# SCREENING FOR SALT STRESS TOLERANCE, *IN VITRO* REGENERABILITY AND RELATIVE GROWTH AMONG SELECTED KENYAN SWEETPOTATO *Ipomoea batatas L. Lam* GENOTYPES

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# DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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## DEDICATION

I dedicate this work to my late parents Mr Omar Makenzi and Mrs. Zainab Omar.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **ABA** | Abscisic acid |
| **AOS** | Active oxygen species |
| **APX** | Ascorbate perioxidase |
| **ASAL** | Arid and semi-arid areas |
| **CAT** | Catalase |
| **CBP** | Cytokinin- binding protein |
| **CIP** | International Potato Center |
| **DAP** | Days after planting |
| **DMSO** | Dimethyl Sulphoxide |
| **DNA** | Deoxyribose Nucleic acid |
| **dS/M** | Deci semions per meter |
| **EC** | Electrical conductivity |
| **$GA_3$** | Gibberellic acid |
| **GDP** | Gross domestic product |
| **GPX** | Glutathione peroxidase |
| **GR** | Glutathione reductase |
| **HKT** | High Affinity Potassium transporters |
| **IBA** | Isobutyric acid |
| **KARLO** | Kenya Agricultural Research and Livestock Organization |
| **Kj** | Kilo joules |
| **LEA** | Late Embryogenesis Abundant |
| **MAS** | Marker assisted selection |
| **NAA** | Naphthalenic acetic acid |
| **NADPH** | Nicotinamide Hydrogen Phosphate |
| **NHX** | Sodium transporters |
| **OFSP** | Orange fleshed sweetpotato |
| **PEG** | Polyethylene glycol |
| **ROS** | Reactive oxygen species |
| **RWC** | Relative Water Content |

| | |
|---|---|
| **SOD** | Superoxide Dismutase |
| **SOS** | Salt Overly Sensitive |
| **SSA** | Sub Saharan Africa |
| **TDZ** | Thidiazuron |

## ABSTRACT

Salinity affects about 40% of the global area mainly the arid and semi-arid regions. In Kenya the ASALs cover approximately 80% of the total area where agricultural production constraints include water scarcity, salinity and sodicity. Sweetpotato *Ipomoea batatas* L. (Lam.) is the third most economically important root crop after potato and cassava in the world cultivated for human consumption, animal feed and industrial uses. The production of sweetpotato by smallholder farmers in the ASALs is affected by abiotic stresses including salinity. However, data on levels of salinity stress tolerance among Kenyan sweetpotato genotypes is limited. The objective of the study was to determine physiological response of sweetpotato to salinity stress and assess *in vitro* regeneration among selected Kenyan sweetpotato genotypes. Fifteen Kenyan selected sweetpotato genotypes *Ksp 36, Ksp 20, Ksp28, Kemb 36, Kemb 10, Kemb 23, Kalamb Nyerere, Mweu Mutheki, Enaironi, Mugande, Zambezi, Spk 004, Spk 013, Spk203* and *Jewel* were used for the present study. *In vitro* shoot organogenesis using TDZ was used in detrmining regenrability while sudden shock treatment and an incremental stress regime were used for studying the physiological resposes of sweetpotato genotypes to osmotic and salinity stresses. Physiological responses was assessed by measuring the leaf photosynthetic pigment content, vine and leaf length, relative water content and yield. All data collected were analyzed using ANOVA at 95 % confidence interval using SAS statistical software. Mean separation was carried out using pairwise comparison test at 5 % probability level. Results shows that the highest number of adventitious bud; 8.00 (*Kalamb nyerere)* was produced in the dark at 0.25 mg/l TDZ hormone level. Regeneration frequencies of adventitious buds recovered in the dark was the highest, 83.20% (*Jewel*) at 0.10 mg/l NAA hormone level. The best genotypes for direct shoot organogenesis were *Kalamb nyerere, Kemb 36* and *Spk 004*. Growth analysis shows that the sweetpotato genotypes with the highest mean growth rates were *Kalamb Nyerere, Spk203, Enaironi,* and *Mweu Mutheki*. Results show that at high *in vitro* osmotic and salinity stresses all genotypes had reduced amount of photosynthetic pigments. Best performing genotypes under *in vitro* osmotic and salinity stress were *Ksp 36, Ksp 28* and *Zambezi.* Results of *in vivo* salinity stress shows that all the genotypes had reduced vine length except *Spk 013, Spk 203* and *Kemb 23*. Yield was negatively affected by *in vivo* salinity stress but was lowest in *Spk004* (-31.13%), Mweu Mutheki (-31.43%) and *Ksp 36* (-35.29%). Using the combined morphophysological approach the following genotypes were found to be salt tolerant *Spk 004, Mweu Mutheki, Ksp 36, Kemb 36* and *Kalamb Nyerere* and can be incooperated in breeding programs so as to introgress tolerance to sensitive genotypes.

# CHAPTER ONE

# INTRODUCTION

## 1.1 SWEETPOTATO PRODUCTION

Sweetpotato *Ipomoea batatas* L. (Lam.), also known variously as batata, boniato, camote (Spanish); kumara (Polynesian) and viazi vitamu (Kiswahili) is a harbatious plant grown for its edible roots in the tropics sub tropics and temperate regions of the world. Sweetpotato and its wild relatives are members of the family Convolvulaceae whose genetic composition is hexaploid, with a basic chromosome number X=15 while the wild *Ipomoea* species can be diploid through hexaploid. The *Ipomoea* genus includes 600-700 species of which sweetpotato is the only cultivated (Huaman and Zhang, 1997).

*Ipomoea batatas* is a self-incompatible species that is pollinated by insects. Self incompatibility barriers including saprophytic incompatibility and partial sterility are the major constraints facing the propagation of *Ipomoea* landraces. In Kenya, for example, less than 10% of small holder farmers had access to improved seed sweetpotato varieties in 2001 (Roy-Macauley, 2003). Indeed both macro (stem cuttings) and micro vegetative propapagation techniques such as meristem and shoot tip culture have now become a proposed method for reproduction, storage and distribution of sweetpotato germplasm (Panta *et al*., 2007).

Sweetpotato is a tropical perennial crop, cultivated as an annual crop in temperate climates. It is grown in more than 100 countries worldwide. Sub-Saharan Africa produces more than 11 million tons of sweetpotatoes annually, which constitutes 5% of global production whose average yield is below that of the world

(FAOSTAT, 2012). Sweetpotato is a staple food in Kenya, grown primarily by resource poor smallholder farmers in marginal lands, where about half of the harvest is kept for home use. Low yields partly result from mono-cropping, lack of improved planting material and biotic stress. Besides, water, salinity stress or drought stress are the most important abiotic stresses that limits sweetpotato crop production in Africa (Rashid *et al*., 2013).

The arid areas and semi-arid (ASAL) or drylands cover approximately 80% of the total area in Kenya. The major agricultural production constraints in the drylands include rainfall scarcity, salinity and sodicity (Wanjogu *et al*., 2004; Ndegwa and Kiiru, 2009). Water scarcity in the ASALs necessitated the use of irrigation to increase the cropping fields thereby incresing food production in the country (Kaluli *et al.*, 2011). On the basis of surface water availability, Kenya has irrigation potential of 539,000 hectares and 600,000 hectares drainage or flood protection potential. However, currently only 110,000 hectares (20%) of irrigation and 30,000 hectares (5%) of drainage area have been developed (ROK, 2009).

Agriculture is importance in Kenya because it contributes 55% of the GDP, 80% of employment and 60% of exports and generates about 40% of government revenue (Blank *et al.,* 2002). On the other hand, irrigation directly contributes 3% of the total GDP and provides 18% of the value of all agricultural produces (ROK, 2009).

The ASAL areas having saline sodic soils originating from natural, geological, hydrological and pedological processes that occupy about 40% of Kenya's land surface aproximately 25 million hectares. Salinization in Kenya occurred mainly

on young volcanic sediments in areas of high evaporation rates (Wanjogu *et al*., 2004). The type of salts present determines the degree of salinity in a given area which in turn is affected by the soil permeability and climatic conditions.

Irrigation combined with poor drainage leads to ground water table rising therby salts accumulating in the rooting zone of the soil leading to poor production after a period of time (Wanjogu *et al*., 2004). The productivity of the soils in the irrigation schemes becomes low and economically unviable hence are abandoned due to salinisation and sodification problems. Suggested measures includes leaching excess salts with fresh water, husbandry practices to improve the depth of rooting zone and introduction of improved crop species (Wanjogu *et al*., 2004).

