EVALUATION OF SALT TOLERANCE IN SORGHUM (*Sorghum bicolor*) GENOTYPES

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Agronomy of Egerton University

EGERTON UNIVERSITY

JANUARY 2024

DECLARATION AND RECOMMENDATION

Declaration

This research proposal is my original work and has not been presented in this university or any other for the award of a degree.

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Recommendation

This proposal has been submitted with our approval as University Supervisors.

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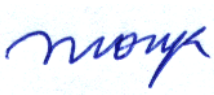
Signature  Date 08/11/2023

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DEDICATION

This work is dedicated to my grandmother Pauline, who taught me to work hard, to my husband Peter and my son Mark who were strengths that kept me pushing even better to the completion of this work.

ACKNOWLEDGEMENTS

I thank the Almighty God for keeping me to the completion of my Masters' degree. I acknowledge Egerton University for giving me a platform to undertake my Master's degree. I am indebted to the World Bank and Government of Kenya through the Kenya Climate Smart Agriculture Project (KCSAP) for their financial support. My sincere thanks goes to Kenya Gene Bank (GBK), International Crops Research Institute for Semi-Arid Tropics (ICRISAT) and Egerton University Sorghum Team for providing planting materials. My great thanks goes to my able supervisors; Prof. Erick K. Cheruiyot and Prof. Samuel Mwonga for their dedication, guidance and academic mentorship throughout the study period. This work was also made possible by the facilities in soil microbiology and biotechnology laboratory of Egerton university through the support of Prof. Nancy Mungai. I also acknowledge Mr. Simon Misoi and Mr. Amos Kitur who are members of staff from the Department of Crops, Horticulture and Soils of Egerton University for their consistent support and guidance on the laboratory and greenhouse work. In a special way, I thank my husband Mr. Kirui Peter who held my hand throughout my study period. Lastly this work came to completion with support from my friends and classmates who provided moral and spiritual support throughout the study period.

ABSTRACT

Sorghum is a drought-tolerant crop with high potential to improve productivity and livelihood of communities living in Arid and Semi-Arid lands (ASALs). While sorghum is largely adapted to diverse agro-ecological conditions, saline soils which is largely predominated by sodium chloride (NaCl) is a major abiotic threat to production in the ASALS. Laboratory and greenhouse studies were conducted at Egerton University, Njoro ($0^{o}22'11.0$" S, $35^{o}55'58.0$" E) to screen sorghum genotypes for salt tolerance. The experiments were laid out in a Completely Randomized Design (CRD) with treatment combinations of sorghum genotypes tested over four levels of NaCl concentrations 0, 3, 5 and 7 dSm^{-1}. The salt solutions treatments were prepared in hydro-A and hydro-B nutrient solutions to provide the required plant nutrients. A laboratory study to test for the effects of NaCl on seed germination and seedling establishment was determined for 250 sorghum genotypes in a growth incubator maintained at a temperature of $23^{o}C$ using Petri dishes lined with Whatman's filter papers. The data from this experiment were subjected to R4.2.0 for ANOVA and SPSS V. 20.0 for hierarchical cluster analysis of sorghum for salt tolerance. The results from this experiment showed a significant correlation ($P \leq 0.001$) between number of root hairs, root length and shoot length among the genotypes and NaCl concentrations. Cluster analysis showed BM 17, GBK 000049, GBK 000038, and BM 29 genotypes to be highly salt tolerant. Root hairs were shown to be a good parameter for screening for salt tolerance. Three salt tolerant and three salt sensitive genotypes were selected from the laboratory experiment and planted in plastic pots in the green-house using vermiculite as a growth media to determine the morphological and ion uptake responses of sorghum genotypes to NaCl concentrations. The data from this experiment were subjected to R4.2.0 for ANOVA. Sorghum genotypes showed significant variation ($P \leq 0.001$) in their growth and shoot/root Na^{+} uptake at the different NaCl concentrations. BM 17 genotype was significantly tolerant to NaCl concentrations with low salt injury index of below 2 when compared to the highly salt sensitive EST 41. The salt tolerant genotypes seemed to absorb and store more Na^{+} in their roots and restrict their excess transit to the shoots while salt sensitive genotypes transported more Na^{+} to the shoots. The results suggest salt exclusion as a mechanism of salt tolerance in sorghum. BM 17, BM 17, GBK 000049 and GBK 000038 were identified as the salt tolerant genotypes which provide a basis for crop improvement for enhanced resilience and improved productivity in ASAL regions.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASALs	Arid and Semi-Arid Lands
CRD	Completely Randomized Design
EABL	East Africa Breweries Limited
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics
GDP	Gross Domestic Product
GoK	Government of Kenya
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
KCSAP	Kenya Climate Smart Agriculture Project
MLND	Maize Lethal Necrosis Disease
SDGs	Sustainable Development Goals
UN-INWEH	United Nations University Institute of Water, Environment and Health
USDA	United States Department of Agriculture

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Sorghum (*Sorghum bicolor*) has been categorized a climate smart crop because of its resilience to climate changes, diseases resistance and its multiple uses. The crop can be used as food for humans from the grains, feed for animals from the grain and biomass, fuel from ethanol production, paper from its fiber, fertilizer utilization through incorporation of its residues as a source of organic carbon to the soil among other uses (ICRISAT, 2013). Although it is an indigenous crop to Africa, its largest production and consumption occurs in the United States of America (FAOSTAT, 2021). In Africa, it is mainly grown in the Arid and Semi-Arid areas (ASALs) of Eastern and Western Africa. The crop is grown in many parts of Western, Eastern, and North Eastern Kenya by smallholder farmers with land size of about 0.02 to 0.55 ha mainly for subsistence use (Chepng'etich *et al*., 2014). Most of the production by farmers are mainly for supplementation purposes because of their associated nutritional value. It has been identified as the most important crop as a source of protein, vitamin B complex, phosphorus, iron and energy (Khaton *et al.*, 2016).

Sorghum is a C_4 crop and therefore, is able to adapt to varied ago-ecological zones. However, salinity also known as "*quiet crisis*" is the major abiotic challenges limiting its productivity mostly in the ASALs (Haw *et al*., 2000). It is approximated that, 90 million hectares globally are affected by salinity and this constitute about 41% of the total earth's land (Turkan & Demiral, 2009). Kenya's ASALs covers about 80% of the total area (Mburu *et al*., 2019) with 50% of this predominated by saline soils (Mugai, 2004). Most of these saline ASALs regions are largely the stretches of the Northern to the Eastern parts of Kenya whose main economic activity is livestock production. The effects of climate changes have since increased the coverage of these ASALs to some parts of the Western and Central regions which are generally considered as the most productive agro-zones (Mburu *et al*., 2019; Mugai, 2004). The high salt accumulation maybe attributed to highly salt rich parent material from soil formation, poor soil drainage, poor soil management by human through continuous use of sodic fertilizers and use of saline ground water for irrigation. The increasing changes in climate pattern and irrigation with low quality water are projected to increase saline land. According to Robin *et al*. (2016), most of the irrigation water contain about 3% Sodium chloride (NaCl) salts which when used in excess leads to increased accumulation of toxic ions of Na^+, and Cl^- in and on the soils.

The increasing world's population has led to increased land pressure and demand for food. There is therefore, need to embrace sustainable crop production systems through adoption of climate resilient crops such as sorghum which are known to do well in harsh environment (GoK, 2021). It has been demonstrated that sorghum utilizes less water compared to the widely grown maize and wheat, and can withstand relatively high temperatures, commonly associated with the ASALs. Sorghum production has widely been promoted among smallholder farmers because of its potentiality to thrive well in ASALs and its low input requirements as opposed to other cereals. This promotion is done as a government strategy to enable the country meet its vision 2030 development goals of increasing household food, nutrition, energy security needs and increased rural income (GoK, 2021; Ochieng' *et al.,* 2011) in the marginal lands. However, there is little focus on breeding of sorghum genotypes for salinity stress even for indigenous lines to Kenya.

Superior genetic resources with improved tolerance to salinity are essential so as to reduce the yield losses from salinity. This requires identification of easily measurable traits related to salinity tolerance. This study therefore, evaluated sorghum genotypes for salinity tolerance; the identified genotypes can be recommended for adoption in ASALs and use by breeders working on salt tolerance.

1.2 Statement of the problem

Climate change has impacted negatively on agricultural production system due to prolonged drought, flash floods and poor rainfall distributions. This has exacerbated the negative effect of salinity on crop production and other dependable enterprises. Salinity is largely associated with the ASALs conditions and this has further worsened its productivity. Most farmers the saline areas have abandoned food and fodder crops production because they are susceptible to salt and engage in other enterprises. This shift has then expose them to runaway food and feed insecurity leading to acceleration of other related factors like malnutrition and conflicts. Saline soils hinder crop emergence and if they emerge, growth is retarded and can result in total crop loss in severe cases. This condition has to be addressed in order to increase agricultural productivity and improve food security for the communities living in the ASALs through adoption of climate resilient crops like sorghum. Sorghum is one of the climate smart cereal suitable for the ASALs with multiple role of satisfying food, feed needs and industrial uses. There is therefore, need to evaluate, select and recommend salt tolerant sorghum genotypes for the wider saline prone ASALs in Kenya.

1.3 Objectives of the study

1.3.1 General objective

To contribute to increased food security, nutrition and enhanced resilience of households to the effects of climate change through development of saline tolerant sorghum genotypes.

1.3.2 Specific objectives

i. To determine the effect of Na^+ and Cl^- ions on seed germination and seedling establishment of sorghum genotypes
ii. To determine physiological and biochemical processes of salt tolerance in sorghum genotypes.

1.4 Hypotheses

i. Na^+ and Cl^- have no significant effect on seed germination and seedling establishment in sorghum genotypes
ii. Physiological and biochemical processes of salt tolerant and salt sensitive sorghum genotypes shows no significant differences

1.5 Justification

Climate change and salinity are the major limiting factors to sorghum production and sustainable food security in the ASALs of Kenya given that their soils are fragile and predominated by salinity. The ASALs livelihood also depend on livestock as alternative source of food and other income resulting in high demand for feed. In order to meet the United Nations 2030 Sustainable Development Goals (SDGs) 1, 2, 3, 5 12, 13 and 17 (2015), there is need to adopt the GoK policies of 2021 which aims to promote climate resilient crops like sorghum so as to increase the productivity of the ASALs. However, to realize high yield and quality produce of sorghum, soil is a critical factor. Evaluation, determination and selection of saline tolerant sorghum genotypes is critical to substantial and sustainable agricultural productivity. Besides, the identification of traits related to saline tolerance will be useful in development of new varieties with desirable attributes for ASALs. There is thus a need to provide saline tolerant sorghum genotypes for utilization by the ASALs communities apart from as feeds and food but also, for other diverse uses. Sorghum genotypes with such attributes will result in enhanced grain yield for human consumption and biomass for feed, leading to improved livelihoods of the ASAL communities.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and Botany of sorghum

Sorghum is believed to have originated from Africa particularly South Sudan, Ethiopia and Kenya, where it is a major food crop and where wide variability of wild and cultivated forms still exists (Vavilov, 1951). It then spread to the rest of the world along people's movements, trade and shipping routes (Shewale & Pandit, 2011) where it has been adopted for domestic and commercial uses. Sorghum is ranked 5th most important cereal after rice, wheat, maize and barley and is currently cultivated in tropical, semi-tropical, arid and semi-arid areas in over 100 countries of the world (FAO, 2021). Its demand for grain is increasing due to numerous benefits, among which being gluten free cereal (Shewale & Pandit, 2011). This crop has been widely adopted because of its ability to withstand harsh environmental conditions such as low available soil water and low productive soils. Its resilience to limited soil water conditions is promoted by its extensive and aggressive root system, waxy bloom on the leaves surfaces to reduce water loss and its ability to stop growth during extreme environmental conditions and regenerate during favourable conditions (ICRISAT, 2013).

Sorghum is a cereal crop of the grass family *Poaceae*, sub-family *panicoideae* and shares the same with maize and sugarcane. Sorghum is also called great millet, Indian millet, milo, durra or shallu (Shewale & Pandit, 2011). It has approximately 25 species which are divided into 5 sub-genera: Eusorghum, Stiposorghum, Chaetosorghum, Heterosorghum and Parasorghum. The widely cultivated sorghum species belong to the sub-genera Eusorghum. The species *bicolor* L. Moench is grown for grains, forage and industrial uses while *halepense* L. Pers and *propinquum* (Kunth) Hitchc is grown exclusively for forage. Wild relatives of sorghum include, *S. bicolor* subspecies. *verticilliflorum*, *S. purpureosericeum* and *S. versicolor*, *S. arundinaceum* and *S. sudanese* (ICRISAT, 2013; Price *et al*., 2005; Vavilov, 1951). Sorghum has a haploid chromosome number of 10, containing 33,000 protein coding genes and its genetic integrity is maintained by self-pollination. Most of its cultivars are annual while some are perennials (ICRISAT, 2015). In Kenya, sorghum diversity is mainly represented by *Sorghum bicolor* which is the commonly cultivated species.

The crop has a wide phenotypic diversity which enables it to fix carbon through the C4 pathways for it to tolerate the harsh environmental conditions thus making it an ideal model grass compared to other grasses. It can grow under high temperatures of about 24^{o}C to

30^{o}C and low rainfall of below 100 mm per annum with little variation on yield (Mbinda *et al.*, 2019). Sorghum can tolerate moisture of up to 50% field capacity at even at germination. As a predominant rainfed crop, its yield is dependent on its drought tolerance ability. Sorghum can grow to a height of about 2.5 to 5 metres with leaf area of approximately 380 cm^2 and produce grain size of 2-3 mm in diameter (Britannica, 2022). Their stalks and leaves are coated with white wax that helps them reduce water loss. Their seeds vary widely in colour, shape and size among different genotypes ranging from loose branches to compact (Shewale & Pandit, 2011). Varieties grown for grains are relatively shorter in height with short panicles and their branches less than those grown for forage which are leafier, late maturing with long stems. Sorghum is among 8 cereals that provide 56% of food energy and 50% of protein consumed worldwide (ICRISAT, 2015). Varieties widely grown for grain has high nutritive value with protein content of close to 11.3% and average starch of between 55-74%. The nutrient composition is comparable to that of the widely consumed cereals like maize and wheat (ICRISAT, 2013).

2.2 Production and challenges affecting sorghum production

2.2.1 Global sorghum production

Sorghum is a stable food for over 500 million people and a source of direct and indirect income to a larger population. It is widely grown because of its numerous uses which include; feed as fodder and forage, grain for making flour for soft and hard porridge and baked food products, stalk juice for industrial ethanol production and brewing (ICRISAT, 2013, 2015). Globally, 50% of the domesticated sorghum is grown for human consumption. Under favourable conditions, sorghum has been proven to have high yield potential of upto 27 tonnes per ha as compared to other cereals. Its yield is also determined by the cultivar and management practices. Mostly farmers select cultivars that best suit their environment and meet their preferred needs. Continued selection has led to increased number of genotypes in their possession (Mundia *et al*., 2019; NRC, 1996).

Sorghum is widely grown in ASALs with limited water and frequent occurrence of drought. About 120 countries of the world grow sorghum making about 20% of the total world's cereal production (FAOSTAT, 2021). Africa contribute 39.2% with approximate yield of 27M tonnes followed by United States of America at 38.2% with approximate yield of 9.4M tonnes. In Africa, it is mainly grown in Western Africa, Eastern Africa with production and yield per unit area standing at 5.8M tonnes and 1,398.9 kg ha^{-1} respectively

(FAOSTAT, 2021). This production has been increasing slowly at a rate of about 3% annually since 1960s.

The area under production in countries across the world has recorded a mixed trend over the last decade. In Africa, there has been an increase in Eastern and Western Africa and has remained the most important cereal in these regions (Deb & Bantilan, 2003). With the current climate change and the increasing population, there has been increase in the world's drought stress and sorghum production is expected to increase by 20% by 2030 because of its adaptability to diverse ecological zones (Chepng'etich *et al*., 2014; ICRISAT, 2013, 2015; Mbinda *et al*., 2019; Mundia *et al*., 2019). The projected increase aimed at feeding the growing population and livestock in the ASALs.

2.2.2 Sorghum production in Kenya

Kenya's agricultural sector contributes 53% of the gross domestic product (GDP) (GoK, 2021) with 26% direct and about 27% through linkages with manufacturing, processing, distribution and other services related to this sector. Crop production contributes about 18% of GDP with 4% being contributed by sorghum value chain and it employs over 50% of the total agricultural labor force (GoK, 2021). Crops play a pivotal role in most areas in developing countries including Kenya. The government has promoted crops sector through policy changes and financial support since 1930, whose results has led to a growth rate of 19.98% per annum which is double the rate of many African countries (Oluoch, 2016).

Sorghum is grown in many parts of the country by smallholder farmers mainly for subsistence. It is tolerant to a wide range of soils with an average pH levels of between 5-8.5 (Balole & Legwaila, 2006; ICRISAT, 2013). Its production in Kenya has been low and this is largely attributed to the introduction of maize as an alternative food crop by the white settlers (Chepng'etich *et al*., 2014). Before 2007, its production average was estimated at 10,000 kg per hectare after which, it increased to about 13,500 kg per hectare in 2018 (FAOSTAT, 2021). The increase in production was occasioned by the introduction of sorghum-brewed beer by the East Africa Breweries Limited (EABL) around 2007 (Njagi *et al*., 2019).

2.2.3 Challenges to sorghum production in Kenya

Several interventions like plant breeding, distribution of high-yielding sorghum varieties (HYSVs) that are highly resistant and tolerant to pest and diseases have been harnessed by the GoK and other international stakeholders in crops production like ICRISAT

with an aim of improving sorghum productivity (Chepng'etich *et al*., 2014). Despite these efforts, there has been observed variability in potential and actual yield per unit area. These variations have been attributed to the unfavorable weather as a result of climate change. The changes have led to poor rains and severe dryness of the ASALs increasing salt accumulation in the soils due to increased evaporation. It is also attributed to short rains, severe droughts, genotype, poor soil fertility, reduced land sizes, and change in production to other enterprises (FAO, 2020; ICRISAT, 2015).

The ASALs land coverage in Kenya is to about 80%, limiting agricultural production because soils in these areas are fragile and predominated by salinity. Low agricultural productivity due to effects of prolonged droughts and low moisture has led to increased cases of hunger and prevalence of food insecurity by 4% (Ezenwa *et al*., 2018; FAOSTAT, 2019). This condition is expected to increase if agricultural production approaches are not modified. Sorghum production is being encouraged in most of the ASALs due to its adaptability to low rainfall (FAO, 2013, 2020). This promotion is done to increase household food, nutrition, energy security needs and increased rural income (GoK, 2021; Ochieng' *et al.,* 2011). However, these areas have been experiencing increased salinity due to increased evaporation, occasioned by climate change.

2.3 Proportion and contribution of saline soils to crop production

Salinity has been a major abiotic challenge limiting crop productivity mostly in ASALs. A total land area coverage of approximately 65 million hectares globally is highly affected by salinity accounting for approximately 41% of the total earth's land (Munns & Tester, 2008; Turkan & Demiral, 2009; Xu *et al*., 2021; Zheng *et al*., 2020). Most of the ASALs are dominated by highly saline soils, *solonchaks* (FAO, 1988), which accounts for 80% of the total salinity coverage (Mugai, 2004; USDA, 2015). The high salt accumulation maybe attributed to highly salt rich parent material for soil formation, poor soil drainage, poor soil management by human through continuous use of sodic fertilizers and use of saline ground water for irrigation. It is estimated that an average of 2,000 ha of irrigated land in the global ASALs is being degraded by salts daily (UN-INWEH, 2014) limiting crops productivity since water has high amount of salts (Kausar *et al*., 2012; Robin *et al*., 2016). This occurs mostly in ASALs where rainfall is too low to maintain regular percolation of rainwater through the soil.

The total area covered by saline soils from low to high is estimated to cover about 18 million hectares which is equivalent to 40% of the total ASALs coverage in Kenya based on

the Explanatory Soil Map of Kenya (Sombroek *et al*., 1982). Kenya has a very wide range of soils as a result of variation in geology, relief and climate. Its soil resources vary from sandy to clay, shallow to deep and low to high fertility (Mugai, 2004). The current variation in climate and land use management, has increased the area coverage under salinity. Increased evaporation of soil water leaves a lot of salt in the soil occasioned by the high correlation of aridity to the degree of salinity (Mugai, 2004). The major soils used in agriculture are *ferralsols, vertisols, acrisols, lixisols, luvisols and nitisols* with *Vertisols* and *ferralsols* being associated with saline areas (USDA, 2015).

Kenyan soils are divided into four zones based on the predominant salt types; The Southern Rift Valley (Zone I), the Turkana area (Zone 2), the Mandera-Wajir area (Zone 3) and the coastal area (Zone 4). The zones have been found to be predominated by significant amount of sodium chloride salts. Zone 1 has high agricultural productivity but is highly dominated by Na^+ from weathering of sodium mineral (feldspathoids) and Cl^- because of the high solubility of these salts and poor drainage of the land scape (Mugai, 2004). The surrounding soda lakes in these regions have also had salinizing effect on the surrounding soils because their water over runs their banks and the possibility of soil particle movements during rainy season. Saline soils are known to have high concentration of salts of chloride and sulfates of Sodium (Na^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}) and Potassium (K^+). Among all these salts, Na^+ and Cl^- are the most dominant and toxic ions. Sodium Chloride (NaCl) has a measurable negative effect on crops growth and development that results in severe reduction in crop production leading to losses in agriculture and poor economic growth (Roy *et al.,* 2018).

2.4 Effects of salt stress on plants

Crop tolerance to salinity varies from low to high depending on genotype. Salinity tolerance in crop plants is linked to growth response to salt stress (Sagar *et al*., 2019). Salt tolerance in crops is controlled by a number of complex traits. These traits are involved in numerous physiological processes, which are influenced by the interaction between genotype and the environment at different plant growth stages (Guan *et al*., 2013). Salt affect plant growth in a number of ways, among them is by increasing osmotic potential (Farooq *et al*., 2015; Meneguzzo *et al*., 2000; Sagar, 2017). The salts can also induce toxicity effects of Na^+ and Cl^+ (Niu *et al.,* 2012). When these ions are taken up in high amounts, water and nutrient uptake are compromised leading to poor growth and development (Islam & Karim *et al*., 2010; Netondo *et al.,* 2004a, 2004b; Roy *et al*., 2018). In order to combat the stresses, plants

modify their morphological, physiological and biochemical adaptive strategies to salt concentration, thereby compromising their growth and productivity (Attia, 2016, Sagar *et al*., 2019). Most of plant's physiological processes like water uptake, photosynthesis, transpiration, are highly sensitive to salt stress (Tari *et al*., 2013) leading to significant influence on their biochemical processes and ionic relations. Salinity tolerance in plants is a complex mechanism which is not highly explored because it requires long term experiments. The adaptation mechanisms differ from plants and genotypes.

An increase concentration of NaCl causes a negative impact in crop physiological processes. It leads to a reduction in photosynthesis due to low water availability which then impedes nutrient uptake causing ion toxicity to crops (Netondo *et al.,* 2004a, 2004b; Niu *et al.,* 2012). Under saline stress, Na^+ is a major ion known to accumulate in plants roots (Meneguzzo *et al*., 2000). High concentrations of Na^+ limits the absorption of other ions like K^+ and Nitrogen (N) which are essential for plant growth (Farooq *et al*., 2015; Sagar, 2017; Sagar *et al*., 2019). As NaCl concentrations increases in the solution, the accumulation of Mg^{2+} also increases while that of Ca^{2+} reduces both in the shoots and roots of plants (Farooq *et al.,* 2015; Flowers, 2004; Yasmeen *et al.,* 2013). Salinity causes osmotic stress, ion toxicity and essential mineral deficiency to the plant due to reduced mineral uptake, cell turgor, transpiration and growth, limiting crop yield and quality. Halophytes accumulate more inorganic ions in the vacuoles by excluding Na^+ from their aerial parts (Serraj *et al*., 2007). High accumulation of Na^+ in the roots than shoots can be a strategy of salt tolerance in plants. They respond by reducing cell water loss potential and restricting the spread of Na^+ to the shoot, maintaining normal enzymatic activities of the cell (Renault *et al*., 2001).

