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Research Article

Impact of Salinity Stress on Ion Homeostasis of Some Selected Groundnut (*Arachis hypogaea L.*) Varieties

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ABSTRACT
Groundnut (*Arachis hypogaea L.*) is one of the most important leguminous crops in Africa. Nigeria is the largest groundnut producing country in West Africa. Salinity stress is one of the major abiotic constraints hindering groundnut production. This study was carried out to analyze the impact of salinity stress on ion homeostasis of some selected Groundnut varieties. Ten groundnut varieties (*Samnu*t 22, 23, 24, 25, 27, 28, 29, *Bahaushiya*, *Maibargo* and *Kwankwasiyya*) were collected from Center for Dryland Agriculture (CDA) Bayero University, Kano. The groundnut varieties are planted and irrigated with four different concentrations of salt-water solutions (0 dsm/m^2, 3.5 dsm/m^2, 6.5 dsm/m^2 and 9.5 dsm/m^2) for three months. The varieties treated with 9.5 dsm/m^2 was observed to record significantly higher Na^+ and lower K^+ concentrations, while Na^+/K^+ ratio was observed to be higher with the increase in salinity concentration. *Maibargo* consistently exhibited low Na^+ concentrations across all salinity levels, indicating effective Na^+ exclusion mechanisms. However, its K^+ concentration dropped significantly under salinity stress, suggesting that its tolerance is primarily due to Na^+ exclusion rather than K^+ retention. *Kwankwasiyya* maintained high K^+ concentrations under salinity stress, indicating strong K^+ retention mechanisms. *Samnut 23* and *Samnut 29* accumulated high Na^+ concentrations and showed significant reductions in K^+ concentrations, indicating poor ion homeostasis. The study demonstrated that salinity stress significantly increased Na^+ concentration, Na^+/K^+ ratio and reduced K^+ concentration. Varietal differences played a crucial role in determining tolerance to salinity, with *Maibargo* and *Kwankwasiyya* showing the most promise for cultivation under saline conditions.

Keywords: Groundnut; Homeostasis; Ion; Salinity; Samnut; Stress; Varieties

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INTRODUCTION

Groundnut (*Arachis hypogaea L.*) is one of the most important leguminous crops in tropical cropping systems in Africa in which it is grown mostly by small scale farmers Nyabyenda (2005). Nigeria is the largest groundnut producing country in West Africa, accounting for 51% of production in the region. Nigeria contributes 10% of total global production and 39% in Africa. Between 1956 and 1967, groundnut was the country's most valuable single export crop, exemplified by the famous Kano groundnut pyramids (Ajeigbe *et al.,* 2014). However, in the later period, the combined effects of soil salinity as a result of excessive use of fertilizers and irrigation system of farming in some areas and also inadequate rain in semi-arid regions of Nigeria to leach out salt, have caused a decline in groundnut production (Mensah *et al.,*2006).

The significant increase in Nigerian population raises groundnut demand which led Nigerian farmers to adopt dry season irrigation system of cultivation (Ajeigbe *et al.,* 2014). The practice continues increasing significantly over the last few

years. (Ajeigbe *et al*., 2014) also suggested that dry season groundnut production is usually done where there is source of water for irrigation, the major problem is the heavy accumulation of salt in the soil due to irrigation which is a serious threat to the future groundnut productivity. (Mensah *et al.,* 2006) suggested that: soil salinity and drought are the major abiotic constraints responsible for low yield of groundnut in Nigeria. Kafi and Goldani (2000) has earlier reported that irrigated lands in the arid northern parts of the country are also increasingly becoming saltier due to over fertilization and may soon be faced with the salinity problem and become unfavorable for groundnut cultivation in the near future.

The constraints of groundnut production include the decrease in harvested area due to the competition with other commodities and land conversion Sumarno (2015).The conversion of optimal agricultural land to non-agricultural land in Northern Nigeria per every year should be seriously taken into consideration (Mulyani *et al*., 2016) and scientific research should be done to develop new varieties, so that groundnut development in future should be more focused to the sub-optimal land, including saline soils. Saline soil is a soil which contains soluble salt or their ions at least in one horizon at above the toxicity threshold with electrical conductivity (EC) of above 4 dS/m (Vargas *et al*., 2018). Salinity is known to induce stress in groundnut; hence the ability of groundnut to tolerate and thrive in saline soils is of great importance in agriculture, since it indicates that the groundnut plant has genetic potential for salt tolerance, (Mahmood *et al*., 2000). Salinity which causes reductions in yield is one of the important abiotic constraints to groundnut production.

