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Germination, growth and physiological responses of *Senegalia senegal* (L.) Britton, *Vachellia seyal* (Delile) P. Hurter and *Prosopis juliflora* (Swartz) DC to salinity stress in greenhouse conditions

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Salinity is among the most widespread environmental threats to global plant production, especially in arid and semi-arid climates. Thus, the selection of salt tolerant species is necessary for sustainable plant productivity. The purpose of this study was to measure and understand the salt tolerance of three multipurpose trees used in reforestation programs in many Sahelian countries (*Senegalia senegal*, Syn. *Acacia senegal*; *Vachellia seyal*, Syn. *A. seyal*, and *Prosopis juliflora*). The effect of salinity was evaluated at seed germination stage on Petri dishes containing water agar (0.9%, w/v) with seven concentrations of NaCl (0, 86, 171, 257, 342, 428, and 514 mM). Our results showed that all the species had a germination rate higher than 85% at 257 mM. However, it decreased at 342 mM with a reduction of 70 and 20%, respectively for *S. senegal* and *V. seyal*. For plants growth and physiological responses, seedlings were individually cultivated in plastic bags (25×12 cm) containing non-sterile soil and watered with four salt solutions (0, 86, 171 and 257 mM NaCl). Four months after the plants' cultivation, the results showed that for all species, the salinity reduced significantly the height, the collar diameter, the shoot and root dry biomass as well as the total chlorophyll, K^+ and K^+/Na^+ ratio. In the meantime, proline content, Cl^- and Na^+ accumulation in leaves were increased. It was also found that *S. senegal* and *V. seyal* tolerated high concentrations of NaCl (257 mM) and developed physiological and molecular mechanisms, such as salt tolerance genes (NHX1), which allow them to be considered as moderated salt tolerant species and seemed to be potential species for the restoration of salt-affected land as *P. juliflora*.

Key words: Multipurpose leguminous trees, abiotic stress, salt tolerance, Senegal.

INTRODUCTION

Soil salinization is an emerging environmental problem around the world and represents a major limiting factor for plants production and ecological environment, especially in arid and semi-arid regions (Zahran, 1999). Over 953 million ha of land are salt-affected throughout the world, covering about 8% of the world's land surface (Singh, 2009). In Senegal, 1 700 000 ha of the 3 800 000 ha of the agricultural lands are salt-affected (FAO-LADA, 2009). Numerous studies have shown that high NaCl concentrations in the growth medium of plants generate primary and secondary effects that negatively affect plant growth and development. Several physiological functions, including photosynthesis, mineral nutrition and carbohydrate metabolism have been shown to be affected by the high salinity (Chen et al., 2008). Primary effects are ionic toxicity and osmotic stress. Ionic toxicity occurs because of the high Na^+ and Cl^- concentrations in the cytoplasm of cells. The lowering of the water potential that causes turgor reduction and cellular water loss also induces osmotic stress. Secondary effects of NaCl stress include inhibition of K^+ uptake, membrane dysfunction and generation of reactive oxygen species (ROS) in the cells (Rout and Shaw, 2001; Ghoulam et al., 2002; Agarwal and Pandey, 2004; Upadhyay and Panda, 2005). Salinity modifies photosynthetic parameters, including osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content (RWC); salt also affects. The components of photosynthesis such as enzymes, chlorophylls, and carotenoids are also affected by the salt. Changes in these parameters depend on the severity and duration of stress (Misra et al., 1997) and on plant species (Liu et al., 2011).

Senegalia senegal (L.) Britton*, Vachellia seyal* (Delile) P. Hurter and *Prosopis juliflora* (Swartz) DC are trees species widely found in arid and semi-arid zone of Senegal. *S. senegal* is a multipurpose perennial legume, widespread in the semi-arid zone of tropical Africa and the Middle East. *S. senegal* has huge potential in agroforestry systems, fuelwood production, forage, medicinal products and gum production (Von Maydell, 1986). In this respect, more attention is given to *S. senegal* which is among the selected forest species for the Great Green Wall, from Senegal to Djibouti (GMV, 2009). *V. seyal* contributes to soil fertility in agroforestry systems. It produces fodder and gum and is also an important source of rural energy because of its role in the production of both firewood and charcoal. In Senegal, *V. seyal* is widely distributed in the salt-affected coastal steppes. *P. juliflora* is used in agroforestry systems. This legume tree is an important component of the system, because it serves as a source of high quality animal feed. It can be sold for fuel and timber, as well as it improves soil physiochemical and biological properties, generating "fertility islands" or "resource islands" beneath its canopy (Reyes-Reyes et al*.,* 2002; Diedhiou et al., 2009; Dossa et al., 2010). It also has been described as salt-tolerant species (Basavaraja, 2007).

Adaptation of plants during germination and early seedling stages in saline environments is crucial for the establishment of species (Debez et al., 2004; Vicente et al., 2004). After seeds germination, seedlings are the most vulnerable in the life cycle of plants (Kolb and Barsch, 2010). Thus, for the successful establishment of plants in saline environments, seeds must remain viable at high salinity in an imposed secondary dormancy and germinate when salinity decreases (Vicente et al., 2004). Furthermore, seedlings will be able to grow in these conditions. One key mechanism of salinity tolerance is the ability to remove Na^+ ions from the cytosol and its sequestration into the vacuole to limit cell damage. The transport of Na^+ into vacuoles is thought to be mediated by vacuolar Na^+/H^+ antiporters of the NHX family, which are driven by the electrochemical gradient of protons (Gaxiola et al., 1999; Pardo et al., 2006). Several studies showed that NHX1 overexpression increased salinity tolerance in plants (Apse et al., 1999; Tian et al., 2006; Brini et al., 2007; Chen et al., 2015).

In Senegal, like in most arid and semi-arid regions, reforestation of salty lands has become a priority. *S. senegal*, *V. seyal* and *P. juliflora* are leguminous multipurpose trees selected in many reforestation programs. However, few studies have been done on the adaptation of these species to environmental conditions in relation to climate change such as drought and salinity. The objective of our study was to evaluate the effect of NaCl on *S. senegal*, *V. seyal* and *P. juliflora* germination, growth and some physiological and molecular traits in order to measure and understand their salt tolerance; with the aim to facilitate the on-going initiatives of Senegalese Forest Department to find suitable species for plantation in the salt affected areas in a context of climate change.

MATERIALS AND METHODS

Effect of NaCl on *S. senegal*, *V. seyal* and *P. juliflora* seeds germination

Seeds of *S. senegal* (Provenance Dahra-2013), *V. seyal*

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Table 1. Physical and chemical characteristics of soils collected in non-saline zone at 0 to 25 cm layer.

Soil parameter	Values
Clay	05.5%
Silt	11.5%
Sand	83.0%
Chemical characteristics	
pH H_2O	5.5
Electrical conductivity (at 25°C)	27 µS/cm
Salinity	0.00‰
Total nitrogen	0.05%
Total carbon	0.56%
Total phosphorus	52.00 mg/kg
Calcium (Ca)	0.78 méq%
Magnesium (Mg)	0.25 méq%
Sodium (Na)	0.09 méq%
Potassium (K)	0.15 méq%
Cation exchange capacity	2.99 méq%

(Provenance Ndiaffate-2013) and *P. juliflora* (Provenance Ndiaffate-2013) were provided by the National Centre for Forestry Research (CNRF) of the Senegalese Institute of Agricultural Research (ISRA). *P. juliflora* was chosen as a reference of wood species for salt tolerance. Seeds scarification and germination were performed as described by Fall et al. (2008). Seeds (n=20) were germinated on Petri dishes containing water agar (0.9% w/v) with the different concentrations of sodium chloride (NaCl). Seven concentrations of NaCl were tested: 0, 86, 171, 257, 342, 428 and 514 mM (Fall et al., 2009). The experiment was repeated three times (20 seeds × 3 = 60 seeds) for each salt treatment. Seeds were considered as germinated when emerging radical was visible. The number of germinated seeds was counted daily for 10 days. The germination rate was expressed in mean final percent germination, calculated from cumulative germinated seeds on the final day of assessment to that of the total number of seeds in the sample at different salinity levels.