2.4.1 Effects of salt stress on seedling establishment

Salt tolerance of a crop at the mature stage is best expressed during an early stage of plant growth and development (Kausar *et al.,* 2012; Khan *et al*., 2003; Rahnama *et al*., 2010) making seedlings important character for screening (Islam & Karim *et al*., 2010). Plant ability to tolerate salt is controlled by multiple genes involved in many physiological processes. Their tolerance is determined by salt ion concentrations and distribution (Guan *et al*., 2013) as influenced by the environment, genotype or interaction of the two. As salt stress increases, the ability of plants to coordinate their own growth is destroyed. When seed germination and seedling elongation is intensified, roots functions and other physiological and morphological

processes are affected lowering or even inhibiting general plant growth and development (Ran *et al*., 2021). Plant life start at seed germination, emergence and seedling establishment.

Salinity stress causes toxicity of Na^+ and Cl^- in the soil and on its surface, lowering the osmotic potential of the solution. Low osmotic potential leads to reduced water uptake by the seed which then reduces or delays germination (Roy *et al*., 2018). Germination starts at water imbibition by the seed but increased NaCl concentrations increases absorption of Na^+ and Cl^- which causes ion stress and toxicity in the embryo limiting biochemical processes of germination even in salt tolerant plants (Mwando *et* a*l*., 2020; Tobe *et al*., 2000). Low seedling emergence reported by most farmers may be due to high mortality of epicotyl and hypocotyl as a result of low nutrient and water uptake by the seed occasioned by high accumulation of salt (Mbinda *et al.,* 2019; Roy *et al.,* 2018; Sagar, 2017; Sun *et al.,* 2014). The activity of cell division and elongation required for seed germination is reduced due to osmotic stress. At high osmotic stress condition, protein synthesis is altered due to reduced plant available water leading to low turgor pressure and cell growth (Farooq *et al.,* 2015, Roy *et al.,* 2018).

Seedling establishment is a critical growth stage in plants growing in saline conditions. Seedling emergence, root activity, shoot and root biomass is reduced with increasing salt concentration due to increased osmotic stress causing low water and nutrient uptake (Kausar *et al*., 2012; Li *et al.,* 2010; Sui *et al*., 2019). This leads to poor crop establishment and poor nutritional status for growth (Tari *et al*., 2013). The poor plant growth can be attributed to inhibitory effect of salt stress on crops' physiological processes by destroying the chloroplast. Chloroplasts are required for food manufacturing for the growing plant's parts through accumulation of chlorophyll. It has been determined that, chlorophyll content for the salt sensitive plants decreases with increasing salt stress (Guan *et al*., 2013; Mbinda *et al.,* 2019). In *Artemisa,* the low amount of chlorophyll content was due to increased degradation by increased activity of chlorophyllase and inhibited photoassimilate (Guan *et al*., 2013). This was also observed in maize (Niu *et al*., 2012) and cotton (Zhang *et al*., 2014) among other cereals. This is associated with the high accumulation of Na^+ and Cl^- in the plant tissues, reducing the photosynthetic efficiency (Sun *et al*., 2019; Wei *et al*., 2006) by promoting enzymatic activities which are toxic to the plant's physiological processes. Serious effects of these conditions are highly evident from visual effects on plant growth rate and biomass productivity.

High salt inhibits crop development causing reduction in overall plant growth and production (Niu *et al*., 2012; Sui *et al*., 2018). Long term exposure of plants to salt stress

causes chlorosis due to salt injury on the chloroplast and reduced photosynthetic activities and efficiencies on older leaves (Guan *et al*., 2013; Netondo *et al*., 2004a, 2004b). The death of chloroplast induces low content of chlorophyll and damage to mesophyll cells causing stomatal closure. This is a strategy to cushion the plant from salt damage by reducing water loss through transpiration and improving plant water use efficiency (WUE). Reduction in photosynthesis causes slow plant growth or even death over longer period of exposure, reducing its biomass (Guan *et al*., 2013). At high salt concentrations, photo-assimilation is inhibited causing reduction in tissue nutrients which is directly proportion to plant's stress strength. When plants are subjected to high salt stress, some above ground growth like biomass, leaf area, shoot length and internode will be compromised compared to those plants in the same growth stage but at low stress (Castillo *et al*., 2007; Ran *et al*., 2021). The poor growth will then increase symptoms of salt injury which include wilting, dying of leaf tips, stunted shoot, increasing number of death leaves due to low metabolic activity under stress (Ran *et al*., 2021). High salt concentration reduced the biomass of beans and cotton by 59% and 14%, respectively (Kausar *et al*., 2012). Reduction in aboveground growth maybe, due to shoot dependence on the roots for mineral ion absorption and uptake and for support.

2.4.2 Effects of salt stress on root growth

Reduction in growth occurs highly in the roots since it is the first organ to develop and come in contact with salts leading to increase in accumulation of Na^+ which limits the uptake of other important ions thus suffering from ion toxicity (Khan *et al*., 1990; Li *et al*., 2010; Sagar *et al*., 2019). However, low salt concentration stimulates root germination and elongation and its intensity is positively correlated with salt concentration (Ran *et al*., 2021). Under the saline condition, the amount of oxygen is reduced, depriving the plant of an energy source, while increasing the accumulation of ethylene hormone that inhibits root growth and development (Akram *et al.,* 2010; Roy *et al*., 2018; Sagar *et al.,* 2019). This hormone has inhibitory effect on plant growth and has been found to be in high concentration in saline soils (Akram *et al*., 2010) thus affecting root growth which is the basis of crop development. High salt concentrations and high accumulation of ethylene hormone causes a reduction of Gibberellic acid (GA) which promote growth and inhibit salt stress (Iqbal *et al*., 2010) through cell division and elongation. These effects of hormone imbalance are best manifested in the root hairs at the root. Root hairs forms the basis of overall root growth since they are primarily involved in mineral nutrients and water absorption from the growth media. Root

hairs growth is tip-based achieved through deposition of new plasma membrane and cell materials (Peterson & Farquhar, 1996). Regulation of cell division and elongation at the root tip appears to be linked to increased concentration of free Ca^{2+} at the cytosolic whose function is largely inhibited by salt stress (Shabala *et al*., 2003). When roots are exposed to high NaCl stress, there is increased absorption and accumulation of Na^+ in the cytosol limiting cell division (Halperin & Lynch, 2003) causing slow growth or death of root hairs. The content of salt ion and priority sequence of absorption and transportation to different parts of the plant under salt stress determine its tolerance to salt (Guan *et al*., 2013; Lin *et al*., 2004; Zhang *et al*., 2008).

Salt tolerant plants tend to absorb and accumulate more Na^+ in their roots under high salt concentrations. Increase in NaCl concentration increases Na^+ concentrations in different plant organs with high increase in the roots than other parts (Guan *et al*., 2013) limiting growth. Increased accumulation of Na^+ in the roots of salt tolerant plants decreases the concentration of K^+ (Castillo *et al*., 2007; Ran *et al*., 2021; Zhao *et al*., 2014) while there in no difference with Ca^{2+} concentration (Dashti *et al*., 2009; Niu *et al*., 2012). Ions play important role in normal plant growth but too much or less salt destroys dynamic ion balance in plant tissues. High salt concentration in plants leads to ion toxicity causing changes in membrane permeability. Interruption of ion and water absorption balance leads to water and ion imbalance near root surface lowering physiological functions and even destroy the structure of the roots (Ran *et al*., 2021). High salt concentrations offset Na^+ to K^+ balance in the cytoplasm because large amounts of Na^+ are taken up by the plant limiting K^+ absorption (Munns, 2002). Excess Na^+ may lead to loss of K^+ as a result of membrane depolarization and displacement by Na^+ because K^+ transporters have low integrity on levels of Na^+ toxicity (Castillo *et al*., 2007). Salt tolerance is highly associated with plant's efficiency for high ion compartmentalization restricting their entry to young and actively dividing plant organs. Crop plants therefore, restrict Na^+ uptake through selective Na^+ root absorption which promote their efflux and maintain high ratio of K^+:Na^+, Ca^{2+}:Na^+ and Mg^{2+}:Na^+ balance (Castillo *et al*., 2007; Guan *et al*., 2013; Ran *et al*., 2021; Zhao *et al*., 2014). The difference in adaptation is due to genotype, environment and the interaction of the two. Maize (Niu *et al*., 2012) and cotton (Zhang *et al*., 2014) adapt to salinity by increasing Na^+ concentration in the roots, stems and leaves while rice adopt by excluding Na^+ absorption (Zhao *et al*., 2014).

2.4.3 Effects of salt stress on plant ion uptake

The ability of a crop to maintain low Na^+ to other ion ratios in all organs explains its strong tolerance to salinity. At low salt stress, selective absorption of K^+, Ca^{2+} and Mg^{2+} and translocation to other plant organs is high. This increases with increasing salt concentrations up to a point where the plant cannot tolerate high NaCl concentration then it reduces (Dashti *et al*., 2009; Serraj *et al*., 2007; Zhao *et al*., 2014). High ion ratios are effective in enhancing salt tolerance. At high salt stress, there is competitive absorption and translocation of K^+ and Na^+ because their ionic radius and hydration energy are similar (Guan *et al*., 2013). Potassium ion is not only a key ion related to salt stress (Zhu, 2003) but a key ion in most higher plants required for regulation of physiological functions (Sano *et al*., 2007; Xu *et al*., 2021). High Na^+ concentrations compete for binding site of K^+ inhibiting enzymatic and metabolic processes which are dependent on K^+ (Munns & Tester, 2008; Ran *et al*., 2021). Salt tolerant crops tend to have high Ca^{2+}, Mg^{2+} and K^+ concentrations in their shoots than roots (Sun *et al*., 2019). This is a mechanism of tolerance where they exclude Na^+ absorption from the media and compartmentation by restricting their translocation (Dashti *et al*., 2009; Niu *et al*., 2012). They also have a high selectivity for K^+ in their symplasm channel at the point of entry into the xylem which helps to reduce damages by excess Na^+ (Dashti *et al*., 2009). Maintaining high K^+ concentrations and K^+/Na^+ ratios under salt stress help reduce damages to plant metabolic processes. The relationship between salt tolerance and K^+/Na^+ rather than Na^+ alone is a trait adopted by most glycophytes as a mechanism to tolerate salt stresses (Ding & Zhu, 1997; Iseki *et al*., 2017; Maathius & Amtmann, 1999; Zhu *et al*., 2001). Salt tolerance in plants can be increased by increasing concentration of the external calcium ion.

High concentration of Ca^{2+} help increase plant growth by increase Na^+ exclusion while increasing K^+ accumulation in the roots of ghycophytes (Dashti *et al*., 2009). Ca^{2+} is required by plants for cell division and elongation (Shabala *et al*., 2003). Increased Na^+ accumulation in the membrane system displaces Ca^{2+} causing damage to integrity of the membrane and its function leading to inorganic solutes leakage (Ran *et al*., 2021). As Na^+ influx increase, it activates Ca^{2+} signal transduction by enhancing increased Ca^{2+} absorption and transportation into the exchange system which then reduces Na^+ damages by eliminating it. Increased concentration of Ca^{2+} in the intracellular cells maintains K^+ and Na^+ balance. This helps the plant to establish ion homeostasis and adaptation to salt stress (Ran *et al*., 2021; Shabala *et al*., 2003). In order to prevent nutrient deficiency and ion toxicity in the shoots under saline stress, *Salix alba* compensate the change in concentration by regulating

upward transport of K^+ and Ca^{2+} through selective absorption and accumulation in the roots (Ran *et al*., 2021). Most halophytes accumulate more Na^+ in their roots under high salt stress and exclude them from the shoots (Castillo *et* al., 2007; Li *et al*., 2010; Niu *et al*., 2012; Zhao *et al*., 2014) while others exclude them by limiting absorption into the root system as an adaptation trait to salt tolerance. Salt sensitive crops absorb and transport excess Na+ to their shoots causing damage to their physiological activities (Guan *et al*., 2013; Niu *et al*., 2012). Cations selective absorption and transport ratios from source to sink provide a basis of giving a representative degree of salt damage to plants (Iseki *et al*., 2017; Munns & Tester, 2008). These cations are not only relative to salt tolerance but also important in regulation of physiological and biochemical functions of the plant such as photosynthesis, osmosis, ion balance, cell turgor and protein synthesis.

2.5 Mechanisms of salt tolerance in plants

Plants adaptation traits to salinity is described as either osmotic stress tolerance, Salts of Na^+ and Cl^- exclusion or tissue tolerance of high concentrations of Na^+ and or Cl^- mechanism. (Munns & Tester, 2008; Rogers & Noble, 1992).

2.5.1 Osmotic tolerance

This is the ability of a plant to tolerate salt stress and maintain normal physiological functions. Growth of salt-stressed plants is largely limited by the osmotic effect of salinity that results to retarded growth and reduced stomatal conductance (Farooq *et al*., 2015; Munns, 2002). High stomatal conductance is related to increased CO_2 assimilation rate which lead to increased growth rate. The ability of a salt-treated plant to have high stomatal conductance is an indicator of its osmotic tolerance to salinity (Munns, 2002; Roy *et al*., 2018). Halophytes have shown to accumulate more proline and glycine betaine (GB) proteins in the leaves leading to increased osmotic pressure in the mesophyll cell (Farooq *et al*., 2015). The increased osmotic pressure helps the plant increase its water and nutrient uptake, cushioning them from salinity shock. When salt accumulation overcome the toxic concentrations, the old leaves die and the young leaves will then undergo a reduced growth rate due to reduced photosynthates (Munns & Tester. 2008). The salt tolerant plant modifies their adaptive strategies to high salt concentrations which then compromise on their productivity over prolonged periods. The visual plants responses to osmotic stress like reduced leaf area, reduced plant height, wilting, dying back, poor emergence among others starts to manifest on the salt stressed genotypes (Attia, 2016; Netondo *et al.,* 2004a, 2004b). These responses are often independent of nutrients level in the growth media.

2.5.2 Salts exclusion

This involves the up and down regulation of the expression of specific channels and transporters allowing control of salt transport in the plant. Na^+ and Cl^- are the major ions which are known to be toxic when are in excess. However, Na^+ is highly considered because, it has been found to reaching toxic concentration before Cl^- amongst plants species under salinity (Niu *et* al., 2012). The ability of a plant to reduce ionic stress by minimizing the amount of Na^+ that accumulates in the cytosol of the cells of actively transpiring leaves and roots is associated with their tolerance to salt (Ran *et al*., 2021; Zhao *et al*., 2014). Plants achieve Na^+ exclusion in the leaves through its low uptake by the cells in the root cortex and restricted flow to the xylem by the parenchyma cells (Dashti *et* al., 2009; Iseki *et al*., 2017; Zhu *et al*., 2001;). These cells ensure that Na^+ doesn't accumulate to toxic levels within the leaf blades and failure to which, toxic effects like slow growth rate wilting, dying back and low biomass accumulation starts to manifest after few days of growth. This is due to the fact that, salt sensitive plants have low ability for K^+/Na^+ discrimination (Munns, 2002). Increased uptake of K^+ by plants under salt stress compensate for Na^+ toxicity, allowing the plant to delay or postpone the problem as a mechanism of salt tolerance (Iseki *et al*., 2017; Maathius & Amtmann, 1999). Details on how Na^+ is discriminated and where it is stored is scanty.

2.5.3 Tissue tolerance

This is a process which is achieved through synthesis and accumulation of compatible solutes. This involves compartmentalization of Na^+ and Cl^- at the cellular and intracellular level to avoid toxic concentrations in the cytoplasm (Castillo *et al*., 2007; Ran *et al*., 2021; Shabala *et al*., 2003). Accumulation of compatible solutes like amino acids, amines, organic acids and sugars by plants plays a role in tissue tolerance to salt-stress. These solutes have been found to increase greatly under salt and drought stress (Munns, 2002). They help the plant by protecting enzymes from denaturation, stabilizing the membrane and mediating osmotic adjustment to salinity (Renant *et at*., 2001; Serraj *et al*., 2007; Sun *et al*., 2019; Wei *et al*., 2006).

CHAPTER THREE

EFFECTS OF SODIUM CHLORIDE ON SEED GERMINATION AND SEEDLING ESTABLISHMENT OF SORGHUM GENOTYPES

Abstract

Salinity and climate change are major threats affecting crop productivity in arid and semi-arid fields globally. Sorghum is a climate smart crop but wide range of sorghum genotypes grown are sensitive to salt. Sorghum genotypes were screened for salt tolerance using sodium chloride (NaCl) at different concentrations. There were 250 evaluated sorghum genotypes using factorial arrangement in a Completely Randomized Design (CRD) with 4 levels of NaCl concentrations; 0, 3, 5 and 7 dSm^{-1} and three replications. The seeds were put in Petri dishes lined with Whatman's paper and saturated with NaCl solutions. The set Petri dishes were then put in a growth chamber maintained at $23^{o}C$ for four days to allow for germination analysis. Germinated seeds were transferred on the 4^{th} day after planting to plastic cups in a greenhouse at Egerton University, Njoro ($0^{o}22'11.0"$ S, $35^{o}55'58.0"$ E) with day and night temperatures being $30^{o}C$ and $25^{o}C$ respectively. The plants grew for 6 days in the cups that were half filled with Hydro-A and B nutrient solution with specific NaCl concentrations as were in the growth chamber. Data obtained were subjected to R4.2.0 for ANOVA and SPSS V. 20.0 for cluster analysis. There were significant differences in root hair numbers, root length and shoot length among the genotypes and NaCl concentrations at $P \leq 0.001$. Pearson correlation coefficient showed a high positive correlation at $P \leq 0.001$ between root length and root hair numbers. The results revealed presence of tolerance among local sorghum genotypes and roots as the key trait for use in crop improvement.

3.1 Introduction

Salinity is a major abiotic challenge to crop productivity with a total affected area approximated to be 800 M ha globally (Munns & Tester, 2008; Xu *et al*., 2021; Zheng *et al*., 2020). The increasing drought and irrational irrigation are expected to rapidly expand the saline land (Robin *et al*., 2016). Sustainable crop production remains a concern as the world's population and food demand increase (Griffiths & York, 2020; Hunter *et al*., 2017). Strategies to improve food production in response to increasing demand are threatened by climate change occurrences. This calls for a deliberate effort to identify and promote climate-smart crops, among them being sorghum (*Sorghum bicolor* L. Moench). Sorghum is a cereal crop ranked 5^{th} among major crops globally and is grown on approximately 44 million

hectares with a production of about 58 million tonnes (FAOSTAT, 2021). It produces adequately well even under conditions of relatively low soil moisture. However, salinity which is also referred to as a 'quiet crisis' (Haw *et al*., 2000) limits the promotion and utilization of sorghum in ASAL regions.

Kenya's arid and semi-arid lands (ASALs) covers about 80% of the total area (Mburu *et al*., 2019) with 50% of this predominated by saline soils (Mugai, 2004). The saline condition in the ASALs has been increasing over the years due to increased population and adverse encroachment to virgin lands for food and livelihood. This has led to the current accentuated climate change effects and reduction in food and feed crops production. The effects of climate change are compounded by salinity in the ASALs causing crop yield losses of about 35-100% (GoK, 2021; Robin *et al*., 2016). The government has widely promoted sorghum production among smallholder farmers owing to its resilience to soil water deficit unlike other cereals. The promotion of sorghum as a climate smart crop is a strategy to meet the 2030 sustainable development goals (SDGs) of improved food security, nutrition and increased rural income (FAO, 2020; GoK, 2021; Ochieng' *et al*., 2011) in the marginal lands. Although ASALs are associated with salinity, there are no sorghum varieties that have been recommended for these saline conditions in Kenya.

The main aim of this study was therefore, to identify sorghum genotypes that are tolerant to salt for the development of suitable and adaptable varieties to saline environments. The results are expected to generate useful information for sorghum improvement and selection for the saline environments.

3.2 Materials and Methods

3.2.1 Evaluated sorghum genotypes

A total of 250 diverse sorghum genotypes were evaluated for salt sensitivity. The genotypes were sourced from the Kenya Gene Bank, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Farmers' collection and a few from Eastern Africa Region (Appendix A). The genotypes were selected based on their yield, performance and uses.

3.2.2 Experimental procedures

The 250 genotypes were subjected to 3 levels of NaCl concentration: 0, 3, 5, and 7 deciSiemens per metre (dSm^{-1}) with the 0 dSm^{-1} concentration as the control. The experiment was laid in a Completely Randomized Design (CRD) and replicated 3 times. Different levels

of NaCl were prepared by dissolving solid NaCl in distilled water until the desired treatment level was attained as measured with an Electrical Conductivity (EC) meter. Ten seeds per genotype were placed on Whatman's filter papers which were placed on Petri dishes and soaked with the different NaCl treatments. The seeded petri dishes were placed in a multi-layered incubator chamber at a temperature maintained at 23^{o}C for four days. The number of seeds germinating were counted daily for four days after placement.

After four days, three seedlings of each of the germinated genotypes were transplanted into plastic cups (diameter 4 cm × 7 cm height) containing NaCl treatments levels as their Petri dishes and enriched with Hydro A and Hydro B hydroponic nutrients (Table 3.1). The nutrient solutions were prepared by taking and dissolving 2g each of Hydro A and Hydro B nutrients in 1L of distilled water. A known amount of NaCl was added to the prepared nutrient solution while checking the EC to ensure attainment of the desired level of concentration. The procedure was repeated for each of the salinity levels except for the control which was saturated with nutrients only. Enough quantity of the solution was added to each of the plastic cup to cover the roots of germinated seedlings and topped-up daily when necessary. The transplanted plants in the plastic cups were transferred to a greenhouse whose average day and night temperatures were 30^{o}C and 25^{o}C respectively. The plants were allowed to grown for 7 days after which, three seedlings were analyzed for root and shoot growth. The seedlings were gently washed with water over a 0.2 mm sieve to avoid loss of roots and shoots.

Table 3.1: The full spectrum composition of Hydro-A and Hydro-B hydroponics nutrients.

Mineral Ion	**Symbol**	**%Error**	**ppm**
Potassium	K2O	0.02	153
Copper Proteinate	Cu	0.03	0.2
Zinc Proteinate	Zn	1.8671	.03
Boron Proteinate	B	0.04	0.7
Calcium	Ca	1.075	126
Manganese Proteinate	Mn	1.97	
Phosphate	P		50
Iron Proteinate	Fe		28
Magnesium Sulphate	MgSO4		48
Nitrogen	N		120

ppm- parts per million, 126(98)-, and (P205)-. *Source:* Hydroponics Africa (2021)

3.2.3 Data collection

Final seed germination percentage was calculated from the final germination count on the fourth day using the following formula as adopted by Sagar *et al*. (2019).

$$\text{FGP (\%)} = \frac{S_G}{S_T} \times 100\% \quad \text{..... (Equation 1)}$$

Where, FGP (%) = Final germination percentage, S_G = Total number of seeds germinated, and S_T= Total number of seeds taken for germination.

After growing in the greenhouse for 7 days, root length per seedling was determined by measuring the root from its base to the tip in centimeters. Root hairs were counted with the help of magnifying hand lenses. Shoot length for each treatment was determined by measuring the shoot length from crown to the topmost visible leaf color in centimeters. The average shoot length for each treatment was determined.