Salinity and physiology of groundnut plant

Salinity interferes with water absorption leading to osmotic stress; it enhances accumulation of Na and Cl ions which at higher concentration may lead to cytotoxicity, impaired enzymatic function and imbalance of other elements. Under high salinity conditions, ion imbalance takes place by disturbing the osmotic homeostasis in salt sensitive plants, which can be sensed rapidly. As a result, these plants are not able to manage an optimal ion transport ratio, which should be high potassium ions and low sodium ions for normal growth of plants (Munns and Tester, 2008). Primarily, roots are affected by osmotic imbalances or water deficit created by high salt concentration which restricts nutrients entrance (Munns, 2002). Excessive soluble salt in the soil, mainly sodium (Na^+) and chloride (Cl^-) ions (Ismail *et a*l., 2014; Chang *et a*l., 2020), lead to both osmotic stress and ion stress during plant growth and development (Yang and Guo, 2018b). The osmotic stress not only compromises the ability to take water, but also leads to rapid closure of stomata, which reduces the assimilation of carbon dioxide (Hedrich and Shabala, 2018). The ion stress caused by over accumulation of Na^+ and Cl^- in plant cells is harmful to plant metabolism and the physicochemical properties of the cell wall (Munns and Tester, 2008; Cheeseman, 2013; Endler *et a*l., 2015; Zhang *et a*l., 2016). Both osmotic and ionic stress can promote secondary stress such as oxidative damage in plants (Genisel *et a*l., 2014; Hazman *et a*l., 2015; Li *et a*l., 2015). Moreover, osmotic stress-induced stomatal closure and ion stress impair the photosynthetic machinery (Zhao *et a*l., 2020), which is the major mechanism by which salt-stress inhibits plant growth (Bose *et a*l., 2017). Prolonged high salt soil exposure then leads to leaf necrosis, chlorosis, senescence and enzymatic degradation resulting in the loss of yield (Munns and Tester, 2008, Rahnama *et a*l*.,* 2010).

The tolerance to salinity was likely related to the ability of the groundnut genotypes to inhibit the Na uptake and its translocation to the shoot, resulting in high K/ Na ratio in the shoot thus would increase photosynthetic process of the plants. The ratio of Na-root/Na-shoot was also an effective indicator as K/Na ratio in the shoot, therefore it can be used as a new selection criteria for groundnut tolerance to salinity stress.

The high salinity environments can be combated by growing salt tolerant groundnut variety developed to have various tolerance mechanisms, such as by the exclusion of excess sodium ions from the cytoplasm, or their accumulation in vacuoles by their tissue using Atomic Absorption Spectroscopy (AAS). The main objective of this study is to determine the effects of different levels of salinity on different groundnut varieties and also to identify the variety that is tolerant to salinity through evaluation of their ability to exclude Na^+ and K^+ retention. This may provide the information to the groundnut breeders for their breeding activities, where a salinity tolerant variety may be developed so that our saline soils can be utilized.

MATERIALS AND METHODS

Experimental Site

The phenotypic experimental trials were carried out at the screen house of the Department of Plant Biology Bayero University, Kano old campus. The area is savannah zone with long dry season (November-April) and wet season (May-October) and total annual rainfall of 700-980mm, and elevated approximately 480 meters above the sea level. The low temperature season start from December-February with temperature range of 29-

33^0C and peak hot season in April (39-42^0C). While the physiological parameters were evaluated at the Center For Dry Land Agriculture Laboratory Bayero University, Kano New campus.

Germplasm Collection

Ten groundnut varieties, *Samnut 22, Samnut 23, Samnut 24, Samnut 25, Samnut 27, Samnut 28, and Samnut 29, Maibargo, Bahaushiya*, and *Kwankwasiyya*, were collected from the Center for Dryland Agriculture (CDA), Bayero University, Kano Nigeria. The dry pods of the groundnut varieties were packed in a paper envelop and taken to the screen house for planting.