Salinity affects plants in number of specific ways depending on the type of plants amount of salt and duration of exposure. Most of the agricultural important crops belong to the glycophytes of plants that have no adaptive mechanisms to survive in varying saline environments. On the other hand halophytes are plants that have adaptive mechanisms that enable them to survive in salty environmental conditions. However, glycophytes share most tolerance mechanisms with halophytes but not well adapted to moderate and high levels of salt, resulting in a greater degree of sensitivity (Moller and Tester, 2007).

Consequently, there is need to develop salt tolerant crop (glycophytes) suitable for the ASAL regions. To this end the identification of salt tolerant crops is the first step in the improvement of genotypes through breeding technologies for introgression of novel salt tolerant traits and transformation with genes that confers either tolerance or resistant mechanism to sweetpotato genotypes. Such

crops with enhanced physiological capabilities to survive in salt and or osmotic stress conditions at the same time ensuring the farmer does not suffer total crop failure are urgently needed to make the country food secure.

*In vitro* culture of plant cells, tissues or organs on a medium containing selective agents offers the opportunity to select and regenerate plants with desirable phenotypes. The technique has been used to induce tolerance by using selective agents that permit the preferential survival and growth of desired phenotypes (Purohit *et al.,* 1998). The selecting agents added to the culture media for *in vitro* selection include NaCl (salt-tolerance) and PEG or mannitol (drought/osmotic tolerance). Only the explants capable of survival in the long run are selected. These methods induces genetic variation known as somaclonal variation among cells, tissues and/or organs in cultured and regenerated plants (Mohamed *et al.,* 2000).

Tissue culture induces variation in regenerated plants, (somaclonal variation), which can result in a range of genetically stable variations useful in crop improvement (Larkin and Scowcroft, 1981). Desirable traits are selected within a considerably shorter time with minimal environmental interaction and can complement field selection (Jain, 2001).

In this study, sweetpotato (*Ipomoea batatas* L. Lam) was selected since it is an autopolyploid showing polysomic inheritance and self-sterility thus propagated vegetatively. Sweetpotato is one of the promising neglected food crops whose performance data in saline environments either *in vitro* or *in vivo* is unavailable. Research therefore needs to be undertaken to identify sweetpotato genotypes for

direct planting in areas with saline soils and improve the food availability to smallholder subsistence farmers in dryland areas affected by salinization.

**1.2 Problem statement and justification**

Soil salinization is a major process of land degradation that causes decreased productivity in many irrigation schemes in the Arid and Semi-Arid Lands (ASALS). Over irrigation, excess water seepage during irrigation periods and lack of sub surface drainage leads to accelerated waterlogging problems and rapid rise of ground water table (Ndegwa and Kiiru, 2009). This leads to salt build-up in the soil profile that affects both recent established and older irrigation schemes as a result of hydrological disturbances where soils not initially saline, with deep water tables becomes saline and or sodic under irrigation. Increased salinity levels in the soil becomes less suitable for salt sensitive crops leading to loss of plant stand, reduced rates of plant growth, reduced yields and even total crop failure (Sijali *et al*., 2003).

Field evaluations of sweetpotao crops developed by MAS approaches are now required to determine the effect of salt stress on crop yield and the best strategy to improve the salinity tolerance of specific sweetpotato genotypes. This far, very few studies have been able to examine the performance of Kenyan sweetpotato genotypes in saline vis a vis non saline environments and identifying effect of modifying salinity tolerance traits on the yield of field grown crop plants, and assess their yield penalty in non-saline soils. Transferring the tolerance mechanisms identified in other plants into crops such as sweetpotato may significantly increase salinity tolerance of sweetpotato.

Modern plant biotechnology is a promising option since it does not suffer from the pitfalls of time suffered by conventional breeding. New sweetpotato genotypes with improved qualities suited to the changing local climate and salinity can be developed within a short period of time. These improved sweetpotato genotypes are needed to increase food production to meet the projected food demands to feed the increased population and enhance food security to the resource poor families in the rural areas of Kenya.

To this end firstly, data on levels of salinity tolerance among the Kenyan sweetpotato genotypes need to be availed to enable their use in developing new sweetpotato genotypes and secondly resources should be made available to make the research on new genotype development a reality. Lastly, it is recommended that plant breeders use the available technologies like conventional breeding, marker assisted selection and genetic engineering to develop salinity tolerant crops.

**1.3 Null Hypotheses**

i) It is not possible to develope a reproducible *in vitro* regeneration protocol for selected Kenya sweetpotato genotypes (*I. batatas*) through organogenesis.

ii) There are no differences of growth rates in selected sweetpotato genotypes under natural conditions.

iii) Salt and osmotic stresses have no *in vitro* effects on pigment content in selected Kenyan sweetpotato (*I. batatas*) genotypes.

iv) Salt stress has no *in vivo* effect on growth parameters, pigment content and yield of selected sweetpotato (*I. batatas*) genotypes.

## 1.4 Objectives

### 1.4.1 General Objective

To determine salt stress tolerance, *in vitro* regenerability and relative growth among selected Kenyan sweetpotato genotypes

### 1.4.2 Specific Objectives

i) To develop a reproducible *in vitro* regeneration protocol for selected Kenyan sweetpotato genotypes via organogenesis.

ii) To determine the growth rate of selected Kenyan sweetpotato genotype under natural environmental conditions.

iii) To determine the *in vitro* effect of salt and osmotic stresses on pigments of selected Kenyan sweetpotato genotypes.

iv) To determine the *in vivo* effect of salt stress on growth parameters, pigment content and yield of selected Kenyan sweetpotato genotypes.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 SWEETPOTATO (*Ipomoea batatas* L.)

### 2.1.1 Origin and Distribution

Sweetpotato is the English name for *Ipomoea batatas L.* which is also known variously as batata, boniato, camote in Spanish; Kumara in Polynesian and Viazi vitamu in Kiswahili. Sweetpotato is native to the tropical parts of South America, and was domesticated there nearly 5000 years ago. Sweetpotato is cultivated in areas with sufficient water to support its growth as a perennial or an annual crop in tropical or temperate climates respectively in more than 100 countries worldwide (Huaman and Zhang, 1997).

Sweetpotato and its wild relatives are members of the family Convolvulaceae. Within the genus *Ipomoea* are 600-700 species of which sweetpotato is the only one cultivated. Sweetpotato together with 13 wild *Ipomoea* are closely related and belong to the section Batatas (Huaman and Zhang, 1997).

### 2.1.2 Genetics

Sweetpotato is an autopoliploid derived from the wild species *Ipomoea trifida* or an allopolyploid involving *Ipomoea trifida* (diploid) and an unidentified tetraploid parent resulting in an hexaploid, whose basic chromosome number is x=15 (Collins and Qualset, 1998). Sweetpotato (*Ipomoea batatas* L: 2n= 6x= 90) and its close relatives are annuals, perennials, autogamous and polyploidy out-crossing plants.

For ease of identification, Sweetpotato species are classified into three sub groups, namely A, B and X. The members in the A- sub group are self-

incompatible and cross-compatible with each other and include *Ipomoea triloba* and closely related species such as *I. lacunose, I. trichocarpa* and *I. Ramoni* (Nishiyama, 1982).

The B-group comprises species that are self-incompatible but cross-compatible with one another and include *I. batatas, I. littorals* and *I. trifida*. Unlike the A and B subgroups, the X- sub group comprises of tetraploid species; *I. tiliacea* and *I.gracilis*, which are self-incompatible but are cross-compatible with one another.

In terms of cross compatibility of the three sub groups; the B-sub group is cross-incompatible with A- and X- sub groups whereas the A- sub group is cross-compatible with the X-sub group using the latter as pollen parent. In the natural environment cross-pollination is performed by insects such as honey bees, bumble bees and butterflies (Austin, 1988).