Growth and physiological responses of *S. senegal*, *V. seyal* and *P. juliflora* seedlings under saline conditions

Growth conditions and salt treatment

Seeds were germinated as described on water agar. Seedlings were then individually cultivated in plastic bags (25×12 cm) containing 1.3 kg of non-sterile soil (Table 1) collected from Sadioga (Centre of the groundnut basin of Senegal, 16° 23' 18 W, 14° 03' 53 N). The experiment was carried out in greenhouse conditions at the Laboratoire Commun de Microbiologie IRD/ISRA/UCAD (LCM) of Dakar (Senegal) in Bel-Air (14°44'N, 17°30'W) under natural sunlight (35°C day, 27°C night, with 14 h photoperiod). The relative humidity was about 75%. Accordingly to the results on the species germination rate and the level of salinity of our study site (EC = 0.414 to 34.3 mS/cm), four concentrations of NaCl (0, 86, 171, and 257 mM) were tested. A randomized experimental design was used with four treatments and 10 replicates per treatment. Salt stress treatment was performed one month after transplantation. Seedlings were gradually exposed to NaCl in order to minimize any salinity shock. NaCl concentrations were increased by 43 mM per day until reaching the required final concentration. The salinity of the leachate from representative pots was monitored regularly with a salinometer (Digit 100 ATC Salinity pocket refractometer, CETI, Optical Instruments, Belgium) to ascertain actual NaCl concentrations within the rooting medium.

Plants growth measurement

Three months after salt stress application, plants growth was evaluated by measuring plant height, collar diameter, shoot (leaves + stems) and root dry biomass.

Physiological traits measurements

Relative water content: Relative water content (RWC) estimation was done by incubating stem fragment (5 cm) in 15 ml distilled water for 24 h and calculated according to Yamasaki and Dillenburg (1999) after 96 h at 80°C in a stove.

Leaf water potential: Water potential is defined as the potential energy per unit mass of water with reference to pure water at zero potential (atmospheric pressure and 20°C), (Campbell, 1977). Leaf water potential (LWP) was measured using a Scholander pressure chamber (Scholander et al., 1965).

Total chlorophyll content: The total chlorophyll content was evaluated from 100 mg of fresh leaves according to Arnon (1949) method. The total chlorophyll content was calculated as follow: C = [20.2 (A645) + 8.02 (A663)] × V/M; where, V and M are the extraction volume (L) and weight (mg) of crushed leaves, respectively.

Proline content: Free proline content was determined by spectrophotometry from 100 mg leaf samples according to Monnevaux and Nemmar (1986). The proline concentration on a fresh-matter basis was obtained from a calibration graph prepared with a series of standard proline solutions.

Concentration of Na and K in roots and leaves: Concentrations of Na and K were determined by atomic absorption spectrophotometer after HNO_3-H_2O_2 digestion. Chloride was extracted by contact with boiling deionized water and colorimetric assay was done using the method of mercuric thiocyanate and ferric nitrate.

Salt tolerance index of *S.senegal, V. seyal* and *P. juliflora* at germination and growth in greenhouse conditions: Salt tolerance index (STI) was calculated as the ratio of the parameters salt stressed plants versus those of control plants (Cano et al., 1998).

$$STI\ (\%) = (MV \text{ at } C_x / MV \text{ at } C_0) \times 100$$

Where, MV is the measured variable, C_0 is the control, and C_x is a given concentration of salt.

Identification of a vacuolar antiport Na^+/H^+ (NHX1) in *S. senegal, V. seyal* and *P. juliflora*

Total RNA extraction

The seedlings of *S. senegal*, *V. seyal* and *P. juliflora* were grown on

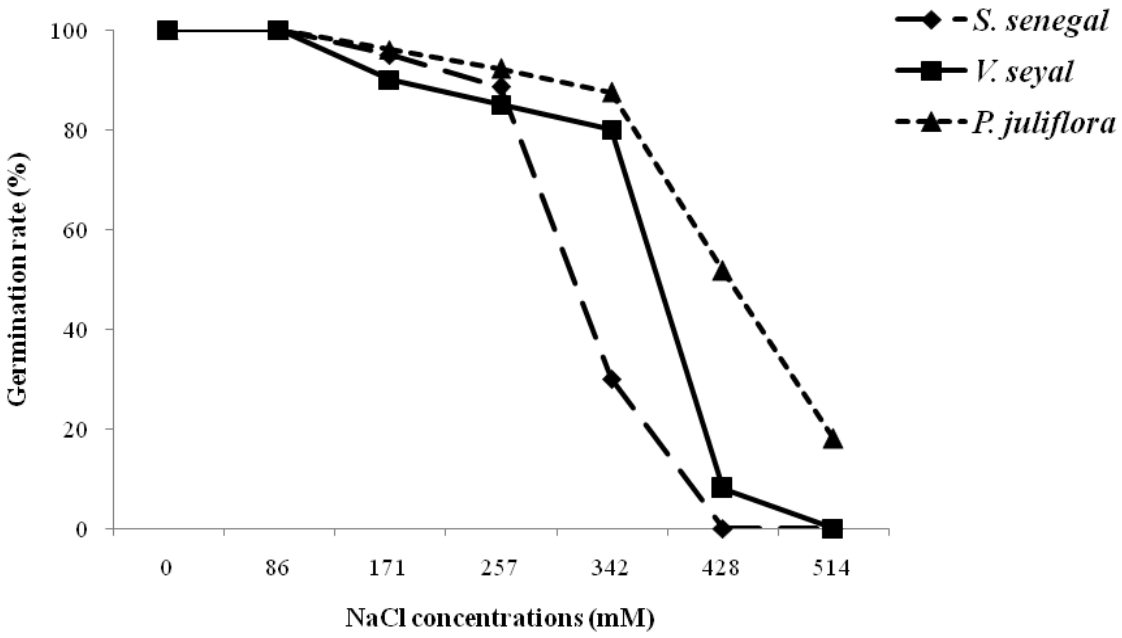

Figure 1. NaCl effect on final percent germination (%) of *S. senegal, V. seyal* and *P. juliflora*. The mean final percent germination was calculated from cumulative germinated seeds in water-agar (0.9%, w/v) after 10 days on the indicated NaCl concentrations (0, 85, 171, 256, 342, 428, and 514 mM) to that of the total number of seeds in the sample. Each value represented the mean of three replications (20 seeds × 3 = 60 seeds).

a mix of peat and vermiculite (2/1) during three weeks. Total RNA was extracted from young leaves using the RNeasy total RNA isolation kit (Qiagen). About 100 mg of fresh leaves were excised and ground using liquid nitrogen to fine powder. One milliliter of RTL buffer containing 10 mg of polyvinylpyrrolidone (PVPP) and 10 µl de Mercaptoethanol was added to the powder. The mix was incubated at 56°C for 2 min in a water bath. The lysate (650 µl) was transferred to a QIAshredder column and centrifuged at room temperature (25°C) at 18000 rpm for 2 min. The supernatant was collected in a fresh Eppendorf tube and 0.5 volumes of alcohol (96%) were added and mixed very well. The mixture was transferred on a spin column and centrifuged at 10 000 rpm for 45 s. Total RNA were washed with 700 µl of RW1 buffer by centrifugation at 10 000 rpm for 20 s and with 500 µl of RPE buffer at 10 000 rpm for 20 s. Total RNA was eluted with 30 µl of RNase-free water. To remove contaminating DNA, RNAs were treated with RNase-free DNase. The integrity of total RNA was estimated by a bioanalyzer before reverse transcription. One microgram of RNA was reverse-transcribed using Supercript II reverse transcriptase. The reverse transcription (RT) reactions were performed at 42°C for 50 min and using oligo-dT by following the instruction of the manufacturer.

