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Response of *Grewia tenax* (Forssk.) Fiori to NaCl-induced Salinity

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Summary

With the rapid intensification of agriculture in Sudan salinity problems are increasingly hampering efforts to meet the food demands of a growing population. To increase income and subsistence needs of farmers and to counteract land degradation increased reliance on salt-tolerant fruit trees are of particular interest for many marginal areas. 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 four different levels of NaCl salinity for a period of 7 weeks. Treatments were 0, 20, 40 and 80 mM in the irrigation solution corresponding to an EC of 1.8, 3.8, 5.6 and 8.6 dS m^{-1} for the first four weeks of the experiment, representing the early seedling stage of *G. tenax*. In an effort to mimic salt stress under field conditions the subsequent three weeks salt concentrations were doubled reflecting EC values of 1.8, 5.6, 8.6 and 15.2 dS m^{-1}.

Plants treated with 40/80 mM and 80/160 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 20/40 mM, 40/80 mM and 80/160 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 the moderate and high salt levels, 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.

Key words. leaf gas exchange – mineral concentration – salinity tolerance – wild fruit species

Introduction

Globally, land degradation is one of the factors most limiting food production. 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). 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 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.

In Sudan saline soils amount to 2.1 million ha, the majority of which are located in the low rainfall regions, affected by the weathering of salt bearing rocks, poor soil and water management and insufficient drainage (Fao 2000).

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 (Vogt 1995; El-Siddig et al. 2003). 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, 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 are lacking.

Materials and Methods

Plant material and growth conditions

Seeds of *G. tenax* were obtained from El Obeid (13° 20' N, 30° 15' E), Central Sudan 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 a native population and germination was spread over a period of time.

Salt treatments

To simulate conditions that young seedlings may encounter when planted under amended conditions in a salt affected soil where after a few weeks their roots grow into the natural, higher salinity soil, seedlings received during the first four weeks every two days 200 ml of Hoagland solution to which 0 (control), 20 (low salinity), 40 (moderate salinity) or 80 mM NaCl (high salinity) was added. This corresponded to an electrical conductivity (EC) of 1.8, 3.8, 5.6 and 8.6 dS m^{-1}, respectively. Thereafter, for the next three weeks (until harvest), the salt concentrations were doubled and plants received 0 (control), 40 (low salinity), 80 (moderate salinity) and 160 mM NaCl (high salinity) corresponding to an EC of 1.8, 5.6, 8.6 and 15.2 dS m^{-1}, respectively.

Measurements

Plant height was measured and leaf number was counted every week starting from week 0, the time just before the onset of the salt treatments.

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 % 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 ground 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 *G. tenax* roots for different minerals was estimated by dividing the mineral concentration accumulated in the entire plant by root DM at final harvest.

Statistical analyses

All experimental data were analyzed with SPSS-statistical package (SPSS, Chicago, USA). Data were tested for normal distribution of residuals and variance homogeneity and means were separated by Tukey test at $P < 0.05$. Residuals of Cl concentration in roots were not normally distributed, therefore for these variable F-values and results of mean separation tests are only approximate.

Results

Blocking, which represented in this case the pre-treatment size of seedlings had an effect on plant height, 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 (80/160 mM). However, mature leaves of three out of the eight plants treated with the high salt level, showed leaf chlorosis. The yellowing started six weeks after the onset of the salt application at the leaf tip and advanced progressively along the leaf margins to the base. Visual damage to other plant parts was not observed.

Weekly leaf counts showed that leaf number was unaffected by salt treatments until week 5 (Fig. 1). Starting from week 6, plants treated with moderate (40/80 mM) and high (80/160 mM) salt levels had significantly lower leaf numbers than control plants (Fig. 1). These differences became obvious at week 7, when these plants started to shed their leaves. Plant height seemed to be less affected by salt stress (Fig. 2). It was significantly reduced at the high (80/160 mM) salt level, but this reduction was only observed from week 6 onwards (Fig. 2).

The presence of NaCl in the growing medium reduced total leaf area per plant by 33, 42 and 54 % at the low (20/40 mM), moderate (40/80 mM) and high (80/160 mM) NaCl levels, respectively (Table 1). Area per leaf, however, remained unaffected by salt treatments. Specific leaf area (SLA) rose significantly at the high (80/160 mM) salt level, whereas it remained unchanged at the other salt treatments (Table 1). A consistent decline in specific root length (SRL) was observed in plants at higher salinity as compared with control plants (Table 1).

