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Effect of NaCl Salinity on Growth and Mineral Composition of *Ziziphus spina-christi* (L.) Willd.

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Abstract

Ziziphus spina-christi (L.) Willd. 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 contribute to filling 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 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 $\mathrm{K/Na}$ ratio, in contrast, was hardly affected by increasing salinity indicating adaptation or tolerance of *Z. spina-christi* to low or moderate NaCl salinity. These results suggest that *Z. spina-christi* could be an interesting species for re-vegetation of moderately degraded saline lands.

Keywords: foliar injury, fruit tree, ion content, neglected species, salt stress-tolerance

1 Introduction

Soil salinity is becoming an increasingly serious constraint to plant growth in many parts of the world (FAO, 2005). This is particularly the case in semi-arid and arid zones, where already over a decade ago 50% of the cropland was salt affected (Flower and Yeo, 1995). This can lead to a reduction of biodiversity and land degradation (Ghassemi *et al.*, 1995). In many plant species soil salinity is known to reduce growth and 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 previous studies focused on annual species' ability to tolerate salinity and only limited information is available on multipurpose fruit trees that

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

Ziziphus spina-christi (L.) Willd. is a multipurpose fruit tree that is ubiquous in arid and semi-arid Asia and Africa (ARBONNIER, 2004). The fruits, leaves, bark and the wood are intensively used by the rural population. 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.*, 2008b). The plant is also well adapted to dry and hot climates which makes it suitable for re-vegetation of degraded lands (SAIED *et al.*, 2008b), 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 *Z. spina-christi*.

2 Materials and Methods

2.1 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.*, 2008a). 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 was determined. Plants were grouped into ten blocks (replications) 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.

2.2 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 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 and increasing it to the final concentration for another eight weeks. Total duration of the salt stress was therefore 12 weeks.

2.3 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). SPAD (Soil-Plant Analysis Development) readings are significantly related to extracted chlorophyll of leaves both on a fresh weight and leaf area basis (AZIA and STEWART, 2001).

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, plant samples were oven-dried at 65°C for 48 hours to measure their dry weight. The difference between fresh and dry weight divided by the fresh weight yielded the relative water contents of the leaves, stems and roots.

2.4 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 spectrophotometry (UVIKON 930, Kontron Instruments Ltd, Bletchley, UK). A continuous flow analyzer with potentiometric detection (AutoAnalyzer II, Technicon Instruments, NY, 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 60 °C.

2.5 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.

3 Results

3.1 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 treatments, respectively (Table 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 1). Compared to the control treatment SPAD values of seedling leaves were 27, 27 and 32% lower at 40, 80 and 160 mM NaCl, respectively (Table 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 1).

Table 1: Effect of different $NaCl$ salt levels on growth parameters of *Z. spina-christi* seedlings 12 weeks after the initiation of the treatments.

	$NaCl$ *concentration (mM)*				*F-probability*	
Growth parameter	*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
Leaf chlorophyll (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

Each value is a mean of 10 replicates $\pm$ standard error and different letters within a row specify significant difference ($p < 0.05$; Tukey test).

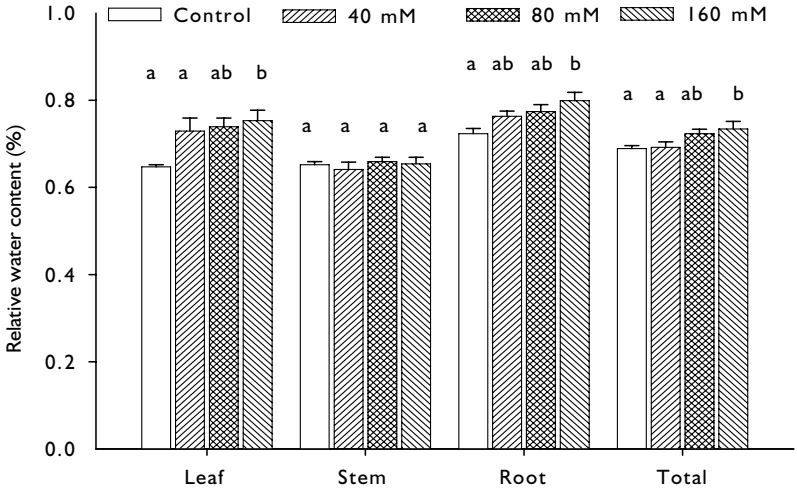
Salt stress of 80 and 160 mM $NaCl$ induced reductions in total dry matter of $>$ 50% which was equally reflected in leaf, stem and root dry matter (Table 1). Shoot / root ratio was not significantly effected by the salinity treatments (Table 1). Increasing salt concentrations also led to a significant 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 (Fig. 1).