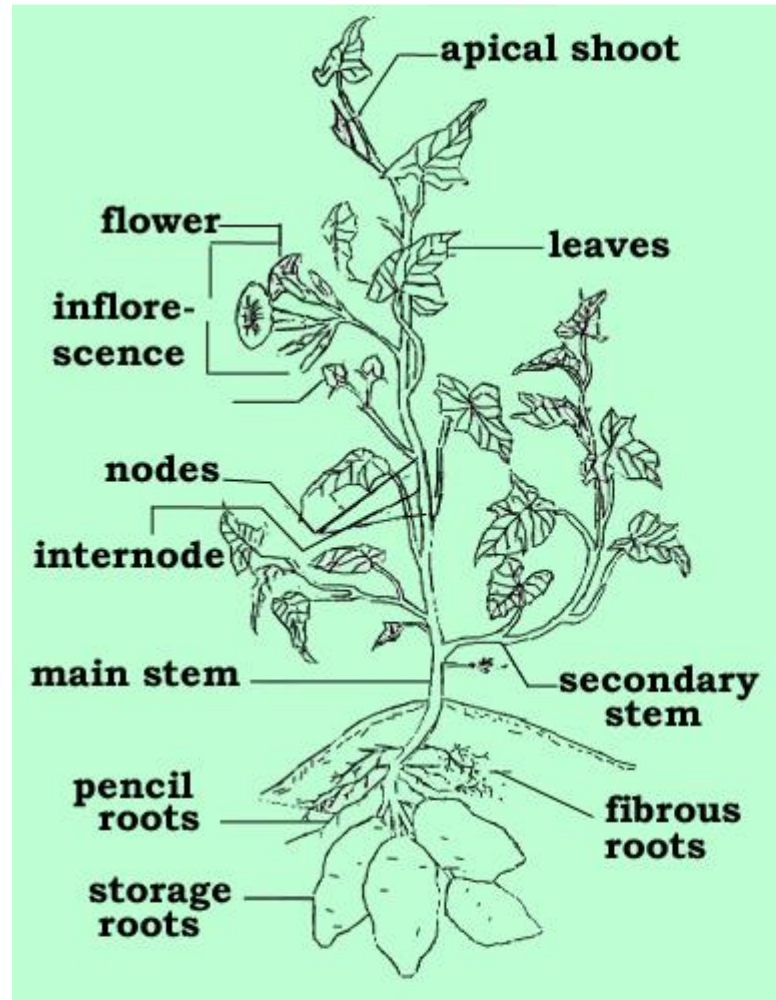


**Figure 2.1: Sketch of a sweetpotato plant. Source: CIP**

### 2.1.3 Cultivation

In general sweetpotato is a perennial crop, but managed agriculturally as an annual with a growing period varying from 3 to 8 months depending on the environmental conditions and genotype. The edible root is long and covered with a smooth skin which ranges in colour from red, purple, brown to white. Its flesh root colour ranges in gradations from white, yellow, orange to purple. Sweetpotato is a good source of energy more than wheat (*Triticum aestivum L*), rice (*Oryza sativa L.*) or cassava (*Manihot esculenta* Crantz) and is able to grow

in arid conditions with limited water supply once it is established (Bovell-Benjamin, 2007).

Most of the sweetpotato varieties grown in Africa are diverse clones selected by farmers on the basis of adaptation and taste. Accessibility to improved planting material still remains a problem in most African countries (Roy-Macauley, 2003). In Kenya, for example, only 7% of the smallholder farmers had access to improved crops such as sweetpotatoes in 2001 (Roy-Macauley, 2003). Cultivation of sweetpotato is considered to be environmentally friendly because of the low nitrogen inputs required and its ability as a fast-growing crop to cover the land and prevent erosion as well as ability to grow under shady conditions (Jayasinghe *et al*., 2003).

### 2.1.4 Production

Sweetpotato is the third most economically important root crop after potato and cassava with global production of more than 100 million metric tons per year (FAOSTAT, 2012). Asia is the world's largest sweetpotato producing region, with 85 million metric tons/year; China with 77 million metric tons per year (Figure 2.3) accounting for 70% of world production.

African farmers produced about 18 million metric tons per year accounting for approximately 17% of world production (Figure 2.2). However, Africa's sweetpotato yield of six tons per hectare is below the global average yield of fourteen tons per hectares (FAOSTAT, 2012). Africa's top producers of sweetpotatoes are: Nigeria, the second largest producer in the world (3.4 million metric tons); Tanzania (3.0 million metric tons); Uganda (2.6 million metric tons);

and Ethiopia (1.1 million metric tons) (FAOSTAT, 2012). (Table 2.1)

**Table 2.1: World sweetpotato production 2012, ranking of selected countries and regions in the world based on the quantity of crop produced.**
Source: (Faostat, 2012)

| Area | Production (MT) |
|---|---|
| **Africa** | 18,318,588 |
| **Angola** | 644,854 |
| **Asia** | 85,304,354 |
| **Burundi** | 659,593 |
| **China, mainland** | 77,375,000 |
| **East Africa** | 11,600,056 |
| **Ethiopia** | 1,185,050 |
| **Kenya** | 859,549 |
| **Madagascar** | 1,144,000 |
| **Mozambique** | 900,000 |
| **Nigeria** | 3,400,000 |
| **Rwanda** | 1,005,305 |
| **Uganda** | 2,645,700 |
| **Utd Republic of Tanzania** | 3,018,175 |
| **West Africa** | 4,638,664 |
| **World** | 108,004,174 |

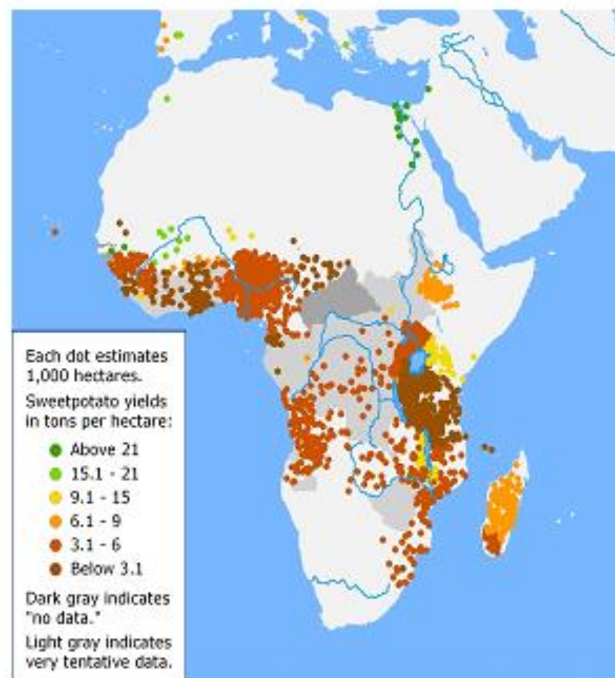


**Figure 2.2: Sweetpotato production areas in Africa.**
Source: International Potato Center: World Sweetpotato Atlas

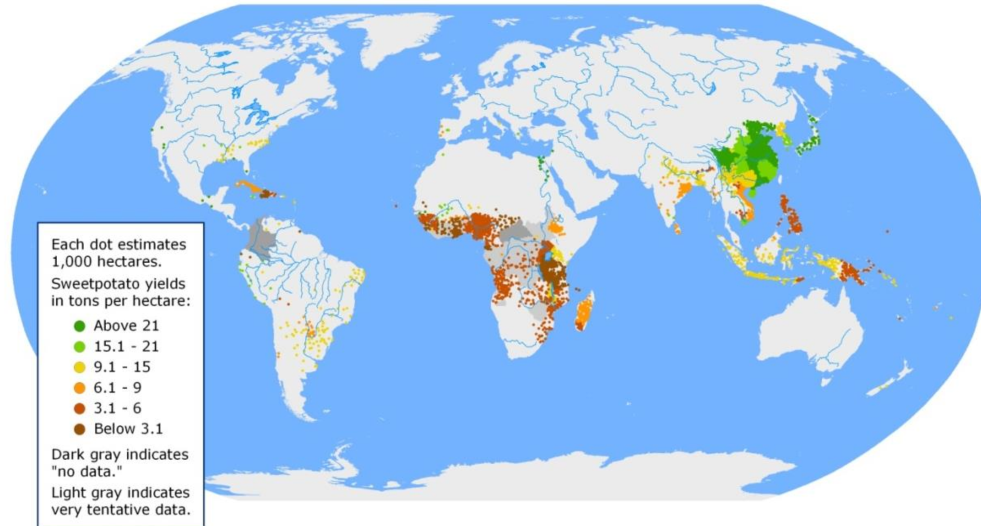


**Figure 2.3: World sweetpotato production. Areas of cultivation and average yields**
Source: International Potato Center: World Sweetpotato Atlas

### 2.1.5 Economic importance

Most of the global sweetpotato is produced in developing countries where it is a staple or alternative style food. In Kenya, the sweetpotato is a staple food grown primarily by resource poor smallholder farmers who keep for home use about half of the harvest. The sweetpotato is a reliable crop because it can grow and stay underground and provides a "larder" of food if other foods are in short supply (Qaim, 1999).

