. It is hard and heavy and

is known to resist termites (Sudhersan and Hussain 2003). Flowers are an important source for the production of excellent flavored wild bee honey, which is normally sold at high price (Sudhersan and Hussain 2003). The genus *Ziziphus* is known for its medicinal properties as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, antitumor, liver protective agent and as an immune system stimulant (Saied et al. 2008). Almost all parts of the plant (fruits, leaves, roots and bark) are important ingredients in folk medicine (Saied et al. 2008). Fruits and crashed kernels are eaten to treat chest pains, respiratory problems and as a tonic (Jongbloed 2003). Fruits are regarded as having purifying properties such as cleansing the stomach, removing impurities from blood as well as being a restorative for the whole system (Saied et al. 2008). Leaves and young branches are used as anti-inflammatory for eye wash, treat toothache and stomachache and as an antirheumatic (Ali-Shtayeh et al. 1998).

Figure 5 Leaves, spines, seeds and fruits of *Z. spina-christi* with scale in cm (Photo: J. Gebauer).

References

Ali-Shtayeh MS, Yaghmour MR, Faidi YR, Salem K and Al-Nuri MA (1998) Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. Journal of Ethnopharmacol 60, 265–271

Anonymous (2006) Agro Forestry Tree Database: A tree species reference and selection guide of the International Center for Research in Agroforestry, 1-2, http://www.worldagroforestry.org/sea/Products/ AFDbases/AF/asp/SpeciesInfo.asp? Sp ID = 17952, cited 15.01.2007.

Arbonnier M (2004) Trees, shrubs and lianas of West African dry zones. CIRAD, Margraf Publishers GmbH, Paris, France

Bredenkamp CL (2000) Tiliaceae In: Leistner OA (ed) Seed plants of southern Africa: families and genera. Strelitzia 10. National Botanical Institute, Pretoria, South Africa

Bunderson TW, El Wakeel A, Saad ZA and Hashim I (1990) Agroforestry practices and potentials in Western Sudan. In: Budd WW, Duchhart I, Hardesty LH, Steiner F (eds) Planning for agroforestry. *Elsevier Science Publishers B.V., Amsterdam*, pp 227–246

El Amin HM (1990) Trees and shrubs of the Sudan. Ithaca Press Exeter, Ithaca

El Ghazali GEB, El Tohami MS and El Egami AAB (1994) Medicinal plants of the Sudan: medicinal plants of the White Nile provinces. Khartoum University Press, Khartoum

El Ghazali GEB, El Tohami MS, El Egami AAB, Abdalla WS and Mohammed MG (1997) Medicinal plants of the Sudan: medicinal plants of northern Kordofan. Omdurman Islamic University Printing and Publishing House, Omdurman

El-Siddig K, El Tahir BA and Ebert G (2003) *Grewia tenax* – a potential new small fruit for the Sudan. Deutscher Tropentag, Conference on International Agricultural Research for Development in Göttingen, Book Abstr:309.

Gebauer J, Patzelt A, Hammer K and Buerkert A (2007) First record of *Grewia tenax* (Forssk.) Fiori in northern Oman, a valuable fruit producing shrub. Genetic Resources and Crop Evolution 54, 1153-1158.

Hammer K, Heller J and Engels J (2001) Monographs on underutilized and neglected crops. Genetic Resource and Crop Evolution 48, 3–5.

Jawanda JS, Bal JS (1978) The ber, highly paying and rich in food value. Indian Horticulture 23, 19–21.

Johnston MC (1963) The species of *Ziziphus* indigenous to United States and Mexico. American Journal of Botany 50, 1020–1027.

Jongbloed M (2003) The comprehensive guide to the wild flowers of the United Arab Emirates. Environmental research and wildlife development agency (ERWDA), Abu Dhabi

Maxwell S (1991) To cure all hunger: food policy and food security in Sudan. Short Run Press, London

Maydell HJ (1986) Trees and shrubs of the Sahel: their characteristics and uses. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn

Miehe S (1986) *Acacia albida* and other multipurpose trees on the fur farmlands in the Jebbel Marra highlands, western Darfur, Sudan. *Agroforestry Systems* 4, 89–119.

Miller AG and Morris M (1988) Plants of Dhofar, the southern region of Oman traditional, economic and medicinal uses. Adviser for Conservation of the Environment, Diwan of Royal Court Sultanate of Oman, Muscat

Montagnini F and Sancho F (1990) Impacts of native trees on tropical soils: a study in the Atlantic lowlands of Costa Rica, Central America. Ambio 19, 386-390.

National Academy of Sciences (1980) Firewood crops: shrub and tree species for energy production. National Academy Press, Washington.

Saied AS, Gebauer J, Hammer K and Buerkert A (2008) *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. Genetic Resource and Crop Evolution 55, 929–937.

Sohail M, Saied A, Gebauer J and Buerkert A (2008) Effect of NaCl-induced salinity on seedling growth of *Ziziphus spina-christi* (L.) Willd: a wild fruit tree species. Tropentag 2008*: conference on "competition for resources in changing world: new drive for rural development"* Hohenheim, Germany, 7-9 October 2008.

Sudhersan C and Hussain J (2003) In vitro propagation of a multipurpose tree, *Ziziphus spina-christi* (L.) Desf. Turkish Journal of Botany 27, 167–171.

Verinumbe I (1993) Soil and *Leucaena leucocephala* L. growth variability under *Faidherbia albida* Del. and *Ziziphus spina-christi* (L.) Desf. Agroforestry Systems 21, 287–292.

Vogt K (1995) A field worker's guide to the identification, propagation and uses of common trees and shrubs of dryland Sudan. SOS Sahel International, London, UK

Von Maydell HJ (1990) Criteria for the selection of food producing trees and shrubs in semi-arid regions. In: Wickens GE, Haq N, Day P (eds) New crops for food and industry. Chapman and Hall, London, pp 66–75.

C h a p t e r *3*

Seed Science and Technology (will be submitted)

Effect of seed stratification on germination of
Grewia tenax (Forssk.) Fiori

M. Sohail, A. S. Saied, J. Gebauer, and A. Buerkert

Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics, University of Kassel, Steinstr. 19, 37213 Witzenhausen, Germany

Abstract

The aim of this study conducted under controlled conditions was to compare different dormancy breaking methods for *G. tenax* seeds which were collected from the wild stands in Pakistan. In a first experiment, treatments were an untreated-control, constant heat exposure at 40°C, constant cold exposure at 4°C and alternate heat and cold exposure at 4 and 40°C. Seeds were treated for one week before sowing. In a second experiment, seeds were subjected to constant heat exposure (40°C) for 0, 1, 2, 3, 4, 5 and 6 weeks before sowing. The results of the first experiment showed that exposure of seeds to dry heat at 40°C for one week significantly improved germination up to 42% as compared to control (20%). The results of the second experiment provided evidence for a linear increase in total seed germination with in time of seed incubation at constant heat. Maximum germination (70%) was achieved, when seeds were incubated for 4 weeks. Seeds exposed to constant heat for 4 weeks also took only 4 and 5 days to reach first and 50% emergence, respectively as compared to untreated seeds, which took 10 and 14 days to reach first and 50% emergence, respectively. Moreover, emergence spread (duration between emergence of first and last seedling) lasted only 4 days as compared to untreated seeds with 21 days. Our results indicate that seeds of *G. tenax* possess a limited physiological dormancy which can be overcome by heat stratification.

3.1   Introduction

Increasing average temperatures combined with rise in rainfall variability and recurrent drought-spells (Houghton et al. 2001; Walsh and Ryan 2000; Easterling et al. 2000) are affecting stands of sensitive plant species in arid and semi-arid tropical areas of the world. Under these conditions indigenous multipurpose fruit tree species can play an important role to combat land degradation and minimize the risks of food scarcity (Teketay 1996). *G. tenax* is one of those underutilized indigenous fruit tree species that grows wild in many arid and semi-arid tropical regions of the world (Bredenkamp 2000) and has potential to rehabilitate degraded land and overcome malnutrition in rural population. Despite its ability to grow on wide array of soil and withstand harsh climatic condition, wild stands of the species are often threatened by overuse. Many of perennial plants species have developed mechanisms for seed survival under unfavorable climatic conditions. Dormancy is one way that enables seeds to survive, often for a number of years in the soil seed bank, until conditions are suitable for germination. On the other hand seed dormancy is considered a big hurdle to the effective use of many species in land revegetation programs (Merritt et al. 2007).

As there is little information on seed dormancy and germination characteristics of *G. tenax,* the purpose of this study was to identify practical pre-sowing seed treatments to promote the germination of *G. tenax.*

3.2   Materials and Methods

Seeds of *G. tenax* were collected from wild stands in the surrounding of Dera Ismail Khan (31° 48' N; 70° 37' E), Pakistan. Seeds were extracted from the pulp and stored in paper bags at room temperature until the start of experiment. Before sowing seeds were disinfected by soaking in sodium hypochloride solution (2%) for 15 minutes followed by three rinses with distilled water (Saied et al. 2008). Two successive experiments were conducted to identify appropriate seed treatment to improve their germination.

In the first experiment, treatments were untreated seeds (T_0), constant heat exposure of the seeds to 40°C (T_1), constant cold exposure of the seeds to 4°C (T_2) and alternate heat (40°C) and cold (4°C) exposure of the seeds (T_3) for 12 hours each day. Seeds were treated for one week before sowing for all treatments applied. In the second experiment, seeds were subjected to constant heat exposure at 40°C for 0 (T_0), 1 (T_1), 2 (T_2), 3 (T_3), 4 (T_4), 5 (T_5) and 6 weeks (T_6) before sowing.

The pretreated seeds were sown separately in a silica sand-based medium contained in inverted pyramid cells of 50-cell plastic trays, placed in plastic pans. Seeds were allowed to germinate under 30/25 °C (day/night) temperature and 50% relative air humidity. To minimize evaporation, each plastic tray was covered with a thin transparent plastic sheet with minute holes to facilitate gas exchange. Each experiment was composed of 8 replications with 8 seeds per experimental unit.

Emergence was assessed daily for a period of 30 days. A seed was considered germinated when the hypocotyls hook emerged above the surface. The number of days to the first emergence (E_{1st}), 50% emergence (E_{50}), emergence spread (Es) and total emergence percentage (E_t) were recorded. At the end of the experiment seedlings were harvested and cleaned of sand. After taking fresh weight (FW) of the seedlings, samples were oven dried at 70 °C for 48 hours for dry weight (DW).

The SPSS statistical package (SPSS Inc. Chicago, USA) was used to analyze experimental data using one way analysis of variance (ANOVA). Means were separated by a Tukey-test (P = 0.05).

3.3 Results

Experiment 1

The results of the first experiment showed that constant heat exposure of seeds to 40°C for one week significantly improved emergence (E_t) from 20% (control) to 42% (Figure 3.1). At the same time, the number of days to first

emergence (E_{1st}), 50% emergence (E_{50}), emergence spread (E_s) were reduced slightly (data not shown). Alternate cold and heat exposure (T_2) and constant cold exposure (T_3) of seeds for one week before sowing did not yield any significant increase in total emergence (Figure 3.1).
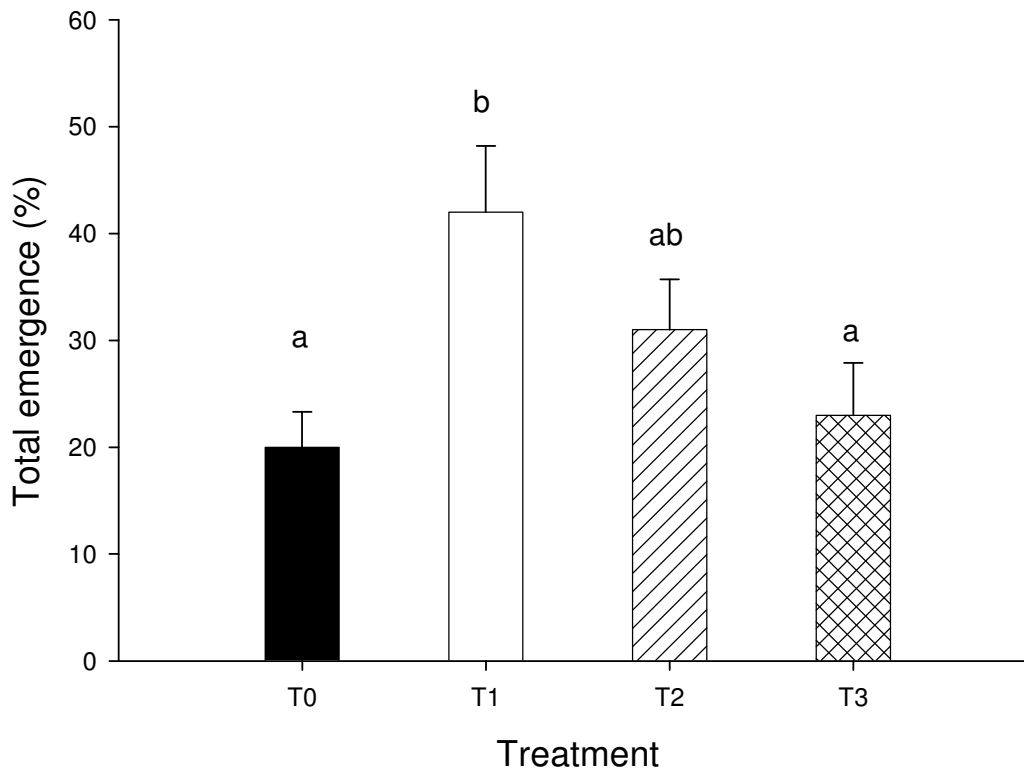
Figure 3.1 Effect of different pre-sowing seed stratification treatments on total emergence (E_t) percentage of *G. tenax* seeds, where T_0 = control, T_1 = constant heat exposure, T_2 = alternate heat and cold exposure and T_3 = constant cold exposure. Bars show means of eight replicates and letters indicate significant difference among treatment means ($P<0.05$; Tukey test).

