EVALUATION OF MORPHOPHYSIOLOGICAL, BIOCHEMICAL AND YIELD RESPONSES OF BAMBARA GROUNDNUT LANDRACES TO SODIUM CHLORIDE SALINITY

BY

JANE GAYALWA AMBEDE

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DECLARATION

I certify that this thesis has not been previously submitted for a degree at any one university. The work contained herein is original and has been carried out by myself and all sources of information have been specifically acknowledged by means of references.

Jane Gayalwa Ambede

PHD/SC/00032/2016

Sign……………………………. Date…………………………

Declaration   by the Supervisors

This thesis has been submitted for examination with our approval as the University supervisors.

Prof. Godfrey Wafula Netondo, PhD

School of Physical and Biological of Sciences

Department of Botany

Maseno University

Sign……………………………. Date…………………………

Prof. David Mutisya Musyimi, PhD

School of Physical and Biological of Sciences

Department of Botany

Maseno University

Sign……………………………. Date…………………………

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DEDICATION

This work is devoted to God; without whom I can do nothing.

ABSTRACT

Soil salinity limits crop growth, development and productivity in agricultural soils worldwide and contributes to food insecurity. It is induced by accumulation of high levels of particularly sodium and chloride ions within the rooting zone. Bambara groundnut {*Vigna subterranea* (L.) Verdc} is underutilized and fairly neglected indigenous African food legume with potential to alleviate food and nutritional insecurity in tropical regions of Africa. The crop has potential to be grown in semi-arid areas or under irrigation, both of which offer potentially saline conditions. The effect of sodium chloride (NaCl) salinity on growth and physiology of this plant continues to attract research, more so on the locally grown landraces. There are still unanswered questions on the effect of NaCl salinity on photosynthetic pigments, compatible solutes, nodulation and yield. Therefore, the overall objective of the study was to evaluate morphophysiological, biochemical and yield responses of Bambara groundnut landraces in response to NaCl salinity. The specific objectives were to determine the effects of NaCl salinity on growth, gas exchange (transpiration rate (Tr), stomatal conductance (gs) and CO_2 assimilation rate(Cr)), mineral nutrients (sodium, potassium and calcium), proline, leaf pigments (chlorophyll a, b, total chlorophyll and carotenoids), nodulation and yield parameters in Bambara. Experiments were laid out in a greenhouse at Maseno University in a completely randomized design, involving 15 factors of 5 NaCl salinity treatments: (0/control, 2, 4, 6 and 8) in dSm^{-1}, and 3 Bambara groundnut landraces. There were 3 replications. Ten large similar sized seeds of three local landraces: red seed coat (RSC), white (WSC) and black (BSC) were each sown in a 20-liter pot containing moist loam soil with pH of 4.7. All the seeds were coated with *Bradyrhizobium* strain USDA 110, to enhance biological nitrogen fixation of the seedlings. Thinning was done 7 days after emergence, leaving 5 plants per pot. NaCl salinity treatments commenced 7 days after thinning. Data collection commenced on the 3rd day after initiating salinity treatments and was repeated after every 2 or 4 weeks. Plant height (PH), root length (RL), width and length of leaf were measured using a meter rule, and leaves and branches were counted. Seedling fresh and dry weights were measured using electronic weighing balance. Leaf area (LA), root to shoot ratio (R:S) and percent water content (%WC) were calculated. Gas exchange parameters were determined using portable infra-red gas analyzer. Plant mineral nutrient, proline and leaf pigment content were determined using atomic absorption spectrometry, colorimetric assay and spectrophotometer respectively. Nodules, pods and seeds were counted. Data were subjected to analysis of variance using the SAS Statistical Computer Package and separation of means using the Least Significance Difference at 5% level. Plant growth parameters, PH, LA, leaf number, shoot and root fresh weights of the three landraces significantly ($p \leq 0.05$) reduced as NaCl salinity increased. Increase in salinity had adverse effect on leaf pigments, and number of pods and seeds in all the landraces. Salinity did not influence root dry weight (RDW) of the RSC and BSC landraces. There were significant ($p \leq 0.05$) interactions between NaCl salinity treatments and Bambara such that the number of branches and Na^+ content increased in all the landraces. Salinity increased shoot dry weight (SDW) in salinity treatments of 2, 4 and 6 dSm^{-1} in RSC landrace, RDWin salinity treatments of 4, 6 and 8 dSm^{-1} in WSC landrace, RL in salinity treatment of 2 dSm^{-1}in all the landraces, R: S ratio in salinity treatment of 4 dSm^{-1} in WSC and BSC land races, and 2, 4 and 6 dSm^{-1} in RSC landrace, and %WC in salinity treatment of 2 dSm^{-1} in WSC and 4 dSm^{-1}in BSC landrace. Salinity also increased K^+ and Ca^{2+}in salinity treatment of 2 dSm^{-1}in all the landraces, and carotenoid content in salinity treatment of 2 dSm^{-1} in WSC landrace. Cr, gs, Tr and number of nodules significantly increased in salinity treatment of 2 dSm^{-1} in all the landraces however Cr, Tr and number of nodules also increased in RSC landrace atsalinity treatment of 4 dSm^{-1}. Increased proline content in all the landraces under salinity could be an indicator of salt tolerance due to osmotic adjustment. Salinity negatively influenced morphophysiological, biochemical and yield responses of Bambara groundnut landraces. The RSC landrace responded better to NaCl salinity on average followed by BSC and least was WSC. The three landraces can do well where soil NaCl salinity reaches 4dSm^{-1} electrical conductivity.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

ABA	:	Abscisic acid
ANOVA	:	Analysis of variance
BSC	:	Black seed coat
Chl a	:	Chlorophyll a
Chl b	:	Chlorophyll b
DAT	:	Days after Treatment
DAS	:	Days after Sowing
DNA	:	Deoxyribonucleic acid
EC	:	Electrical Conductivity
^{0}C	:	Degrees Celsius
KNBS	:	Kenya National Bureau of Statistics
KSB	:	Potassium solubilizing bacteria
Lsd	:	Least significant difference
NaCl	:	Sodium chloride
pH	:	Hydrogen concentration
PS11	:	Photosystem 11
ROS	:	Reactive oxygen species
RSC	:	Red seed coat
RuBP	:	Ribulose bisphosphate
RUBISCO	:	Ribulose bisphosphate carboxylase/oxygenase
t Chl	:	Total chlorophyll
WC	:	Water content
WSC	:	White seed coat

LIST OF SYMBOLS

Ca^{2+} -   Calcium ions

Cl^- -   Chloride ions

CO_2 -   Carbon (iv) oxide

cm   -   Centimeter

dSm^{-1} / dS/m   -   Desi Siemens per meter

HCO_3^- -   Hydrogen carbonate ions

g   -   Gram

$CaSO_4.2H_2O$ -   Gypsum

K^+ -   Potassium ions

Kg   -   Kilogram

Km^2 -   Kilometer square

mg   -   Milligram

Mg^{2+} -   Magnesium ions

N_2 -   Nitrogen

Na^+ -   Sodium ions

Na_2SO_4 -   Sodium sulphate ions

O_2 -   Oxygen

%   -   Percent

SO_4^{2-} -   Sulphate ions

LIST OF TABLES

LIST OF APPENDICES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Agriculture production in Kenya has stagnated since 1980s resulting in poverty and malnutrition in over 50% of Kenya's population (KNBS, 2014). Food insecurity has been identified as the prime cause of malnutrition in many Kenyan households (Kimani-Murage *et al*., 2015). Despite the rich variety of plant genetic resources, serious food insecurity and malnutrition problems persist in Kenya(Ambede *et al*., 2012). Land for agriculture is an increasingly shrinking resource and the entire country depends on only about 20% suitable arable land (Attibu, 2014). As the population of the world continues to rise (World Population Prospects, 2019)global farming is tested in meeting its food requirements. Kenya's equally rapidly increasing population is currently estimated at 47.7million (KNBS, 2019). Growth in population, poor living standards and lack of employment necessitate that the country's agricultural potential be fully developed hence need to diversify production of food and increase yield per given land area for the consumption by family or as income source which is a basic pre-requisite for improved household security (Chapagain and Raizada, 2017).

Salinity affects crop production in various parts of the world. The most common inorganic ions that cause soil salinity include Na^+, Mg^{2+}, Ca^{2+}, K^+, Cl^-, $SO_4^{2-}$and HCO^{3-}.The soluble salts accumulate in the rooting regions to levels that interfere with growth and survival of plants(Harmon and Daigh, 2017).Higher levels of ionic salts, in particular Na^+ and Cl^- make up almost 50% to 80 % of total soluble salts in such soils. Low water potential and elevated electrical conductivity (EC) are also features of saline soils (Kumar *et al*., 2020).The EC of such soils may exceed 4 dSm^{-1} because of excessive levels of NaCl and sodium sulphate (Na_2SO_4) salts (Egermerdieva *et al*., 2019). The acceptable limit of EC that does not harm crops is <0.7 dSm^{-1}. However, when the EC is above 3.0 dSm^{-1}crop productivity is severely affected.

Salinity restricts crop growth and productivity by altering morpho-physiological and biochemical processes (Egamberdiyeva *et al*., 2019). Key processes in plants including photosynthesis, lipid and energy pathways, growth and synthesis of proteins are therefore affected (Parida and Das, 2005). This is revealed via ion imbalances, osmotic stress, deficiency in nutrients and disturbances incarbon and nitrogen metabolism (Kumar *et al*., 2020). Soil respiration, nitrogen fixation, biodiversity as well as microbiological activity are also affected by salinity (Egermerdieva *et al*., 2019). The ultimate result is retarded crop growth and yield (Kumar *et al*., 2020).Salinity may also lead to agricultural losses that are hard to quantify, however believed to

be considerable and likely to rise over time (Ghassemi-Golezani *et al*., 2010). The cost of salinity to agriculture globally is around $US 12 billion per year and it is likely to rise as soils deteriorate further (Drammeh, 2015). Additional grave effects of salinity occur on social structure, infrastructure, steadiness of communities and supplies of water.

Soils containing excessive soluble salts or high quantities of Na^+, become unproductive as well as unmanageable. To ease influences of salinity on crop productivity in saline soils several attempts have been made however with varying achievement (Dimkpa *et al*., 2009). Use of inorganic (synthetic) and organic fertilizers on saline soils may be expensive. Synthetic fertilizers may also be a source of pollution. Munns and Tester (2008) suggested development of crops tolerant to salt as one of the desired scientific goal of alleviating salinity stress, but this has attained little success (Fita *et al*., 2015). Other approaches involve conventional physical methods of remediation of salt degraded soils. Such include flushing, scraping, leaching which have equally proved to be ineffective (Dimkpa *et al*., 2009). Similarly, chemical methods including applying gypsum ($CaSO_4.2H_2O$), one of the major sources of Ca^{2+}that improves soil water infiltration and reclaims toxic Na^+ soil, and lime as neutralizing agents are unsustainable. Although restricted worldwide, efforts have also turned on to growing salt tolerant crops like canola and barley in salt stressed areas (Egermerdieva *et al*., 2019). Currently, microorganisms involved in symbiotic relationships with plant roots significantly ease crop stress in saline environments because they tolerate extremities, interact with agricultural plants in addition to their potential utilization strategies (Dimkpa *et al*., 2009). These microbes improve nitrogen fixation and crop productivity, as a result reclaim the saline agroecosystems. Increased NaCl salt in the soil may be well or practically managed by 'physiological basis of tolerance' of crop plants for enhanced salt tolerance (Flowers and Colmer, 2015**;** Munns and Gilliham, 2015). Therefore, selecting and breeding of crops that are able to grow providing economic yield under saline areas provide more stable complementary solution to reduce negative impacts of salinity.

As an indigenous African food crop, Bambara groundnut, {*Vigna subterranea* (L.) Verdc} (Mohammad *et al*., 2020)remains a fairly neglected and underutilized legume (Ambede *et al*., 2012). The crop is drought tolerant, relatively resistant to diseases and pests, produces reasonable yields on relatively low fertile soils and has high nutritional value (Ambede *et al*., 2012). Small holder farmersin Western, Nyanza and Coastal parts of Kenya grow it. Indeed, its nutritional value is quite high with seeds containing nearly 63 % carbohydrate, 19 % proteins including essential amino acids (lysine, cysteine and methionine), 6.5 % fat and high in fiber and minerals such as calcium, potassium and iron (Ogodo *et al*., 2018). Though greatly valued for its food and nutritional security (Harouna *et al*., 2018)in rural areas, it is occasionally considered as food for

poor persons. The seed fetches good prices for the farmers who grow it for commercial purposes. Bambara plant can also withstand up to moderate sodium chloride salinity (Ambede *et al*., 2012). In the symbiotic relationship with stress tolerant *Bradyrhizobium* strains in nodulation process, the plant may fix atmospheric nitrogen therefore enhancing fertility of the soils (Bationo *et al*., 2018) and in the course of the crop microbe interaction, plant productivity is improved under saline environments (Dimkpa *et al*., 2009).Despite its usefulness, there is limited scientifically documented information on growth and physiology of the plant under NaCl salinity. It is thus of interest to assess the influence of subjecting Bambara groundnuts to sodium chloride salinity which may help in part to expound on the extent of tolerance when the crop is cultivated in potentially salty environments.

The worldwide demand for Bambara groundnuts is increasing compared to the production (Tan *et al*., 2020)). Sodium chloride salinity may hinder Bambara crop production (Ambede *et al*., 2012) particularly in areas potentially affected by salinity. The plant grows in regions exposed to different stresses such as soil salinity leading to very low variable harvests(Tadele, 2018)**.**Seed yield in African countries, including Kenya, is still low and it is affected by both biotic and non-biotic changes in cultivated regions and the landraces (Bonny *et al*., 2019). Since Bambara is grown in areas that may be affected by NaCl salinity such as the coastal region, the landraces currently being grown in less salt affected areas such as Kakamega and Mumias, could also be grown in saline soils of the Kenyan Coast (Ambede *et al*., 2012). It also has potential to be grown in drier regions under irrigated or rainfed cultivation. The potentially saline areas in Kenya have been mapped to be in North Eastern, Coastal, Nyanza, Eastern and Rift Valley (Netondo, 1999; Mwai, 2001; Musyimi, 2007) that may support Bambara production and improve on food security in the country.

Salt stress affects general legume growth and productivity by disrupting hormonal interactions, nutritional stability and via osmotic and toxicity of ions (Patil *et al*., 2016). In legumes, growth is reduced when salts interfere with the plant itself or with nutrient assimilation consequently yield decrease (Nadeem *et al*., 2019).Growth parameters were reduced in Bambara on exposure to NaCl salinity (Ambede *et al*., 2012). Furthermore, growth of shoot and root, biomass of plant, and internode and pod numbers in soybean were reduced (In-Jung Lee*et al*., 2019). However, under salt stress, growth and yield in French beans were improved (Kumar *et al*., 2020). Symbiotic interactions may help maintain balance of the hormones, like auxin to cytokinin levels in the course of germination and earlier plant growth; hence play a major part in ordering the genes regulating growth even in saline environments (Kumar *et al*., 2020; Chu *et al*., 2019). The extent to which sodium chloride salinity affects growth parameters remains inconclusive in most

plants including Bambara groundnuts. Mineral nutrient accumulation within plants are affected by salt tress. The high quantities of salts particularly, Na^+ and Cl^- within the rooting region under salinity leads to imbalances in nutrients such as calcium, potassium as well as magnesium in plants (Nadeem *et al*., 2019). Absorption and uptake of K^+ and Ca^{2+} by crops is prevented because of the surplus Na^+ ions leading to sodium-potassium antagonism (Toffauo *et al*., 2010). In Bambara, salt stress led to an increase in Na^+ ions in plant organs of all the landraces (Toffaou *et al*., 2014) with toxicity site in most crop plants being mainly leaf blades where Na^+ accumulates. The high levels of Na^+ in shoots for most crop varieties brings about deleterious effects such that ionic stress leads to early senescence, and necrosis and chlorosis toxicity signs in older and mature leaves of crops respectively (Munns and Tester, 2008).Sodium chloride salinity led to high levels of Na^+ ions that compete with K^+ binding proteins (In-Jung Lee *et al*., 2019) reducing synthesis of proteins and thus K^+ influx and Na^+ expulsion is the most significant plant strategy for reducing salt stress. It is worthwhile to assess the impact of salinity on distribution or accumulation of mineral nutrients in different parts of the plant body. This may help to further the understanding of effects of NaCl salinity on mineral nutrient accumulation in most crops including Bambara when grown in potentially saline conditions.

Gas exchange parameters are a common practice in investigations of plants exposed to salinity. Stomatal conductance is a very sensitive stress indicator in wheat and sorghum exposed to salt stress (Acosta-Motos *et al*., 2017).Stomatal conductance, internal CO_2 concentration and rate of transpiration reduced when bean plants (Amira *et al*., 2015) and *Brassica juncea* (Arif *et al*., 2013) were subjected to NaCl salinity leading to reduction in photosynthesis, a factor controlling productivity in plants(Arif *et al*., 2013).Stomatal conductance, photosynthetic rate and activities of enzymes are disturbed by excessive salt levels (Kumar and Verma, 2018).Non-stomatal (including injury to the photosynthetic machinery) and stomatal (closing of stomata) factors may be connected with the lowering of CO_2 assimilation (Xu *et al*., 2016). Studies involving salinity have to be extended to gas exchange parameters in plants including Bambara groundnuts in order to further understand the mechanisms involved.

Plant leaf pigments such as chlorophyll content is a useful indicator for overall plant vigor and potential photosynthetic efficiency (Golan *et al*., 2015). Salinity alters chlorophyll and carotenoid content and hence reduces photosynthetic capacity of the plant (Abdelhamid, *et al*., 2011). The salt may induce degradation of pigment synthesizing enzymes (D'souza and Devaraj, 2013). The decreased chlorophyll content and feeble leaves observed are indicators of salt induced chlorophyll damage accounting for the low photosynthesis in salt stressed plants (Ahmed and Ahmad, 2016; Avila-Sakar *et al*., 2018). Reduction in leaf pigments i.e. Chl a, b and total

chlorophyll under salt stress indicate its possible degradation as a caused by increased cytosol Na$^+$ levels (Kumar *et al*., 2017). According to Sharma *et al*., (2012) low salinity had no effect on carotenoid but increased chlorophyll content in chick pea suggesting that carotenoids may play a significant role in protecting photosynthetic apparatus. Such contradicting findings point to inconclusive understanding of the effect of NaCl salinity on photosynthetic pigments. Thus, Bambara groundnuts may be a good candidate for further investigation.

Osmolytes are produced in response to salinity. One of the osmolytes produced is proline. Water and salt stress are known to induce accumulation of proline in plants (Verbruggen and Hermans 2008). The elevated proline levels may aid in the maintenance of plant cell water status and thus assist plant survive under stress. Microorganisms too improve symbiotic nitrogen fixation in crops, more so allow the plants produce osmolytes like proline more easily easing the consequences of salt stress enhancing tolerance in leguminous species (Avila-Sakar *et al*., 2018). Proline may promote activity of different enzymes, destroy reactive oxygen species and thus sustain antioxidant activity, and stabilize cell pH (Verbruggen and Hermans 2008). Due to the important physiological role played by proline, it may be necessary to determine the extent of its accumulation in Bambara groundnuts. Plants subjected to NaCl salinity help to expound partly on the crop's level of tolerance when cultivated in potentially saline soils.

Various researches give account of how salinity interferes with nodulation in leguminous plants. For instance, salinity was shown to interrupt various stages of symbiosis initiation, production and functionality of the nodule thus interfering with the entire nodulation mechanism(Dwivedi *et al*., 2015).Salts damage symbiotic interactions, restricting production of nodules, resulting in reduced nodule number and plant growth(Avila-Sakar *et al*., 2018). Salinity affects biological nitrogen fixation and uptake of nitrogen in legumes hence interfered with density and activity of nodules resulting in early nodule senescence. Bambara groundnuts being leguminous plant may be affected by NaCl salinity in a similar or different way. This needs to be ascertained.

Yields in plants are generally reduced under salinity. Salinity affects physiological functions impeding crop production (Khan *et al*., 2017). For instance, reproductive parameters (number of flowers and weight of seed) were significantly reduced in *Cajanus cajan* under salt stress (Ahmed and Ahmad, 2016) and ultimately the yield. Salinity stress reduced the final yield in chick pea because of shriveled seeds and low grain weight (Serraj *et al*., 2007).The impact of salinity on the absorption and movement of nutrients disturbs yield in crops in saline conditions (Shi-Ying *et al*., 2018). Thus, salt stress leads to yield losses in various legumes (Farooq *et al*., 2018). The extent of reduction in yield as for leguminous plants subjected to salinity varies. It

may also vary among varieties/landraces. This does not exclude Bambara groundnuts, hence needs to be established.

1.2 Statement of the Problem

Salinity is one of the major problems limiting crop productivity in Kenya. Salinization of agricultural areas is extensive occurring mainly in semi-arid, irrigated and low-lying poorly drained regions, such as parts of North Eastern, Coastal, Nyanza, Eastern and Rift Valley in Kenya (Attibu, 2014; Ambede *et al*., 2012; Netondo, 1999).The effect of climate change threatens to increase the problem further by causing areas that were initially not saline to be in the group. If these areas could be exploited by the production of salt tolerant food crops, then Kenya's food security would be improved. Thus, as the arable land continue to decrease, other than those growing in potentially saline areas like parts of the coastal region, Bambara landraces growing at present in the non-saline areas could also be grown in such areas. Salinity restricts growth, development and productivity by altering morphological, physiological and biochemical processes (Ambede *et al*., 2012). It interferes with major plant processes such as photosynthesis, energy and lipid metabolism, and protein synthesis ultimately growth, survival and yield of plants. However, plants differ greatly in the way they tolerate salinity. Although Bambara has sustained human nutrition for generations, the crop remains underutilized and fairly neglected as an African indigenous food legume.

