African Journal of Biotechnology Vol. 10(31), pp. 5820-5830, 29 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2351
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Random amplified polymorphic DNA (RAPD) marker associated with salt tolerance during seeds germination and growth of selected *Acacia senegal* provenances

Hussien M. Daffalla[1], Rami S. Habeballa[1]*, Elsadig A. Elhadi[2] and Mutasim M. Khalafalla[1]

[1]Commission For Biotechnology and genetic Engineering, Ministry of Science and Technology, Mohammed Najeeb Street, Khartoum, P. O. Box 2404, Sudan.
[2]Desertification Research Institute, National Centre for Research, P.O. Box 2404, Khartoum, Sudan.

Accepted 2 May, 2011

Seed germination and seedling growth of 20 provenances of *Acacia senegal* were evaluated under salinity conditions. For germination study, seeds irrigated with NaCl in five concentrations had electrical conductivities (EC) of 0 (only distilled water), 2, 4, 6 and 10 dSm^{-1}. Final germination percentage (FG) and germination rate index (GRI) were recorded during 14 days. The result showed significant effect of salinity and provenance on FG and GRI. Seed germination was significantly reduced in all provenances with the increase in NaCl concentrations. Provenance Al Feel (FE, clay+ high rainfall) from Eastern Sudan was more tolerant than the other provenances at seed emergence stage. Greenhouse experiment examined the growth response of 8 provenances *A. senegal* chosen as the most tolerant provenances at seeds germination study. Potted seedlings aged 3 weeks were irrigated with 0, 4, 6 and 10 dSm^{-1}. Seedling height, root length, shoot dry weight, root dry weight and root/shoot ratio were measured for 4 weeks. Provenances showed a large variability in growth characteristics. No correlation between seed sources (soil type and rainfall) and growth response was found. However, provenance MH shows tolerance to salt stress at seedling stage. The genetic polymorphism between the provenances was detected by RAPD analysis. Forty four (44) out of 51 bands detected were polymorphic for the provenances of *A. senegal* and the dissimilarity indices between the studied provenances were less than 39%.

Key words: *Acacia Senegal*, provenance variation, random amplified polymorphic DNA (RAPD) marker, salt tolerance, seed germination, seedling growth.

INTRODUCTION

Salinity is the major factor limiting plants growth, widely spread and has more severe impact in arid and semi-arid environments (Manchanda and Garg, 2008; Meloni et al., 2008). Water and salt stresses are correlated, as excess of soluble salts reduces soil water and apoplastic water potential obstructing water absorption by seeds (Tobe et al., 2000; El-Keblawy and Al-Rawai, 2005). Habitually, there is a critical level of hydration that seeds must reach before germination can proceed (Bakke et al., 2006). Low external water potential due to high levels of salts, especially sodium chloride (NaCl) can inhibit enzymatic activity of seed and delay radical emergence and seeds appear spotty (Dell'aquilla, 1992; Rhoades et al., 1992). On other hand, salts ions can affect seeds germination through toxity of their embryos (Khajeh-Hosseini et al., 2003). Salinity has different effects on both seed germination and seedling growth (Ramoliya and Pandey, 2003). Sensitivity during early seedling growth indicates the survival and subsequent trends of mature plant

*Corresponding author. E-mail: ramialsir@yahoo.com. Tel: +249-9-12605428. Fax: +249-183-770701.

in arid environments (Elfeel and Warrag, 2004; Raddad, 2007), although, plant species are substantially more salt-tolerant as a mature plant than during seed-lings stage (Maas, 1993). Furthermore, plants are more tolerant to salt stress during seed germination than the seedling stage (Maas, 1993; Cony and Trione, 1998; Meloni et al., 2008). However, tolerance to salinity during seeds germination is the most viable criteria used for selection of salt tolerant plants (El-Keblawy and Al-Rawai, 2005; Muhammad et al., 2006). Consequently, adaptations during these stages can determine their natural distribution (Raddad, 2007; Meloni et al., 2008).

Acacia are key element in arid areas; as browse plants, provide gums, resins and firewood (Or and Ward, 2003). *Acacia senegal* (L.) willd. var. *senegal* Brenan is an important multipurpose tree species well adapted to extreme drought (Raddad, 2007). The distribution patterns of *A. senegal* specified to a geographic zone spread from East to West between 16° to 10° N latitude and the gum production mainly between latitudes 11° and 14°N in Sudan (Kananji, 1994; Elfeel and Warrag, 2004). This gum belt covering three types of soil: clay, sand and gardod (hard compacted sand soil) under two levels of annual rainfall; high (900 mm) and low of 200 mm (Elfeel and Warrag, 2004; Raddad and Luukkanen, 2006). The wide range of environments formulates high variability of the tree, with four distinct varieties or provenances were recognized (Aelbaek and Kananji, 1995; Wickens et al., 1995). However, Elfeel and Warrag (2004) reported differences in growth traits on seedlings originated from gardod soil stands which supplement adding up as a fifth distinct provenance. Estimation of the quantity and type of variation between genetically controlled geographical sources is an important factor for estimating genetic gains in adaptability and productivity with the changing environments (Elfeel et al., 2009). The high level of genetic variation between geographically structured *A. senegal* provenances or populations has been already analyzed using molecular markers (Chiveu et al., 2008; Habeballa and El Gaali, 2009; Habeballa et al., 2010). Nevertheless, the potential of this tree species provenance to grow and survive in the saline soil is scanty. Provenance-specific differences in salt-tolerant have been observed in many tree species (Park, 1995).

This study was undertaken to determine the variation between *A. senegal* provenances in response to NaCl application during seeds germination and seedlings growth, so as to decide which provenance can tolerate saline environments. To determine the source of variation in salinity tolerance between the provenances, random amplified polymorphic DNA (RAPD) marker was used.

MATERIALS AND METHODS

Plant material

Mature seeds were collected in March 2006 from 20 *A. senegal* provenances of trees growing on a non-saline soil within the gum Arabic belt in Sudan. The selection was made to cover the area of distribution on three soil types: sandy, clay and gardod soil, and two annual rainfalls regimes such as: high (400 to 500 mm) and low rainfall (200 to 300 mm), based on Aelbaek and Kananji (1995) (Table 1). In each geographical location, freshly pods were collected from 20 randomly selected and widely separated (> 100 m) trees that were presumed to be un-related clonally. Seeds were subsequently extracted and bulked together for each location as one seeds lot. Undamaged seeds that have no markings on their seed coat were retained for the experiment. The seeds were pretreated by soaking into concentrated. H_2SO_4 for 5 min then rinsed three times in sterile distilled water with shaking to remove the acid. The experiment was carried out at Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan.

