RESEARCH ARTICLE

NaCl salinity affects germination, growth, physiology, and biochemistry of bambara groundnut

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ABSTRACT

The effects of NaCl salinity on seed germination, growth, physiology, and biochemistry of two bambara groundnut landraces (*Vigna subterranea* (L.) Verdc), Kakamega (white seed coat) and Mumias (red seed coat), were investigated with the aim of establishing traits, which can provide a basis for breeding to salt tolerance in groundnuts. A study was conducted under laboratorial and greenhouse conditions. Bambara groundnut seeds and plants were subjected to five concentrations of NaCl solutions with several electrical conductivities: 0 (control), 6.96, 12.93, 19.89, and 25.86 dS m^{-1}. Germination percentage, growth, chlorophyll fluorescence, and leaf chlorophyll content were determined. Sodium chloride salinity ($p<0.05$) significantly decreased germination and plant growth in both landraces. Mumias had significantly higher total chlorophyll, chlorophyll a and b content compared to Kakamega landrace. Salinity significantly decreased Fv/Fm ratio and electron transport rate in the two landraces, however there were no significant ($p>0.05$) differences in the Fv/Fm values for Mumias' landrace, as compared to the Control. Overall, Mumias' landrace seeds seemed to be more salt-tolerant at higher salinity levels compared to Kakamega. A greater reduction in growth in Mumias than in Kakamega is a possible indicator for salt tolerance. The chlorophyll fluorescence parameters may not be used to identify salt sensitivity between the two landraces. The results indicated that leaf area and seed germination were suitable parameters for screening the two bambara landraces for salt tolerance.

Keywords: salinity, *Vigna subterranea*, growth physiology, saline water.

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an indigenous African crop (Vurayai et al., 2011), which has been cultivated for centuries (Heller et al., 1997) and is mainly grown by women as a source of protein and rural income (Tafouo et al., 2010). It is one of the most neglected and underutilized crops in Kenya, despite having sustained human nutrition for generations in Western Kenya (Heller et al., 1997; Musotsi et al., 2005). It is a highly nutritious food crop that plays a crucial role in people's diet and is currently grown throughout Africa. Such groundnut is grown in contrasting agroecological zones of Kenya, mainly in the high and medium potential zones of Western Kenya, some parts of South Nyanza, and in the drought prone coastal areas. It is ranked the second most important underground pod legume in much of Africa after groundnut, *Arachis hypogea* (Ntundu et al., 2006).

Bambara's groundnut potential of contributing to food security has fueled an increasing research interest (European Union STD-3, 1997), including its tolerance to environmental stresses. Salinity is one of the major environmental problems affecting plant's growth, development, and productivity in agricultural soils, and an increase in this menace poses a serious threat to agriculture globally (Alam et al., 2004). Salinization

of agricultural lands is widespread and occurs in semiarid, low-lying, irrigated, and poorly drained areas (Musyimi, 2005; De Abreu et al., 2008). Such salinization of the soil hugely contributes to low-crop productivity worldwide (Alam et al., 2004), and a drop of crop yield over large areas due to physicochemical constraints in the subsoil (Rengasamy, 2010). Minimal successes have been achieved when using irrigation and drainage as viable management options to improve productivity of saline-sodic soils (Tavakkoli et al., 2011). In Kenya the potentially saline areas include some regions of Coastal, North Eastern, Rift Valley, and Nyanza provinces (Musyimi, 2005). Saline soils are mainly dominated by Na^+ and Cl^- anions, in which NaCl constitutes from 50 to 80% of the total soluble salts (Rengasamy, 2010). Salt stress affects plant growth in three ways: osmotic stress by reducing soil water potential; induction of ion imbalance in cells, especially lowering concentrations of K^+, Ca^{2+}, and NO^{-3}; and causing ion (Na^+ and/or Cl^-) toxicity. Since salt stress involves both osmotic and ionic stresses, the growth suppression is directly related to the total concentration of soluble salts and osmotic potential of the soil solution (Tavakkali, 2011).

The detrimental effect is observed at the whole-plant level with the death of plants or a decrease in productivity (Munns and Tester, 2008). Therefore, understanding the mechanisms of tolerance to high-soil concentration of NaCl is essential to improve crop salt tolerance. The mechanisms of salinity tolerance in plants have been widely studied and categorized into three: tolerance to osmotic stress, Na^+ exclusion, and tissue tolerance (Munns and Tester, 2008). It has been suggested that salinity tolerance is particularly associated with the ability of excluding Na^+ from the shoot by reduced loading into the xylem (Garthwaite et al., 2005), and is a crucial feature of restricting salt accumulation in plants (Munns and Tester, 2008).