3.2.4 Statistical analysis

Data on germination, root and shoot growth were subjected to Analysis of Variance (ANOVA) using R Statistical Package Version 4.2.0. The effects of NaCl concentration were

determined for seed germination and seedling establishment. Salt tolerance indices were analyzed by hierarchical cluster analysis using SPSS V. 20.0 (SPSS Inc. 2007 Chicago, IL, USA) statistical software. Cluster analysis identifies variables which were further clustered into groups using Ward's Method (Kumar *et al*., 2014; Singh *et al*., 2015) to identify genotypes with certain levels of salt tolerance.

3.3 Results

3.3.1 Influence of NaCl concentration on germination of sorghum genotypes

Germination of sorghum genotypes was negatively affected by salt concentration. Germination of sorghum progressively declined with increasing salt concentration **(**Figure 3.1**)**. However, there was no significant difference between main factor and interaction effect. Despite the general decline in germination with increase in salt concentration, there was no significant difference between the control and low NaCl concentrations (3 and 5 dSm^{-1}). Some sorghum genotypes sustained relatively high germination at 7 dsm^{-1} (high concentration) though their significance was negligible. Others showed a rapid decline in germination even under mild salt concentration indicating that they are susceptible to salt. Lesser decrement in germination percent (0%) from control to high salt concentration was evident in Seredo, BM 29, and BM 17, and high decrement of up to 25% was expressed in IESV 94076 DL, GBK 000382, IS 21158, and LARSVYT 58 85.

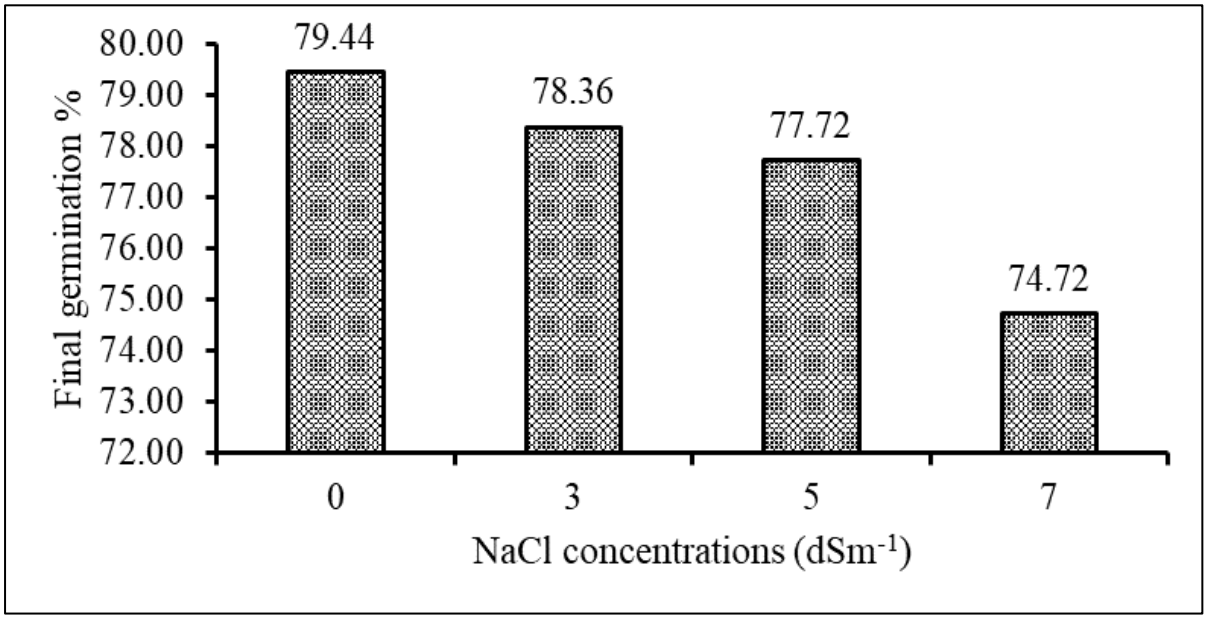


Figure 3.1: Effects of NaCl concentrations on germination of 250 sorghum genotypes

3.3.2 Effects of NaCl concentrations on shoot growth

Shoot length for all the genotypes was negatively affected by salt concentration at P ≤ 0.001 (Appendix B). High shoot performance was evident in the control but progressively reduced with increasing NaCl concentration (Figure 3.2). The most susceptible genotypes showed a rapid reduction with some not showing the possibility of shoot emergence while others had a lesser reduction. Higher shoot length reduction was evident in BUSIA 28-1, IS 21158, IESV92022/1SH, EST 20 and SHAMBUKO (PP 290) all of which showed 100% reduction and low in AINAMOI (0%), IESH 22002 (0%), GBK 000098 (2%), IESV 23006 DL (5%), GBK 000070 (5%) and GBK 000111(10%). The rest of the genotypes had medium reduction percentages.

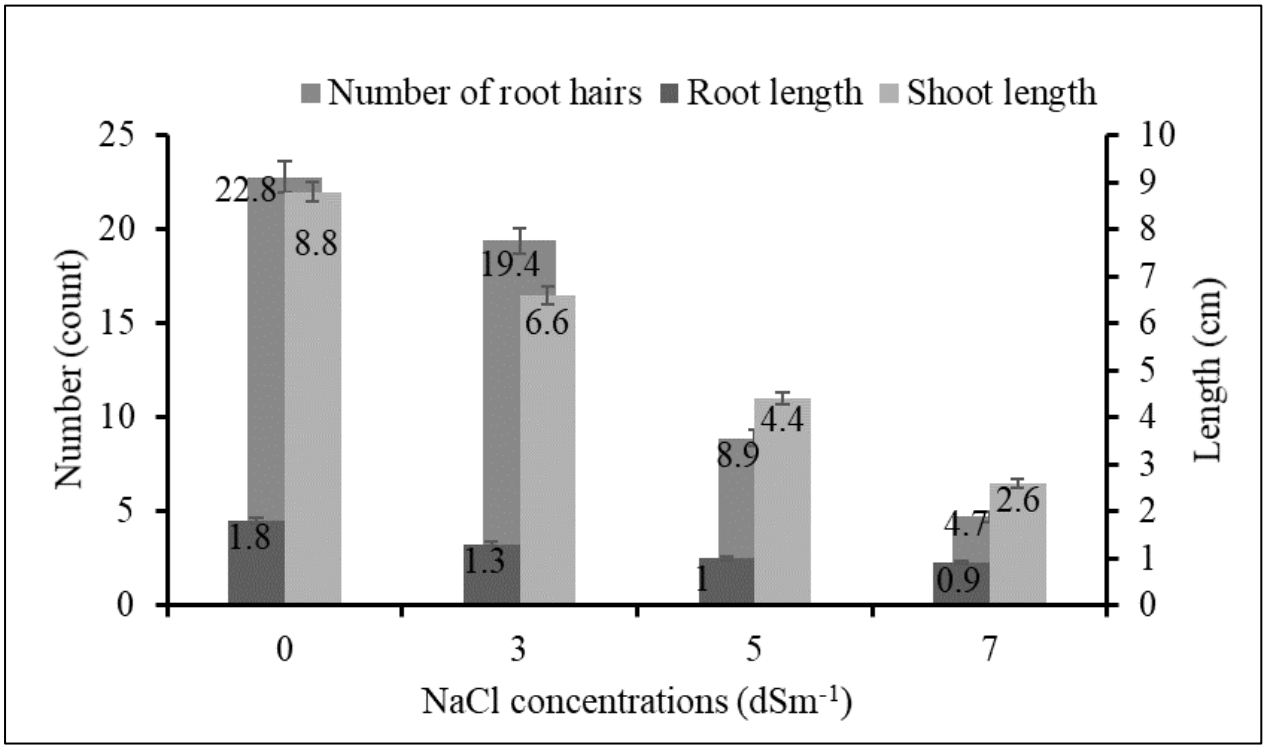


Figure 3.2: Effects of NaCl concentrations on number of root hairs, root length and shoot length for 250 sorghum genotypes. The error bars represent standard error of the means

3.3.3 Effect of NaCl on number of root hairs and root length

Root length and number of root hairs were significantly affected by NaCl concentration (Figure 3.2). The number of root hairs and root length reduced with increasing salt concentration at P≤0.001 (Appendix B). The reduction was genotype-specific where some genotypes showed drastic reduction compared to gradual response by others.

The reduction in root length as per cent of the control from 0-7 dSm^{-1} was noticeably small in GBK 000366 (0%), LABA (MW 5003) (5%), GBK 000027 (9%), EST 26 (10%) and

ICSR93034 (12%). A high reduction in root length of up to 100% under the same treatments was observed in SIAYA 42, IS 21158, SIAYA 27 3, EST 20, and ICSR 24008. The rest exhibited medium reduction ranges. Root length ranged from 0.2-18.1 cm in the control and 0-14.5 in the highest salt concentration treatment.

The number of root hairs for different genotypes varied dependent on root length at different salt concentrations. Using the number of root hairs as an indicator, EST 26(0%), MAKUENI LOCAL (1%), PP 290 (10%), BM 27 (13%), and IKINYARUKA (15%) were some of the genotypes which had high root hairs number and small percent reduction as compared to the control. IESV23008DL, GBK 000387, IS 2558, LARSVYT 58 85 and IS 25557 had 100 % root hair reduction relative to the control. The number of root hairs ranged from 1-90 in control and 0-55 in high concentration (7 dSm^{-1}).

3.3.5 Correlation of root hair numbers, root length, shoot length and final germination percentage

The Pearson Correlation Analysis using respective means from ANOVA showed a positive relationship between the variables at $P \leq 0.001$ (Table 3.2). A high positive correlation was observed between root length and number of root hairs. This positive correlation was expected given that increase in length is indicative of growth which means cell differentiation was taking place and hence the production of root hairs.

Table 3.2: Pearson's Correlation matrix of number of root hairs, root length, shoot length and final germination percentage

Variables	Number of root hairs	Root length	Shoot length	Final germination percentage
Number of root hairs	1			
Root length	0.73***	1		
Shoot length	0.27***	0.37***	1	
Germination	0.23***	0.25***	0.13***	1

***, significant at $P \leq 0.001$

3.3.6 Cluster analysis of sorghum genotypes for salt tolerance

Mean Membership Function Value (MFV) was used to aggregate the genotypes into salt-sensitive and salt-tolerance groupings. The methods have been used widely in this regard (Ding *et al*., 2018; Wu *et al*., 2019). Data for root length, shoot length, and the number of root hairs were analyzed by cluster for salt tolerance indices. The mean MFV for each of the

physiological parameters taken was calculated. The results were used to classify genotypes in a hierarchy for salt tolerance and sensitivity at different salt levels. The genotypes were divided into five clusters: highly salt tolerant (HST), salt tolerant (ST), moderately salt tolerant (MST), salt sensitive (SS) and highly salt sensitive (HSS) (Figure 3.3). Hierarchical analysis for the 250 genotypes used showed that BM 17 was the highly consistent for salt tolerance at all levels of salt concentration used followed by GBK 000049, GBK 000038, and BM 29, while EST 41, IESV 23007 DL, and GBK 000073 were highly consistent for salt sensitivity respectively.

The results showed that using individual parameters and cluster analysis to classify genotypes for salt tolerance has different outcomes which may or may not tally. However, using one parameter to classify crops for salt tolerance may not give enough information because the mechanisms and parameters for tolerance could be diverse and interrelated. These parameters therefore, need to be pooled and screened together to give a clear direction.

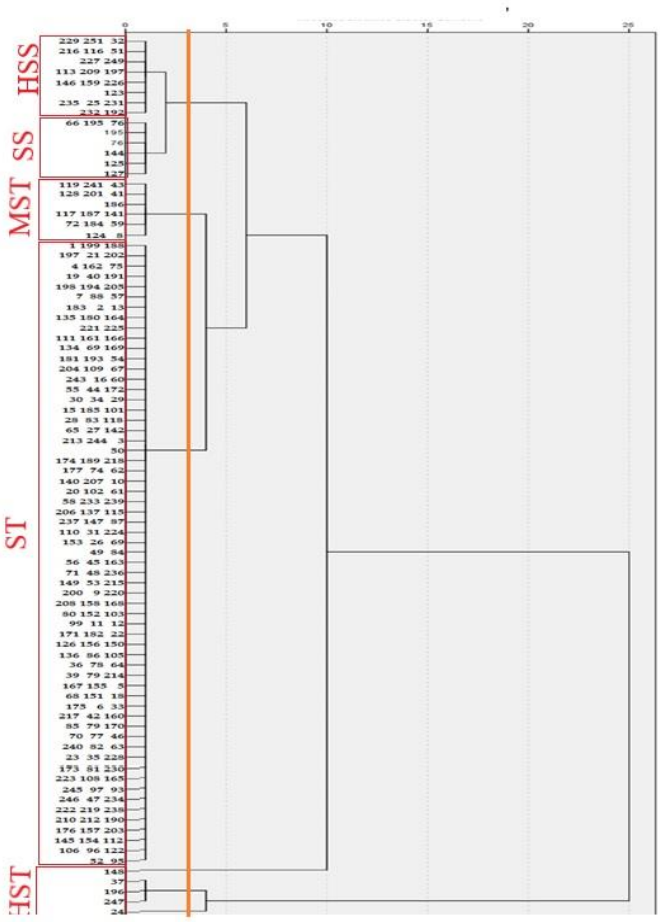


Figure 1.3: Hierarchical cluster analysis based on Ward's Method to evaluate 250 sorghum genotypes for salt tolerance at 7 dSm^{-1} NaCl concentration using MMFV. HSS-highly salt sensitive; SS-salt sensitive; MT-moderately tolerant; ST-salt tolerant; HST-highly salt tolerant.

After cluster analysis, a comparison analysis on the trend of root hair growth for the salt tolerant and salt sensitive genotypes at different NaCl concentrations was drawn (Figure 3.4). It is clear that, salt have a significant effect on root hairs. The number of root hairs dropped rapidly from an average of 28 per root in control to zero at 7 dSm^{-1} of NaCl indicating 100% reduction in number of root hairs in the salt sensitive genotypes. The salt tolerant genotypes had a low reduction in number of root hairs from an average of 21 in control to 12 in 7 dSm^{-1} indicating a 50% reduction. However, there was a slight decline in number of root hairs at 3 dSm^{-1} of NaCl for the tolerant and rapid decline for the sensitive genotypes respectively. This may imply that salt influences some physiological process responsible for root formation and elongation leading to the observed trend (Figure 3.4).

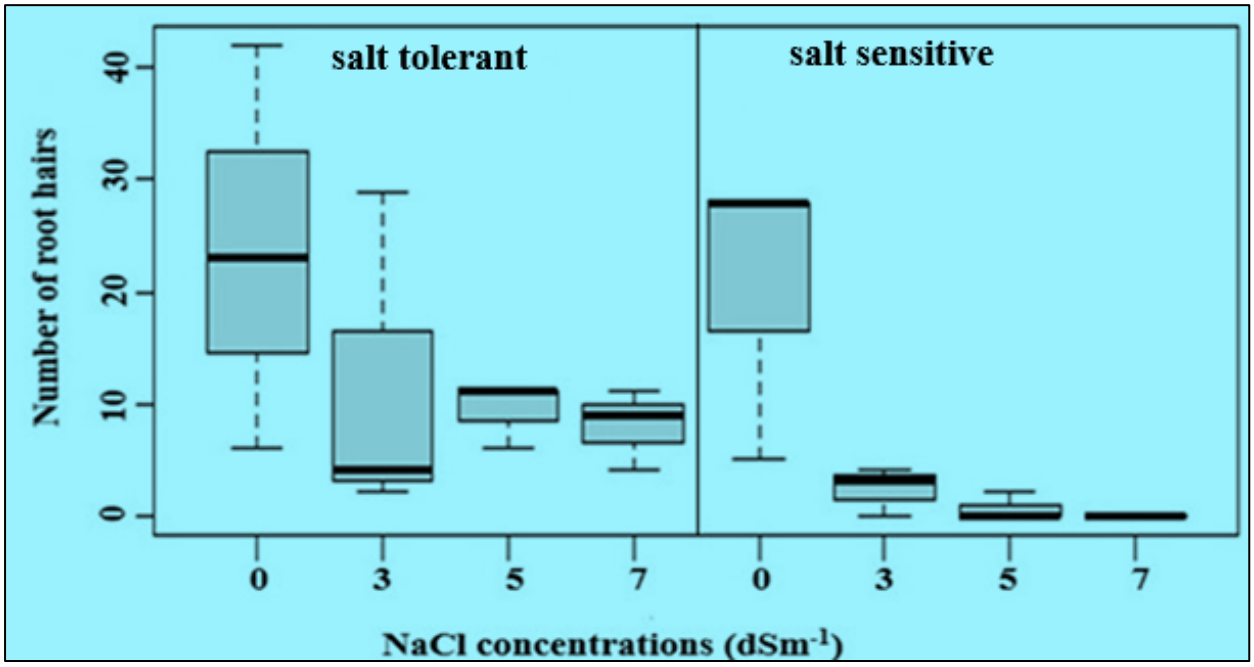


Figure 3.2: Box plots showing comparison of the effects of NaCl concentrations on number of root hairs for the salt tolerant and salt sensitive sorghum genotypes at 0, 3, 5 and 7 dSm^{-1}, respectively. The bars show the maximum and minimum range

3.4 Discussion

The hierarchical clustering of 250 sorghum genotypes emphasized the genetic variability of sorghum to salt tolerance (Sagar *et al.*, 2019) with a unique response being expressed by the highly tolerant genotypes. The ability of sorghum to adjust to different NaCl concentrations as shown in Figure 3.4 seems to point to a unique trait associated with salt tolerance in sorghum. However, a salt tolerance mechanism is yet to be fully understood. In this study, we found that salt tolerance in sorghum genotypes cannot be evaluated using a

single parameter as done on *Brassica napus* (Wu *et al*., 2019). Based on the outcome of this study, root hairs appear to be a reliable screening trait for salt tolerance in sorghum.

Germination and seedling growth of sorghum had varied responses to NaCl concentrations. Results show germination of sorghum as being largely unaffected by salt concentrations. These results disagree with other studies that salinity limits seeds germination by limiting water imbibition by the seed leading to increased absorption of sodium (Na^+) and chloride (Cl^-). Increased uptake of Na^+ and Cl^-creates ionic stress and toxicity (Mwando *et al*., 2020; Tobe *et al*., 2000) causing disruption of biochemical processes of germination. It was only at a higher NaCl concentration (7 dSm^{-1}) that a decline in germination was observed. In this regard, and due to the limitation of salt on growth, the seeds in the farmers' field may germinate but fail to emerge under saline soil. The adverse effect of NaCl was more visible at seedling stage and therefore, data on germination was not used in clustering genotypes for salt tolerance since it showed no significant contribution.

Though both shoot and root growth was limited by salt at the seedling stage, there was noticeable reduction in the roots. Root growth progressively reduced with increase in salt concentrations. Most of the sorghum genotypes tolerated salt concentration below 5 dSm^{-1} but a few could survive in NaCl concentration above 5 dSm^{-1}. Past studies points to a salt threshold of 1.9 to 4.5 dSm^{-1} for most crops that can allow about 70% yield output and a significant reduction with progressive salt increase above the range (Grieve *et al*., 2012; Maas & Hoffman 1977). Sorghum genotypes that survived at 7 dSm^{-1} were considered tolerant to salt. Roots are the primary organs exposed to salt in the growth media. Therefore, the observed effect of salt on roots is no surprise since increased concentrations of salt retard root growth even in halophytes (Li *et al*., 2010). A more salt-sensitive root component in this study was the root hairs. A rapid decline in root hairs on exposure to increasing NaCl concentrations could point to a process in root hair initiation in the trichoblast. Root exposure to NaCl leads to increased Na^+ in cytosol of root hairs (Halperin and Lynch, 2003). Root uptake of Na^+ in Arabidopsis has been shown to reduce potassium (K^+) in root cells with a greater reduction in salt sensitive cultivars (Guan *et al*., 2013). It has also been shown that high uptake of Na^+ in barley reduces the concentration of calcium (Ca^{2+}) in the cytosol (Shabala *et al*., 2003). Both K^+ and Ca^{2+} influence cell division and growth. It has been demonstrated that K^+ play a significant role in cell division and cell-cycle progression in plants among other functions (Sano *et al*., 2007; Xu *et al*., 2021) while Ca^{2+} is involved in cell division and elongation (Shabala *et al*., 2003). The reduction in Ca^{2+} and K^+ as occasioned by root exposure to NaCl could partly explain the reduced root hair numbers with

increased salt concentrations. Besides the possible limitation of growth due to reduced Ca^{2+} and K^{+} in root cells, direct toxicity of emerging root hairs by NaCl salt was likely (Nxele *et al*., 2017). However, specific information on the ratios of these ions and root hair growth and/or inhibition as a mechanism of salt tolerance or sensitivity is scanty.

Reduction in shoot growth with increased salt concentration can be associated with shoot dependence on roots for nourishment. The transpiration stream causes increased concentration of Na^{+} in the shoot (Tobe *et al*., 2000). High concentration of Na^{+} is known to limit the transport of other ions such as K and nitrogen (N) which are essential for growth (Farooq *et al*., 2015; Sagar, 2017; Sagar *et al*., 2019). However, shoot ion component and translocation in relation to root uptake as a mechanism of salt tolerance in sorghum is yet to be determined.

3.5 Conclusion

The present study demonstrates the existence of large genetic variation for salt tolerance among sorghum genotypes that can be used for the development of varieties suitable for the marginal areas. The variability can be explored during early seedling developmental stage. The root hairs appear to be a reliable parameter for screening for salt tolerance in sorghum. This may present a significant contribution to breeding for salt tolerance in sorghum.

CHAPTER FOUR

EFFECTS OF SODIUM CHLORIDE CONCENTRATIONS ON GROWTH AND ION UPTAKE IN SORGHUM

Abstract

Salt tolerance in plants is controlled by multiple genes involved in many physiological and biochemical processes. Six sorghum genotypes were used to determine the morphological and ion uptake responses to NaCl concentrations. A greenhouse experiment was laid down at Egerton University, Njoro ($0^{o}22'11.0"$ S, $35^{o}55'58.0"$ E) in a Completely Randomized Design (CRD) with factorial treatment arrangement and 3 replications. The day and night temperatures in the green house were $35^{o}C$ and $20^{o}C$ respecively The treatments were six sorghum genotypes, four levels of NaCl concentration; 0, 3, 5 and 7 dSm^{-1} with 0 dSm^{-1} being control and enriched with full strength Hydro-A and Hydro-B nutrients. Data obtained were subjected to R4.2.0 for ANOVA. There were significant differences in number of roots, plant height, total leaf area, biomass accumulation and ion uptake between genotypes and NaCl concentrations at $P \leq 0.001$. Pearson correlation coefficient between number of roots, total leaf area, biomass accumulation and ion uptake showed a high positive correlation at $P \leq 0.001$. The results revealed that, sorghum adopt to salt stress by reducing ion uptake from the roots to the shoots. This could be a tolerance mechanism by avoiding ion toxicity through ion compartmentalization.

4.1 Introduction

Sorghum is ranked 4th cereal globally after maize, wheat and rice in production and is grown in approximately 44 million hectares with an approximate production of 58 million tonnes per hectare (FAOSTAT, 2021). The increased production has been occasioned by the fact that sorghum is adopted to harsh environments experienced in the ASALS where most cereals do not perform (GoK, 2021; ICRISAT, 2015). The ASALs communities are largely pastoralist whose major concern is feed and fodder for their livestock. However, the strategies to increase food and feed production in the ASALs are limited by the current climate change being experienced across the globe (Griffiths & York, 2020; Hunter *et al*., 2017). As the world's population increases, there is an increased demand for food and therefore, sustainable food production remains a concern. In order to realize high yields and utilization of sorghum, soil remains a critical concern as soil salinity is the major threat.