Effect of NaCl on seed germination

Scarified seeds were surface sterilized by immersing in a 10% solution of commercial sodium hypochlorite for 5 min and rinsing repeatedly with sterilized distilled water. Twenty sterilized seeds were germinated in 9 cm diameter plastic Petri dishes lined with two Whatman No.1 filter papers and moistened with 5 ml NaCl solution. The electrical conductivity (EC) values of NaCl solutions were, 0, 2, 4, 6 and 10 dSm^{-1}. The experiment was incubated at 25 ± 2°C under 12 h light and 8 h dark conditions. Germination was recorded at two-day intervals until no more germination occurred for a period of 14 consecutive days. A seed was considered germinated when there was radical protrusion through the seed coat to 5 mm or longer. Swollen seeds that did not germinated but covered with fungi for at least 5 days were considered lost.

The percentage of final germination (FG %) of seeds on the control was calculated as the proportion of germinants (*a*) as a percentage of the number of viable seeds originally set to germinate (*b*) whereas for NaCl treatments, the FG % for each salinity level was expressed as the proportion of germinants (*a*) with normal radicals as a percentage of the number of seeds germinate in control treatment (*b*), as:

$FG = (a/b)\ 100$, at day 14.

The germination rate index (GRI) was calculated as a cumulative number from the formula described by Fowler (1991):

$GRI = G_4 + G_6 + G_8 + G_{10} + G_{12} + G_{14}$,

Where, G4, G6, G8, G10, G_{12} and G_{14} are germination percentages at 4, 6, 8, 10, 12 and 14 days after initiation of germination. The greater the value, the more rapid is the germination.

The salinity index (Table 2) for sensitivity of seeds to NaCl application was classified to three levels according to Rhoades et al. (1992) as a germination percentage in different degree of salinity of the control germination value in non-saline (distilled) water.

Effect of NaCl on seedling growth

Scarified *A. senegal* seeds of 8 provenances chosen as the most tolerant from the germination experiment were sown directly in 5 × 10 cm plastic pots, filled with a 48 g mixture of soil silt: sand (2:1). The planted seeds were irrigated to field capacity (50 ml) with tap water on alternate days for 3 weeks under greenhouse condition. A salinization experiment was conducted on 3-weeks-seedlings using the following solutions: control (NaCl-free), 4, 6 and 10 dSm^{-1}. The seedlings were irrigated with saline water every day through the experiment. The following parameters were studied: seedling height, root length, shoot dry weigh (stem + leaves), root dry weight

Table 1. *A. senegal* provenances studied and sites of origin in Sudan.

Soil classification	Average annual rainfall (mm)	Collection site	Code	Seed source
Clay	450	Eastern	GD	Algadamblia
Clay	385	Eastern	SM	Simmer belt
Clay	390	Eastern	MG	Macrm grees belt
Clay	390	Eastern	MG1	Macrm grees 2
Clay	385	Eastern	SM1	Simmer 3
Clay	385	Eastern	SM2	Simmer 3.1.2
Clay	600	Eastern	FE	Al Feel
Clay	390	Eastern	MG2	Macrm grees 3.1
Clay	390	Eastern	MG3	Macrm grees 3.1.2
Clay	385	Eastern	SM3	Simmer 3.1.2.3
Clay	736	Blue Nile	KD	Khor Donia
Sand	200	Western	GB	Al Ghabsha
Sand	200	Western	HA	Hamrat Algoz
Sand	325	Western	ED	El Rahad
Sand	300	Western	DK	Al Damokeya
Sand	200	Western	GB1	Al Ghabsha
Sandy clay	345	Western	MH	Al Semaih
Sandy clay	350	Western	NA	Nabag
Sandy clay	374	Western	GR	Abualgur
Sandy clay	374	Western	NW	Nawa

Table 2. Salinity index due germination percentages induction.

EC (dSm^{-1})	Salinity degree	Species seed germination (%)	Index
2 to 4	Low	< 60	Sensitive (S)
4 to 8	Moderate	60 - < 90	Moderate (M)
> 8	High	90 - 100	Tolerant (T)

and root/shoot ratio. The parameters were recorded, every week, for 3 weeks. The leaves, stems and roots of 10 randomly selected seedlings from all seed provenances per treatment were harvested and weighed for fresh weights and then air-dried for days at laboratory (30°C) to determine dry weights.

DNA extraction

Total DNA isolation was based on a modified phenol: chloroform protocol as described by Doyle and Doyle (1990). The modification made in intention to improve the quantity and the quality of the DNA. In this method the fine powdered plant materials were transferred into 13 ml Falcon tubes containing 4 ml of pre-warmed lysis solution. The samples were then incubated in a water path at 65°C with gentle shaking for 30 min and left to cool at room temperature. Phenol-chloroform mixture (1:1) was added and the phases were mixed gently for 5 min to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 10 min and the resulted clear aqueous phases containing DNA. The aqueous phase was mixed with chloroform: isoamyl alcohol mixture (24:1) followed by centrifugation at 5000 rpm for 5 min and repeated twice. The final supernatants were transferred to 1.5 ml Eppendorf tubes (400 µl/tube). The nucleic acids in the aqueous phase were precipitated by adding 800 µl of deep cooled isopropanol and 50 µl of 5 M NH4 acetate. The contents were mixed gently for 5 min by inversion manually and collected by cooled centrifugation (5°C) at 12000 rpm and for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning. The pellet was dissolved in Tris-EDTA. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis, according to Sambrook et al. (1989).

RAPD analysis

The PCR reaction mixtures were prepared in 25 µl volumes containing 2.5 µl of 10X Taq buffer, 1.5 µl MgCl2 (50 mM), 2.5 µl dNTPs (2 mM/µl), 2 µl random primer (10 pmol/µl), 0.5 µl Taq DNA polymerase (5 U/µl) and 1 µl of the extracted DNA (10 ng). The mixture was made up to 25 µl by addition of sterilized distilled water. RAPD/PCR reactions were initiated using an Applied Biometra thermalcycler programmed to repeat the thermal profile. Setting of the PCR program was based on three steps. Step one, was an initial denaturation step at 94°C for 5 min. Step two, was run for 40 cycles, each starting with denaruration at 94°C for 1 min, followed by annealing 36°C for 1 min and ended by extension at 72°C for 1 min. Step three, was a final extension cycle that performed at 72°C for 7 min. The PCR machine was adjusted to hold the product at 4°C. The amplified DNA fragments and the standard marker (λ Hind III digested DNA) were then separated in

Table 3. Mean final germination percentage (FG) and salinity tolerance of *A. senegal* seeds as influenced by NaCl levels at the end of 14 days. Seeds sources are ranked from most to least salt tolerant due to salinity index.