It has received little attention through scientific research despite its potential to alleviate poverty, malnutrition and contribution to food security. As Kenya's population continue to grow, there is pressure on land for diversified food production and increased yield. To realize this objective, research on Bambara groundnuts that have the potential to be grown in many agroecological zones including salinized areas become significant. Researches on growth in crops subjected to salinity have yielded contradictory outcomes. This necessitates further investigation to understand better the effects of NaCl salinity in crops including Bambara groundnuts. Salinity affects uptake and final accumulation of mineral nutrients in most crops. Differential accumulation of these nutrients in Bambara leaves may partly explain salinity tolerance mechanisms when cultivated in potentially saline environments. To understand better the mechanisms involved, studies on salinity should be extended to gas exchange parameters in plants such as Bambara groundnuts. The effect of NaCl salinity on photosynthetic pigments in most crop plants including Bambara groundnuts is not clear. Even though, plants tolerate salt stress through synthesis of osmotic balancing proteins such as proline, it may be necessary to determine the extent of proline accumulation in Bambara groundnuts. This helps to partially explain the tolerance level of plants when grown in potentially saline soil. Salinity interferes with

nitrogen fixation, thus disrupting nodule activity and numbers. The extent to which Bambara groundnut, a legume, is affected by NaCl salinity requires further investigation. As with legumes exposed to salt stress, the extent of yield reduction varies between varieties and landraces. This must also be established in Bambara groundnuts. The study intends to evaluate morphophysiological, biochemical and yield responses of Bambara groundnut landraces to sodium chloride salinity.

1.3 Justification of the Study

The increasing demand for food as a result of the rapid population growth necessitate full development of the country's agricultural potential. Salinity is a serious impediment to crop productivity in agricultural regions round the world (Ambede *et al*., 2012; Egermerdieva *et al*., 2019) resulting in food shortage and production. Salt affected land compriseover 800 million hectares of agricultural land around the world (Kumar *et al*., 2020) and this increase is posing a serious threat to global agriculture. Food security would be improved in Kenya when salt-tolerant food crops are grown. Research on salt tolerance of the crop is one of the possible approaches to bring saline or potentially saline areas under cultivation. Furthermore, this will enhance the productivity of subsistence farmers and improve on their standards of living.

Bambara groundnut though regarded as a food for poor people, is highly valued in parts of Kenya /Africa. It has positive qualities such as being drought tolerant, production of reasonable yields on low fertile soil and resistant to pests and diseases (Mohammad *et al*., 2020). Its nutritional value is quite high, especially in proteins. It is a highly valued crop for its food and nutritional security in rural areas. Salinity limits plant growth and restricts physiological responses of plants hence the need to evaluate the effects of sodium chloride salinity in Bambara landraces commonly grown in Kenya. Due to their great genetic variation and adaptability, the plant is a good candidate for research in its salt tolerance, which could assist identify cultivars suitable to be grown in the saline agro-ecological zones and as a result advice on the growing of the most tolerant and high yielding landrace.

Salinity may enhance accumulation of proline, potassium and calcium in plants. These assist plants to increase performance and survive in hostile conditions. Research has not sufficiently reflected on their accumulation in Bambara groundnut landraces when exposed to sodium chloride salinity.

Data on growth, mineral nutrition and gaseous exchange parameters of Bambara groundnuts may help in understanding the relationship between mechanisms of salt tolerance and nitrogen fixation thereby providing new avenues for better crop productivity. Research on Bambara groundnuts

addressing nodulation, pigment content and yield parameters aspects of NaCl salinity tolerance is also required.

Results obtained from this research may help understand effects of sodium chloride salinity hence the mechanisms employed by Bambara groundnuts in their tolerance and provide a basis for breeding and improvement on crop salt tolerance. The findings from the study may be used by plant breeders to generate sodium tolerant landraces.

1.4 Objectives

1.4.1 General Objective

To investigate the effects of sodium chloride salinity on morphophysiological, biochemical and yield responses of Bambara groundnut landraces.

1.4.2 Specific Objectives

i. To determine the effects of sodium chloride salinity on growth parameters in Bambara groundnut landraces.

ii. To determine the effects of sodium chloride salinity on mineral nutrient content and gas exchange parameters in Bambara groundnut landraces.

iii. To determine the effects of sodium chloride salinity on leaf pigment, proline content and nodulation in Bambara groundnut landraces.

iv. To investigate the effects of sodium chloride salinity on yield in Bambara groundnut landraces.

1.5. Hypotheses

i. Sodium chloride salinity has no effect on growth parameters of Bambara groundnut landraces.

ii. Sodium chloride salinity has no effect on physiological parameters of Bambara groundnut landraces.

iii. Sodium chloride salinity has no effect on biochemical parameters of Bambara groundnut landraces.

iv. Sodium chloride salinity has no effect on yield of Bambara groundnut landraces.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bambara Groundnut

Bambara groundnut belongs to the Genus: Vigna and Species: *Vigna subterranea* (L.) Verdc (Grönemeyer *et al*., 2016)**.**Some of its local names are njugu mawe (Kiswahili), tsimbande (Luhya) and bande (Luo) (Ambede *et al*., 2012). Although tolerant to drought, Bambara groundnut food crop indigenous to Africa (Mohammad *et al*., 2020) remains underutilized and fairly neglected legume. It is a legume that can withstand up to moderate sodium chloride salinity (Ambede *et al*., 2012). The landraces vary in many ways, including colors of pods and seeds as well as growth habits that differ from bunching to semi-bunching to spreading. Lack of seeds, unsuitable varieties, pod losses during harvesting and superstitions related to traditional preferences and practices have contributed to the limited cultivation of this crop in Africa (Tsamo *et al*., 2018). Hybrid cultivar breeding is challenging because of small blooms. Its cultivation on large scale is rare. Other major challenges associated with low production of the crop include drought, diseases, low germination as a result of poor seed storage and un-improved cultivars (Ambede *et al*., 2012). The crop continues to attract scientific research in various aspects such as NaCl salinity as findings have never been conclusive.

2.2 Ecological Requirements and Production of Bambara Groundnuts

Bambara groundnut grows well on light sandy loam well-drained soil with a pH of 5.0 to 6.5 (Ambede *et al*., 2012). Calcareous soils discourage its growth. An altitude not exceeding 1600 m and daily temperature range of 20 ^{o} C to 28 ^{o} C together with evenly distributed precipitation of 600mm to 700 mm optimize growth of the cultivar. However, crop damage occurs at times of harvest if there is excessive rain (Umesha, 2015). Large and healthy seeds are recommended for sowing at 20mm to 30 mm depth and a spacing of 100mm to 150 mm in a row 450mm to 900 mm apart (Tsamo *et al*., 2018). Good yields are obtained from well levelled seedbeds in deeply tilled grounds (Masideni, 2006). Excessive nitrates may encourage vegetative growth at the expense of the grain yield (Ikenganyia *et al*., 2017). Information on fertilizer requirements is limited, while rhizobial inoculation is practiced in some areas (Laurette *et al*., 2015).Studies on Bambara symbioses in many regions of the globe show its non-selectivity in its nutritional requirements. It also has unrestricted nodulation ability with diverse groups of rhizobia (Onyango *et al*., 2015) making it a 'promiscuous' host. This promiscuity in symbiosis is advantageous as legumes form efficient associations with several rhizobia strains, allowing them to adapt freely to various niches (Onyango *et al*., 2015).

West Africa accounts for about 150,000-160,000 tones or 45-50 percent of global Bambara groundnut production (Ambede *et al.*, 2012)). Nigeria is the largest producer of Bambara groundnuts, with an annual mean of 0.1 million tones, followed by Burkina Faso (44,712 tones) and Niger (30,000 tones) (Tan *et al.*, 2020).In Kenya, Bambara is cultivated mainly in Western, Nyanza and Coastal regions by small holder farmers (Ambede *et al.*, 2012). In Kenya, it is grown in areas that may be affected by sodium chloride salinity. It is potentially suitable for cultivation in areas that are dry or semi-arid under rainfed or irrigated cultivation. Effects of NaCl salinity remain unclear in crops including Bambara groundnuts calling for further research.

2.3 Usesof Bambara Groundnuts

As a nourishing food, Bambara groundnut contains adequate amounts of proteins, carbohydrates, lipids and iron in the range of 2.0-10.0 mg/100g unlike most legumes(Oyiga, 2015). Traditionally Bambara products like cakes or biscuits are baked from seed flour. Furthermore, porridge is produced from a mixture of cereals and the flour. The seeds together with maize may be boiled or roasted (Honi *et al.*, 2016). In Kenya the beans are roasted, minced and used to make soup. Fresh seeds may be eaten or seeds may be grilled while young. The flour is used in bread making. Research has established its potential use in different food products like weaning food, vegetable milk and processed products, and it has even turned out that mashed Bambara seeds can be used as coagulants in solar water disinfection (Wambete and Mpotokwane, 2003). The seeds have been used to feed poultry while their haulms, vegetative plant part also give nutritious hay for livestock feeding. After groundnut (*Arachis hypogea*) and cowpea (*Vigna unguilata*), Bambara is rated the third important leguminous food crop and second underground pod after the groundnut in Africa (Smýkal *et al.*, 2015). In nodulation process, exposure to strains of *Bradyrhizobium* improve soil fertility as atmospheric nitrogen is fixed, thus Bambara is useful in crop rotations and as an intercrop with cereals (Babalola *et al.*, 2017). Bambarais a significant food crop in African traditional agriculture. However, no substantial technological efforts to improve it have taken place and as a result farm yields are still low. It remains fairly ignored and under-utilized food legume in Kenya and more often it is regarded to as a food for poor persons. It has also received little consideration through research.

2.4 Definition, Origin and General Distribution of Salinity

Salinity may refer to the presence of inorganic ions in high levels within the soil. The salts accumulate within the root zone to levels that interfere with productivity of plants (Harmon and Daigh, 2017). Salinization of cultivated lands is caused mainly by high

Na^+ and Cl^- levels in the soil (Bharti *et al*., 2016; Shi-Ying *et al*., 2018). Such soils usually have high pH above 7, low soluble Ca and occur in poorly drained areas where soils are shallow and precipitation is limited. Soil minerals in the open earth's crust slowly release the salts through chemical weathering involving hydrolysis, hydration, solution, oxidation, reduction and carbonation (Netondo, 1999; Vance *et al*., 2016).The salts also originate from volcanic eruptions, discharge from deep thermal sources and oceans. These salts are distributed from their areas of origin mainly by water either as surface run-off or as ground water streams usually accumulating in the poorly drained valley basins. Soils also become saline through saline seeps, tidal waves, salt sprays, irrigation using saline water or irrigation and accumulation of salts in areas of poor drainage (Netondo, 1999; Shrivastava and Kumar 2015).

Natural salt accumulation (primary salinization) is caused by excessive salts in the parental matter whereas secondary salinization results from activities of man such as unsuitable practices in irrigation. In arid and semi-arid regions where crop production involves irrigation, secondary salinization (Nadeem *et al*., 2019) is particularly common hence a major concern for global food production. Regardless of the current improved management techniques, enormous pieces of land are and continue to be salinized globally (Ritzema, 2016). Salinity problem is intensified by malpractices in agriculture. This therefore continues to harshly decrease crop productivity.

Worldwide, more than 800,000,000 hectares of cultivated land is possibly saline (Kumar *et al*., 2020), about 20% under irrigation (Egamberdieva *et al*., 2019;Nadeem *et al*., 2019) nearly 43.6 million hectares in Africa (Flowers, *et al*., 2010) and almost 25 million hectares in Kenya (Attibu, 2014). The situation is expected to worsen as more areas of the semi-arid and arid lands are increasingly being put under cultivation. Out of a total area of 582,646 km^2,Kenya's land area is almost 80% dry or semi-arid, with the bulk of it being saline. Salinity in these regions might arise from: (i) excessive evaporation (ii) vicinity to the sea (iii) over-irrigation (iv) irrigation water containing dissolved inorganic ions (v) poor water management and (vi) low precipitation (Shrivastava and Kumar, 2015). Even some areas that receive adequate rainfall have the potential to become saline. Irrigation and drainage have had limited success in improving the productivity in these soils (Wichelns and Qadir, 2015). Therefore, crop plants including Bambara groundnuts that have the potential can be manipulated for growing in salinized soils become important.

2.5 Effects of Salinity on Crop Growth and Productivity

Usually, salinity produces dwarfed, stunted plants with dull colored leaves frequently covered with deposits of wax (Ambede *et al*., 2012). High salt levels in the root zone may induce: cell

turgor loss followed by growth reduction or direct plant death through marginal burns, necrotic spots, defoliation depending on severity of salinity (Bawa, 2016). In pigeon pea, growth parameters reduced with salinity possibly through imbalances in nutrients, injurious ions and shortage of water (Ahmed and Ahmad, 2016).Also, soybean exposed to increasing salinity reduced or inhibited nodulation consequently plant growth. Productivity in crops is thus influenced by salinity (Parihar, *et al*., 2015). High NaCl levels restrict water and air movement, and porosity of the soil (Egamberdiyeva *et al*., 2019). Additionally, it controls physio-chemical properties of soil decreasing soil health. Thus, poor growth in plants subjected to salinity results from decrease in circulation of nutrients, ROS generation, imbalances in hormones among others (Kumar *et al*., 2020).

The level of injury caused by salts and possible death varies from one species to another and even between varieties in the same species subject to factors like age, concentration of salt, species level of salt tolerance (Yu *et al*., 2016). However, bean plant height substantially increased (Egamberdieva, 2011) and Faba bean improved growth under saline conditions (Metwal *et al*., 2015) as a result of enhanced growth of roots promoting enormous surface area that improved absorption of nutrients. Similarly, under saline conditions productivity in crops and fertility of the soils increased (Grover *et al.*, 2011). Although salinity adversely affects growth responses varying leaf morphology, root length and shoot to root ratio in plants (Ambede *et al*., 2012), symbiotic interactions may amend the effect of salinity and improve plant growth. They increase growth in plants through production and regulation of phtyto-hormones such as auxins and cytokinin (Qin *et al*., 2009). Higher auxins and cytokinins quantities are associated with enhanced plant growth, cytokinins maintain totipotency in cells within the growing regions and gibberellins promote growth and yield in plants under salinity (Howell *et al*., 2003). For instance, more gibberellins and auxins were produced in rice leading to greater crop productivity (Bottini *et al*., 1989). This may assist to expound on crop salt tolerance when cultivated in possibly saline environments.

Growth is crucial in plants because survival and reproduction are dependent on plant size, and thus on the rate of growth. Salt interference on growth and productivity in plants is therefore a complicated process relating to water stress, toxicity of ions as well as nutritional effects (Negrão *et al*., 2016). Thus, sodium and chloride ions may cause considerable damage substantially decreasing growth and yield in crops (Lodeyro *et al*., 2016). Therefore, investigating the effects of osmotic, ionic stress, and nutritional imbalances in plants like Bambara groundnuts could enhance the understanding of the effects of NaCl salinity.

2.5.1 Osmotic Effects of Salinity

Plants subjected to salt stress suffer osmotic stress involving disruption in water relations. Soil salinity reduces water accessibility by plants (Munns, 2002). High levels of salt in the growth media interfere with or damage growth and physiology in plants through water, ionic stress and imbalances in nutrients (Egamberdieva *et al*., 2019). Growth inhibition was consequently reported under salt stress in peas, chickpea, faba, mung beans and in *Cicer arietinum* (Ahmed and Ahmad, (2016). High salinity disturbs vital structures in cells (Egamberdieva *et al*., 2019) subsequently reducing growth in plants. The high quantities of salts in cytosol challenge vacuole capability to compartmentalization, disturb division of cells and cell elongation, injure the cells upsetting more processes in growth (Julkowska and Testerink, 2015). For instance, salt accumulation in the root cell walls cause cell protoplasts dehydration (Munns, 2002). However, when the accumulation of salts surpasses the capacity of cells to store, it causes dehydration of tissues and ultimately death of plant (Kang *et al*., 2014).

The osmotic potential of the soil medium and the content of the salts directly inhibit growth in plants (Parihar *et al*., 2015). On exposure to salt stress, the plant immediately suffers osmotic dehydration and shock. The water in the plant tends to move out, the cells decrease in their volume and their water potentials fall leading to decreased cell turgor and growth. Osmotic dehydration therefore leads to depressed growth, reduced rate of photosynthesis, transpiration and limited carbon dioxide fixation (Munns, 2005). However, with time accumulation of salts within leaves to toxic levels results in necrosis decreasing the leaf photosynthetic surface causing further reduction in growth (Farooq*et al*., 2018). Salinity also decreases water supply to the roots because of osmotic effects resulting in decreased plant growth and yield (Parihar *et al*., 2015). Although most salt tolerant species control the accumulation of inorganic ions as a basic mechanism to adjust their internal tissues osmotic potential against external salinity, further research would clarify the extent to which Bambara groundnuts employ this mechanism as a survival response.

2.5.2 Nutritional Effects (Mineral Deficiencies) of Salinity

Under saline conditions, the large quantities of Na^+ and Cl^- ions lead to imbalances in nutrients (Talei *et al*., 2012). Salinity affects accessibility of other nutrients, plant mineral uptake, movement and partitioning causing imbalances in nutrients (Cardi *et al*., 2015) eventually impedes growth. Ionic imbalances caused by the presence of salts, lead to reduced osmotic potential that affect the physiology and biochemistry of plants resulting in general growth reduction (Parihar *et al*., 2015).Toxicity by Na^+ and Cl^- ions under saline environments disrupts normal soil ratios of different mineral nutrients (Dong *et al*., 2015; Turan *et al*., 2007). The

excessive Na^+ ions, can lead to K^+ shortage and cause harmful effects by upsetting K^+ controlled processes and stimulate toxic alterations in protein structure (Kärkönen and Kuchitsu, 2015). The decrease in growth due to salinity has been partly explained by a suppression of nutrient absorption caused by Na^+ and Cl^- ion uptake in competition with more nutrient ions on the plasma membrane (Munns and Tester, 2008) which may arise from Na^+ and K^+ competing over binding sites that are important in physiology of cells. Over 50 enzymes are made active by use of ion K^+ hence its role cannot be taken by ion Na^+ (Karkonen and Kuchitsu, 2015). In plant cells, sustaining cytosol K^+ionsin a surrounding with elevated Na^+ion levels are a factor defining the capability to tolerate salinity (Munns and Tester, 2008).Hence, the key mechanism of salt tolerance employed by salt tolerant plants generally depend on their abilities to sequestrate toxic ions i.e. Na^+ and Cl^- in the vacuoles (Munns, 2002) and the consequence of these salts is believed to decrease biomass and yields in plants. Excessive Na^+ and Cl^- ions in the soil led to reduced nutrients in *Brassica napus* (Zadeh and Naeini, 2007). Moreover, ion homeostasis interruptions were noted in poor Na^+ excluding and Cl^- sensitive crops when cultivated in saline soils (Munns, 2002; Tester and Davenport, 2003).

Reduced uptake of nutrients has partially been attributed to the osmotically induced restricted growth of roots limiting nutrient absorption (Porcel *et al*., 2016) thus uptake of K^+, Cl^- and Na^+ in peanut was reduced (Taffaou *et al*., 2010).Salinity has been seen to inhibit ion absorption and translocation, and the high pH of sodic soils depresses nutrient uptake by precipitating micronutrients (Mwai, 2001).Increased soil Na^+ and Cl^- ion levels in plants like spinach is followed by a reduction in growth and nutrient uptake (Flowers *et al*., 2010).

Nutrient deficiency induced salt tolerance can result from replacing a deficient element by the excessively absorbed one (Munns and Tester, 2008). The observations of increased K^+ absorption by some salt resistant plants may also be evidence of salt tolerance due to avoidance of salt induced nutrient deficiency (Farooq *et al*., 2018). Excessive ion influx may control nutrient exchange and raise plant nutrient obtainability via improved generation of siderophores and pH stabilizing (Lugtenberg *et al*., 2013). Salinity influences mineral nutrient status in plants, however, further research on crops like Bambara groundnuts would help us understand better the effects of NaCl salinity while varying salinity.

2.5.3 Specific Ion Toxicity Effects of Salinity

Salt injury to plant through toxicity by specific ions is effective at the organ, tissue, cell and sub-cellular levels and it involves inhibited growth, development and metabolic disturbances. Injury increases with time as more salts are absorbed leading to ionic disturbance of the whole plant.

Sodium toxicity may induce cell injury and turgor reduction (Shrivastaya *et al*., 2017). Excessive amounts of Cl^- ions are injurious to certain species of plants thus interfere with plant growth (Dong *et al*., 2015). Formation of harmful radicals under salinity also lead to abnormal growth in plants by starting processes producing surplus peroxyl radicals (Egamberdieva *et al*., 2019).

Ion toxicity causes injury to the plasma membrane proton pump or H^+-ATpase (Ambede *et al*., 2012) which produces the force propelling the trans-cell membrane fluxes. The ions directly penetrate the cells and cause injury to the internal contents of the protoplasts causing decline in enzyme activity and synthesis of proteins, cell death through DNA destruction and degradation of chloroplast and mitochondrial membranes decreasing growth (Kumar *et al*., 2020). In case the ions build up in the cytoplasm, then they cause death to the cell due to cell poisoning or induce dehydration (Ambede *et al*., 2012). Sodium ions accumulate in the leaf blades because of transport and deposition as a result of transpiration and thus the main site for Na^+ toxicity is the leaf blade and not root tips for most plants (Munns and Tester, 2008). Therefore, excessive quantities of Na^+ ions should not reach leaf blades since its removal from leaves to roots is possibly small portion compared to what was taken to the leaf (Toffouo *et al*.,2014). Furthermore, ionic stress leads to early ageing in old leaves and chlorosis and necrosis in mature leaves (Munns and Tester, 2008).However, plants enhanced bio-protection' against biotic stress and root bacteria increased stress tolerance (Dimkpa *et al*., 2009). The extent to which Bambara groundnuts employ this tolerance mechanism when subjected to NaCl salinity remain questionable in most plants including Bambara groundnuts.