NHX1 gene amplification

Since any specific primers were described in the literature to amplify NHX1 gene for the three species, degenerated primers were used. Thus, the complete sequences of NHX1 gene in several salt tolerant and non-tolerant species were searched in Genbank. After alignment of the obtained sequences, primers were designed in the highly conserved regions. Thus, one forward primer (NHX1F_5'-TTYAATGCHGGSTTTCARG-3') and 3 reverse primers (NHX1R1_5'-GABGTDGCATCATTHACAACWCC-3'; NHX1R2_5'-ACCTCDCGATCWGTNGART GC-3' and NHX1R3_5'-TGRGACRTVACAATMCCAC-3') were designed and theoretical sizes for three combinations were estimated. Two microliters of cDNAs were used as templates for PCR amplification with degenerated primers. Three couples of primers were tested (NHX1F/NHX1R1, NHX1F/NHX1R2 and NHX1F/NHX1R3). Samples were denatured for 3 min at 94°C and then run for 30 cycles of 30 s each at 94 and 50°C, 45 s at 72°C with a final extension of 7 min at 72°C. The PCR products were separated by 1% agarose gel electrophoresis (Figure 2).

Data statistical analysis

A one-way analysis of variance (ANOVA) analysis was performed for all data sets using XLSTAT™ for Windows statistical data analysis package (version 2009, Addinsoft, Paris, France). Student-Newman-Keuls´s post-hoc test was employed to determine if significant ($P \leq 0.05$) differences occurred in parameters measured between salinity treatments, and also the STI of species.

RESULTS

Effect of NaCl on *S. senegal, V. seyal* and *P. juliflora* seeds germination

The effect of NaCl on *S. senegal*, *V. seyal* and *P. juliflora* seeds germination, evaluated by the percentage of germinated seeds after 10 days, is as shown in Figure 1.

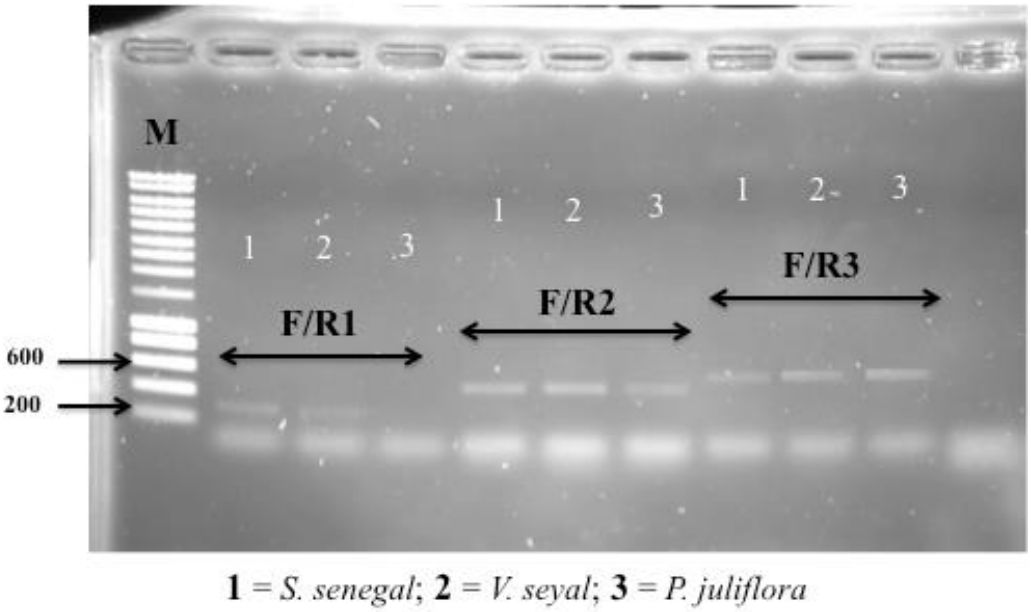

Figure 2. PCR profiles obtained after amplification of NHX1 gene with three couples of primers in leaves of *S. senegal, V. seyal* and *P. juliflora*. F = forward primer: NHX1F_5'-TTYAATGCHGGSTTTCARG-3'. R1 = Reverse primer 1: NHX1R1_5'-GABGTDGCATCATTHACAACWCC-3'. R2 = Reverse primer 2: NHX1R2_5'-ACCTCDCGATCWGTNGARTGC-3'. R3= Reverse primer 3: NHX1R3_5'-TGRGACRTVACAATMCCAC-3'. M = Molecular weight marker (200 pb).

The results show that for all species, the germination rate decreased with an increase of the NaCl concentration. Nevertheless, the negative effect of NaCl varied according to species. Below 257 mM, the germination rate of species was nearly equal to 100%. However, from this concentration, a difference in salinity tolerance was observed between species. For example, at 428 mM of NaCl, a reduction was observed in the germination rate of 100 (no germination), 92 and 48%, respectively for *S. senegal*, *V. seyal* and *P. juliflora*. At 514 mM NaCl, no germination was observed in *V. seyal* while a germination rate of 18% was observed for *P. juliflora*.

Effect of NaCl on *S. senegal, V. seyal* and *P. juliflora* seedlings growth

Salinity reduced height, shoot and root dry biomass of *S. senegal, V. seyal* and *P. juliflora* seedlings as shown in Table 2. However, the collar diameter seemed to increase with NaCl concentration. The low concentrations of NaCl (86 mM) seemed to increase the growth of *P. juliflora*. No significant negative effect of salinity was observed on seedlings height of the three species and on *P. juliflora* shoots dry biomass (SDB). In contrast, a significant negative effect was noted on SDB of *S. senegal* and *V. seyal* seedlings. For *S. senegal*, the average SDB was 0.95 g plant^{-1} at control and this value gradually decreased throughout the increasing salt concentrations, and reached to 0.28 g plant^{1} at 257 mM NaCl. In *V. seyal*, the SDB was 1.16 gplant^{-1} at 0 mM NaCl against 0.28 g plant^{-1} at 257 mM NaCl. At 257 mM, when compared to the control, the species had approximately the same percentages reduction in height with 24, 21 and 21%, respectively for *P. juliflora, S. senegal* and *V. seyal*. The percentages reduction in shoots dry weights were 76, 71 and 37%, respectively for *V. seyal, S. senegal*, and *P. juliflora* (Table 2). RDB at 257 mM NaCl decreased by 71, 57 and 51%, respectively for *P. juliflora, S. senegal* and *V. seyal* when compared with the seedlings control.