Soil Sampling

The soil was sampled from Janguza correctional centre farms, located at Janguza Tofa Local Government Area Kano State. One hundred and twenty empty plastic bags were filled with 25kg of the soil and taken to the screen house for planting. Some portion of the soil was taken to laboratory for soil analysis.

Experimental Design

Ten groundnut varieties were laid in a Completely Randomized Design (CRD) with four replications (where one replication was used as destructive sampling), four treatments including control. Four seeds were planted in each pot, and the seedlings was later thinned to three per each pot at 2 weeks after emergence. The whole experiment was conducted with four different concentrations (treatments) of salt-water solutions as irrigation water, i.e. 0 ds/m^{-1} (control), 3.5 ds/m^{-1}, 6.5 ds/m^{-1}, and 9.5 ds/m^{-1} of NaCl.

Treatment and Preparation of Salt Solution

Four different concentrations of salt-water solutions (0 dsm/m^{-1}, 3.5 dsm/m^{-1}, 6.5 dsm/m^{-1} and 9.5 dsm/m^{-1}) was prepared and imposed to the groundnut plants in the amount sufficient to saturate the soil at field capacity. Salt-water solution (irrigation water) was prepared by using standard method of salt solution preparation. To confirm the accuracy of the concentration, Electric Conductivity (EC) Meter was used to measure the concentration of the solution. This procedure adopted the method of Skoog *et a*l., (2017)

Seed Planting

Four seeds were planted in each pot and later thinned to 3 seedlings. One groundnut seed were planted per hole at 5-6cm depth in the soil in a plastic bag of 50kg capacity. This was practiced by Vadez *et,al*., (2005)

Measurement of Leaf Na^+ and K^+ concentration

This was measured to determine the extent at which groundnut variety absorb Na^+ from the soil. Higher Na^+ concentration in the leaf tissue indicates higher sensitivity to salinity. Na^+ concentration in leaves was determined as follows: 150 mg of finely groundnut leaf was harvested from each pot at 35 days after planting. The surface of the leaves was rinsed in distilled water to wash away the contamination by handling or by splashing of saline solution. The leaves was transferred to a small envelopes by handling with rubber gloves and then dried in an oven at 60◦C– 70◦C for 2 days. The dried leaves was later grounded using grinding machine (KN 295 Knife tcc ™) at Center for Dry Land Agriculture (CDA) Laboratory, Bayero University, Kano. It was made to a fine powder to have a homogenous representative sample for ion analysis. The grounded leaf samples was digested by sampling 0.5g using weighing scale (COLE-PALMER INSTRUMENT PA 120×0.0001g) and transferred to digestion tubes, and 6ml of Nitric Acid, 2ml of H_2SO_4 and 2m of H_2O_2 was added. The mixture was gently shaken and transferred to digestion machine (2006 Digestor Foss Tecator) at 200^OC for 75 minutes and the digest was diluted to 75ml. Filter paper was used to filtrate the digest in a plastic bottle. Prior to using AAS machine, the machine was checked to ensure that the "U" tube is full of water (checked by giving a squeeze) and make sure that the nebulizing tube is in a beaker full of de-ionized (DI) water. The air and gas power was switched on and the ignition switch was held until the flame is strong. Then the machine was set to Na^+ and allowed for 30 min for the machine to warm up and stabilize. All the standards was aspirated to obtain a linear standard curve calibrated by measuring a 'blank' solution consisting of only the dilute acid and no tissue at the start and end of each set for the determination of baseline absorption measurement. The absorption solution was measured to 4, 6, and 8mg/L of Na^+ and calibration curve was created. The calibration curve determines the relationship between the absorbance of the light and the concentration of the element in the solution. This curve follows the Beer-Lambert Law. The samples was aspirated until a steady reading is obtained. Approximately after every 10 samples standard was repeated so as to check that the machine has not drifted. After finishing, the tube was left in DI water for about 10 min. Then, the gas switch was switched off, then air, followed by the power. For the determination of K^+ the same procedure was followed, but the machine was switched or set to K^+ detection. This was done following the procedure of (Muuns *et,al.,* 2010; and Vadez; *et,al*., (2005)

Determination of Leaf K^+ /Na^+ Ratio

This was calculated from the result of Na^+ and K^+ concentrations obtained from AAS analysis. The calculation was done using online Na^+ and K^+ calculator. This was reported by Hnilickova *et al*., (2019).