2.6 Physiological Effects of Salinity

2.6.1 Effects of Salinity on Plant Mineral Nutrient Content

The influence of salinity and mineral nutrient on photosynthesis, growth and productivity is vital in different crops (Taffaou *et al*., 2010; Arif *et al*., 2013). Sodium and Cl^- ions produce varied effects on different physiological traits and development of plants (Acosta-Motos *et al*., 2017). Salinity decreased K^+, Ca^{2+} and Mg^{2+} ions in cotton leaves but increased Na^+ and Cl^- ions (Narbaeva and Babina, 2015). Salinity significantly reduced K^+ uptake in barley leaves (Wue *et al*., 2015). Sodium chloride salinity increased Na^+ concentrations in plant organs of the peanut and Cl^- uptake by plant (Taffouo *et al*., 2010) with Na^+ accumulating in the root, stem, leaf and also gynophore while the uptake of K^+ was hindered.

High levels of Na^+ within the soil, affects cytosol K^+ influx, yet K^+ is a major nutrient in growth of plants. Potassium significantly regulates productivity in plants as it controls membrane turgor and potential, pH homeostasis, activates intracellular enzymes and improves photosynthetic rates (Koksal *et al*., 2016).Plants thus need extra nutrients for sustained growth during stress (Sharma

and Archana, 2016). Adequate quantities of K^+ are required by crops for maximum yields however, salt stress reduces its accessibility for direct uptake by plants. In such situation, potassium solubilizing bacteria are very effective in fulfilling the crop K requirements (Mukherjee *et al*., 2019). A good example, *Burkholderia* microbe stimulates release of potassium from soil minerals (Kang *et al*., 2014).NPK contents significantly increased in wheat leaves exposed to bacteria under salinity (Upadhyay and Singh, 2015).Extracellular Polymeric Substance (ESP) producing microbes caused ameliorative effects on Na^+, K^+ and Ca^2 uptake by plants (Ashraf *et al*., 2004). Bacteria generating EPS may trap cations in their matrix, rendering them unobtainable for plant absorption (Egermerdieva *et al*., 2019). The production of EPS by soil bacteria around roots also enhances water potentials and nutrient uptake by plants (Ashraf *et al*., 2004; Naseem and Bano, 2014). When wheat plants were subjected to EPS producing bacteria Na^+ ions were trapped and hence not transported to the leaves (Ashraf *et al*., 2004).Thus, symbiotic inter-relations reduced ion accumulation by promoting root Na^+ expulsion and K^+ transporters functioning which eventually decreased Na^+ accumulation in aerial portions, thus supporting ion homeostasis in crops (Pliego *et al*., 2011).

Under salt stress, there is an influx of sodium ions into roots, moved to aerial portions eventually settling in the photosynthetic organ/ leaf (Tester and Davenport, 2003). Its removal is difficult leading to plant damage. However, under salt stress plant calcium signals formed assist in maintaining the high Na^+/K^+ ratio via sustained K^+ transporters hence reduce the harmful effects of NaCl salinity (Torabi, 2014). Symbiotic microbes may assist in restricting absorption of sodium and conversely increasing absorption of other nutrient elements like calcium and potassium. When *Arabidopsis thaliana* was subjected to salinity absorption of Na^+ reduced (Qin *et al*., 2009). When Na^+ absorption is limited at the roots, its recirculation to the roots may assist in sustaining suitable plant K^+/Na^+ ratios (Ali *et al*., 2019). Maize plants under microorganisms boosted extrusion of Na^+ and uptake of K^+ which increased contents of proline and chlorophyll (Rojas-Tapias *et al*., 2012). Thus, in plant cells, maintaining cytosolic K^+ in a high Na^+ environment is a vital factor in salinity tolerance (Abduallah Al-Amoudi and Abduallah Rashed, 2012).There is progress in researches concerning the effects of sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) in salt tolerance in plants however, further research on crops like Bambara groundnuts would help us understand better this phenomenon.

2.6.2 Effects of Salinity on Gas Exchange Parameters

When studying the effects of salinity in plants, gas exchange parameters are a common consideration. Salinity limits growth because of water potential reduction within cells indicating reduced water supply to the cells (Garg and Bhandari, 2016). This leads to decline in

photosynthesis, closure of stomata and growth reduction (Garg and Manchanda, 2009). *Brassica juncea* subjected to NaCl salinity reduced stomatal movements, internal CO_2 concentration and rates of transpiration and photosynthesis (Martínez-Ballesta *et al*., 2016). Comparable observations were made on bean plants (Brewster, 2018) and barley (Roche *et al*., 2005).

Increasing levels of harmful ions are connected with reduced levels of phytohormones such as gibberellins, auxins and cytokinins, and increased abscisic acid (Barnawal *et al*., 2016). Carotenoids precursors for abscisic acid (ABA) hormone are synthesized within roots and leaves of plants (Anuradha and Rao, 2003) and under water deficit in the rooting zone, ABA formed within roots is transported to the plant shoots finally reach guard cells, where it controls stomatal closure involving calcium ions (Shahid *et al*., 2019). Thus, stomatal closure limits CO_2 assimilation and decreases the rate of photosynthesis (Montero *et al*., 2018). Increased ABA within plant tissues including leaves interferes with activity of stomata, improves stress protein formation and adaptation to salt through osmotic adjustment (Singh *et al*., 1987). Closure of the stomata also decreases CO_2 to O_2 ratio in the leaves and prevents CO_2 fixation (Gao *et al*., 2015). Reduced stomatal conductance decreasesCO_2 assimilation and reflects stomatal limitation thus the plant makes use of the CO_2 from respiratory activities in order to maintain least photosynthetic rate when CO_2 assimilation is quite low (Dias *et al*., 2020). Stomatal closure as a response to salinity in one way is a constrain to photosynthetic capacity however, it also in another way, offers a shielding mechanism which helps the survival of plants that are exposed to salt stress by minimizing salt loading in leaves and conserving water (Julkowska and Testerink, 2015). A plant with better compromise between opening the stomata to allow water to evaporate (hence more salts enter) and the closure to minimize the entry of salts will do better. In the process water is conserved in order to keep a high plant water status (Kumar and Verma, 2018). This complements the reduced leaf area which minimizes the rate of transpiration. Reduced rate of photosynthesis during salt stress limits productivity in plants (Kalaji *et al*., 2016).Photosynthetic capability is reduced because of decreased chlorophyll content and leaf area caused by disturbances from harmful ions on stomatal movements and water stress (Kang *et al*., 2014).Salinity decreased gas exchange parameters in mung bean through enhanced levels of ethylene which impeded growth and physiology of plants (Ahmad *et al*., 2013). However, microbes improved growth in plants under salinity and also enhanced crop productivity through lowered ethylene levels (Glick, 2014). For instance, exposure to *Pseudomonas* lowered levels of ethylene, improved ionic balance and protein content in mung bean grains.Under salt stress, there was also promoted uptake of nutrients, photosynthesis and

growth in soybean (Han and Lee, 2005).It is not clear to what extent Bambara groundnuts employ this mechanism as a protective measure for its survival when subjected to salinity.

2.7 Biochemical Effects of Salinity

2.7.1 Effects of Salinity on Leaf Chlorophyll and Carotenoid Content

Chlorophyll content of leaves can be used as a sign of the likely photosynthetic efficiency and overall strength in plants (Golan *et al*., 2015).Salinity reduces leaf pigments, chlorophyll and carotenoid content and hence reduction in photosynthetic capability of plants (Abdelhamid, *et al*., 2011). This may be caused by salts damaging the lipo-protein pigment complex and or the destruction of enzymes responsible for pigment synthesis (D'souza, and Devaraj, (2013).High levels of the salt reduced leaf pigment (chlorophyll) content, leaf area and the photosynthetic efficiency (Ambede *et al*., 2012).Salt stress reduced soybean chlorophyll content (In-Jung Lee*et al*., 2019). Salt stress also decreased leaf total chlorophyll in groundnut (Otitoloju, 2014) whereas in maize and beans it lead to increased destructive enzymes, chlorophyllases (Rahdari, 2012). The elevated level of salts promotes chlorophyll breakdown, inhibit CO_2 fixation and damage the photosynthetic apparatus (Praveena, 2016). Reduction in total leaf chlorophyll due to salinity stress in beans and maize may be attributed to increased destructive enzyme, chlorophyllase (Dawood and El-Awadi, 2015) and decreased absorption of essential nutrients causing decrease in content of chlorophyll (Abou-Leila *et al*., 2012). Research has also shown that chlorophyll a/b ratio and total leaf chlorophyll increased in pigeon pea at low salinity while higher salinity adversely affected all chlorophyll measurements (Ahmed and Ahmad, 2016). Kumar *et al*. (2017) noted comparable decrease in leaf pigments (Chl a, b and total chlorophyll) in chickpea cultivars exposed to high Na^+ levels   indicating its possible degradation. Reduction in chlorophyll content a n d weak leaves indicate salt induced chlorophyll damage. This explains the low photosynthetic rates in saline plants (Ahmed and Ahmad, (2016). Furthermore, these effects affect the capacity to produce additional biomass or to sustain defensive mechanisms in *Vicia faba* (Fufa, 2018). Chlorophyll b is less sensitive to salt stress compared to chlorophyll a and it may be changed into chlorophyll a while a reduction intotal chlorophyll is mainly due to decreased chlorophyll a content (Ahmed and Ahmad, 2016).

Under salt stress Indian mustard enhanced carotenoid and glutathione levels when exposed to putrescine and its supplementation reduced production of ROS by accelerating antioxygenic enzymes, hence assisting in the maintenance of chloroplastic membranes (Sofo *et al*., 2015). Low salinity did not affect chick pea carotenoid content but increased its chlorophyll content (Sharma *et al*., 2012). Furthermore, salinity caused leaf yellowing, indicating substantial damage to the chlorophyll pigment. These findings imply that carotenoids have a function in the

protection of photosynthetic apparatus. The activity of ACC (Amino cyclopropane carboxylate) deaminase in symbiotic microorganisms is also widespread, particularly when subjected to increasing salinity. The enzyme changes ACC into forms used by bacteria as sources nitrogen and carbon (Glick, 2014), affect different biochemical aspects of plant cells such as stability of the membrane, formation of biologically compatible solutes and synthesis of photosynthetic pigment under salinity (Tiwari *et al*., 2018). Iron is required for chlorophyll synthesis during plant growth. Siderophore generating bacteria increase plant biomass as caused by increased iron supply (Rungin *et al*., 2012). Salinity also enhanced iron-related deficiencies, i.e. chlorosis in plants, and reduced its availability in saline environments due to proton pump inhibition (Ferreira *et al*., 2019). Studies suggest that symbiotic microbes stimulate root iron accumulation as well as its translocation to the photosynthetic organ (Kumar *et al*., 2020). Increased root exudates caused by microbe-induced root development might improve the availability of minerals to plants (Kang *et al*., 2014). Subjecting plants including Bambara to sodium chloride salinity may provide further information on the influence of salinity on leaf pigments and photosynthesis.

2.7.2 Effects of Salinity on Proline Content

On exposure to salinity, plants increase production of compatible solutes (Slama *et al*., 2015). Proline production is evident in water and salt stressed plants (Verbruggen and Hermans 2008) where it maintains the cell osmotic balances without interfering with metabolic activities even when the levels increase (Slama *et al*., 2015).Proline accumulated in bean plants as salt stress increased (Amira *et al*., 2015). Rice seedlings grown from proline exposed seeds grew faster under saline conditions (Deivanai *et al*., 2011). Similarly, proline levels increased on exposure to salinity where it protected tissues through osmotic balancing (Qados Abdul., 2015). Furthermore, it accumulates in the leaves of halophytes from different families (Parihar *et al*., 2015).

Organic solutes, such as proline, accumulate in various microbes causing enhanced salt tolerance in legumes through contained water status of cells and so assisting the plant cope with salt stress as well serve as a nitrogen source as plants recover from salinity stress (Ali *et al*., 2007). Some symbiotic bacteria enhance protection from salt stress using bioactive components in plants (Kumar *et al*., 2020) consequently initiate production of compatible osmolytes after exposure to salinity stress. These osmolytes enable microbes develop the ways for coping with stress and provide plants with some resistance to abiotic stress like salinity.

Although plants withstand salt stress via synthesis of osmotic balancing proteins such as proline this might not be universal. For instance, proline accumulation and salt tolerance were inversely

proportional in chickpea cultivars (Soussi *et al*., 1998). In rice plants proline may act as a symptom of salt stress damage (Ashraf and Foolad, 2007) and sorghum (Huang *et al*., 2013).

Under salinity stress, oxidative stress damages cellular structures promoting death of cells (Kumar *et al*., 2020). Proline promote antioxidant defense system enzymes activity in *Nicotiana tabacum* enhancing salt tolerance (Murata *et al*., 2008).Antioxidant activities and homeostasis in plants were promoted under salt stress resulting in salinity tolerance and improved productivity in plants (Ali *et al*., 2014). Antioxidants may as well play an important part in the mechanism of defense by regulating levels of reactive oxygen species {ROS} (Ansari *et al*., 2019). Plants exposed to microorganisms generate antioxidative enzymes that reduce the harmful effects of oxidative stress (Islam *et al*., 2016). Proline accumulation in plants differ under salinity stress and studies have to be extended to plantssuch as Bambara groundnuts in order to understand the mechanisms involved.

2.7.3 Effects of Salinity on Nodulation

Salinity reduces rhizobia establishment, inhibits nodule infection and growth thus reduces activity of nitrogenase, consequently N_2 fixation in leguminous plants (AbdAllah *et al*., 2015).Several cellular enzymes involved in the synthesis of proteins and nitrogen metabolism are salinity sensitive (Siddiqui *et al*., 2008).Reduced nodulation in legumes under salinity can prevent legume establishment, growth, as well as reduce crop production (Junior and Andrade, 2015).Salinity also affects soil processes and microbial activity (Egermerdieva *et al*., 2019) resulting in retarded growth and yield of crops (Kumar *et al.*, 2020). The salts further induce distortions in nodule morphology leading to production of non-functional nodules with degraded peribacterial membrane and abnormal structure (Bandyopadhyay *et al*., 2015). Salts may interfere with and alter nodule ultra-structure in soybean nodules (Singleton and Buhlool, 1983)**,** in addition toroot hair growth and deformation, and the reduction in leghemoglobin content. The salt-induced abnormalities in nodule structure could possibly explain the decrease in legume nitrogen fixation rate (Zahran and Abu-Gharbia, 1995). Salinity affects growth and survival of rhizobia by inhibiting infection process and nitrogenase activity, limiting the ability of plants to photosynthesize consequently reducing growth and yield in plants (Avilar Sakar *et al*., 2018). Thus, salinity limits legume productivity through decreased nodule nitrogen fixation and respiration (Al-Saedi *et al*., 2016).

Plant productivity in legumes is limited by salt stress due to reduced photosynthetic rates, fixation of nitrogen and carbon metabolism (Jez *et al*., 2016).The carbon provided by the host legume fuels the nodulation process (Ahmed and Ahmad, 2016). Energy needed in the process is mainly supplied by dicarboxylic acids which are absorbed by the bacteroids (Mus *et al*., 2016).

Salinity may decrease bacteroids' nitrogen fixation capacity by lowering carbohydrate supply (malate content in nodules) for nodule respiration and cytosolic protein formation, notably leghemoglobin, by nodules (Ahmed and Ahmad, 2016) and also reducing photosynthetic activity (Garg and Chandel, (2015).Decrease in nitrogen fixation in salt stressed nodules is accompanied by a corresponding decrease in nodule respiration (Kenenil, 2010).

Chick peas exposed to strains of *Bradyrhizobium* under salt stress reduced nodulation (Soussi *et al.*, 1998) suggesting that a greater performance of symbiosis appear to be primarily determined by legume tolerance. Furthermore, salt affected chick pea cultivars had more efficient nodulation and supported symbiotic nitrogen fixation (Garg and Rangu, 2004) suggesting that salinity tolerance may be linked to biochemical characteristics like increased nitrogenase activity and nodule number. In addition, (Predeepa and Ravindran, 2010) revealed a decrease in salt tolerance of the symbiotic system by 1 dS/m. Among forage crops, alfalfa responds to low salinity by decreasing nodule number and size (Bandyopadhyay *et al.*, 2015) however, soybean subjected to strains of bacteria improved nodule formation and quantities of nitrogen under salinity (Al-Saedi *et al.* 2015).When *Bradyrhizobium* strains were exposed to Bambara under salinity stress nodulation and nitrogen fixation capacities improved (Laurette *et al.*, 2015). Under salinity, 1-aminocyclopropane-1-carboxylate (ACC) deaminase improved persistence of infecting threads thereby assisted in production of nodules (Nascimento *et al.*, 2016).Plant nodulation and biomass improved when chickpea was subjected to rhizobia under saline environment (Chaudhary and Sindhu, 2015).Salinity stress may thus inhibit symbiosis however the process may assist plants tolerate salt stress. Nodulation is a complex and diverse process not well established in different crops under salinity stress. Research on Bambara groundnuts under sodium chloride salinity would provide additional information on nodulation in legumes.

2.8 Effects of Salinity on Yield

Generally, salinity has a depressive influence on productivity in plants. It disturbs morphology, physiology, and plant biochemistry, resulting in significant yield loss of crops (Kumar *et al.*, 2020). Salinity disturbs composition of grains and grain yield (Khan *et al.*, 2017). For example, increased leaf necrosis and chlorosis under salinity causes leaf senescence and reduced photosynthesis in grain legumes (Khan *et al.*, 2017).Furthermore, high Na^+ levels inside the cell induce a variety of physiological disorders (Singh *et al.*, 2015) that negatively affected yield in plants such as *Brassica napus* (Rossi *et al.*, 2016). Salt stress disturbs grain and grain yield composition (Manchanda and Garg, 2008). Many disorders in the reproductive phases occurred in barley plants subjected to salinity (Ramegowda *et al.*, 2015) that ultimately reduced the yield. Under salinity, there were considerable losses in yield in soy bean (Khan *et al.*, 2017). All yield-

related characteristics in soybean decreased under salt stress (Ghassemi-Golezani *et al*., 2010). *Arachis hypogaea* may survive and produce a reasonable yield in soil with a NaCl concentration of up to 50 mM, but it is not suitable for soil with a NaCl content of more than 150 mM (Otitoloju, 2014). Under salinity, the common bean suffers yield losses at soil EC of less than 2 dS/ m (Bayuelo-Jiménez *et al*., 2003).

Salinity also inhibits reproductive growth by reducing the growth of flowers, pollen grains, and embryos, resulting in ineffective ovule fertilization and fewer seeds and fruits (Sadeghirad *et al*., 2017). In *Brassica juncea*, the yields also reduced substantially when the soil NaCl level rose (Rossi *et al*., 2016). This decrease in seed yield and other related characteristics could be linked to poor plant growth caused by a slower rate of photosynthesis (Parihar *et al*., 2015). When exposed to salinity, the thickness of the phloem elements controlling assimilates decreases affecting the sink -source movements (Wani *et al*., 2017) thus assimilate transport towards developing reproductive organs is inhibited, resulting in their poor development and seed setting. In groundnut, a reduction in number of pods and seeds may be associated with an increase in ABA and pollen death as well as decreased pod size (Parihar *et al*., 2015). There were many disorders in reproductive stages in barley plants under salt stress (Ramegowda and Senthil-Kumar, 2015) which may have been due to inhibiting influence of salinity on growth of plant and interference on absorption of nutrients (Abou-Leila *et al*., 2012) and/or Na^+ and Cl^- ion injury (SH Sadak *et al*., 2015). Limited resources for normal seed development cause reduced nutrient component of *A. hypogaea* seeds, seed quality in and cherry gold plant (El-Hindi and El-Ghamry, 2005) and *Foeniculum vulgare* (Abd El- Wahab, 2006). Maximum production /total yield was reported in inoculated plants including groundnut cultivars (Sajid *et al*., 2010). This was caused by the symbiotic association between rhizobia and leguminous roots, where atmospheric nitrogen is fixed and thus increased yield. Increased number of leaves and growth in these plants is in line with, the source sink relationship, where extra carbohydrates were synthesized in the leaves, translocated to the root zone consequently increased growth.

The role of EPS generating microbes in yield improvement is very important since they are used as seed priming agents to improve germination (Tewari and Arora, 2014).When bacteria were exposed to French bean under salinity stress, growth and yield improved (Kumar *et al*., 2020). A similar approach can be used on Bambara groundnuts, by subjecting them to sodium chloride stress to establish the response in terms of yield.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The study was done at Maseno University in western part of Kenya under polythene enclosed greenhouse located at the university research farm between January 2018 and October 2019. Maseno University soils are acidic with pH 4.65, low nutrient content i.e. nitrogen 0.16%, phosphorus 2.57mg/kg, potassium 46.8mg/kg, calcium 105mg/kg, magnesium 22.3mg/kg and alminium 1.88mg/100g (D. Simiyu, 2018, unpublished data). The area receives short and long rains averaging 1750mm yearly with an average temperature of 28.7^0C. Greenhouse temperatures varied 25 ± 3° C (day/night) with 27-99% relative humidity.

3.2 Plant material, experimental design and reatment

The soil was collected from the Maseno University research farm. It was filled into 20-liter PVC pots (20 Kg soil per pot) after solarization for 3 days mainly to prevent fungal growth. The pots were perforated to ease drainage and avoid flooding. Bambara groundnut landrace seeds were obtained from Kakamega, Mumias and Mombasa. The seeds were categorized according to locality they were collected from. Red seed coat (RSC) seeds were collected from Kakamega, white seed coat (WSC) from Mumias and black seed coat (BSC) from Mombasa. The RSC and WSC landraces were previously found to tolerate moderate NaCl salinity (Ambede *et al*., 2012). The salinity tolerance of BSC landrace is yet to be determined.