Experiment 2

On the basis of results of the first experiment, seeds were subjected to increasing duration of constant heat to identify the most effective period. A linear increase in total emergence percentage with duration of heat exposure from 1 to 4 weeks ($T_1 – T_4$) at 40°C was observed (Figure 3.2).
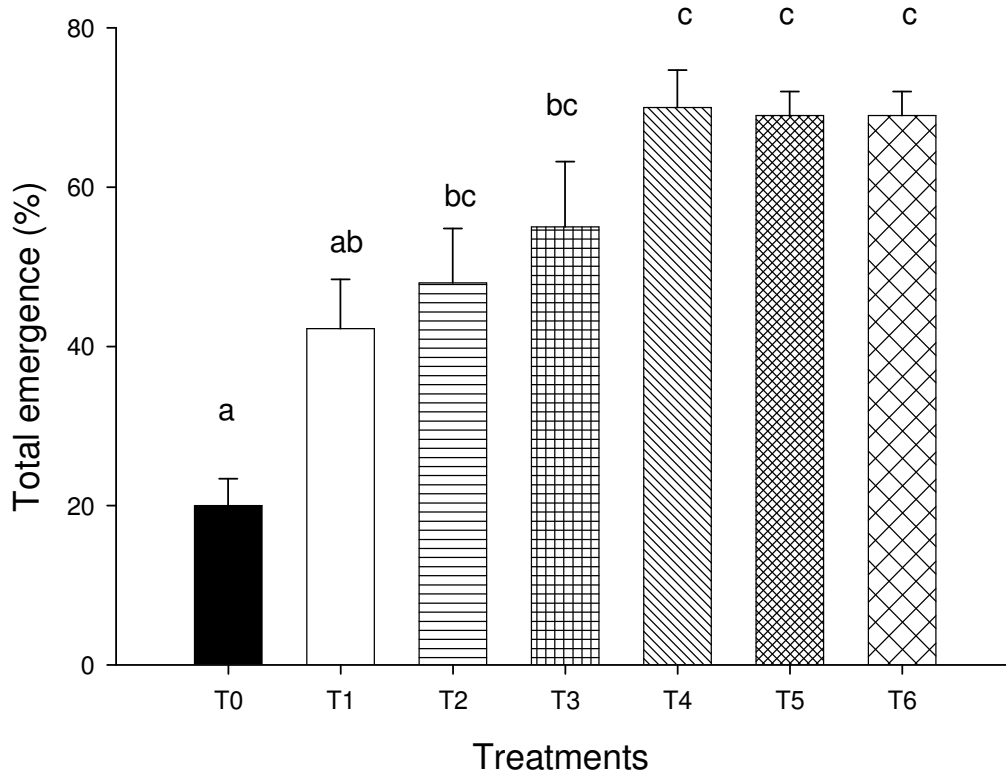
Figure 3.2 Effect of different pre-sowing heat stratification periods on total emergence (E_t) percentage of *G. tenax* seeds, where T_0 = control and $T_1 - T_6$ correspond to seeds exposed to constant heat at 40°C for 1, 2, 3, 4, 5, and 6 weeks, respectively. Bars show means of eight replicates and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

Constant heat exposure for 4 weeks prior to sowing not only significantly improved the seedling emergence (E_t) of *G. tenax* up to 70% as compared to the untreated control (20%) but also significantly reduced mean days to first emergence (E_{1st}), 50% emergence (E_{50}) and emergence spread (E_s). Seeds exposed to constant heat for 4 weeks, started to emerge (E_{1st}) after 4 days of sowing as compared to the control seeds which showed first emergence after 10 days. These seeds also achieved 50% emergence (E_{50}) after 5 days of sowing while control seeds took 14 days. At the same time, emergence

spread (E_s) in these seeds that is duration between emergence of the first and the last seedlings lasted only 4 days as compared to untreated seeds with 21 days (Table 3.1). Further increases in exposure time did not yield any significant improvement in total emergence (E_t) percentage, days to first emergence (E_{1st}), 50% emergence (E_{50}) and emergence spread (E_s). Constant heat exposure for 4 to 6 weeks before sowing also significantly increased fresh and dry weight of the seedlings (Table 3.1.)

Table 3.1   Effect of different pre-sowing heat stratification (at 40°C) treatments on days to first emergence (E_{1st}), 50% emergence (E_{50}), emergence spread (E_s) , fresh weight (FW) and dry weight (DW) of *G. tenax*.

Heat exposure (weeks)	E_{1st} (days)	E_{50} (days)	E_s (days)	FW (g)	DW (g)
Control	10 ± 1.5 a	14 ± 2.8 a	21 ± 2.5 a	1.3 ± 0.2 a	0.7 ± 0.4 a
1	08 ± 0.4 ab	12 ± 0.5 ab	14 ± 0.8 ab	2.1 ± 0.3 ab	1.0 ± 0.4 ab
2	08 ± 0.4 ab	11 ± 0.5 ab	10 ± 0.8 ab	2.6 ± 0.3 bc	1.3 ± 0.4 bc
3	06 ± 0.3 bc	07 ± 0.6 bc	08 ± 0.8 b	2.7 ± 0.2 bc	1.3 ± 0.3 bc
4	04 ± 0.2 c	05 ± 0.2 c	04 ± 0.4 b	3.3 ± 0.2 c	1.8 ± 0.3 c
5	04 ± 0.2 c	05 ± 0.2 c	04 ± 0.4 b	3.1 ± 0.2 bc	1.7 ± 0.2 bc
6	04 ± 0.2 c	05 ± 0.2 c	04 ± 0.4 b	3.1 ± 0.2 bc	1.7 ± 0.2 c

Values show means of 8 replicates ± s.e. different letters indicate significant difference among treatment means ($P < 0.05$; Tukey test).

3.4   Discussion

Since only a few weeks of heat stratification significantly improved germination percentage and rate, seeds of *G. tenax* are physiologically dormant, which is the most common form of dormancy found in seeds (Baskin and Baskin 1998; Baskin and Baskin 2003). Our results are in agreement with the findings of Turner et al. (2006), who reported breaking of seed dormancy by exposure to heat in *Acanthocarpus preissii*. Four weeks of continuous heat exposure also broke the physiological dormancy of *G. fascicularis* (Hoyle et al. 2008).

After-ripening changes in seeds can also occur at room temperature but can be enhanced by exposure to heat. The molecular mechanisms of after-ripening in seeds are not well understood (Finch-Savage and Leubner-Metzger 2006) but are thought to be non-enzymatic reactions removing inhibitors, membrane alterations within the seed and protein degradation (Bell 1999; Finch-Savage and Leubner-Metzger 2006). Exposure of seeds to low or high temperature has been shown to stimulate germination by inducing physical and physiological changes within the seeds and breaking dormancy (Willis and Groves 1991; Bewley and Black 1994; Copeland McDonald 2004; Baskin et al. 2005).

It is well documented that seeds of many tropical plant species require either cold or warm stratification to germinate, depending on a number of factors especially environmental conditions during seed development and storage time (Hartmann et al. 1997; Srivastava 2002; Copeland and McDonald 2004). Factors such as relative humidity, temperature, soil moisture and internal seed morphology and embryo size also influence a seed's lifespan and ability to germinate (Copeland and McDonald 2004). Seeds with under-developed or immature embryos may not germinate even under highly favorable germination conditions (Srivastava 2002; Baskin and Baskin 2001).

In conclusion, seeds of *G. tenax* appear to exhibit physiological dormancy like some forb species of semi-arid tropical Queensland (Hoyle et al. 2008) and

Acanthocapus preissii, a common perennial herb of Western Australia (Turner et al. 2006). Our results indicate how this dormancy can be broken in *G. tenax* and therefore have important implications for revegetation programs of degraded lands. Where this species is used for revegetation.

References

Anonymous (2006) AgroForestryTree Database: A tree species reference and selection guide of the International Center for Research in Agroforestry, 1-2,

http://www.worldagroforestry.org/sea/Products/AFDbases/AF/asp/SpeciesInfo.asp? Sp ID = 17952, cited 15.01.2007.

Baskin CC and Baskin JM (1998) Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. Academic press, San Diego, 666 pp.

Baskin CC and Baskin JM (2001) Seeds: Ecology, biogeography, and evolution of dormancy and germination. Academic Press*: San Diego*, California.

Baskin CC and Baskin JM (2003) Overview and recommendations for future research priorities on native seed dormancy and germination of Australian plants. Australian Plant Conservation 11, 2-9.

Baskin CC, Baskin JM and Yoshinaga A (2005) Morphophysiological dormancy in seeds of six endemic lobeliod shrubs (Campanulaceae) from the montane zone in Hawaii. Canadian Journal of Botany 83, 1630-1637.

Bell DT (1999) The process of germination in Australian species. Australian Journal of Botany 47, 475-517.

Bewley D and Black DJ (1994) Seeds: physiology of development and germination 2nd edn. Plenum Press: New York.

Bredenkamp CL (2000) Tiliaceae In: Leistner OA (ed) Seed plants of southern Africa: families and genera. Strelitzia 10. National Botanical Institute, Pretoria, South Africa

Copeland LO and McDonald MB (2004) Seed Science and Technology, Kluwer Academic Publishers: Massachusetts.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR and Mearns LO (2000) Climate extremes: observations, modelling, and impacts. Science 289, 2068–2074.

Finch-Savage WE and Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytologist 171, 501-523.

Hartmann HT, Kester DE, Davies FT and Geneve RL (1997) Plant Propagation: principles and practices.' (Prentice-Hall International Inc.: Upper Saddle River, New Jersey.

Hoyle GL, Daws MI, Steadman KJ and Adkins SW (2008) Mimiking a semi-arid tropical environment achieves dormancy alleviation for seeds of Australian native Goodeniaceae Asteraceae. Annals of Botany 101, 701-708.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K and Johnson CA (2001) IPCC Third assessment report: climate change 2001. Cambridge University Press.

Merritt DJ, Turner SR, Clarke S and Dixon KW (2007) Seed dormancy and germination stimulation syndromes for Australian temperate species. Australian Journal Botany 55, 336–344.

Saied AS, Gebauer J and Buerkert A (2008) Effect of different scarification methods on germination of *Ziziphus spina-christi* seeds. Seed Science and Technology 36, 201-205.

Srivastava L (2002) Plant growth and development; Simon Fraser University: Burnaby, British Columbia, Canada.

Teketay D (1996) Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethopia. Forest Ecology and Management 80, 209-223.

Turner SR, Merrit DJ, Ridley EC, Commander LE, Baskin MJ, Baskin CC and Dixon KW (2006) Ecophysiology of seed dormancy in the Australian

endemic species *Acanthocarpus preissii* (Dasypogonaceae). Annals of Botany 98, 1137-1144.

Walsh KJE and Ryan BF (2000) Tropical cyclone intensity increase near Australia as a result of climate change. Journal of Climate 13, 3029–3036.

Willis AJ and Groves RH (1991) Temperature and light effects on the germination of seven native forbs. Australian Journal of Botany 39, 219-228.

C h a p t e r 4

Response of *Grewia tenax* (Forssk.) Fiori

seedlings to NaCl-induced salinity

A. S. Saied, M. Sohail, J. Gebauer, and A. Buerkert

Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics, University of Kassel, Steinstr. 19, 37213 Witzenhausen, Germany

Abstract

With the rapid intensification of agriculture salinity problems are increasingly hampering efforts to meet the food demands of a growing population in arid and semi-arid regions of the world. To increase income and subsistence needs of people and to counteract land degradation increased reliance on salt-tolerant fruit trees are of particular interest for many marginal areas. *Grewia tenax* (Forssk.) Fiori is a wild growing fruit tree species intensely used by the rural population in multiple ways. Given lacking knowledge about tolerance of *Grewia tenax* (Forssk.) Fiori to NaCl salinity, this study aimed at investigating the tolerance of this species to NaCl stress. Seedlings of *G. tenax* were exposed to 0, 40, 80 and 160 mM levels of NaCl salinity. Experiment was conducted under controlled conditions to assess growth response of G. tenax to salinity. Plants treated with 80 and 160 mM NaCl dropped 6 and 10% of their leaves, respectively. Leaf area per plant decreased due to a decreased number of leaves rather than a reduction in area per leaf. The reduction in leaf area was 33, 42 and 54% at 40, 80 and 160 mM NaCl, respectively, whereas the corresponding reduction in total dry matter was 25, 36 and 49% of the control. Net photosynthesis was reduced by 29 and 42% at 80 and 160 mM NaCl, respectively. Similarly, stomatal conductance and transpiration decreased with increasing salt level, however, internal CO_2 increased. Given the accumulation of Na and Cl in plant tissues, *G. tenax* classifies as a salt-includer species whereby leaves managed to maintain the level of K constant under high NaCl. Our results specify *G. tenax* a salt tolerant fruit tree species.