Seed source	Soil type	NaCl treatment EC (dS m^{-1})								
		0	2		4		6		10	
		FG (%)	FG (%)	Salinity index	FG (%)	Salinity index	FG (%)	Salinity index	FG (%)	Salinity index
FE	Clay	100	100	T	100	T	100	T	100	T
MG2	Clay	100	100	T	100	T	100	T	100	T
MG	Clay	100	100	T	100	T	95	T	95	T
GB1	Sand	100	95	T	95	T	95	T	95	T
DK	Sand	100	100	T	95	T	90	T	95	T
MH	Sandy clay	65	95	T	92	T	92	T	92	T
ED	Sand	100	95	T	95	T	95	T	85	M
HA	Sand	95	95	T	95	T	89	M	89	M
KD	Clay	85	94	T	94	T	88	M	88	M
NA	Sandy clay	85	100	T	96	T	89	M	82	M
SM2	Clay	100	95	T	90	T	85	M	85	M
MG3	Clay	100	95	T	85	M	85	M	85	M
GB	Sand	70	93	T	72	M	86	M	79	M
GR	Sandy clay	60	92	T	83	M	75	M	58	S
GD	Clay	100	90	T	60	M	55	S	40	S
SM	Clay	60	92	T	89	M	50	S	33	S
NW	Sandy clay	55	90	M	37	S	46	S	18	S
MG1	Clay	20	75	M	50	S	50	S	50	S
SM1	Clay	15	68	M	50	S	50	S	34	S
SM3	Clay	30	50	S	50	S	50	S	50	S

T, Tolerant; M, moderate; S, sensitive.

1.5% ethidium bromide-stained agarose gels. The separated fragments and their patterns were then visualized with an ultraviolet (UV) transilluminator.

Experimental design and data analysis

The experiment was designed in a completely randomized design and each treatment was replicated three times. All parameters were subjected to analysis of variance (ANOVA) to test effects of main factors (salinity and provenance). The significant differences between treatments means were separated using LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Seeds germination

Radical emergence was noted on the 3rd to 4th day after sowing and 100% seed germination was obtained after 14 days (Table 3). The analysis of variance showed a highly significant ($P < 0.05$) variation in germination percenttage and salinity tolerance between different acacia provenances. Final germination (FG) was significantly high (70 to 100 %) under the control (0 dS m^{-1} salinity) (Table 3; Figure 1). At the end of the experiment (day 14), the control gave the highest GRI (300). In general, seeds with a high final germination percentage in distilled water had a rapid germination rate, shown as a higher GRI, and indexed as salinity tolerant (Figure 1). Similar results were obtained for other acacia species (Rehman et al., 1996; Ndour and Danthu, 1999) and other trees and shrubs (Villagra, 1997; Agboola, 1998; Gul and Weber, 1999; Tobe et al., 2000; Ghorbanli et al., 2001). Thus, differences in ion content and leakage from different species of *Acacia* and from different accessions of the same *Acacia* species are not unexpected.

In this study, some *A. senegal* seeds showed an increase in germination percentage and rate index (GRI) under NsaCl concentration of 2 dSm^{-1} over the control (Table 3; Figure 1a). This may be interpreted in the light of the statement mentioned by Crawford (1978) that there are a number of species which show improved growth in the presence of limited concentrations of salt. Duan et al. (2007) reported that low concentrations of NaCl promote seeds germination of *Suaeda salsa*. However, the cumulative percentage germination was significantly ($P < 0.05$) reduced with the increase in NaCl concentration from 0 to 10 dSm^{-1} (Figure 1). However, the germination rates at a given NaCl concentration varied according to provenance. It is a well-known phenomenon that natural