Total dry matter (DM) per plant was reduced by 25, 36 and 49 % at the low (20/40 mM), moderate (40/80 mM) and high (80/160 mM) salt levels, respectively (Fig. 3). The reduction was most distinct for leaves, whereas root DM was unchanged by salt level. In all treatments including the control shoot DM accounted for more than 50 % of total DM, resulting in shoot/root ratios of 8–9 (Fig. 3).

Addition of NaCl to the nutrient solution also led to an increase in relative water content of *G. tenax* (Fig. 4). This effect was most distinct in leaves, where water content increased by 6 % at low (20/40 mM), 12 % at moderate (40/80 mM) and 21 % at high (80/160 mM) salt levels. The corresponding values for stems were 1, 3 and 4 %, respectively, with a significant increase only at the high (80/160 mM) NaCl concentration. The increases in root water contents were not significant.

Leaf chlorophyll and gas exchange

Measurements of leaf chlorophyll concentration 3 weeks after the onset of the salt treatments did not reveal any difference between control and salt-stressed plants (Fig. 5). However, at week 7, leaf chlorophyll of plants treated with high (80/160 mM) salt concentration decreased by 21 % as compared to control plants. Similarly to chlorophyll, measurements of net photosynthesis (P_N), internal CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration (E) 3 weeks after salt application showed no differences between salt-stressed and control plants (data not shown).

At week 7, P_N of salt-treated plants decreased by 29 % at the moderate (40/80 mM) and by 42 % at the high (80/160 mM) salt levels compared to the control plants (Table 1). The low (20/40 mM) salt level decreased g_s by 50 %, the moderate (20/40 mM) by 72 % and the high (80/160 mM) by 83 %. This decrease in g_s reflected the reduction in E. As a consequence of stomata closure, E of salt-stressed leaves decreased to 41, 63 and 77 % at the low (20/40 mM), moderate (40/80 mM) and high (80/160 mM) salt levels, respectively (Table 1).

Concentrations of mineral elements

Addition of NaCl to the nutrient solution increased Na and Cl concentrations in leaves, stems and roots (Fig. 6a

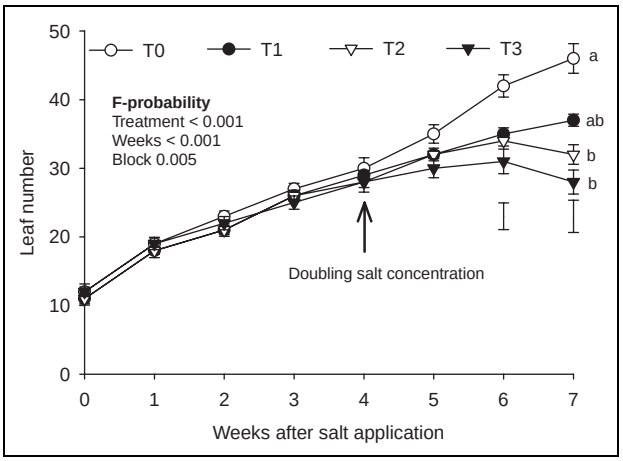


Fig. 1. Time course of leaf number of pot-grown *Grewia tenax* seedlings as affected by different NaCl concentrations (T0 = 0 mM, T1 = 20/40 mM, T2 = 40/80 mM, T3 = 80/160 mM) in the irrigation solution. Note that salt concentrations were doubled after the first four weeks of the experiment as shown by the arrow. Symbols with bars show treatment means ± one SE and different letters indicate significant differences at the 5 % level by a Tukey test.