4.1 Introduction

Globally, land degradation is one of the factors most limiting food productions. In arid and semi-arid regions, apart from soil erosion due to overgrazing and deforestation, the main factor contributing to land degradation and desertification is salinization (Pazira and Sadeghzadeh 1998) whereby already ten years ago salinity was estimated to affect about 7% of the world's total land area and 5% of its arable land (Ghassemi et al. 1995). This area is continuously increasing due to the effects of improper irrigation and land clearing (Munns et al. 2002). Wang et al. (2003) pointed out that salinity and drought may cause serious salinization of more than 50% of all arable lands by the year 2050.

Countries affected by salinization are mainly located in arid and semi-arid areas (Alaoui-Sossé et al. 1998; Salama et al. 1999). The major reasons of excessive salt ion accumulation in the soil are insufficient drainage and the use of saline irrigation water. In many areas the high cost of water make reclamation of saline soils prohibitive (Peel et al. 2004). In such case, an alternative reclamation procedure is to grow salt tolerant species (Chartzoulakis and Klapaki 2000). According to Norlyn and Epstein (1984), plant breeding for tolerance to salt stress has been difficult because a variety of physiological parameters appears to contribute to such tolerance, which is regarded to be a quantitative trait.

Trees with their dense and deep root systems can play an important role in overcoming salinity effects on land productivity (Niknam and McComb 2000) as some have been reported to be more tolerant to saline conditions than agricultural crops (Fung et al. 1998). Annual crops may allow more water to escape their root systems than perennial plants thereby leading to a rise of the water table and carrying salts that have accumulated in the soil to the surface (Munns et al. 2002).

Fruit trees are generally regarded as very sensitive to salt salinity but a significant variation in their salt tolerance was reported by Ebert et al. (1997).

Grewia tenax (Forssk.) Fiori is a fruit-producing tropical shrub or small tree that widely grows in semi-arid and sub-humid climates such as in the semi-arid savanna zone in Sudan where it is very important to the local population (El-Siddig et al. 2003; Vogt 1995). Its fruits are eaten fresh or prepared as porridge with millet flour. Because of their iron content of up to 7.4 mg (100 g)[-1] (Freedman 1998) these fruits are often used in special diets for pregnant women and anemic children, leaves and twigs are used as fodder, wood as fuel and leaves, and roots and woods in folk medicine (Gebauer et al. 2007). In addition, because of its dense fast growing root system, *G. tenax* is promising as a dune fixing species (Anonymus 2006). Reportedly, the species can tolerate seasonal drought and temperatures of more than 50°C (Gebauer et al. 2007). However, quantitative data about its potential to resist saline conditions is lacking.

4.2   Materials and methods

Plant material and growth conditions

Seeds of *G. tenax* were obtained from Dera Ismail Khan (31° 48' N, 70° 37' E), Pakistan and transferred to Germany. Six weeks after germination *G. tenax* seedlings were transplanted into 2.5 l plastic pots containing silica sand as the growing medium. All plants were placed in a growth chamber at 30/25°C (day/night) temperature and 50% relative air humidity. During the initial two weeks establishment period, seedlings were supplied with Hoagland solution every second day. To avoid branching lateral buds were thinned. Before the commencement of salt treatments, initial measurements of leaf number and plant height were taken; these data were used to group plants into eight blocks of four homogenous plants each to which treatments were applied. Such development-based blocking was done to separate potential effects of seedling size from treatment effects as all plants were raised from seeds of native population and germination was spread over a certain period of time.

Salt treatments

The final salt treatments applied consisted of 0, 40, 80 and 160 mM NaCl, corresponding to electrical conductivities (EC) of 1.8, 5.6, 8.6 and 15.2 ds m^{-1}, respectively, dissolved in a full strength Hoagland solution. To simulate conditions that young seedlings may encounter when planted under amended conditions in a salt affected soil and after a few weeks their roots grow into the natural, higher salinity environment, seedlings received half of the final salt concentration over four weeks and increasing it to the final concentration for the rest of period. Total duration of the salt stress was 7 weeks.

Measurements

After the initiation of salt treatments, plant height and number of leaves per plant were measured weekly. Light absorption-based chlorophyll readings (SPAD-502 chlorophyll meter, Konica-Minolta Corporation, Osaka, Japan) of fully expanded leaves (leaf number 5-6 from above) were taken at week 3 (after the osnet of salt applications) and week 7 (just before harvest). These readings were considered to be well related to extractable chlorophyll (Azia and Stewart 2001; Samsone et al. 2007). On the same leaves net assimilation rate (P_N), leaf internal CO_2 (C_i), stomatal conductance (g_s) and transpiration rate (E) were determined in the growth chamber at 1000 µmol m^{-2} s^{-1} light intensity, 24-30°C temperature and humidity of 22-28 mbar using a portable photosynthesis system LI-6400 (LI-COR Biosciences Inc., Lincoln, NE, USA).

At the end of the experiment the plants were harvested and separated into leaves, stems and roots. Fresh weights of different plant parts were determined and leaf area per plant was measured using a leaf area meter (LI-3000A Portable Area Meter, LI-COR Biosciences Inc., Lincoln, NE, USA) Area per leaf was calculated by dividing the total leaf area per plant by the number of leaves. Specific leaf area (SLA) was determined as the amount of leaf area per unit of plant dry matter (DM). Root length was measured using

the line intersect method of Tennant (1975) after cleaning the roots in KOH and staining with blue ink.

Dry weights of different plant organs were determined after oven drying to constant weight at 65°C for 48 h. Relative water content was calculated as the difference between fresh and dry weight divided by fresh weight.

After drying the samples were finely grounds before measuring their concentrations of sodium (Na), potassium (K), phosphorus (P), chloride (Cl) and nitrogen (N). Concentrations of Na, K and P where measured after dissolving the samples in concentrated hydrochloric acid by flame photometry (AutoCal 743, Instrumentation Laboratories, Lexington, MA, USA), while P was measured using a spectrophotometer (UVIKON 930, Kontron Instruments Ltd, Bletchley, UK). Chloride concentration was measured after dissolving the samples in distilled hot water, using a continuous flow analyzer with potentiometric detection (AutoAnalyzer II, Technicon Instruments, NY, USA) and total N was determined using a protein/nitrogen analyzer (FP-328, LECO Instruments GmbH, Mönchengladbach, Germany). Uptake efficiency of Grewia roots for different minerals was estimated by dividing the mineral concentration accumulated in the entire plant by root DM at final harvest.

Statistical analysis

All experimental data were analyzed with SPSS 12.0 (SPSS, Chicago, USA) using analysis of variance (ANOVA). Tukey-tests ($P<0.05$) were used to separate means.

4.3   Results

Blocking, which represented in this case the pre-treatment size of seedlings had an effect on plant size, leaf number, leaf area, dry matter content and leaf water content. Gas exchange parameters, transpiration and stomatal conductance were also affected by initial seedling size.

Plant growth

NaCl addition had no effect on the survival of *G. tenax* seedlings up to the high salinity level (160 mM), nevertheless, visible chlorosis and necrosis symptoms were noticed on the surface of mature basal leaves at high salinity (160 mM) after 6 weeks of salt application. Significant reduction in number of leaves per plant was observed at relatively higher salt levels. Salt treatments of 80 and 160 mM reduced number of leaves per plant by 30 and 39%, respectively (Table 4.1). Significant reduction in plant height was only observed at 160 mM NaCl salinity which is reduced by 34% in comparison to control (Table 4.1).

The presence of NaCl in the growing medium reduced total leaf area per plant by 33, 42 and 54% at 40, 80 and 160 mM NaCl levels, respectively (Table 4.1). Area per leaf, however, remained unaffected by salt treatments indicating that the reduction in leaf area per plant was due to a lower number of leaves and/or slower rate of leaf production, rather than reduced area per leaf. Specific leaf area (SLA) rose significantly at the high (160 mM) salt level, whereas it remained unchanged at the other salt treatments (Table 4.1). A consistent decline in specific root length (SRL) was observed in plants at higher salinity as compared with control plants (Table 4.1).

Table 4.1   Effect of different NaCl salt levels on seedlings growth of *G. tenax* 7 weeks after the initiation of the treatment

Growth parameters	NaCl salinity (mM)				F-probability	
	0	40	80	160	Treatment	Block
Plant height (cm)	189 ± 4.5 a	160 ± 6.1 ab	137 ± 7.5 ab	125 ± 6.9 b	<0.001	0.004
Leaves per plant	46 ± 2.15 a	37 ± 1.0 ab	32 ± 1.4 b	28 ± 1.7 b	<0.001	0.005
Total leaf area (cm^2)	709 ± 51 a	478 ± 33 b	409 ± 24 bc	327 ± 34 c	<0.001	0.034
Area per leaf (cm^2)	15.4 ± 0.7 a	13.1 ± 1.0 a	12.8 ± 0.8 a	12.0 ± 1.5 a	0.104	0.147
Specific leaf area (cm^2 g^{-1})	133 ± 5.9 a	141 ± 6.5 a	150 ± 3.9 a	176 ± 9.9 b	0.004	0.824
Specific root length (cm g^{-1})	43 ± 3.3 a	34 ± 1.9 b	32 ± 2.3 b	28 ± 0.8 b	0.002	0.654
Leaf chlorophyll (SPAD value)	50.6±1.2 a	50.4±1.6 a	47.7±1.9 a	39.7±1.9 b	0.001	0.573

Values represent means of 8 replicates ± s.e. and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

Total dry matter per plant was reduced by 25, 36 and 49% at 40, 80 and 160 mM salt levels, respectively (Figure 4.1). The reduction was most distinct for leaves, whereas root dry matter was unchanged by salt stress. In all treatments including the control shoot dry matter accounted for more than 50% of total DM, resulting in shoot/root ratios of 8 - 9 (Figure 4.1).
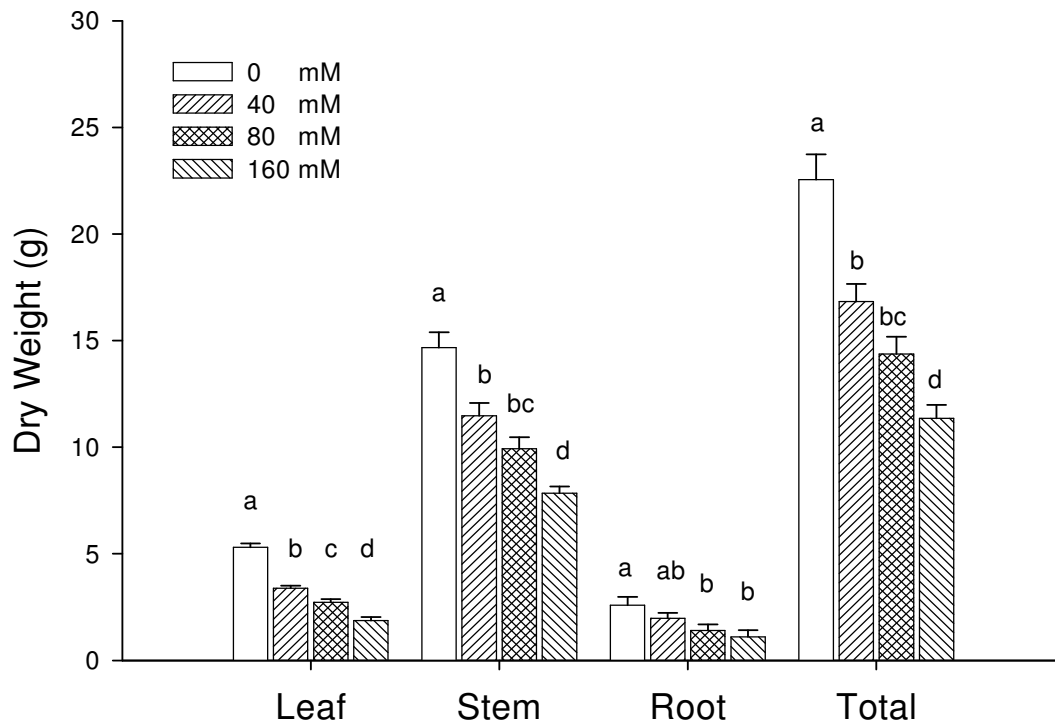
Figure 4.1. Effect of different NaCl salt levels on leaf, stem and root dry matter production (g) of *G. tenax* seedlings after 7 weeks of treatments initiation. Bars represent means of 8 replicates and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