Sweetpotato is cultivated primarily for the enlarged edible storage roots which provide high amounts of starch, and in some countries tender leaves are consumed as vegetable. The vegetative parts of sweetpotato are commonly used as feed for cattle, pigs, goat, sheep and rabbit. For this purpose, it is used as a raw material or ingredient in the feed processing industries (Lebot, 2009).

Due to their high anthocyanin content, sweetpotato is used to obtain natural food coloring agents which are superior to synthetics and also for preparation of noodles, pastries and cakes. Sweetpotato also forms an important component of biodegradable plastics and feed stock for the production of biofuels (Santa-Maria *et al*., 2011).

Nutritionally, sweetpotato roots (orange and non-orange fleshed) are a valuable source of vitamins B, C, E, and moderate levels of iron and zinc as well as unique starch properties of interest to the food industry; vine tops have excellent micronutrient contents and adequate protein content (3–4%; quantity and quality) for use as feed or food. The orange-fleshed sweetpotato varieties (OFSP) have high levels of beta carotene, the precursor to vitamin A, in the roots. The high flavonoid content in the purple fleshed varieties have strong antioxidant properties important in reducing cardiovascular diseases (Yamakawa and Yoshimoto, 2001; Yang *et al*., 2004). Recent research in Japan indicates that consumption of sweetpotatoes can suppress stomach diseases (Yoshimoto *et al*., 2001).

## 2.2 Role of roots in plants

Plant roots perform many important functions such as water and nutrient uptake, storage of reserves, synthesis of specific compounds such as cytokinins, anchorage to the soil, and the establishment of biotic interactions in the environment (Lo´pez-Bucio *et al.,* 2003). The development of plant rooting systems architecture is an important agronomic trait that correlates to the resistance to water stress in several crop plants such as maize (Tuberosa *et al.,* 2002).

Rooting depth is one of the most important traits required for optimal plant function under low water availability conditions and account for the differences in drought resistance among genotypes (Sayar *et al.,* 2007). Deep rooting system in any cultivar is attractive to breeders because it permits such plants to extract soil moisture at greater depths, important when the unwetted soil layer is thicker than the maximum root depth (Zuo *et al.,* 2004).

### 2.2.1 Breeding for root traits

Most studies on organ size control in agricultural crop investigate growth of aerial organs with minimal consideration of the roots. But, the size and the architecture of the root system determine the plant's ability to access water and nutrients in many agricultural ecosystems. Plants with larger and deeper rooting systems are able to compete for nutrients and to survive under conditions of nutrient deficiency. Since nutrients and water shortages are the major problems limiting agriculture, the knowledge of root system architecture is increasingly important for the manipulation and optimization of the same in new crop varieties (Coque and Gallais, 2006).

Irrespective of their importance in salt resistance, relatively little progress has been made in breeding for root traits because of the difficulty in screening large numbers of plants. However, there is a correlation between plant height and DRL (Bchini *et al.,* 2010) as well as an overlap between quantitative trait loci determining root features and those for plant productivity that can be manipulated in the development of salt tolerant genotypes (de Dorlodot *et al.,* 2007). To this end, breeding efforts have focused on obtaining genotypes with larger root

systems for example; drought-resistant rice (*Oryza sativa*) varieties have a deeper and more highly branched root system than drought-sensitive varieties (Price *et al.,* 1997). The same approaches can be used to develop sweetpotato crops resistance to salt stress.

### 2.3 Salt stress

Stress is defined as mechanical force acting on a unit area of an object leading to a change in dimensions referred to as strain. Form the biological stand point stress can be defined as an adverse force or a condition, which inhibits the normal functioning and well-being of biological systems such as plants (Mahajan and Tuteja, 2005). Salt stress on the other hand can be defined as a condition in which salt concentration is high enough to make the water potential in the plant excessively negative leading to osmotic stress and ultimately ion toxicity. Salinity can also be defined as the excess of ions of soluble salts in soil that affect plant growth and development (Lewis, 1984). Soil salinity is measured by its electrical conductivity whose SI unit (EC) is dS/m. Saline soils having an electrical conductivity of above 4 dS/m are categorized into saline, sodic and saline sodic soils depending on EC, soil pH, exchangeable sodium percentage and soil physical condition (Etehadnia, 2009).

#### 2.3.1 Categories of soil salinity

Salinity can be categorized as primary or secondary where primary salinity refers to accumulation of soluble salts through natural phenomena such as weathering of rocks containing salts and deposition of salts from oceans carried by wind and rain (Manchanda and Garg, 2008). Secondary salinity refers to the accumulation

of soluble salt in the soil, due to a raised water table and anthropogenic practices such as agriculture (Manchanda and Garg, 2008).

Soil salinity can further be subdivided into irrigated land salinity or dry land salinity. Irrigated land salinity occurs when salt-contaminated ground water rises leading to accumulation of soluble salt in crop fields. Some of the causes of salinity in irrigated farm lands include inefficient water use, poor drainage deposition of salts in water channels, drains and water stores. Dry land salinity mainly occurs in the arid regions through evapotranspiration, making the land unsuitable for agricultural purposes (Bauder *et al.,* 2011).

### 2.3.2 Salinity problems in the world

Over 800 million hectares of land are affected by high levels of salinity worldwide accounting for more than 6% of the total land area (Table 2.2). Nearly 40% of the world's land surface confined to the tropics and Mediterranean region, can be categorized as having potential salinity problems (FAO, 2008). In general the world over, there is a strong negative correlation between agricultural production and soil salinity. Salinity limitation on crop productivity and production worldwide and is expected to worsen over the next 30-50 years (Tuteja, 2007). The high demand for agricultural based products to feed the increasing global population can be met by increasing productivity from cultivated land and the use of marginal land for agricultural production. Agricultural productivity in salt affected environments can be potentially raised by growing crops with high tolerance to salt stress (O'Leary, 1995).

**Table 2.2: World variation in salinity levels (million hectares)**

| Regions/Country | Total area | Saline soils | | Sodic soils | |
|---|---|---|---|---|---|
| | **Mha** | Mha | % | Mha | % |
| **Africa** | 1841.5 | 34.29 | 1.92 | 33.05 | 1.79 |
| **Kenya** | 57.6 | 4.41 | 0.08 | 0.45 | 0.01 |
| **Asia and the pacific and Australia** | 3107.2 | 195.1 | 6.3 | 248.6 | 8 |
| **Europe** | 2010.8 | 6.7 | 0.3 | 72.7 | 3.6 |
| **Latin America** | 2038.6 | 60.5 | 3 | 50.9 | 2.5 |
| **Near East** | 1801.9 | 91.5 | 5.1 | 14.1 | 0.8 |
| **North America** | 1923.7 | 4.6 | 0.2 | 14.5 | 0.8 |
| **Total** | 12781.3 | 397.1 | 3.10% | 434.3 | 3.40% |

Source: FAO Land and plant nutrition service, 2008

### 2.3.3 Arid lands and salinity in Kenya

Kenya has a total of 582,646 sq km, approximately 60 million ha, of which 4.5 million are arable and with permanent crops. About 80% of the land constitutes the semi-arid and arid areas (ASAL). The major agricultural production constraints in the ASALS include availability of water, salinity and sodicity (Figure 2.4). The ASALs have salinity and sodicity problems formed under the prevailing climatic conditions and due to high rates of evapotranspiration. The areas are located in Eastern, Northern, North Eastern (NE), North Western (NW), Coastal and Rift Valley (RV) regions of the country comprising of about 40% or about 47 million ha (Table 2.5).

Data available indicate that 10% of Kenya uplands covering about 6 million hectares and about 25% of plateaus and plains covering approximately 16 million hectares have saline and sodic soils. Floodplains, swamps and badlands with saline and sodic soil account for 5% with a coverage of 3 million hectares.

### 2.3.4 Irrigation and soil salinity

Arid environments of Kenya have largely been used for pastoralism and partly for subsistence arable agriculture with a high production risk due to variation in annual precipitation. Kenya's potential for irrigation is estimated to be between 200,000 ha and 500,000 ha but the actual land under irrigation in Kenya is estimated to be about 84,000 ha (Ngigi, 2002).

Most irrigation schemes are located in the arid environments where the evapotranspiration rates are very high. The pressure exerted on the fragile ecosystems such as the ASAL with the introduction of irrigation with poor quality

water and unsuitable soils, has seen an increase on salinization and sodification in the soils. According to Mugwanja et al (1995), about 26,000ha is considered salt degraded hence cannot be used for any agricultural production. Many irrigation schemes in Kenya have been abandoned after less than twenty years of project establishment mainly due to salinization and sodification problems (Wakindiki, 1993).