Physiological responses of *S. senegal, V. seyal* and *P. juliflora* seedlings under saline conditions

Chlorophyll, proline, leaves Cl^-, Na^+ and K^+ contents were not evaluated in *S. senegal* at 257 mM NaCl because seedlings had no leaves. Table 3 showed total chlorophyll and proline contents, RWC and LWP of *S. senegal, V. seyal* and *P. juliflora* seedlings. The chlorophyll content decreased in *S. senegal* and *V. seyal* with the increase in NaCl concentration. However, the chlorophyll content seemed to be increased in *P. juliflora*.

Table 2. Effect of NaCl concentrations (0, 86, 171 and 257 mM NaCl) on collar diameter, height, shoot and root biomass of *S. senegal, V. seyal* and *P. juliflora* seedlings grown during four months in greenhouse conditions on non-sterile sandy soil.

Species	NaCl (mM)	Col. dia. (mm plant^{-1})	Height (cm plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})
S. senegal	0	4.25±0.43^{a}	19.97±1.60^{a}	0.95±0.06^{b}	2.41±0.30^{b}
	86	4.42±0.29^{a}	18.57±1.29^{a}	0.92±0.03^{b}	2.03±0.14^{ab}
	171	4.89±0.45^{a}	16.43±0.75^{a}	0.75±0.24^{b}	1.69±0.48^{ab}
	257	4.98±0.13^{a}	15.83±3.33^{a}	0.28±0.03^{a}	1.04±0.64^{a}
V. seyal	0	2.60±0.36^{a}	26.90±5.91^{a}	1.16±0.03^{c}	1.62±0.04^{b}
	86	2.33±0.49^{a}	24.13±4.23^{a}	0.62±0.12^{b}	1.46±0.13^{b}
	171	2.34±0.52^{a}	20.47±4.68^{a}	0.55±0.15^{b}	0.94±0.11^{a}
	257	2.77±0.42^{a}	21.27±1.54^{a}	0.28±0.03^{a}	0.79±0.14^{a}
P. juliflora	0	2.18±0.17^{a}	25.87±1.40^{a}	0.67±0.07^{a}	0.49±0.12^{b}
	86	2.51±0.52^{a}	27.80±5.30^{a}	0.73±0.27^{a}	0.35±0.18^{ab}
	171	2.35±0.25^{a}	23.13±2.67^{a}	0.42±0.10^{a}	0.26±0.07^{ab}
	257	2.32±0.06^{a}	19.73±2.25^{a}	0.42±0.08^{a}	0.14±0.02^{a}

For each species, values within a column sharing same letter comparing NaCl treatments are not significantly different at *P<0.05* (Student-Newman-Keuls test). Each value represented the mean of three replications. Col. dia.: Collar diameter; SDW: shoot dry weight; RDW: root dry weight.

Table 3. Total chlorophyll (a + b) and proline contents, relative water content (RWC) and leaf water potential (LWP) of *S. senegal, V. seyal* and *P. juliflora* seedlings grown under greenhouse on non-sterile sandy soil and exposed during four months to four salinity levels (0, 86, 171 and 257 mM NaCl).

Species	NaCl (mM)	Chlorophyll a+b (mg g FW^{-1})	Proline (µg g FW^{-1})	RWC (%)	LWP (MPa)
S. senegal	0	2.17±0.13^{c}	1.08±0.02^{a}	83.1±1.8^{a}	-1.03±0.15^{b}
	86	1.41±0.05^{b}	1.09±0.01^{a}	84.1±2.4^{a}	-1.13±0.14^{b}
	171	0.75±0.03^{a}	2.11±0.14^{b}	89.1±6.2^{ab}	-1.24±0.10^{ab}
	257	nd	nd	90.8±3.6^{b}	-1.30±0.05^{a}
V. seyal	0	2.31±0.13^{c}	0.11±0.01^{a}	86.2±1.1^{a}	-1.17±0.04^{b}
	86	2.78±0.15d	0.12±0.04^{ab}	85.9±4.3^{a}	-1.20±0.10^{b}
	171	2.10±0.07^{b}	0.19±0.03^{b}	78.4±0.4^{a}	-1.18±0.08^{b}
	257	1.84±0.03^{a}	0.55±0.02^{c}	75.6±11.9^{a}	-1.43±0.10^{a}
P. juliflora	0	1.79±0.04^{a}	1.01±0.01^{a}	90.7±1.4^{a}	-0.67±0.10^{b}
	86	2.52±0.02^{c}	1.02±0.01^{a}	82.6±7.7^{a}	-1.00±0.11^{a}
	171	1.92±0.05^{b}	3.11±0.01^{b}	87.1±1.5^{a}	-1.15±0.12^{a}
	257	1.99±0.03^{b}	5.00±0.05^{c}	85.1±2.3^{a}	-1.17±0.05^{a}

For each species, values within a column sharing same letter comparing NaCl treatments are not significantly different at *P<0.05* (Student-Newman-Keuls test). Each value represented the mean of three replications. nd: Not determined (no leaves).

The reduction rate in chlorophyll content at 171 mM NaCl was 65 and 9%, respectively in *S. senegal* and *V. seyal* when compared with controls, while an increase of 11% was observed in *P. juliflora* at the same concentration of NaCl. In contrast to chlorophyll, free proline content increased with the NaCl concentration. This increase of proline content was more pronounced, respectively in *P. juliflora, S. senegal* and *V. seyal* for NaCl concentrations tested, with respectively 208, 95 and 73%. No significant difference (excepted *S. Senegal* at 257 mM) was observed in RWC of seedlings grown under salinity stress compared to those grown in non-saline soil.

Table 4. Mineral elements accumulation (g kg^{-1} dry weight) in roots and leaves of *S. senegal, V. seyal* and *P. juliflora* seedlings grown under greenhouse on non-sterile sandy soil and exposed during four months to four salinity levels (0, 86, 171 and 257 mM NaCl).