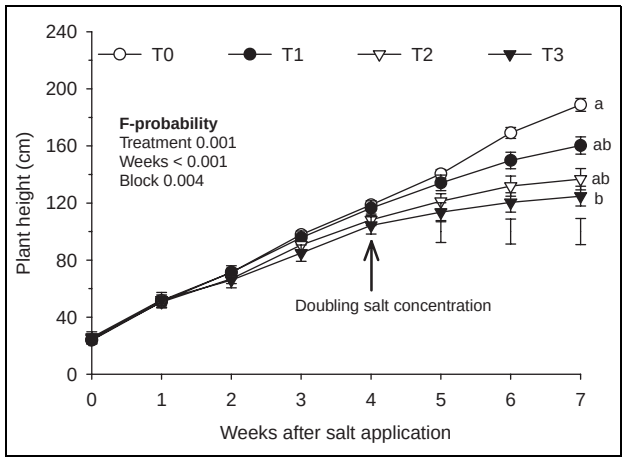


Fig. 2. Time course of plant height of pot-grown *Grewia tenax* seedlings as affected by different NaCl concentrations (T0 = 0 mM, T1 = 20/40 mM, T2 = 40/80 mM, T3 = 80/160 mM) in the irrigation solution. Note that salt concentrations were doubled after the first four weeks of the experiment as shown by the arrow. Symbols with bars show treatment means ± one SE and different letters indicate significant differences at the 5 % level by a Tukey test.

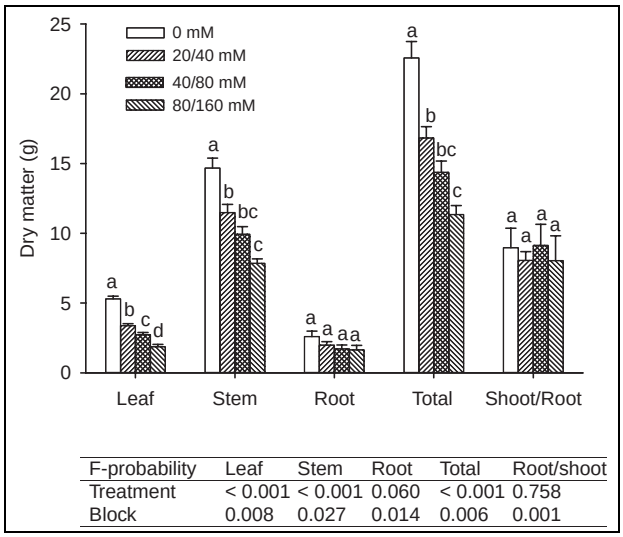
Table 1. Leaf area, area per leaf, specific leaf area (SLA) and specific root length (SRL), net photosynthesis (P_N), internal CO_2 (C_i), stomatal conductance (g_s) and transpiration (E) of leaves of pot-grown *Grewia tenax* seedlings treated with different concentrations of NaCl in the irrigation solution. Measurements were taken 7 weeks after initiation of salt treatments.

Salt level (mM)	Leaf area (cm^2)	Area per leaf (cm^2)	SLA ($cm^2\ g^{-1}$)	SRL ($cm\ g^{-1}$)
0	708.5 ± 51.4 a	15.4 ± 0.7 a	133.0 ± 5.9 b	43.3 ± 3.3 a
20/40	477.9 ± 33.0 b	13.1 ± 1.0 a	141.0 ± 6.5 b	34.2 ± 1.9 b
40/80	409.3 ± 24.2 bc	12.8 ± 0.8 a	149.8 ± 3.9 b	32.2 ± 2.3 b
80/160	327.0 ± 34.0 c	12.0 ± 1.5 a	175.9 ± 10.6 a	28.1 ± 0.8 b
F-probability				
Treatment	< 0.001	0.104	0.004	0.002
Block	0.034	0.147	0.824	0.654

Salt level (mM)	P_N ($\mu mol\ m^{-2}\ s^{-1}$)	C_i (ppm)	g_s ($mmol\ m^{-2}\ s^{-1}$)	E ($mmol\ m^{-2}\ s^{-1}$)
0	24.7 ± 1.2 a	115 ± 13 b	0.18 ± 0.013 a	5.8 ± 0.5 a
20/40	22.1 ± 0.9 a	207 ± 47 b	0.09 ± 0.008 b	3.4 ± 0.3 b
40/80	17.4 ± 0.7 b	213 ± 37 b	0.05 ± 0.003 c	2.1 ± 0.1 c
80/160	14.3 ± 1.6 b	418 ± 49 a	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