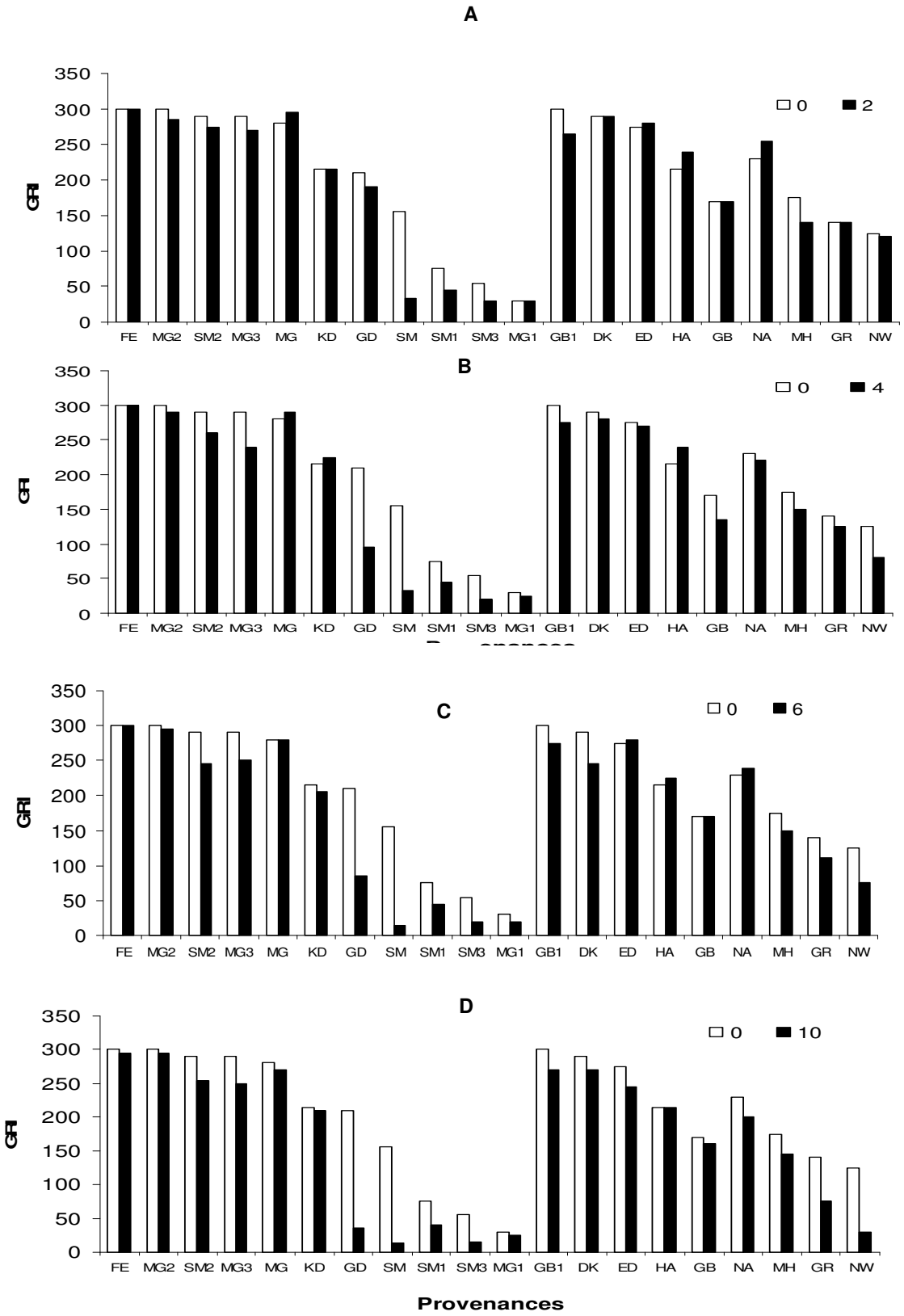

Figure 1. Germination rate index (GRI) of *A. senegal seeds* as influenced by the control (white bars) and irrigation with different level of NaCl as: (A) 2 dSm^{-1}, (B) 4 dSm^{-1}, (C) 6 dSm^{-1} and (D) 10 dSm^{-1} (black bars) after 14 days. The high GRI value, the high growth rate.

populations of plants grow well in different soils varies inherently in their ability to absorb and/or utilize mineral nutrients. The seed sources from different soil and rainfall presented large variable behavior in their response to NaCl application. Among the examined provenances, six seed sources: FE, MG2, MG (clay), GB1, DK (sand) and MH (gardod) appears as the most tolerant sources (Tolerant index: T), they exhibit high germination percentage (92 to 100%) and rate index (295) at 10 dSm^{-1} (Table 3; Figure 1d). FE provenance, and to a less extent MG2, are the seed sources that germinate 100% in all NaCl level and have the high germination rate (300) in all NaCl level except for 10 dSm^{-1} (295). ED, HA, GB (sand), KD, SM2, MG3 (clay) and NA (Gardod) provenance showed a moderate FG (82 to 89%, tolerant index: M) (Table 3). GD, SM, MG1, SM1, SM3 (clay) and GR, NW (Gardod) provenance showed a relatively weak FG (18 to 58%, tolerant index: S). All of this provenance are sensitive to high NaCl level (10 dSm^{-1}) with FG percentage less than 60%. GR (Garwood) provenance show moderate tolerance to 6 dSm^{-1} salinity level, whereas SM3 (clay) was sensitive to NaCl even at a 2 dSm^{-1} Ec. No sand provenance appears within this NaCl salinity sensitive group.

In general, this plant species is moderately salt tolerant at seed germination phase. Generally, the acacia classified as moderately salt tolerant. Similar result was reported by Hardikar and Pandey (2008) indicating *A. senegal* as relatively salt tolerant at seed germination level. Also, Rehman et al. (1996) screened seed germination of 10 *Acacia* species included *A. senegal* mentioned that it is intermediate in salt tolerance. Using eight NaCl concentrations included five concentrations similar to those used in this study. Tomar et al. (2003) reported that *Acacia nilotica* var. *cupressiformis* should be rated as moderately tolerant to salinity since it can successfully raised using irrigation water of salinity up to 4 dSm^{-1}. Also Mahmood (2007) indicated that *A. nilotica* can tolerate moderately saline soil. Aziz et al. (2001) in an *in vitro* experiment with *Acacia raddiana* and *A. nilotica* under different concentration of NaCl classified both species as moderately salt tolerant. Among species studied in field trail on saline soil, *A. nilotica, A. senegal, Acacia tortilis* and *Acacia mellifera* record the greater growth rate and with 100% survival (Oba et al., 2001).

Germination of halophytes shows a characteristic pattern in response to increased salt levels, with higher resistance up to a certain critical concentration and then a rapid decrease in final germination beyond this. Glycophytes, on the other hand, show a concomitant reduction in germination with increasing salinity (Rogers et al., 1995). The present germination data therefore suggests that *A. senegal* is a salt-tolerant glycophyte rather than a halophyte.

The variations were random and showed no significant correlation with sites. This variability, which is likely to be related to the different geographical origins of the seeds, may confirm the existence of polymorphism of the species (Chiveu et al., 2008; Habeballa and El Gaali, 2009). Khalil and Siam (2003) reported the effect of geographical origin on seed germination of four *A. senegal* provenances. Seed growth variation within provenance have been reported for a number of species, including *Acacia albida* (Sniezko and Stewart, 1989), *A. nilotica* sp. *indica* (Krishan and Toky, 1996), *A. nilotica* ssp. *Tomentosa* (Wolde-Meskel and Sinclair, 2000) and *Balanites aegyptiaca* (Elfeel et al., 2009). Large random variations between provenances may be mainly due to the genotypes. This study has suggested the significant role that the geographical origin may play in the germination behavior of seeds before and after exposure to salinity.

Seedlings growth

The effect of irrigation with saline water on stem length and root length is shown in Figure 2a, b, respectively. There were variations in growth response of all seedlings to NaCl levels. MH, NA provenances (Gardod + low rainfall) showed significant decreases in height and root growth with the increase in NaCl concentration in the irrigation water. Reduction in plant height due to salt stress may be attributed to the effects of salts through ion toxicity which retarding the processes of cell division and cell extension upon which growth depends (Netondo, 1999). The maximum reduction in height plant growth (less than 50% of control) was observed in the provenance NA at higher NaCl concentration (10 dSm^{-1}). However, other group of provenances including FE, GD, KD (clay+ high rainfall) and GB1, DK (sand) showed increase in height and root growth with the increase in NaCl levels up to 4 dSm^{-1} and then decrease when the level reach 6 dSm^{-1}. However, provenance FE show decrease in root length with application of NaCl while DK decrease in stem length. In contrast, provenance HA (sand+ low rainfall) showed an increase in growth with the increase in ECe level even to 10 dSm^{-1}. Reduced growth was probably caused by the high NaCl level, which increased the osmotic potential of the irrigation solution and also created salt stress from excessive uptake of ions (Greenway and Munns, 1980). Provenance HA gave higher root length (19.6 cm) on 10 dSm^{-1} and also the lowest root length (9.9 cm) on nonsaline condition (Figure 2b). As salinity increased to 10 dSm^{-1}, provenance NA have the lowest shoot length (15 cm) compared with other tree provenances whereas provenance KD gave the higher stem length (27.5 cm) when irrigated with 4 dSm^{-1}. It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman, 1977; Munns, 2002; Hardikar and Pandey, 2008).