F-probability	Leaf	Stem	Root	Total
Treatment	<0.001	<0.001	0.040	<0.001
Block	0.008	0.027	0.014	0.006

Addition of NaCl to the nutrient solution also led to an increase in relative water content of *G. tenax* seedlings. This effect was most distinct in leaves, where water content increased by 6% at 40 mM, 12% at 80 mM and 21% at

160 mM salt levels. The corresponding values for stem were 1, 3 and 4%, respectively, with a significant increase only at the high (160 mM) NaCl treatment (Figure 4.2). The increases in root water contents were not significant.
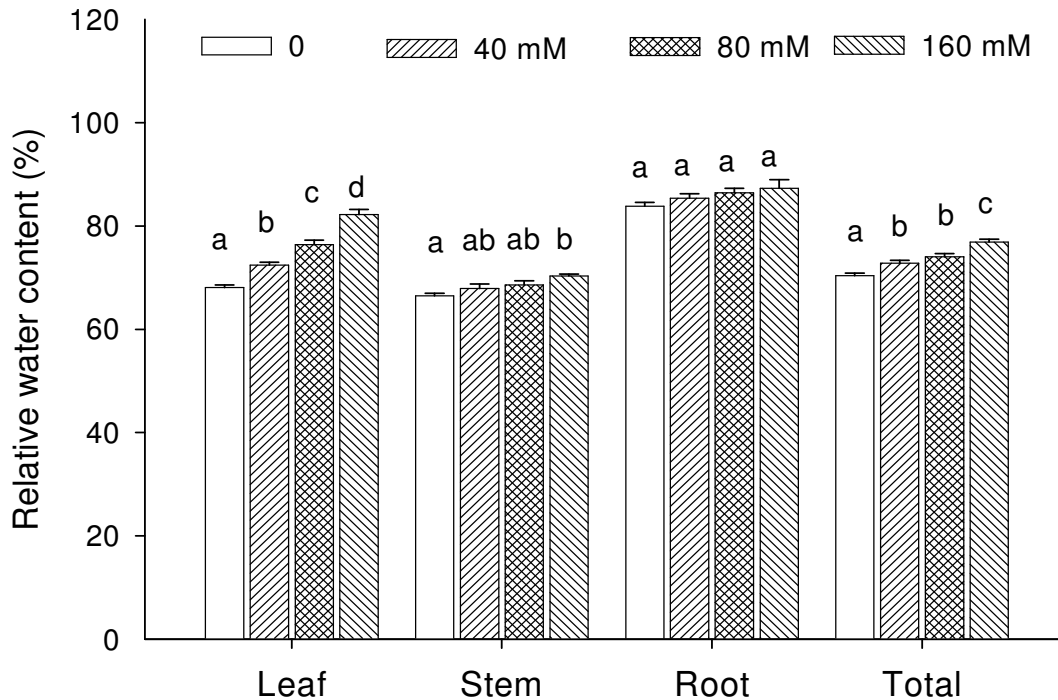
Figure 4.2. Effect of different NaCl salt levels on relative water content (%) of leaf, stem and root tissues of *G. tenax* seedlings after 7 weeks of treatments initiation. Bars represent means of 8 replicates and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

F-probability	Leaf	Stem	Root	Total
Treatment	<0.001	0.003	0.156	<0.001
Block	0.025	0.142	0.405	0.157

Leaf chlorophyll and gas exchange

After 7 weeks of salt application, significant reduction in leaf chlorophyll (SPAD value) was only observe in plants treated with 160 mM salinity. It showed 21% lower SPAD value as compare to control plants (Table 4.1). Net photosynthesis (P_n) was only decreased significantly by 29 and 42% at 80 and 160 mM, respectively in comparison to untreated plants (Table 4.2).

Stomatal conductance (G_s) and transpiration (E) were also reduced significantly at all salt levels applied, while significant increase in internal CO_2 concentration (C_i) was only observed at 160 mM salinity (Table 4.2).

Table 4.2. Effect of different NaCl salt levels on net photosynthesis (P_n), internal CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration (E) of *G. tenax* seedlings after 7 weeks of treatments initiation

NaCl conc. (mM)	Gas-exchange parameters			
	P_n (μmol m^{-2} s^{-1})	C_i (ppm)	G_s (mmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})
0	24.7 ± 1.2 a	115 ± 13 a	0.18 ± 0.013 a	5.8 ± 0.5 a
40	22.1 ± 0.9 a	207 ± 47 a	0.09 ± 0.008 b	3.4 ± 0.3 b
80	17.4 ± 0.7 b	213 ± 37 a	0.05 ± 0.003 c	2.1 ± 0.1 c
160	14.3 ± 1.6 b	418 ± 49 b	0.03 ± 0.004 c	1.3 ± 0.2 c
F-probability				
Treatment	<0.001	<0.001	<0.001	<0.001
Block	0.055	0.055	0.022	0.005

Values represent means of 8 replicates ± s.e. and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

Concentrations of mineral elements

Addition of NaCl to the nutrient solution increased Na and Cl concentrations in leaves, stems and roots whereby the concentration of Na ion increased from 0.5 (0 mM) to 14.6 mg g^{-1} dry matter in leaf tissues of the plants treated with 160 mM NaCl salt. The corresponding values in stem dry matter ranged from 0.6 to 6.2 and in root from 1.2 to 7.2 mg g^{-1} dry matter (Figure 4.3). Regarding the distribution of Na ion in various plant parts at highest level of salinity (160 mM), leaf tissues accumulated 41% of the total absorbed Na ions followed by root (33%) and stems (26%) tissues (Figure 4.3).

Concentration of Cl ions in leaf tissues increased from 5.2 in control plants to 28.8 mg g^{-1} dry matter in plants treated with 160 mM NaCl salt. The

corresponding values in stem ranged from 2.7 to 18.2 and in root from 3.4 to 20.1 mg g^{-1} dry matter (Figure 4.4). The distribution of absorbed Cl closely followed that of Na. (Figure 4.4).

Figure 4.3 Effect of different NaCl salt levels on Na concentration (mg g^{-1} dry matter) of leaf, stem and root tissues of *G. tenax* seedlings after 7 weeks of treatments initiation. Bars represent means of 8 replicates and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

F-probability	Leaf	Stem	Root
Treatment	<0.001	<0.001	0.001
Block	0.540	0.916	0.117

Our results showed a strong correlation (r^2 = 0.94) between the rate of photosynthesis and the Cl concentration in the leaves of *G. tenax* (Figure 4.5).

Salt application significantly reduced K concentration in dry matter of stem and root tissues, however reduction of K concentration in leaf tissues was insignificant. Salt treatments of 40, 80 and 160 mM reduced K concentration in stem tissues by 19, 28 and 29%, respectively in comparison to control plants. While, root tissues showed significant reduction in K concentration by 34 and 35% only at 80 and 160 mM salinity, respectively (Table 4.3).

Similarly, salt concentrations of 80 and 160 mM also significantly decreased N concentration in leaf and stem tissues, while root tissues showed significant change in N concentration only at 160 mM salinity (Table 4.3). Salt treatments also reduced P concentration in dry matter of all tissues as compare to control but significant reduction in P concentration was only observed in root tissues at 160 mM salinity which is decreased by 22% as compare to control (Table 4.3).
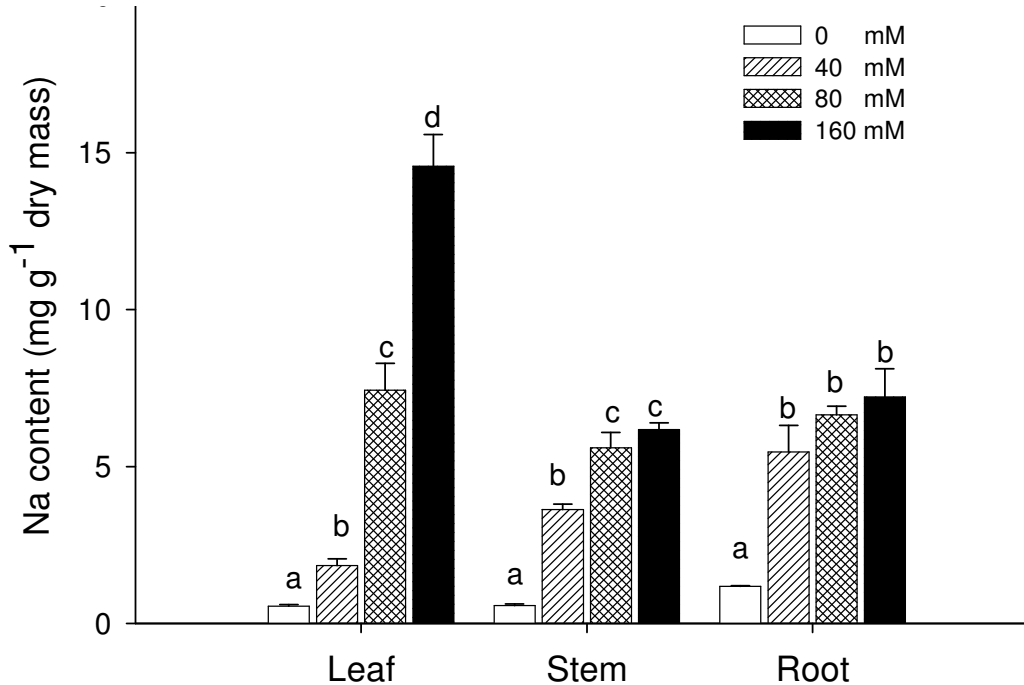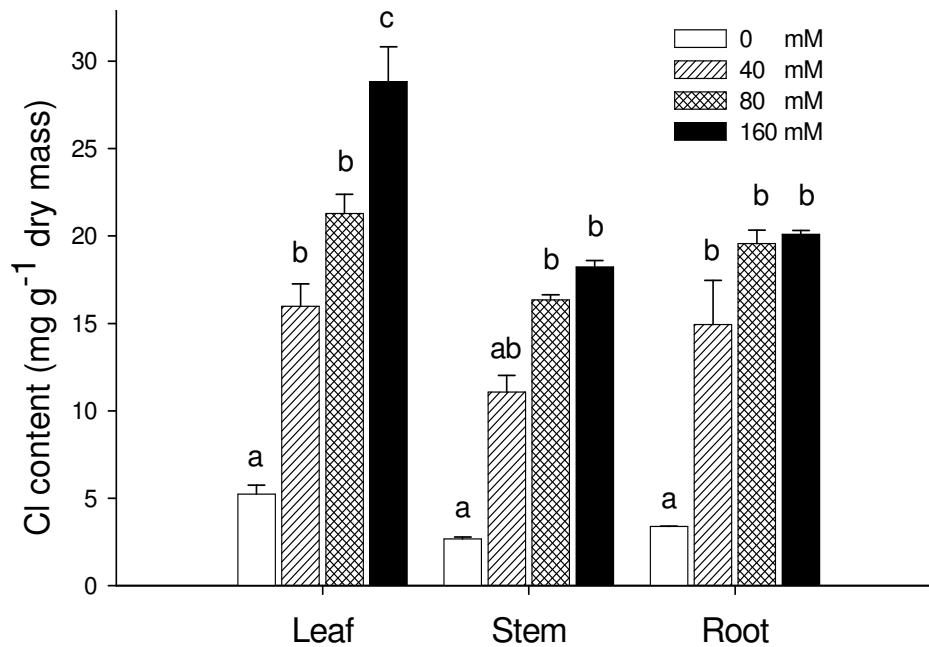

Figure 4.4 Effect of different NaCl salt levels on Cl concentration (mg g^{-1} dry matter) of leaf, stem and root tissues of *G. tenax* seedlings after 7 weeks of treatments initiation. Bars represent means of 8 replicates and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

F-probability	Leaf	Stem	Root
Treatment	<0.001	<0.001	<0.001
Block	0.477	0.436	0.242

Table 4.3. Effect of different NaCl salt levels on macronutrients (K, N, and P) concentrations (mg g^{-1} dry matter) of *G. tenax* seedlings after 7 weeks of treatments initiation

Tissue	NaCl salinity (mM)	Ion concentrations (mg g^{-1} dm)		
		K	N	P
Leaf	0	14.0 ± 0.6 a	3.82 ± 0.07 a	0.20 ± 0.01 a
	40	13.9 ± 0.7 a	3.58 ± 0.05 ab	0.18 ± 0.01 a
	80	13.5± 0.5 a	3.56 ± 0.04 b	0.18 ± 0.01 a
	160	12.0 ± 1.2 a	3.24 ± 0.14 b	0.17 ± 0.00 a
Stem	0	15.9 ± 0.9 a	1.4 ± 0.03 a	0.32 ± 0.05 a
	40	12.8 ± 0.3 b	1.3 ± 0.03 ab	0.25 ± 0.02 a
	80	11.5 ± 0.3 b	1.2 ± 0.04 b	0.21 ± 0.04 a
	160	11.3 ± 0.6 b	1.2 ± 0.05 b	0.21 ± 0.01 a
Root	0	13.1 ± 0.4 a	2.0 ± 0.09 a	0.23 ± 0.002 a
	40	11.8 ± 0.6 a	1.9 ± 0.13 ab	0.20 ± 0.01 ab
	80	8.6 ± 0.2 b	1.8 ± 0.09 ab	0.18 ± 0.02 ab
	160	8.5 ± 0.3 b	1.6 ± 0.06 b	0.17 ± 0.00 b
F-probability				
Treatment	Leaf	0.289	0.001	0.146
	Stem	0.003	0.011	0.057
	Root	0.001	0.027	0.019
Block	Leaf	0.433	0.209	0.672
	Stem	0.984	0.685	0.324
	Root	0.913	0.070	0.266