Large, similar sized seeds (plate 1) were sterilized in 10% sodium hypochlorite for 5 minutes and then rinsed thoroughly in distilled water prior to sowing. All the seeds were inoculated with *Bradyrhizobium* strain USDA 110 obtained from Kenya Forestry Research Institute to enhance biological nitrogen fixation of the seedlings according to Soussi *et al*. (1998; 1999). Ten seeds from each landrace were planted at 2 cm depth and at 10 cm spacing according to Ambede *et al*. (2012). All the pots were irrigated daily with sufficient tap water of pH 6.5 to guarantee emergency and growth of the seedlings. Thinning was done 7 days after emergency, leaving five uniformly spaced similar height seedlings in each pot. Sodium chloride salinity treatments were imposed from 14 days after emergence. The experimental design was a completely randomized design with three landraces and five NaCl salinity treatments replicated three times. There were two sets of experiments: non-destructive and destructive. The non-destructive experiment was for the determination of growth (plant height, root length, number of leaves and branches, leaf area), gas exchange and yield (number of pods), while the destructive experiment was for determination of photosynthetic pigments, proline content, mineral nutrient, fresh and dry weights, % water content, root to shoot ratio, root nodules and yield (number of seeds).The

salinity treatments were: 0/control, 2 dSm^{-1}, 4 dSm^{-1}, 6 dSm^{-1} and 8 dSm^{-1}. The control experiment (0 dSm^{-1}) was irrigated with a liter of distilled water up to the end of the experiment. The salinity treatments were prepared by dissolving sodium chloride in water to make NaCl solutions, 1.28g/liter, 2.56g/liter, 3.84g/liter and 5.12g/liter which were equivalent to EC 2, 4, 6 and 8 dSm^{-1} respectively. The calculation factor for salinity treatments, 1 dSm^{-1} is equivalent to640 parts per million (0.64g/liter). To minimize the osmotic shock, the solutions were applied incrementally i.e., by increasing the concentration every second day starting from salinity treatment of 2 dSm^{-1}, until the final salinity treatment of 8 dSm^{-1} was reached (Ambede *et al.*, 2012). The pot soil EC was determined using conduct meter at a temperature of 25^{0}C in saturated paste methods. The soil matrix effects that may have arisen from drying soil were removed by irrigating the pots after every 3 days with a liter of their respective solutions to field capacity.

3.3 Measurement of Parameters

One plant from every pot was randomly sampled, tagged and used on the day of measurement. The percentage increase in a particular parameter in relation to the control was expressed as:

$$Percentage\ increase\ =\ FS - CS/\ CS\ X\ 100$$

Where, FS-final salinity treatment mean and CS-control salinity treatment mean.

3.3.1 Growth Parameters

3.3.1.1 Plant Height

Plant height was measured from the base of stem to the shoot apex using a meter rule according to Musyimi (2011) after every 14 days from 0/zero days after treatment (DAT).

3.3.1.2 Number of Branches

Fully expanded branches were counted after every 14 days from 0 DAT to the end of the experiment.

3.3.1.3 Number of Leaves

Fully expanded leaflets were counted after every 14 days from 0 DAT to the end of the experiment.

3.3.1.4 Leaf Area

The middle-leaflet length and width were measured with a meter rule. Area of plant leaves (A $_{plant}$) was determined after every 14 days from 0 DAT to end of experiment using formula by Cornelissen *et al.* (2005);

$$A_{Plant} = 0.74 \times 3 \times N_1 \left\{ L \times W \times \left(\frac{\pi}{4} \right) \right\}$$

Where: (A $_{plant}$) - Area of leaves, L - Middle-leaflet length in cm, W- Middle leaflet width in cm, $\pi = 3.1416$ and N_1 - total Number of leaflets.

3.3.1.5 Plant Root and Shoot Fresh and Dry Biomass, Root Length and Root: Shoot Ratio

A single plant per pot was cautiously uprooted with all its roots in place, washed with tap water over a fine sieve and all the roots collected after every 28 days from 0 DAT to end of experiment. Each plant was then separated into shoot and root. Fresh weights of shoots and roots were measured after which they were oven dried at 72^0C for 2 days and dry weights measured following method by (Sikuku *et al.*, 2012). An electronic weighing balance (Denver Instrument Model XL-31000, Germany) was used to measure the weights (Sikuku *et al.*, 2012). A meter rule was used to measure root length from the base of the stem to the farthest root tip every after 2 weeks from 0 DAT. The data on root and shoot dry biomass was used to calculate the % root shoot ratio according to Sikuku *et al.* (2012).

$$Root: Shoot\ ratio = \frac{Root\ dry\ weight}{Shoot\ dry\ weight} \times 100$$

3.3.1.6 Percentage Water Content (%WC)

A single plant from each pot was carefully uprooted and washed in tap water with all its roots in place after every 4 weeks from 0 DAT to end of experiment. Each plant was divided into shoot and root. Fresh weights of shoots and roots were measured using an electronic weighing balance (Denver Instrument Model XL-31000, Germany) after which they were oven dried at 72^0C for 2 days and dry weights measured (Sikuku *et al.*, 2012). The water content for each plant was calculated as a % (Ambede *et al.*, 2012).

$$\%\ WC = \frac{(Fresh\ weight - Dry\ weight)}{Fresh\ weight} \times 100$$

3.3.2 Physiological Parameters

3.3.2.1 Plant leaf Mineral Nutrient (Na$^+$, K$^+$ and Ca^{2+}) Content Determination

Plant mineral nutrient content were determined at 120 days after planting, from leaves picked from a single plant per replicate. About 0.1g each of the leaf sample was weighed after careful cleaning in distilled water. The sample was oven-dried at 72^0C for 2 days. It was finely ground and acid digested in 25 ml of 65% ultra-pure nitric acid at 270^0C for 6 hours. The total cation

concentrations of Na^+, K^+ and Ca^{2+} were determined by atomic absorption spectrophotometer (DW-AA320NR)(L'vov, 2005).

3.3.2.2. Gas exchange Parameters

Leaf CO_2 assimilation rate, stomatal conductance and rate of transpiration were measured using a portable infra-red gas analyzer system (CIRAS, 1 PP Systems, Herts, UK) connected to a plant leaf after every 4weeks from 0 DAT. The effect was measured from an area of 2.5 cm^2 on wholly expanded leaf between 09:00 am and 12:30 pm. After leaf chamber was closed, readings were taken under steady-state conditions for 60 to 90 seconds. The measurements were done under the following specifications: air flow rate to the cuvette, varied from 200 μmols^{-1}to 400 μmols^{-1}, cuvette air temperature from (25.9 - 37.4) ^{0}C, vapor pressure deficit 1.2 to 2.4 kPa and photosynthetic active radiation (PAR) from (700-1200) μm^{-2}s^{-1}.

3.3.3 Biochemical Parameters

3.3.3.1 Chlorophyll Content Determination

The third youngest leaf was sampled from all treatments and chlorophyll content was determined according to Coombs *et al*., (1987) as described by Netondo (1999) after every 30 DAT. A half a gram (0.5g) of the fresh leaf tissue was weighed and reduced into small pieces. Ten milliliters of 80% acetone was added and the set up placed in the dark for a week for the leaf pigment to be dissolved by the acetone. Absorbance of the leaf pigment solution was measured at 645 nm and 663 nm to determine chlorophyll a and b contents respectively using a spectrophotometer (Novaspec II, Pharmacia Biotech, and Cambridge, England). The respective chlorophyll content in mg of chlorophyll per gram of the leaf collected was calculated using the formula of Arnon (1949) as below: -

$$Chl\ a\ =\ 12.7\ (D663) - 2.69\ (D645) \times \frac{V}{1000} \times W\ [mg\ Chl\ a\ g^{-1}\ leaf\ tissue]$$

$$Chl\ b\ =\ 22.9\ (D645) - 4.68\ (D663) \times \frac{V}{1000} \times W\ [mg\ Chl\ b\ g^{-1}\ leaf\ tissue]$$

$$total\ Chl\ =\ 20.2\ (D645) + 8.02\ (D663) \times \frac{V}{1000} \times W\ [mg\ Chl\ a\ g^{-1}\ leaf\ tissue]$$

3.3.3.2 Carotenoid Content Determination

The procedure for chlorophyll content determination was followed in carotenoid content determination. Absorbance of the leaf pigment solution was measured at 480 nm to determine carotenoid content using a spectrophotometer (Novaspec II, Pharmacia Biotech, and Cambridge, England). Carotenoids content in mg per gram of the leaf was calculated according to Yadegari *et al*., (2007) in Musyimi (2011) as follows: -

$$C_{X+C} = 1000 \, (D480) - 2.270 \, (chl \, a) - 81.4 \, (chl \, b)/227 \, [mg \, C_{X+C} \, g^{-1} leaf \, tissue]$$

Where:

Chl a and chl b are concentrations of chlorophylls a and b respectively; C_{X+C} are contents of carotenoids (x is xanthophylls and c is carotenes); D were absorbances measured at wavelengths 645 nm, 480 and 663 nm; V was volume in ml of acetone extract used while W was fresh weight (g) of leaf from which the extract was made.

3.3.3.3 Proline Content Determination

Measurements of proline was done from the largest youngest leaf taken from plant per treatment per landrace after every 28 DAT. Portions of ground material were heated at 95^0Cfor 20 min in pure ethanol as well as in water. The resultant mixture was left overnight at 4°C, and centrifuged at 14000 rpm 5 min. The cold extraction method was repeated on the pellet and supernatants collected and used for the analysis according to Carillo *et al*.(2008). A thousand (1000) μl of reaction mixture with 500 μl ethanolic extract was pipetted into 1.5 ml screw-cap tubes. Proline standard was finalized with up to 400μl of ethanol to water (40:60 v/v). The sealed tubes, were mixed and heated at 95°C in water bath for 20 min and centrifuged (1 min, 10000 rpm). Contents were transferred to 1.5 ml cuvette tubes and read at 520 nm in a spectrophotometer (Novaspec II, Pharmacia Biotech, and Cambridge, England). The content of proline in the extracts was calculated according to Carillo *et al*.(2008);

$$Proline \, content = (absExact - blank) \div slope \, (Vol. \, extract \div Vol. \, aliquot) \times 1 \div FW$$

Where: Abs extract is the absorbance determined with the extract, blank (expressed as absorbance) and slope (expressed as absorbance·nmol^{-1}) are determined by linear regression, Vol. extract is the total volume of the extract, Vol. aliquot is the volume used in the assay, FW is fresh weight (expressed in mg), the amount of plant material extracted. It was assumed that Abs extract is within the linear range.

3.3.3.4 Nodulation Parameters

3.3.3.4.1 Number of Nodules

A single plant per pot was uprooted cautiously with its roots intact, washedwith tap water over a fine sieve after 28 days from 0 DAT. Nodules were counted after they were collected from the roots.

3.3.4 Yield Parameters

3.3.4.1 Number of Pods and Seeds

At harvest (120 days after planting), 2 plants from each pot in salinity treatments of 0, 2 and 4 were uprooted cautiously after wetting the soil to field capacity. The total pod and seed number per pot per landrace were counted.

3.4 Statistical Data Analysis

Data collected were subjected to factorial analysis of variance (ANOVA) using SAS Statistical Computer Package (Steel *et al*., 2006) to assess the effects of NaCl salinity on three Bambara groundnut landraces. This was intended to determine if the saline treatment had any significant effect on the parameters that were measured and if there were any landrace differences in the responses. Salinity treatment means were separated using Fishers protected t-test least significant difference test at 5% level.

CHAPTER FOUR

RESULTS

4.1 Plant Growth Parameters

4.1.1 Plant Height

Shoot height (PH) in all the Bambara groundnut landraces was significantly (p ≤ 0.05) reduced by sodium chloride salinity at salinity treatment of 6dSm^{-1} (Table 4.1). As NaCl salinity concentration increased, plants in salinity treatments of 6 and 8 dSm^{-1} were significantly shorter compared to those in the control in all the landraces however, PH did not significantly differ among the plants in control and salinity treatments of 2 and 4 dSm^{-1}. Salinization showed a tendency to increase PH growth in salinity treatment of 2 dSm^{-1}, as 0.95% in WSC landrace. The three landraces were not significantly (p≥0.05) different in PH in response to NaCl salinity. There was significant (p≤0.05) interaction in PH between salinity treatments and Bambara groundnut landraces (Table 4.1).

Table 4.1: Plant height for Bambara groundnut landraces subjected toNaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1} for 70 days

Landrace	Plant height(cm) under NaCl salinity treatments					
	0 dSm^{-1} /control	2dSm^{-1}	4dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	**28.50a**	**28.47a**	**27.61a**	**22.18b**	**22.10b**	**25.77a**
White seed coat (WSC)	**28.31a**	**28.58a**	**27.93a**	**22.13b**	**22.13b**	**25.82a**
Black seed coat (BSC)	**28.48a**	**28.47a**	**27.90a**	**22.24b**	**22.12b**	**25.84a**
LSD (p≤0.05)for Salinity treatment=**3.020**						
LSD (p≤0.05)for Landrace = **0.0608**						
LSD (p≤0.05)for interaction salinity treatments and landrace = **0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.2 Number of Branches

Sodium chloride salinity treatment decreased the number of branches(NB) in all Bambara land races at salinity treatment of 8 dSm^{-1}(Table 4.2).The NB in control was significantly lower compared to that in salinity treatments of 2, 4 and 6 dSm^{-1}in the RSC and WSC landraces. The NB in control was also not significantly different compared to that in salinity treatment of 6 dSm^{-1}, however, it was significantly lower compared to that in salinity treatments of 2 and 4dSm^{-1}in BSC landrace. The NB increased by 12%, 38% and 20% at salinity treatments of 2, 4 and 6 dSm^{-1} respectively in RSC landrace, by 08 %, 05%, 05 % at salinity treatments of 2,4 and 6 dSm^{-1} respectively in WSC landrace, and by 12 % and 18% at salinity treatments of 2 and 4 dSm^{-1} respectively in BSC landrace. The highest NB was observed in BSC landrace, followed by RSC

and least was WSC landrace. There was significant ($p \leq 0.05$) interaction in the NB between salinity treatments and Bambara landraces (Table 4.2).

Table 4.2: Number of branches of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1} for 56 days

Landrace	Number of branches under NaCl salinity treatments					
	0 dSm^{-1} /control	2dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	7.4b	8.27ab	10.20a	8.90ab	6.53c	8.26ab
White seed coat (WSC)	7.33b	7.93ab	7.73ab	7.67ab	6.00c	7.33b
Black seed coat (BSC)	8.33b	9.33ab	9.87a	7.93b	7.40bc	8.57a
LSD at ($p \leq 0.05$)for Salinity treatment = **0.5000**						
LSD at ($p \leq 0.05$)for Landrace = **0.05**						
LSD at ($p \leq 0.05$) for interaction salinity treatments and landrace = **0.0039**						

Means followed by the same letter(s) a long the rows are not significantly differently, at ($p \leq 0.05$) level using Lsd test. Values represent means of three replicates.

4.1.3 Number of Leaves

Sodium chloride salinity significantly ($p \leq 0.05$) reduced the number of leaves (NL)at salinity treatments of 6, 2 and 4dSm^{-1}in RSC, WSC and BSC landraces respectively (Table 4.3). The NL in control was significantly higher compared to all other salinity treatments in WSC landrace however, the NL in the control was significantly lower compared to that in salinity treatments of 2and 4 dSm^{-1} in RSC landrace. On overall, salinity treatment of 2dSm^{-1} had a higher NL compared to other salinity treatments in BSC landrace The NL increased by 08% and 12% at salinity treatments of 2 and 4 dSm^{-1}respectively in RSC landrace, and by 05% in salinity treatment of 2 dSm^{-1}of BSC landrace. In the overall, landraces were not significantly different ($p \geq 0.05$) in number of leaves in response to NaCl salinity treatments. There was significant ($p \leq 0.05$) interaction in number of leaves between salinity treatments and Bambara groundnut landraces (Table 4.3).

Table 4.3: Number of leaves for Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1} for 70 days

Landrace	Number of leaves under NaCl salinity treatments					
	0 dSm^{-1} /control	2dSm^{-1}	4dSm^{-1}	m^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	34.00ab	36.66a	38.06a	56bc	24.10c	31.9a
White seed coat (WSC)	37.00a	31.39ab	30.56ab	72bc	22.72c	29.28a
Black seed coat (BSC)	42.39ab	44.67a	26.56b	94b	23.89c	32.69a
LSD at ($p \leq 0.05$)for Salinity treatment = **1.5010**						
LSD at ($p \leq 0.05$)for Landrace = **3.450**						
LSD at ($p \leq 0.05$)for interaction salinity treatments and landrace =**0.0019**						

Means followed by the same letter(s) a long the rows are not significantly differently, at ($p \leq 0.05$) level using Lsd test. Values represent means of three replicates.

4.1.4 Leaf Area

Sodium chloride salinity significantly ($p \leq 0.05$) reduced leaf area (LA) of RSC landrace at salinity treatment of 4 dSm^{-1}, and WSC and BSC landraces at salinity treatment of 2 dSm^{-1} (Table 4.4). The LA in the control was significantly higher compared to all the other salinity treatments among the WSC and BSC landraces. The LA in salinity treatment of 2 dSm^{-1} was significantly higher compared to control that was not significantly different from that in salinity treatment of 4 dSm^{-1} in RSC landrace. The increase in LA in salinity treatment of 2 dSm^{-1} was 07% for the RSC landrace. The landraces were not significantly different in LA in response to salinity treatments. There was significant ($p \leq 0.05$) interaction in LA between salinity treatments and Bambara groundnut landraces (Table 4.4).

Table 4.4: Leaf area of Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1} for 70 days

Landrace	Leaf area(cm^2) under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	1496.4b	1593.6a	1424.6b	1032.3bc	966.9c	1302.76a
White seed coat (WSC)	1949.1a	1509.2b	1154.2bc	1095.8c	1085.7c	1358.8a
Black seed coat (BSC)	1949.1a	1509.2b	1154.2bc	1095.6c	1089.7c	1321.80a
LSD at (p≤0.05)for Salinity treatment =**11.020**						
LSD at (p≤0.05)for Landrace = **58.53**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0099**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.5 Shoot Fresh Weight

Shoot fresh weight (SFW) of all the Bambara groundnut landraces was significantly ($p \leq 0.05$) reduced by sodium chloride salinity at salinity treatment of 2 dSm^{-1}(Table 4.5). There were no significant differences in SFW among the landraces at salinity treatments of 2, 4 and 6 dSm^{-1} as NaCl salinity increased. The control was significantly higher in SFW compared to all other salinity treatments in all the landraces. The landraces were not significantly different in SFW in response to salinity. There were no significant ($p \geq 0.05$) interactions in SFW between salinity treatments and Bambara groundnut landraces (Table 4.5).

Table 4.5: Shoot fresh weight for RSC, WSC and BSC Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days

Landrace	Shoot fresh weight(g) under NaCl salinity treatments					
	0 dSm^{-1}/control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	17.20a	11.29b	11.28b	10.98b	10.14b	12.18a
White seed coat (WSC)	11.83a	9.69b	9.22b	9.10b	5.97c	9.16a
Black seed coat (BSC)	12.60a	9.81b	9.13b	8.69b	8.38b	9.72a
LSD at (p≤0.05)for Salinity treatment = **1.010**						
LSD at (p≤0.05)for Landrace =**3.067**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.1438**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates

4.1.6 Root Fresh Weight

Root fresh weight (RFW) of the RSC and BSC landraces was significantly (p ≤ 0.05) reduced by sodium chloride salinity at salinity treatment of 2 dSm^{-1}(Table 4.6). There were no significant differences in RFW of the WSC landrace with increase in salinity. The control had a significantly higher RFW compared to all other salinity treatments in RSC and BSC landraces. The RFW in salinity treatments of 2 and 4dSm^{-1} in RSC landrace were not significantly different. Also, the RFW in salinity treatments of 4 and 6 dSm^{-1} in BSC landrace were not significantly different. The landraces were significantly different in RFW in response to salinity with the BSC landrace having highest RFW followed by RSC and least WSC landrace. There was significant (p ≤ 0.05) interaction in RFW between salinity treatments and Bambara groundnut landraces (Table 4.6).

Table 4.6: Root fresh weight of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days

Landrace	Root fresh weight(g) under NaCl salinity treatments					
	0 dSm^{-1}/control	2 dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	0.52a	0.44ab	0.43ab	0.41b	0.27c	0.40ab
White seed coat (WSC)	0.38b	0.39b	0.40b	0.37b	0.34b	0.38b
Black seed coat (BSC)	0.74a	0.51ab	0.41b	0.41b	0.33c	0.48a
LSD at(p≤0.05)for Salinity treatment = **0.040**						
LSD at (p≤0.05)for Landrace = **0.01**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0091**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.7 Shoot Dry Weight

The results for shoot dry weight (SDW) are shown in table4.7. Increasing sodium chloride salinity significantly (p ≤ 0.05) reduced SDW in salinity treatments of 8, 6 and 2dSm^{-1} in RSC, WSC and BSC landraces respectively. The SDW was significantly higher in salinity treatments of 2, 4 and 6 dSm^{-1}compared to control that was not significantly different from that in salinity treatment of 8 dSm^{-1} in RSC landrace. The SDW in the control and salinity treatments of 2 and 4 dSm^{-1} were not significantly different in WSC landrace. The SDW in the control was significantly higher compared to salinity treatments of 2,4 and 6 dSm^{-1} that were not significantly different in BSC landrace. The SDW increased by 30%, 22% and 14% at salinity treatments 2, 4 and 6 dSm^{-1} respectively in RSC landrace, and 08% in salinity treatment of 2 dSm^{-1}of WSC landrace. The landraces were significantly different in SDW in response to salinity. The highest SDW was observed in BSC landrace, followed by RSC and least was WSC landrace. There was significant (p ≤ 0.05) interaction in SDW between salinity treatments and Bambara groundnut landraces (Table 4.7).

Table 4.7: Shoot dry weight of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days.