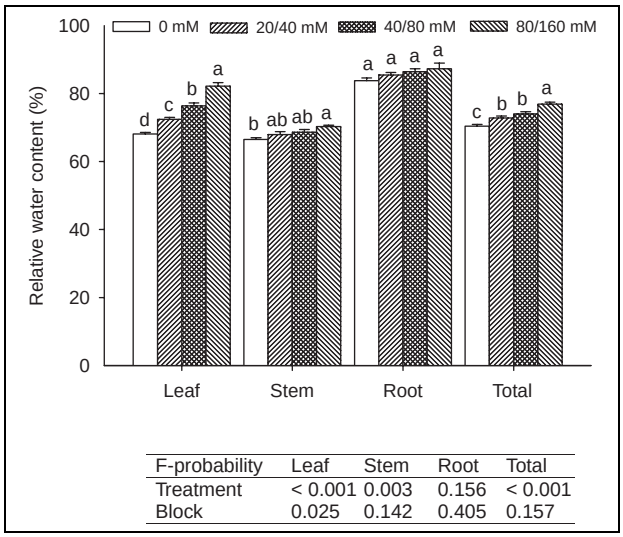
Note that salt concentrations were doubled after the first four weeks of the experiment. Data shown are means with standard errors and different letters within a column indicate significant differences at the 5 % level by Tukey test.

and 6b) whereby the concentration of Na ranged from 0.5 in leaves of control plants to 14.6 mg g^{-1} DM in plants of the high (80/160 mM) salt treatment. The corresponding values in stems ranged from 0.6 to 6.2 and in roots



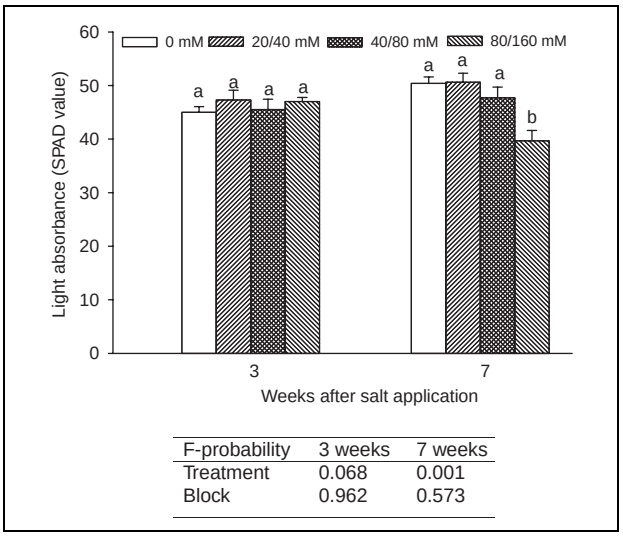
F-probability	Leaf	Stem	Root	Total	Root/shoot
Treatment	< 0.001	< 0.001	0.060	< 0.001	0.758
Block	0.008	0.027	0.014	0.006	0.001

Fig. 3. Dry matter of different plant parts and shoot/root ratio of *Grewia tenax* seedlings at final harvest in a pot experiment with different concentrations of NaCl in the irrigation solution. Bars show one SE of the mean and letters indicate significant differences at the 5 % level by a Tukey test.



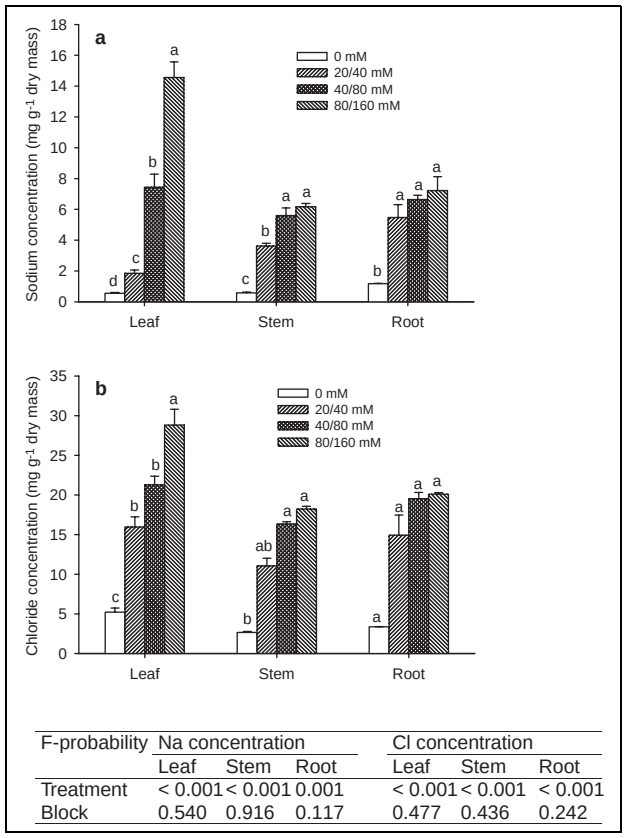
F-probability	Leaf	Stem	Root	Total
Treatment	< 0.001	0.003	0.156	< 0.001
Block	0.025	0.142	0.405	0.157