Whereas methods of screening for salt tolerance based on yield response measurements have been employed with good results, they are time consuming and expensive, thus needing the use of indirect ones (Belkhodja et al., 1999). These include physiological traits such as photosynthesis, which are equally important and quite specific in indicating salt tolerance. Chlorophyll fluorescence is now a very powerful noninvasive mean of obtaining quickly semiquantitative information on photosynthesis in the field and in the laboratory (Netondo et al., 2004), using intact leaves still attached to the plants (Netondo et al., 2004; Bacarin et al., 2011; Tavakkoli, et al., 2011).

Direct salt injury to the functioning of the photosystem II (PSII) has been reported in various plants (Tavakkoli et al., 2011), contributing to inhibition of photosynthesis and biomass production, together with stomatal closure. A recent study on salinity tolerance in bambara groundnuts (Tafouo et al., 2011) involved mineral uptake, water content of leaves, as well as growth and yield components. However, developing a salt-tolerant crop is not an easy task because salt tolerance is a polygenic trait. Integration of knowledge on morphological, physiological, biochemical, and genetic aspects of salt tolerance is essential to make any progress in this regard (Ashraf and Foolad, 2007). Therefore, there is a need to assess effects of salinity on commonly cultivated bambara landraces in Kenya, with the purpose of identifying parameters that may confer the tolerance to salinity hence may extend the cultivation to areas with varying salinity. The aim of the present study was to evaluate the growth and physiological responses of two landraces of bambara groundnuts to NaCl salinity. A possible difference in response to salinity would indicate variable tolerance to NaCl stress, may coincide with the wide agroecological areas of growth, and reflect the genetic diversity of bambara groundnuts.

MATERIAL AND METHODS

Experimental materials and growth conditions: The experiment was conducted under greenhouse conditions at Maseno University, starting in October 2009 through January 2010. The experimental area lies at latitude 0º1'N-0º12'S and longitude 34º25'E-34º47'E. It is approximately 1,500 m above the sea level and receives an annual mean precipitation of 1,750 mm with bimodal pattern of distribution. The mean air temperature is 28.7ºC with a 40% relative humidity. The soils are classified as acrisol, deep reddish brown friable clay with pH ranging from 4.5 to 5.5, soil organic carbon and phosphorus contents are 1.8% and 4.5 mg kg^{-1}, respectively (Netondo, 1999). There is no salinity problem in soils in Maseno area. The soils are acidic, well-drained and deep, with high extractable Ca and K. The minimum and maximum temperatures inside the greenhouse were 26±6ºC and 35±6ºC respectively. The relative humidity of air ranged between 50 and 95% during the experiment. The experiment involved two bambara groundnut landraces, which were originally collected from Kakamega – Kk (white seed coat) and Mumias – Mm (red seed coat) areas of Western Kenya, and were labeled as Kk and Mm, respectively. The seeds were bulked and harvested during the long rains (from February to May, in 2009) by researchers at Maseno University in the Department of Botany.

Germination test: Large and similar sized seeds of Kk and Mm were sterilized for five minutes in 10% sodium hypochlorite, and then rinsed five times with distilled water. The seeds were germinated in sterile 90 mm diameter plastic petri dishes lined with Whatman number 1 filter papers. The petri dishes were arranged in a completely randomized design (CRD) consisting of five treatments and three replicates. The petri dishes were then moistened with 10 mL of the respective treatment solutions. Treatments consisted of five levels of NaCl concentrations with electrical conductivity (EC) of: 0 (Control), 6.96, 12.93, 19.89, and 25.86 dS m^{-1} in the growth media. Each petri dish containing 30 seeds was then covered to minimize microbial contamination and water loss. The filter paper linings were constantly moistened until the seeds had been germinated. Observations were made by counting the number of seeds germinating each day during a 14-day period.

Greenhouse pot experiment: A total of 20 L PVC pots was filled with soil from the botanic garden, which had been solarized (sun sterilized) for at least two days mainly to prevent fungal growth. The bottom of each pot was perforated to facilitate drainage. Large, similar sized seeds of the two bambara groundnut landraces, Kk and Mm, were sterilized for 5 minutes in 10% sodium hypochlorite and then rinsed several times with distilled water before planting. Ten seeds were planted per pot at the depth of 20 to 30 mm and at the recommended spacing of 100 to 150 mm. Each pot was irrigated daily with tap water to ensure a successful germination and establishment of the crop in readiness for treatment. Seedlings emerged as from the sixth day after sowing. Thinning was done 20 days after sowing to leave six uniformly spaced plants per pot. Salinity treatment started 20 days after sowing. The experiment was laid out as a completely randomized design, including two landraces, five treatments, and three replicates. To reduce osmotic shock, saline treatment was imposed incrementally, increasing the concentration by EC of 6.96 dS m^{-1}, every second day until the final electrical conductivity was reached. The experiment was carried out in a greenhouse under natural conditions with the following mean values: approximately 700 µmol m^{-2} s^{-1} of maximum photosynthetically active radiation (PAR), 55% relative humidity, and temperature ranging from 29 (day) to 24°C (night).