Values represent means of 8 replicates ± s.e. and different letters indicate significant difference among treatment means (P<0.05; Tukey test).
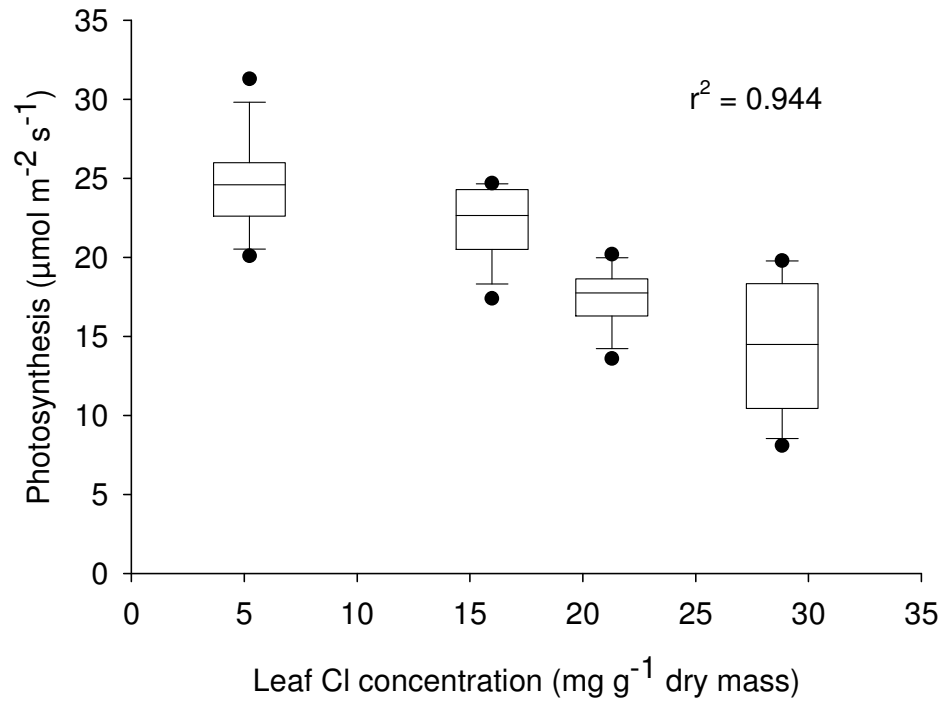
Figure 4.5. Relationship between photosynthetic activity and leaf Cl concentration of *G. tenax* seedlings after 7 weeks of treatments initiation

4.4 Discussion

The results of this study showed that all plants survived salt levels of up to 160 mM NaCl. Salt damage symptoms were only observed in a few plants treated with the high NaCl level (160 mM), indicating likely genetic differences among seedlings that were raised from a wild plant population. Genetic variation in salt tolerance of genotypes within one species was previously observed by different authors (Ghassemi et al. 1995; Kozlowski 1997; Martínez Barroso and Alvarez 1997; Saied et al. 2005) indicating the potential for selection and breeding of tolerant varieties.

The drop in leaves observed at the moderate and high salt level in the last week of our experiment might be the classical mechanism by which many plants eliminate accumulated salt ions. It was also previously reported by Wahome et al. (2001) for Rose (*Rosa chinensis* Jacq. and *R. rubiginosa* L.), Saied et al. (2003) for Strawberry (*Fragaria × ananassa* Duch.) and Gebauer et al. (2004) for Tamarind (*Tamarindus indica* L.).

Among the measured growth parameters, the reduction in leaf area per plant was significant in salt treated plants. This is in agreement with the findings of Chartzoulakis et al. (2002), who reported that effects of salinity on leaf area of olive seedlings (*Olea europaea* L.) were the greatest among all observed growth parameters. At least for the moderate and high salt treatment this could be partially explained by leaf shedding. The area per leaf of treated plants tended to decrease with increasing salinity, but this reduction was not statistically significant. Saied et al. (2003) explained the reduction in leaf area per plant as a result of leaf senescence and eventually leaf drop, therefore a reduction in area per leaf may indicate a more severe stage of salinity stress. The increase in specific leaf area (SLA), which reflects a decrease in dry matter per unit leaf area, at the high salt level may be explained by a reduced net assimilation rate, a reduction of mesophyll cell size and/or a decrease in palisade cell layers in the leaves (Chen et al. 1997). Similarly, Parida et al.

(2004) found a decrease of mesophyll thickness and intercellular spaces in leaves of NaCl-treated plants of *B. parviflora* compared with untreated plants.

In the present study, salinity reduced the specific root length (SRL). Alaoui-Sossé et al. (1998) explained the reduction in root length under salinity stress by either a decrease in daily taproot increment or by a temporary cease of root elongation due to an abrupt supply of NaCl, thereby reflecting plant adaptation.

Reduction in dry matter due to salt was most distinct in leaves and stems, whereas reduction in root was insignificant. This has previously been reported by Chartzoulakis et al. (2002) for olive. Netondo et al. (2004) reported that the reduction in dry matter reflected the increased metabolic energy cost and reduced carbon gain associated with salt adaptation. Ebert et al. (1999) reported from a survey of more than 50 studies done by different authors, that on average, a short-term salinity of 60 mM NaCl in the root medium resulted in a 40% growth reduction in fruit species. Ali-Dinar et al. (1998) recorded a reduction of 54% of dry matter in the moderately tolerant Guava (*Psidium guajava*) seedlings at a salinity level of 30 mM NaCl and Gebauer et al. (2004) a reduction of less than 50% in moderately tolerant Tamarind (*Tamarindus indica*)at 40 mM level. Comparing this with our findings, *G. tenax* could be therefore, classified as salt tolerant fruit species as reflected by the 49% reduction in total dry matter at 160 NaCl treatment.

Like increase in relative water content of *G. tenax* seedlings with increase in salinity especially in leaves was also observed by other authors in different plant species. Saied et al. (2003) explained it as a way to partially compensate possible morphological changes to salinity. On the other hand, Larcher (2003) and Maas and Nieman (1978) stated that increased succulence is a morphological feature of some plants to avoid excessive ion concentrations in their tissues. According to Tal and Gavish (1973) and Kleinkopf et al. (1975) the increased water content is either due to a high

osmotic effectiveness of the absorbed salt or due to a salt-induced decrease in transpiration.

Leaf chlorophyll of plants treated with the high salt level decreased by 21% compared to control plants at the end of the experiment. Different authors reported a reduction in chlorophyll due to NaCl salinity (Ebert et al. 2002; Netondo et al. 2004; Parida et al. 2004; Sultana et al. 1999). Salinity can affect chlorophyll concentration of leaves through accumulation of salt ions in leaves (Yeo and Flowers 1983) which inhibit its synthesis (Miteva et al. 1992) or through an acceleration of chlorophyll degradation by an increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy and Vora, 1986).

Our study also showed that the moderate and high salinity level led to a decrease in net photosynthesis compared with that of control plants. Although it is difficult to conclude that reduction in net photosynthesis in the seedlings of *G. tenax* at relatively higher salt levels was caused by partial stomatal closure or non-stomatal factors but linear decrease in net photosynthesis associated with reduction in stomatal conductance and transpiration rate is a strong indication that photosynthetic activity hampered by partial closure of stomata. Such salt-induced concurrent reductions in net photosynthesis associated with reduction in stomatal conductance have also been reported in leaves of olive (Bongi and Loreto 1989; Tattini et al. 1997) and cotton plants (Brugnoli and Björkman 1992) under salt stress. Moreover, it could be partially due to leaf chlorophyll degradation at high salinity. De Lucia et al. (2003) and Flexas et al. (2004) reported that the capacity to conduct CO_2 from the intercellular spaces in leaves to the site of fixation or mesophyll conductance may pose a significant limitation to photosynthesis. The level of internal CO_2 concentration (C_i) could be high; nevertheless, the CO_2 concentration at the chloroplasts could be low due to low mesophyll conductance. Reduction in transpiration rate (E) due to partial closure of stomata might be used by the plant as adaptive response to cope stressed environment. Net photosynthesis of plants exposed to salt stress could also be reduced by non-stomatal factors

such as impairment of metabolic processes (Bethke and Drew 1992; Lawlor 1995; Massacci and Loreto 2001; Sultana et al. 1999).

In contrast to the findings of Chartzoulakis et al. (2002) in olives, we found a strong relationship between the rate of photosynthesis and the Cl and Na concentration in the leaves of *G. tenax* (r^2 of 0.94 and 0.93, respectively), suggesting that the reduction in photosynthesis was due to the accumulation of salt ions. Such a relationship was also found by Bethke and Drew (1992) in Bell pepper (Capsicum annuum L.), Chartzoulakis and Loupassaki (1997) in Eggplant (Solanum melongena L.), Chartzoulakis and Klapaki (2000) in Pepper (Capsicum annuum).

Remarkable increase in Na and Cl ions concentrations in all plant tissues with increase in salinity indicates that *G. tenax* is an ion accumulator. Moreover, maximum concentration of salt ions was observed in leaf tissues as compare to stem and root. In agreement with the findings of Fostad and Pedersen (2000) and Wahome et al. (2001), the accumulation of Na in plant leaves was lower than that of Cl. Accumulation of Cl and Na ions in plant tissues under salinity has been associated with osmotic adjustment (Chartzoulakis and Loupassaki 1997; Martínez-Ballesta et al. 2004). The strategy to employ mainly ions for osmotic adjustment is energetically favorable since the synthesis of compatible solutes is metabolically expensive, whereas an ion such as Na is a cheap and abundant osmoticum (Ottow et al. 2005).

Salt tolerance is generally correlated with the capacity of plants to keep their K level in a defined range (Blumwald et al. 2000). In our study, while K concentration was reduced in stem and root dry matter of *G. tenax* with increasing salt level, leaves managed to maintain their K status, this was also observed by other authors in different species (Chartzoulakis and Klapaki 2000; Parida et al. 2004 and Ruiz et al. 1999). At the cellular level, plants respond to elevated Na concentrations by maintaining a low cytosolic Na concentration and a high cytosolic K/Na ratio (Blumwald 2000). This is achieved either by sodium extrusion and/or intracellular compartmentalization

of sodium in the vacuole by an active transport operated mainly by Na^+/H^+ antiporters (Beritognolo et al. 2007).

A general shortage of metabolic energy, which will occur under severe salinity stress, might impair several metabolic processes, such as the assimilation of macronutrients and protein synthesis (Helal and Mengel, 1981). In our study, the uptake efficiencies of the root for N, P and K remained fairly constant at different levels of NaCl salinity, indicating that their assimilation was not impaired. Nevertheless, the pattern of their concentration changed within organs. While N decreased in all plant parts, P concentration in leaves and stems remained unaffected but that of roots decreased. The former could be due to the reduction in chlorophyll content, while the later could be due to mobilization of P from roots to leaves, where it is needed for photosynthesis and energy release.

In literature fruit trees are reported to be generally fairly susceptible to salts with a few exceptions such as Date palm (*Phoenix dactylifera* L.), which is rated as tolerant and Guavas (*Psidium guajava* L.), Olives (*Olea europaea* L.) and Tamarind (*T. indica*) rated as moderately tolerant (Maas 1993; Ali-Dinar et al. 1998; Ebert et al. 2002; Gebauer et al. 2004). From our study we can conclude that seedlings of *G. tenax* are tolerant to NaCl-induced salinity. Nicknam and McComb (2000) reported that most woody species show barley any salt tolerance at germination, low tolerance at the seedling stage, but greater tolerance to salinity at 4 to 6 months of age. This means that one might expect *G. tenax* to be more tolerant to salinity after the seedling stage, which therefore makes it suitable for afforestation in marginal soils. The partial closing of stomata can also be an adaptive response to reduce water loss through evapotranspiration and, therefore keep toxic ions diluted. Another adaptive response of plants to salinity may be through maintaining the K level constant in leaves allowing this to act as the major monovalent cationic osmoticum.

References

Alaoui-Sossé B, Sehmer L, Barnola P and Dizengremel P (1998) Effect of NaCl salinity on growth and mineral partitioning in *Quercus robur* L., a rhythmically growing species. Trees 12, 424-430.

Ali-Dinar HM, Ebert G and Lüdders P (1998) Biomass production and partitioning in guava (*Psidium guajava* L.) in response to salinity and nitrogen nutrition. Angewandte Botanik 2, 56-60.