Species	NaCl (mM)	Cl^-		Na^+		K^+		K^+/Na^+	
		Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
S. senegal	0	$^{*A}11.0\pm3.1^{a**}$	$^{A}12.4\pm2.5^{a}$	$^{A}1.6\pm0.7^{a}$	$^{A}0.4\pm0.2^{a}$	$^{A}9.3\pm2.8^{a}$	$^{A}27.4\pm1.9^{b}$	$^{D}5.8\pm1.7^{a}$	$^{C}68.5\pm4.6^{b}$
	86	$^{AB}17.0\pm3.6^{a}$	$^{B}26.2\pm2.8^{b}$	$^{B}4.2\pm1.4^{a}$	$^{B}6.9\pm1.1^{b}$	$^{A}7.7\pm1.6^{a}$	$^{A}26.9\pm2.8^{b}$	$^{C}1.9\pm0.2^{a}$	$^{B}3.9\pm0.2^{b}$
	171	$^{B}18.0\pm1.5^{a}$	$^{C}32.7\pm1.1^{b}$	$^{BC}7.0\pm1.8^{a}$	$^{C}12.6\pm3.3^{b}$	$^{A}7.2\pm0.7^{a}$	$^{A}24.3\pm3.5^{b}$	$^{B}1.0\pm0.1^{a}$	$^{A}1.9\pm0.1^{b}$
	257	$^{B}22.2\pm3.6$	nd	$^{C}11.2\pm4.4$	nd	$^{A}7.1\pm0.2$	nd	$^{A}0.6\pm0.12$	nd
V. seyal	0	$^{A}14.6\pm0.6^{b}$	$^{A}8.8\pm1.2^{a}$	$^{A}5.8\pm0.9^{b}$	$^{A}1.4\pm1.0^{a}$	$^{A}11.9\pm3.2^{a}$	$^{C}24.5\pm2.5^{b}$	$^{B}2.1\pm1.0^{a}$	$^{C}17.5\pm2.9^{b}$
	86	$^{B}29.1\pm4.9^{a}$	$^{B}25.8\pm4.1^{a}$	$^{A}7.1\pm0.7^{a}$	$^{B}12.8\pm1.5^{b}$	$^{A}10.0\pm1.3^{a}$	$^{A}17.2\pm2.6^{b}$	$^{A}1.4\pm0.4^{a}$	$^{B}1.3\pm0.1^{a}$
	171	$^{B}24.9\pm4.2^{a}$	$^{B}33.3\pm2.5^{b}$	$^{A}7.3\pm1.5^{a}$	$^{B}16.7\pm2.2^{b}$	$^{A}9.5\pm1.9^{a}$	$^{A}16.7\pm2.5^{b}$	$^{A}1.3\pm0.2^{a}$	$^{AB}1.0\pm0.2^{a}$
	257	$^{B}24.2\pm2.2^{a}$	$^{B}32.2\pm3.0^{b}$	$^{A}8.2\pm2.8^{a}$	$^{C}23.4\pm0.9^{b}$	$^{A}8.7\pm2.3^{a}$	$^{A}15.9\pm1.1^{b}$	$^{A}1.1\pm0.1^{b}$	$^{A}0.7\pm0.2^{a}$
P. juliflora	0	$^{A}14.6\pm2.6^{a}$	$^{A}17.3\pm1.9^{a}$	$^{A}4.1\pm0.1^{b}$	$^{A}2.1\pm1.5^{a}$	$^{A}14.3\pm0.3^{a}$	$^{A}28.9\pm2.4^{b}$	$^{C}3.5\pm0.1^{a}$	$^{C}13.8\pm1.3^{b}$
	86	$^{B}29.1\pm4.9^{b}$	$^{B}21.1\pm1.7^{a}$	$^{B}15.2\pm 2.9^{b}$	$^{B}9.7\pm1.0^{a}$	$^{A}11.6\pm0.6^{a}$	$^{B}17.7\pm1.6^{b}$	$^{B}0.8\pm0.2^{a}$	$^{B}1.8\pm0.3^{b}$
	171	$^{C}36.8\pm2.6^{b}$	$^{AB}18.3\pm2.4^{a}$	$^{C}20.0\pm1.9^{b}$	$^{B}9.9\pm1.2^{a}$	$^{A}10.2\pm1.6^{a}$	$^{B}17.5\pm4.1^{b}$	$^{AB}0.5\pm0.1^{a}$	$^{A}1.8\pm0.25^{b}$
	257	$^{C}40.9\pm2.1^{b}$	$^{AB}18.9\pm0.7^{a}$	$^{C}21.6\pm4.2^{b}$	$^{B}12.0\pm1.1^{a}$	$^{A}9.4\pm1.6^{a}$	$^{B}17.2\pm0.6^{b}$	$^{A}0.4\pm0.1^{a}$	$^{A}1.4\pm0.1^{b}$

*****For each species, values within a column sharing same upper case letter comparing NaCl treatments are not significantly different at *P<0.05* (Student-Newman-Keuls test). ******For each element, values within a line sharing same lower case letter comparing the repartition of element between roots and leaves are not significantly different at *P<0.05* (Student-Newman-Keuls test). Each value represented the mean of three replications after pooling seedlings. nd: Not determined (no leaves).

However, RWC seemed to increase in *S. senegal* and decrease in *V. seyal* and *P. juliflora* seedlings when increasing NaCl concentration. LWP was more negative under salinity stress for all species. Significant difference was noted between controlled and stressed plants for all species (Table 3). At 171 mM NaCl, LWP was negatively increased by 75, 26 and 22%, respectively in *P. juliflora, S. senegal* and *V. seyal.* Our results indicated that Cl^- and Na^+ content in leaves and roots increased with salinity (Table 4). The accumulation of Cl^- and Na^+ was higher in leaves than roots in *S. senegal* and *V. seyal*, while it is higher in roots than leaves in *P. juliflora*. However, the accumulation of K^+ was higher in leaves than roots for all species (Table 4). At 171 mM NaCl, leaf Cl^- content was increased by 6, 164 and 278%, respectively in *P. juliflora, S. senegal* and *V. seyal*. However, leaf Na^+ content was increased by 471, 1093 and 3050%, respectively in *P. juliflora, V. seyal* and *S. senegal.* The K^+ content and the K^+/Na^+ ratio were reduced in roots and leaves of *S. senegal, V. seyal* and *P. juliflora*. The K^+ content was significant higher in leaves than roots for all species.

Salt tolerance index of *S. senegal, V. seyal* and *P. juliflora* at germination and growth

Results show that salt tolerance index (STI) decreased with increasing NaCl concentration at germination and growth (Table 5). For germination, no significant difference was noted between STI of species for NaCl concentrations less than or equal to 257 mM NaCl with 89, 85 and 92%, respectively in *S. senegal, V. seyal* and *P. juliflora*. Nevertheless, when the NaCl concentration increased, a difference in STI occurred. For *S. senegal*, STI was 30% at 342 mM and became 0% from 428 mM of NaCl, while *V. seyal* and *P. juliflora* had an STI of 80 and 88%, respectively at 342 mM. At 514 mM, the STI of *V. seyal* became 0%; however, *P. juliflora* had a STI of 18% (Table 5). For growth (shoot dry biomass), STI of *S. senegal* was higher than *V. seyal* for all NaCl concentrations. At 86 mM NaCl, no significant difference was observed between *S. senegal* and *P. juliflora* which had STI significantly higher than *V. seyal*. At 257 mM NaCl, STI was 24, 29 and 63%, respectively for *V. seyal, S.*

Table 5. Salt tolerance index (%) of *S. senegal, V. seyal* and *P. juliflora* at germination and growth after four months under greenhouse.

Parameter	Species	NaCl concentrations (mM)						
		0	86	171	257	342	428	514
Germination	*S. senegal*	100±0^{a}	100±0^{a}	95±5^{a}	89±4^{a}	30±4^{a}	0±0^{a}	0±0^{a}
	V. seyal	100±0^{a}	100±0^{a}	90±3^{a}	85±3^{a}	80±5^{b}	8±1^{b}	0±0^{a}
	P. juliflora	100±0^{a}	100±0^{a}	96±4^{a}	92±4^{a}	88±4^{b}	52±3^{c}	18±2^{b}
Growth (shoot biomass)	*S. senegal*	100±0^{a}	97±4^{b}	79±5^{c}	29±4^{a}	nd	nd	nd
	V. seyal	100±0^{a}	53±3^{a}	47±5^{a}	24±3^{a}	nd	nd	nd
	P. juliflora	100±0^{a}	109±9^{b}	63±6^{b}	63±5^{b}	nd	nd	nd

For each column, values with same letter comparing the salt tolerance index of species are not significantly different at $P<0.05$ (Student-Newman-Keuls test). nd: Not determined.

senegal and *P. juliflora*.

Identification of NHX1 gene in *S. senegal, V. seyal* and *P. juliflora*

Results showed that the three primers generated amplicons for all species except for *P. juliflora* with NHX1F/NHX1R1 couple. NHX1F/NHX1R1, NHX1F/ NHX1R2 and NHX1F/NHX1R couples produced good amplicons with the expected size with an approximately size of 300, 500 and 600 pb, respectively.

Any difference in terms of amplicon size was obtained among species for the same primer.