Plant growth: Leaf number, leaf area, root length, root and shoot biomasses were determined at seven-day intervals from the day the treatment was initiated (DAT). Plant leaf area (A_{plant}) was measured according to Cornelissen et al. (2005). The length and width of the middle leaflet were measured, and leaf area was calculated using the Equation 1:

$$A_{plant}=0.74 \times 3 \times N_1 (L \times W \times \pi /4) \quad (1)$$

Where,

A_{plant}: plant's leaf area; L: length of the middle leaflet (mm); W: width of the middle leaflet (mm); ∏: 3.1416; and N_1: total number of leaves.

One plant from each pot was carefully scooped with all its roots intact using a trowel and hand washed over a fine sieve with tap water collecting all roots one at a time. The roots were then separated from the shoots and oven dried at 72ºC for 48 h, and their dry weights were determined using a weighing balance (Denver Instrument Model XL-3100D, Denver Instrument, USA).

Chlorophyll fluorescence: Chlorophyll fluorescence measurements were carried out using a portable fluorescent monitoring system (Hansatech model FMS 2; Hansatech Instruments, England) on the first fully opened and exposed leaf at an interval of two weeks. Leaves were dark-adapted for 15 minutes, using the dark adaptation clips and then illuminated for six seconds to induce fluorescence. The leaves were continuously illuminated with a white actinic light (200 µmol m^{-2} s^{-1}). The initial fluorescence (F_0) and the maximum fluorescence (FM) were measured, and the variable fluorescence (F_v=FM-F_o) and the F_v/F_o ratio were calculated. The potential minimum efficiency of PSII (F_v/FM) of dark-adapted leaves was calculated as F_v/FM=(FM-F_o)/FM. The parameters of fast chlorophyll fluorescence, which were maximum fluorescence yield from PSII following a saturating pulse of photons in a light-adapted plant (FM'), steady state yield of PSII fluorescence in the light (F_s), and electron transport rate through PSII (ETR) (Belkhodja et al., 1999; Maricle et al., 2007), were determined during the day between 11:00 am and 1:00 pm. Data were collected between 9:30 am and 1:00 pm at light intensity (PAR) between 500 and 700 µmol photons m^{-2} s^{-1}.

Chlorophyll content: Chlorophyll content was determined using the methods of Arnon (1949) and Coombs et al. (1987). It involved 0.5 g of a fresh leaf tissue from the fourth youngest fully expanded leaf extracted in 10 mL of 80% acetone during seven days in the dark. The absorbance of the extract was measured using a spectrophotometer (Model Novaspec II, Pharmacia Biotech, Cambridge, England) at 645 and 663 nm in order to determine the content of chlorophylls *a* and *b* respectively. Total chlorophyll was calculated by adding chlorophylls *a* and *b*.

Statistical data analysis: Data were subjected to analysis of variance (ANOVA) using Costat statistical package for comparison of means. Significant means

were separated using the least significant difference (LSD) test at 5% level, and correlation analysis was performed to determine the relationship between variables at 5% level.

RESULTS

Salinity effects on seed germination: The salt treatments significantly ($p<0.01$) reduced germination as compared to the Control in both landraces (Figure 1A and B). Although both landraces did not attain 100% germination in the control, the effect of NaCl salinity was evident with increase in the electrical conductivity. Low salinity of 6.96 dS m^{-1} had less effect in Kk landrace than Mm, however the reverse occurred at higher salinity of 12.93 dS m^{-1} where Kk landrace was more affected. The highest salinity of 25.86 dS m^{-1} was so severe that no germination was recorded in both landraces.

Effects of salinity on root length: Salinity had a significant ($p<0.01$) effect on root lengths in both landraces. There were also significant ($p<0.01$) differences in the root lengths between the two bambara groundnut landraces. The root lengths in Kk landrace were more sensitive to salinity than in the Mm one. The effect started to be significant ($p<0.001$) at seven DAT (Table 1) and continued after 14, 21 and 28 DAT. Plants died at higher salinity treatments of 12.83 dS m^{-1} and above, hence there were no data recorded. However, the 6.96 dS m^{-1} treatment had low effect on Mm compared to the Kk landrace.

Effects of salinity on root dry weight: Salinity treatment had significant effect on the root dry weight (RDW) in both landraces at 21 and 28 DAT (Table 1). The control and the 6.96 dS m^{-1} plants showed no significant ($p>0.05$) RDW differences in Mm landrace from 35 to 42 unlike in the Kk landrace. There were significant ($p<0.05$) differences in RDW between the two landraces (Table 1).