Promotion of salt tolerant crops through breeding is one of the interventions to salinity challenges aimed at improving dryland productivity.

Salinity is a major environmental stress limiting plants growth and development and which can cause up to 100% yield losses. The current global land area affected by salinity is estimated to be about 800 Mha (Munns & Tester, 2008; Xu *et al*., 2021). The area under saline soils are expected to increase due to increased irrigation and irrational use of salty water (Robin *et al*., 2016). Most of the affected areas are the Arid and Semi-Arid Lands (ASALs) because of the increased evaporation leaving a lot of salts on and in the soil. The current climate change has increased Kenya's land cover of ASALs form about 40% to about 80-85% and about 50% of these being saline (Mburu *et al*., 2019; Mugai, 2004). There is therefore, a need to identify and promote under-utilized high-value crops that are climate resilient and among which is sorghum.

Salt tolerance in plants is controlled by multiple genes which are involved in many physiological and biochemical functions (Guan *et al*., 2013). The mechanisms involved are complex and are not well documented. Salt stress causes plant water loss, osmotic stress, and ion imbalance which leads to ion toxicity and nutrient deficiency (Ran *et al*., 2021). Long term exposure of crops to salt can cause total crop loss which may lead to food and nutrition insecurity in areas with 100% dependence on crop farming. In order to meet its vision 2030, the government of Kenya has been promoting sorghum production by small holder farmers (GoK, 2021) because of its resilience to soil water deficit and salinity (Ran *et al*., 2021). However, there are no sorghum varieties which have been recommended for the saline soils in Kenya.

This study used six genotypes to determine the morphological and biochemical responses of salt tolerance in sorghum. It was hypothesized that, salt stress has no effect on the morphological and biochemical processes of sorghum genotypes. The aim was to obtain the salt-tolerant genotypes that can be used in breeding programs of *Sorghum bicolor* for the saline ASALs.

4.2 Materials and Methods

4.2.1 Selected sorghum genotypes

A total of six sorghum genotypes were used to determine the effect of NaCl concentration on whole-plant sorghum response and ion uptake. The genotypes were selected from experiment 1 (Section 3.2), and they included three salt tolerant and three salt sensitive sorghum genotypes. The genotypes were selected on the basis of parameters performance

across salt treatments. Genotypes that performed better beyond 5 dSm^{-1} were considered salt tolerant while those whose performance did not go beyond 5 dSm^{-1} were considered salt sensitive. The selected salt tolerant genotypes were BM 17, GBK 000049, GBK 000038 and salt sensitive were EST 41, IESV 23007 DL, GBK 000073.

4.2.2 Experimental procedures

A greenhouse experiment was laid down in Completely Randomized Design (CRD) with factorial treatment arrangement and replicated 3 times. The six genotypes were subjected to three levels of NaCl concentration, 0, 3, 5 and 7 dSm^{-1}, with the control being the 0 dSm^{-1} treatment. Each of the different NaCl concentration treatments were enriched with a Hydro-A and Hydro-B nutrient solutions. The different levels of NaCl were prepared by dissolving 2 g of Hydro-A and Hydro-B nutrient solution in 1L of distilled water and its electrical conductivity (EC) was determined using an EC meter. The pre-determined NaCl levels of 3, 5, and 7 dSm^{-1} were each prepared by slowly adding and stirring solid NaCl into the already prepared nutrient solution until the required EC reading is attained.

Ten seeds of each of the selected genotypes were sown in 3L plastic pots measuring 20 cm × 15 cm containing vermiculite. Immediately after sowing, each pot was irrigated with sufficient nutrient solution with respective NaCl concentration. Each treatment was irrigated with its specific salt concentration daily for four weeks. The seedlings emerged after five days.

The germinated seedlings were thinned to three plants per pot on the 10^{th} day after sowing (DAS). The three plants were then allowed to grow for 40 days to allow for an adequate growth of leaves and roots for subsequent analyses.

4.2.3 Data collection

Data on the growth performance of the seedlings were collected for plant height, Leaf area, root characteristics, biomass, ion uptake and on salt injury.

All the three plants per pot were used for repeated growth measurements. Plant height was taken on the 10^{th} 14^{th}, 21^{st}, 30^{th} and at 40^{th} DAS by measuring the length in centimeters from the crown of the plant to the leaf collar of the uppermost leaf. Leaf area for fully unfolded leaves in each plant was determined at the 30^{th} day after sowing (Bueno, 1979).

Leaf area = Leaf length x Leaf width…… (Equation 2)

Root characteristics were determined by scanning three plants samples per pot on the 10^{th} DAS and at 40 DAS. The roots of the sampled plants were gently but thoroughly washed

with distilled water to remove foreign media attached. Roots architecture indices were scanned for root length, root numbers, and root volume using the root scanning image analysis system WinRHIZO Pro 2012b (Regent Instruments, Inc., Québec, QC, Canada) (Du *et al.*, 2019).

After 40 days, biomass yield for all the 3 plants in each pot were determined. The plants were harvested and separated into roots and shoots. The divided samples were then oven dried for 3 days with temperature maintained at 65^0C. The dry weight of both roots and shoots for each treatment were measured in grams. Shoot to root ratios and Dry matter accumulation per day for each pot was calculated using the following formulas;

a) Shoot to root ratio

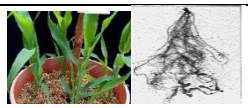
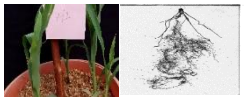
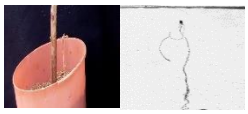
$$SRR = \frac{S_W}{R_W}$$

Where SRR = shoot to root ratio, S_W = Shoot weight and R_W = Root weight

b) Dry matter accumulation per day = $Dm\ acc = \frac{\text{mass at 40th DAS}}{\text{40 DAS}}$

Each treatment was scored for salt injury at a scale of 1-5 (Table 4.1) using modified standard evaluation system (SES) of visual salt injury at seedling stage (Gregoria, 1997) with modification to include salt injury on the roots.

Table 4.1: Modified standard evaluation system (SES) of visual salt injury at seedling stage

Score	Observation	Degree of tolerance	Visual
5	Normal growth, no root, leaf and stem symptoms	Highly tolerant	
4	Nearly normal growth, but brownish roots, whitish shoot or leaf tips or few leaves whitish and rolled	Tolerant	
3	Growth severely retarded; short roots and shoots; most leaves rolled; reduced number of roots; only a few are elongated	Moderately tolerant	
2	Complete cessation of growth; most leaves and roots dry; some plants dying	Susceptible	
1	Almost all plants are dead or dying	Highly susceptible	

Source: Gregoria, 1997

Data for ion uptake for the seedling in the 40 days of growth were determined from the samples taken for the biomass measurement. The oven dried samples in (iv) above were milled using an electric motor and 50 mg of each sample taken into tubes for digestion in aid of ion analyses. The samples were digested by concentrated nitric acid (HNO_3) and concentrated hydrochloric acid (HCl) mixed with distilled water (H_2O) at a ratio of 1:1. From the diluted acids, 20 cm^3 of HNO_3 and 10 cm^3 of HCL were pipetted into the digesting tube containing plant samples. Using Kjeldahl method, the samples were digested for 40 minutes with temperature maintained at 180^oC. After all the samples were fully digested, that is with the presence of brown fumes and clear solution, they were allowed to cool for 15 minutes before filtering to remove residues. The filtered solution was then transferred into sample bottles and topped up to 100 ml with distilled H_2O.

The contents of K^+, Na^+, Mg^{2+} and Ca^{2+} in the nutrient solutions of each treatment were measured using Atomic Absorption Spectrophotometer (AAS).

4.2.4 Data analyses

Data on root architecture, plant height, total leaf area, biomass yield and ion uptake were subjected to a two-way analysis of variance in R Statistical Package Version 4.2. The means of treatments and interaction were separated using Tukey's Honestly significant difference (HSD) (Tukey, 1949) test at a significant level of 5%.

4.3 Results

4.3.1 Effects of NaCl concentrations on shoot growth and biomass accumulation in sorghum genotypes

Results showed that, even though plant can grow at high salt stress, plant height (Figure 4.1, 4.2), total leaf area (Figure 4.3) and biomass (Figure 4.4 and Table 4.2) differed between treatments and the trend is the same across genotypes.

The results showed that, the overall plant height for all the 6 sorghum genotypes evaluated were significantly affected by salt (Figure 4.2). The effect due to NaCl concentrations were significant at $P \leq 0.001$ while the effect due to genotypes were significant at $P \leq 0.001$, however, there was no effect due to interaction between NaCl concentrations and genotypes (Appendix C). At different NaCl concentrations, the plant height for both salt tolerant and salt sensitive genotypes increased from 10 DAS to 21 DAS. Past 21st DAS, the height for the salt sensitive genotypes was stunted while salt tolerant showed gradual increase (Figure 4.1). The average increase in height relative to the control between 10th to 40th DAS was 2.8, 1.8, 2 and 1.8 times at 3, 5 and 7 dSm^{-1} respectively. When the salt treated plants were compared to their controls, highest increase in height from 10th to 40th DAS was recorded for BM 17 while the lowest increase was recorded for EST 41.

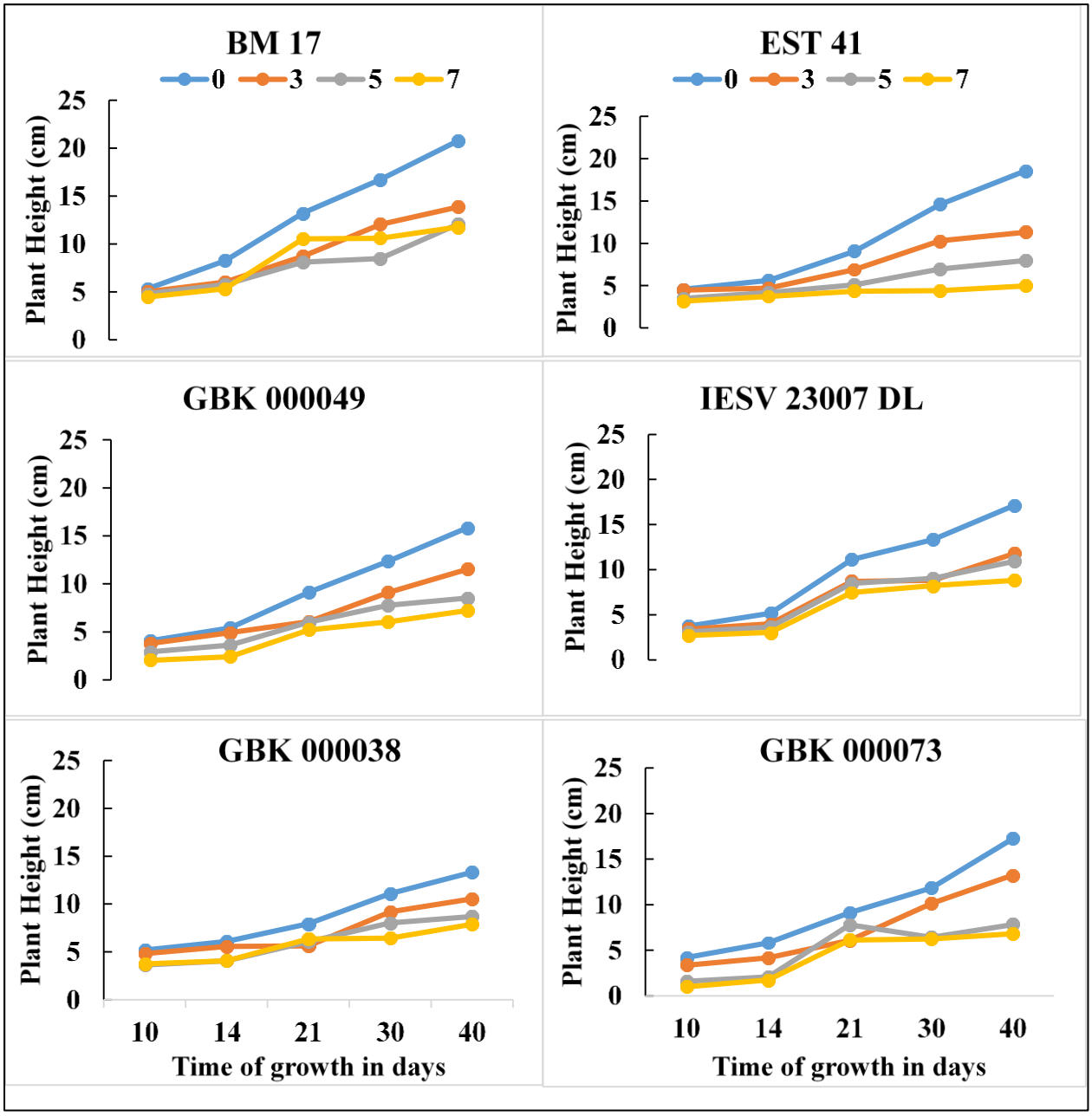


Figure 4.1: Plant height growth of six sorghum genotypes as influenced by salt stress of 0, 3, 5 and 7 dSm-1 from 10 to 40 DAS

The plant height increase per day for all the six evaluated sorghum genotypes reduced with increasing salt concentrations (Figure 4.2). The average increase in plant height per day ranged from 0.42 cm in the control to 0.16 cm at 7 dSm^{-1} of NaCl concentration from the 10^{th} to 40^{th} DAS. BM 17 increased by 52% in control and 18% at 7 dSm^{-1} while EST 41 showed the lowest increase with 47% in the control and 6% at 7 dSm^{-1}. There was a sharp reduction in height for the salt sensitive genotypes even at the low NaCl concentrations of 3 dSm^{-1} compared to the salt tolerant genotypes (Figure 4.2). The slow progressive growth in plant

height of the salt tolerant genotypes showed that, salt stress has negative effect on plant growth but the effects were genotype specific.

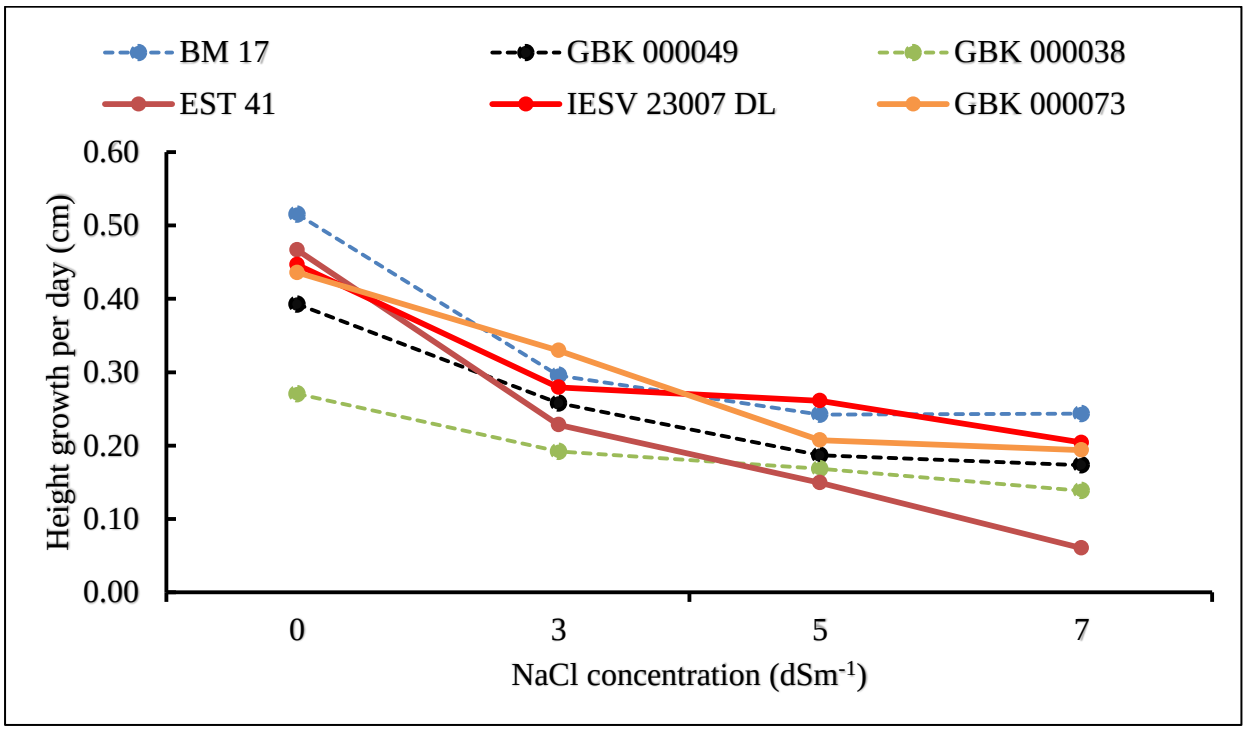


Figure 4.2: Plant height increase per day from 10DAS to 40DAS as influenced by NaCl concentrations. Broken lines represent salt tolerant and solid lines represent salt sensitive sorghum genotypes

The total leaf area for the selected sorghum genotypes reduced with increasing NaCl concentrations. There was significant difference between NaCl concentrations ($P \leq 0.001$), genotypes ($P \leq 0.01$) and no interaction effect (Appendix C). All the genotypes showed a reduction from the control to 5 dSm^{-1} beyond which, the salt tolerant genotypes maintained their leaf area while that of the salt sensitive dropped drastically (Figure 4.3). The reduction in leaf area for the tolerant genotypes relative to the control were 76%, 72% and 71% and that of the salt sensitive were 37%, 60% and 86% in 3, 5 and 7 dSm^{-1} NaCl concentrations respectively. Larger leaf area was shown by BM 17 with 37%, 61% and 64% reduction relative to the control while smaller leaf area was shown by EST 41 with 28%, 49% and 91% reduction.

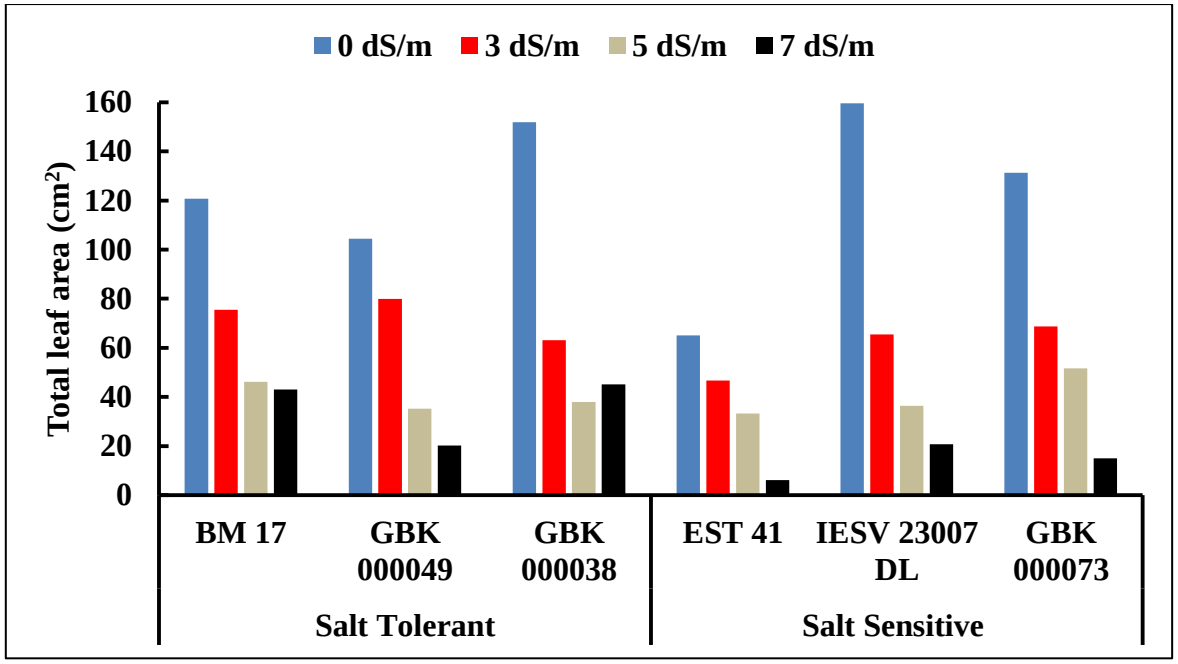


Figure 4.3: Total leaf area of salt tolerant and salt sensitive sorghum genotypes as influenced by different NaCl concentrations

The effects due to NaCl concentrations was significantly different for dry matter (DM) ($P \leq 0.001$), DM accumulation per day ($P \leq 0.001$) and for SRR ($P \leq 0.01$). The results also showed that, DM accumulation per day differed significantly between genotypes ($P \leq 0.05$). However, there was no main factor interaction effect on DM, SRR and DM accumulation per day (Appendix C). The total DM (Table 4.2) and DM accumulation per day (Figure 4.4) for both salt tolerant and salt sensitive sorghum genotypes reduced with increasing NaCl concentration while shoot to root ratio (SRR) (Table 4.2) increased with increasing concentrations of NaCl. Among the salt tolerant genotypes, BM 17 showed a relatively higher amount of DM while EST 41 showed a relatively low amount of DM across the NaCl concentration (Table 4.2). The total DM accumulation per day for the salt tolerant genotypes increased gradually while those for the salt sensitive sorghum genotypes increased up to 3 dSm^{-1}, then there was stagnant growth (Figure 4.4).

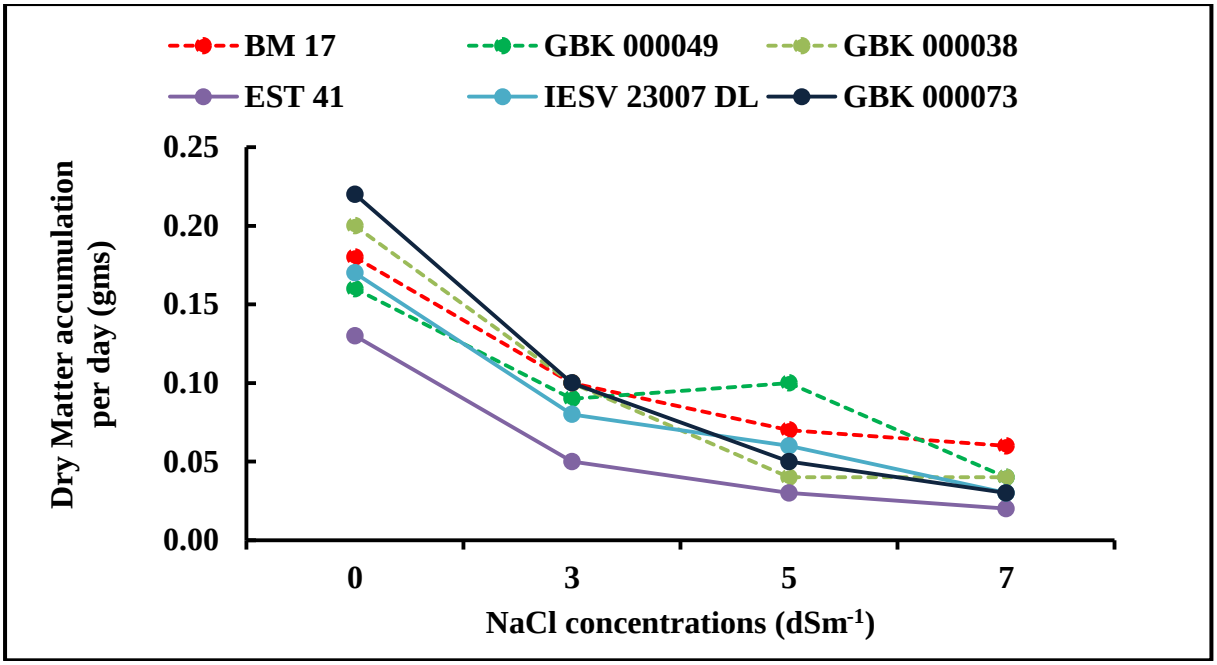


Figure 4.4: Effects of NaCl concentration on dry matter accumulation per day from. Broken lines represent salt tolerant and solid lines represent salt sensitive sorghum genotypes

Shoot to root ratio for all the 6 evaluated genotypes increased with increasing NaCl concentrations by 35%, 15% and 105% at 3, 5 and 7 dSm^{-1} respectively, relative to the control (Table 4.2). There was a gradual increase in SRR at the low NaCl concentration (3 dSm^{-1}) when compared to the control and beyond which, the tolerant genotypes showed almost constant ratio while the sensitive showed a sharp increase. Relative to the control, the salt tolerant genotypes showed an increase by 4% and 19% in 3 and 7 dSm^{-1}, and 17% reduction in 5 dSm^{-1} while the salt sensitive genotypes showed an increase by 75%, 59% and 215% in 3, 5 and 7 dSm^{-1}. Beyond 5 dSm^{-1}, EST 41 showed a higher increase in its SRR (217%) while BM 17 showed a lower increase (35%), when both were compared with their respective controls.