DISCUSSION

Our results show that for all species, the percentage of germination decreased as the degree of salinity increased. Reduction in germination by increasing salinity levels has been described by numerous studies (El-Tayeb, 2005; Abari et al., 2011; Tsegay and Gebreslassie, 2014; Sharma and Vimala, 2016). High concentration of NaCl in the salt solution increases its osmotic potential and the germination rate reduction could be attributed to the osmotic effect of NaCl, which limits the seed hydration (Tobe et al., 2000; El-Keblawy and Al-Rawai, 2005). It could also be due to the toxic effect of NaCl on seed embryo or endosperm cell membranes (Bliss et al., 1986; Khajeh-Hosseini et al., 2003). The inhibition of germination can be due to high absorption of Na and Cl ions during seed germination (Taiz and Zeiger, 2002). On the basis of their salt tolerance at germination, the species can be arranged as follows: *P. juliflora* > *V. seyal* > *S. senegal*.

It was found that the seedlings growth (height, shoot and root dry weight) was affected by salinity and that the salt effect depends on salinity level and species. Results for height, shoot and root dry weight in response to increasing salinity level suggested that there was maximum reduction in shoot dry weight for *V. seyal* and *S. Senegal*, while the highest reduction of root dry weight was observed in *P. juliflora*. According to Alam et al. (2004), it is possible that the decrease in plant growth in saline condition was due to several reasons. One possibility is that salinity reduced photosynthesis, which in turn limited the supply of carbohydrates needed for growth (da Silva et al., 2011). A second possibility is that salinity reduced shoot and roots growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium. Third, a disturbance in mineral supply, either an excess or deficiency, induced by changes in concentrations of specific ions in the growth medium, might have affected directly the growth (Lazof and Bernstein, 1998; Zhu, 2002). The decrease in shoot biomass and the yellow leaves might be due to disturbance in carbohydrates allocation in shoots. Our results for reduction of seedlings growth of all species with increase in NaCl concentration are in conformity with the finding of several authors (Abari et al., 2011; Saini et al., 2012; Sharma and Vimala, 2016). The low concentration of NaCl (86 mM) seemed to increase *P. juliflora* seedlings growth. This result corroborated those obtained by Viégas et al. (2004) on *P. juliflora* seedlings in hydroponic condition. The absence and the falling leaves at 257 mM NaCl, observed respectively in *S. senegal* and *V. seyal*, reduced the photosynthetic area which result in the reduction of plant growth and yield. On the basis of tolerance at growth (shoot biomass production), the species can be arranged as follows: *P. juliflora* > *S. senegal* > *V. seyal*. This ranking was confirmed by STI, which were 63, 29 and 24%, respectively in *P. juliflora, S. senegal* and *V. seyal* at 257 mM NaCl. The total chlorophyll (a + b) content decreased significantly with increasing NaCl concentration for all species. The decrease in chlorophyll content under salt stress is a commonly reported phenomenon. It is related

to its adverse effects on membrane stability (Ashraf and Bhatti, 2000), the weakening of protein-pigment-lipid complex (Ayala-Astorga and Alcaraz-Meléndez, 2010) and the increasing chlorophyllase (EC: 3.1.1.14) activity (Lakhdar et al., 2008). Also, Santos (2004) pointed out that the induced decrease in chlorophyll content in severely NaCl stressed leaves is mainly due to a decrease of 5-aminolinolic acid (ALA) synthesis, and therefore to limitations of chlorophyll synthesis. These observations corroborated with the results obtained by several authors (Hardikar and Pandey, 2008; Turan et al., 2009; Molazem et al., 2010; Heidari, 2012; Taibi et al., 2016). In contrast, the increase in NaCl concentration seemed to increase leaf total chlorophyll of *P. juliflora* suggesting that it was more tolerant in saline medium than *S. senegal* and *V. seyal* whose chlorophyll content decreased.

Free proline content increased with NaCl concentration for all species. Salt tolerant plants are distinguished by their capacity to accumulate high concentrations of compatible osmotica such as glycine betaine, proline, in cytoplasm to balance the osmotic pressure of ions in the vacuoles. Increasing leaf proline content under salinity stress might be caused by induction or activation of proline synthesis from glutamate or decrease in its utilization in protein synthesis or enhancement in protein turnover. The high accumulation of proline indicated that *S. senegal* (95%) and *V. seyal* (73%) seemed to have the capacity to tolerate salinity as *P. juliflora* (208%). Similar results were observed in many plant species such as *Acacia auriculiformis* (Diouf et al., 2005), date palm (Sané et al., 2005), rice (Shereen et al., 2007), maize (Cha-um and Kirdmanee, 2009), walnut (Akça and Samsunlu, 2012), and potato (Jaarsma et al., 2013).

Relative water content (RWC) and leaf water potential (LWP) are the basic parameters of plant water status. Water status is the main factor affecting the plants growth and development. Even if no significant difference was observed, the decrease of RWC in *V. seyal* and *P. juliflora* seedlings grown under salinity stress, compared to those grown in non-saline soil, indicated that salinity resulted in dehydration at cellular level and dehydration symptoms were greater in higher NaCl concentration treatment because of the increase in cellular water loss. This is a common reaction to salinity similar to those reported for other species such as pepper plants (Navarro et al., 2003), *Avicennia germinans* (L.) (Suárez and Medina, 2008). Therefore, plants should have osmotic adjustment inside the cell, since turgor maintenance is required for cell expansion and the biochemical, physiological and developmental processes (Flowers, 2004). Even so, the RWC seemed to increase in *S. senegal* with NaCl concentrations. This exception can be explained by the reduction and/or the absence of leaves, which reduced the transpiration area along the water retention in cells. Water potential in *S. senegal, V. seyal* and *P. juliflora* became increasingly more negative with the corresponding increase in media salinity, indicating that these species osmotically adjust in response to increases in salinity. Water potential was more negatively increased by 75, 26 and 22%, respectively in *P. juliflora, S. senegal* and *V. seyal*, suggesting that *P. juliflora* adjust better its osmotic pressure followed by *S. senegal* and *V. seyal*.

According to Greenway and Munns (1980), NaCl, the predominant form of salt in most saline soils, enhances Na^+ and Cl^- contents and consequently affects the uptake of other mineral elements. Our results showed that Cl^- and Na^+ accumulation increased in roots and leaves when increasing NaCl concentration while K^+ accumulation and K^+/Na^+ ratio decreased, but more Na^+ was taken up than Cl^-, indicating that *S. senegal*, *V. seyal* and *P. juliflora* are an ion accumulators. The strong Na^+ accumulation in leaves could be responsible for their loss as observed in *S. senegal* and *V. seyal*. Previous studies have shown that salinity increases Na^+ and Cl^- and decreased K^+ and K^+/Na^+ in plant leaves (Saghir et al., 2002; Hosseini and Thengane, 2007; Taffouo et al., 2010; Silini et al., 2016) found. This implies a competition between Na^+ and K^+ absorption in plants, resulting in a Na^+/K^+ antagonism (Mori et al., 2011). The reduction in K^+ uptake caused by Na^+ is likely to be the result of the competitive intracellular influx of both ions (Tripathi and Müller, 2015). It is well established that many K transport systems have significant affinity for Na^+ (Rodriguez-Navarro and Rubio, 2006).