Effects of salinity on shoot dry weight: The effects of NaCl treatment on shoot dry weight (SDW) of Kk and Mm landraces were significant ($p<0.01$), as seen in Table 1. Significant ($p<0.001$) differences began to be exhibited at 21 DAT for Kk and Mm landraces with control and 6.96 dS m^{-1} plants showing significantly ($p<0.01$) higher dry matter accumulation. By 42 DAT, there were generally no significant ($p>0.05$) differences in SDW of the control and the 6.96 dS m^{-1} plants of both landraces.

Effects of salinity on leaf area: The impact of salinity was significant ($p<0.001$) at 14 DAT in both landraces (Table 1). The plants in the control experiment in Mm landrace recorded the highest leaf area compared to all other treatments in the two landraces. Whereas plants under 6.96 dS m^{-1} treatment of Kk had no significant ($p>0.05$) differences in leaf area compared to control, those of Mm had a significant one. There was more salinity effect on 12.93 dS m^{-1} plants in Mm landrace as compared to Kk. Leaf area of plants subjected to the high electrical conductivity of 19.69 and 25.89 dS m^{-1} behaved similarly throughout the experimental period and were the most affected.

Effects of salinity on chlorophyll fluorescence parameters: The Kk landrace had higher F_V/F_M ratios at most treatments, however, the impact of the salt on the photosynthetic apparatus was significant ($p<0.001$) at 28 DAT (Figure 2A). The Mm landrace indicated lower ratios that were not significantly ($p>0.05$) different from the control (Figure 2B). The ETR was significantly affected by salinity in both landraces (Figure 3). Both Kk and Mm landraces had significant ($p<0.01$) differences by 14 and 28 DAT, respectively. The control maintained higher ETR compared to all other treatments for both landraces. All the plants in the 12.93, 19.89, and 25.86 dS m^{-1} treatments died by 28 DAT.

Effects of salinity on chlorophyll content: Salinity caused significant ($p<0.01$) reduction in chlorophylls *a* and *b*, and total chlorophyll content in both landraces, respectively (Table 2). Mm landrace recorded higher chlorophylls *a* and *b* and total chlorophyll content than Kk landrace. Mm landrace had significantly ($p<0.05$) more chlorophylls *a* and *b* and total chlorophyll content at the highest NaCl salinity level than Kk. Significant ($p<0.001$) differences in chlorophyll *a* content of Kk and Mm and chlorophyll *b* of Kk occurred at 14 DAT, while the same occurred in chlorophyll *b* and total chlorophyll of Mm and of Kk from 28 DAT. At the end of the experiment, the Kk control plants were generally significantly ($p<0.05$) different in chlorophylls *a* and *b* and in total chlorophyll content from the 6.96 dS m^{-1} plants, while in Mm the differences were not significant ($p>0.05$).

DISCUSSION

The observed reduction in percentage germination in both bambara groundnut landraces is attributed to the lowered osmotic potential of the germination medium, which makes the water less available for extraction by the seeds. The seeds were therefore exposed to a salt-induced physiological drought stress. The results indicate that both bambara groundnut landraces can tolerate

Table 1. The effect of NaCl salinity on root length, root dry weight, shoot dry weight, and leaf area in Kakamega and Mumias bambara groundnut landraces. Values are means of three replicates.