Landrace	Shoot dry weight(g) under NaCl salinity treatments					
	0 dSm^{-1}/control	2dSm^{-1}	4dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	1.28b	1.66a	1.56a	1.46a	1.21bc	1.43ab
White seed coat (WSC)	1.31ab	1.42ab	1.31ab	1.01b	0.99b	1.21b
Black seed coat (BSC)	2.24a	1.53ab	1.52ab	1.50ab	1.15c	1.59a
LSD at(p≤0.05)for Salinity treatment =**0.150**						
LSD at (p≤0.05)for Landrace =**0.010**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0406**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.8. Root Dry Weight

There were no significant differences in RDW of the RSC and BSC landraces as sodium chloride salinity increased (Table 4.8). The RDW in the control was not significantly different from that in salinity treatment of 2dSm^{-1} however, the RDW of the salinity treatments of 4,6 and 8dSm^{-1} was significantly higher compared to control in WSC landrace. The landraces were not significantly different in RDW in response to salinity. There was no significant (p ≤ 0.05) interaction in RDW between salinity treatments and Bambara groundnut landraces (Table 4.8).

Table 4.8: Root dry weight of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days

Landrace	Root dry weight(g) under NaCl salinity treatments					
	0 dSm^{-1}/control	2dSm^{-1}	4dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	0.14a	0.13a	0.14a	0.14a	0.13a	0.14a
White seed coat (WSC)	0.10b	0.10b	0.13a	0.12a	0.11a	0.11a
Black seed coat (BSC)	0.12a	0.13a	0.11a	0.11a	0.11a	0.12a
p≤0.05)for Salinity treatment =**0.025**						
LSD at (p≤0.05)for Landrace =**0.4**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.5725**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.9 Root Length

Sodium chloride salinity significantly (p ≤ 0.05) reduced root length (RL) of all the landraces at salinity treatment of 6dSm^{-1}(Table 4.9). The RL in the control and salinity treatment of 4dSm^{-1}were not significantly different however was significantly lower compared to that in salinity treatment of 2dSm^{-1}in all the landraces. Root length increased by 14%, 09% at salinity treatments of 2 and 4 dSm^{-1}respectively in RSC landrace, by 06% and 04% at salinity treatments of 2 and 4 dSm^{-1}respectively in WSC landrace and by 07% at salinity treatment of 2 dSm^{-1}in BSC landrace. The landraces were not significantly different in RL in response to salinity. There was significant (p ≤ 0.05) interaction in RL between salinity treatments and Bambara groundnut landraces (Table 4.9).

Table 4.9: Root length of Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days

Landrace	Root length(cm) under NaCl salinity treatments					
	0 dSm^{-1}/control	2dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	11.52b	13.12a	12.52b	11.48c	10.68d	11.86a
White seed coat (WSC)	11.99b	12.76a	12.47b	11.94c	11.19d	12.07a
Black seed coat (BSC)	13.27b	13.53a	13.24b	10.22c	10.17c	12.09a
LSD at (p≤0.05)for Salinity treatment = **0.0601**						
LSD at (p≤0.05)for Landrace =**0.85**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0025**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.10 Root: Shoot Ratio

Sodium chloride salinity significantly (p ≤ 0.05) reduced root: shoot ratio (RS) ratio of WSC and BSC landraces at salinity treatments of 8 and 6dSm^{-1}respectively (Table 4.10).Salinity significantly (p ≤ 0.05) increased RS ratio of RSC landrace in salinity treatments of 2, 4 and 8

dSm^{-1}however, the ratio in the control and salinity treatment of 8dSm^{-1} were not significantly different. The RS ratio in the control and salinity treatments of 2and 6 dSm^{-1}were not significantly different but significantly lower compared to that in salinity treatments of 4 dSm^{-1}inthe WSC landrace. The RS ratio in salinity treatment of 4 dSm^{-1} was significantly higher compared to control that was not significantly different from salinity treatment of 2 dSm^{-1}in BSC landrace. Root to shoot ratio increased by 14%, 29% and 27% at salinity treatments of 2,4 and 6 dSm^{-1}respectively in RSC landrace, by 06%, 42 % and 15 % at salinity treatments of 2, 4 and 6 dSm^{-1}respectively in WSC landrace and by 05 % and 14 % at salinity treatments of 2 and 4 dSm^{-1}respectively in BSC landrace. The landraces were significantly different in RS ratio in response to salinity. The highest RS ratio was in observed in RSC landrace followed by WSC and least was BSC landrace. There was significant ($p \leq 0.05$) interaction in RS ratio between salinity treatments and Bambara groundnut landraces (Table4.10).

Table 4.10: Root to shoot ratio of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days

Landrace	Root: shoot ratio under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	20.82b	23.69ab	26.40a	26.53a	19.16b	23.32a
White seed coat (WSC)	17.47ab	18.45ab	24.74a	20.05ab	14.66c	19.07ab
Black seed coat (BSC)	18.18ab	19.13ab	20.75a	14.42b	12.68c	17.0b
LSD at (p≤0.05)for Salinity treatment = **1.011**						
LSD at (p≤0.05)for Landrace = **0.02**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.1.11 Percent (%) Water Content

Percent water content (% WC) of the RSC landrace decreased significantly ($p \leq 0.05$) as sodium chloride salinity increased (Table 4.11). Percent water content (% WC)significantly ($p \leq 0.05$)decreased in salinity treatment of 6 dSm^{-1} in both WSC and BSC landraces as salinity increased. Percent WC in salinity treatment of 4 dSm^{-1}and control were also not significantly different however, significantly lower compared to that of salinity treatment of 2 dSm^{-1} in the WSC landrace. In the BSC landrace, percent WC in salinity treatment of 2 dSm^{-1}and control were not significantly different however, significantly lower compared to that of salinity treatment of 4 dSm^{-1}.Percent WC increased by 01% at salinity treatment of 2 dSm^{-1}in WSC landrace, and 0.4 % and 1.5% at salinity treatments of 2 and 4 dSm^{-1}respectively in BSC landrace. The landraces were not significantly different in % WC in response to salinity. There were no significant (p ≤

0.05) interactions in % WC between salinity treatments and Bambara groundnut landraces (Table 4.11).

Table 4.11: Percent water content of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 56 days

Landrace	% Water content under NaCl salinity treatments					
	0 dSm^{-1} /control	2dSm^{-1}	4dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	84.31a	82.81ab	82.34b	81.92bc	79.88c	82.25a
White seed coat (WSC)	83.41b	84.49a	83.35b	81.15bc	80.82c	82.64a
Black seed coat (BSC)	80.20ab	80.52ab	81.41a	75.92b	67.12c	77.03a
LSD at (p≤0.05)for Salinity treatment = **0.4010**						
LSD at (p≤0.05)for Landrace =**5.71**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.7772**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.2 Physiological Parameters

4.2.1. Plant mineral Nutrient Content

4.2.1.1. Leaf Sodium Content

Sodium chloride salinity treatment significantly(p ≤ 0.05) increased sodium (Na$^+$) content of leaves in all the landraces(Table4.12).   Symptoms of NaCl salinity treatment/toxicity on Bambara groundnut leaf (plate 2 and 3). Sodium content in the control was significantly lower compared to all the other salinity levels in all the landraces. The highest significant sodium content was noted in salinity treatment of 8 dSm^{-1} in the RSC and BSC landraces and salinity treatment of 4 dSm^{-1} in the WSC landrace. The landraces were not significantly different in Na$^+$ content in response to salinity treatments. There was significant (p ≤ 0.05) interaction in Na$^+$ content between salinity treatments and Bambara groundnut landraces (Table 4.12).

Table 4.12: Leaf sodium content of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 100 days

Landrace	Leaf sodium content (mg g -1) under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	0.30bc	2.00ab	1.60b	1.90ab	2.40a	1.64a
White seed coat (WSC)	0.35bc	2.33ab	2.83a	1.50b	2.00ab	1.80a
Black seed coat (BSC)	0.30bc	1.33b	2.63ab	2.57ab	3.20a	2.01a
LSD at (p≤0.05)for Salinity treatment = **0.0650**						
LSD at (p≤0.05)for Landrace = **0.240**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0278**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.2.1.2 Leaf Potassium Content

As shown in table 4.13, there was significant($p \leq 0.05$) decrease potassium (K^+) content in salinity treatment of 6 and 8 dSm^{-1}of RSC and BSC, and WSC landrace leaves respectively. Potassium content in salinity treatment of 2 dSm^{-1}was significantly higher compared to control that was not significantly different from that in salinity treatment of 4 dSm^{-1} in the RSC and BSC landraces however, potassium content in salinity treatment of 2 dSm^{-1} was significantly higher compared to control that was not significantly different from that in salinity treatments of 4 and 6 dSm^{-1} in WSC landrace. Potassium content in salinity treatment of 2 dSm^{-1}was significantly higher compared to control by 127% and 54% in RSC and WSC landraces respectively. Potassium content increased by 06% and 02 % in salinity treatments of 2 and 4 dSm^{-1}respectively in the BSC landrace. The landraces were not significantly different in K^+ content under salinity. There was significant ($p \leq 0.05$) interaction in K^+ content between salinity treatments and Bambara groundnut landraces (Table 4.13).

Table 4.13: Leaf potassium content of Bambara groundnut landraces, subjected to NaCl salinity treatments   of 0, 2, 4, 6 and 8 dSm^{-1}at 100 days

Landrace	Leaf potassium content (mg g -1) under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	17.30b	39.23a	17.25b	14.15c	14.05c	20.40a
White seed coat (WSC)	22.10ab	34.05a	20.00ab	19.80ab	13.25c	21.84a
Black seed coat (BSC)	27.08ab	28.60a	27.75ab	18.95b	17.95c	24.07a
LSD at (p≤0.05)for Salinity treatment =**0.2101**						
LSD at (p≤0.05)for Landrace =**2.34**						
D at (p≤0.05)for interaction salinity treatments and landrace =**0.0086**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.2.1.3 Calcium Content

Sodium chloride salinity treatment caused a significant ($p \leq 0.05$) increase in calcium (Ca^{2+}) content of all Bambara groundnut landrace leaves at salinity treatment of 2dSm^{-1}(Table 4.14). Also calcium content in salinity treatments of 2 and 4 dSm^{-1} were not significantly different but significantly higher compared to the control that was not significantly different from that in salinity treatments of 6 and 8dSm^{-1}in BSC landrace. The Ca^{2+} content in the control was not significantly different from that in salinity treatments of 4,6 and 8dSm^{-1} in the RSC and WSC landraces. The BSC landrace had a significantly higher Ca^{2+}content compared to RSC and BSC landraces that were not significantly different in response to salinity. There was significant ($p \leq$ 0.05) interaction in Ca^{2+} content between salinity treatments and Bambara groundnut landraces (Table 4.14).

Table 4.14: Leaf calcium content of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 100 days

Landrace	Leaf calcium content (mg g -1) under NaCl salinity treatments					
	0 dSm^{-1} /control	2dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	0.03b	0.10a	0.02b	0.02b	0.01b	0.04b
White seed coat (WSC)	0.04b	0.10a	0.03b	0.03b	0.02b	0.04b
Black seed coat (BSC)	0.02b	0.10a	0.10a	0.01b	0.01b	0.05a
LSD at (p≤0.05)for Salinity treatment =**0.0201**						
LSD at (p≤0.05)for Landrace = **0.005**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.2.2. Gas Exchange Parameters

4.2.2.1 Carbon Dioxide Assimilation Rate

Sodium chloride salinity significantly ($p \leq 0.05$) reduced CO_2 assimilation rate (Cr) at salinity treatments of 4, 6and 8 dSm^{-1} in BSC, WSC and RSC landraces respectively (Table 4.15). Cr in the control and salinity treatment of 6 dSm^{-1}were not significantly different in the RSC landrace however, the rate increased by 11% and 19% in salinity treatments of 2 and 4 dSm^{-1}respectively in RSC landrace. Cr was also significantly higher in salinity treatment of 2dSm^{-1} compared to the control by 38% however, the control and salinity treatment of 4 dSm^{-1} in the WSC landrace were not significantly different. The control Cr was not significantly different from that in salinity treatment of 4dSm^{-1}however Cr in salinity treatment of 2 dSm^{-1} was higher compared to control by 04% in BSC landrace. The landraces were not different in response to NaCl salinity. There were significant ($p \leq 0.05$) interactions in Cr between salinity treatments and Bambara groundnut landraces (Table 4.15).

Table 4.15: Carbon dioxide assimilation rate of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 56 days

Landrace	CO_2 assimilation rate (mmol m^{-2} s^{-1}) under NaCl salinity treatments					
	0 dSm^{-1} /control	2dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	7.57b	8.41ab	9.00a	6.57b	6.48c	7.61a
White seed coat (WSC)	8.26ab	11.36a	7.73ab	6.68b	6.57c	8.12a
Black seed coat (BSC)	9.94ab	10.34a	7.83ab	7.81b	7.5bc	8.68a
LSD at (p≤0.05)for Salinity treatment=**0.0501**						
LSD at (p≤0.05)for Landrace =**0.55**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0474**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.2.2.2 Stomatal Conductance

Sodium chloride salinity significantly ($p \leq 0.05$) reduced stomatal conductance (gs) of RSC landrace, and WSC and BSC landraces at salinity treatments of 6 and 4 dSm[-1] respectively (Table 4.16). Stomatal conductance in the control and salinity treatment of 4 dSm[-1] in the RSC landrace were not significantly different however the increase in gs at salinity treatment of 2 dSm[-1] for both RSC and WSC landraces was 11%. Stomatal conductance in the control and salinity treatment of 2 dSm[-1] in the BSC landrace were not significantly different. The landraces were not significantly different in gs under salinity. There was significant ($p \leq 0.05$) interaction in gs between salinity treatments and Bambara groundnut landraces (Table 4.16).

Table 4.16: Stomatal conductance of Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm[-1] at 56 days

Landrace	Stomatal conductance (mmol m^{-2} s^{-1}) under NaCl salinity treatments					Mean
	0 dSm[-1] /control	2 dSm[-1]	4 dSm[-1]	6 dSm[-1]	8 dSm[-1]	
Red seed coat (RSC)	14.8ab	16.42a	14.63ab	13.91bc	13.76c	14.70a
White seed coat (WSC)	16.9ab	18.71a	14.37b	14.17bc	13.14c	15.46a
Black seed coat (BSC)	15.33a	15.35a	13.48b	11.47c	10.61d	13.25a
LSD at ($p \leq 0.05$) for Salinity treatment = **0.050**						
LSD at ($p \leq 0.05$) for Landrace = **2.46**						
LSD at ($p \leq 0.05$) for interaction salinity treatments and landrace **0.0490**						

Means followed by the same letter(s) a long the rows are not significantly differently, at ($p \leq 0.05$) level using Lsd test. Values represent means of three replicates.

4.2.2.3 Transpiration Rate

Transpiration rate (Tr) significantly ($p \leq 0.05$) reduced at salinity treatments of 6 and 8 dSm[-1] in WSC landrace, and RSC and BSC landraces respectively as sodium chloride salinity increased (Table 4.17).In the RSC landrace, Tr was significantly higher in salinity treatments of 2 and 4 dSm[-1] compared to control by 56% and 37% respectively however, the Tr in control and salinity treatment of 6dSm[-1] were not significantly different. The Tr in control and salinity treatment of 4dSm[-1] of WSC landrace were also not significantly different however, Tr in salinity treatment of 2 dSm[-1] was significantly higher compared to control by 44%. Tr in salinity treatment of 2 dSm[-1] was also significantly higher compared to control by 16% in the BSC landrace however, the control and salinity treatments of 4 and 6 dSm[-1] were not significantly different. The landraces were not significantly different in the rate of Tr under salinity. There were significant ($p \leq 0.05$) interactions in Tr between salinity treatments and Bambara groundnut landraces (Table 4.17).

Table 4.17: Transpiration rate of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm[-1] at 56 days

Landrace	Transpiration rate (μ mol m^{-2} s^{-1}) under NaCl salinity treatments					
	0 dSm^{-1}/control	2dSm^{-1}	4dSm^{-1}	6 dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	6.73b	10.53a	9.25ab	8.55b	8.06c	8.62a
White seed coat (WSC)	7.50b	10.83a	6.3b	5.97bc	4.58c	7.04a
Black seed coat (BSC)	8.51b	9.89a	8.01b	7.93b	7.63c	8.39a
LSD at (p≤0.05)for Salinity treatment =**0.1011**						
LSD at (p≤0.05)for Landrace = **1.601**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0440**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.3 Biochemical Parameters
4.3.1 Chlorophyll Content
4.3.1.1 Chlorophyll a

Sodium chloride salinity significantly (p ≤ 0.05) reduced chlorophyll (Chl) a content of the three landraces (Table 4.18). The control was significantly higher in Chl a content compared to all the other salinity treatments. There were no significant differences in Chl a content among the landraces in each salinity treatment. The landraces were not significantly different in chlorophyll a content in response to NaCl salinity. There was significant (p ≤ 0.05) interaction in Chl a between salinity treatments and Bambara groundnut landraces (Table 4.18).

Table 4.18: Chlorophyll a content of Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 90 days

Landrace	Chlorophyll a content (mg g^{-1} leaf tissue) under NaCl salinity treatments					
	0 dSm^{-1}/control	2dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	4.9a	2.67b	1.67c	0.72d	0.68d	2.13a
White seed coat (WSC)	4.34a	1.77b	1.15c	0.65d	0.61d	1.70a
Black seed coat (BSC)	4.28a	1.94b	1.47c	1.08d	1.07d	1.97a
LSD at (p≤0.05)for Salinity treatment = **0.0500**						
LSD at (p≤0.05)for Landrace = **0.53**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.3.1.2 Chlorophyll b

Sodium chloride salinity significantly (p ≤ 0.05) reduced chlorophyll (Chl) b of all the Bambara groundnut landraces (Table 4.19). There were significant differences in Chl b content among the landraces at salinity treatments of 2 d Sm^{-1} where Chl b content in WSC landrace was significantly lower compared to RSC and WSC landraces. The highest significant Chl b content in the landraces was observed in the control among the landraces. There were no significant differences in Chl b content   among the landraces in salinity treatments of 4, 6 and 8 dSm^{-1}.The

landraces were not significantly different in Chl bcontent under NaCl salinity. There was significant (p ≤ 0.05) interaction in Chl b content between salinity treatments and Bambara groundnut landraces (Table 4.19).

Table 4.19: Chlorophyll b content of Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 90 days

Landrace	Chlorophyll b content (mg g $^{-1}$ leaf tissue) under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	3.5a	1.62ab	1.47b	0.77c	0.31d	1.53a
White seed coat (WSC)	3.59a	0.74b	0.73b	0.46c	0.45d	1.19a
Black seed coat (BSC)	3.66a	1.94ab	1.81b	1.48c	1.29d	2.04a
LSD at (p≤0.05)for Salinity treatment = **0.0202**						
LSD at (p≤0.05)for Landrace =**0.901**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0012**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.3.1.3 Total Chlorophyll

Sodium chloride salinity significantly(p ≤ 0.05) reduced total (t) Chl content of all the Bambara groundnut landraces (Table 4.20). The control had significantly higher t Chl content compared to all other salinity treatments among the landraces. There were no significant differences in t Chl content   among the landraces in each salinity treatment except in salinity treatment of 8 dSm^{-1}. The landraces were not significantly different in t Chl content in response to NaCl salinity. There was significant (p ≤ 0.05) interaction in t Chl content between salinity treatments and Bambara groundnut landraces (Table 4.20).

Table 4.20: Total chlorophyll content of Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}for 90 days

Landrace	Total chlorophyll content (mg g $^{-1}$ leaf tissue) under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	3.7a	0.69b	0.44c	0.4d	0.37d	1.12a
White seed coat (WSC)	3.18a	0.68b	0.64c	0.44d	0.42d	1.07a
Black seed coat (BSC)	3.27a	1.40b	0.77c	0.41d	0.31e	1.23a
LSD at (p≤0.05)for Salinity treatment = **0.031**						
LSD at (p≤0.05)for Landrace = **0.190**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.3.1.4 Carotenoid Content

Sodium chloride salinity significantly($p \leq 0.05$) reduced carotenoid content of all the Bambara groundnut landraces except in WSC landrace at salinity treatments of 2 dSm^{-1}(Table 4.21). The control had significantly higher carotenoid content compared to other salinity treatments among the RSC and BSC landraces. Carotenoid content in salinity treatment of 2 $dSm^{-1}$was significantly higher compared to the control by 06% in the WSC landrace. The landraces were not significantly different in carotenoid content in response to NaCl salinity. There was significant ($p \leq 0.05$) interaction in carotenoid content between salinity treatments and Bambara groundnut landraces (Table 4.21).

Table 4.21: Carotenoid content of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 $dSm^{-1}$for 90 days

Landrace	Carotenoid content under NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
Red seed coat (RSC)	5.74a	2.99b	2.47c	0.93d	0.77e	2.58a
White seed coat (WSC)	2.98b	3.17a	2.62c	1.64d	1.56e	2.39a
Black seed coat(BSC)	4.32a	3.14b	2.59c	1.13d	1.13d	2.46a
LSD at ($p \leq 0.05$)for Salinity treatment =**0.0101**						
LSD at ($p \leq 0.05$)for Landrace =**0.21**						
LSD at ($p \leq 0.05$)for interaction salinity treatments and landrace =**0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at ($p \leq 0.05$) level using Lsd test. Values represent means of three replicates.

4.3.1.5 Proline Content

Sodium chloride salinity treatment significantly($p \leq 0.05$) increased proline content in salinity treatment of 6dSm^{-1}in the RSC landrace, 4 and 6 dSm^{-1} in WSC landrace, and 2,4 and 6 dSm^{-1}in BSC landrace (Table 4.22). Proline content in control and salinity treatments of 2,4 and 8dSm^{-1} in RSC landrace, control and salinity treatment of 2 and 8dSm^{-1} in WSC landrace, and control and salinity treatment of 8dSm^{-1} in BSC landrace were not significantly different. However, proline content in salinity treatment of 6$dSm^{-1}$was significantly higher compared to control by 47% in the RSC landrace. Proline content increased in salinity treatments of 4, 6 and 8 dSm^{-1}by30%,41% and 30% respectively in WSC landrace, and in salinity treatments of 2, 4 and 6$dSm^{-1}$by 30%, 14% and 22% respectively in BSC landrace. The highest proline was observed in RSC landrace, followed by BSC and least was WSC landrace. There was significant ($p \leq 0.05$) interaction in proline content between salinity treatments and Bambara groundnut landraces(Table 4.22).