Table 4.2: Effects of NaCl concentrations on dry matter (DM) and shoot to root ratio (SRR) of salt tolerant and salt sensitive sorghum genotypes

	Total dry matter (g/plant)					Shoot to root ratio				
	NaCl Concentration (dSm^{-1})					NaCl Concentration (dSm^{-1})				
Sorghum genotype	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^{T}	5.5^{a}	3.0^{a}	2.2^{a}	1.9^{b}	**3.1^{a}**	2.0^{a}	1.9^{a}	1.7^{b}	2.7^{a}	**2.1**
GBK 000049^{T}	4.7^{a}	2.7^{a}	3.1^{a}	1.8^{a}	**2.8^{ab}**	1.8^{a}	1.7^{a}	0.8^{a}	1.9^{a}	**1.6**
GBK 000038^{T}	6.0^{a}	3.2^{a}	1.1^{b}	1.1^{b}	**2.8^{ab}**	1.5^{a}	2.0^{a}	1.8^{a}	1.7^{a}	**1.8**
EST 41^{S}	3.9^{a}	1.5^{a}	1.0^{b}	0.5^{b}	**1.7^{b}**	1.8^{b}	1.9^{b}	3.6^{a}	5.8^{a}	**3.3**
IESV 23007 DL^{S}	5.2^{a}	2.3^{a}	1.1^{b}	1.0^{b}	**2.6^{ab}**	1.3^{b}	2.3^{a}	1.9^{a}	2.6^{a}	**2.0**
GBK 000073^{S}	6.7^{a}	2.9^{b}	1.5^{b}	1.0^{b}	**3.0^{ab}**	1.1^{b}	3.2^{a}	1.2^{b}	4.9^{a}	**2.6**
Means	**5.3^{a}**	**2.6^{b}**	**1.8^{bc}**	**1.0^{c}**		**1.6^{b}**	**2.2^{ab}**	**1.8^{b}**	**3.3^{a}**	

Means in a row and column followed by the same letter(s) are not significantly different for total dry matter ($P \leq 0.001$) and SRR ($P \leq 0.01$) using Tukey's HSD test. Sorghum genotypes followed by superscript T are salt tolerant and those followed by S are salt sensitive

4.3.2 Effects of NaCl concentrations on root architecture and growth in sorghum genotypes

Results showed that root architecture is highly influenced by NaCl concentrations. Root length (Table 4.3), root volume (Table 4.4), root diameter (Table 4.5), root surface area (Table 4.6) and number of roots (Table 4.7) differed between NaCl concentrations and between genotypes.

The results showed that, there was significant difference between NaCl concentrations both at 10DAS ($P \leq 0.05$) and 40DAS ($P \leq 0.001$) (Appendix D). However, there was no effect due to sorghum genotypes and main factor interaction effect. The root lengths for all the six evaluated sorghum genotypes reduced with increasing NaCl concentrations (Table 4.3). The average root length ranged from 37.8 cm to 657.6 cm between 10th to 40th DAS. As the growth time increased, the effect of salt progressively reduced root lengths for both tolerant and sensitive genotypes. Significant reduction was observed as salt concentrations increased up to 5 dSm^{-1}, beyond which, there was no effect (Table 4.3). Relative to their control, BM 17 showed longer root length across treatments while EST 41 showed shorter root length across treatments (Table 4.3).

Table 4.3: Effects of NaCl concentrations on root length at 10DAS and 40DAS for the salt tolerant and salt sensitive sorghum genotypes

Sorghum genotypes	Root length (cm)									
	10DAS					40DAS				
	NaCl concentration (dSm^{-1})					NaCl concentration (dSm^{-1})				
	0	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^{T}	38.7^{a}	60.5^{a}	64.9^{a}	45.4^{a}	**52.4**	1442.7^{a}	933.0^{a}	297.1^{a}	361.9^{a}	**758.7**
GBK 000049^{T}	28.5^{ab}	47.0^{a}	26.9^{ab}	20.1^{b}	**30.6**	863.2^{a}	852.3^{a}	300.9^{b}	204.8^{b}	**555.3**
GBK 000038^{T}	48.0^{a}	61.1^{a}	34.6^{a}	27.7^{a}	**42.9**	712.3^{a}	556.4^{a}	493.3^{a}	259.8^{a}	**505.4**
EST 41^{S}	32.1^{ab}	15.2^{b}	35.7^{a}	23.4^{ab}	**26.6**	1450.8^{a}	592.1^{ab}	268.4^{b}	349.2^{b}	**665.1**
IESV 23007 DL^{S}	36.0^{ab}	63.0^{a}	23.8^{b}	34.7^{ab}	**39.4**	1214.3^{a}	942.9^{a}	638.8^{a}	708.8^{a}	**876.2**
GBK 000073^{S}	80.2^{a}	29.5^{b}	30.8^{b}	0.0^{c}	**35.1**	887.5^{a}	752.0^{a}	223.4^{b}	475.4^{ab}	**584.5**
Means	**43.9^{ab}**	**46.0^{a}**	**36.1^{ab}**	**25.2^{b}**		**1095.1^{a}**	**771.4^{b}**	**370.3^{c}**	**393.3^{c}**	

Means in a row and column followed by the same letter(s) are not significantly different for root length at 10DAS ($P \leq 0.05$) and at 40 DAS ($P \leq 0.001$) using Tukey's HSD test. DAS-Days After Sowing. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive

The results for root volume showed that there was significant difference between NaCl concentrations ($P \leq 0.05$) and between genotypes ($P \leq 0.01$) at 10DAS but there was no interaction effect. The results also showed that there was significant difference between NaCl concentrations ($P \leq 0.001$) at 40DAS. However, there was no effect due to genotype and interaction at 40DAS. The reduced root volumes of all the six evaluated sorghum genotypes reduced with increasing NaCl concentrations (Table 4.4). The average increase from 10th to 40th DAS ranged from 0.05 to 2.7 cm^3. However, the rate of increase per day reduced with increasing salt concentrations. The salt sensitive genotypes showed a drastic drop while the salt tolerant genotypes showed a gradual reduction across treatment. The average reduction for the salt tolerant relative to the control were, 48%, 70% and 81% while for the salt sensitive genotypes it was 57%, 84% and 92% at 3, 5, 7 dSm^{-1}, respectively. Despite the similar trend, the salt sensitive showed a dramatic drop even at low salt concentrations as evident by EST 41 while salt tolerant showed gradual reduction evident by BM 17 (Table 4.4).

Table 4.4: Effects of NaCl concentrations on root volume at 10DAS and 40DAS of salt tolerant and salt sensitive sorghum genotypes

	Root volume (cm^3)									
	10DAS					**40DAS**				
Sorghum	**NaCl concentration (dSm^{-1})**					**NaCl concentration (dSm^{-1})**				
genotypes	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^T	0.05^a	0.07^{ab}	0.08^{bc}	0.10^{bc}	**0.07^a**	7.07^a	3.40^a	0.90^b	0.96^b	**3.08**
GBK 000049^T	0.04^a	0.05^a	0.04^a	0.01^b	**0.04^b**	3.80^a	2.25^a	0.82^b	0.52^b	**1.84**
GBK 000038^T	0.09^a	0.07^{ab}	0.06^b	0.03^c	**0.06^{ab}**	5.22^a	2.79^{ab}	1.29^{bc}	0.72^c	**2.50**
EST 41^S	0.06^a	0.03^b	0.05^a	0.03^b	**0.04^b**	10.45^a	2.58^b	0.55^c	0.79^c	**3.59**
IESV 23007 DL^S	0.05^a	0.07^a	0.03^b	0.02^b	**0.05^{ab}**	6.86^a	3.59^{ab}	1.90^c	2.10^{bc}	**3.61**
GBK 000073^S	0.07^a	0.05^a	0.04^a	0.00^b	**0.04^b**	3.10^a	2.75^a	0.57^b	0.98^b	**1.85**
Means	**0.06^a**	**0.06^{ab}**	**0.05^{ab}**	**0.03^b**		**6.08^a**	**2.89^b**	**1.00^c**	**1.01^c**	

Means in a row followed by the same letter(s) are not significantly different between NaCl concentrations for root volume at 10DAS ($P \leq 0.05$) and at 40 DAS ($P \leq 0.001$) and means is a column followed by the same letter(s) are not significantly different between genotype for root volume at 10DAS ($P \leq 0.01$) using Tukey's HSD test. DAS-Days After Sowing. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive

The results showed that there was significant difference in root diameter between genotypes ($P \leq 0.01$) and interaction effect ($P \leq 0.05$) at 10DAS. The results also showed significant difference in root diameter between NaCl concentration ($P \leq 0.001$), sorghum genotypes ($P \leq 0.05$) and interaction ($P \leq 0.05$) at 40DAS (Appendix D). The root diameter for all the six evaluated sorghum genotypes reduced significantly with increasing NaCl concentrations (Table 4.5). Both the salt tolerant and salt sensitive sorghum genotypes showed similar trend across salt treatments. The average increase in root diameter from 10^{th} to 40^{th} DAS was 0.35 cm to 0.66 cm. The root diameter for the salt tolerant genotypes increasing from 0.39 to 0.67 while for the salt sensitive genotypes increased from 0.31 cm to 0.65 cm. However, the diameter for the salt tolerant genotypes reduced from low salt concentrations to 5 dSm^{-1} and beyond which there was a slight increase while salt sensitive had a progressive drop across treatments. Their daily increases in root diameter were equal across treatments except for EST 41 that recorded larger increase between control and low concentrations (Table 4.5).

Table 4.5: Effects of NaCl concentrations on root diameter at 10DAS and 40DAS for salt tolerant and salt sensitive sorghum genotypes

Sorghum genotypes	Root diameter (cm)									
	10DAS					40DAS				
	NaCl concentrations (dSm^{-1})					NaCl concentrations (dSm^{-1})				
	0	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^{T}	0.40^{a}	0.39^{a}	0.38^{a}	0.44^{a}	**0.41^{a}**	0.77^{a}	0.67^{ab}	0.56^{b}	0.60^{ab}	**0.65^{ab}**
GBK 000049^{T}	0.44^{a}	0.35^{a}	0.37^{a}	0.38^{a}	**0.37^{ab}**	0.74^{a}	0.59^{b}	0.58^{b}	0.77^{ab}	**0.67^{ab}**
GBK 000038^{T}	0.42^{a}	0.39^{a}	0.37^{a}	0.40^{a}	**0.39^{ab}**	0.93^{a}	0.80^{ab}	0.56^{b}	0.59^{b}	**0.72^{a}**
EST 41^{S}	0.27^{a}	0.29^{a}	0.27^{a}	0.27^{a}	**0.28^{b}**	0.94^{a}	0.74^{b}	0.51^{c}	0.53^{c}	**0.68^{ab}**
IESV 23007 DL^{S}	0.35^{a}	0.37^{a}	0.36^{a}	0.40^{a}	**0.37^{ab}**	0.83^{a}	0.70^{ab}	0.61^{b}	0.62^{b}	**0.69^{ab}**
GBK 000073^{S}	0.39^{a}	0.38^{a}	0.42^{a}	0.00^{b}	**0.30^{ab}**	0.66^{a}	0.59^{a}	0.57^{a}	0.51^{a}	**0.58^{b}**
Means	**0.37**	**0.36**	**0.36**	**0.31**		**0.81^{a}**	**0.68^{b}**	**0.57^{c}**	**0.60^{bc}**	

Means followed by the same letter(s) in a row are not significantly different between genotype at 10DAS ($P \leq 0.01$) and at 40 DAS ($P \leq 0.05$), means is a column followed by the same letter(s) are not significantly different between NaCl concentrations at 40DAS ($P \leq 0.001$) and the interactions effect are not significantly different ($P \leq 0.05$) at 10DAS and at 40 DAS using Tukey's HSD test. DAS-Days After Sowing. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive

The results showed that, the root surface area for all the sorghum genotypes reduced with increasing NaCl concentrations. At 10 DAS, the significant difference was shown between genotypes ($P \leq 0.05$) with no significant difference between NaCl concentration and interaction effect. The results also showed that, there was significant difference on root surface area between NaCl concentration ($P \leq 0.001$), however, there was no effect due to sorghum genotypes and main factor interaction at 40DAS (Appendix D). The average increase in surface area from the 10^{th} to 40^{th} DAS was 9.1 to 168.7 cm^2. The trend was the same across concentrations and genotypes. When compared with the control, the salt tolerant genotypes recorded smaller area than their salt sensitive counterparts. Relative to the control, the rate of reduction for the salt tolerant genotypes was 24%, 47% and 57% while for the salt sensitive was 48%, 74% and 80% at 3, 5 and 7 dSm^{-1} respectively. BM 17 showed a larger

root surface area across the treatments while EST 41 showed larger root surface area than all the other evaluated genotypes in control but when was subjected to salt concentrations its surface area reduced dramatically than all the genotypes (Table 4.6).

Table 4.6: Effects of NaCl concentrations on root surface area at 10DAS and 40DAS for the salt tolerant and salt sensitive sorghum genotypes

	Root surface area (cm^2)									
	10DAS					40DAS				
	NaCl concentrations (dSm^{-1})					NaCl concentrations (dSm^{-1})				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^{T}	10.8^{a}	11.1^{a}	13.4^{a}	11.8^{a}	**11.8^{a}**	357.0^{a}	199.4^{a}	56.7^{b}	65.8^{b}	**169.7**
GBK 000049^{T}	7.4^{a}	9.7^{a}	8.7^{a}	6.8^{a}	**8.2^{bc}**	202.2^{a}	155.1^{a}	55.6^{b}	35.8^{b}	**112.2**
GBK 000038^{T}	9.8^{a}	10.7^{a}	10.0^{a}	9.6^{a}	**10.0^{a}**	215.6^{a}	138.5^{a}	88.7^{b}	48.5^{b}	**122.8**
EST 41^{S}	7.8^{a}	6.1^{a}	6.8^{a}	7.9^{a}	**7.2^{c}**	435.8^{a}	138.3^{ab}	43.1^{c}	58.7^{bc}	**169.0**
IESV 23007 DL^{S}	9.2^{a}	12.3^{a}	7.8^{a}	6.6^{a}	**9.0^{ab}**	322.6^{a}	205.6^{a}	123.4^{a}	135.8^{a}	**196.9**
GBK 000073^{S}	12.7^{a}	9.6^{a}	6.6^{a}	0.0^{b}	**7.2^{c}**	185.4^{a}	160.2^{a}	39.7^{c}	76.0^{b}	**115.3**
Means	**9.6**	**9.9**	**8.9**	**7.1**		**286.4^{a}**	**166.2^{b}**	**67.9^{c}**	**70.1^{c}**	

Means in a column and row followed by the same letter(s) are not significantly different between sorghum genotypes ($P \leq 0.05$) and NaCl concentrations ($P \leq 0.001$) at 10DAS and 40DAS respectively, using Tukey's HSD. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive. DAS-Days After Sowing.

The results for number of roots showed that there was significant difference between NaCl concentrations ($P \leq 0.01$) and sorghum genotypes ($P \leq 0.05$) at 10DAS. The results also showed that, the significant difference at 40 DAS were due to NaCl concentration ($P \leq 0.001$) and sorghum genotypes ($P \leq 0.05$) (Appendix D). However, there was no interaction effect at 10DAS and 40DAS. Increase in NaCl concentrations reduced the number of roots per plant across the genotypes (Table 4.7). As the plants progressed in growth, the salt sensitive genotypes showed higher numbers of roots in the control compared with the salt tolerant genotypes. However, when the salt sensitive genotypes were subjected to salt concentrations, the number of roots reduced dramatically even at the low salt concentrations as opposed to the salt tolerant genotypes that showed a gradual reduction across treatments. The change in the number of roots between 10 to 40 DAS for the salt tolerant was 970, 820, 528 and 419 with daily increase of 32, 27, 17 and 13 at 0, 3, 5 and 7 dSm^{-1} while the change for the salt sensitive genotypes was 1133, 748, 523 and 345 with daily increase of 38, 25, 17 and 12 at 0, 3, 5 and 7 dSm^{-1} respectively. Relative to the control, the salt tolerant genotypes recorded a

reduction of 15%, 46% and 57% while salt sensitive showed a decline by 34%, 54% and 70% at 3, 5 and 7 dSm^{-1}. When the salt treated plants were compared with their respective control, GBK 000049 recorded a lesser reduction of 11%, 34% and 44% while EST 41 recorded a larger reduction of 50%, 71% and 78 at 3, 5 and 7 dSm^{-1} (Table 4.7).

Table 4.7: Effects of NaCl concentrations on the number of roots at 10DAS and 40DAS for the salt tolerant and salt sensitive sorghum genotypes

	Number of roots									
	10DAS					40DAS				
	NaCl concentrations (dSm^{-1})					NaCl concentrations (dSm^{-1})				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^{T}	77.0^{a}	72.0^{a}	63.0^{a}	50.0^{a}	**65.5^{a}**	1510.0^{a}	1249.0^{a}	553.0^{a}	494.0^{a}	**951.5^{ab}**
GBK 000049^{T}	53.0^{a}	51.0^{a}	45.0^{a}	26.0^{a}	**43.8^{ab}**	952.0^{a}	850.0^{a}	640.0^{a}	533.0^{a}	**743.8^{ab}**
GBK 000038^{T}	81.0^{a}	81.0^{a}	59.0^{a}	44.0^{a}	**66.3^{a}**	660.0^{a}	566.0^{a}	559.0^{a}	350.0^{a}	**533.8^{b}**
EST 41^{S}	53.0^{a}	26.0^{a}	48.0^{a}	30.0^{a}	**39.3^{b}**	1402.0^{a}	705.0^{b}	440.0^{bc}	321.0^{c}	**717.0^{ab}**
IESV 23007 DL^{S}	52.0^{ab}	58.0^{a}	44.0^{b}	19.0^{c}	**43.3^{ab}**	1248.0^{a}	978.0^{a}	746.0^{b}	499.0^{b}	**867.8^{a}**
GBK 000073^{S}	82.0^{a}	55.0^{a}	45.0^{a}	0.0^{b}	**45.5^{ab}**	937.0^{a}	700.0^{a}	522.0^{b}	265.0^{c}	**606.0^{ab}**
Means	**66.3^{a}**	**57.2^{a}**	**50.7^{ab}**	**28.2^{b}**		**1118.2^{a}**	**841.3^{b}**	**576.7^{c}**	**410.3^{c}**	

Means in a column followed by the same letter(s) are not significantly different between genotypes at 10DAS ($P \leq 0.05$) and 40DAS ($P \leq 0.05$) and means in a row are not significantly different between NaCl concentrations at 10DAS ($P \leq 0.01$) and 40DAS ($P \leq 0.001$) using Tukey's HSD. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive. DAS-Days After Sowing

4.3.3 Effects of NaCl concentrations on ion uptake in the roots and shoots of salt tolerant and salt sensitive sorghum genotypes

The content of Sodium (Na^{+}) (Table 4.8), Potassium (K^{+}) (Table 4.9), and Calcium (Ca^{2+}) (Table 4.10) in the roots and shoots increased with increasing NaCl concentrations for both the salt sensitive and salt tolerant sorghum genotypes. However, the amount of each ion differed between genotypes and plant part.

The effect due to NaCl concentrations on the amount of Na^{+} in the roots of the six evaluated sorghum genotypes were not significantly different while that of the shoots was significantly different at $P \leq 0.001$ (Appendix E). Salt tolerant genotypes showed a continuous increase in Na^{+} content with increasing NaCl concentration. Salt sensitive genotypes showed increase in Na^{+} content in the roots up to 5 dSm^{-1} then there was a drop at 7 dSm^{-1}.

Contrary to its roots, the Na^+ accumulated in the shoots was much higher for all the six sorghum genotypes. The amount of Na^+ accumulated in the shoots increased with increasing NaCl concentrations. From the results, the salt tolerant genotypes had lower Na^+ accumulation compared to the salt sensitive genotypes. The salt tolerant genotypes showed a gradual increase across the concentrations showing significant difference at 7 dSm^{-1} while the salt sensitive genotypes showed a drastic increase with significant difference realized at that 3 dSm^{-1}. Among the salt tolerant genotypes, GBK 000049 showed are relatively low accumulation of Na^+ in their shoots while EST 41, among the salt tolerant, showed a relatively higher amount of Na^+ accumulation in the shoots (Table 4.8).

Shoot to root ratios of the accumulated Na^+ for the salt tolerant and salt sensitive genotypes were largely influence by NaCl concentrations but showed no significant difference. Except for BM 17 that showed an increase up to 5 dSm^{-1} before it dropped, an increase was recorded for all the other genotypes across the concentrations (Table 4.8). From the results, salt tolerant genotypes had high ratios even at low salt concentrations while salt sensitive genotypes had smaller ratios up to 3 dSm^{-1} beyond which, there was a sharp increase.

Table 4.8: Effects of NaCl concentrations on Na^+ accumulation in the shoots and Na^+ SRR for the salt tolerant and salt sensitive sorghum genotypes

	Shoots Na^+ concentration (µg/g)					**Na^+ shoot to root ratio**				
	NaCl concentrations (dSm^{-1})					**NaCl concentrations (dSm^{-1})**				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^T	2.7^b	3.7^a	4.5^a	4.6^a	**3.8**	0.6^a	0.7^a	0.8^a	0.8^a	**0.7**
GBK 000049^T	2.8^b	3.6^a	4.0^a	4.4^a	**3.7**	0.6^a	0.6^a	0.7^a	0.8^a	**0.7**
GBK 000038^T	2.4^b	2.6^a	3.4^a	4.9^a	**3.2**	0.5^b	0.6^b	0.7^b	0.9^a	**0.6**
EST 41^S	2.5^b	2.5^b	5.2^a	5.4^a	**4.0**	0.5^b	0.5^b	1.0^a	1.1^a	**0.8**
IESV 23007 DL^S	2.5^b	3.1^a	3.1^a	4.3^a	**3.2**	0.5^b	0.5^b	0.6^b	0.9^a	**0.6**
GBK 000073^S	2.7^b	3.2^a	4.2^a	4.7^a	**3.7**	0.6^b	0.6^b	0.8^b	1^a	**0.7**
Means	**2.5^c**	**3.1^c**	**4.1^b**	**4.7^a**		**0.5^b**	**0.6^b**	**0.7^a**	**0.9^a**	

Means in a row and column followed by the same letter(s) are not significantly different at $P \leq 0.001$ using Tukey's HSD test. Sorghum genotypes followed by superscript T are salt tolerant and those with S are salt sensitive

The amount of K^+ in different plant parts were variably affected by NaCl concentrations. The effect due to NaCl concentrations was significantly different in the roots at $P \leq 0.05$ (Appendix E) while the effect on the shoots and SRR were genotype specific with significant difference at $P \leq 0.05$ and $P \leq 0.001$ (Appendix E), respectively. Salt tolerant genotypes had higher accumulation than the salt sensitive sorghum genotypes. The significant accumulation of K^+ content in roots of the salt tolerant genotypes was shown from 5 dSm^{-1} while the salt sensitive sorghum genotypes showed significant difference only at 7 dSm^{-1}. GBK 000073 recorded high reduction while GBK 000038 recorded high increase (Table 4.9).