Data Analysis

The pot experiment was arranged in Completely Randomized Design with three different salt concentrations and three replicates. Data was presented in term of mean (± standard deviation). Multiple comparisons of several means were used using analysis of variance (ANOVA) and LSD test at 5%. Multiple comparisons of data in experimental groups versus those recorded in the single control group was used using DMRT (Duncan's Multiple Range Test).

RESULTS

Results of the effects of different levels of salinity and variety on Leaf Na^+ concentration and Leaf K^+ concentration of different groundnut varieties are presented in Table 1. Significant difference were observed for both salinity levels and groundnut varieties on different parameters. Groundnut variety treated with 9.5 dsm/m^2 was observed to record significantly higher Leaf Na^+ concentration (24.52mmol/L). However, Leaf K^+ concentration was observed to record significantly higher in groundnut varieties treated with 3.5 dsm/m^2 (61.53mmol/L).

With respect to varietal responses, *Samnut 23* has significantly recorded a greater concentration of Na^+ (15.64mmol/L) when compared to other varieties. Leaf K^+ concentration was also observed to be significantly higher in *Kwankwasiyya* variety with (81.66mmol/L).

The results of the salt stress and varietal interaction on Leaf Na^+ concentration and Leaf K^+ concentration are presented in Table 2. The results showed that interaction for Na^+ was significant ($P \leq 0.05$) when varieties were treated with 9.5 dsm/m^2 for *Samnut 23* and *Kwankwasiyya*. For Leaf K^+ concentration, the interaction was significant ($P<0.005$) in *Kwankwasiyya* when treated with 9.5 dsm/m^2. Treating varieties with 6.5 dsm/m^2, produced significantly higher leaf K^+ concentration in *Kwankwasiyya* ($P \leq 0.05$). While treating varieties with 3.5 dsm/m^2 concentration, the interaction was significant in the *Kwankwasiyya* variety also.

Table 1: The effects of salinity and variety on Leaf Na^+ Concentration (mmol/L), Leaf K^+ concentration (mmol/L) and Leaf Na^+K^+ Ratio

Factor	LF.Na^+(mmol/L)	LF.K^+(mmol/L)	LF.Na^+K^+RATIO
Salinity(dsm/m^2)			
Control	4.46 ^{c}	74.18 ^{a}	0.056 ^{d}
3.5	4.75 ^{c}	61.53 ^{b}	0.070 ^{c}
6.5	11.31 ^{b}	60.17 ^{b}	0.176 ^{b}
9.5	24.52 ^{a}	53.37 ^{c}	0.404 ^{a}
Varieties			
SAMNUT 22	9.38 ^{d}	63.94 ^{bcd}	0.162 ^{d}
SAMNUT 23	15.64 ^{a}	59.74 ^{d}	0.267 ^{a}
SAMNUT 24	12.54 ^{b}	67.64 ^{bc}	0.192 ^{c}
SAMNUT 25	12.50 ^{b}	59.06 ^{d}	0.150^{d}
SAMNUT 27	11.22 ^{c}	68.55 ^{b}	0.162 ^{d}
SAMNUT 28	10.67 ^{c}	61.81 ^{cd}	0.170 ^{d}
SAMNUT 29	11.12 ^{c}	68.64 ^{b}	0.155 ^{d}
BAHAUSHIA	11.10 ^{c}	62.61 ^{bcd}	0.197 ^{c}
MAIBARGO	6.07 ^{e}	49.44 ^{e}	0.090 ^{e}
KWNWASIYA	12.37 ^{b}	81.66 ^{a}	0.217^{b}
Interaction			
TRT x VAR	0.001	0.001	0.001
S.E	0.4544	3.978	0.013

Means followed by the same superscripts within the same column do not differ significantly ($P \geq 0.05$).
KEY: LF.Na^+ Conc.= Leaf Sodium Ion Concentration, LF.K^+ Conc.= Leaf Potassium Ion Concentration.