Landrace	Parameters	NaCl (dS m^{-1})	Days after salt application			
			0	14	28	42
Kakamega	Root length (mm)	0	103	170	210	210
		6.96	103	170	188	191
		12.93	102	160	185	-
		19.89	102	155	110	-
		25.86	102	137	92	-
		LSD (0.05)	0.4 ns	4.17 ns	1.88***	2.16 ns
	Root dry weight (g $plant^{-1}$)	0	0.16	0.43	0.60	0.83
		6.96	0.16	0.40	0.53	0.70
		12.93	0.15	0.37	0.20	-
		19.89	0.13	0.30	0.10	-
		25.86	0.12	0.30	0.10	-
		LSD (0.05)	0.11 ns	0.25 ns	0.09***	0.19 ns
	Shoot dry weight (g $plant^{-1}$)	0	0.4	1.0	1.2	1.4
		6.96	0.4	1.0	1.2	1.4
		12.93	0.3	1.0	0.4	-
		19.89	0.3	0.9	0.4	-
		25.86	0.2	0.6	0.2	-
		LSD (0.05)	0.2 ns	0.3 ns	0.22***	0.5 ns
	Leaf area (mm^2)	0	2247	8500	8620	8700
		6.96	2237	8000	8600	8670
		12.93	2233	7950	6000	-
		19.89	2223	4200	3810	-
		25.86	2220	4000	3800	-
		LSD (0.05)	75.51***	36.50***	32.04***	9.07 ns
Mumias	Root length (mm)	0	99.0	190	214	220
		6.96	99.0	160	205	216
		12.93	98.0	140	148	-
		19.89	98.0	139	127	-
		25.86	98.0	135	100	-
		LSD (0.05)	0.63	1.64***	1.8***	4.25ns
	Root dry weight (g $plant^{-1}$)	0	0.25	0.60	0.73	0.90
		6.96	0.23	0.60	0.73	0.90
		12.93	0.23	0.27	0.10	-
		19.89	0.23	0.20	0.10	-
		25.86	0.22	0.20	0.10	-
		LSD (0.05)	0.6	0.12***	0.07***	0.23 ns
	Shoot dry weight (g $plant^{-1}$)	0	0.4	0.4	1.4	1.4
		6.96	0.4	0.7	1.0	1.4
		12.93	0.3	0.7	0.5	-
		19.89	0.3	0.6	0.2	-
		25.86	0.3	0.6	0.1	-
		LSD (0.05)	0.14 ns	0.24 ns	0.38***	0.6 ns
	Leaf area (mm^2)	0	1727	9720	9460	9520
		6.96	1700	5620	7800	7843
		12.93	1693	5320	4680	-
		19.89	1693	4550	2930	-
		25.86	1680	4420	2920	-
		LSD (0.05)	59.34 ns	22.44***	101.4***	10.42***

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ns: $p > 0.05$, i.e., non-significant difference.

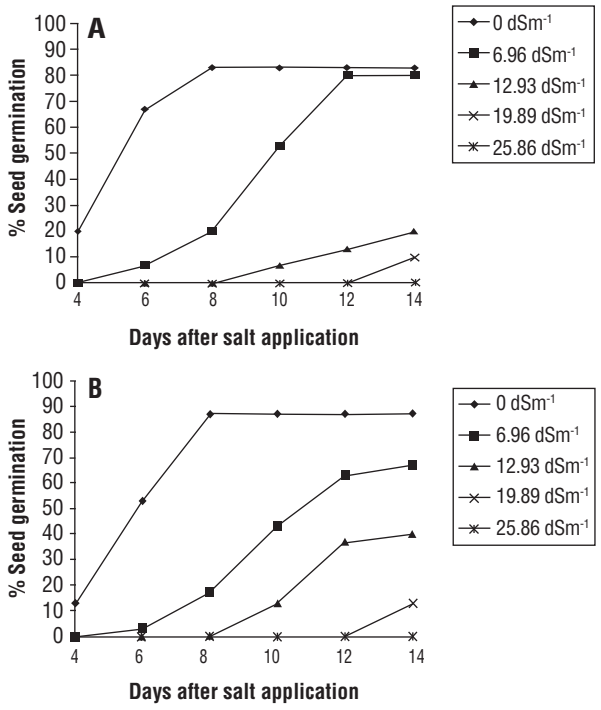

Figure 1. The effect of sodium chloride salinity on percentage (%) seed germination in Kakamega (A) and Mumias (B) bambara groundnut landraces. Values are means of three replicates. In A: LSD (5%): 0.81 at 4 DAT; 1.15 at 6 and 8 DAT; 1.41 at 10 and 12 DAT; and 1.63 at 14 DAT. In B: LSD (5%): 0.47 at 4 DAT; 1.15 at 6 DAT; 0.81 at 8 DAT; 1.69 at 10 DAT; 1.41 at 12 DAT; and 1.63 at 14 DAT. All LSD values are significant at $p \leq 0.001$.

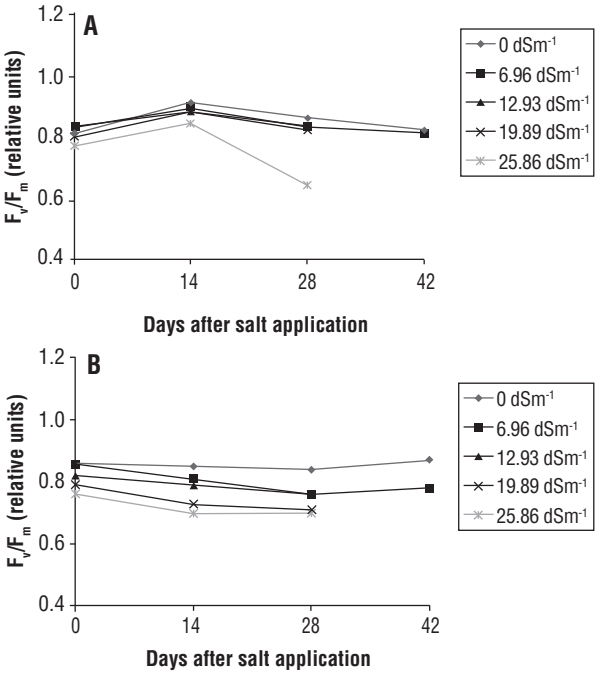

Figure 2. The effect of NaCl salinity on F_v/F_m ratio in Kakamega (A) and Mumias (B) bambara groundnut landraces. Values are means of three replicates. In A: Significant changes were found only at 28 DAT (LSD=0.07, $p \leq 0.001$). In B: Non-significant differences were found ($p > 0.05$).