The amount of K^+ accumulated in the shoot as NaCl concentrations increased varied with genotype. The salt tolerant genotypes showed an increase up to 3 dSm^{-1} before it dropped while salt sensitive genotypes recorded a varied trend across salt concentrations (Table 4.9). GBK 000038 recorded a higher reduction while IESV 23007 DL recorded the lowest reduction.

Table 4.9: Effects of NaCl concentration on accumulated K^+ in the shoots and roots of the salt tolerant and salt sensitive sorghum genotypes

	Roots K^+ concentration (µg/g)					Shoots K^+ concentration (µg/g)				
	NaCl concentrations (dSm^{-1})					NaCl concentrations (dSm^{-1})				
Genotype	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^T	4.5^a	3.3^a	3.6^a	3.8^a	**3.8^{ab}**	4.6^a	5.2^a	4.8^a	4.3^a	**4.7**
GBK 000049^T	3.5^a	3.7^a	3.7^a	2.5^a	**3.4^{ab}**	4.1^a	4.6^a	4.2^a	4.7^a	**4.4**
GBK 000038^T	3.8^a	3.9^a	4.3^a	4.0^a	**4.0^a**	4.9^a	4.2^a	4.0^a	3.8^a	**4.2**
EST 41^S	3.1^a	3.8^a	3.0^a	2.6^a	**3.1^b**	5.0^a	4.3^a	4.6^a	4.7^a	**4.7**
IESV 23007 DL^S	4.0^a	3.9^a	3.9^a	3.7^a	**3.9^{ab}**	4.0^a	3.6^a	4.2^a	4.6^a	**4.1**
GBK 000073^S	4.5^a	3.9^a	3.8^a	3.4^a	**3.9^{ab}**	4.5^a	3.7^a	4.6^a	4.0^a	**4.2**
Means	**4.0**	**3.7**	**3.7**	**3.3**		**4.5**	**4.3**	**4.4**	**4.3**	

Means in a column followed by the same letter(s) are not significantly different for K^+ concentrations in the shoots ($P \leq 0.05$) and roots ($P \leq 0.01$) using Tukey's HSD test. Sorghum genotypes followed by superscript T are salt tolerant and those with S are salt sensitive

The shoot to root ratio of accumulated K^+ was highly genotype specific. The salt tolerant genotypes showed a drop of shoot to root ratio from 0 dSm^{-1} to 5 dSm^{-1} NaCl concentration beyond which, there was a slight increase while the salt sensitive genotypes showed a drop at 3 dSm^{-1} beyond which there was an increase (Table 4.10).

Table 4.10: Effect of NaCl concentrations on accumulated K^+ shoot to root ratio of the salt sensitive and salt tolerant sorghum genotypes

	K^+ shoot to root ratio				
	NaCl concentrations (dSm^{-1})				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Mean**
BM 17^T	1.0^{b}	1.6^{a}	1.36^{ab}	1.1^{ab}	**1.3^{ab}**
GBK 000049^T	1.1^{b}	1.3^{ab}	1.1^{b}	1.8^{a}	**1.3^{ab}**
GBK 000038^T	1.3^{a}	1.1^{a}	0.9^{b}	1.0^{b}	**1.1^{b}**
EST 41^S	1.7^{ab}	1.3^{b}	1.5^{ab}	1.8^{a}	**1.5^{a}**
IESV 23007 DL^S	1.0^{ab}	0.9^{b}	1.1^{ab}	1.3^{a}	**1.1^{b}**
GBK 000073^S	1.0^{ab}	0.9^{b}	1.3^{a}	1.3^{a}	**1.1^{b}**
Means	**2.5**	**3.1**	**4.1**	**4.7**	

Means in a column followed by the same letter(s) are not significantly different for genotype ($P \leq 0.001$) and for the main effect interaction ($P \leq 0.05$) using Tukey's HSD test. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive

The effect of NaCl concentration on accumulated Ca^{2+} in the shoots of sorghum genotypes were significantly different at $P \leq 0.05$ (Appendix E) while between genotypes was differently significant at $P \leq 0.001$ (Appendix E). The Ca^{2+} content for the salt tolerant genotypes increased with increasing NaCl concentrations while for the salt sensitive genotypes, there was a reduction as the NaCl concentration increased (Table 4.11). BM 17 maintained an almost equal amount of Ca^{2+} content in their shoots as the salt concentration increase while GBK 000073 reduced its accumulation across the treatments. The results also showed that there were no effect due main factor and interaction in the shoots of sorghum genotypes.

The results for shoot to root ratio of the total accumulated Ca^{2+} showed that there was significant difference between NaCl concentrations at $P \leq 0.001$. However, there was no effect due to genotypes and interaction. The salt tolerant genotypes recorded an increase as the concentrations increases while the salt sensitive genotypes recorded a gradual reduction. GBK 000049 showed the highest increase while GBK 000073 showed the highest reduction (Table 4.11).

Table 4.11: Effects of NaCl concentration of the total accumulated Ca^{2+} in the roots and shoots of salt tolerant and salt sensitive sorghum genotypes

	Shoots Ca^{2+} concentration (µg/g)					Ca^{2+} shoots to roots ratio				
	NaCl concentrations (dSm^{-1})					NaCl concentrations (dSm^{-1})				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^{T}	4.9^{a}	5.03^{a}	5.05^{a}	5.07^{a}	**5.02^{ab}**	0.96^{a}	0.97^{a}	0.99^{a}	0.99^{a}	**0.98**
GBK 000049^{T}	4.95^{a}	4.87^{a}	4.87^{a}	4.83^{a}	**4.88^{bc}**	0.98^{a}	0.97^{a}	0.98^{a}	0.96^{a}	**0.97**
GBK 000038^{T}	5.13^{ab}	4.87^{b}	5.11^{ab}	5.18^{a}	**5.07^{ab}**	1.04^{a}	0.97^{b}	1.02^{ab}	1.02^{ab}	**1.01**
EST 41^{S}	5.09^{ab}	4.79^{b}	4.96^{ab}	5.11^{a}	**4.98^{abc}**	1.0^{a}	0.94^{b}	0.98^{ab}	1.0^{a}	**0.98**
IESV 23007 DL^{S}	5.22^{a}	5.07^{b}	5.05^{b}	5.16^{ab}	**5.12^{a}**	1.04^{a}	1.0^{ab}	1.02^{ab}	0.99^{b}	**1.01**
GBK 000073^{S}	5.07^{a}	4.72^{a}	4.69^{a}	4.70^{a}	**4.80^{c}**	0.97^{a}	0.91^{a}	0.9^{a}	0.92^{a}	**0.93**
Means	**5.06^{a}**	**4.89^{b}**	**4.95^{ab}**	**5.00^{ab}**		**1.0**	**0.96**	**0.98**	**0.98**	

Means in a row and column followed by the same letter(s) are not significantly different for shoot Ca^{2+} concentrations between genotype ($P \leq 0.001$) and NaCl concentrations ($P \leq 0.05$) and Ca^{2+} SRR ($P \leq 0.05$) using Tukey's HSD test. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive

4.3.4 Effects of NaCl concentrations on ion ratios in the shoots and roots of the salt tolerant and salt sensitive sorghum genotypes

The K^{+}/Na^{+} for all the six evaluated sorghum genotypes increased with increase in NaCl concentrations. The results showed that there was significant difference between NaCl concentrations in the roots ($P \leq 0.05$) and shoots ($P \leq 0.001$), and between sorghum genotypes in the roots ($P \leq 0.05$) and shoots ($P \leq 0.01$). However, there was no effect due to interaction both in the roots and shoots. The salt tolerant genotypes had high ratios in their roots compared to the salt sensitive genotypes that had relatively low ratios. The average root ratios for the salt tolerant genotypes at 0 3, 5 and 7 dSm^{-1} were 12.18, 15.06, 14.40 and 17.38 while for the salt sensitive genotypes were 13.40, 13.61, 15.67 and 15.56. GBK 000049 had the highest increase reaching 67% at 7 dSm^{-1} and IESV 23007 DL had the lowest increase reaching 6% at 7 dSm^{-1} (Table 4.12).

The average shoots ratios for the salt tolerant and sensitive sorghum genotypes were 0.18, 0.14, 0.11 and 0.9 at 0 3, 5 and 7 dSm^{-1} (Table 4.12). However, relative to the control of individual genotypes at 3, 5 and 7 dSm^{-1}, GBK 000038 showed a high ratio reduction of 24, 43 and 62% compared to IESV 23007 DL whose reduction was 29, 24 and 35%. These results revealed that, salt tolerant genotypes accumulate more K^{+} in the roots and shoots than the salt sensitive genotypes.

Table 4.12: Effects of NaCl concentrations on K^+/Na^+ in the roots and shoots of salt tolerant and salt sensitive sorghum genotypes

	Roots K^+ to Na^+ ratios					Shoots K^+ to Na^+ ratios				
	NaCl concentrations (dSm^{-1})					NaCl concentrations (dSm^{-1})				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Mean**	**0**	**3**	**5**	**7**	**Mean**
BM 17^T	10.44^a	16.58^a	15.61^a	15.39^a	**14.51^{ab}**	0.17^a	0.14^a	0.11^b	0.09^b	**0.13^{ab}**
GBK 000049^T	13.38^b	15.91^b	15.43^b	22.35^a	**16.77^a**	0.15^a	0.13^a	0.11^b	0.10^b	**0.12^b**
GBK 000038^T	12.72^a	12.69^a	12.16^a	14.40^a	**12.99^{ab}**	0.21^a	0.16^a	0.12^b	0.08^b	**0.14^a**
EST 41^S	17.13^a	13.95^a	17.87^a	18.77^a	**16.93^a**	0.19^a	0.17^a	0.09^b	0.09^b	**0.13^{ab}**
IESV 23007 DL^S	12.63^a	13.13^a	14.46^a	13.35^a	**13.39^b**	0.17^a	0.12^a	0.13^a	0.11^b	**0.13^{ab}**
GBK 000073^S	10.44^a	13.74^a	14.68^a	14.56^a	**13.36^{ab}**	0.17^a	0.12^{ab}	0.11^b	0.09^b	**0.12^b**
Means	**12.79^b**	**14.33^{ab}**	**15.04^{ab}**	**16.47^a**		**0.18^b**	**0.14^b**	**0.11^a**	**0.09^a**	

Means in a row followed by the same letter(s) are not significantly different between genotypes for roots ($P \leq 0.05$) and shoot ($P \leq 0.01$) K^+/Na^+ while means in a column followed by the same letter(s) are not significantly different between NaCl concentrations for roots ($P \leq 0.05$) and shoot ($P \leq 0.001$) K^+/Na^+ using Tukey's HSD test. Sorghum genotypes with superscript T are salt tolerant and those with S are salt sensitive

The results showed that, there was significant difference on shoot Ca^{2+}/Na^+ due to NaCl concentrations ($P \leq 0.001$). However, there was no effect due to sorghum genotypes and main factor interaction. The results also showed that, there was no significant difference in the roots ration for all the main factor and interaction effect. Shoots Ca^{2+}/Na^+ increased with increasing NaCl concentrations across the treatments for all the six evaluated sorghum genotypes (Table 4.13). Despite the increase in Ca^{2+}/Na^+, the salt tolerant genotypes showed a gradual change while the salt sensitive genotypes showed a drastic increase when the concentrations were beyond 3 dSm^{-1}. The average ratios at 0, 3, 5 and 7 dSm^{-1} for the salt tolerant was 0.52, 0.66, 0.79 and 0.91 while for the salt sensitive genotypes was 0.50, 0.59, 0.85 and 0.98. When the salt treated treatments were compared to their respective controls, the salt tolerant genotypes increased their ratios by 26, 5 and 74% as opposed to the salt sensitive genotypes whose increase was 17, 70 and 98%. EST 41 had the highest Ca^{2+}/Na^+ ratios while GBK 000049 had the lowest ratios.

Table 4.13: Effects of NaCl concentrations on Ca^{2+}/Na^{+} in the roots and shoot of salt tolerant and salt sensitive sorghum genotypes

	Ca^{2+}/Na^{+} ratio in the shoots				
	NaCl concentrations (dSm^{-1})				
Sorghum genotypes	**0**	**3**	**5**	**7**	**Means**
BM 17^{T}	0.54^{a}	0.72^{a}	0.89^{a}	0.90^{a}	**0.76**
GBK 000049^{T}	0.57^{a}	0.74^{a}	0.82^{a}	0.89^{a}	**0.75**
GBK 000038^{T}	0.45^{b}	0.51^{ab}	0.66^{ab}	0.93^{a}	**0.64**
EST 41^{S}	0.53^{b}	0.49^{b}	1.05^{a}	1.14^{a}	**0.80**
IESV 23007 DL^{S}	0.45^{b}	0.59^{ab}	0.62^{ab}	0.85^{a}	**0.63**
GBK 000073^{S}	0.53^{b}	0.68^{ab}	0.89^{a}	1.00^{a}	**0.77**
Means	**0.51^{b}**	**0.62^{ab}**	**0.82^{a}**	**0.95^{a}**	

Means in a row followed by the same letter(s) are not significantly different at $P \leq 0.001$ using Tukey's HSD test.

4.3.5 Visual effects of salt injury on the roots and shoots of the salt tolerant and salt sensitive sorghum genotypes

Visual effect of salt injury on roots and shoots of sorghum increased as NaCl concentrations increased, When the salt treated plants were compared to their control, most of the leaves, shoot and roots for the salt sensitive withered, had stunted growth, turned brown, white bloom in the shoots, dying back, salt burns at the intersection between roots and shoots and even dead. Salt tolerant genotypes showed little or no signs of injury with only a few leaves rolled and near stunted shoot and root growth (Figure 4.5).

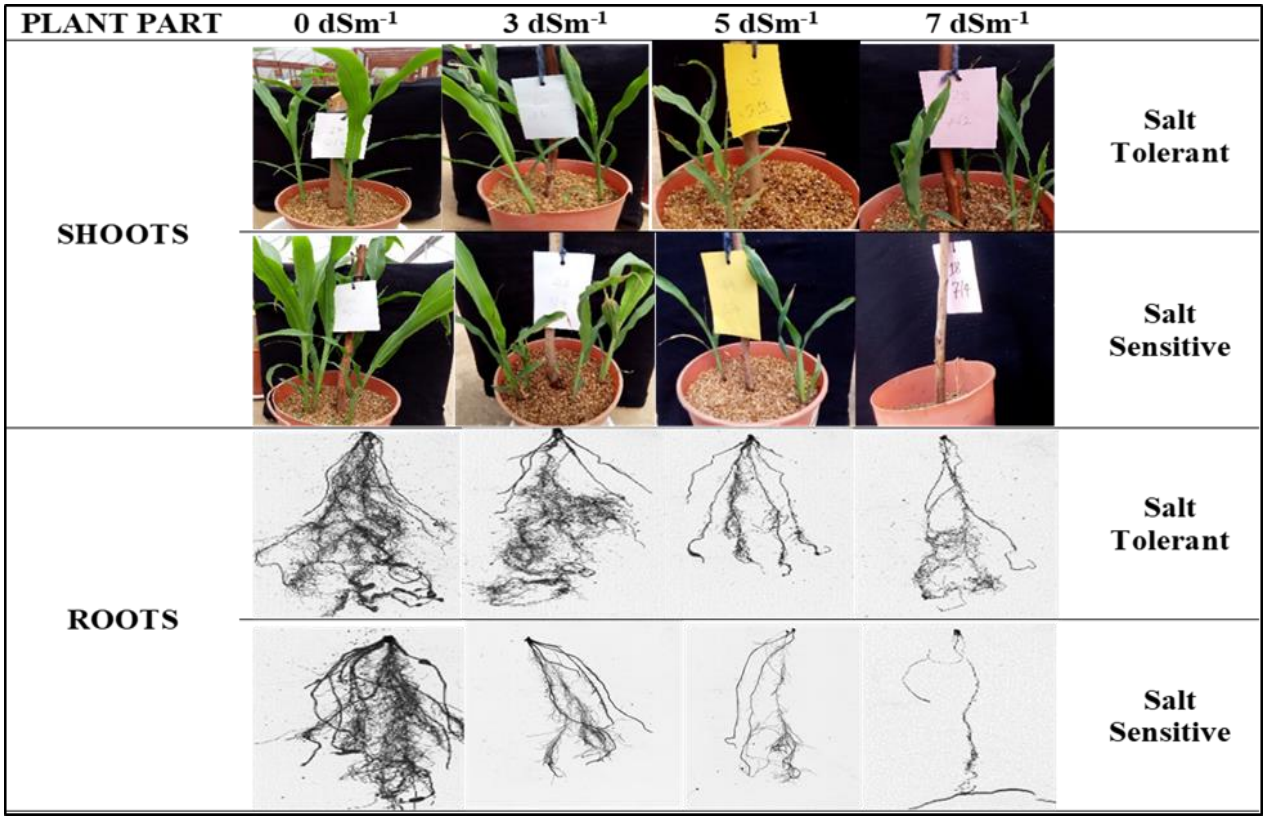


Figure 4.3: Observed effects of NaCl concentration injury on the shoots and roots of salt tolerant and salt sensitive sorghum genotypes. Salt tolerant genotypes were represented by BM 17 and salt sensitive genotypes were represented by EST 41

4.3.6 Correlation of root architecture, shoot growth biomass yield and ion uptake

The Pearson Correlation Analysis using respective means from ANOVA showed a positive relationship between the variables at $P \leq 0.05$ (Table 4.14). A high positive correlation was observed between total leaf area and total dry matter, and high negative correlation was observed between root morphology, aboveground growth, K^+, SRR and shoot Na^+ concentrations (Table 4.14). The high negative correlation between Na^+ concentrations and plant growth parameters was expected since amount of Na^+ taken up by the plant is indicative of growth since ions influence cell division and differentiation and hence production of new plants parts.

Table 4.14: Correlation analysis between root architecture, shoot growth, biomass yield and ion uptake for the salt tolerant and salt sensitive sorghum genotypes

	Root length	**Root surfaces area**	**Root volume**	**Number of roots**	**Plant height**	**Total leaf area**	**Total dry matter**	**Shoot Na^+**	**Na^+ SRR**	**Shoot K^+**	**Root K^+**	**K^+ SRR**
Root length	1.00											
Root surfaces area	0.97	1.00										
Root volume	0.91	0.98	1.00									
Number of roots	0.98	0.93	0.85	1.00								
Plant height	0.60	0.61	0.60	0.61	1.00							
Total leaf area	0.53	0.53	0.53	0.50	0.69	1.00						
Total dry matter	0.53	0.57	0.57	0.48	0.72	0.78	1.00					
Shoot Na^+	-0.50	-0.54	-0.53	-0.50	-0.58	-0.53	-0.56	1.00				
Na^+ SRR	-0.42	-0.45	-0.44	-0.42	-0.54	-0.49	-0.48	0.89	1.00			
Shoot K^+	0.10	0.15	0.19	0.63	0.57	0.02	0.04	0.02	0.07	1.00		
Root K^+	0.25	0.22	0.19	0.25	0.29	0.31	0.26	-0.31	-0.33	-0.14	1.00	
K^+ SRR	-0.16	-0.11	-0.69	-0.16	-0.20	-0.26	-0.23	0.27	0.30	0.54	-0.86	1.00
Shoot Ca^{2+}	0.18	0.23	0.26	0.15	0.08	0.23	0.16	-0.14	-0.10	-0.06	0.16	-0.15

4.4 Discussion

The ability of plants to tolerate salt is controlled by multiple genes involved in different morphological and biochemical functions (Guan *et al*., 2013). The influence of NaCl on shoot growth, root growth, biomass yield and ion uptake indicates the presence of a unique trait to tolerance in sorghum. This study has shown that salt tolerance in sorghum is influenced by genotype and may be attributable to more than one mechanism involving a number of processes. Root growth and ion uptake and balance in plant tissues seem to point to exclusion and tissue tolerance mechanisms of salt tolerance in sorghum.

4.4.1 Morphological responses of sorghum to increasing salt stress

Shoot growth, biomass yield, root growth and ion balance responded differently to increasing NaCl concentrations. The results showed that, NaCl concentrations has negative effect on plant growth and development. These responses are in agreement with work done elsewhere showing that increased NaCl concentrations affect plant growth through ion toxicity leading to low water and nutrient uptake (Kausar *et al*., 2012; Li *et al.,* 2010; Sui *et al*., 2019). When the available water and nutrient is limited for plant metabolic processes, the plant experiences poor nutritional balance leading to retarded growth and development (Tari *et al*., 2013). When sorghum genotypes were subjected to NaCl stress, biomass accumulation, leaf area, plant height, root growth, growth increase per day and ion uptake were inhibited. These toxic salts are known to limit plant's physiological processes by reducing photosynthetic efficiency leading to retarded growth (Sun *et al*., 2019; Wei *et al*., 2006). The observed visual symptoms of salt injury included wilting, dying back, stunted root and shoot growth, rolling leaves, and death of some seedlings occurred as the salt concentrations increased. When sorghum genotypes were subjected to high salt concentrations over longer period, their growth was compromised compared to those in the same growth stage but at low concentrations (Castillo *et al*., 2007; Guan *et al*., 2013; Ran *et al*., 2021). At high salt concentration, biomass accumulation of sorghum reduced, an observation similar to what is reported in beans and cotton (Kausar *et al*., 2012). The negative effect of high salt concentrations has been linked to inhibition of photo-assimilates causing a reduction in tissue nourishment which is directly proportional to plant's ability to withstand stress (Attia, 2016). The low nutrient uptake reduces the metabolic activities of the plant causing a limitation to their growth and biomass productivity (Kausar *et al*., 2012; Ran *et al*., 2021). Plants combat salt stresses by modifying their morphological, physiological and biochemical adaptive

strategies to salt. The pronounced negative effect of NaCl on the shoot from the visual effect of salt injury may be due to reduced uptake of mineral ion and water.

The negative effect of high salt concentration on growth was more evident in the roots than shoots. This was expected since roots are the first organ to come in contact with salt manifesting the symptoms due to excess absorption of Na^+. The visual effect of salt injury on the roots were more visible on the salt sensitive genotypes even at low salt concentration compared to the salt tolerant whose mild effects were slightly visible at 7 dSm^{-1}. The ability of a plant to withstand ion imbalance determines its tolerance to salt (Guan *et al*., 2013; Halperin & Lynch, 2003; Li *et al*., 2010; Zhang *et al*., 2008). As the salt concentration increases, root length, number of roots, root surface area and root diameter reduced drastically. The increased root growth and development at low concentration and drastic reduction as the concentrations increases suggest that, salt may be stimulating growth hormones in sorghum. Increasing salt concentration is known to increase accumulation and inhibitory effect of ethylene hormone on plant growth (Akram *et al*., 2010; Roy *et al*., 2018) by reducing the amount and activity of Gibberellic Acid (GA) (Iqbal *et al*., 2010). These observations are in agreement with the study on *Salix alba* where low salt concentrations stimulated root growth but as the concentration increased their growth was retarded (Ran *et al*., 2021). Roots forms the basis of overall crop growth and development. Their growth meristem is towards the root tip and growth is achieved through regular cell division and elongation for deposition of new plasma membrane and cell organelles (Iqbal *et al*., 2010; Peterson & Farquhar, 1996; Roy *et al*., 2018; Shabala *et al*., 2003). The process is regulated by phytohormones.