Reports indicate that there is a relationship between irrigation water salinity and soil salinity (Tedeschi and Menenti, 2002; Anikwe *et al*., 2002). In fact, Nikos *et al.* (2002) proved that the salinity of soil water is approximately three times that of irrigation water assuming relatively or no leaching occurring. Sijali *et al*. (2003) also found out that, soil initially not saline and with deep water tables became saline and or sodic by hydrological disturbance caused by excessive irrigation and changes in land use.

However, it is worth noting that the effect of salinity varies depending on the type of salts present, soil permeability, climatic conditions, and the kind of crops grown. Indeed, these problems occurs as an influence of ground water table rising after irrigation, poor production after a period of time and above all lack of appropriate crops suitable for saline agriculture.

**Table 2.5: Physiographic regions of Kenya**

| Zone | Appr. Area (km2) | % Total |
|---|---|---|
| **I. Agro-Alphine** | 800 | 0.1 |
| **II. High Potential** | 53,000 | 9.3 |
| **III. Medium Potential** | 53,000 | 9.3 |
| **IV. Semi-Arid** | 48,200 | 8.5 |
| **V. Arid** | 300,000 | 52.9 |
| **VI. Very arid** | 112,000 | 19.8 |
| **Rest (waters etc)** | 15600 | 2.6 |

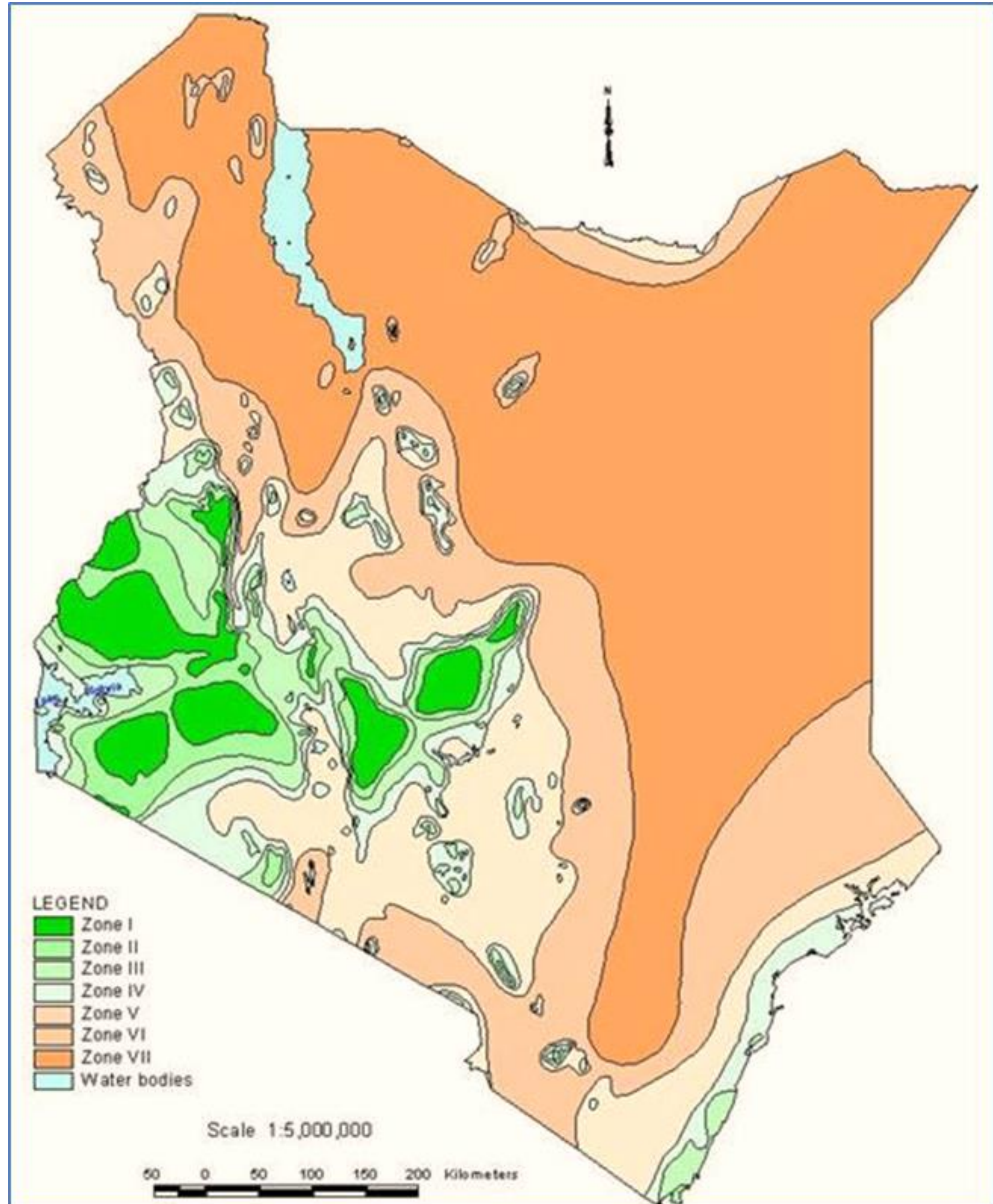


***Figure 2.4: Agroclimatic-zones of Kenya:***
Zone I Zone II: High potential;
Zone III Medium Potential;
Zone IV Semi-arid;
Zone V Arid; Zone
VI very arid and Water bodies.

All irrigation waters irrespective of their sources including those considered of very good quality, contain some dissolved salts. In fact, salts are a common and necessary component of soil, and many salts (for example nitrates and potassium) are essential plant nutrients. The soils contain salts that originate from mineral weathering, inorganic fertilizers, agricultural practices such as soil amendments, and irrigation waters. Soil salinization is dramatically increased and accelerated by crop irrigation importing large quantities of new salts to the soil that were not there before (Kotuby-Amacher *et al.,* 2000).

The irrigation water contains calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), and sodium ($Na^{+}$) of which upon evaporation, $Ca^{2+}$ and $Mg^{2+}$ precipitates into carbonates, leaving large amounts of $Na^{+}$ in the soil. The resulting high concentrations of $Na^{+}$ in the soil solution may depress nutrient-ion activities due to imbalances in ratios of $Na^{+}/Ca^{2+}$ or $Na^{+}/K^{+}$ (Grattan and Grieve, 1998). Increases in cations and their salts in the soil generate external osmotic potential that can prevent or reduce the influx of water into the root. The resulting water deficit from high salt ratio imbalances is similar to drought conditions compounded by the presence of $Na^{+}$ ions (Oh *et al.,* 2010).

### 2.3.5 Crosstalk between water and salt stress

#### 2.3.5.1 Low water potential

Salinity and drought stress show similarity in terms of physiological, biochemical, molecular and genetic effects on plants (Sairam and Tyagi, 2004). When soluble salt amounts in the soil solution are higher it leads to physiological drought that limit water uptake by plants due to low water potential. The total amount of water available as results of salinity or drought differs due to the mechanism that brings about the low water potential (Carrow and Duncan, 1998).

During drought, ever decreasing soil water potential is established creating a minimal amount of water available in the soil profile for plants. In contrast for saline environments, a large amount of water is available but under low water potential. As such, plants may adjust their osmotic potential, to prevent loss of turgor and generate a lower water potential to access water in the soil solution for growth. From the above, both stresses lead to cellular dehydration, causing osmotic stress and removal of water from the cytoplasm into the intracellular space hence reducing the cytosolic and vacuolar volumes (Taiz and Zeiger, 2006).

#### 2.3.5.2 Reduction in photosynthetic pigments

At the molecular level, water and salt stresses accelerate the production of active oxygen species (AOS) in plants that cause damage to membrane systems and other cellular processes (Mittler *et al.,* 2004). Again salinity and drought cause a deterioration of thylakoid membranes and reduction in contents of important photosynthetic pigments such as chlorophyll (Chl) and Carotenoids (Car) (Anjum *et al.,* 2011). Interference with photosynthetic membranes and pigments results in

the inhibition of membrane-associated electron carriers and enzymes activities, which ultimately leads to a reduced rate of photosynthesis and capacity in most plants (Rexroth *et al.,* 2011). Specifically the reduction of Chl b is greater than that of Chl a, thus, transforming the ratio in favor of Chl a in crops such as wheat and brassica. This reduction occurs due to impairment in pigment biosynthetic pathways or in pigment degradation which varies according to the species, variety, duration of plant exposure, and tolerance to the stress (Jain *et al.,* 2010).