up to 19.89 dS m^{-1} salinity during seed germination. It is noticeable that Mm landrace was less affected by higher NaCl salinity (12.93 and 19.89 dS m^{-1}) than the Kk one, indicating that seeds of Mm landrace were more osmotolerant compared to those of Kk. Reduced leaf growth due to salinity has been observed in plant species, such as beans and maize (Cramer et al., 1994).

The primary effect of salinity in many species is to reduce leaf growth rate, leaf emergence rate, and overall shoot development (Netondo, 1999). The reduction in leaf growth of plants exposed to salinity has been attributed to reduced turgor or reduction in extensibility of expanding cell walls (Neumann, 1993). This inhibition of leaf growth in the short-term may be due to water stress, while on long-term scale, leaf growth is affected by ion toxicity when the ions move through the transpiration stream and accumulate in the leaves (Yeo et al., 1991), which eventually leads to increased leaf mortality and senescence. Lack of vasculature to the meristems reduces transport of Na^+ and Cl^- ions to these cells and the fully expanded leaves that are ion sinks may abscise. Plants hence minimize exposure of these cells to the ions in the tissues. The reduced leaf area is an adaptation to reduce ion uptake by roots (Neumann, 1993). Plant development is affected since the reduced leaf area contributes to less photosynthesis, and hence less dry matter accumulation. Leaf growth in both landraces was generally higher during the first 14 days from the beginning of the experiment, which could suggest osmotic tolerance in these plants. Plants with high osmotic tolerance maintain high growth rates, particularly over the first few days after exposure to Na^+ (Munns and Tester, 2008). Salt-tolerant plants usually have lower cytoplasmic Na^+ concentrations than sensitive ones because of their efficient ability to sequester Na^+ into the vacuole (Mansour et al., 2005). Greater accumulation of Na^+ in plants could as well serve to increase the cell solute potential and hence increase their osmotic adjustment.

Shoot and root dry weights decreased with an increasing level of salt stress. Comparable results were obtained in sorghum (Netondo, 1999) and spider plants (Mwai, 2001), and in white seed coat bambara at high-salt treatment (200 mM NaCl) (Tafouo et al., 2008; 2010). In

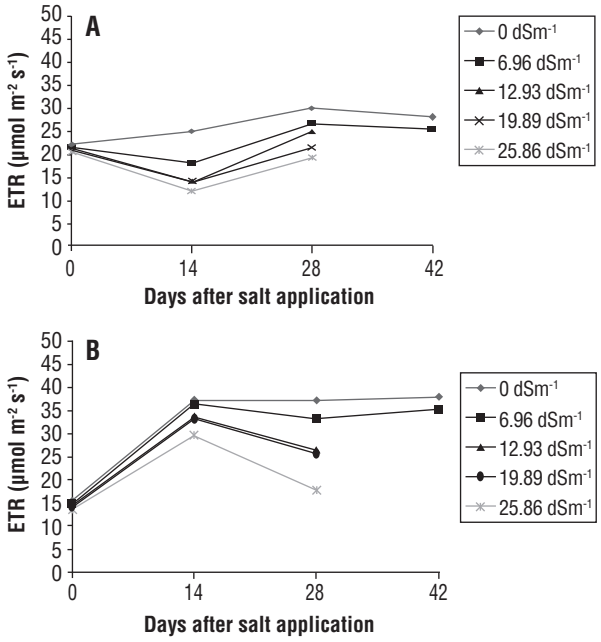

Figure 3. The effect of NaCl salinity on electron transport rate (ETR) in Kakamega (A) and Mumias (B) bambara groundnut landraces. Values are means of three replicates. In A: Significant changes were found 14 DAT (LSD=6.06, p≤0.01), at 28 DAT (LSD=5.90, p≤0.05), and at 42 DAT (LSD=3.91, p≤0.05). In B: Significant changes were found only at 28 DAT (LSD=7.29, p≤0.01).

this study, root dry weights significantly ($p<0.01$) declined in the highly stressed plants prior to death in plants exposed to 12.93, 19.89, and 25.86 dS m^{-1} treatments. The reduction of the plant dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl^- and Na^+ (Turan et al., 2007; Tafouo et al., 2010). A greater inhibition in growth in Mm than in Kk is an indicator of salt tolerance, since it in plants is well correlated with greater growth reduction in different tolerant plant genotypes (Mansour et al., 2005), which may help plants to save energy for maintenance of the processes. According to Alam et al. (2004), many nutrients have an essential role in the process of cell division and extension, and those would cease soon after the supply was halted, especially in tissues with little nutrient storage.