Anonymus (2006) AgroForestryTree Database: A tree species reference and selection guide of the International Center for Research in Agroforestry, 1-2,
http://www.worldagroforestry.org/sea/Products/AFDbases/AF/asp/SpeciesInfo.asp? Sp ID = 17952, cited 15.01.2007

Azia F and Stewart KA (2001) Relationships between extractable chlorophyll and SPAD values in muskmelon leaves. Journal of Plant Nutrition 24, 961-966.

Beritognolo I, Piazzai M, Benucci S, Kuzminsky E, Sabatti M, Mugnozza GS and Muleo R (2007) Functional characterisation of three italian *Populus alba* L. genotypes under salinity stress. Trees 21, 465-477.

Bethke PC and Drew MC (1992) Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. Plant Physiology 99, 219-226.

Blumwald E, Aharon GS and Apse MP (2000) Sodium transport in plant cells. Biochimica et Biophysica Acta (BBA) - Biomembranes 1465, 140-151.

Bongi G and Loreto F (1989) Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves. Plant Physiology 90, 1408-1416.

Brugnoli E and Björkman O (1992) Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatat components of photosynthesis and dissipation of excess light energy. Planta 187, 335-347.

Chartzoulakis KS and Klapaki G (2000) Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Scientia Horticulturae 86, 247-260.

Chartzoulakis K and Loupassaki MH (1997) Effects of NaCl salinity on germination, growth, gas exchange and yield of greenhouse eggplant. Agricultural Water Management 32, 215-225.

Chartzoulakis K, Loupassaki M, Bertaki M and Androulakis I (2002) Effects of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars. Scientia Horticulturae 96, 235-247.

Chen K, Hu G, Keutgen N and Lenz F (1997) Effects of CO_2 concentration on strawberry. I. Plant growth analysis. Angewandte Botanik, 71: 168-172.

Craig GF, Bell DT and Alkyds CT (1990) Response to salt and water logging stress of 10 texa of acacia selected from naturally saline areas of Western Australia. Australian Journal of Botany 38, 619-630.

De Lucia EH, Whitehead D and Clearwater MJ (2003) The relative limitation of photosynthesis by mesophyll conductance in co-occurring species in a temperate rainforest dominated by the conifer *Dacrydium cupressinum*. Functional Plant Biology 30, 1197-1204.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR and Mearns LO (2000) Climate extremes: observations, modelling, and impacts. Science 289, 2068–2074.

Ebert G, Ali-Dinar H and Lüdders P (1997) Food production in salinity affected areas - causes, consequences and possible solutions. Tropentag, University of Hohenheim, Germany, Book of Abstracts pp 77-81.

Ebert G, Casierra F and Lüdders P (1999) Influence of NaCl salinity on growth and mineral uptake of lulo (*Solanum quitoense* L.). Journal of Applied Botany 73, 31-33.

Ebert G, Eberle J, Ali-Dinar H and Lüdders P (2002) Ameliorating effects of $Ca(NO_3)_2$ on growth, mineral uptake and photosynthesis of NaCl-stressed guava seedlings (*Psidium guajava* L.). Scientia Horticulturae 93, 125-135.

El-Siddig K, El Tahir BA and Ebert G (2003) *Grewia tenax*- a potential new small fruit for the Sudan. Tropentag, Conference on International Agricultural Research for Development in Göttingen, Germany, Book of Abstracts pp 311.

Fathi RA and Prat D (1989) Effects of saline stress on *Eucalyptus* seedlings. Annals of Science Forest 46, 376–378.

FAO (2005) Global Network on Integrated Soil Management for Sustainable Use of Salt-affected Soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/spush

Flexas J, Bota J, Loreto F, Cornic G and Sharkey TD (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biology 6, 1-11.

Fostad O. and P.A. Pedersen (2000) Container-grown tree seedling responses to sodium chloride applications in different substrates. Environmental Pollution 109, 203-210.

Freedman R (1998) Famine foods, Tiliaceae.
http://www.hort.purdue.edu/newcrop/famineFoods/ff_families/TILIACEAE.html, Cited 23 April 2008.

Fung LE, Wang SS, Altman A and Hütterman A (1998) Effect of NaCl on growth, photosynthesis, ion and water relations of four poplar genotypes. Forest Ecology and Management 107, 135-146.

Gebauer J, El-Siddig K, Salih AA and Ebert G (2004) *Tamarindus indica* L. seedlings are moderately salt tolerant when exposed to NaCl-induced salinity. Scientia Horticulturae 103, 1-8.

Gebauer J, El-Siddig K, El Tahir BA, Salih AA, Ebert G and Hammer K (2007b) Exploiting the potential of indigenous fruit trees: *Grewia tenax* (Forssk.) Fiori in Sudan. Genetic Resources and Crop Evolution 54, 1701-1708.

Ghassemi FA, Jackman AJ and Nix AH (1995) Salinization of Land and water resources. CAB International, Wallingford, England.

Helal HM and Mengel K (1981) Interaction between light intensity and NaCl salinity and their effects on growth, CO_2 assimilation and photosynthate conversion in young broad beans. Plant Physiology 67, 999-1002.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K and Johnson CA (2001) IPCC Third assessment report: climate change 2001. Cambridge University Press.

Javanshir K and Ewell K (1993) Salt resistance of bald cypress. In: Leith H, Al Masoom A (Eds.) Towards the Rational Use of High Salinity Tolerant Plants, Kluwer Academic, Netherlands 2:285–291

Kleinkopf GE, Wallace A and Cha AW (1975) Sodium relations in desert plants: 4. Some physiological responses of *Atriplex confertifolia* to different levels of sodium chloride. Soil Science 120, 45-48.

Kozlowski TT (1997) Responses of woody plants to flooding and salinity. Tree Physiology Monograph No. 1. Heron Publishing, Victoria, pp 29.

Larcher W (2003) Physiological plant ecology. Springer, Berlin, pp 513.

Lawlor DW (1995) The effects of water deficit on photosynthesis. In: Smirnoff N (ed) Environment and plant metabolism: Flexibility and acclimation. BIOS Scientific Publisher, Oxford, pp 129-160.

Maas EV (1993) Testing crops for salinity tolerance. In: Maranville JW, Baligar BV, Duncan RR, Yohe JM (eds) Proceedings of a workshop on adaptation of plants to soil stresses. INTSORMIL Publication, University of Nebraska, Lincoln, pp 234-247.

Maas EV and Nieman RH (1978) Physiology of plant tolerance to salinity. In: Jung GA (ed) Crop tolerance to suboptimal land conditions. ASA Spec. Publ., Amer. Soc. Agron. Spez., Madison, Wisconsin, pp 277-299.

Martínez-Ballesta MC, Martínez V and Carvajal M (2004) Osmotic adjustment, water relations and gas exchange in pepper plants grown under NaCl or KCl. Environmental and Experimental Botany 52, 161-174.

Martínez Barroso MC and Alvarez EC (1997) Toxicity symptoms and tolerance of strawberry to salinity in the irrigation. Scientia Horticulturae 71, 177-188.

Massacci A and Loreto F (2001) Diffusive resistances to CO_2 entry in the leaves and their limitation to photosynthesis. In: Pessarakli M (ed) Handbook of plant and crop physiology. Kluwer, Dordrecht, pp 327-336.

Miteva TS, Zhelev NZ and Popova LP (1992) Effect of salinity on the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase in barley leaves. Journal of Plant Physiology 140, 46-51.

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses, Plant, Cell and Environment 16, 5–24.

Munns R, Husain S, Rivelli AR, James RA, Condon AGT, Lindsay MP, Lagudah ES, Schachtman DP and Hare RA (2002) Avenues for increasing salt tolerance of crops and the role of physiologically based selection traits. Plant and Soil 247, 93-105.

Munns R (2007) Utilizing genetic resources to enhance productivity of salt-prone land. Published as part of a theme on Salt-Prone Land Resources. CAB Reviews: erspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2, No. 009.

Netondo GW, Onyango JC and Beck E (2004) Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Science 44, 806-811.

Niknam SR and McComb J (2000) Salt tolerance screening of selected Australian woody species - a review. Forest Ecology and Management 139, 1-19.

Norlyn JD and Epstein E (1984) Variability in salt tolerance of four Triticale lines at germination and emergence. Crop Science 24, 1090-1092.

Ottow EA, Brinker M, Teichmann T, Fritz E, Kaiser W, Brosché M, Kangasjärvi J, Jiang X and Polle A (2005) Populus eupratica displays apoplastic sodium accumulation, osmotic adjustment by decreases in

calcium and soluble carbohydrates, and develops leaf succulence under salt stress. Plant Physiology, 139: 1762-1772.

Parida AK, Das AB and Mittra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees 18, 167-174.

Pazira E and Sadeghzadeh K (1998) Soil desalinization and land reclamation in Iran - A case study: Khuzistan province. Proceedings of the international symposium: New technologies to combat desertification. Tehran, Iran 80-88.

Peel MD, Waldron BL, Jensen KB, Chatterton NJ, Horton H and Dudley LM (2004) Screening for salinity tolerance in Alfalfa: a repeatable method. Crop Science 44, 2049-2053.

Reddy MP and Vora AB (1986) Changes in pigment composition, Hill reaction activity and saccharides metabolism in Bajra (*Pennisetum typhoides* S & H) leaves under NaCl salinity. Photosynthetica 20, 50-55.

Ruiz D, Martinez V and Cerdá A (1999) Demarcating specific ion (NaCl, Cl$^-$, Na$^+$) and osmotic effects in the response of two citrus rootstocks to salinity. Scientia Horticulturae 80, 213-224.

Saied AS, Keutgen N and Noga G (2003) Effects of NaCl stress on leaf growth, photosynthesis and ionic contents of strawberry cvs 'Elsanta' and 'Korona'. In: Paradossi A, Serra G, Tognoni F (eds) Managing greenhouse crops in saline environment. ISHS international symposium in Pisa, Acta Horticulturae 609, 67-73.

Saied AS, Keutgen N and Noga G (2005) The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs "Elsanta" and "Korona". Scientia Horticulturae 103, 289-303.

Saied AS, Gebauer J and Buerkert A (2008) Effect of different scarification methods on germination of *Ziziphus spina-christi* seeds. Seed Science and Technology 36, 201-205.

Salama RB, Otto CJ and Fitzpatrick RW (1999) Contributions of groundwater conditions to soil and water salinization. Hydrogeolgy Journal 7**,** 46-64.

Samsone I, Andersone U, Vikmane M, Ieviņa B, Pakarna G and Ievinsh G (2007) Nondestructive methods in plant biology: an accurate measurement of chlorophyll content by a chlorophyll meter. Acta Universitatis Latviensis 723, 145-154.

Saneoka H, Ishiguro S and Moghaieb RE (2001) Effect of salinity and abscisic acid on accumulation of glycinebetaine and betaine aldehyde dehydrogenase mRNA in Sorghum leaves (*Sorghum bicolor*)**.** Journal of Plant Physiology 158, 853-859.

Sultana N, Ikeda T and Itoh R (1999) Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environmental and Experimental Botany 42**,** 211-220.

Teketay D (1996) Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethopia. Forest Ecology and Management 80, 209-223.

Tal M and Gavish U (1973) Salt tolerance in the wild relatives of the cultivated tomato: water balance and abscisic acid in *Lycopersicon esculentum* and *L. peruvianum* under low and high salinity. Australian Journal of Agricultural Research 24**,** 353-361.

Tattini M, Lombardini L and Gucci R (1997) The effect of NaCl stress and relief on gas exchange properties of two olive cultivars differing in tolerance to salinity. Plant and Soil 197**,** 87-93.

Tennant D (1975) A test of a modified line intersect method of estimating root length. The Journal of Ecology 63, 995-1001.

Van der Moezel PG, Watson LE, Pearce-Pinto GVN and Bell DT (1988) The response of six Eucalyptus species and *Casuarina obesa* to the combined effect of salinity and waterlogging. Australian Journal of Plant Physiology 15, 465–474.

Vogt K (1995) A field worker's guide to the identification, propagation and uses of common trees and shrubs of dryland Sudan. SOS Sahel International, London, England.

Wahome PK, Jesch HH and Grittner I (2001) Mechanisms of salt stress tolerance in two rose rootstocks: *Rosa chinensis* 'Major' and *R. rubiginosa*. Scientia Horticulturae 87, 207-216.

Walsh KJE and Ryan BF (2000) Tropical cyclone intensity increase near Australia as a result of climate change. Journal of Climate 13, 3029–3036.

Wang W, Vinocur B and Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218, 1-14.

Weimberg RH, Lerner HR and Poljakoff-Mayber A (1984) Changes in growth and water soluble solute concentration in *S. bicolor* with sodium and potassium salts. Physiologia Plantarum 62, 472-480.

Yang YW, Newton RJ and Miller FR (1990) Salinity tolerance in sorghum.1. Whole plant response to sodium chloride in *S.bicolor* and *S.helepense*. Crop Science 30, 775-781.

Yeo AR (1998) Molecular biology of salt tolerance in the context of whole-plant physiology. Journal of Experimental Botany 49, 915-929.

Yeo AR and Flowers TJ (1983) Varietal differences in the toxicity of sodium ions in rice leaves. Physiologia Plantarum 59, 189-195.