Fig. 4. Relative water content of different plant parts of *Grewia tenax* seedlings at final harvest in a pot experiment with different concentrations of NaCl in the irrigation solution. Bars show one SE of the mean and letters indicate significant differences at the 5 % level by a Tukey test.



F-probability	3 weeks	7 weeks
Treatment	0.068	0.001
Block	0.962	0.573

Fig. 5. Light absorbance corresponding to the chlorophyll content of leaves of *Grewia tenax* seedlings in a pot experiment with different concentrations of NaCl in the irrigation solution. Bars show one SE of the mean and letters indicate significant differences at the 5 % level by a Tukey test.



F-probability	Na concentration			Cl concentration		
	Leaf	Stem	Root	Leaf	Stem	Root
Treatment	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001
Block	0.540	0.916	0.117	0.477	0.436	0.242

Fig. 6. Concentration of Na (a) and Cl (b) in different plant parts of *Grewia tenax* seedlings at final harvest in a pot experiment as a function of different concentrations of NaCl in the irrigation solution. Bars show one SE of the mean and letters indicate significant differences at the 5 % level by a Tukey test.

from 1.2 to 7.2 mg g^{-1} DM (Fig. 6a). Leaves contained 41 % of the absorbed Na, stems 26 % and roots 33 %. For Cl, the values in the control and the high salt plants (80/160 mM) varied from 5.2 to 28.8 in leaves, from 2.7 to 18.2 in stems and from 3.4 to 20.1 mg g^{-1} DM in roots (Fig. 6b). The distribution of absorbed Cl closely followed that of Na.

Leaf K concentration was unaffected by salinity, whereas stem and root K was reduced by NaCl addition (Table 2). The reduction in stem K varied from 19 % at the low (20/40 mM), 28 % at the moderate (40/80 mM) to 29 % at the high (80/160 mM) salt level. In roots the reduction in K concentration was not significant at the low salt concentration (20/40 mM), but the moderate (40/80 mM) and high (80/160 mM) level led to reductions in K concentrations by 35 and 34 %, respectively. Root N concentration was only affected at the high (80/160 mM) salt concentration, whereas in leaves it was significantly reduced by 7 % at the moderate (40/80 mM) and 15 % at the high (80/160 mM) salt level compared to control plants (Table 2). In the stem the reduction in N concentration was also significant at the moderate (40/80 mM) and high (80/160 mM) salt level and accounted for 13 % in both salt treatments. In contrast to the roots where the high (80/160 mM) salt level decreased the P concentration by 22 % compared to the control, P concentration in leaves and stems was not affected by NaCl addition (Table 2). The uptake efficiency of roots for N, P, K and Na was unaffected by the salt treatments but it significantly increased for Cl when controls and salt-affected plants were compared (Fig. 7).

A plot of P_N against leaf Cl concentration indicated an inverse relationship (r^2 = 0.94; Fig. 8). The same was found when plotting P_N against leaf Na concentration (data not shown), indicating that the reduction in photosynthesis was partly due to the accumulation of both Na and Cl ions in leaves.

Discussion

The results of this study showed that all plants survived salt levels of up to 80/160 mM NaCl. Salt damage symptoms were only observed in a few plants treated with the high (80/160 mM) NaCl level, 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 (40/80 mM) and high (80/160 mM) salt level in the last week of our experiment might be the classical mechanism by which many plants eliminate accumulated salt ions. Such was 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.). However, Cram et al. (2002) in their work with mangroves (*Bruguiera cylindrical* (L.) Blume., *Avicennia rumphiana* Hallier f. and *A. marina* (Forssk.) Vierh.), argued that mature leaves may serve as temporary deposits of salts within the shoots, but when leaves fall this func-

Table 2. Concentrations of potassium (K), nitrogen (N) and phosphorus (P) in different parts of pot-grown *Grewia tenax* seedlings. Analysis was done 7 weeks after initiation of salt treatments. K and P concentrations are in mg g^{-1} dry mass and N concentration is as percent of dry mass.