Table 4.22: Proline content of Bambara groundnut landraces, subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 56 days

Landrace	Proline content (mg g^{-1} fresh leaf tissue) under NaCl salinity treatments					
	0 dSm^{-1}/control	**2** dSm^{-1}	**4** dSm^{-1}	**6**dSm^{-1}	**8**dSm^{-1}	**Mean**
Red seed coat (RSC)	**1.52b**	**1.50b**	**1.53b**	**2.24a**	**1.53b**	**1.67a**
White seed coat (WSC)	**1.01b**	**0.99b**	**1.31ab**	**1.42a**	**1.31b**	**1.21c**
Black seed coat (BSC)	**1.28b**	**1.66a**	**1.46ab**	**1.56a**	**1.21b**	**1.43b**
LSD at (p≤0.05)for Salinity treatment = **0.050**						
LSD at (p≤0.05)for Landrace = **0.01**						
LSD at (p≤0.05)for interaction salinity treatments and landrace =**0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.3.1.6 Number of Nodules

The number of nodules (NN) in all the Bambara groundnut landraces was significantly (p ≤ 0.05) reduced at salinity treatments of 8 and 6 dSm^{-1} in RSC, and WSC and BSC landraces respectively (Table 4.23). The NN was significantly higher in salinity treatment of 2 and 4 dSm^{-1}compared to the control in the RSC landrace. In salinity treatment of 6 dSm^{-1},the NN were not significantly different from the control in RSC landrace however the increase in NN for salinity treatments of 2 and 4 dSm^{-1}was 43% and 19% respectively. Salinity treatment of 4 dSm^{-1}was also not significantly different in NN compared to control in WSC and BSC landraces however, salinity treatment of 2 dSm^{-1}had significantly higher NN compared to the control. The RSC and BSC land raceshad a significantly higher NN compared to WSC landrace. There was (p ≤ 0.05) significant interaction in number of nodules between salinity   treatments and Bambara groundnut landraces (Table 4.23).

Table 4.23: Number of nodules of Bambara groundnut landraces, subjected toNaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 28 days

Landrace	Number of nodules under NaCl salinity treatments					
	0 dSm^{-1}/control	**2** dSm^{-1}	**4** dSm^{-1}	**6** dSm^{-1}	**8**dSm^{-1}	**Mean**
Red seed coat (RSC)	**3.50b**	**5.00a**	**4.16ab**	**2.80b**	**1.67c**	**3.43a**
White seed coat (WSC)	**2.67b**	**8.00a**	**2.50b**	**2.00c**	**1.50d**	**3.33b**
Black seed coat (BSC)	**2.67b**	**8.00a**	**3.50b**	**2.00c**	**1.41d**	**3.52a**
LSD at (p≤0.05)for Salinity treatment =**0.40**						
LSD at (p≤0.05)for Landrace =**0.091**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

4.4 Yield

4.4.1 Number of Pods

Sodium chloride salinity significantly($p \leq 0.05$) reduced number of pods in all the Bambara groundnut landraces (Table 4.24). The number of pods in the control was significantly higher compared to all other salinity treatments in all the landraces. The number of pods in salinity treatments of 2 and 4 dSm^{-1} were not significantly different in the BSC landrace. There were no pods in salinity treatments of 6 and 8 dSm^{-1}in all the landraces. The landraces were highly different in number of pods in response to NaCl salinity treatments. The highest number of pods was observed in RSC landrace, followed by BSC and least was WSC landrace. There was significant ($p \leq 0.05$) interaction in number of pods between salinity treatments and Bambara groundnut landraces (Table 4.24).

Table 4.24: Number of pods for Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 100 days

Landrace	Number of pods under NaCl salinity treatments					
	0 dSm^{-1} /control	**2** dSm^{-1}	**4** dSm^{-1}	**6**dSm^{-1}	**8**dSm^{-1}	**Mean**
Red seed coat (RSC)	**40.67a**	**23.67ab**	**3.00c**	**0**	**0**	**22.45a**
White seed coat (WSC)	**20.00a**	**7.7b**	**1.00c**	**0**	**0**	**9.57c**
Black seed coat (BSC	**30.00a**	**8.33b**	**8.00b**	**0**	**0**	**15.44b**
LSD at ($p\leq0.05$)for Salinity treatment =**0.4001**						
LSD at ($p\leq0.05$)for Landrace =**0.05**						
LSD at ($p\leq0.05$)for interaction salinity treatments and landrace =**0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at ($p\leq0.05$) level using Lsd test. Values represent means of three replicates.

4.4.2 Number of seeds

Sodium chloride salinity significantly ($p \leq 0.05$) reduced number of seeds in all the Bambara groundnut landraces (Table 4.25). There were significant reductions in number of seeds in all the landraces at salinity treatment of 2 dSm^{-1}. The highest number of seeds was noted in the control for the three landraces. There were no seeds in salinity treatments of 6 and 8 dSm^{-1}in all the landraces. The landraces were significantly different in number of seeds in response to NaCl salinity. The highest number of seeds was observed in RSC landrace followed by BSC and least was WSC landrace. There was significant ($p \leq 0.05$) interaction in number of seeds between salinity treatments and Bambara groundnut landraces (Table 4.25).

Table 4.25: Number of seeds for Bambara groundnut landraces subjected to NaCl salinity treatments of 0, 2, 4, 6 and 8 dSm^{-1}at 100 days

Landrace	Number of seeds under fifteen NaCl salinity treatments					
	0 dSm^{-1} /control	2 dSm^{-1}	4 dSm^{-1}	6dSm^{-1}	8dSm^{-1}	Mean
Red seed coat (RSC)	50.33a	20.67b	3.00c	0	0	24.67a
White seed coat (WSC)	25.33a	7.00bc	1.00c	0	0	11.11c
Black seed coat (BSC)	35.67a	20.00b	8.33bc	0	0	21.33b
LSD at (p≤0.05)for Salinity treatment =**5.001**						
LSD at (p≤0.05)for Landrace = **0.05**						
LSD at (p≤0.05)for interaction salinity treatments and landrace = **0.0001**						

Means followed by the same letter(s) a long the rows are not significantly differently, at (p≤0.05) level using Lsd test. Values represent means of three replicates.

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CHAPTER FIVE

DISCUSSION

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5.1 Effect of Sodium Chloride Salinity on Growth Parameters of Bambara Groundnut Landraces

The results from this study show that plant height in the three Bambara groundnut landraces reduced with increasing sodium chloride salinity level. These results are in agreement with those reported by other researchers in many other plant species (Soussi *et al*., 1999; Garg and Ranju, 2004;Alam*et al*., 2004; Ambede *et al*., 2012; Fontenele *et al*., 2014). Plant height reduced significantly ($p \leq 0.05$) at salinity treatment of 6 dSm^{-1} in all the landraces. This response has previously been attributed to osmotic and ion toxicity effects which interfere with cell growth, cell division, enlargement and differentiation ((Netondo, 1999; Munns and Tester, 2008; Toffauo *et al*., 2010). Reduced plant height for plants subjected to salinity has also been linked to inefficient circulation of nutrients causing reductions in photosynthetic activity which in turn limits the supply of carbohydrate needed for growth (In-Jung Lee*et al*., 2019). The previous reasons may equally apply to the current study. The high concentrations of leaf Cl^{-} ions may also reflect the changes in nutritional balances of the plant, alter water relations consequently reduce growth (Piñero *et al*., 2016).

The slight increase in plant height observed in WSC landrace at salinity treatment of 2dSm^{-1}may be attributed to stimulated cell division and expansion under NaCl salinity.This may further be linked to higher chances of osmotic tolerance, and presumably normal metabolic activity at a lower water potential than would have been possible. Plants are known to grow and survive under saline conditions (Musyimi, 2005). Increase in growth under saline conditions has been reported to be enhanced by the supply of fixed nitrogen to plants for cell expansion and maturation (Pii *et al*., 2015; Gupta and Pandey, 2019; Nadeem *et al*., 2019).

Reduction in number of branches as a result of salinity treatment may be attributed to salinity toxicity, which may interfere with cell growth processes and reduce nutrient availability (Alam *et al*., 2004). Inhibition of cell expansion is usually followed by a reduced cell wall synthesis reducing growth further (Garg and Ranju, 2004; Ambede *et al*., 2012). Reduced photosynthesis and nitrogen fixation may also contribute to the reduction in the number of branches. High salinity disturbs vital biological structures including membranes where salinity-induced membrane peroxidation promoted membrane integrity loss and leakage of vital cellular components, further reducing growth in plants (Torabi, 2014). Thus, there is a wide variety of physio-biochemical changes that inhibit general plant growth (Kumar *et al*., 2020).

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46

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The observed increase in the number of branches at salinity levels of 2 and 4 dSm^{-1} for all the Bambara landraces could be as a result of osmotic adjustment involving accumulation of internal solutes such as proline. The increase in number of nodules and leaves observed at mild salinity in the landraces in this study may also suggest improved nitrogen fixation, nutrient uptake, photosynthesis and therefore growth by the Bambara plants. Legumes are known to improve N$_2$ fixation, nutrient uptake and growth under salinity (Jaiswal *et al*., 2016). Plants may produce phytohormones including auxins that support plant growth through cell division and expansion to cope with salinity stress (Torabi, 2014).

Increase in NaCl salinity reduced leaf growth of Bambara plants in the current study. This may be attributed to osmotic and ion toxicity effects which interfere with leaf development processes, delayed leaf emergence, reduced leaf growth, leaf senescence and death whose overall effect is malformed leaves that offer less leaf area leading to reduced leaf size of the plants. Reduced leaf growth limit light interception and CO$_2$ absorption, lower net photosynthesis and result in the overall slow growth in plants. Reduction in leaf area in WSC and BSC landraces at salinity treatment of 2 dSm^{-1}may suggest that leaf growth was sensitive at low salinity. The reduction in leaf growth of plants exposed to salinity has been attributed to reduced turgor or reduction in extensibility of expanding cell walls (Munns and Tester, 2008).The limited area of leaves was attributed to the death of old leaves due to toxicity of ions that in hibiedt nutrient and hormone movement to the young leaves leading to reduced chlorophyll content (Ahamd *et al*., 2014).A restricted leaf growth in RSC and WSC Bambara landraces under NaCl salinity was previously attributed to reduction in water loss by transpiration, a plants survival response in saline environments (Ambede *et al*., 2012).The reduced leaf area is an adaptation to reduced ion uptake by roots (Munns and Tester, 2008), where transpiration that aids in transport of mineral salts is minimized. Plant development is affected since the reduced leaf area contributes to less photosynthesis hence less dry matter accumulation. The general leaf destruction in form of burns on leaf margins, necrotic spots and death of Bambara leaves observed in lower leaves especially in salinity treatments of 6and 8 dSm^{-1}and the premature senescence of leaves could be attributed to ion toxicity. Early ageing, and yellowing and necrosis in older leaves and mature leaves respectively result from influences of ionic stress since excessive Na$^+$ levels interfere with synthesis of proteins and inhibit activities of enzymes (Munns *et al*., 2006).

The increase in leaf growth observed in (salinity treatments of 2 and 4 dSm^{-1}in RSC landrace and salinity treatment of 2dSm^{-1}in BSC landrace) could suggest osmotic tolerance in these plants. Plants with high osmotic tolerance maintain high growth rates particularly over the first

few days after being subjected to sodium ions. Increase in photosynthesis allows increased photosynthate allocations to other plant organs enhancing supply of carbohydrate needed for growth (Nadeem *et al*., 2019). Enhanced leaf growth has been reported under low NaCl salinity in saffron plants (Mzabri *et al*. (2017).

Shoot and root dry and fresh weights decreased with increasing level of salt stress however, there were no significant differences in root dry weights in RSC and BSC landraces. Increasing salinity through a combined effect of osmotic and ion toxicity reduced plant biomass in this study as it altered several plant physiological processes. The reduced Bambara plant growth under salt stress could also be due to disturbed and imbalanced nutrition. Thus, the vegetative growth of crops in saline areas may be severely restricted leading to reduction in total dry matter (Ambede *et al*., 2012). Reduced plant dry weights was further attributed to osmotic and/or specific ion effects of Cl^- and Na^+ (Taffouo *et al*., 2010). The reduction in shoot dry weight could as well be associated with reduced rate of leaf production hence low number of leaves leading to reduced photosynthesis and accumulation of dry matter. Root damage and death due to salinity may have affected water and mineral salt absorption from the soil hence decreased water and minerals in the transpiration stream reaching the leaves resulting in decreased net photosynthesis which in turn may have affected shoot growth. Decreased plant biomass in response to salinity has been reported in many species (Netondo, 1999: Mwai, 2001: Taffouo *et al*., 2010: Ambede *et al*., 2012; In-Jung Lee*et al*., 2019).

Shoot and root damage caused by ion toxicity, osmotic effects or both may have contributed to the observed drop in fresh and dry weights (shoot fresh weight in all the landraces, root fresh in RSC and BSC landraces and shoot dry weight in WSC and BSC landraces) especially in the highly stressed plants. Inhibition of long-distance transport of nutrient ions by salinity may explain reduced nutrient content in the shoot due to displacement of K^+ and Ca^{2+} by Na^+ on the membranes (Taffouo *et al*., 2010) hence reduced shoot growth. Plant damage resulting from toxicity of ions, osmotic loss or imbalances in nutrients may contribute to further decline in plant biomass in the extremely stressful conditions (Ahmed and Ahmad, 2016)

Accumulation of shoot dry matter in salinity treatments of 2, 4 and 6 dSm^{-1} in RSC landrace and root dry weight in salinity treatments of 4, 6 and 8 dSm^{-1}in WSC landraces were significant. This growth increase may reflect increased carbon gain associated with salt concentration tolerated by the plant leaves in addition to increased photosynthesis, nodulation (nitrogen fixation), root growth and nutrient uptake in Bambara. Soybean also enhanced plant biomass under salinity *(*Zeffa *et al*., 2020). Furthermore, fenugreek improved dry matter accumulation (Miransari and Smith, 2007).

The significant reduction in root to shoot ratios and root length under salinity may be as a result of a reduction in plant growth and increased root death. The reduction in root growth reduce plant growth since the available surface area for absorption of water and mineral salts is reduced. The reduction in plant growth depends on cells that would be the first to be affected directly by ionic toxicity of the salt and hence the roots die (Ambede *et al*., 2012). The roots are directly in contact with saline soil hence the root epidermal cells exposed to higher salt concentrations. 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 (Taffouo *et al*., 2010). The reduced cell length as a result of salinity may also result from reduced cell extension rates and or in the duration of extension period (Acosta Motos *et al*., 2017). Salt induced death of root cells has been attributed to osmotically induced turgor loss and Na^+ ion toxicity in root meristem, causing reduced instant cell extension rates (Ahmed and Ahmad, 2016). 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.

Roots enable plants absorb nutrients from their surroundings, and a robust rooting system is essential for vigorous plant growth. The increased root to shoot ratio noted in all the landraces in this study may indicate healthier plants under salinity, a response for survival since increase in root surface area allows more water plus nutrients to be taken from the soil. The increased ratio also suggests higher chances of osmo-tolerance involving maintenance of turgor at a lower water potential than would have been possible. This study is in agreement with previous findings by Aisha and Anjum (2011); Garg and Ranju, (2004) and Li *et al*. (2020) where salinity increased root to shoot ratio. Similarly, studies by Acosta Motos *et al*. (2017) suggest that increased root to shoot ratio or reduced shoot to root ratio is a normal occurrence to salinity and it is linked to characteristics related to osmotic other than toxicity of ions. The increased root length observed in salinity treatment of 2 dSm^{-1}in all the landraces may have aided plant growth resulting in an improved plant water status and allowed the plants to access more nutrients. Roots will continue to grow until the plant's water requirements are met (Li *et al*., 2020).Furthermore, a higher percentage of roots under salt stress may also conserve harmful ions and regulate their transport to aerial portions. Khan *et al*. (2018) noted a considerable increase in plant growth, provided greater access to soil nutrients and encouraged plant development under salinity and substantially alleviated negative effects due to salinity in soybean.

All the landraces showed an overall general decrease in percent WC in response to salinity. The decrease was very significant at salinity treatment of 6 dSm^{-1}. The plant water potential may have reduced as soil water was less available for absorption by roots in the landraces. The decrease in

percent WC may have resulted in insufficient water in the cells indicating that plants exposed to salinity experienced water stress, hence plants suffered turgor loss and subsequent depressed growth. Similarly, Tsoata *et al*. (2017) suggested that water stress occurs throughout the time plants are exposed to salinity, leading to turgor loss and subsequent depressed growth, transpiration and yield. The %WC in the RSC landrace was generally much higher compared to WSC and BSC landraces. This may suggest accumulating of more dry matter as evidenced through increased R: S ratio and, a response for survival under saline conditions since increased root surface area allows more water to be absorbed from the soil resulting in an improved plant water status under the saline conditions. Furthermore, the increase in % WC at salinity treatment of 2 dSm^{-1}in WSC landrace and salinity treatment of 4 dSm^{-1}in BSC landrace may be interpreted as evidence that these landraces are capable of osmotic adjustment under NaCl salinity hence better water relations under saline environments and improved growth through cell turgor maintenance. The higher % WC in BSC landrace may indicate enhanced capacity for osmotic adjustment that may also be explained by the degree of salt tolerance as different plant species are reported to accumulate different types of organic solutes for this purpose. The high salt tolerance of the halophytes is believed to involve their ability to absorb large quantities of inorganic ions for osmotic adjustment (Tsoata *et al*., 2017).

The differences observed in the number of branches, root fresh weight, shoot dry weight and root to shoot ratio among the Bambara groundnut landraces in response to salinity treatments could be partly explained through osmotic, ionic and nutritional imbalances effects which influenced plant growth as they partly influenced the level of salt tolerance among the landraces. The differences could further be associated with genetic variations among the landraces, environmental conditions and the stage of plant growth. The RSC landrace on average responded better on growth parameters followed by BSC and least was WSC in response to NaCl salinity.

5.2 Effect of Sodium Chloride Salinity on Physiological Parameters of Bambara Groundnut Landraces

5.2.1 Plant Mineral Nutrient

Based on the current study, Na$^+$ content in leaves significantly increased in all the landraces under NaCl salt stress. The results imply that there was uptake, movement and final accumulation in the Bambara leaves. The results further imply that there is no clear mechanism of preventing its entry and accumulation in the roots. This is further aided by the transpiration pull. Sodium entry into the plant plays a negative role as was manifested in necrotic areas, marginal burns, leaf fall or plant death especially in salinity treatments of 6 and 8 dSm^{-1}. Abou-Leila *et al*. (2012) associated sodium toxicity with disturbances in nutrient absorption, water stress development

and turgor decrease. Once the Na^+ enter into the leaves, plants must consequently limit it in the cytosol in order that it does not interfere with normal working of enzymes. When the potentially harmful ions begin to accumulate in the tissues of leaves, they must either be extruded or tolerated (Munns and Tester, 2008). According to Muhammad *et al.* (2020), the sodium which enters leaf cells must be extruded or pumped into vacuoles before it reaches to toxic levels affecting enzymatic activities. Findings by Zhang *et al.* (2001) show that improved transport of Na^+ to shoot vacuoles or particular cell types ease storage of Na^+ and eventually confers salinity tolerance through reduction of Na^+ content in *Brassica napus*. Recently, Muhammad *et al.* (2020) reported that under saline conditions, toxic cytoplasmic ions are vacuole sequestered, triggering high K^+ levels and other compatible solutes that correct the osmotic force of the ions within vacuoles. Torabi, (2014) concluded that the high concentration of Na^+ salt in the cytoplasm of both glycophytes and halophytes is injurious and to overcome the challenge, plants must contain the extra salts in the vacuole or sequester them in various cells and tissues to enable metabolism. As a result, ion uptake and compartmentalization are imperative in normal and saline growth. However, ion sequestration in vacuoles or any other cell component in Bambara is yet to be determined.

Potassium and Ca^{2+} significantly decreased at salinity treatment of 6 and 4 dSm^{-1} in RSC and BSC, and RSC and WSC landraces respectively in leaves of Bambara landraces. High amounts of Na^+ and Cl^- within the root zone may have led to imbalances in these nutrients (K^+ and Ca^{2+}) in these plants. Potassium and Ca^{2+} ionic imbalances under salt stress can have damaging effects on the plant growth and physiology. Plants therefore must sustain significantly higher levels of these ions if they are to live in saline environments (Etesami, 2018). Increasing NaCl salinity prevented uptake of Ca^{2+} and K^+ causing antagonism in Na^+/K^+ (Turan *et al.*, 2007). High amounts of Na^+, prevented absorption of Ca^{2+} and K^+ in plants (Toffauo *et al.*, 2010) and high sodium displaces Ca^{2+} from root cell walls, which causes leakage of K^+ as well as other plant metabolites from the root (Musyimi, 2005). Furthermore, increasing Na^+ levels disturb Na^+ to K^+ ratio thus inhibiting photosynthetic and respiratory enzyme activities in the cytosol (Baral *et al.*, 2015).

With the uptake of Na^+ ions, the absorption of K^+ ions through the roots are reduced. This causes water shortage, generating an osmotic effect that is detrimental to plant growth. This is manifested through reduced leaf chlorophyll, photosynthetic rates, low stomatal conductance as well as reduced rates of transpiration (In- Jung Lee *et al.* 2019). This infers that Na^+ and K^+ ions could be one of the factors causing a decrease in biomass and yield components under salinity.