Our preliminary results on molecular studies show the presence of salt tolerant gene NHX1 in *S. senegal, V. seyal* and *P. juliflora*. To our knowledge, this is the first study of the tonoplast associated Na^+/H^+ antiporter in these species. This result indicated that these species have the ability to sequester the Na^+ in the vacuole which in turn, will enhance their adaptation to salinity. The overexpression of NHX1 in saline conditions will increase their tolerance to salinity as shown by several studies (Gouiaa et al., 2012; Baltierra et al., 2013; Hasegawa, 2013; Panahi et al., 2013; Hu and Wu, 2014; Chen et al., 2015).

Conclusion

The results show that salt stress decreased germination rate, growth, total chlorophylls content, relative water content, leaf water potential, K^+ accumulation and K^+/Na^+ ratio in *S. senegal*, *V. seyal* and *P. juliflora*. Salt stress increased proline, Cl^- and Na^+ accumulation in all species. Nevertheless, results showed that species maintained a good germination rate and an overall growth at high level of salinity. In summary, the results show that *S. senegal, V. seyal* and *P. juliflora* accumulated Na^+ to achieve a negative water potential gradient and also accumulate

proline as an osmoprotectant or to achieve osmotic balance in the cytoplasm. According to our results, *S. senegal* and *V. seyal* should be considered as species that might be used to phytoremediate degraded saline lands as *P. juliflora*. However, molecular studies will be continued to evaluate the expression salt tolerant gene (NHX1) in saline conditions and field trials will be conducted to confirm their ability.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Abari A, Nasr MH, Hojjati M, Bayat D (2011). Salt effects on seed germination and seedling emergence of two *Acacia* species. Afr. J. Plant Sci. 5:52-56.

Agarwal S, Pandey V (2004). Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol. Plant 48:555-560.

Akça Y, Samsunlu E (2012). The effect of salt stress on growth, chlorophyll content, proline and nutrient accumulation, and K/Na ratio in walnut. Pak. J. Bot. 44:1513-1520.

Alam MZ, Stuchbury T, Naylor REL, Rashid MA (2004). Effect of salinity on growth of some modern rice cultivars. J. Agron. 3:1-10.

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999). Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. Sci. 285:1256-1258.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta Vulgaris*. Plant Physiol. 24:1-15.

Ashraf MY, Bhatti AS (2000). Effect of salinity on growth and chlorophyll content of Rice. Pak. J. Sci. Ind. Res. 43:130-131.

Ayala-Astorga GI, Alcaraz-Meléndez L (2010). Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold & Zuccarini) and *Paulownia fortunei* (Seemann & Hemsley) grown in vitro. Elect. J. Biotechnol. 13:5-15.

Baltierra F, Castillo M, Gamboa MC, Rothhammer M, Krauskopf E (2013). Molecular characterization of a novel Na^+/H^+ antiporter cDNA from *Eucalyptus* globules. Biochem. Biophys. Res. Commun. 430:535-540.

Brini F, Hanin M, Mezghani I, Berkowitz GA, Masmoudi K (2007). Overexpression of wheat Na^+/H^+ antiporter *TNHX1* and H^+-pyrophosphatase *TVP1* improve salt and drought-stress tolerance in *Arabidopsis thaliana* plants. J. Exp. Bot. 58:301-308.

Campbell GS (1977). The relationship between vapor pressure, relative humidity, and wet and dry bulb temperature at sea level. An Introduction to Enviornmental Biophysics, Springer-Verlag.

Cano EA, Perez-Alfocea F, Moreno V, Caro M, Bolarin MC (1998). Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. Plant Cell Tissue Org. Cult. 53:19-26.

Cha-um S, Kirdmanee C (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. Pak. J. Bot. 41:87-98.

Chen X, Bao H, Guo J, Jia W, Li Y (2015). Overexpression of SeNHX1 improves both salt tolerance and disease resistance in tobacco. Plant Signal. Behav. 10(4): e993240.

da Silva EN, Ribeiro RV, Ferreira-Silva SL, Viégas RA, Silveira JAG (2011). Salt stress induced damages on the photosynthesis of physicnut young plants. Sci. Agric. 68(1):62-68.

Debez A, Karim Ben H, Grignon C, Abdelly C (2004). Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. Plant Soil 262:179-189.

Diedhiou S, Dossa EL, Badiane AN, Diedhiou I, Sène M, Dick RP (2009). Decomposition and spatial microbial heterogeneity associated with native shrubs in soils of agroecosystems in semi-arid Senegal. Pedobiologia 52:273-286.

Diouf D, Duponnois R, Ba AT, Neyra M, Lesueur D (2005). Symbiosis of *Acacia auriculiformis* and *Acacia mangium* with mycorrhizal fungi and *Bradyrhizobium* spp. Improves salt tolerance in greenhouse conditions. Funct. Plant Biol. 32:1143-1152.

Dossa EL, Diedhiou S, Compton J, Assigbetse K, Dick RP (2010). Spatial patterns of P fractions and chemical properties in soils of two native shrub communities in Senegal. Plant Soil 327:185-198.

El Keblawy A, Al-Rawai A (2005). Effect of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. J. Arid Environ. 61:555-565.

El-Tayeb MA (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul. 45:215-224.

Fall D, Diouf D, Ourarhi M, Faye A, Abdelmoumen H, Neyra M, Sylla SN, Missbah El Idrissi M (2008). Phenotypic and genotypic characteristics of root-nodulating bacteria isolated from *Acacia senegal* (L.) Willd. in the dryland part of Senegal. Lett. Appl. Microbiol. 47:85-97.

FAO-LADA (2009). Field Manual for Local Level Land Degradation Assessment in Drylands. LADA-L Part 1: Methodological Approach, Planning and Analysis, Rome: FAO. 76p.

Flowers TJ (2004). Improving crop salt tolerance. J. Exp. Bot. 55:307-319.

Flower TJ (2004). Improving crop salt tolerance. J. Exp. Bot. 55:307-319.

Gaxiola RA, Rao R, Sherman A, Grisafi P, Alper SL, Fink GR (1999). The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. Proc. Natl. Acad. Sci. USA. 96(4):1480-1485.

Ghoulam C, Foursy A, Fares K (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ. Exp. Bot. 47:39-50.

Gouiaa S, Khoudi H, Leidi EO., Pardo JM, Masmoudi K (2012). Expression of wheat Na^+/H^+ antiporter TNHXS1 and H^+-pyrophosphatase TVP1 genes in tobacco from a bicistronic transcriptional unit improves salt tolerance. Plant Mol. Biol. 79:137-155.

Greenway H, Munns R (1980). Mechanisms of salt tolerance in non-halophytes. Ann. Rev. Plant Physiol. 31:149-190.

Hasegawa PM (2013). Sodium (Na^+) homeostasis and salt tolerance of plants. Environ. Exp. Bot. 92:19-31.

Hardikar SA, Pandey AN (2008). Growth, water status and nutrient accumulation of seedlings of *Acacia senegal* (L.) Willd. in response to soil salinity. Anal Biol. 30:17-28.

Hosseini G, Thengane RJ (2007). Salinity tolerance in cotton (*Gossypium hirsutum* L.) genotypes. Int. J. Bot. 3:48-55.

Hu LH, Wu YX (2014). Effect of salt stress on the expression of NHX-type Na^+/H^+ antiporters in *Populus euphratica* and *P. pruinosa* calli. Sci. Cold Arid Reg. 6(1):66-72.