Salt level (mM)	Leaf			Stem			Root		
	K	N	P	K	N	P	K	N	P
0	13.5 ± 0.6 a	3.82 ± 0.07 a	0.20 ± 0.013 a	15.9 ± 0.9 a	1.4 ± 0.03 a	0.32 ± 0.05 a	13.1 ± 0.4 a	2.0 ± 0.09 a	0.23 ± 0.002 a
20/40	13.9 ± 0.7 a	3.58 ± 0.05 ab	0.18 ± 0.006 a	12.8 ± 0.3 b	1.3 ± 0.03 ab	0.25 ± 0.02 a	11.8 ± 0.6 a	1.9 ± 0.13 ab	0.20 ± 0.006 ab
40/80	14.0 ± 0.5 a	3.56 ± 0.04 b	0.18 ± 0.007 a	11.5 ± 0.3 b	1.2 ± 0.04 b	0.21 ± 0.04 a	8.5 ± 0.2 b	1.8 ± 0.09 ab	0.18 ± 0.017 ab
80/160	12.0 ± 1.2 a	3.24 ± 0.14 b	0.17 ± 0.003 a	11.3 ± 0.6 b	1.2 ± 0.05 b	0.21 ± 0.01 a	8.6 ± 0.3 b	1.6 ± 0.06 b	0.17 ± 0.003 b
F-probability									
Treatment	0.289	0.001	0.146	0.003	0.011	0.057	0.001	0.027	0.019
Block	0.433	0.209	0.672	0.984	0.685	0.324	0.913	0.070	0.266

Note that salt concentrations were doubled after the first four weeks of the experiment. Data shown are means with standard errors and different letters within a column indicate significant differences at the 5 % level by a Tukey test.

tion ceases and therefore leaf fall is far from being a salt elimination mechanism.

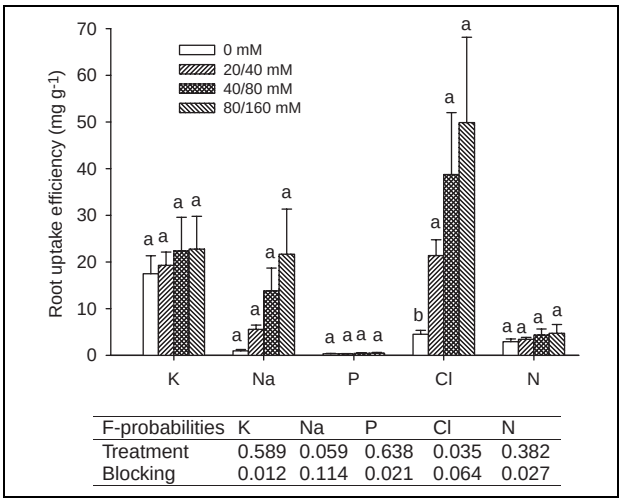
Among the measured growth parameters, the reduction in leaf area per plant was significant in salt treated plants. This is in agreement with the findings of Chartzoulakis et al. (2002), who reported that effects of salinity on leaf area of olive seedlings (*Olea europaea*) were the greatest among all observed growth parameters. At least for the moderate (40/80 mM) and high (80/160 mM) 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 DM 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 *Bruguiera parviflora* (Roxb.) Wt. & Arn. ex Griff. 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.

Reduction in DM 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 DM 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 DM in the moderately tolerant seedlings of guava (*Psidium guajava* L.) at a salinity level of 30 mM NaCl and Gebauer et al. (2004) a reduction of less than 50 % in moderately tolerant tamarind at 40 mM level. Comparing this with our findings, *G. tenax* could be therefore, classified as a salt tolerant fruit species as reflected by the 49 % reduction in total DM at the high (80/160 mM) NaCl treatment.

The observed increase in relative water content due to salinity especially in leaves was also observed by other authors. Saied et al. (2003) explained it as a way to partially compensate possible morphological changes to salinity. On the other hand, Maas and Nieman (1978) and Larcher (2003) 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 to 21 % compared to control plants at the end of the experiment. Different authors reported a reduction in chlorophyll due to NaCl salinity (Sultana et al.