From the study, increased K^+ content in salinity treatment of $2dSm^{-1}$ for RSC, WSC and BSC landraces may be connected with plant root nodule bacteria helping solubilize potassium and promoting K^+ channel proteins controlling K^+ transport across membranes preventing and probably reducing Na^+ uptake. This regulates salt ion translocation from roots to aerial parts and so mediates salt tolerance in different plant species (Kaundal *et al*., 2019) consequently preservation of ion homeostasis in plants. This may further result from increased competition for K^+ and Na^+ uptake, and the fact that Na^+ competes with K^+ for the binding sites on the high affinity K^+ channels (Sharmin *et al*., 2021). By accumulating K^+ in vacuoles, Pessarakli *et al*. (2015) observed adjusted cell's osmotic potential via K channels and transporters, a common plant strategy in stress response. In-Jung Lee *et al*. (2019) noted improved K^+ levels and Farooq *et al*. (2018) attributed increased K^+ absorption by some plants to salt tolerance due to avoidance of salt induced nutrient deficiency. Under saline conditions plant bacterial interactions maintained selective uptake of ions K^+ to Na^+ in order to keep higher ratio of K^+: Na^+ (Etesami, 2018).Potassium ions significantly regulate stomatal conductance hence diffusion of carbon dioxide into and water out of the plant, and promote synthesis of proteins and compatible solutes (Koksal *et al*., 2016).The elevated K^+ concentrations in salinity treatment of $2dSm^{-1}$ in Bambara leaves is a halophytic plant mechanism (Farooq *et al*., 2018).

Sharmin *et al*. (2021) noted that sodium and calcium interactions are crucial under saline stress since Na^+ may displace Ca^{2+} from its binding sites, resulting in decreased uptake of Ca^{2+} ions. The reduced Ca^{2+} in the roots and leaves decrease stomatal movements and transpiration rates. The observed increase in Ca^{2+} content in salinity treatment of 2 dSm^{-1} in RSC and WSC, and in salinity treatments of 2 and $4dSm^{-1}$ in BSC landraces could be a response for osmotic adjustment. Moreover, Ca^{2+} is known to offset harmful influences of NaCl by increasing uptake for K^+. Salinity may have also stimulated its translocation to the Bambara leaves. Calcium supplements ameliorated the injurious aspects of salt stress in beans and the plants' ability to live under osmotic stress is based on their ability to sustain a higher Ca^{2+} to Na^+ ratio as well as Na^+ extrusion (Sharmin *et al*., 2021).Likewise, calcium helps in transport of solutes, stomatal conductance and plant cell protection as salinity increases. Although Ca^{2+} is known to be relatively immobile within the plant, its shortage occurs in plants cultivated in saline soil and its transport to the young tissues heavily rely on its uptake from the growth substrate and movement through the transpiration stream. Numan *et al*. (2018) explained that water deficits in the rooting zone promote production of the stress hormone abscisic acid which helps in the accumulation of Ca^{2+} and K^+ reducing the toxic influences of excess salinity. However, calcium content of the landraces differed significantly, with the BSC landrace having the highest calcium content. This

could be attributed to the sensitivity of the landraces to osmotic effects of salinity.Thus, the observed increase in potassium and calcium content in the landraces may serve a supplemental role in sodium chloride salinity tolerance.

5.2.2 Gas Exchange Parameters

Results from this study show that sodium chloride stress reduced CO_2 assimilation rate, an indication that high salt concentration interfered with the photosynthetic system in the leaves of Bambara. Decrease in stomatal conductance in reaction to salinity stress lowers net CO_2 uptake for photosynthesis. Studies by Gao *et al*. (2015) show that stomatal closure lowers the carbon dioxide oxygen ratio in the leaves and prevents CO_2 fixation under saline conditions. As a result, under low CO_2 assimilation rate, the plant assimilates CO_2 from respiratory activities and supports least photosynthesis (Dias *et al*., 2020). In addition, movement of cytokinins to the aerial portions from roots reduced under salt stress since they take part in the restoration of RuBP or RUBISCO in the process of photosynthesis (Dias *et al*., 2020).Musyimi, (2007) attributes reduction in CO_2 fixation to high levels of leaf chloride ions increasing leaf burns, necrotic margins, and defoliation. Injury due to salt exposure on tips of roots may have also removed a major sink (roots) for photosynthesis thus lowering photosynthetic requirements. Results of the present study are in agreement with studies by Martínez-Ballesta *et al*. (2016) who showed that *Brassica juncea* subjected to salinity decreased net photosynthesis, stomatal movements and internal CO_2 content and rate of transpiration. Wang *et al*. (2012) also observed comparable trends in tomato. Long-term decreases in net CO_2 uptake associated with salinity could be linked to stomatal closure, decreased leaf pigments and concomitant non-stomatal effects like lower protein content (Acosta Motos *et al*., 2017).

As salinity increased, Bambara plants may have been unable to store salts in vacuoles, causing salt to accumulate rapidly in the cytosol and cause photosynthetic decline. However, in salinity treatment of 2 dSm^{-1} or mild salinity all the landraces increased CO_2 assimilation rate, an indication of elevated CO_2 and nutrient uptake leading to enhanced photosynthesis that increased root carbohydrates required to support respiration driven ion transport at root surface. Likewise, Han and Lee (2005) reported enhanced photosynthetic rate, uptake of nutrients and growth in soybean under salt stress. The RSC landrace appears to strike compromise between stomatal conductance and transpiration rate at salinity treatments of 2 and 4 dSm^{-1} as both were positively correlated. This could enable this Bambara landrace plant to open the stomata to allow water to evaporate and close to minimize the entry of salts hence better CO_2 assimilation rate.

Stomatal conductance significantly reduced on exposure to salinity. Salinity induced osmotic and ionic stress, and interfered with stomatal behavior. Salinity interferes with water relations, ionic

concentrations, and induces the synthesis of abscisic acid leading to reduction in stomatal pore (Sharmin *et al.*, 2021). This reduces CO_2 movement to the carboxylation site resulting in drop in CO_2 uptake and net photosynthesis. Stomatal closure caused by high salt levels reduced internal CO_2 concentration as reported by Gray *et al*. (2016) and consequently CO_2 assimilation rate/ carbon fixation that controls metabolism of energy and photosynthate production (Dias *et al.*, 2020; Gama *et al.*, 2007). Furthermore, Singh *et al.* (1987) noted that increased levels of ABA within leaf tissues disrupted the functioning of stomata in plants, promoted formation of stress proteins and salt adaptation through osmotic adjustment.

Salinity-induced stomatal closure in one way is a limitation to photosynthetic capacity however, it also in another way implies a survival response for managing high levels of salt by lowering leaf salt load and improving water conservation (Julkowska and Testerink, 2015). Stomatal conductance is also indirectly affected by sodium ions through decreased K^+ to Na^+ ratios (Acosta Motos *et al.*, 2017).This study indicated an increase in stomatal conductance at salinity treatment of 2 dSm^{-1}in all the landraces which may be linked to improved absorption of nutrients. This could enable the Bambara plant to open the stomata to allow water to evaporate and close to minimize the entry of salts hence better CO_2 assimilation rate.

Increasing salinity reduced transpiration rate due to direct influence on the stomata, whose aperture determines how much water is lost. Reduced transpiration has the tendency to lower the rate of leaf salt loading. This is because salts reach leaves through the transpiration stream. This tends to maintain the salts at sub toxic levels long enough for water to be conserved within the plant upholding high water status (Razzaghi *et al.*, 2011; Ambede *et al.*, 2012). This complements the reduced leaf area which minimizes the amount of water lost per unit leaf area in Bambara. The observed improvement in the rate of transpiration in salinity treatments of 2 and 4 dSm^{-1}in RSC landrace and salinity treatment of 2 dSm^{-1}in WSC and BSC landraces may be attributed to improved water and nutrient uptake in Bambara plants under salinity. The landraces may also minimize water movement and loss through reduction of the transpiring surface area by the plant in form of reduced leaf area and number. Decrease in the total soil water potential lead to a reduction in leaf water potential, consequently decrease in transpiration rate under salinity (Razzaghi *et al.*, 2011). Studies by Alon Ben-Gal *et al.* (2008) show that high transpiration rate at low salinity may be attributed to the accumulation of potassium ions that increased stomatal conductance and improved plant biomass production. It has been suggested that salinity tolerance of Bambara groundnut is a result of osmotic adjustment, reduction of leaf area index and low water loss through the stomata (Ambede *et al.*, 2021).

5.3 Effect of Sodium Chloride Salinity on Biochemical Parameters of Bambara Groundnut Landraces

5.3.1. Leaf Pigment Content

There were significant reductions in all the leaf pigments on exposure to salinity which may be attributed to salinity injuring the photosynthetic apparatus including the biosynthesis of pigments and activity of enzymes. This could be further linked with the effects of imbalances in Na $^+$ and K$^+$ plant nutrients under salinity. Similar to the findings of D'souza and Devaraj (2013), the salts could have induced the weakening of protein-pigment-lipid complex, enzyme degradation or enhanced activity of chlorophyllase. Under salinity, free oxygen radicals may also destroy biological molecules and cellular structures interfering with membrane stability. Kumar *et al*. (2020) explains that chlorophyll breakdown and peroxidation of lipids are also connected with decreased photosynthetic capacity of the plant affecting the amount of assimilates translocated from leaves to growing tissues further limiting growth. Furthermore, the high levels of salts directly toxify or/and osmotic stress negatively influence soil microbes consequently reduce nutrient uptake by plants. Other studies have also shown that salinity reduced leaf pigments in chickpea (Garg and Ranju, (2004) and soybean (Avila-Sakar *et al*., 2018).

From this study, the influence of salinity on leaf pigments in the landraces was significant. The control had significantly higher chlorophyll a, chlorophyll b, total chlorophyll and carotenoids which significantly decreased as salinity increased in all the Bambara landraces. The reduction in chlorophyll content in salt stressed plants may be due to interference with absorption of magnesium component of chlorophyll or/and reduced lamellar content of the light harvesting chlorophyll a/b protein. This process could be associated with salinity causing metabolic disturbances associated with photosynthesis such that the absorbed ions (Na$^+$ and Cl$^-$) interfered with the synthesis of leaf pigments. Under salt stress, Chl a, b and total chlorophyll of leaf pigments in chickpea cultivars reduction was attributed to degradation because of excess Na$^+$ within the cytoplasm (Kumar *et al*., 2017). Osmotic stress reduces Chl a synthesis and may cause breakdown of already formed chlorophyll (Ambede *et al*., 2012).Chlorophyll b is mainly associated with PS 11 antenna, a decrease in its content could suggest a structural modification of the antenna (Musyimi 2005). Sharma and Hall(1992) however, attributed decreases in total carotenoids in plants under salinity to change of carotenes to Zeaxanthin, that protected the plants against photo inhibition. Bambara groundnuts are slightly salt sensitive legumes. Salinity did not allow these plants to compartmentalize the salts resulting in reduced rates of photosynthesis. Photosynthesis is thus reduced by salt stress over time as salts accumulate in the young leaves decreasing contents of leaf pigments even in halophytic plants (Acosta Motos *et*

al., 2017). The landraces showed a considerable increase in carotenoid content for salinity treatment of 2dSm^{-1}in WSC landrace. Salinity may induce carotenoids synthesis in WSC landrace through enhanced availability of some nutrients including iron for pigment formation. Improved carotenoid levels partly protected the photosynthetic apparatus by scavenging the ROS (Muhammad *et al*., 2020). Carotenoids also aid in light capture for photosynthesis.

Although the landraces were not significantly different, theRSC landrace appeared to have a higher Chl a content as may be attributed to its photosynthetic fluorescence system that may withstand salinity to some extent.The BSC landrace also appear to have highe rChl b content as its PS11 antenna system structure may not have been severely modified by osmotic and/ or ion toxicity whereas a reduction in Chl a content simultaneously reduces total chlorophyll content however a higher total chlorophyll may indicate tolerance to salinity. The WSC landrace had a higher carotenoid, an implication of a fairlyactive photosynthetic apparatus.

5.3.2 Proline Content

Salt stress caused an increased in proline content in the three Bambara groundnut landraces. Proline may have contributed to the plants' ability to cope with salinity through osmotic adjustment. Increased proline content might also be associated with improved nutrient circulation, accumulation of osmolytes and stimulated enzyme synthesis that assist in the breakdown of ROS produced in response to salinity. Furthermore, proline helps in degradation of ROS thus sustaining antioxidant activity and balancing cell pH thus allowing plants such as Bambara to perform normal physiological functions in spite of degradation of its internal water state caused by salinity (Tsoata *et al*., 2017). El Moukhtari *et al*. (2020) attributed improved plant biomass growth to the role of proline in strengthening cell's capability for ionic adjustments.

Proline content substantially increased in Bambara plants in most of the treatmentsin relation to the control. The Bambara plant accumulated proline through enhanced nitrogen fixation and intake of nutrients when subjected to NaCl stress during plant growth. Under salinity increased synthesis of osmolytes like proline was reported (Kumar *et al*., 2020).El Moukhtari *et al*. (2020) attributes proline biosynthesis to accessibility to relevant amino acids with the ornithine pathway predominating in these plants. This phenomenon may be associated with increased N_2 fixation that consequently influence accumulation of proline and directly stimulate productivity in plants under salinity. According to Bremer and Kramer, (2019) this process might thus support Bambara plant to be more adaptable to NaCl stress when water availability decreases. From studies done by Teh *et al*. (2016) it is clear that proline relieved the undesirable salinity effects in O. *sativa* by increasing enzyme activity.

Proline too, reduced salt stress effects in plants through increased antioxidant activities, restricted absorption and transport of toxic ions and increased uptake of K^+ as suggested by El Moukhtari *et al*. (2020). Thus, tolerant species amassed more proline and its synthesis in plants was directly associated with stress tolerance (Kumar *et al*., 2013).On contrary, production of proline increased in the sensitive cultivars compared to the tolerant ones on exposure to stress from studies by Soussi *et al*. (1999). This suggested that in the chick pea, an undesirable relationship existed between proline accumulation and salt stress tolerance.

The accumulated proline, may serve to supplement salt tolerance in the three landraces by counteracting the water deficiency effect in the saline soil thus enhancing the activity of various enzymes and detoxification of free oxygen radicals. Furthermore, the RSC landrace accumulated more proline followed by BSC and least was WSC landrace. Kandowangko *et al*. (2009) attributed increased leaf proline content to lowered solute potential that supported the plants withstand and to recover after water stress. Moreover, studies by Ben Ahmed *et al*. (2010) showed that proline complements improved olives' salt tolerance through increased activity of antioxidative enzymes, photosynthesis and plant productivity.

5.3.3 Effect of Sodium Chloride Salinity on Nodulation of Bambara Groundnut Landraces

Based on the current study increasing salinity significantly reduce the number of nodules. This may be due to interferences on nodulation mechanism. Salinity reduced the number of nodules due to failure in the infection process, rhizobia establishment and nitrogen fixation. The legume and soil microbes might have been directly poisoned by the salts or affected through osmotic effects. Salts may encourage malfunctions, disturbed the structure of nodules and reduce nitrogen fixation in legumes (Fahmi *et al*., 2011). In a symbiosis that is stable , López *et al*. (2008) reports that salts lower symbiotic activities though decreased activity of nitrogenase and production of leghemoglobin. Furthermore, lesser amounts of photosynthates translocated to the roots in the landraces could also account for the decreased number and size of nodules. In a similar study, Dwivedi *et al*. (2015) reported reduced symbiosis initiation, production, development and functioning of nodules under salinity. In addition, salt stress reduced bacterial establishment, growth of nodules, decreased legume fixation of nitrogen and activity of enzymes (Egamberdieva *et al*., 2019). Salinity also suppressed initiation of the symbiosis through decreased root hair numbers (Zahran and Sprent, 1986).Studies by El Moukhtari *et al*. (2020) revealed that increasing salinity limits infection, existence and dispersal of rhizobia through decreased volume and weight of nodules, synthesis of leghemoglobin and nodule respiratory activities leading to reduced activity of nitrogenase, consequently nitrogen fixation. Moreover, Abd-Alla *et al*. (2016) confirmed that salinity undesirably interfered with Legume-*Rhizobium*

when it controlled growth and survival of rhizobia, slowed the process of infection, inhibited function of nodule, and decreased plant photosynthetic potential. Other studies have shown that salt stress decreased nodule numbers in chick peas (Soussi *et al*.,1999) and soy bean (Avila-Sakar *et al*., 2018).

Salinity treatments of 2 and 4dSm^{-1} in the RSC landrace, and salinity treatment of 2dSm^{-1} in WSC and BSC landraces significantly enhanced the number of nodules. This could result from enhanced nitrogen fixation, root growth and improved uptake of nutrients. The symbiotic relationship resulting in formation of nodules is a complicated process (Sanjay *et al*., 2021) during which the symbionts release phytohormones and enzymes that direct and control growth and adaptability in plants (Sanjay *et al*., 2021) under salinity. Thus, the N$_2$-fixing bacteria promote plant growth when they solubilize phosphorus bound on to the soil within the root zone while the siderophore releasing microbes may attack and degrade harmful effects resulting from salinity at the same time increase root accessible iron under salt stress (Kumar *et al*., 2020). Nitrogen fixation is apparently achieved by symbiotic bacteria (Etesami, 2018). The production of nodules by RSC landrace at salinity treatments of 4 and 6 dSm^{-1} could be associated with the ability of plant rhizobia growing and thriving in high levels of NaCl, imparting high competition in the rooting zone in order for the host plants to live producing nodules. This salt tolerance property might be associated with the ability of microbes to alleviating harmful effects caused by accumulation of protective osmolytes (Vriezen *et al*., 2007). In addition, ionic stress may be evaded by microbes making ionic adjustments in saline environments. The rhizobia from *Medicago ciliaris* and bean survived (4%) NaCl harmful salinity effects because of their capability to synthesize and accumulate protective osmolytes (Laurette *et al*., 2015).

The relatively higher number of nodules produced in the RSC and BSC landraces could also be attributed to genetic differences among landraces as the legume species- microsymbionts interactions are highly specific. Similar findings were reported by Qiang *et al*. (2003). Laurette *et al*. (2015) observed that nodulation potential significantly differed in Bambara depending on such factors as composition of soil minerals and bacterial populations in the growth media. Furthermore, the potassium ions that accumulated may contribute to good root growth, improved number and size of nodules on roots. According to Nascimento *et al*. (2016), on exposure to salinity, plant microbes improved infection and supported production of nodules. Checcucci *et al*. (2017) also observed that through nitrogenase activity, micro-organisms were capable of transiting sufficient nitrogen to the legume in exchange for different photosynthates. Other studies done on soybean under salinity showed improved nodulation (Al-Saedi *et al*., 2016) and nitrogen fixation in soybean (Sibpokrung *et al*., 2020). The highest number of

nodules noted in RSC landrace implied greater rates of symbiotic nitrogen fixation in saline environments.

5.4 Effect of Sodium Chloride Salinity on Yield in Bambara Groundnut Landraces

The decrease in number of pods and seeds of Bambara exposed to NaCl stress in this study might be due to the depressing effect of the salts on the plant itself or on growth of plant that lead to lowered photosynthesis, disrupted uptake of mineral nutrients and toxicity from the ions. Khan *et al*. (2017) attributes this to salinity induced abortion of flowers, lowered bloom numbers, hindered pod set, and ultimately impeded productivity in crops. In chickpea, salt induced yield decline was attributable to wrinkled seeds and decreased weight of grains (Serraj *et al*., 2007). Salt stress thus reduces yields in different legumes (Farooq *et al*., 2018). Studies show that salt stress reduced yield in *Brassica juncea* (Rossi *et al*., 2016), *C. cajan* (Ahmed and Ahmad, 2016) and barley plants (Ramegowda *et al*., 2015).

The control had significantly higher number of pods and seeds which reduced substantially as salt stress increased in all the landraces. This process may be attributed to NaCl salinity causing metabolic disturbances associated with photosynthesis such that the absorbed ions interfered with synthesis of leaf pigments, establishment of the microorganisms and nutrient uptake. Considerable reduction in yield when exposed to salt stress in cowpea, were partially linked to decreased leaf chlorophyll concentration above 50%, which is important in fruit production (Taffouo*et al*., 2010). According to Nadeem *et al*. (2019) salinity influenced nutritional imbalances, the complex hormonal interactions, specific ion toxicity as well as osmotic effects that affected the general plant growth and yield. Increasing NaCl salinity did not improve the yield in Bambara landraces as may be attributed to reduction in leaf pigment content.

Sodium chloride salinity may also affect microbial activities leading to slow growth culminating in decreased crop yield. Poor nodulation of Bambara may also have contributed to reduced pod numbers. Ahmed and Ahmad, (2016) observed that high salinity could hinder the signal exchange processes between the symbionts, resulting in poor nodulation and nitrogen fixation leading to significant yield loss in legumes. As a result, salinity inhibits nitrogen fixation through osmotic stress depressing activity of microbes and growth of plants. In addition, Wani *et al*. (2017) noted that the transport of assimilates decreases under salinity and leaves begin to act as sinks but not sources ultimately assimilate mobility into growing reproductive organs is inhibited, resulting in reduced development and seed setting. Al-Saedi *et al*. (2016) infers that soil salinity limits growth in plants, rates of photosynthesis, yield and nodulation in legume as caused by reduction in nodule nitrogen fixation and respiration. At later stages of plant growth, Nadeem *et al*. (2019) attributes further decrease in the rate of photosynthesis to effects of salinity

on the photosynthetic parameters. Reduced photosynthesis generates less photosynthetic products under these stressful conditions. This, coupled with increased respiration under salinity may increase breakdown of metabolites, directly reduce growth and yield. The differences in number of pods and seeds in Bambara landraces in response to NaCl salinity were noteworthy and could be attributed to decreased number of nodules, chlorophyll content, water stress adaptation and disturbances in nutrients. The result suggests further that WSC landrace as indicated by low yields at salinity treatment of $2\,dS\,m^{-1}$ was more sensitive to sodium chloride salinity. There were no pods/seeds in salinity treatments of 6 and 8 dSm^{-1}in all the landraces as these plants were unable to control ion content because of extreme physiological malfunctions that lead to lower growth rates and eventual death of cells and plants (Kumar*et al*., 2020).