3.2 Concentration of minerals

When exposed to salinity stress, leaf tissue of *Z. spina-christi* 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 (Table 2). 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 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 40, 80 and 160 mM $NaCl$ stems showed a significant decrease in K concentration (Table 2). With an increase in salt concentrations to 80 mM $NaCl$ we observed a significant increase of the P concentration 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 2). The K/Na ratio in leaf, stem and root tissues was hradly reduced by $NaCl$ salinity (Table 2).

Figure 1: Effect of different NaCl salt levels on relative water content (%) of leaf, stem and root tissues of *Z. spina-christi* seedlings after 12 weeks of treatment application.*



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

* Each bar shows the mean of 10 replicates ± one standard error and different letters specify significant difference (p <0.05; Tukey test).

4 Discussion

4.1 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 and Misopolinos, 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 that remove toxic salts from their transpiration stream (Munns, 2005). As indicated by the SPAD values, leaf chlorophyll concentration in seedlings declined with increasing salt level 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 the increase in water contents (succulence)

Table 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 treatments.

Tissue	*Treatments*	*Ion concentrations (mg g dm^{-1})*					
	NaCl *(mM)*	Na	Cl	N	P	K	K/Na *ratio*
Leaf	0	0.36±0.3^{d}	2.10±0.1^{c}	1.57±0.0^{a}	0.24±0.0^{c}	17.10±1.8^{a}	67.40±2.90^{a}
	40	17.60±2.1^{c}	26.96±1.3^{b}	2.14±0.2^{a}	0.26±0.0^{bc}	14.93±1.0^{a}	0.85±0.03^{b}
	80	24.63±3.4^{b}	42.85±2.2^{a}	1.84±0.2^{a}	0.35±0.0^{a}	15.80±2.1^{a}	0.64±0.02^{b}
	120	32.40±0.8^{a}	44.64±2.3^{a}	1.89±0.2^{a}	0.33±0.1^{ab}	14.06±1.2^{a}	0.43±0.02^{b}
Stem	0	0.60±0.1^{d}	2.75±0.1^{c}	1.59±0.1^{b}	0.17±0.0^{d}	12.56±0.1^{a}	21.32±1.96^{a}
	40	4.10±0.1^{c}	10.33±0.2^{b}	1.52±0.1^{b}	0.34±0.0^{c}	11.60±0.2^{b}	2.83±0.05^{b}
	80	5.90±0.1^{b}	12.87±0.1^{a}	2.05±0.1^{a}	0.55±0.0^{a}	9.96±0.1^{c}	1.69±0.02^{b}
	120	6.33±0.0^{a}	13.16±0.1^{a}	1.98±0.1^{a}	0.53±0.0^{b}	9.36±0.1^{c}	1.47±0.02^{b}
Root	0	1.03±0.0^{c}	3.34±0.2^{c}	2.93±0.1^{a}	0.16±0.0^{c}	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^{a}	15.80±0.7^{a}	2.96±0.2^{a}	0.39±0.0^{a}	8.10±0.4^{a}	1.19±0.04^{b}
	120	7.73±0.2^{a}	17.60±0.5^{a}	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

Each value is a mean of 10 replicates ± standard error and different letters with in column specify significant difference ($p < 0.05$; Tukey test).

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 can also be attributed to increases in spongy mesophyll cells as a response to salt stress (ZEKRI and PARSONS, 1990).

4.2 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 MOEZEL *et al.*, 1988). However, despite large accumulation of Na and Cl 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 ROBERT *et al.* (1984) that moderate salinity may enhance P uptake, when sufficient P is available in the substrate.

Over all, our results allow to conclude that *Z. spina-christi* can tolerate salinity up to 40 mM at the seedling stage. Being well adapted to arid climatic conditions, the species has the potential for re-vegetation of moderately degraded saline lands. However, further investigations are needed to screen ecotypes for genetic variation in salt tolerance which if existing would provide scope for selection towards enhanced salinity tolerance of this multipurpose fruit tree species.

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