4.4.2 Influence of salt stress on ion uptake and ion ratios in sorghum

Under high salt concentration, the salt tolerant genotypes absorbed and accumulated more Na^+ in their roots than the salt sensitive genotypes. It was observed that, as NaCl concentrations increased, the amount of Na^+ accumulated in the roots increased while K^+ decreased while Ca^{2+}remained unchanged. High accumulation of Na^+ limits the uptake of other important ions which could lead to ion imbalance and toxicity (Khan *et al*., 1990; Li *et al*., 2010; Sagar *et al*., 2019). The increased uptake of Na^+ against other ions maybe due to the fact that Na^+ is more mobile (Roy *et al*., 2018) because its ionic radius and hydration energy is similar to that of water (Guan *et al*., 2013). Excess uptake of Na^+ causes ion imbalance near the root surface lowering physiological functions and even destroy the

structure of the roots as salt stress intensify (Castillo *et al*., 2007; Munns, 2002; Ran *et al*., 2021; Zhao *et al*., 2014). Increased Na^+ accumulation in the cell membrane system displaces Ca^{2+} and K^+ causing damage to integrity of the cell membrane. This causes damage to the membrane functions due to Na^+ toxicity from inorganic solutes leakage (Castillo *et al*., 2007: Ran *et al*., 2021). These ions play important roles in normal plant's metabolic activities. An alteration in their absorption interferes with the dynamic ion balance in plant tissues (Dashti *et al*., 2009; Guan *et al*., 2013; Niu *et al*., 2012). Potassium ion is required for the regulation of plant cell turgidity (Sano *et al*., 2007; Xu *et al*., 2021; Zhu *et al*., 2003) while Ca^{2+} is required for cell division and elongation (Shabala *et al*., 2003). Altering the amount of these ions interferes with the physiological and morphological processes that influence plant growth and development. The difference in crop adaptability to salinity is due to genotype, environment and the interaction of the two.

Based on the results in this study, sorghum seems to tolerate salt through exclusion and tissue tolerance mechanism. The salt tolerant genotypes restricted transport of excess Na^+ from the roots to the shoots compared to the salt sensitive genotypes. The ability of salt tolerant genotypes to restrict uptake of excess Na^+ helped them maintain low Na^+ to other ion ratios than salt sensitive genotypes. Increased ion balance by the tolerant genotypes also helps them escape the impact of salt stress while promoting their growth and development. The ability of plants to maintain low Na^+ ratios to other ions by restricting its entry to young and actively dividing plant organs is associated with salt tolerance (Guan *et al*., 2013; Munns, 2002; Xu *et al*., 2021). Absorption and uptake of Na^+ is restricted through ion compartmentalization, promoting its efflux and maintain high ratio of K^+:Na^+ and Ca^{2+}:Na^+ balance (Castillo *et al*., 2007; Guan *et al*., 2013; Ran *et al*., 2021; Zhao *et al*., 2014). However, tissue tolerance to high Na^+ concentration is also an important salt tolerance mechanism that could contributed to salt tolerance in the tolerant sorghum genotypes.

4.5 Conclusion

The ability of sorghum to adjust to NaCl stress as shown in the results on shoot growth, root growth and ion uptake and balance indicates unique trait to tolerance or sensitivity to salt. Salt tolerance in sorghum seems to be controlled by multiple traits involved in morphological, physiological and biochemical processes. BM 17 and EST 41 exhibited significant signs for salt tolerance and are thus promising genotype for future screening for salt-tolerance**.**

CHAPTER FIVE
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General Discussion

Soil salinity is becoming a major limitation to crop productivity and the situation has been exacerbated by the changes in climate pattern. Salinity is mainly experienced in the ASALs and this has partly contributed to increased food and nutrition insecurity (Robin *et al*., 2016). There is a need to adopt high valued crops that can escape the harsh environmental changes in the ASALs. Sorghum is considered a climate smart crop owing to its tolerance to soil moisture deficit. It is also believed to have moderate tolerance to salt (Sagar, 2017; Sagar *et al*., 2019). However, extreme salt concentration remains a major challenge to its production and utilization. The current work concentrated on evaluating sorghum genotypes for salt tolerance and to provide useful information that can facilitate the establishment of an associated genetic link in salt tolerance for crop improvement.

The responses of sorghum to different salt concentrations at seedling stage points to their ability to grow to maturity if improved. The current study has shown that salt tolerance in sorghum is best expressed in their roots. The response of root hairs to different salt concentrations varied between genotypes. The salt tolerant genotypes showed a relatively high number of roots across NaCl concentrations. Root hairs are actively involved in water and nutrient uptake in plants. Reduced root hairs implies a general reduction in plant's growth and development, an observation also reported in *Arabidopsis thaliana* where root hairs reduced with increasing salt concentrations (Xu *et al*., 2021). The ability of some genotypes to maintain their growth even at high salt concentrations suggests the presence of genetic variation to salt tolerance in sorghum. The root hairs seemed to be the possible parameter to be considered during future breeding programs.

Ion uptake by the selected salt sensitive and salt tolerant sorghum genotypes revealed a possible mechanism of salt tolerance in sorghum. Generally, the root architecture was severely altered by increased salt stress by virtue of being the first plant part to come in contact with the salty solutions. The adverse effects of salt stress on the roots were pronounced by the reduced crop biomass yield and shoot growth, and imbalance of ion uptake. The relationship between root/shoot growth and ion uptake explains the morphological, physiological and biochemical processes of plants growth regulation and ability to tolerate stress. These result are consistent with Ran *et al* (2021) who reported a stable growth and development and ion balance when *Salix alba* was exposed to salt stress to

be attributed to salt tolerance. The roots of the salt tolerant sorghum genotypes absorbed more salt but less was detected in the shoot, unlike the salt sensitive which translocated more salts to the shoot, suggesting a mechanism to restrict them from reaching the actively dividing and young cells. Such mechanism of salt exclusion was also observed in rice by Zhao *et al*. (2014) and in *Artemisia* by Guan *et al*. (2013). The ability of the salt tolerant genotypes to balance the ratios of ion uptake both in the roots and shoots, helps them to escape the stress and maintain their growth to maturity with limited compromise on yield.

Improving sorghum productivity in salt-prone environment may require identification of genetic traits responsible for salt tolerance for use in variety development. Such move will increase the uptake of sorghum in the ASALs and provide resilience to affected communities in the face of climate change. The positive outcome of which will be an increase in food and nutrition security and economic growth of the resource-poor farmers. The following are the conclusion and recommendations from the current study.

5.2 Conclusions

Based on the results of this study, the conclusions are as follows:

i. There exists a large expression of variation for salt tolerance in sorghum genotypes which can be observed distinctively at early seedling stage. This can be exploited to obtain suitable varieties for the salt-prone marginal areas.
ii. The results revealed that BM 17, GBK 000049, GBK 000038, and BM 29 are tolerant to salt while EST 41, IESV 23007 DL, and GBK 000073 are sensitive to salt.
iii. Sorghum seems to tolerate salt stress through Na^+ exclusion and tissue tolerance mechanism by restricting transport of excess Na^+ from the roots to the shoots. Roots seems to be reliable parameters for screening for salt tolerance in sorghum

5.3 Recommendations

The following are the recommendations from these studies:

i. There is need to subject the segregated highly salt tolerant genotypes such as BM 17, GBK 000049, GBK 000038, and BM 29 and highly sensitive genotypes such as EST 41, IESV 23007 DL, and GBK 000073 to field observation under saline soils.
ii. Gene analysis and gene locus sequencing should be conducted to identify gene(s) associated to salt tolerance in sorghum.
iii. Introgression of salt tolerance genes to desirable sorghum varieties should be carried out for improved grain and fodder production in salt.

The outputs of the study point to a huge gap on development of superior sorghum varieties for the saline areas and therefore, suggested the following areas of further research:

i. Research on differential gene expression profile for the salt tolerant and salt sensitive sorghum genotypes be conducted
ii. Studies on incorporation of salt tolerance gene(s) to desirable sorghum varieties for specific uses be conducted for improved grain and fodder production in the saline ASALs.

REFERENCES

Akram, M., Ashraf, M. Y., Ahmad, R., Rafiq, M., Ahmad, I., & Iqbal, J. (2010). Allometry and yield components of maize (*Zea mays* L.) hybrids to various potassium levels under saline conditions. *Archives of Biological Sciences*, *62*(4), 1053-1061

Attia, M. A. (2016). Performance of some sorghum genotypes under salinity conditions. *IOSR Journal of Agricultur and Veterinary Sciences, 9*(4), 8-12

Balole, T. V., & Legwaila, G. M. (2006). *Sorghum bicolor* (L.) Moench; Record from Protabase. *PROTA Plant Resources of Tropical Africa, Wageningen. http://database. prota. org/search. Htm*

Britannica, T. Editors of Encyclopedia (2022). Sorghum. Encyclopedia Britannica. https://www.britannica.com/plant/sorghum-grain. Retrieved on September, 2022.

Bueno, A. (1979). Leaf area estimation, growth analysis, and yield evaluation in grain sorghum (*Sorghum bicolor* L. Moench). *Iowa State University*.

Canton, H. (2021). Food and Agriculture Organization of the United Nations—FAO. In Europa publications (Eds), *The Europa directory of international organizations* (pp. 297-305). Routledge. https://doi.org/10.4324/9781003179900

Castillo, E. G., Tuong, T. Ph., Ismail A. M., & Inubushi, K. (2007). Response to salinity in Rice: comparative effects of osmotic and ionic stresses. *Plant Production Science, 10*(2), 159-170

Charrondière, U. R., Stadlmayr, B., Rittenschober, D., Mouille, B., Nilsson, E., Medhammar, E., Olango, T., Eisenwagen, S., Persijn, D., Ebanks, K., Vowak, J. D., & Burlingame, B. (2013). FAO/INFOODS food composition database for biodiversity. *Food chemistry*, 140(3), 408-412

Chepng'etich, E., Bett, E. K., Nyamwaro, S. O., & Kizito, K. (2014). Analysis of Technical Efficiency of Sorghum Production in Lower Eastern Kenya: A Data Envelopment Analysis (DEA) approach. *Journal of Economics and Sustainable Development 5*(4), 8-65

Council, N. R. (1996). *Lost crops of Africa: volume I: grains*. National Academies Press

Dashti, A., Khan, A. A., & Collins, J. C. (2009). Effects of salinity on growth, ionic relations and solute content of Sorghum bicolor (L.) Monench. *Journal of Plant Nutrition, 32*(7), 1219-1236

Deb, U. K., & Bantilan, M. C. S. (2003). Impacts of genetic improvement in sorghum. In: R. E. Evenson and D. Gollin (Eds.) *Crop variety improvement and its effect on*

productivity: the impact of international agricultural research (pp. 183-213). Wallingford UK: CABI Publishing

Ding, L., & Zhu, J. K. (1997). Reduced Na+ uptake in the NaCl-hypersensitive sos1 mutant of *Arabidopsis thaliana*. *Plant Physiology, 113*(3), 795-799

Ding, T., Yang, Z., Wei, X., Yuan, F., Yin, S., & Wang, B. (2018). Evaluation of salt-tolerant germplasm and screening of the salt-tolerance traits of sweet sorghum in the germination stage. *Functional Plant Biology*, *45*(10), 1073-1081. https://doi.org/10.1071/FP18009

Du, Y., Zhao, Q., Li, S., Yao, X., Xie, F., & Zhao, M. (2019). Shoot/root interactions affect soybean photosynthetic traits and yield formation: a case study of grafting with record-yield cultivars. *Frontiers in Plant Science, 10*(445), 1-14

Ezenwa, L. I., Omondi, P. A., Nwagbara, M. O., Gbadebo, A. M., & Bada, B. S. (2018). Climate variability and its effects on gender and coping strategies in Baringo County, Kenya. *Journal of Applied Sciences and Environmental Management*, *22*(5), 699-706

FAO (1988). FAO-UNESCO Soil Map of the World, revised legend, FAO World Soil Resources Report, 60, UNESCO, Paris

FAOSTAT (2019). *FAO statistical yearbook of 2019*. World food and Agriculture. Statistical Division of Food and Agriculture Organization of United Nations

FAOSTAT (2021). *FAO statistical yearbook of 2021*. Word food and Agriculture. Statistical Division of Food and Agriculture Organization of United Nations

Farooq, M., Hussain, M., Wakeel, A., & Siddique, K. H. M. (2015). Salt stress in maize: effects, resistance mechanisms, and management. *Agronomy for Sustainable Development, 35*, 461–481. https://doi.org/10.1007/s13593-015-0287-0

Government of Kenya. (2021). *Arid and Semiarid lands*; Northern Kenya and other Arid Lands Ministry report, Government Printer, Nairobi, Kenya

Gregoria, G. B., Senadhira, D., & Mendoza, R. D. (1997). *Screening rice for salinity tolerance* (No. 2169-2019-1605). International Rice Research Institute (IRRI)

Grieve, C. M., Grattan, S. R., & EV Maas (2012). *Plant salt tolerance*. In: W.W. Wallender and K.K. Tanji (Eds.) *ASCE Manual and Reports on Engineering Practice, 71*, 405-459

Griffiths, M., & York, L. M. (2020). Targeting root ion uptake kinetics to increase plant productivity and nutrient use efficiency. *Plant Physiology 182*(4), 1854-1868. http://dx.doi.org/10.1104/pp.19.01496

Guan, Z. Y., Su, Y. J., Teng, N. J., Chen, S. M., Sun, H. N., Li, C. L., & Chen, F. D. (2013). Morphological, physiological, and structural responses of two species of *Artemisia* to NaCl stress. *The Scientific World Journal, 13*, 1-10 https://doi.org/10.1155/2013/309808

Halperin, S. J., & Lynch, J. P. (2003). Effects of salinity on cytosolic Na^+ and K^+ in root hairs of *Arabidopsis thaliana*: *in vivo* measurements using the fluorescent dyes SBFI and PBFI. *Journal of Experimental Botany, 54*(390), 2035-2043. https://doi.org/10.1093/jxb/erg219

Haw, M., Cocklin, C., & Mercer, D. (2000). A pinch of salt: landowner perception and adjustment to the salinity hazard in Victoria, Australia. *Journal of Rural Studies, 16*(2), 155-169. http://dx.doi.org/10.1016/S0743-0167(99)00055-8

Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., & Mortensen, D. A. (2017). Agriculture in 2050: recalibrating targets for sustainable intensification. *Bioscience, 67*(4), 386-391

Hydroponic Africa limited (2021). Full Spectrum of Hydroponics nutrients (Hydro-A and Hydro-B). https://www.hydroponicsafrica.org/

ICRISAT (2013). CGIAR Research Programme on Climate Change, Agriculture and Food Security (CCAFS). *International Crops Research Institute for the Semi-Arid Tropics*. Copenhagen, Denmark, pp 50

ICRISAT (2015). Consumer demand for sorghum and millets in eastern and Southern Africa: In Gierend, A., and Orr, A (Eds). *Priorities for the CGIAR Research Programme for Dryland Cereals*; Socioeconomics Discussion Paper Series, *35*

Iqbal, M., & Ashraf, M. (2010). Gibberellic acid mediated induction of salt tolerance in wheat plant: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environmental and Experimental Botany, 86*, 76-85. Doi. 10.1016/j.envexpbot.2010.06.002

Iseki, K., Marubodee, R., Ehara, H., & Tomooka, N. (2017). A rapid quantification method for tissue Na^+ and K^+ concentrations in salt-tolerant and susceptible accessions in *Vigna vexillata* (L.) A. Rich. *Plant Production Science, 20*(1), 144-148

Islam, M. M., & Karim, M. A. (2010). Evaluation of rice (*Oryza sativa* L.) genotypes at germination and early seedling stage for their tolerance to salinity. *The Agriculturists, 8*(2), 57-65

Kausar, A., Ashraf, M. Y., Ali, I., Niaz, M., & Abbass, Q. (2012). Evaluation of sorghum varieties/lines for salt tolerance using physiological indices as screening tool. *Pakistan Journal of Botany*, *44*(1), 47-52

Khan, A. A., Rao, S. A., & McNeilly, T. (2003). Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica*, *131*, 81-89

Khan, A. H., Ashraf, M. Y., & Azmi, A. R. (1990). Effect of sodium chloride on growth and nitrogen metabolism of Sorghum. *Acta Physiologiae Plantarum*, *12*(3), 233-238

Khaton, M. A., Sagar, A., Tajkia, J. E., Islam, M. S., Mahmud, M. S., & Hossain, A. K. M. Z. (2016). Effect of moisture stress on morphological and yield attributes of four sorghum varieties. *Progressive Agriculture*, *27*(3), 265-271

Kumar, S., Dwivedi, S. K., Singh, S. S., Jha, S. K., Lekshmy, S., Elanchezhian, R., Singh, O. N., & Bhatt, B. P. (2014). Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. *SABRAO Journal of Breeding & Genetics*, *46*(2), 217-230

Li, R., Shi, F., & Fukuda, K. (2010). Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte *Spartina alterniflora* (Poaceae). *Environmental and Experimental Botany*, *68*(1), 66-74

Lin, H. X., Zhu, M. Z., Yano, M., Gao, J. P., Liang, Z. W., Su, W. A., Ren, H. Z., & Chao, D. Y. (2004). QTLs of Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. *Theoretical and Applied Genetics*, *108*, 253-260

Maas E.V., & Hoffman G. J. (1977). Crop salt tolerance expressed as the decrease in yield: evaluation of existing data. *In international conference on managing saline water for irrigation, planning for the future, Lubbock*, Tex.(USA), 1976. ICASALS

Maathuis, F. J., & Amtmann, A. (1999). K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Annals of Botany*, *84*(2), 123-133

Mbinda, W., and Kimtai, M. (2019). Evaluation of Morphological and Biochemical Characteristics of sorghum (*Sorghum bicolor*. L. Moench) varieties in response salinity stress. *Annual Research & Review in Biology*, *33*(1), 1-9.

Mburu, J., Maina. F. W., Ackello-Ogutu, C., & Egelyng, H (2019). Producers' valuation of geographical indications-related attributes of agri-food products from semi-arid lands in Kenya. *Heliyon*, *5*, e01218. http://dx.doi.org/10.1016/j.heliyon.2019. e0121

Meneguzzo, S., Navavi-Izzo, F., & Izzo, R. (2000). NaCl effects on water relations and accumulation of minerals in shoots, roots and cell sap of wheat seedling. *Journal of Plant Physiology*, *156*(5-6), 711-716

Mugai, E. N. (2004). Salinity characterization of the Kenyan saline soils. *Soil Science and Plant Nutrition*, *50*(2), 181-188. http://dx.doi.org/10.1080/00380768.2004.10408467

Mundia, C. W., Secchi, S., Akamani, K., & Wang, G. (2019). A regional comparison of factors affecting global sorghum production: the case of North America, Asia and Africa's Sahel. *Sustainability*, *11*(7), 2135

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment*, *25*(2), 239-250

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651-681

Mwando, E., Han, Y., Angessa, T. T., Zhou, G., Hill, C. B., Zhang, X. Q., & Li, C. (2020). Genome-wide association study of salinity tolerance during germination in Barley (*Hordeum vulgare.* L). *Frontiers in Plant Science*, *11*, 118. http://dx.doi.org/10.3389/fpls.2020.00118

Netondo, G. W., Onyango, J. C., & Beck, E. (2004a). Sorghum and salinity: I. Response of growth, water relations, and ion accumulation to NaCl salinity. *Crop Science*, *44*(3), 797-805

Netondo, G. W., Onyango, J. C., & Beck, E. (2004b). Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science*, *44*(3), 806-811.

Niu, G., Xu, W., Rodriguez, D., & Sun, Y. (2012). Growth and physiological responses of maize and sorghum genotypes to salt stress. *International Scholarly Research Network in Agronomy*, *12*, 1-2

Njagi, T., Onyango, K., Kirimi, L., & Makau, J. (2019). *Sorghum production in Kenya: Farm-level characteristics, constraints and opportunities*. Tegemeo Institute. http://41.89.96.81:8080/xmlui/handle/123456789/2396

Nxele, X., Klein, A., Ndimba, B. K. (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. *South African journal of Botany*, *108*, 261-266. https://doi.org/10.1016/j.sajb.2016.11.003

Ochieng, L. A., Mathenge, P. W., & Muasya, R. J. A. J. O. F. (2011). A survey of on-farm seed production practices of sorghum (*Sorghum bicolor* L. Moench) in Bomet District

of Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *11*(5), 5232-5253

Oluoch, W. K. (2016). Performance of Kenya's Agriculture: A Macro-Economic Perspective. *Department of Agricultural Economics University of Nairobi* (unpublished)

Peterson, R. L., & Farquhar, M. L. (1996). Root hairs: specialized tubular cells extending root surfaces. *The Botanical Review*, *62*, 1-40

Price, H. J., Dillon, S. L., Hodnett, G., Rooney, W. L., Ross, L., & Johnston, J. S. (2005). Genome evolution in the genus Sorghum (*Poaceae*). *Annals of Botany*, *95*(1), 219-227

Rahnama, A., James, R. A., Poustini, K., & Munns, R. (2010). Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil. *Functional Plant Biology*, *37*(3), 255-263

Ran, X., Wang, X., Gao, X., Liang, H., Liu, B., & Huang, X. (2021). Effects of salt stress on the photosynthetic, physiological and mineral absorption and distribution in white wallow (*Salix alba* L.). *Public Library of Science*, *16*(11), e0260086. https://doi.org/10.1371/journal.pone0260086

Renault, S., Croser, C., Franklin, J. A., & Zwiazek, J. J. (2001). Effects of NaCl and Na_2SO_4 on red-osier dogwood (*Cornus stolonifera.* Michx) seedlings. *Plant and Soil*, *233*, 261-268

Robin, A. H. K., Matthew C., Uddin M. J., & Bayazid, K. N. (2016). Salinity-induced reduction in root surface area and changes in major root and shoot traits at the phytomer level in wheat. *Journal of Experimental Botany*, *67*(12), 3719-3729. http://dx.doi.org/10.1093/jxb/erw064

Rogers, M. E., & Noble, C. L. (1992). Variation in growth and ion accumulation between two selected populations of *Trifolium repens* L. differing in salt tolerance. *Plant Soil*, *146*, 131-136

Roy, R. C., Sagar, A., Tajkia, J. E., Razzak, M. A., & Hossain, A. Z. (2018). Effect of salt stress on growth of sorghum germplasms at vegetative stage. *Journal of the Bangladesh Agricultural University*, *16*(1), 67-72

Sagar, A. (2017). Screening of sorghum germplasms for salinity tolerance based on morphophysiological and biochemical traits. *Bangladesh Agricultural University*. Mymensingh, Bangladesh

Sagar, A., Tajkia, J. E., Haque, M. E., Fakir, M. S. A., & Hossain, A. Z. (2019). Screening of sorghum genotypes for salt-tolerance based on seed germination and seedling stage. *Fundamental and Applied Agriculture*, *4*(1), 735-743

Sano, T., Becker, D., Ivashikina, N., Wegner, L. H., Zimmermann, U., Roelfsema, M. R. G., Hedrich, R. (2007). Plant cells must pass a K^+ threshold to re-enter the cell cycle. *The Plant Journal*, *50*(3), 401-413

Serraj, R., Krishnamurthy, L., Hash, C. T., Dakheel, A. J., & Reddy, B. V. (2007). Screening sorghum genotypes for salinity tolerant biomass production. *Euphytica*, *156*(1), 15-24

Shabala, S., Shabala, L., & Van Volkenburgh, E. (2003). Effect of calcium on root development and root ion fluxes in salinized Barley seedlings. *Functional Plant Biology*, *30*(5), 507-514. https://doi.org/10.1071/FP03016

Shewale, S. D., & Pandit, A. B. (2011). Uses of sorghum and value addition. Sorghum: Cultivation, varieties and uses. In TD Pereira Edition, *Sorghum Cultivation, varieties and uses*. *Nova Science Publishers*, Incorporated New York, 181

Shrestha, A., Cox, R., Wu, Y., Robles, O., Desouza, L. L., Wright, S. D., & Dahlberg, J. A. (2016). Moisture and salt tolerance of a forage and grain sorghum hybrid during germination and establishment. *Journal of Crop Improvement*, *30*(6), 668-683

Singh, S., Sengar, R. S., Kulshreshtha, N., Datta, D., Tomar, R. S., Rao, V. P., Garg, D & Ojha, A. (2015). Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). *Journal of Agricultural Science*, *7*(3), 49. http://dx.doi.org/10.5539/jas.v7n3p49

Sombroek, W. G., Braun, H. M. H., & Van der Pouw, B. J. A. (1982). *Exploratory soil map and agro-climatic zone map of Kenya, 1980. Scale 1: 1,000,000*. Kenya Soil Survey.