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

6.1 Conclusions

The results obtained in this study have brought out various growth, physiological, biochemical and yield responses of Bambara groundnuts to sodium chloride salinity. The results are significant since the knowledge gained will form a basis for further research and development of salt tolerant Bambara landraces. Although some conclusions can be made from these results, some questions remain to be answered.

Plant growth parameters among the three landraces were significantly reduced by increasing NaCl salinity. The decrease was more evident in higher salinity treatments of 6 and 8 dSm^{-1} and during the final growth stages. This may be attributed to the toxic effect of the salts.

i. There was significant interaction between the salinity treatments and landraces such that : (a) The number of branches increased up to salinity treatment of 4 dSm^{-1} in all the landraces as was attributed to increased cell division and elongation, and maintenance of cell turgor in the growing regions as caused by osmotic adjustment (b) Shoot and root dry weight increased up to salinity treatments of 6 and 8 dSm^{-1} in RSC and WSC landraces respectively as may be attributed to accumulation of dry matter through improved photosynthesis, nitrogen fixation and uptake of nutrients (c) Root length increased at salinity treatment of 2 dSm^{-1} in all the landraces, and root to shoot ratio up to salinity treatments of 6 and 4 dSm^{-1} in RSC, and WSC and BSC landraces respectively leading to increased root surface area offering better access to nutrients (d) Percent WC increase in salinity treatments of 2 and 4 $dSm^{-1}$of WSC and BSC landraces respectively could be attributed to osmotic adjustment.

ii. Bambara plants significantly accumulated sodium in the leaves as the salinity treatments increased. Sodiumions were translocated through transpiration stream, accumulated and caused ion toxicity in leaves. Plants therefore, have to restrict the free movement of Na^{+} in order to prevent sodium from interfering with normal functioning of the cells. The significant increase in K^{+} content in salinity treatment of 2 dSm^{-1} in all the landraces may suggest increased uptake and possible role in supplementing salt tolerance while the increase in Ca^{2+} content in salinity treatment of 2 dSm^{-1} in all the landraces and salinity treatment of 4 dSm^{-1}

in the BSC landrace could also be a response for osmotic adjustment, and given that Ca^{2+} may mitigate the harmful effects of NaCl salinity. Salinity may also have stimulated calcium translocation to the leaves.

iii. Salinity significantly reduced photosynthetic parameters, CO_2 assimilation rate, stomatal conductance and transpiration rate as may be attributable to osmotic and ionic stress, and stomatal closure in all the landraces. However, the increase in CO_2 assimilation rate, stomatal conductance and transpiration rate at salinity treatment of 2 dSm^{-1} in all the landraces, and CO_2 assimilation and transpiration rates at salinity treatment of 4 dSm^{-1} in RSC landrace could be attributed to improved water and nutrient uptake, photosynthesis and stomatal opening in Bambara plants.

iv. Leaf pigment content was significantly reduced by salinity. There was substantial reduction in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids among the landraces. The influence of salinity on the leaf pigment content was attributed to damage of the photosynthetic apparatus.

v. Proline content significantly increased with salinity in all the landraces. Proline contributed to osmotic adjustment and possibly supplemented in NaCl salinity tolerance.

vi. Salinity significantly reduced number of nodules due to failure to infect, form rhizobia and nitrogen fixation in the landraces. However, the enhanced number of nodules in salinity treatments of 2 and 4, and 2 dSm^{-1} in the RSC landrace, and WSC and BSC landraces respectively could result from enhanced nitrogen fixation and nutrient uptake.

vii. Pods and seeds significantly reduced in the Bambara groundnut landraces as salinity increased due to reduction in number of nodules, nitrogen fixation, chlorophyll content and nutritional disturbances.

viii. Red seed coat landrace responded better to sodium chloride salinity followed by BSC and WSC landraces respectively.

ix. This result confirms that Bambara tolerated NaCl salt stress in most parameters up to salinity treatment of 4 dSm^{-1}, therefore encouraging its cultivation in saline regions will increase the value of those that are difficult to grow because of sodium chloride salinity and assist in the support of low-input agricultural systems hence improved family incomes and limited rural urban migration.

6.2 Recommendations

1.Leaf pigment content in Bambara was quite sensitive to NaCl salinity. This parameter could be suitable in determining effects of salinity in Bambara and therefore it is recommended for future biochemical studies.

2. Salinity increased proline content in Bambara plants. Proline may contribute to improved performance and survive in stressful conditions. This parameter could help improve the understanding of the effects of sodium chloride salinity hence the mechanisms employed by Bambara groundnuts in their tolerance and provide a basis for breeding and improvement on crop salt tolerance.

6.3 Suggestions for further studies

i.   Further study to focus on effects of rhizobium inoculation and sodium chloride salinity on growth and physiology of different locally grown Bambara groundnut landraces from varied ecological zones.

ii. Even though physiological parameters were significantly reduced because of salt damage on the photosynthetic apparatus or stomata, further studies on chlorophyll fluorescence of the leaves would partly explain why photosynthesis reduced on exposure to salinity.

iii. Determine plant mineral nutrients including nutrients like magnesium, phosphorus, iron within the roots, stems and leaves at different plant growth stages. This will provide further information on nutrient partitioning and the extent of damage due to salinity.

iv. The link between nitrogen fixation and mechanisms of salt tolerance was limited. Enzyme activity including nitrogenase and nitrate reductase need to be explored further.

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APPENDICES

Appendix 1: Summary tables for analysis of variance results for growth parameters of three Bambara groundnut landraces exposed to different saline solution levels over a 120 days period in chapter 4.

Plant height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	73	16818.43941	230.38958	21.76	<.0001
Error	196	2074.84	10.58592		
Corrected total	269	18893.27941			
	R-Square	Coeff Var	Root MSE	PH Mean	
	0.890181	12.91036	3.253601	25.81	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	5	6979.710519	1395.942104	131.87	<.0001
Landraces	2	60.128963	30.064481	2.84	0.0608
Salinity treatments in dsm	4	1823.858296	455.964574	43.07	<.0001
Salinity treatments *DAT	20	6869.726148	343.486307	32.45	<.0001
Salinity treatments*Landrace	8	444.408815	55.551102	5.25	<.0001

Number of branches

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	64	5036.257778	78.691528	10.79	<.0001
Error	160	1166.871111	7.292944		
Corrected total	224	6203.128889			
	R-Square	Coeff Var	Root MSE	NB Mean	
	0.811890	33.49629	2.700545	8.062222	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	4	4327.351111	1081.837778	148.34	<.0001
Landraces	2	62.995556	31.497778	4.32	0.0149
Salinity treatments in dsm	4	41.128889	10.282222	1.41	0.0233
Salinity treatments *DAT	16	95.315556	5.957222	0.82	0.6651
Salinity treatments*Landrace	8	173.804444	21.725556	2.98	0.0039

Number of leaves

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	73	385635971.0	5282684.5	10.28	<.0001
Error	196	100693612.3	513742.9		
Corrected total	269	486329583.3			
	RSquare	Coeff Var	Root MSE	LA Mean	
	0.792952	52.64175	716.7586	1361.578	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	5	269376405.4	53875281.1	104.87	<.0001
Landraces	2	646042.8	323021.4	0.63	0.5343
Salinity treatments in dsm	4	29987653.1	7496913.3	14.59	<.0001
Salinity treatments *DAT	20	58695003.0	2934750.2	5.71	<.0001
Salinity treatments*Landrace	8	10711131.9	1338891.5	2.61	0.0099

Shoot fresh weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	10484.71960	227.92869	37.84	<.0001
Error	88	530.12189	6.02411		
Corrected total	134	11014.84149			

	R-Square	Coeff Var	Root MSE	Shoot Fresh Weight Mean	
	0.951872	23.70440	2.454407	10.35422	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	8061.907324	4030.953662	669.14	<.0001
Landraces	2	231.506258	115.753129	19.21	<.0001
Salinity treatments dsm	4	477.826590	119.456647	19.83	<.0001
Salinity treatments*DAT	8	1120.728557	140.091070	23.26	<.0001
Salinity treatments*Landrace	8	75.906957	9.488370	1.58	0.1438

Shoot dry weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	98.6736296	2.1450789	6.85	<.0001
Error	88	27.5696296	0.3132912		
Corrected total	134	126.2432593			

	R-Square	Coeff Var	Root MSE	Shoot Dry Weight Mean	
	0.781615	39.03036	0.559724	1.434074	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	79.50325926	39.75162963	126.88	<.0001
Landraces	2	4.62459259	2.31229630	7.38	0.0011
Salinity treatments in dsm	4	1.10474074	0.27618519	0.88	0.0478
Salinity treatments *DAT	8	1.62859259	0.20357407	0.65	0.7338
Salinity treatments*Landrace	8	5.34948148	0.66868519	2.13	0.0406

Root fresh weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	8.86340741	0.19268277	8.62	<.0001
Error	88	1.96592593	0.02234007		
Corrected total	134	10.82933333			

	R-Square	Coeff Var	Root MSE	Root Fresh Weight Mean	
	0.818463	35 .21449	0.149466	0.424444	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	4.57911111	2.28955556	102.49	<.0001
Landraces	2	0.26133333	0.13066667	5.85	0.004
Salinity treatments in dsm	4	0.75229630	0.18807407	8.42	<.0001
Salinity treatments *DAT	8	1.74237037	0.21779630	9.75	<.0001
Salinity treatments*Landrace	8	0.49348148	0.06168519	2.76	0.0091

Root dry weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	0.10592593	0.00230274	1.47	0.0625
Error	88	0.13822222	0.00157071		
Corrected total	134	0.24414815			

R-Square	Coeff Var	Root MSE	Root Dry Weight Mean
0.433859	32.03796	0.039632	0.123704

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	0.03614815	0.01807407	11.51	<.0001
Landraces	2	0.01837037	0.00918519	5.85	0.4000
Salinity treatments in dsm	4	0.00192593	0.00048148	0.31	0.8729
Salinity treatments *DAT	8	0.01718519	0.00214815	1.37	0.2686
Salinity treatments*Landrace	8	0.01051852	0.00131481	0.84	0.5725

Root length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	634.406815	13.791452	3.30	<.0001
Error	88	367.897185	4.180650		
Corrected total	134	1002.304000			

R-Square	Coeff Var	Root MSE	RL Mean
0.632949	17.02940	2.044664	12.00667

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	201.8831111	100.9415556	24.14	<.0001
Landraces	2	1.3724444	0.6862222	0.16	0.8489
Salinity treatments in dsm	4	43.9632593	10.9908148	2.63	0.0397
Salinity treatments *DAT	8	117.4887407	14.6860926	3.51	0.0014
Salinity treatments*Landrace	8	109.9705185	13.7463148	3.29	0.0025

Root shoot ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	154.2551400	5.5091121	31.98	<.0001
Error	16	2.7566711	0.1722919		
Corrected total	44	157.0118111			

R-Square	Coeff Var	Root MSE	SR R Mean
0.982443	7.281407	0.415081	5.700556

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	5.0813678	2.5406839	14.75	0.0002
Landraces	2	2.7476744	1.3738372	7.97	0.0040
Salinity treatments in dsm	4	20.4599111	5.1149778	29.69	<.0001
Salinity treatments *DAT	8	3.7039422	0.4629928	2.69	0.0401
Salinity treatments*Landrace	8	121.5254156	15.1906769	88.17	<.0001

% Water content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	1137.597814	40.628493	2.48	0.0304
Error	16	262.632913	16.414557		
Corrected total	44	1400.230727			

	R-Square	Coeff Var	Root MSE	%WC Mean	
	0.812436	4.873305	4.051488	83.13637	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	46.0055319	23.0027660	1.40	0.2749
Landraces	2	47.5567394	23.7783697	1.45	0.2641
Salinity treatments in dsm	4	593.5858060	148.3964515	9.04	0.0005
Salinity treatments *DAT	8	247.1735922	30.8966990	1.88	0.1340
Salinity treatments*Landrace	8	76.6386621	9.5798328	0.58	0.7772

Appendix 2: Summary table for analysis of variance results for mineral nutrient parameters of three Bambara groundnut landraces exposed to different saline solution levels over a 120 days period in chapter 4.

Sodium (Na^+)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	48.26133333	1.72361905	5.86	0.0003
Error	16	4.70866667	0.29429167		
Corrected total	44	52.97000000			

R-Square	Coeff Var	Root MSE	Na^+ Mean
0.911107	29.86165	0.542487	1.816667

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Landraces	2	1.01233333	0.50616667	1.72	0.2106
Salinity treatments in dsm	4	27.80000000	6.95000000	23.62	<.0001
Salinity treatments*Landrace	8	7.15933333	0.89491667	3.04	0.0278

Potassium (K^+)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	3569.725111	127.490183	2.87	0.0152
Error	16	710.197333	44.387333		
Corrected total	44	4279.922444			

R-Square	Coeff Var	Root MSE	K^+ Mean
0.834063	30.14501	6.662382	22.10111

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Landraces	2	102.550778	51.275389	1.16	0.3399
Salinity treatments in dsm	4	943.583000	235.895750	5.31	0.0064
Salinity treatments*Landrace	8	1429.150333	178.643792	4.02	0.0086

Calcium (Ca^{2+})

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	0.09955556	0.00355556	16.00	<.0001
Error	16	0.00355556	0.00022222		
Corrected total	44	0.10311111			

R-Square	Coeff Var	Root MSE	Ca^{2+} Mean
0.965517	41.92627	0.014907	0.035556

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Landraces	2	0.00577778	0.00288889	13.00	0.0004
Salinity treatments in dsm	4	0.02088889	0.00522222	23.50	<.0001
Salinity treatments*Landrace	8	0.06977778	0.00872222	39.25	<.0001

Appendix 3: Summary table for analysis of variance results for gas exchange parameters of three Bambara groundnut landraces exposed to different saline solution levels over a 120 days period in chapter 4.

Transpiration rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	6074.284233	83.209373	4.77	<.0001
Error	88	3418.594945	17.441811		
Corrected total	134	9492.879178			
	R-Square	Coeff Var	Root MSE	Tr Mean	
	0.639878	83.38059	4.176339	8.016667	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	4547.663199	909.532640	52.15	<.0001
Landraces	2	41.778506	20.889253	1.20	0.3041
Salinity treatments in dsm	4	165.075199	41.268800	2.37	0.0500
Salinity treatments *DAT	8	296.579516	14.828976	0.85	0.6502
Salinity treatments*Landrace	8	39.734347	4.966793	0.28	0.0440

Stomatal conductance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	1432.911941	19.628931	1.95	0.0001
Error	88	1970.772831	10.054963		
Corrected total	134	3403.684772			
	R-Square	Coeff Var	Root MSE	Sg Mean	
	0.420988	148.2628	3.170956	14.470000	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	939.8379185	187.9675837	18.69	<.0001
Landraces	2	15.3844985	7.6922493	0.77	0.4624
Salinity treatments in dsm	4	6.3770052	1.5942513	0.16	0.0480
Salinity treatments *DAT	8	104.9404704	5.2470235	0.52	0.0553
Salinity treatments*Landrace	8	48.0803570	6.0100446	0.60	0.0490

CO_2 assimilation rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	46	2118.420725	29.019462	2.73	<.0001
Error	88	2082.738402	10.626216		
Corrected total	134	4201.159127			
	R-Square	Coeff Var	Root MSE	CO_2 Mean	
	0.504247	188.3706	3.259788	8.136667	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	2	912.3471941	182.4694388	17.17	<.0001
Landraces	2	6.5816385	3.2908193	0.31	0.0967
Salinity treatments in dsm	4	77.9681496	19.4920374	1.83	0.0127
Salinity treatments *DAT	8	422.2186504	21.1109325	1.99	0.0094
Salinity treatments*Landrace	8	139.3264059	17.4158007	1.64	0.0474

Appendix 4: Summary table for analysis of variance results for biochemical parameters of three Bambara groundnut landraces exposed to different saline solution levels over a 120 days period in chapter 4.

Chlorophyll a

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	55	660.4258237	12.0077422	24.41	<.0001
Error	124	60.9889791	0.4918466		
Corrected total	179	721.4148028			

R-Square	Coeff Var	Root MSE	Chlo a Mean
0.915459	37.73636	0.701318	1.858467

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	3	3.0362220	1.0120740	2.06	0.1093
Landraces	2	4.9152937	2.4576469	5.00	0.0082
Salinity treatments in dsm	4	354.6130813	88.6532703	180.25	<.0001
Salinity treatments *DAT	12	259.4269275	21.6189106	43.95	<.0001
Salinity treatments*Landrace	8	20.8517093	2.6064637	5.30	<.0001

Chlorophyll b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	55	492.8153304	8.9602787	18.29	<.0001
Error	124	60.7450804	0.4898797		
Corrected total	179	553.5604108			

R-Square	Coeff Var	Root MSE	Chlo b Mean
0.890265	46.32534	0.699914	1.510867

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	3	26.5200220	8.8400073	18.05	<.0001
Landraces	2	14.1847396	7.0923698	14.48	<.0001
Salinity treatments in dsm	4	222.0856638	55.5214159	113.34	<.0001
Salinity treatments *DAT	12	181.4567270	15.1213939	30.87	<.0001
Salinity treatments*Landrace	8	13.6661844	1.7082731	3.49	0.0012

Total Chlorophyll

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	55	366.8857536	6.6706501	47.81	<.0001
Error	124	17.2996114	0.1395130		
Corrected total	179	384.1853650			

R-Square	Coeff Var	Root MSE	t Chlo Mean
0.954971	33.59446	0.373514	1.111833

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	3	4.4092838	1.4697613	10.53	<.0001
Landraces	2	1.0409851	0.5204925	3.73	0.0267
Salinity treatments in dsm	4	241.0758655	60.2689664	432.00	<.0001
Salinity treatments *DAT	12	110.3915357	9.1992946	65.94	<.0001
Salinity treatments*Landrace	8	7.1504234	0.8938029	6.41	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	55	981.191120	17.839839	66.51	<.0001
Error	124	33.258930	0.268217		
Corrected total	179	1014.450051			

	R-Square	Coeff Var	Root MSE	Carotenoid Mean	
	0.967215	24.25216	0.517897	2.135467	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	3	241.0551940	80.3517313	299.58	<.0001
Landraces	2	6.0622564	3.0311282	11.30	<.0001
Salinity treatments in dsm	4	417.5507303	104.3876826	389.19	<.0001
Salinity treatments *DAT	12	251.5806205	20.9650517	78.16	<.0001
Salinity treatments*Landrace	8	43.9432291	5.4929036	20.48	<.0001

Carotenoids

Proline

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	55	98.6736296	2.1450789	6.85	<.0001
Error	124	27.5696296	0.3132912		
Corrected total	179	126.2432593			

	R-Square	Coeff Var	Root MSE	Proline Mean	
	0.781615	39.03036	0.559724	1.436667	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	3	79.50325926	39.75162963	126.88	<.0001
Landraces	2	4.62459259	2.31229630	7.38	<.0001
Salinity treatments in dsm	4	1.10474074	0.27618519	0.88	0.0405
Salinity treatments *DAT	12	1.62859259	0.20357407	0.65	0.7338
Salinity treatments*Landrace	8	5.34948148	0.66868519	2.13	<.0001

Appendix 5: Summary table for analysis of variance results for number of nodule parameter of three Bambara groundnut landraces exposed to different saline solution levels over a 120 days period   in chapter 4.

Nodules

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	37	1911.222222	51.654655	2.73	0.0004
Error	52	984.066667	18.924359		
Corrected total	89	2895.288889			

R-Square	Coeff Var	Root MSE	Nods Mean
0.660115	117.9275	4.350214	3.688889

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	1	67.6000000	67.6000000	3.57	0.0643
Landraces	2	52.3555556	26.1777778	1.38	0.0259
Salinity treatments in dsm	4	365.4000000	91.3500000	4.83	0.0022
Salinity treatments *DAT	4	548.0666667	137.0166667	7.24	0.0001
Salinity treatments*Landrace	8	752.2000000	94.0250000	4.97	0.0001

Appendix 6: Summary table for analysis of variance results for yield parameters of three Bambara groundnut landraces exposed to different saline solution levels over a 120 days period in chapter 4.

Seeds

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	11367.86667	405.99524	32.36	<.0001
Error	16	200.71111	12.54444		
Corrected total	44	11568.57778			
	R-Square	Coeff Var	Root MSE	Seeds Mean	
	0.982650	29.95895	3.541814	11.82222	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Landraces	2	674.844444	337.422222	26.90	<.0001
Salinity treatments in dsm	4	9137.911111	2284.477778	182.11	<.0001
Salinity treatments*Landrace	8	958.488889	119.811111	9.55	<.0001

Pods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	37	18780.45556	507.57988	56.90	<.0001
Error	52	463.86667	8.92051		
Corrected total	89	19244.32222			
	R-Square	Coeff Var	Root MSE	Pods Mean	
	0.975896	28.02972	2.986723	10.65556	

Source	DF	Type II SS	Mean Square	F Value	Pr > F
DAT	1	122.50000	122.50000	13.73	0.0005
Landraces	2	1098.68889	549.34444	61.58	<.0001
Salinity treatments in dsm	4	14812.04444	3703.01111	415.11	<.0001
Salinity treatments *DAT	4	193.77778	48.44444	5.43	0.0010
Salinity treatments*Landrace	8	1618.42222	202.30278	22.68	<.0001

***Plate 1* in chapter 3:** RSC, WSC and BSC Bambara groundnut landrace seeds in chapter 3.

***Plate 2*in chapter 4:** Symptoms of sodium chloride salinity (dull colored leaves, marginal burns, death of plants) i.e leaf damage in Bambara groundnut landraces at Maseno University research farm.

***Plate 3* in chapter 4:** Symptoms of sodium chloride salinity treatments inBambara groundnut landraces at Maseno University research farm.