Table 2: Interactive effect of different concentrations of salt and varietal responses on leaf Na^+ concentration (mmol/L) and leaf K^+ concentration (mmol/L).

	LF.Na^+CONC.

Trtm/Var	Control	3.5 dsm/m^2	6.5 dsm/m^2	9.5 dsm/m^2
SAMNUT 22	4.90 $^{n-q}$	4.49 $^{n-r}$	8.60 ^{k}	19.51 ^{ef}
SAMNUT 23	3.90 ^{qr}	6.49 ^{lm}	18.29 ^{fg}	33.87 ^{a}
SAMNUT 24	3.92 ^{qr}	5.43 $^{m-q}$	14.46 ^{i}	26.34 ^{c}
SAMNUT 25	5.47 $^{m-p}$	4.17 ^{pqr}	10.35 ^{j}	30.01 ^{b}
SAMNUT 27	4.61 $^{n-r}$	5.15 $^{m-q}$	10.21 ^{j}	24.92 ^{d}
SAMNUT 28	4.10 ^{pqr}	4.39 $^{o-r}$	13.69 ^{i}	20.51 ^{e}
SAMNUT 29	4.35 ^{pqr}	5.89 $^{l-o}$	18.05 ^{g}	16.17 ^{h}
BAHAUSHIYA	5.95 ^{lmn}	4.45 $^{n-r}$	10.14 ^{j}	23.84 ^{d}
MAIBARGO	3.32 ^{rs}	1.71 ^{t}	2.29 st	16.95 ^{gh}
KWANKWASIYYA	4.05 ^{pqr}	5.29 $^{m-q}$	6.99 ^{l}	33.13 ^{a}
		LF. K$^+$CONC.		
SAMNUT 22	62.00 $^{f-i}$	69.58 $^{c-g}$	78.80 $^{b-e}$	45.39 ^{k}
SAMNUT 23	68.66 ^{efg}	59.62 $^{g-j}$	50.74 ^{ijk}	66.71^{d-g}
SAMNUT 24	82.27 ^{abc}	64.02 $^{f-i}$	61.82 $^{f-i}$	62.47 $^{f-i}$
SAMNUT 25	73.66 $^{b-f}$	42.67 ^{k}	67.18 $^{d-g}$	52.74 $^{h-k}$
SAMNUT 27	79.36 $^{b-e}$	67.74 $^{d-g}$	60.40 $^{f-j}$	49.55^{i-j}
SAMNUT 28	65.88 $^{e-h}$	60.07 $^{f-j}$	62.42 $^{f-i}$	58.86 $^{g-j}$
SAMNUT 29	68.30 $^{d-g}$	61.88 $^{f-i}$	78.11 $^{b-e}$	66.29 $^{e-h}$
BAHAUSHIYA	70.91 $^{c-g}$	70.21 $^{c-g}$	60.82 $^{f-j}$	48.51 ^{jk}
MAIBARGO	92.50 ^{a}	72.68 $^{b-g}$	23.24 ^{l}	9.35^{a-d}
KWANKWASIYYA	93.24 ^{a}	85.64 ^{ab}	82.71 ^{abc}	80.07^{m}

Means followed by the same superscripts within the same column do not differ significantly (P≥ 0.05).
KEY: LF.Na$^+$ Conc.= Leaf Sodium Ion Concentration, LF.K$^+$ Conc.= Leaf Potassium Ion Concentration

Table 3: Interactive effect of different concentrations of salt and varietal responses on leaf Na$^+$/K$^+$ ratio