FE (clay + high rainfall) and NA (Gardod+ low rainfall) show significantly the higher mean root/shoot ratio (2.0)

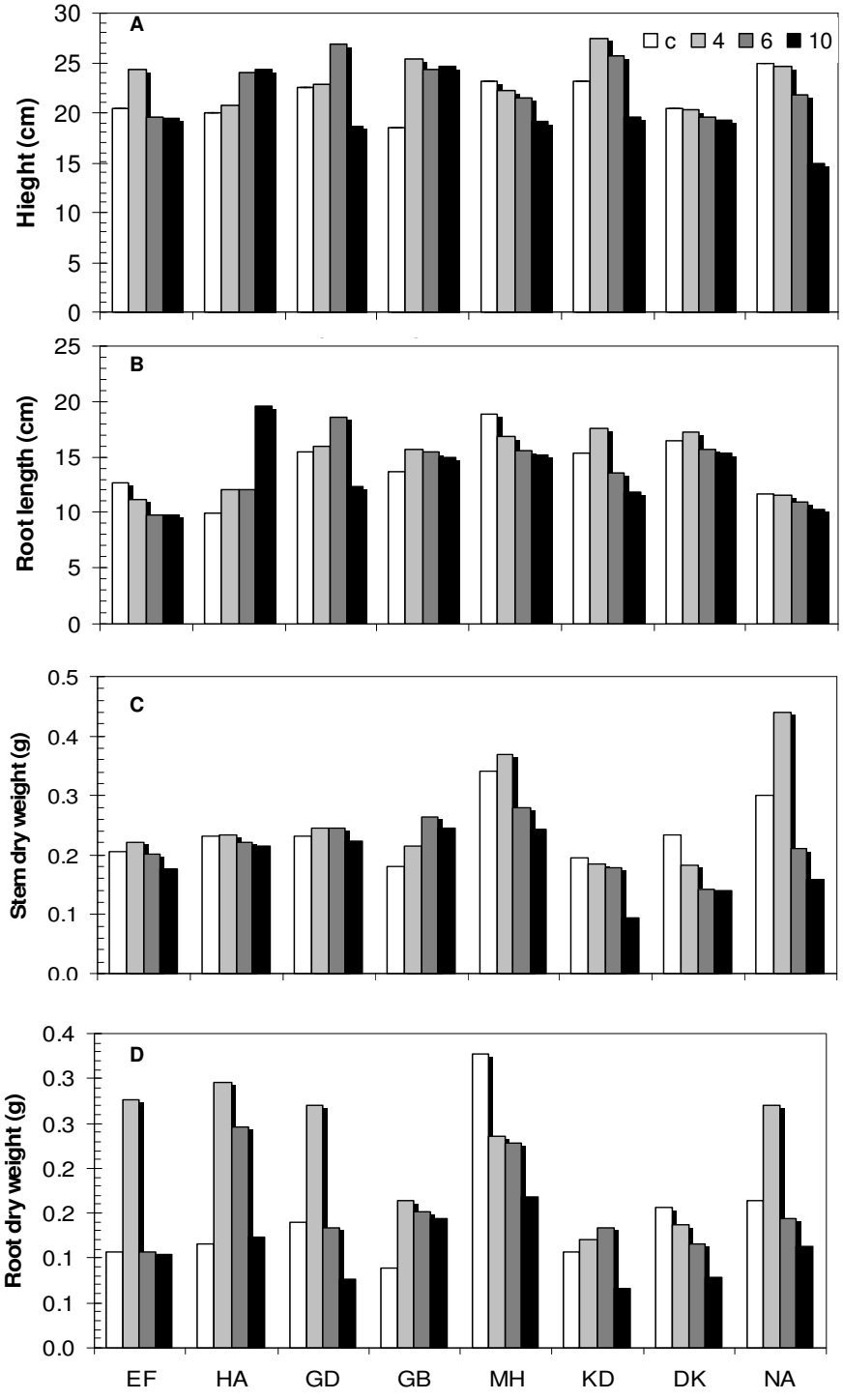

Figure 2. Effect of irrigation with NaCl (EC = 0 □, 4 ▨ 6 ▩, 10 ■ dSm^{-1}) on (A) stem length, (B) root length, (C) shoot dry weight and (D) root dry weight of 8 provenances of *A. senegal* seedlings after 4 weeks under greenhouse condition.

(Table 4). Mean root/shoot ratio (1.3) of MH (Gardod+ low rainfall) and DK (sand+ low rainfall) was significantly the lowest when compared with other provenances at all levels of salinity. Salt stress reduces seedlings growth, inhibited shoot growth more than root growth; as a result root: shoot ratio is increased (Manchanda and Garg, 2008). Moreover, tap root elongation for seedlings grown in control and saline soils both was greater than that of shoot. It is suggested that *A. senegal* has a tendency for rapid root extension as an adaptation to survive and tole-

Table 4. Effect of irrigation with saline water on root/shoot ratio of seedlings of 8 *A. senegal* provenances under greenhouse condition after

Provenance	Salinity concentration ECe (dSm^{-1})				Mean±0.26
	c	4	6	10	
FE	2.80	6.07	2.85	2.97	3.7^{b}
HA	2.39	3.15	2.94	2.94	2.9^{d}
GD	3.42	3.49	2.94	1.68	2.9^{d}
GB	4.07	3.87	3.65	2.90	3.6^{b}
MH	5.17	4.69	3.92	3.65	4.4^{a}
KD	2.90	3.97	3.65	3.50	3.5^{bc}
DK	3.21	4.03	5.10	2.83	3.8^{b}
NA	2.77	3.04	3.31	3.20	3.1^{cd}
Mean±0.18	3.3^{a}	4.0^{a}	3.5^{a}	3.0^{b}	
LSD0.05	Prove: 0.51		Salinity: 0.36		

Means±SE followed with the same latter (s) are not significantly different at P = 0.05.

rant of plants to dry habitats. This appears in a root/ shoot dry weight ratio of *A. senegal* of 1.1 under control conditions (Hardikar and Pandey, 2008). In glycophytes, they may alter root/shoot ratios to adapt salt stress. Slower growth is also observed in salt-conditioned halophytes and some apparently having an obligate requirement for NaCl accumulation (Mahmood, 2007).

There was noticeable variation in stem and root dry weight among provenances in response to salt stress at harvest (Figure 2b, c). Only provenance DK show significant reduction in stem and root dry weight with increasing the level of salt in irrigation water. Provenance KD, however, show reduction in stem dry weight and increasing in root dry weight with increasing NaCl concentration to 6 $dSm^{-1}$and then reduction at 10 $dSm^{-1.}$ While in all other provenances, stem and root dry weight improved over the control at 4 dSm^{-1}NaCl and then reduction. Nevertheless, in provenance GB, the increasing in dry weight was high than control even to 6 dSm^{-1} (Figure 2b, c). Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted in internal water deficit to plants, which in turn, reduced the growth of shoots and roots and finally, general reduction in size and dry matter production. Many investigators have reported retardation of germination and growth of seedlings at high salinity (Garg and Gupta, 1997; Ramoliya and Pandey, 2003; Hardikar and Pandey, 2008). However, plant species differ in their sensitivity or tolerance to salts. In general, seedlings of *A. senegal* survived up to the soil salinity of 10 dSm^{-1} and therefore, this tree species is moderate salt tolerant which it is in conformity with the finding of Hardikar and Pandey (2008). Nevertheless, there was no correlation between shoot and root dry weight with height and root lengths within each provenance.