Jaarsma R, de Vries RSM, de Boer AH (2013). Effect of salt stress on growth, Na^+ accumulation and proline metabolism in potato (*Solanum*

tuberosum) cultivars. PLoS ONE 8(3):e60183.
Khajeh-Hosseini M, Powell AA, Bingham IJ (2003). The interaction between salinity stress and seed vigour during germination of soybean seeds. Seed Sci. Technol. 31:715-725.
Kigel J (1995). Seed germination in arid and semi-arid regions. In: Kigel J, Galili G. (edss), Seed Development and Germination, 645-699. Marcel Dekker Inc. New York.
Kolb A, Barsch K (2010). Environmental factors and seed abundance influence seedling emergence of a perennial forest herb," Acta Oecol. 36(5):507-513.
Lakhdar A, Hafsi C, Rabhi M, Debez A, Montemurro F, Abdelly C, Jedidi N, Ouerghi Z (2008). Application of municipal solid waste compost reduces the negative effects of saline water in (*Hordeum maritimum* L.). Bioresour. Technol. 99:7160-7167.
Lazof DB, Bernstein N (1998). The NaCl-induced inhibition of shoot growth: The case for disturbed nutrition with special consideration of calcium nutrition. Adv. Bot. Res. 29:113-189.
Liu Y, Du H, Wang K (2011). Differential photosynthetic responses to salinity stress between two perennial grasss pecies contrasting in salinity tolerance. Hortsci. 46(2):311-316.
Misra A, Sahu AN, Misra M, Singh P, Meera I, Das N, Kar M, Sahu P (1997). Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biol. Plant 39:257-262.
Molazem D, Qurbanov EM, Dunyamaliyev SA (2010). Role of proline, Na and chlorophyll content in salt tolerance of corn (*Zea mays* L.). American-Eurasian J. Agric. Environ. Sci. 9:319-324.
Monnevaux P, Nemmar M (1986). Contribution à l'étude de la résistance à la sécheresse chez le blé tendre *Triticum aestivunt* L. et chez le blé dur *Triticum durum* Desf.: étude de l'accumulation de la proline au cours du cycle de développement. Agron. 6:583-590.
Mori S, Suzuki K, Oda R, Higuchi K, Maeda Y, Yoshiba M, Tadano T (2011). Characteristics of Na^+ and K^+ absorption in *Suaeda salsa* (L.) Pall. Soil Sci. Plant Nutr. 57(3):377-386.
Navarro JM, Garrido C, Martínez V, Carvajal M (2003). Water relations and xylem transport of nutrients in pepper plants grown under two different salts stress regimes. Plant Growth Regul.41:237-245.
Panahi B, Ahmadi FS, Mehrjerdi MZ, Moshtaghi N (2013). Molecular cloning and the expression of the Na^+/H^+antiporter in the monocot halophyte *Leptochloa fusca* (L.) Kunth. NJAS – Wag. J. Life Sci. 64-65: 87-93.
Pardo JM, Cubero B, Leidi EO, Quintero FJ (2006). Alkali cation exchangers: roles in cellular homeostasis and stress tolerance. J. Exp. Bot. 57:1181-1199.
Reyes-Reyes GL, Baron-Ocampo I, Cuali-Alvarez JT, Frias-Hernandez V, Olalde Portugal V, Varela Fregoso L, Dendooven LC (2002). N dynamics in soil from the central highlands of Mexico as affected by mesquite (*Prosopis* spp.) and huizache (*Acacia tortuoso*): a laboratory investigation. Appl. Soil Ecol. 19:27-34.
Rodriguez-Navarro A, Rubio F (2006). High-affinity potassium and sodium transport systems in plants. J. Exp. Bot. 57(5):1149-1160.
Rout NP, Shaw BP (2001). Salt tolerance in aquatic macrophytes: Ionic relation and interaction. Biol. Plant 55:91-95.
Saghir A, Khan NO, Igbal MZ, Hussain A, Hassan M (2002). Salt tolerance of cotton (*Gossypium hirsutum* L.). Asian J. Plant Sci. 1:715-719.
Sané D, Ould Kneyta M, Diaga D, Diouf D, Badiane FA, Sagna M, Borgel A (2005). Growth and development of date palm (*Phœnix dactylifera* L.) seedlings under drought and salinity stresses. Afr. J. Biotechnol. 4:968-972.
Santos CV (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci. Hortic. 103:93-99.
Sharma S, Vimala Y (2016). Effect of salt stress on germination and growth of *T. foenumgraecum* seedlings. Internat. J. Adv. Res. 4:40-45.
Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965). Sap pressure in vascular plants. Sci. 148:339-346.
Shereen A, Ansari RU, Yasmin S, Raza S, Mumtaz S, Khan MA, Mujtaba SM (2007). Physiological responses of rice (*Oryza sativa* L.) to saline stress. Pak. J. Bot. 39:2527-2534.
Siegel SM, Siegel BZ, Massey J, Lahne P, Chen J (1980). Growth of corn in saline water. Physiol. Plant 50:71-73.
Singh G (2009). Salinity related desertification and management strategies: Indian experience. Land Degrade Dev. 20:367-385.
Silini A, Cherif-Silini H, Yahiaoui B (2016). Growing varieties durum wheat (*Triticum durum*) in response to the effect of osmolytes and inoculation by *Azotobacter chroococcum* under salt stress. Afr. J. Microbiol. Res. 10(12):387-399.
Taffouo VD,Wamba OF, Youmbi E, Nono GV, Akoa A (2010). Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranea* (L.) Verdc.) landraces grown under saline conditions. Int. J. Bot. 6:53-58.
Taïbi K, Taïbi F, Abderrahim LA, Ennajah A, Belkhodja M, Mulet JM (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. South Afr. J. Bot.105:306-312.
Taiz L, Zeiger E (2003). Plant Physiology. 3rd Edn. Sunderland, Sinauer Associates, Inc. 690 p.
Tian L, Huang C, Yu R, Liang R, Li Z, Zhang L, Wang Y, Zhang X and Wu Z (2006). Overexpression AtNHX1 confers salt-tolerance of transgenict all fescue. Afr. J. Biotechnol. 5(11):1041-1044.
Tobe K, Xiaoming L, Omasa KI (2000). Seed Germination and Radicle Growth of a Halophyte, *Kalidium caspicum* (*Chenopodiaceae*). Ann. Bot. 85:391-396.
Tsegay BA, Gebreslassie B (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. Afr. J. Plant Sci. 8(5):225-231.
Tripathi BN, Müller M (2015). Stress responses in plants: Mechanisms of toxicity and tolerance. Springer International Publishing Switzerland.
Turan MA, Elkarim AHA, Taban N, Taban S (2009). Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. Afr. J. Agric. Res. 4:893-897.
Upadhyay RK, Panda SK (2005). Salt tolerance of two aquatic macrophytes *Pistia stratiotes* and *Salvinia molesta*. Biol. Plant 49:157-159.
Vicente O, Boscaiu M, Naranjo MA, Estrelles E, Belles JM, Soriana P(2004). Responses to salt stress in the halophyte *Plantago crassifolia* (*Plantaginaceae*). J. Arid Environ. 58:463-481.
Viégas RA, Fausto MJMF, Silveira JAG, Queiroz JEQ (2004). Growth and total N content of *Prosopis juliflora* (SW) D. C. are stimulated by mild NaCl level. Braz. J. Plant Physiol. 16:65-68.
Yamasaki S, Dillenburg LR (1999). Measurements of leaf relative water content in *Araucaria angustifolia*. R. Bras. Fisiol. Veg. 11:69-75.
Zahran HH (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev. 63:968-989.
Zhu JK (2002). Salt and drought stress signal transduction in plants. Ann. Rev. Plant Biol. 53:247-273.