Zekri M, Parsons LR (1990) Calcium influences growth and leaf mineral concentration of under saline conditions. HortScience 25, 784-786.

C h a p t e r 5

Response of *Ziziphus spina-christi* (L.) Willd. seedlings to NaCl-induced salinity

M. Sohail, A. S. Saied, J. Gebauer, and A. Buerkert

Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics, University of Kassel, Steinstr. 19, 37213 Witzenhausen, Germany

Abstract

Z. spina-christi is a fruit tree species growing wild in arid and semi-arid areas of Asia and Africa where rural populations intensively use its fruits, leaves, bark and wood. However, little is known about the effects of salinity, a widespread problem in these regions, on early growth and mineral composition of this species. This study was conducted under controlled conditions to fill this gap. Six weeks old seedlings of *Z. spina-christi* germinated in a full strength Hoagland solution were subjected to 0, 40, 80 and 160 mM NaCl. Compared to the unstressed control plants, salinity levels of 80 and 160 mM reduced plant height, leaf number, leaf chlorophyll, total leaf area and dry matter by > 50%. Salinity levels of 40, 80 and 160 mM enhanced leaf water contents by 14, 16 and 17%, respectively, and 160 mM NaCl raised the concentration of Na and Cl ions in leaf tissues 81- and 21-fold. The K/Na ratio, in contrast, was hardly affected by increasing salinity indicating adaptation or tolerance of *Z. spina-christi* to low or moderate NaCl salinity.

5.1 Introduction

Soil salinity is becoming an increasingly serious problem in many parts of the world (FAO 2005), particularly in semi-arid and arid zones where already over a decade ago 50% of the cropland was salt affected (Flower and Yeo 1995) and suffered from subsequent reduction of biodiversity and land degradation (Ghassemi et al. 1995). In many species soil salinity is known to reduce plant growth and retard development through osmotic stress, ion toxicity, mineral deficiencies and induced physiological and biochemical disorders in metabolic processes (Hasegawa et al. 2000). However, species are varying widely in their ability to withstand salt stress (Craig et al. 1990; Glenn et al. 1996). Most studies, however, focus on annual species' ability to tolerate salinity and only limited information is available on multipurpose fruit trees that often grow

under harsh environmental conditions and are an important component of local livelihoods.

Z. spina-christi is one of those (neglected) multipurpose fruit trees that is ubiquous in arid and semi-arid Asia and Africa (Arbonnier 2004). The rural population of these areas often meets subsistence requirements through its biomass-based products. Fruits of *Z. spina-christi* are mostly consumed raw, while leaves and twigs are highly palatable and nutritious fodder for sheep and goats (Verinumbe 1993; Sudhersan and Hussain 2003; Saied et al. 2008). The plant is also well adapted to dry and hot climates which makes it suitable for re-vegetation of degraded lands (Saied et al. 2008), but little is known about the physiological basis for these characteristics. This study therefore aimed at studying the effects of different levels of NaCl salinity on seedling growth of *Ziziphus spina-christi* (L.)

5.2   Materials and methods

Plant material and growth conditions

In December 2006 seeds of *Z. spina-christi* were collected from the 'Jabra Saeed' forest (15°37' N, 32°33' E) about 110 km north of Khartoum, Sudan. After extraction from the pulp, the seed surface was sterilized by immersion in 2% sodium hypochloride solution for 15 minutes (Saied et al. 2008). Subsequently, seeds were placed on moist silica sand in inverted cells of plastic trays at 30/25°C (day/night) temperature and 50% (±10%) relative air humidity, the moisture level was periodically readjusted as necessary. After six weeks 40 seedlings were selected and transplanted into 2.5 l sand filled plastic pots. Before application of salt treatments the number of leaves per plant and plant height (cm) was determined. Plants were grouped into ten blocks of four similarly sized plants which were subjected to one of four salinity levels. The purpose of this development-based blocking was to separate potential effects of seedling size from treatment effects.

Salt treatments

The final salt treatments applied consisted of 0, 40, and 80 and 160 mM NaCl, corresponding to electrical conductivities (EC) of 1.8, 5.6, 8.6 and 15.2 dS m^{-1} respectively, dissolved in a full strength Hoagland solution. To avoid early plant death by a sudden salt stress shock of the young transplanted seedlings, the salt stress was imposed gradually by applying half of the salt concentration over four weeks followed by the full concentration applied for another eight weeks. Total duration of the salt stress was 12 weeks.

Growth parameters measured

Data on plant height and number of leaves per plant were recorded weekly. Chlorophyll readings (SPAD value) were taken fortnightly with a SPAD-502 chlorophyll meter (Konica-Minolta Corporation, Osaka, Japan). At the end of experiment, plants were harvested and separated into leaves, stem and roots. Leaf area per plant was measured using a portable leaf area meter (LI-3000A Portable Area Meter, LI-COR Biosciences Inc., Lincoln, NE, USA). Area per leaf was calculated by dividing the total leaf area per plant by the number of leaves. Specific leaf area (SLA) was calculated as leaf area per unit of plant dry matter. After determining the fresh weight of all plant parts, Followed by dry matter determination plant samples were oven-dried at 65°C for 48 hours. The difference between fresh and dry weight divided by the fresh weight yielded the relative water contents of the leaves, stems and roots.

Ion analysis

To determine sodium (Na), phosphorus (P) and potassium (K) concentrations, samples were ashed at 550 °C for 6 hours and the ash dissolved in concentrated HCl. Extracts were filtered and stored in plastic vials until analysis. Concentrations of Na and K were measured by flame photometry (AutoCal 743, Instrumentation Laboratory Co., Lexington, MA, USA) and P was determined by spectrophotometyr (UVIKON 930, Kontron Instruments Ltd, Bletchley, UK). A continuous flow analyzer with potentiometric detection (AutoAnalyzer II, Technicon Instruments, New York, USA) was used to determine the Cl concentration in samples after hot water extraction. A

protein/nitrogen analyzer (FP-328, LECO Instruments GmbH, Mönchengladbach, Germany) was used to measure nitrogen (N) in samples, dried at 105 °C. Nutrient uptake efficiency of roots was determined by dividing the mineral concentration accumulated in the shoot dry matter by root dry matter.

Statistical analysis

All experimental data were analyzed with SPSS 12.0 (SPSS, Chicago, USA) using analysis of variance (ANOVA). Tukey-tests ($P<0.05$) were used to separate means.

5.3 Results

Plant growth

NaCl-induced salinity significantly reduced the vegetative growth of *Z. spina-christi* seedlings. Compared to untreated control plants, after 12 weeks of salt stress seedling height was reduced by 28%, 37% and 57% at 40, 80 and 160 mM salt levels, respectively (Table 5.1). Three weeks after the application of final salt levels, at 80 and 160 mM salinity visible chlorosis and necrosis symptoms on the leaf surface appeared and regular shedding of mature basal leaves was observed. In the 80 and 160 mM treatments leaf number was reduced by 68 and 72%, respectively (Table 5.1). Compared to the control treatment SPAD values of seedling leaves were 27%, 27% and 32% lower at 40, 80 and 160 mM NaCl (Table 5.1). Significant reductions in plant height and number of leaves per plant led to a significant decline in total leaf area and area per leaf with increasing salt stress. Reduction in total leaf area was largest (79%) at 160 mM salt concentration followed by decreases of 76% and 30% at 80 and 40 mM, respectively. Differences in leaf area between seedlings subjected to 40 mM salinity and untreated control plants were not statistically different; however, 80 and 160 mM NaCl led to respective reductions in leaf area by 35 and 38% (Table 5.1).

Salt stress of 80 and 160 mM NaCl induced reductions in total dry matter of > 50% which was equally reflected in leaf, stem and root dry matter (Table 5.1). Stable shoot-root ratios indicated that the extent of salt stress damage to shoot and root growth was similar for all salt concentrations (Table 5.1).

Increasing salt concentrations also led to a significant increase in relative water contents of leaf and root tissues. These tissues contained 15 and 10% more water in the 80 and 160 mM NaCl treatment than in the control (Figure 5.1).

Table 5.1. Effect of different NaCl salt levels on growth parameters of *Z. spina-christi* seedlings 12 weeks after the initiation of the treatment

Growth parameters	NaCl concentration (mM)				F-probability	
	0	40	80	120	Treatment	Block
Plant height (cm)	134±8.2 a	96±9.5 b	84±5.9 b	58±6.0 b	<0.001	<0.001
Leaves per plant	92±2.8 a	73±7.8 a	29±5.2 b	26±4.4 b	<0.001	0.133
SPAD value	44±1.5 a	32±1.4 b	32±2.3 b	30±2.8 b	0.005	0.643
Total leaf area (cm^2)	433±20 a	304±30 b	106±17 c	93±12 c	<0.001	0.532
Area per leaf (cm^2)	4.8±0.3 a	3.7±0.3 ab	3.1±0.7 b	3.0±0.8 b	0.006	0.586
Specific leaf area ($cm^2\,g^{-1}$)	82±3.7 a	93±7.1 a	88±9.3 a	80±5.2 a	0.544	0.338
Leaf dry matter (g)	5.37±0.35 a	2.74±0.46 b	1.45±0.31 bc	1.07±0.15 c	<0.001	0.047
Stem dry matter (g)	9.37±1.00 a	4.42±1.10 b	2.55±0.23 b	2.37±0.51 b	<0.001	0.001
Root dry matter (g)	4.90±0.51 a	3.21±0.35 b	1.71±0.14 c	1.16±0.27 c	<0.001	0.001
Total dry matter (g)	19.6±1.44 a	10.4±1.42 b	5.72±0.56 c	4.60±0.84 c	<0.001	0.002
Shoot / root ratio	3.21±0.36 a	2.19±0.39 a	2.37±0.27 a	3.30±0.43 a	0.445	0.254

Values represent means of 10 replicates ± s.e. and different letters indicate significant difference among treatment means (P<0.05; Tukey test).
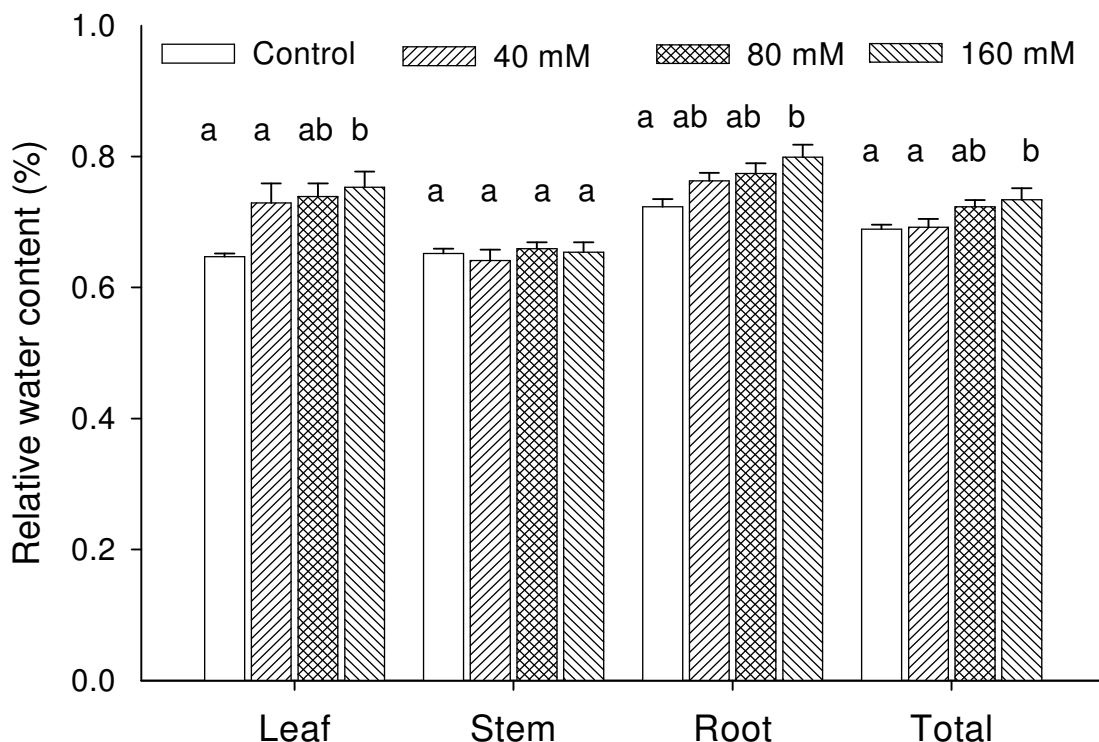
Figure 5.1. Effect of different NaCl salt levels on relative water content (%) of leaf, stem and root tissues of Z.*spina-christi* seedlings 12 weeks after the initiation of treatments. Bars represent means of 10 replicates different letters indicate significant difference among treatment means ($P < 0.05$; Tukey test).