### 2.3.6 ROS production and elimination

Normally, different cellular reactions catalysed by various enzymes such as lipoxygenase, peroxidase, NADPH oxidase and xanthine oxidase produce reactive oxygen species (ROS) (Blokhina *et al.,* 2003). Thus, plants have evolved mechanisms to maintain at equilibrium the production and the elimination of free radicals including the production of antioxidants such as ascorbic acid, glutathione, tocopherols, phenolic compounds as well as ROS-interacting enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Blokhina *et al.,* 2003).

Salinity stress may results in oxidative stress that may exceed the scavenging capacity of the natural defense system of the plant. Plants under salt stress therefore generate increased amount of ROS such as hydrogen peroxide ($H_2O_2$) and superoxide radicles ($O_2^{2-}$) that cause oxidative reactions (Lin *et al.,* 2006). However, serious stress condition leads to accumulation of ROS because of the breakdown of the scavenging and repairing mechanisms within the cell (Lin *et al.,* 2006). The imbalance between the production of reactive oxygen species (ROS)

and their elimination leads to the destruction of lipids, carbohydrates and proteins of cell membrane and cell nucleic acids. It may also leads to the reduction of photosynthetic ability and growth of the plant under salt environment (Blokhina *et al.,* 2003). Therefore, efficient defense mechanism against ROS to survive severe salt and osmotic stress is a prerequisite for a crop genotype to adapt to salinity condition.

**2.4 Salinity stress and plant development**

**2.4.1 Impacts of High Salt on Plants Growth**

Salinity brings about osmotic disruption, specific-ion toxicity and/or nutritional disorders in the plant (Läuchli and Epstein, 1990). High salt concentration in the soil affects plants in two ways such that it upset the ability of roots to extract water, and high concentrations of salts within the plant itself can be toxic, resulting in an inhibition of nutrient uptake and assimilation (Munns and Tester, 2008).

Munns (1993) suggested that plant growth under salinity is inhibited through two phases. Initially (phase 1), growth is affected because of cellular responses to the osmotic effects outside the roots. In the subsequent phase (phase 2), growth is reduced due to the toxic effects of accumulated salts leading to death of old leaves in the sensitive plant and reduce the photosynthetic capacity of the plant.

In phase 1, the soil water around the roots containing high salt concentration reach a critical level making it difficult for the roots to absorb water affecting the shoot growth rate. This effect blocks the ion flux to the shoot and promotes stomatal closure. However this strategy is untenable for long-term salt tolerance

(Hasegawa *et al.,* 2000).

Shoot growth is more sensitive to osmotic stress induced by high salt concentration than root growth. Again, the reduction of leaf area and stunted shoots in plants appears to be a result of inhibition of xylem loading of calcium in the root. Indeed, the reduction in the leaf area relative to root growth would decrease the water use by the plant, conserving soil moisture and prevent salt concentration in the soil (Läuchli and Grattan, 2007; Munns and Tester, 2008).

Leaf initiation is not affected by salt stress in some plant species however all other leaf parameters are salt-sensitive processes. Interestingly, roots apical region of plants growing under salinity have been observed to be more vacuolized and lacking typical organization of apical tissue. Additionally, root tips expansion and root cell growth in plants is affected by salt induced inhibition of the uptake of potassium and calcium ions (Larcher *et al.,* 1990).

Phase II results in much slower effect of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves the so called salt-specific effect (Munns, 2002). $Na^+$ accumulation turns out to be toxic especially in old leaves. As a result, the rate at which older leaf die is more than the rate at which new leaves are produced reducing the supply of photosynthate to the plant, affecting the overall carbon balance necessary to sustain growth (Munns and Tester, 2008). Sodium accumulation in photosynthetic tissue affects photosynthetic components (Munns, 2005).

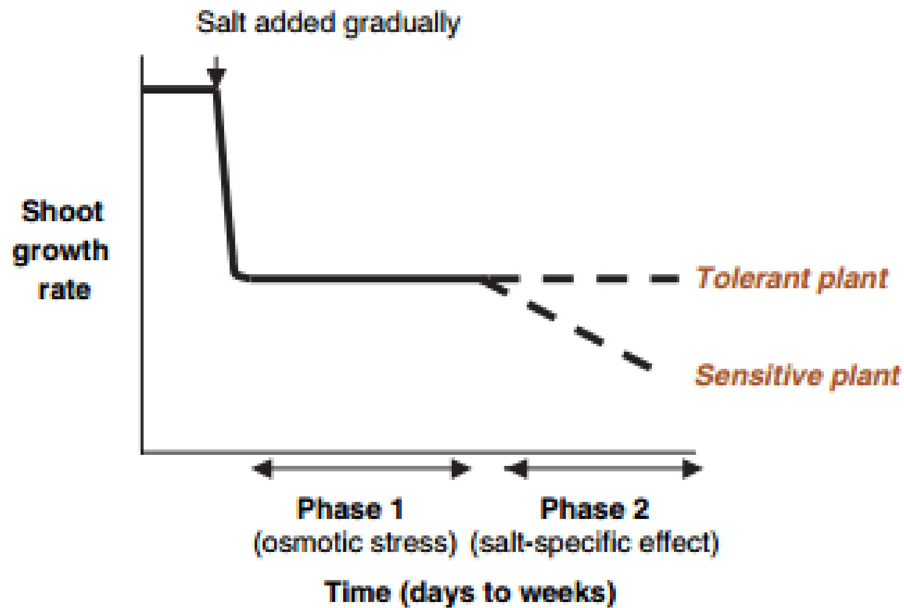


**Figure 2.5: Schematic illustration of the two-phase growth response to salinity for genotypes that differ in the rate at which salt reaches toxic levels in leaves.**
Source; (Munns, 2005)

## 2.5 Mechanisms of salt-tolerance in plants

Plants have evolved over time many biochemical and molecular mechanisms to protect themselves from the detrimental effects of salt-stress. The main biochemical strategies are: (i) induction of antioxidative enzymes, (ii) ion homeostasis and (iii) synthesis of compatible organic solutes.

### 2.5.1 Antioxidant defense system

During salinity induced oxidative stress, a wide range of cytotoxic reactive oxygen species (ROS) are generated in greater proportions in the mitochondria, peroxisomes and cytoplasm. The ROS may lead to the destruction of the normal metabolic reactions through oxidative damage of lipids, proteins and nucleic acids (Turkan and Demiral, 2009). ROS mainly comprises of superoxide radicals ($O^{2-}$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($OH^-$) and singlet oxygen ($1O_2$).

Plant cells have developed complex antioxidant defense system both enzymatic and non-enzymatic to protect themselves against superoxide radicals produced during salt stress (Hossain *et al.,* 2007; Turkan and Demiral, 2009). Tolerant or sensitive plant genotypes have been observed to have differences in expression levels and activity of these defense mechanisms (Munns and Tester, 2008).

Superoxide dismutase (SOD), a metalloenzyme, catalyses the breakdown of superoxide radicals generated during environmental stress to form oxygen and $H_2O_2$ (Parida and Das, 2005). The $H_2O_2$ is removed by the activity of peroxidase and catalase (Hossain et al., 2007) but its accumulation and reactions lead to damage to proteins, DNA and lipid peroxidation. Additionally, $H_2O_2$ is also reduced to water by the enzymatic activity of both ascorbate peroxidase (APX) and catalases (CAT) (Noctor and Foyer, 1998). Glutathione reductase (GR) plays a crucial role in ascorbate–glutathione cycle by maintaining the reduced glutathione/ oxidized glutathione ratio favorable to ascorbate reduction.

### 2.5.2 Ion-homeostasis

Salinity changes the homeostatic ion distribution and water potential within the cell and the whole plant (Tunuturk *et al.,* 2011). Salinity also alters $K^+/Na^+$ ratios resulting in the buildup of $Na^+$ and $Cl^-$ ions that are harmful to plants. This occurs as a result of influx of $Na^+$ through mechanisms involved in the acquisition of $K^+$ions. The required high $K^+/Na^+$ ratio in the cytosol for plant growth under high salinity is maintained by the following strategies: (i) diminishing the entry of $Na^+$ ions into the cells; (ii) extrusion of $Na^+$ ions out of the cell, and (iii) vacuolar compartmentation of $Na^+$ ions.

Salinity tolerance due to ion exclusion mechanism is only restricted to low concentrations of NaCl but not for high concentrations of salts. At high salt concentration the inhibition of key metabolic processes and growth, occurs leading to the death of the plant (Yamaguchi and Blumwald, 2005).