Broadly speaking, the seed sources (soil or rainfall) have no effect on the response of provenances to saline irrigation at seedling level. Consequently, there is large variation among naturally-occurring provenances of *A. senegal* in there growth response to salinity stress. The distribution pattern of *A. senegal* entail adaptation to diverse soil conditions and provides evidence for the presence of some provenances and/or ecotypes of species that are more tolerant to NaCl salinity. Interspecific genetic variation for salt tolerance has been found in many *Acacia* genera (Niknam and Mc Comb, 2000). Thus, to select suitable trees for the afforestation on salt-affected soils in arid and semi-arid Sudan, screening tests involving various genotypes and ecotypes might be promising. Furthermore, plant tolerance to salinity is determined not only by seed source environment (Sands, 1981), but also by genetics (Van der Moezel et al., 1991).

Together, the results in this study and information available up to date, it assumes that the variation for salt tolerance between the examined provenances can not be explained adequately in relation only to plant growth. Therefore, RAPD analysis was carried out to reinforce the evidences of these provenance variations.

Analysis of genetically difference in salt tolerance by RAPD markers

A total of 15 primers were tested with the 8 genotypes of *A. senegal*. The results indicate that 7 primers (60%) showed at least 1 consistent polymorphic band. The seven informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and between all the *Acacia* spp. under study. The selected primers generated distinctive products in the range of 1.584 to 5.148 Kbp. Total of 51 amplified fragments were distinguished across the selected primers and the statistical analysis showed 44 polymorphic bands among the 8 genotypes with an

Table 5. Names and sequences of the primers used and its polymorphic bands.

Primer code number	Primer sequence (5'- 3')	Total number of band	Number of polymorphic band	Polymorphic band (%)
OPA-01	CAGGCCCTTC	5	3	60
OPA-03	AGTCAGCCAC	6	5	83
OPA-09	GGGTAACGCC	11	8	73
OPA-13	CAGCACCCAC	7	7	100
OPA-14	TCTGTGCTGG	6	6	100
OPA-17	GACCGCTTGT	10	10	100
OPA-20	GTTGCGATCC	6	5	83.3
Total		51	44	599.3
Average		7.2	6.3	85.6

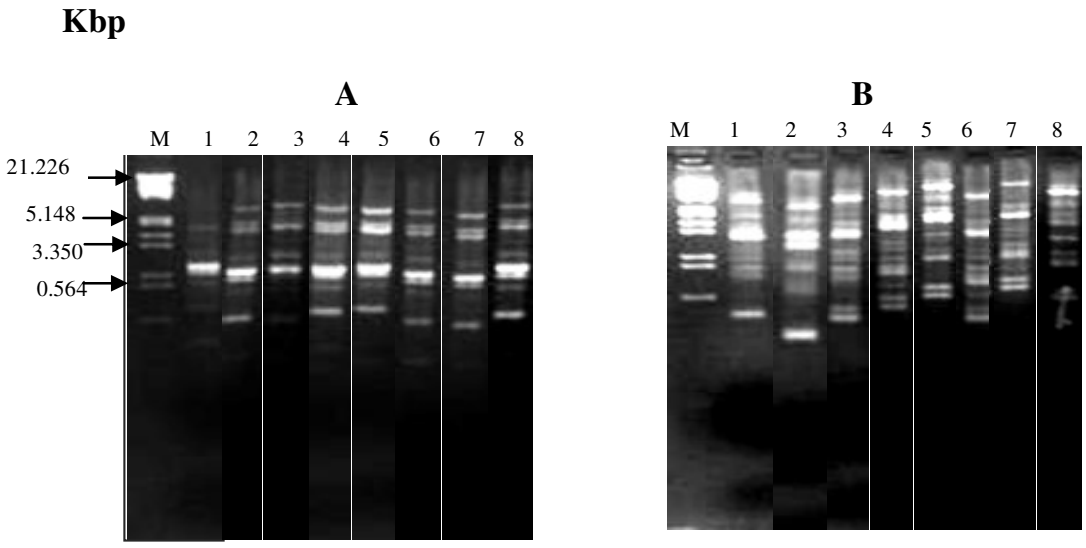

Figure 3. RAPD profiles of *A. senegal* (1-8) obtained with seven primers (Operon Technology Inc., A-OPA-09, B-OPA-17 M- DNA marker λ Hind III digested DNA.

average of 7.2 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primer OPA-09 (11) with 73% polymorphism while the minimum numbers of fragments were produced by the primer OPA-01 (5) with 80% polymorphism Table 5.

The dendrogram showed two main clades that are not related to soil type or rainfall regime as clustered in Figure 3. The first clade restricted to two groups, the first one contain provenances DK (Sand), NA (Gardod) as sisters and MH (Gardod), HA (Sand) as sisters and showed genetic closeness, the second group contain FE (Clay), GB (Sand) as sisters and GD (clay). KD (Clay) came out of group. The high dissimilarity between GD and MH had about 39% and the low dissimilarity found between DK and MH in one hand and NA in other hand had about 14% Table 6.

In this study, among the used primers, some allowed no distinction between the provenances, while others were sensitive enough for differentiation leading to the ability to determine the provenance similarity indices (Figure 4). The high similarities in genetic background between the examined *A. senegal* provenances might for the provenance variation for salt tolerance measured in this study. The high dissimilarity percentage was observed between Al Semaih and Algadmblia, although, Al Semaih has more tolerance to salt stress. The similarity indices were higher compared with *A. senegal*, however, the difference in salt tolerance between the provenances could be related to the difference in the similarity indices experiments that need to be achieved to determine the linkage between these RAPD markers and the gene(s) responsible for salt tolerance in these *Acacia* species.

Conclusion

Sodium chloride is the most soluble component of saline soil that inhibits growth and reduces yield of many plant

Table 6. Dissimilarity among *A. senegal* provenances samples (percent disagreement) by using STATISTCA program.