		LF.Na$^+$K$^+$RATIO		
Trtm/Var	Control	3.5 dsm/m^2	6.5 dsm/m^2	9.5 dsm/m^2
SAMNUT 22	0.070 $^{g-j}$	0.060 $^{g-k}$	0.100 ^{g}	0.420 ^{c}
SAMNUT 23	0.050 $^{h-k}$	0.100 ^{g}	0.360 ^{d}	0.560 ^{a}
SAMNUT 24	0.040 ^{ijk}	0.080 ^{ghi}	0.230 ^{e}	0.420 ^{c}
SAMNUT 25	0.070 $^{g-j}$	0.090 ^{gh}	0.150 ^{f}	0.560 ^{a}
SAMNUT 27	0.050 $^{h-k}$	0.070 $^{g-j}$	0.160 ^{f}	0.370 ^{d}
SAMNUT 28	0.060 $^{g-k}$	0.070 $^{g-j}$	0.210 ^{e}	0.340 ^{d}
SAMNUT 29	0.060 $^{g-k}$	0.090 ^{gh}	0.230 $^{e.}$	0.240 ^{e}
BAHAUSHIYA	0.080 ^{ghi}	0.060 $^{g-k}$	0.160 ^{f}	0.230^{c}
MAIBARGO	0.023 ^{k}	0.030 ^{jk}	0.080 ^{ghi}	0.490^{b}
KWANKWASIYYA	0.050 $^{h-k}$	0.060 $^{g-k}$	0.080 ^{ghi}	0.410 ^{c}

Means followed by the same superscripts within the same column do not differ significantly (P≥ 0.05).
KEY: LF.Na$^+$ Conc = Leaf Sodium Ion Concentration, LF.K$^+$ Conc. = Leaf Potassium Ion Concentration

DISCUSSIONS

Salinity stress disrupts ion homeostasis by increasing Na$^+$ uptake and reducing K$^+$ uptake, leading to a high Na$^+$/K$^+$ ratio. This imbalance affects enzyme activity, protein synthesis, and overall plant growth, as reported by Munns and Tester (2008), and Flowers and Colmer (2015). In the present study, *Maibargo* consistently exhibited low Na$^+$ concentrations across all salinity levels, indicating effective Na$^+$ exclusion mechanisms. However, its K$^+$ concentration dropped significantly under salinity stress, suggesting that its tolerance is primarily due to Na$^+$ exclusion rather than K$^+$ retention. This aligns with findings of Munns and Tester (2008), who highlighted the importance of Na$^+$ exclusion in salinity tolerance. *Kwankwasiyya* maintained high K$^+$ concentrations under salinity stress, indicating strong K$^+$ retention mechanisms. This is consistent with studies of Ashraf and Harris (2013), who reported that

maintaining high K^+ levels is crucial for enzyme activity and osmotic adjustment under salinity stress. *Samnut 23* and *Kwankwasiyy*a accumulated high Na^+ concentrations and high Na^+/K^+ ratios which was indicative of poor salinity tolerance as a result of poor ion homeostasis. These varieties may lack efficient ion exclusion or antioxidant mechanisms, as described by Ashraf and Harris (2013). This is also, consistent with the findings of Hasegawa *et a*l. (2000) who noted that sensitive varieties often lack efficient ion exclusion and retention mechanisms.

The significant interactive effects highlight that varietal responses to salinity were not uniform, and depended on the severity of salt stress. This underscores the importance of evaluating varietal performance under different stress levels. With regard to adaptive mechanism, varieties with better performance under stress likely possessed adaptive mechanisms such as improved osmotic adjustment, antioxidant activity, and ion exclusion.

CONCLUSION

The study demonstrates that salinity stress significantly increases Na^+ concentration, Na^+/K^+ ratio and reduces K^+ concentration. Significant difference ($P \leq 0.05$) was observed among all the varieties, with *Maibargo* showing the lower Na^+ concentration and lower Na^+/K^+ ratio while *Kwankwasiyya* showing the higher K^+ concentration compared with control. The result indicate that *Maibargo* has the superior performance of Na^+ exclusion and *Kwankwasiyya* with K^+ retention and these characters shows that the groundnut varieties possess traits for salinity stress tolerance. These findings highlight the importance of selecting and breeding salt-tolerant varieties to enhance groundnut productivity in saline-affected regions. Traits such as Na^+ exclusion and K^+ retention can be introgressed into high-yielding varieties using marker-assisted selection.. Future research should focus on elucidating the physiological and molecular mechanisms underlying these varietal differences to further improve salinity tolerance in groundnut. And also in saline-affected areas, farmers can adopt MAIBARGO and KWANKWASIYYA to minimize yield losses. Additionally, soil management practices such as leaching of salts, use of organic amendments, and drip irrigation can further mitigate salinity stress.

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