The maintenance of cytosolic $K^+$ balance could be achieved by preventing NaCl-induced $K^+$ efflux from the cell through the use of $H^+$/ATPase making the electron gradient necessary for the ion transport process. Enhanced proton pump on the other hand, provide a driving force to the plasma membrane $Na^+/H^+$ antiporter to expel $Na^+$ out of the cytoplasm into the apoplast and thus reducing cytosolic $Na^+$ load (Turkan and Demiral, 2009). The NHX-type antiporters i.e. $Na^+/H^+$ located in tonoplast are responsible for increased salt-tolerance in many plant species since they favour the $Na^+$ accumulation in vacuole (Leidi *et al.,* 2010).

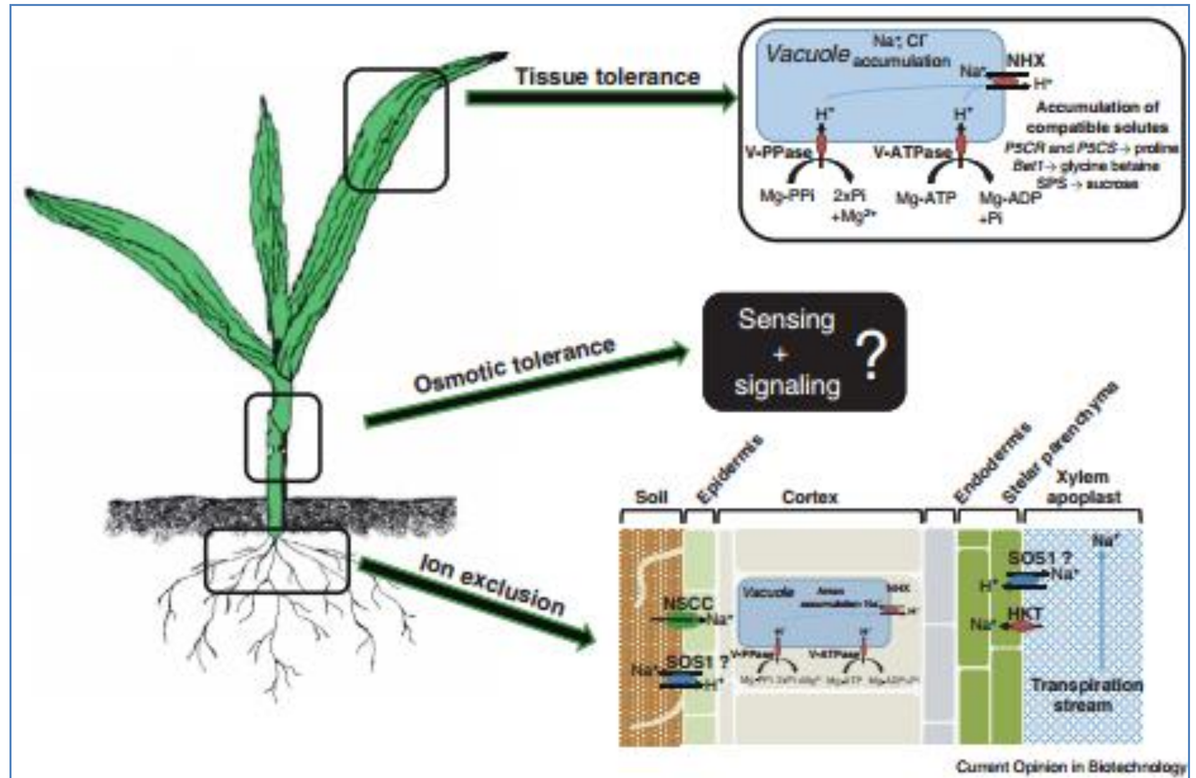


**Figure 2.6: The three main mechanisms of salinity tolerance in a crop plant.**
Source: (Roy *et al*., 2014)

### 2.5.3 Accumulation of compatible solutes

Under salt-stress, plants adjust their osmotic pressure by the synthesis of compatible organic solutes to restrict the uptake of salt. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine, hydroxyprolinebetaine and pipecolatebetaine (Ashraf and Foolad, 2007).

Under salt stress the function of proline accumulation in tissues include osmotic adjustment, carbon and nitrogen reserve for growth after stress resistance, detoxification of excess ammonia, stabilization of membranes, protecting photosynthetic activity and mitochondrial functions, and scavenging of free radicals (Sumithra *et al.,* 2006; Rai *et al.,* 2011).

On the other hand Glycine betaine accumulated by plants under salt stress serves as an osmoregulator. It stabilizes the structure and activities of enzymes and protein complexes and also maintains the integrity of membranes against the damaging effect of salts (Quan *et al.,* 2004; Ashraf and Foolad, 2007).

## 2.6 Salt sensitivity in relation to developmental growth stage

Salinity affects both vegetative and reproductive development depending on whether the harvested organ is a stem, leaf, root, shoot, fruit, fiber or grain. Salinity also reduces shoot growth more than root growth in most species (Läuchli and Epstein, 1990). Since salt-tolerance from an agronomic point of view is based on the yield of the harvestable organ, relative to that in non-stressed environments, understanding how salinity affects vegetative and reproductive development is important for developing management strategies that can minimize stress at critical times.

### 2.6.1 Germination and seedling emergence

#### 2.6.1.1 Germination

Stress due to salinity delays germination leading to the categorization of this developmental stage for most crops as ‘salt tolerant’ (Maas and Poss, 1989). Even though low salinity delays germination, higher salt concentrations have inhibitory effect which eventually reduce the percentage of germinated seeds, (Mauromicale and Licandro, 2002) (Figure 2.6). Interestingly, most crops show enhanced tolerance to salinity during germination but not for others such as sugar beet; a salt tolerant crop but sensitive to salinity at germination (Läuchli and Epstein, 1990).

In contrast salt tolerant barley varieties germinated quickly and recorded higher germination percentage than the more sensitive ones (Tajbakhsh *et al.,* 2006). Surprisingly, salinity is more detrimental to germination of seeds outside their optimal temperature range for germination (Vinizky and Ray, 1988). Regardless, salt tolerance screening in the laboratory at germination provides little basis for assessing crop salt tolerance. While easy to observe germination, such artificially controlled environments cannot mimic that of the field conditions (Esechie *et al.,* 2002).

**2.6.1.2 Emergence**

Severe salinity stress delays emergence and stand establishment (Maas and Grattan, 1999). However, crop tolerance during this growth-stage varies among crops but does not correlate to crop tolerances based on yield-response functions. In addition to salinity, young seedlings near the soil surface in the field are subjected to water stress due to evaporation (Pasternak *et al.,* 1979; Katerji *et al.,* 1994). Indeed, the young salt-stressed seedlings are vulnerable to injury or attack by pathogens (Esechie *et al.,* 2002).

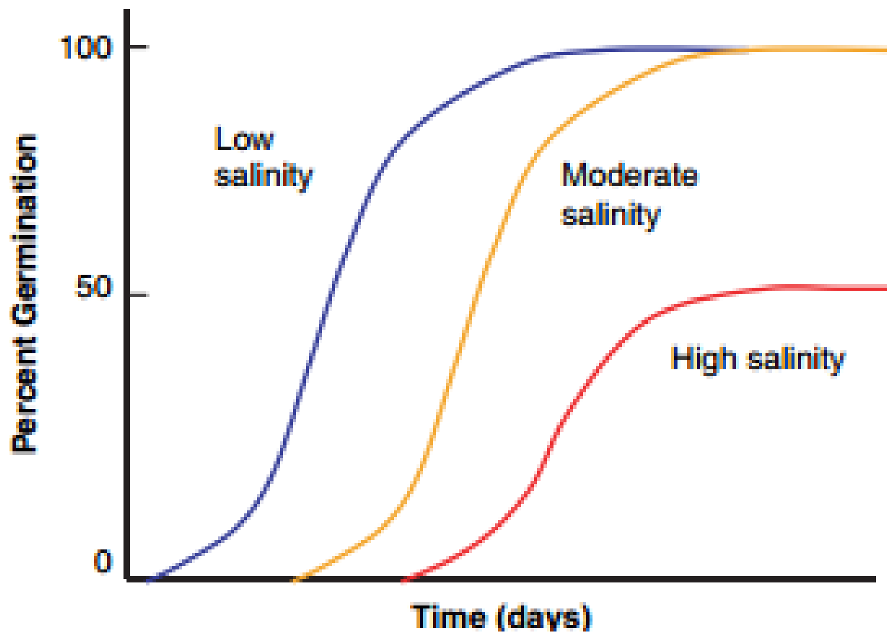


**Figure 2.7: Generalized relationship between percent germination and time after water addition at low, moderate and high salinity.**
Source; (Louchli et al., 2008)

### 2.6.2 Vegetative growth

Early vegetative growth and seedlings are affected by salinity more than seed germination. Total shoot biomass of corn and wheat salt-stressed plants relative to control were much lower than salinity's overall effect on relative grain yield (Maas and Poss, 1989). However, some investigators found that salt tolerance among melon cultivars during early seedling growth correlated with salt tolerance on fruit yield (Nerson and Paris, 1984).