Provenance	GD	EF	GB	MH	HA	DK	NA	KD
GD	0.00	0.33	0.33	0.39	0.33	0.25	0.27	0.25
FE	0.33	0.00	0.16	0.37	0.31	0.27	0.33	0.20
GB	0.33	0.16	0.00	0.29	0.27	0.20	0.22	0.20
MH	0.39	0.37	0.29	0.00	0.25	0.14	0.24	0.37
HA	0.33	0.31	0.27	0.25	0.00	0.16	0.22	0.27
DK	0.25	0.27	0.20	0.14	0.16	0.00	0.14	0.24
NA	0.27	0.33	0.22	0.24	0.22	0.14	0.00	0.18
KD	0.25	0.20	0.20	0.37	0.27	0.24	0.18	0.00

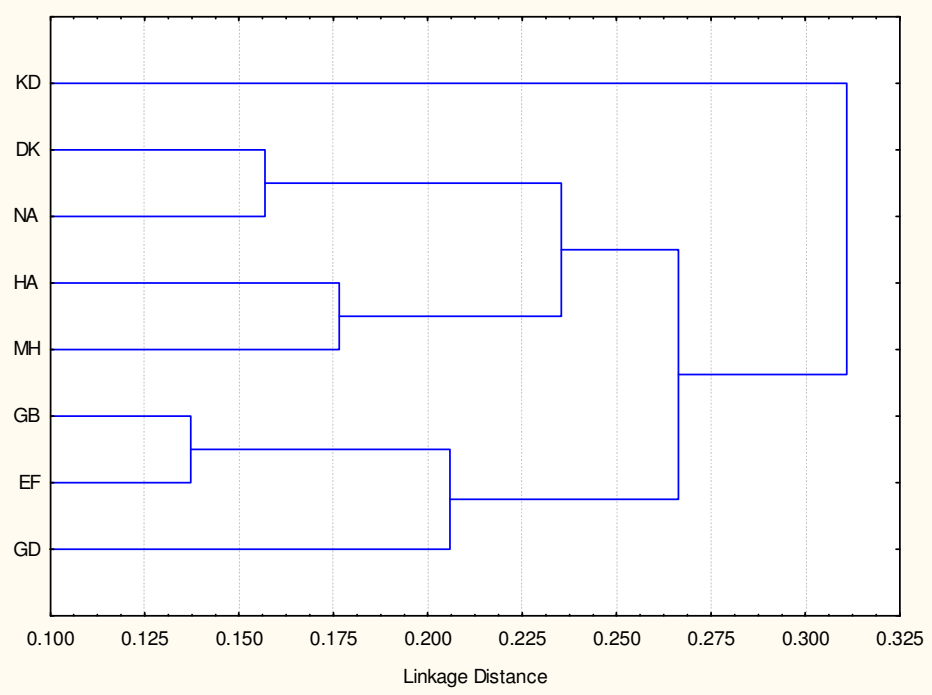

Figure 4. Combined cluster analysis derived from RAPD data by STATISTCA-SPSS to estimate the genetic distance analysis of 8 *A. senegal* from different provenances using 7 RAPD primers.

species. All the provenances tested in this study were improved in growth over control (nonsaline water) when irrigated with 6 dSm^{-1} Furthermore, they are comparatively tolerant to 10 dSm^{-1} NaCl concentration in irrigation water since the reduction in plant height is less than 50%. In general, *A. senegal* is moderately salt tolerant to irrigation water. Provenance MH (Gardod soil + low rainfall) from Al Semaih forest, Western Sudan, appear more tolerant than other seed sources investigated in this study. However, more research in field experiment at plant stage is needed.

REFERENCES

Aelbaek A, Kenanji B (1995). Tree Seed Zones for the Sudan. Sudan Tree Seed Center, Danida Forest Seed Centre.

Agboola DA (1998). Effect of saline solutions and salt stress on seed germination of some tropical forest tree species. Rev. Biol. Trop. 46(4): 1109-1115.

Aziz AAS, Omari MA, Kafawin OM (2001). Germination and seedling growth of *Acacia raddiana* and *Acacia nilotica* at different levels of salinity and drought under *in vitro* conditions. J. Trop. For. Sci. 13(1): 192-200.

Bakke IA, Freire AL, Bakke OA, de Andrade AP, Bruno RL (2006). Water and sodium chloride effects on *Mimosa Tenuiflora* (WILLD.) Poiret seed germination. Rev. Caatinga, 19(3): 261-267.

Chiveu CJ, Dangasuk OG, Omunyin ME, Wachira FN (2008). Genetic diversity in Kenyan populations of *Acacia senegal* (L.) Willd. revealed by combined RAPD and ISSR markers. Afr. J. Biotechnol. 7: 2333-2340.

Cony MA, Trione SO (1998). Inter- and interspecific variability in *Prosopis flexuosa* and *P. chilensis*: seed germination under salt and moisture stress. J. Arid Environ. 40: 307-317.