F-probabilities	K	Na	P	Cl	N
Treatment	0.589	0.059	0.638	0.035	0.382
Blocking	0.012	0.114	0.021	0.064	0.027

Fig. 7. Root uptake efficiencies of the different minerals absorbed by *Grewia tenax* seedlings in a pot experiment with different concentrations of NaCl in the irrigation solution. Bars show one SE of the mean and letters indicate significant differences at the 5 % level by a Tukey test.

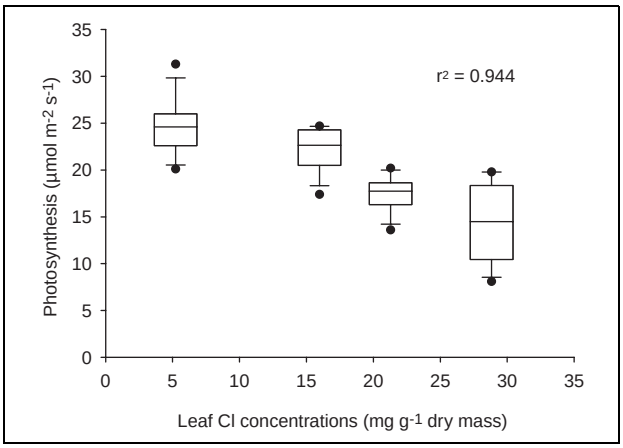


Fig. 8. Net photosynthesis of leaves of pot-grown *Grewia tenax* seedlings as a function of concentration of Cl in leaves. The vertical box plots show the median and the 25 and 75 % percentiles as vertical boxes and 10 and 90 % percentiles as error bars. Outliers of net photosynthesis as measured at the different leaf Cl concentration are indicated by back dots.

1999; Ebert et al. 2002; Netondo et al. 2004; Parida et al. 2004). 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 (40/80 mM) and high (80/160 mM) salinity level led to a decrease in net photosynthesis compared with that of control plants. At the moderate (40/80 mM) salt level, this reduction could not be explained by a decrease in leaf chlorophyll; however, at the high (80/160 mM) salinity level the reduction in P_N could be partially due to a reduction in chlorophyll.

Stomatal conductance decreased with increasing salinity, whereas C_i increased. The reduction in g_s was much greater than the reduction in P_N. Such salt-induced concurrent reductions in P_N and g_s have been reported for a number of species, although it was often difficult to assess the extent to which the reduction in P_N was caused by partial stomatal closure and to which it may have been caused by a reduction in non-stomatal factors (Brugnoli and Björkman 1992). The increase in the C_i level found in olive leaves treated with NaCl salt was due to stomatal closure and reduction in P_N (Tattini et al. 1997). Similarly, Awang et al. (1993) recorded an increased C_i concentration and decreased P_N with increasing salinity. They interpreted this as the result of increased respiration expenditure on maintenance energy needed for osmotic adjustment. 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 (g_m) may pose a significant limitation to photosynthesis. The level of C_i could be high; nevertheless, the CO_2 concentration at the chloroplasts could be low due to low g_m. The low photosynthesis observed in salt-stressed olive (Bongi and Loreto 1989) and cotton (*Gossypium hirsutum* L.) (Brugnoli and Björkman 1992) was due to reduction in g_m. Net photosynthesis of plants exposed to salt stress could also be reduced by non-stomatal parameters such as metabolic impairment, diffuse resistances, leaf water and osmotic potentials, pigments, enzymes and protein (Bethke and Drew 1992; Lawlor 1995; Sultana et al. 1999; Massacci and Loreto 2001). The recorded reduction in g_s, and concurrent reduction in transpiration rate (E) due to partial closure of stomata, could be an adaptive response of stressed plants to reduce water loss through evapotranspiration.

In contrast to the findings of Chartzoulakis et al. (2002) in olives, we found a strong relationship between the rate of photosynthesis in *G. tenax* and the Cl and Na concentration in the leaves (r^2 = 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*), Chartzoulakis and Klapaki (2000) in pepper hybrids.

G. tenax plants accumulated Na and Cl in all plant parts but particularly in leaves. 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 was reduced in *G. tenax* stems and roots with increasing salt level, leaves managed to maintain their K status, this was also

observed by other authors in different species (Ruiz et al. 1999; Chartzoulakis and Klapaki 2000; Parida et al. 2004). 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 compartmentalisation 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, olives and tamarind 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. Niknam and McComb (2000) reported that most woody species show little 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.

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