Sui, N., Wang, Y., Liu, S., Yang, Z., Wang, F., & Wan, S. (2018). Transcriptomic and physiological evidence for the relationship between unsaturated fatty acid and salt stress in peanut. *Frontiers in Plant Science*, *9*(7), 1-12. https://doi.org/10.3389/fpls.2018.00007

Sun, J., He, L., & Li, T. (2019). Response of seedling growth and physiology of *Sorghum bicolor* (L.) Moench to saline-alkali stress. *Public Library of Science*, *14*(7), e0220340

Sun, Y., Niu, G., Osuna, P., Zhao, L., Ganjehunte, G., Peterson, G., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2014). Variability in salt tolerance of *Sorghum bicolor*. L. *Agricultural Science*, *2*(1), 9-21.

Tari, I., Laskay, G., Takács, Z., & Poór, P. (2013). Response of sorghum to abiotic stresses: A review. *Journal of Agronomy and Crop Science*, *199*(4), 264-274.

Tobe, K., Li, X., & Omasa, K. (2000). Effects of sodium chloride on seed germination and growth of two Chinese desert shrubs, *Haloxylon ammodendron* and *H. persicum* (*Chenopodiaceae*). *Australian Journal of Botany*, *48*(4), 455-460. http://dx.doi.org/10.1071/BT99013

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics, 5*(2), 99-105. https://doi.org/10.2307/3001913

Turkan, I., & Demiral, T. (2009). Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany*, *67*(1), 2-9.

United Nations University International Network on Water, environment and Health (UNU-INWEH). http://inweh.unu.edu/, 2014

USDA-NASS, U. S. D. A. (2015). National agricultural statistics service. *USDA-NASS, Washington DC*.

Vavilov, N. I. (1951). The origin, variation, immunity and breeding of cultivated plants. *The origin, variation, immunity and breeding of cultivated plants*.

Wei, Y., Xu, X., Tao, H., & Wang, P. (2006). Growth performance and physiological response in the halophyte *Lycium barbarum* grown at salt-affected soil. *Annals of Applied Biology*, *149*(3), 263-269.

Wu, H., Guo, J., Wang, C., Li, K., Zhang, X., Yang, Z., Li, Maoteng & Wang, B. (2019). An Effective Screening Method and a Reliable Screening Trait for Salt Tolerance of *Brassica napus* at the Germination Stage. *Frontiers in Plant Science*, *10*, 530.

Xu, Y., Jiao, X., Wang, X., Zhang, H., Wang, B., & Yuan, F. (2021). Importin-β from the Recretohalophyte Limonium bicolor enhances salt tolerance in *Arabidopsis thaliana* by reducing root hair development and Abscisic Acid sensitivity. *Frontiers in Plant Science*, *11*, 582459. http://dx.doi.org/10.3389/fpls.2020.582459

Yasmeen, A., Basra, S. M. A., Farooq, M., & Hussain, N. (2013). Exogenous application of Moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Growth Regulation*, *69*(3), 225-233.

Zhang, L., Ma, H., Chen, T., Pen, J., Yu, S., & Zhao, X. (2014). Morphological and physiological responses of Cotton (*Gossypium hirsutum* L.) Plants to Salinity. *Public Library of Science*, *9*(11), e112807. http://dx.doi.org/10.1371/journal.pone.0112807

Zhang, Y. X., Liu, F., Kang, E. X, & Chen, N. L. (2008). Study on ion absorption of Muskemelon seedling under NaCl stress. *Plant Nutrition and Fertilizer Science*, *4*(3), 533-539.

Zhao, X., Wang, W., Zhang, F., Deng, J., Li, Z., & Fu, B. (2014). Comparative metabolite profiling of two rice genotypes with contrasting salt stress tolerance at the seedling stage. *Public Library of Science*, *9*(9), e108020.

Zheng, H., Yang, Z., Wang, W., Guo, S., Li, Z., Liu, K., & Sui, N. (2020). Transcriptome analysis of Maize inbred lines differing in drought tolerance provides novel insights into the molecular mechanisms of drought responses in roots. *Plant Physiology and Biochemistry*, *149*, 11-26. https://doi.org/10.1016/j.plaphy.2020.01.027

Zhu, J. K. (2001). Plant salt tolerance. *Trends in Plant Science*, *6*(2), 66-71.

Zhu, J. K. (2003). Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology*, *6*(5), 441-445.

APPENDICES

Appendix A: List of entry numbers and names of 250 sorghum genotypes evaluated for salt tolerance and sensitivity

ENTRY NUMBER	ENTRY NAME	ENTRY NUMBER	ENTRY NAME	ENTRY NUMBER	ENTRY NAME
1	GBK-000002	35	GBK-000052	69	GBK-000096
2	GBK-000010	36	GBK-000055	70	GBK-000097
3	GBK-000013	37	GBK-000056	71	GBK-000098
4	GBK-000014	38	GBK-000057	72	GBK-000099
5	GBK-000015	39	GBK-000058	73	GBK-000100
6	GBK-000016	40	GBK-000059	74	GBK-000102
7	GBK-000018	41	GBK-000060	75	GBK-000103
8	GBK-000020	42	GBK-000062	76	GBK-000105
9	GBK-000021	43	GBK-000064	77	GBK-000106
10	GBK-000022	44	GBK-000065	78	GBK-000107
11	GBK-000023	45	GBK-000066	79	GBK-000111
12	GBK-000024	46	GBK-000067	80	GBK-000116
13	GBK-000025	47	GBK-000068	81	GBK-000118
14	GBK-000027	48	GBK-000069	82	GBK-000119
15	GBK-000028	49	GBK-000070	83	GBK-000120
16	GBK-000029	50	GBK-000071	84	GBK-000121
17	GBK-000030	51	GBK-000073	85	GBK-000125
18	GBK-000031	52	GBK-000075	86	GBK-000128
19	GBK-000032	53	GBK-000077	87	GBK-000129
20	GBK-000033	54	GBK-000079	88	GBK-000130
21	GBK-000034	55	GBK-000080	89	GBK-000132
22	GBK-000035	56	GBK-000081	90	GBK-000365
23	GBK-000037	57	GBK-000082	91	GBK-000366
24	GBK-000038	58	GBK-000083	92	GBK-000367
25	GBK-000039	59	GBK-000084	93	GBK-000376
26	GBK-000040	60	GBK-000085	94	GBK-000377
27	GBK-000043	61	GBK-000086	95	GBK-000381
28	GBK-000044	62	GBK-000088	96	GBK-000382
29	GBK-000046	63	GBK-000089	97	GBK-000387
30	GBK-000047	64	GBK-000090	98	GBK-000389
31	GBK-000048	65	GBK-000091	99	ASARECA 15-2-1
32	GBK-000049	66	GBK-000092	100	ASARECA 15-3-1
33	GBK-000050	67	GBK-000093	101	ASARECA 24-4-1
34	GBK-000051	68	GBK-000095	102	BUSHUKA (ICSV 210)

ENTRY NUMBER	ENTRY NAME	ENTRY NUMBER	ENTRY NAME	ENTRY NUMBER	ENTRY NAME
103	CR:35:5	137	KARI MTAMA1	171	IS 11159
104	GADAM	138	KIBOKO LOCAL-2	172	IS 11162
105	GAMBELLA 1107	139	KSV 12	173	IS 11187
106	HAKIKA	140	LARSVYT - 58 -85	174	IS 11228
107	ICSH 152010	141	MAKUENILOCAL	175	IS 11257
108	ICSH 152014	142	MEXICO R-LINE #5	176	IS 11338
109	ICSV 111 IN	143	PP290	177	IS 11350
110	IESH 22002	144	S35	178	IS 11353
111	IESH 22007	145	SIAYA_27-3	179	IS 11579
112	IESH 22017	146	SIAYA_50-3	180	IS 11608
113	IESH 22020	147	WAHI	181	IS 1161
114	IESH 28002	148	BM 29	182	IS 11612
115	IESV 23006 DL	149	BM 31	183	ICSH 152002
116	IESV 23007 DL	150	BM 32	184	ICSH 152005
117	IESV 24030 SH	151	BM 39	185	ICSH 152011
118	IESV 92029 DL	152	BM 5	186	ICSH 152016
119	IESV 92170 DL	153	BM 6	187	IS 24484
120	IESV 94076 DL	154	CYATANOMBE	188	IS 26794
121	IS 11167	155	E 1291	189	IS 2853
122	IS 21158	156	GATARAGA	190	SHAMBUKO (PP 290)
123	IS 21282	157	GICAMUNKONI	191	ABALESHYA
124	KHALID	158	IESV 90015 LT	192	AMASUGI
125	LABA (MW 5003)	159	IESV 90042 LT	193	AMATEGA
126	MACIA	160	IESV 91003 LT	194	BAYISHINYIKE
127	MIWALENI LOCAL	161	IESV 91018 LT	195	BM 16
128	SEREDO	162	IESV 91054 LT	196	BM 17
129	SERENA	163	IESV 91069 LT	197	BM 21
130	BUSIA_28-1	164	IESV 91071 LT	198	BM 27
131	IESV91131DL	165	IESV 91073 LT	199	F6YQ212
132	IESV92022/1SH	166	IESV 91075 LT	200	GAMBELLA 107
133	IESV92055/3SH	167	IESV 91105 LT	201	ICSR93034
134	IESV92136DL	168	IKINYARUKA	202	ICSV 112
135	IESV92172DL	169	IS 11076	203	IESV23008DL
136	IS8884	170	IS 11141	204	IESV91048DL

ENTRY NUMBER	ENTRY NAME	ENTRY NUMBER	ENTRY NAME
205	IESV91049DL	240	ARAKUCHOT
206	IESV91063DL	241	RWANDA
207	IESV94025SH	242	EST 15
208	IS11167	243	EST 3
209	IS8193	244	EST 26
210	PLOT_142SUDAN	245	EST 27
211	R8602	246	EST 20
212	SIAYA_42	247	EST 25
213	SIAYA_46-1	248	EST 7
214	SIAYA_81-2	249	EST 41
215	SIAYA_97-1	250	EST 48
216	AIHR 91075		
217	ICSR 160		
218	ICSR 172		
219	ICSR 24008		
220	ICSR 92003		
221	WM89/90#1615		
222	ZSV3		
223	ICSR 43		
224	IESV 93042 SH		
226	MB 27		
227	LITH		
228	IBUNDU		
229	CYURE		
230	IBUNDI		
231	AINAMOI		
232	NYUNDO		
233	MUHIMPUNDU		
234	DHET		
235	NAHADAVA		
236	IS 25557		
237	IS 558		
238	IS 2558		
239	IS 9201		

Appendix B: Mean squares from analysis of variance of 250 sorghum genotypes and four levels of salinity for number of root hairs, root length and shoot length

Source of variation	df	Number of root hairs	Root length	Shoot length
Genotype	249	682.95***	59.84***	6.32***
Salinity level	3	45484.22***	4565.55***	99.96***
Genotype*Salinity level	747	352.31***	26.18***	1.79***
Error	1802	137.94	9.480	0.80
R^2		0.70	0.70	0.69
CV		11.75	3.10	8.90

***, significant at (P≤0.001); CV= Coefficient of variation; d.f= degree of freedom

Appendix C: Mean squares from analysis of variance of six sorghum genotypes and four levels of salinity for plant height, leaf area and biomass yield

Source of variation	df	Plant height	Total leaf area	Total dry matter	Dry matter accumulation (DM)	DM Shoot to root ratio
NaCl Concentrations	3	307.16***	31,663.00***	63.17***	0.07***	10.01**
Genotype	5	28.21***	2366.00**	3.18	0.00*	4.69
NaCl*Genotype	5	8.26	846.00	1.16	0.00	2.61
Error		274.63	28063.73	66.93	0.07	140.33
R2		0.81	0.81	0.77	0.77	0.40
CV		20.76	37.69	44.17	44.03	77.04

***, **, *, significant at P≤0.001, P≤0.01 and P≤0.05 respectively; CV= Coefficient of variation; d.f= degree of freedom

Appendix D: Mean squares from analysis of variance of six sorghum genotypes and four levels of salinity for root length, root surface area, root volume and number of roots

Source of variation	df	Root length	Root surface area	Root diameter	Root Volume	Number of Roots
NaCl Concentrations	3	2,140,528.00***	191,931.00***	0.21***	103.04***	1,914,314.00***
Genotype	5	232,821.00	15,075.00	0.03	7.81	267,753.00*
NaCl*Genotype	15	109,934.00	8297.00	0.02	5.35	123034.00
Error		5360542.20	360427.17	0.57	178.84	4672930.67
R2		0.63	0.68	0.65	0.71	0.66
CV		50.82	58.69	16.37	70.27	45.46

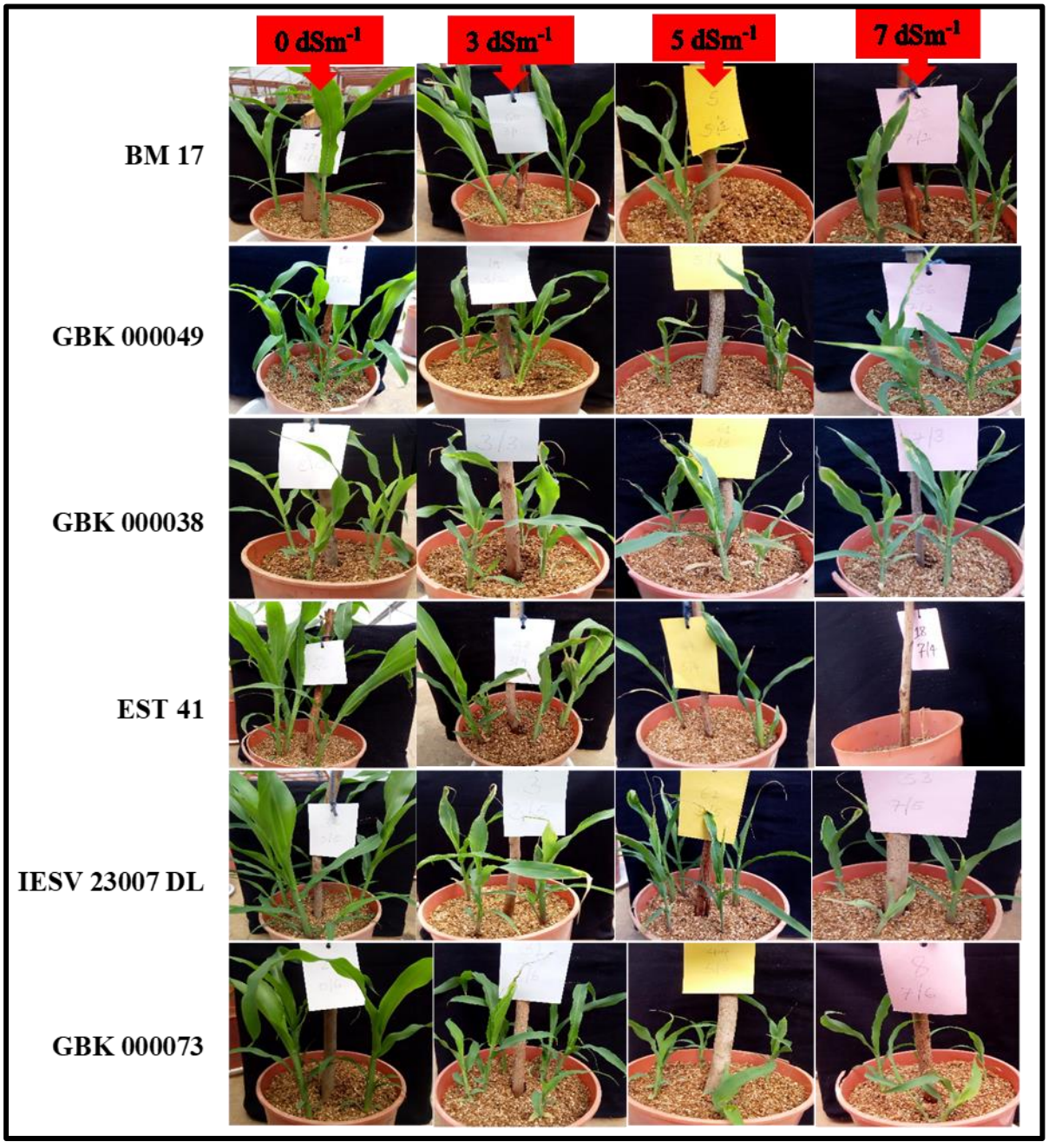
***, *, significant at P≤0.001 and P≤0.05 respectively; CV= Coefficient of variation; d.f= degree of freedom

Appendix E: Mean squares from analysis of variance of six sorghum genotypes and four levels of salinity for ion uptake

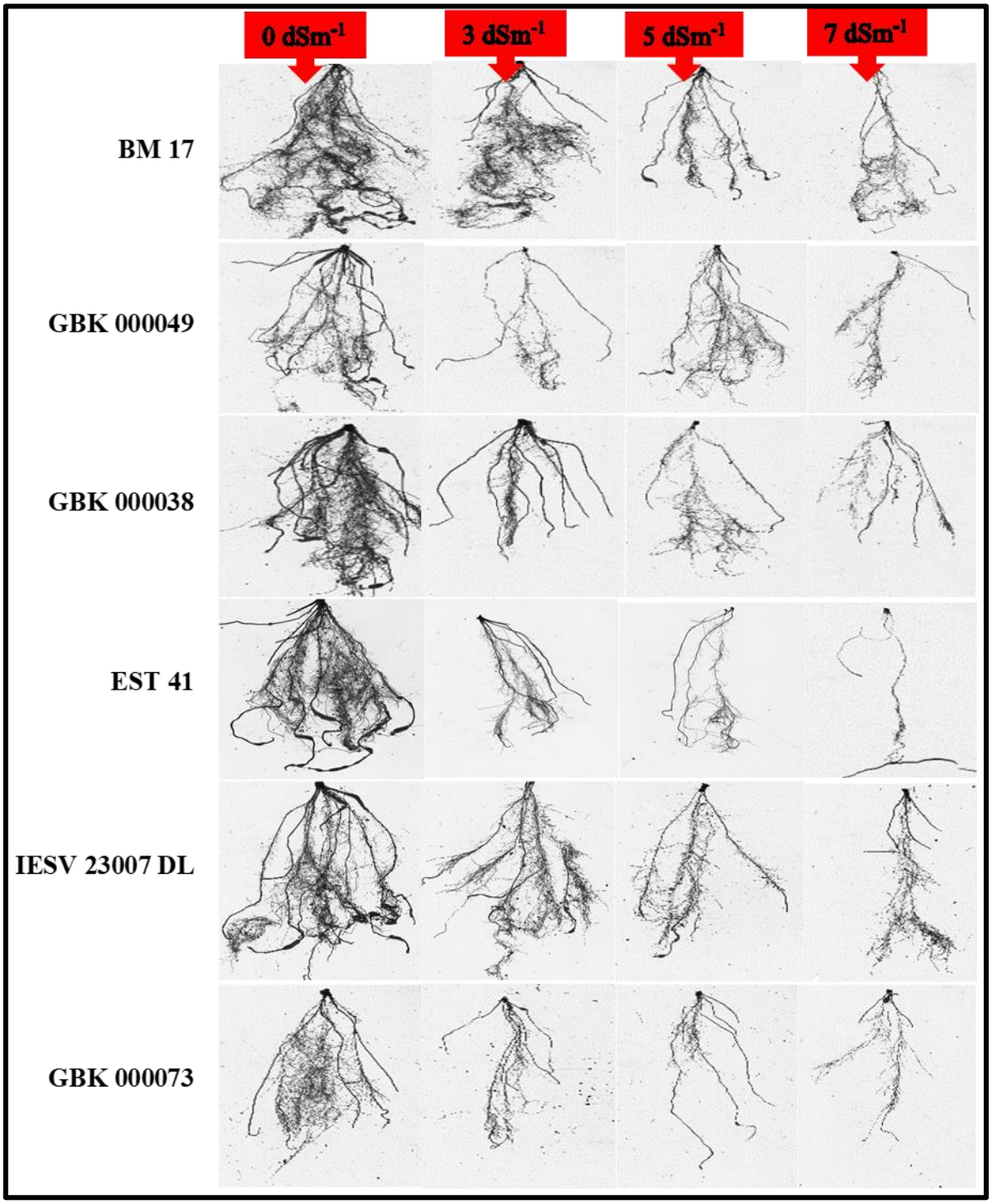
Source of variation	df	Na^+ shoot concentration	Na^+ SRR	K^+ shoot concentration	K^+ root concentration	K^+ shoot to root ratio	Ca^{2+} shoot concentration	Ca^{2+} root concentration	Ca^{2+} shoot to root ratio
NaCl Concentrations	3	0.50***	1.02***	0.00	0.005	0.44	0.003*	0.00	0.001
Genotype	5	0.03	0.07	0.003*	0.00**	1.98***	0.01***	0.00	0.02***
NaCl*Genotype	5	0.02	0.06	0.00	0.00	0.44*	0.00	0.00	0.00
Error		0.78	2.65	0.05	0.07	12.61	0.04	0.05	0.16
R2		0.72	0.62	0.46	0.46	0.59	0.57	0.23	0.51
CV		30.97	39.35	17.41	26.27	35.46	4.58	4.64	6.00

***, **, *, significant at P≤0.001, P≤0.01 and P≤0.05 respectively; CV= Coefficient of variation; d.f= degree of freedom

Appendix F: Visual effects of salt injury on the shoots of salt tolerant and salt sensitive sorghum genotypes.



Appendix G: Visual effects of salt injury on the roots of salt tolerant and salt sensitive sorghum genotypes



Appendix H: Journal Article

Vol. 18(12), pp. 1102-1109, December, 2022
DOI: 10.5897/AJAR2022.16168
Article Number: 6F48B1F70108
ISSN: 1991-637X

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Effects of sodium chloride on seed germination and seedling establishment of sorghum genotypes

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Received 5 August, 2022; Accepted 30 September, 2022

Salinity and climate change are major threats that affect crop productivity in arid and semi-arid fields globally. Sorghum is a climate smart crop but wide range of sorghum genotypes grown is sensitive to salt. Sorghum was screened for salt tolerance using sodium chloride (NaCl) at different concentrations. There were 250 evaluated sorghum genotypes using factorial arrangement in a Completely Randomized Design (CRD) with 4 levels of NaCl concentrations (0, 3, 5 and 7) dSm^{-1} and three replications. Germinated seeds were determined on the 4th day after planting and transferred to nutrient saturated plastic cups in a greenhouse. Data on root hair numbers, root and shoot length were taken on the 6th day after transplanting. Data were subjected to R4.2.0 for ANOVA and SPSS V. 20.0 for cluster analysis. There were significant differences in root hair numbers, root length and shoot length among the genotypes and salt levels (P ≤0.001). Pearson correlation coefficient showed a high positive correlation (P ≤ 0.001) between root length and root hair numbers. The results revealed presence of tolerance among local sorghum genotypes with promise for use in crop improvement.

Key words: Climate change, salinity, tolerance, root length, root hairs.

Appendix I: Research Permit

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