Crawford RMM (1978). Mineral Nutrition (Chapter 6). In: Plant Structure,

Function and Adaptation. Edited by M. A. Hall. The English Language Book Society and Macmillan. London and Basingstocke, p. 243.
Dell'aquilla A (1992). Water uptake and protein synthesis in germinating wheat embryos under the osmotic stress of polyethylene glycol. Ann. Bot. 69: 167-171.
Doyle J, Doyle J (1990). A rapid total DNA preparation procedure for fresh plant tissue. Focus, 12: 13-15.
Duan DY, Li WQ, Liu XJ, Ouyang HPA (2007). Seed germination and seedling growth of *Suaeda salsa* under salt stress. Ann. Bot. Fenn. 44: 161-169.
Elfeel AA, Warrag EI (2004). Geographical source variation in seedling growth and nodulation of *Acacia senegal* var. *senegal* in Sudan. Sudan Silva, 10(1): 18-31.
Elfeel AA, Warrag EI, Musnad HA (2009). Effect of seed origin and soil type on germination and growth of heglig tree (*Balanites aegyptiaca* (DEL.) L. *var. aegyptiaca*). J. Sci. Technol. 10(3): 65-66.
El-Keblawy A, Al-Rawai A (2005). Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. J. Arid Environ. 61: 555-565.
Fowler JL (1991). Interaction of salinity and temperature on the germination of Crambe. Agron. J. 83: 169-172.
Garg BK, Gupta IC (1997). Saline Wastelands environment and plant growth. Scientific Publishers, Jodhpur, India.
Ghorbanli M, Hekmatshear H, Farahvash F (2001). The correlation of temperature and salinity variations on seed germination of two Atriplex species of Azarbijan. J. Agric. Sci. 6(4): 33-46.
Greenway H, Munns R (1980). Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. 31: 149-190.
Gul B, Weber DJ (1997). Effect of salinity and thermoperiod on the seed germination of *Allenrolfea occidentalis* Chenopodiaceae. Am. J. Bot. 84: 60.
Habeballa RS, El Gaali E (2009). Phylogenic analysis in *Acacia senegal* using AFLP molecular markers across the gum Arabic belt in Sudan. Afr. J. Biotechnol. 8 (19): 4817-4823.
Habeballa RS, Hamza NB, El Gaali EI (2010). Genetic variability in Sudanese *Acacia senegal* (L.) assessed by random amplified polymorphic DNA. Afr. J. Biotechnol. 9(30): 4655-4660.
Hardikar SA, Pandey AN (2008). Growth, water status and nutrient accumulation of seedlings of *Acacia senegal* (L.) Willd. in response to soil salinity. Anales de Biología 30: 17-28.
Kananji B (1994). Exploration and seed collection of *Acacia senegal* in Sudan. Palmberg-Lerche C, Souvannavong O, Thomsen A, Forest Genetic Resources, FAO, Rome, p. 22.
Khajeh-Hosseini M, Powell AA, Bingham IJ (2003). The interaction between salinity stress and seed vigor during germination of soybean seeds. Seed Sci. Technol. 31: 715- 725.
Khalil AAM, Siam MJ (2003). Effect of geographic source of seed on morphometric and germination characteristics of *Acacia senegal* (L.) Willd. seeds. U. K. J. Agric. Sci. 11(2): 231-246.
Krishan B, Toky OP (1996). Provenance variation in seed germination and seedling growth of *Acacia nilotica* ssp. *indica* in India. Genet. Resour. Crop Evol. 43: 97-101.
Maas EV (1993). Plant growth response to salt stress. In: Towards the rational use of high salinity tolerant plants, H. Leith and A. Al Masoom (eds.). Kluwer Academic Pub. Netherlands, 1: 279-291.
Maas EV, Hoffman GJ (1977). Crop salt tolerance-current assessment. J. Irrig. Drain Div. ASCE. 103:115-134.
Mahmood K (2007). Salinity/sodicity tolerance of *Acacia ampliceps* and identification of techniques useful to avoid early stage salt stress. PhD Thesis, Kassel University.
Manchanda G, Garg N (2008). Salinity and its effects on the functional biology of legumes. Acta Physiol. Plant, 30(5): 595-618.
Meloni DA, Gulotta MR, Martı´nez, CA (2008). Salinity tolerance in *Schinopsis quebracho* colorado: Seed germination, growth, ion relations and metabolic responses. J. Arid Environ. 72: 1785-1792.
Muhammad J, Lee DB, Jung KY, Muhammad A, Lee SC, Rha, ES (2006). Effect of salt (Na Cl) stress on germination and early seedling growth of four vegetables species. J. Cent. Eur. Agric. 7(2): 273-282.
Munns R (2002). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16: 15-24.
Ndour P, Danthu P (1999). Effects of salinity on the germination of some African acacias. In: Campa C, Grignon C, Gueye M and Hamon S (Edrs). L'acacia au Senegal. Actes de la reunion thematique sur l'acacia au Senegal. Dakar, Senegal, 3-5 December 1998. French Institute of Scientific Research for Cooperative Development, Paris, France, pp. 105-122.
Niknam SR, McComb J (2000). Salt tolerance screening of selected Australian woody species: a review. For. Ecol. Manage. 139: 1-19.
Oba G, Nordal I, Stenseth NC, Stave J, Bjora CS, Muthondeki, JK, Bii WKA (2001). Growth performance of exotic and indigenous tree species in saline soils in Turkana, Kenya. J. Arid Environ. 47: 499-511.
Or K, Ward D (2003). Three way interactions between *Acacia*, large mammalian herbivore and bruchid beetles. Afr. J. Ecol. 41: 257-265.
Park AD (1995). Preconditioning responses of salt-tolerant and salt-sensitive provenances of *Acacia tortilis* (Forsk.) Hayne to high salinity. MScF thesis, Faculty of Forestry, University of Toronto.
Raddad EY (2007). Ecophysiological and genetic variation in seedling traits and in first-year field performance of eight *Acacia senegal* provenances in the Blue Nile, Sudan. New Forest. 34: 207-222.
Raddad EY, Luukkanen O (2006). Adaptive genetic variation in water use efficiency and gum yield in *Acacia senegal* provenances grown on clay soil in the Blue Nile region, Sudan. For. Ecol. Manage. 226: 219-229.
Ramoliya PJ, Pandey AN (2003). Effect of salinization of soil on emergence, growth and survival of seedlings of *Cordia rothii*. For. Ecol. Manage. 176: 185-194.
Rehman S, Harris PJC, Bourne WF, Wilkin J (1996). The effect of sodium on germination and the potassium and calcium contents of Acacia seeds. Seed Sci. Technol. 25: 45-57.
Rhoades JD, Kandiah A, Mashali AM (1992). The use of saline waters for crop production-FAO irrigation and drainage paper FAO, Rome no. p. 48.
Rogers ME, Noble CL, Halloran GM, Nicolas ME (1995). The effect of NaCl on the germination and early seedling growth of white clover (*Trifolium repens* L.) populations selected for high and low salinity tolerance. Seed Sci. Technol. 23: 277-287.
Sambrook J, Fritsch, E, Maniatis T (1989). Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.
Sands R (1981). Salt resistance in *Eucalyptus camaldulensis* Dehnh. from three different seed sources. Aust. For. Res. 11: 93-100.
Sniezko RA Stewart HTL (1989). Range-wide provenance variation in growth and nutrition of *Acacia albida* seedlings propagated in Zimbabwe. For. Ecol. Manage. 27: 179-197.
Tobe K, Li X, Omasa K (2000). Effects of sodium chloride on seed germination and growth of two Chinese desert shrubs, *Haloxylon ammodendron* and *H. persicum* (Chenopodiaceae). Aust. J. Bot. 48(4): 455-460.
Tomar OS, Minhas PS, Sharma VK, Singh YP, Gupta RK (2003). Performance of 31 tree species and soil conditions in a plantation established with saline irrigation. Forest. Ecol. Manage. 177: 333-346.
Van der Moezel PG, Pearce-Pinto GVN, Bell DT (1991). Screening for salt and waterlogging tolerance in Eucalyptus and Meluleucu species. For. Ecol. Manage. 40: 27-37.
Villagra PE (1997). Germination of *Prosopis argentina* and *Prosopis alpataco* seeds under saline conditions. J. Arid Environ. 37: 261-267.
Wickens GE, Seif El Din AG, Sita G, Nahal I (1995). Role of *Acacia* species in the rural economy of dry Africa and the Near East. FAO Conservation Guide, 27: p. 85.
Wolde-Meskel E, Sinclair FL (2000). Growth variability in a senegalese provenance of *Acacia nilotica* ssp. *Tomentosa*. Agrofor. Syst. 48: 207-213.