F-probability	Leaf	Stem	Root	Total
Treatment	0.015	0.869	0.047	<0.001
Block	0.206	0.107	0.662	0.200

Concentrations of mineral elements

When exposed to salinity stress, leaf tissue of *Z. spina-christi* L. had 49-, 68- and 90-fold higher Na and 13-, 20- and 21-fold higher Cl concentrations at 40, 80 and 160 mM NaCl, respectively, compared to control plants. Stem and root tissues followed the same trend in the accumulation of Na and Cl ions, however, the magnitude of ion accumulation was far lower than in leaf tissue (Table 5.2)

Compared to the unstressed control, NaCl application did not lead to changes in the N and K balance of leaf and root tissue; at 80 and 160 mM NaCl stems showed a significant decrease in K concentration (Table 5.2). With increases

in salt concentrations to 80 mM we observed significant increases of P concentrations in all plant parts and of N in stem tissues. Leaf, stem and root tissues contained 1.5-, 3.2- and 2.4-fold higher P in their dry mass at 80 mM NaCl salinity compared to the unstressed control (Table 5.2).

Salinity up to 160 mM NaCl did not hamper macronutrient uptake. As root uptake efficiencies of N, P and K were unaffected by salt application (Table 5.2). However, the K/Na ratio in leaf, stem and root tissues was significantly reduced by NaCl salinity (Table 5.2).

Table 5.2. Effect of different NaCl salt levels on ion concentrations and K/Na ratio of leaf, stem and root dry matter of *Z. spina-christi* seedlings 12 weeks after the initiation of the treatment.

Tissue	Treatments NaCl (mM)	Ion concentrations (mg g^{-1} dm)					K/Na ratio
		Na	Cl	N	P	K	
Leaf	0	0.36±0.3 a	2.10±0.1 a	1.57±0.0 a	0.24±0.0 a	17.10±1.8 a	67.40±2.90 a
	40	17.60±2.1 b	26.96±1.3 b	2.14±0.2 a	0.26±0.0 ab	14.93±1.0 a	0.85±0.03 b
	80	24.63±3.4 c	42.85±2.2 c	1.84±0.2 a	0.35±0.0 c	15.80±2.1 a	0.64±0.02 b
	120	32.40±0.8 d	44.64±2.3 c	1.89±0.2 a	0.33±0.1 bc	14.06±1.2 a	0.43±0.02 b
Stem	0	0.60±0.1 a	2.75±0.1 a	1.59±0.1 a	0.17±0.0 a	12.56±0.1 a	21.32±1.96 a
	40	4.10±0.1 b	10.33±0.2 b	1.52±0.1 a	0.34±0.0 b	11.60±0.2 b	2.83±0.05 b
	80	5.90±0.1 c	12.87±0.1 c	2.05±0.1 b	0.55±0.0 d	9.96±0.1 c	1.69±0.02 b
	120	6.33±0.0 d	13.16±0.1 c	1.98±0.1 b	0.53±0.0 c	9.36±0.1 c	1.47±0.02 b
Root	0	1.03±0.0 a	3.34±0.2 a	2.93±0.1 a	0.16±0.0 a	8.83±0.1 a	8.57±1.02 a
	40	5.10±0.5 b	13.56±1.5 b	2.90±0.1 a	0.30±0.0 b	8.46±0.6 a	1.67±0.03 b
	80	6.86±0.6 c	15.80±0.7 c	2.96±0.2 a	0.39±0.0 c	8.10±0.4 a	1.19±0.04 b
	120	7.73±0.2 c	17.60±0.5 c	3.37±0.2 a	0.35±0.0 b	8.23±0.2 a	1.07±0.01 b
		F-probability					
Treatment	Leaf	<0.001	<0.001	0.397	0.006	0.249	0.014
	Stem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Root	<0.001	<0.001	0.306	<0.001	0.738	<0.001
Block	Leaf	0.200	0.102	0.851	0.276	0.521	0.423
	Stem	0.422	0.210	<0.001	0.595	0.353	0.402
	Root	0.992	0.794	0.702	0.949	0.718	0.377

Values represent means of 10 replicates ± s.e. and different letters indicate significant difference among treatment means (P<0.05; Tukey test).

5.4   Discussion

Growth response

Under the conditions of our experiment, the NaCl stress led to stunted seedling growth. Such decreases in plant height with increasing salinity are typical effects of the accumulation of toxic ions in cells which adversely affect cell division and expansion (Munns 1993). At 80 and 160 mM NaCl severe foliar injuries (chlorosis and necrosis) and shedding of affected leaves resulted in a typical reduction of leaf area per plant (Gupta et al. 2002). Unlike salt stressed olive trees (*Olea europaea* L.) which were found to drop leaves of all size, age and from all positions (Therios et al. 1988), seedlings of *Z. spina-christi* only shed their basal old leaves. Even if this was not measured, such differentiation may reflect removal of salts from the more active young tissues towards older ones, a typical trait of species unable to remove salts from their transpiration stream (Munns 2005). As evidenced by the SPAD measurements leaf chlorophyll concentration in seedlings were observed to decline with increasing salt levels and time of exposure. Such salinity induced reduction of leaf chlorophyll through inhibition of chlorophyll synthesis or accelerated degradation has been well described by Reddy and Vora (1986). The large reduction in seedling dry matter and increases in water contents (succulence) under salt stress likely reflected increased metabolic energy costs and reduced CO_2 gain as a consequence of the seedlings' efforts to cope with salt stress by osmotic adjustment (Maas and Nieman 1978; Yang et al. 1990; Saneoka et al. 2001; Netondo et al. 2004). Leaf succulence has also been attributed to increases in spongy mesophyll cells as a response to salt stress (Zekri and Parsons, 1990).

Mineral composition

Large increases in Na and Cl concentrations of all tissue types with salinity stress indicated that unlike some eucalypt species seedlings of *Z. spina-christi* had little control over the uptake and translocation of salt ions (Flower

and Yeo 1988; Van der Muezel et al. 1988). However, despite large accumulation of NaCl in plant tissues, effects on tissue concentrations of N, P and K were not significant. Lacking decline in root uptake efficiency of these nutrients with salt application may be attributed to internal osmotic adjustment of the seedlings in response to osmotic stress (Yang et al. 1990; Saneoka et al. 2001). At 80 mM NaCl a significant increase in P concentration of plant tissues occurred which confirms results of the classical experiments of Robert et al. (1984).

Over all, our results allow to classify *Ziziphus spina-christi* as a moderately salt tolerant fruit tree species at the seedlings stage similar to Tamarind (*Tamarindus. indica*) and Guava (*Psidium guajava*). Further investigations are needed to screen ecotypes / varieties for genetic variation in salt tolerance which if existing would provide scope for selection towards enhanced salinity tolerance of this species.

References

Arbonnier M (2004) Trees, shrubs and lianas of West African dry zones. CIRAD, Margraf Publishers GmbH, Paris, France

Craig GF, Bell DT and Alkyds CT (1990) Response to salt and water logging stress of 10 texa of acacia selected from naturally saline areas of Western Australia. Australian Journal of Botany 38, 619-630.

FAO (2005) Global Network on Integrated Soil Management for Sustainable Use of Salt-affected Soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/ spush.

Flower TJ and Yeo AR (1988) Ion relations of salt tolerance. In: D. Baker and J. Halls, Editors pp.392–414, Solute Transport in Plant Cells and Tissue, Longman, Harlow.

Flower TJ and Yeo AR (1988) Breeding for salinity resistance in crop plants: where next? Australian Journal Plant Physiology 22, 875-884.

Ghassemi FA, Jackman AJ and Nix AH (1995) Salinization of Land and water resources. CAB International, Wallingford, England.

Glenn EP, Pfister R, Brown JJ, Thomson TL and O'leary JW (1996) Na and K accumulation and salt tolerance of *Atriplex canescens* genotypes. American Journal of Botany 83, 997-1005.

Gupta NK, Meena SK, Gupta S and Khandelwal SK (2002) Gas exchange, membrane permeability and ion uptake in two species of Indian jujube differing in salt tolerance. Photosynthetica 40, 535-539.

Hasegawa PM, Bressen RA, Zhu JK and Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology 51, 463-499.

Maas EV and Nieman RH (1978) Physiology of plant tolerance to salinity. In: Jung GA (ed) Crop tolerance to suboptimal land conditions. ASA Spec. Publ., Amer. Soc. Agron. Spez., Madison, Wisconsin, pp 277-299.

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses, Plant, Cell and Environment 16, 5–24.

Munns R (2005) Genes and salt tolerance bringing them together. New Phytologist 167, 645-663.

Netondo GW, Onyango JC and Beck E (2004) Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Science 44, 806-811.

Reddy MP and Vora AB (1986) Changes in pigment composition, Hill reaction activity and saccharides metabolism in Bajra (*Pennisetum typhoides* S & H) leaves under NaCl salinity. Photosynthetica 20, 50-55.

Robert JKM, Linker CS, Benoit AG, Jardetzky O and Nieman RH (1984) Salt stimulation of phosphate uptake in Maize root tips studied by 31P nuclear magnetic resonance. Plant Physiology 75, 947-950.

Saied AS, Gebauer J and Buerkert A (2008) Effect of different scarification methods on germination of *Ziziphus spina-christi* seeds. Seed Science and Technology 36, 201-205.

Saied AS, Gebauer J, Hammer K and Buerkert A (2008) *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. Genetic Resource and Crop Evolution 55, 929–937.

Saneoka H, Ishiguro S and Moghaieb RE (2001) Effect of salinity and abscisic acid on accumulation of glycinebetaine and betaine aldehyde dehydrogenase mRNA in Sorghum leaves (*Sorghum bicolor*)**.** Journal of Plant Physiology 158, 853-859.

Sudhersan C, Hussain J (2003) In vitro propagation of a multipurpose tree, *Ziziphus spina-christi* (L.) Desf. Turkish Journal of Botany 27, 167–171.

Therios IN, and Misopolinos ND (1988) Genotypic response to sodium chloride salinity of four major olive cultivars (*Olea europea* L.) Plant and Soil 106, 105-111.

Van der Moezel PG, Watson LE, Pearce-Pinto GVN and Bell DT (1988) The response of six Eucalyptus species and *Casuarina obesa* to the combined effect of salinity and waterlogging. Australian Journal of Plant Physiology 15, 465–474.

Verinumbe I (1993) Soil and *Leucaena leucocephala* L. growth variability under *Faidherbia albida* Del. and *Ziziphus spina-christi* L. Desf. Agroforestry Systems 21, 287-292.

Yang YW, Newton RJ and Miller FR (1990) Salinity tolerance in sorghum.1. Whole plant response to sodium chloride in *S.bicolor* and *S.helepense*. Crop Science 30, 775-781.

Zekri M, Parsons LR (1990) Calcium influences growth and leaf mineral concentration of under saline conditions. HortScience 25, 784-786.

Conclusions

Ziziphus spina-christi and *Grewia tenax* are two important fruit tree species; that grow wild in arid tropical regions of Pakistan and posses vital position among rural community for their multipurpose use. Continuous increase in aridity associated with soil salinity is a potential threat to these valuable assets of rural population. Ecologically, these species are well adapted to local conditions but their scattered wild stands raised research questions about awareness for effective propagation techniques to enhance the revegetation of degraded arid lands. Poor germination and susceptibility to soil salinity at the seedling stage were assumed to be important reasons of the poor wild stands of the species.
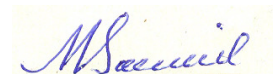
The experimental results presented in this indicated that seeds of *Ziziphus spina-christi* posses physical dormancy (impermeable seed coat), which can be easily overcome through mechanical or acid scarification of seeds before sowing. The results of our investigation to assess the salt tolerance *Z. spina-christi* at seedlings stage allow to classify it as moderately salt tolerant species. *Grewia tenax* proved a salt tolerant fruit tree species and seeds of *G. tenax* posses physiological seed dormancy can be overcome by exposing the seeds to a temperature of 40°C for 4 weeks before sowing (heat stratification).

Being well adapted to arid tropical climate, *G. tenax* is a valuable fruit tree species for the revegetation of salt effected marginal arid lands, whereas *Z. spina-christi* is an appropriate fruit tree species for vegetation of drylands with moderate salinity.

Erklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt und keine anderen als die in der Dissertation angegebenen Hilfsmittel benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden.

Witzenhausen, 15 October 2009   Muhammad Sohail

C U R R I C U L U M   V I T A E

PERSONAL INFORMATION

Name: ***Muhammad Sohail***

Born: February 10, 1974, Dera Ismail Khan, Pakistan

Nationality: Pakistani

EDUCATION

1996-1999   ***Master of Science (Honors) in Agriculture***

Gomal University, Pakistan

1993-1996   ***Bachelor of Science (Honors) in Agriculture***

Gomal University, Pakistan

1990-1992   ***Intermediate Examination***

Board of Intermediate and Secondary Education

Peshawar, Pakistan

1979-1989   ***Secondary School Certificate***

Board of Intermediate and Secondary Education,

Peshawar, Pakistan

RESEARCH INTERESTS

Physiology and biochemistry of mechanisms / traits that confer salt and drought tolerance to crop plants with special emphasis on wild growing underutilized fruit tree species because the species are vital to save the livelihood of rural population and alleviating the effects of malnutrition and land degradation.