The reduction in shoot dry weight could also be associated with reduced rate of leaf production, hence low number of leaves leading to reduced photosynthesis and accumulation of dry matter. Root injury and death due to ionic toxicity may have affected water uptake by the plants and as a result increased water deficit in the plants leading to decreased net photosynthesis, which in turn may have affected shoot growth. Water deficit may as well occur as the result of lowered water potential of the soil solution and restricting root water uptake. Reduction in dry weight of plant tissues reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation (Netondo et al., 2004; Mansour et al., 2005). It also reflects salt impact on tissues, reduction in photosynthetic rates per unit of leaf area (Netondo et al., 2004), and attainment of maximum salt concentration tolerated by the fully expanded leaves (Munns et al., 2006). These findings agree with those from sorghum (Boursier and Läuchli, 1990). At high-salt concentration, 12.93, 19.89 and 25.86 dS m^{-1}, the plants could not regulate ion concentration, since there may have been severe physiological dysfunctions leading to decreased growth rates and eventually cell death leading to death of whole plant. Shoot and root damages caused by ion toxicity, osmotic effects or both may have contributed to the observed sharp drop in dry weights preceding the death of highly-stressed plants. Inhibition of long distance transport of nutrient ions by salinity has been proposed to explain the reduced nutrient content in the shoot due to displacement of K^+ and Ca^{2+} by Na^+ on the membranes, hence reduced shoot growth. Root elongation rate is reduced by salinity due to reduced rates of cell production and growth, reduced final length of epidermal cells, and shorter apical meristem (Zadeh and Naeini, 2007). Salt induced death of root cells has been reported in barley and this has been attributed to osmotically induced turgor loss and Na^+ ion toxicity in root meristem, causing reduced instant cell extension rates. Physiologically, reduction of root epidermal cell elongation and production may be attributed to accumulation of Na^+ to toxic levels in some of the meristematic cells. The reduced cell length as a result of salinity may be a result from reduced cell extension rates and or in the duration of extension period. Neumann et al. (1993) also reported inhibition of root growth in salt stressed maize from reduced extensibility of root tip tissues, due to hardening of the expanding cell walls.

The observed decline F_v/F_m ratio with increase in NaCl treatment may be attributed to the effects of the salts on the reaction centers of PSII system directly or through accelerated senescence (Lazár, 2006). Generally, the leaf photochemical efficiency of the PSII was not severely affected by salinity in both landraces except at the highest salinities (19.89 and 25.86 dS m^{-1}). This indicates that both landraces may withstand higher levels of salinity in the early stages of life, and that the PSII is not substantially damaged in the early stages of salinity exposure. The photosynthetic apparatus of Kk landrace maintained higher F_v/F_m ratios under high salinity except at later stages, when severe damage occurred. On the contrary, Mm landrace F_v/F_m ratio indicates slightly lower

Table 2. The effect of NaCl salinity on chlorophyll *a*, *b* and total content in leaves of Kakamega and Mumias bambara groundnut landraces. Values are means of three replicates.

Landrace	Parameters	NaCl (dS m^{-1})	Days after salt application			
			0	14	28	42
Kakamega	Chlorophyll *a* content (mg g^{-1}fresh weight)	0	2.66	3.30	2.60	3.36
		6.96	2.62	2.60	2.22	2.78
		12.93	2.59	2.60	2.12	-
		19.89	2.56	2.12	2.09	-
		25.86	2.52	1.45	0.85	-
		LSD (0.05)	0.17 ns	0.18*	0.18***	0.23**
	Chlorophyll *b* content (mg g^{-1}fresh weight)	0	1.99	2.38	2.07	2.47
		6.96	1.95	2.04	2.00	2.12
		12.93	1.92	1.93	1.73	-
		19.89	1.89	1.73	1.71	-
		25.86	1.85	1.25	0.69	-
		LSD (0.05)	0.17 ns	0.18***	0.18***	0.23**
	Total chlorophyll content (mg g^{-1} fresh weight)	0	4.65	5.68	4.67	5.83
		6.96	4.54	4.64	4.22	4.90
		12.93	4.48	4.53	3.85	-
		19.89	4.45	3.85	3.80	-
		25.86	4.37	2.70	1.54	-
		LSD (0.05)	0.29 ns	0.18**	0.18***	0.23*
Mumias	Chlorophyll *a* content (mg g^{-1} fresh weight)	0	2.85	3.30	2.88	3.36
		6.96	2.81	2.81	2.87	3.30
		12.93	2.78	2.79	2.20	-
		19.89	2.75	2.78	2.09	-
		25.86	2.71	2.69	1.45	-
		LSD (0.05)	0.20 ns	0.18***	0.18***	0.23 ns
	Chlorophyll *b* content (mg g^{-1} fresh weight)	0	2.09	2.38	2.23	2.46
		6.96	2.05	2.18	2.21	2.38
		12.93	2.02	2.15	1.76	-
		19.89	1.99	2.15	1.71	-
		25.86	1.95	2.12	1.25	-
		LSD (0.05)	0.15 ns	0.18 ns	0.18***	0.23 ns
	Total chlorophyll content (mg g^{-1} fresh weight)	0	4.94	5.68	5.11	5.82
		6.96	4.86	4.99	5.08	5.68
		12.93	4.80	4.94	3.96	-
		19.89	4.74	4.93	3.80	-
		25.86	4.66	4.81	2.7	-
		LSD (0.05)	0.31 ns	0.18 ns	0.18***	0.23 ns

*p≤0.05; **p≤0.01; ***p≤0.001; ns: p>0.05, i.e., non-significant difference.

photochemical efficiency of PSII that was not severely affected by high salinity levels. The decrease in F_v/F_m can be attributed to the downregulation of photosystem II activity and/or impairment of photochemical activity, which indicates damage in the functionality of the photosynthetic apparatus (Redondo-Gómez et al., 2007).

In general, ETR declined with increase in NaCl salinity. The ions could have affected the thylakoid membrane by disrupting the lipid bilayer or lipid-protein complex impairing electron transport activity. Salt stress in plants also induced higher concentration of reactive oxygen species (ROS) intermediates due to the impaired electron transport processes in chloroplast, mitochondria, and photorespiration pathways. The decreased photosystem II activity observed at higher salinity levels may have occurred due to leaves senescence process (Falqueto et al., 2010) leading to loss in photosynthetic pigments. The decrease in chlorophyll content under salt stress, as observed in this study, is commonly attributed to the adverse effects of the salts on membrane stability. Similar results have been reported before in sorghum (Netondo, 1999) and spider plant (Mwai, 2001). All the three (chlorophyll

a, *b* and total) parameters were found to be equally sensitive to increasing NaCl salinity, since they were reduced to comparable levels. Similar findings have been reported for curcubit species (Tafouo et al., 2008), bambara groundnut landraces (white seed coat) (Tafouo et al., 2010) and lentil plants (Turan et al., 2007). The effect of NaCl salinity is usually attributed to salt-induced weakening of protein-pigment-lipid complex and increasing chlorophyllase activity. Chlorophyll content of leaves is a useful indicator of both potential photosynthetic productivity and general plant vigor (Alonso et al., 2002). Chlorophyll content is widely used as a basis for determining photosynthesis, because the reaction components essential for photosynthesis (such as the reaction centers of PSI and PSII, electron carriers, and enzymes related to ATP synthesis and CO_2 fixation) are present in chloroplast at fixed molar ratios to chlorophyll. The maintenance of higher chlorophylls *a* and *b* and total in treatments 12.93 and 19.89 dS m^{-1} in Mm, though not significant, suggests that the chlorophyll pigments in Mm leaves were more tolerant to NaCl salinity. Furthermore, slightly higher chlorophylls *a* and *b* in Mm may be attributed to better light capture hence higher rates of photosynthesis.

Both bambara groundnut landraces seeds have tolerated up to 19.89 dS m^{-1} NaCl salinity during seed germination, with Mm appearing to be more salt tolerant at higher NaCl salinity (12.93 dS m^{-1}), while the Kk landrace was more tolerant at low NaCl salinity (6.96 dS m^{-1}). Increasing NaCl salinity inflicted damage to the plants of the two landraces to different extents. Mm landrace was clearly more salt-tolerant at 12.93 dS m^{-1} as indicated by seed germination, while leaf area was more tolerant in Kk than in Mm. The leaf photochemical efficiency of the PSII and ETR were also not severely affected by salinity in Mm landrace, but both landraces survived in saline environments up to 19.89 dS m^{-1}. The differences in F_v/F_m ratio and ETR between the two landraces from this study may not be conclusive enough to say whether Mm landrace is more tolerant to salinity than the Kk one. The results indicate that leaf area and seed germination may serve as suitable parameters for screening bambara groundnuts to salt tolerance in future breeding programs. This study opens up further perspectives for research in osmotolerance of other locally cultivated landraces in order to understand the mechanisms of tolerance to salt stress, including but not limited to osmotic adjustment.

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