### 2.6.3 Roots

Membrane function and growth of the root are affected by inadequate Ca supply under saline conditions within minutes (Cramer *et al.,* 1991). Reports indicate that increased Ca in salt medium favoured the growth of cotton roots radical whereas the rate of cell production was maintained (Kurth *et al.,* 1986). Interestingly, further research with cotton roots revealed that increased Ca restored cell elongation rate but not the shortening of the growth zone of the root (Zhong and Läuchli, 1993). Indeed, high salt stress increased the amount of Na in the growing region of the root and hence minimizing the selectivity for K versus Na. The conclusion of these studies is that increased Ca in salt medium restored cotton root growth by maintaining plasma membrane selectivity of K over Na (Zhong and Läuchli, 1994).

### 2.6.4 Shoots

Leaf growth and development under salt stress affected the growth zone in sorghum by 20%, and also reduced the maximal relative growth rate, in the youngest region of the leaf. When the external Ca supply was increasing the length of the growing zone of the leaf was restored and relative growth rate increased (Bernstein *et al.,* 1993).

The length of the zone of elongation was not affected when barley leaves were subjected to salt stress (Fricke and Peters, 2002). On the contrary, when sorghum leaves were subjected to salt stress there was a decrease in Ca in the zone of growth. Such reduction resulted in inhibition of leaf growth (Bernstein *et al.,* 1995). From the observations above it was concluded that high Na concentration

in the salt-affected leaf tissue was not the primary cause for growth inhibition (Bernstein *et al,* 1995).

Interestingly, salinity has been shown to reduce the area of proto-and metaxylem in growing leaves of sorghum and wheat, hence decreased amount of water in the growing region of leaves. This could indirectly affect transport of Na and Cl and of nutrient ions to the growing leaves (Baum *et al.,* 2000; Hu *et al.,* 2005).

### 2.6.5 Reproductive growth

In experiments done to investigate the effect of salt stress on wheat (Maas and Poss, 1989) and cowpea (Maas and Poss, 1989), investigators found that the crops were most sensitive during vegetative and early reproductive stages but less sensitive during flowering and seed filling stage. In all these studies similar conclusions regarding growth stage sensitivity were obtained with both determinate crops (the grain crops) and indeterminate (cowpea) crops. If salt stress was applied during the transition between vegetative and reproductive stages, their development is affected significantly.

Salt stress at the reproductive stage, hasten reproductive development, adversely affecting spike development and decrease the yield potential of wheat (Lauchli and Grattan, 2007). While studies on the effect of salt stress on rice varieties found out that it resulted in delayed flowering, a decrease in the number of productive tillers and fertile florets per pinnacle and a reduction in individual grain weight. From the above observations it is clear that salt stress limit the yield of agricultural crops (Lutts *et al.,* 1995; Munns and Tester, 2008).

## 2.7 Plant growth in salt environment

Plants are categorized into glycophytes and halophytes on the basis of their abilities to grow on different salt concentrations. Glycophytes are extremely sensitive whereas halophytes are tolerant to salts in the soil. Glycophytes comprise the majority of plant life and all major crops including important economic cash crops (Flowers *et al.,* 1977).

### 2.7.1 Glycophytes

Glycophytes cannot tolerate salt stress and are extremely sensitive to salt concentrations. Generally all plant processes such as seed germination and photosynthesis are affected by salinity (Malcolm *et al.,* 2003). The majority of glycophytes have salt resistance mechanisms that enables them cope by creating a high $K^+/Na^+$ ratio through active ion transport that is favorable to cytosolic processes (Yadav *et al.,* 2011). The fact that salt accumulates in the reproductive organs and the leaves, survival is the only option to the plant rather than growth or reproduction (Zakharin and Panichkin, 2009).

Research should be done to combat the negative effects of salt stress on glycophytes by developing new crops suitable to increasing soil salinity through natural processes through *in vitro* selection and genetic modification (Zhu, 2001).

### 2.7.2 Halophytes

Halophytes maintain constant potassium concentrations in their tissues through compartmentalization of sodium and accumulation of osmolytes. They can force sodium across the tonoplast with highly Na+/K+ selective protein transporters (Radyukina *et al.,* 2007). Indeed, most halophytes respond to salinity by exclusion, for example mangroves exclude 99% of salts through the roots (Aslams *et al.,* 2011; Yadav *et al.,* 2011). Interestingly, secretion occurs through shedding of salty leaves and specialized cells on the leaves and stems known as salt glands (Aslams *et al.,* 2011).

Similar genes are expressed under salinity stress in both halophytes and glycophytes (Radyukina *et al.,* 2007). Importantly, in halophytes the genes expressed includes the LEA protein, enzymes for biosynthesis of osmolytes, transporters for ions, and regulatory molecules like protein kinases and phosphatases (Aslams *et al.,* 2011). Osmolytes are low molecular weight compounds that help to maintain water potential more negative than the soil to facilitate water uptake (Parida and Das, 2005).

Additionally, halophytes possess a defense mechanism for scavenging Reactive Oxygen Species and eliminating them (Parida and Das, 2005). Transgenic approach could be used to transfer traits from halophytes into glycophyte crops in order to increase salt tolerance, particularly, inserting genes that regulate the production of osmolytes in the cytosol to re-establish an ion and electrochemical gradients (Zhu, 2001).

## 2.8 Approaches for developing salt tolerant crops

The use of modern biotechnology techniques such as genetic engineering to introgression genes that are known to confer salinity tolerance might prove to be a quicker approach towards improving crop varieties and or genotypes. In this section therefore techniques for the development of stress-tolerant crops such as tissue culture based *in vitro* selection and genetic engineering using candidate genes for salinity tolerance in plants are discussed

### 2.8.1 *In vitro* selection technique approach

*In vitro* selection is f one the strategies used to develop tolerance plant lines (Gandonou *et al.,* 2006; Hossain *et al.,* 2007). To achieve this a number of culture systems have been used such as callus, suspension cultures, somatic embryos, shoot cultures systems which are evaluated for their ability to tolerate relatively high levels of salt (NaCl) in media (Woodward and Bennett, 2005). Apart from the use of NaCl, other $Cl^-$ and $SO_2^-$ salts including KCl, $Na_2SO_4$, and $MgSO_4$ have been used for *in vitro* screening.

Additionally, different responses were observed when *Nicotiana tabacum* was grown on seawater, synthetic seawater, manitol, NaCl and other $Cl^-$ and $SO_2^-$ salts. Indeed the use of multiple salts as selection pressure will parallel the salinity under field conditions and may be a better choice in the development of salinity tolerant plant lines.

There are two types of selection methods; (a) stepwise long-term treatment, in which cultures are exposed to salt stress with gradual increase in concentrations of selecting agent and (b) shock treatment, in which cultures are directly subjected to

a shock of high concentration and only those which would tolerate that level will survive (Purohit *et al.,* 1995).

However during in vitro selection, non-tolerant cells sometimes can undergo stable epigenetic alterations that are inherited through mitosis and not through meiosis as observed for rare mutants with true tolerance (Tal, 1994). The genetically stable meiotic heritable tissue culture induced variations in regenerated plants is called somaclonal variation and is useful for salt tolerance (Larkin and Scowcroft, 1981).

Reports indicate that the problem of epigenetic adaptation during in vitro selection can be overcome by the use of one-step selection that may prevent the development of epigenetically adapted cells (Tal, 1994).

This method has been successfully used for developing salt tolerant varieties such as: *Brassica oleracea* –cauliflower (Elavumoottil *et al.,* 2003), *Citrus aurantium* -sour orange (Koc *et al.,* 2009), *Glycine max* -soya bean (Liu and Staden 2000), *Nicotiana tabacum* –tobacco, (Rout *et al.,* 2008) and *Solanum tuberosum* –potato (Queiros *et al.,* 2007)

### 2.8.2 Genetic engineering approach

Many genes are available that can be used to transform crops to improve salinity tolerance such as those conferring shoot ion exclusion, shoot tissue tolerance and osmotic tolerance. It is worth noting that a particular gene or gene family may contribute to more than one trait, just as one trait can be conferred by more than one gene. However, the choice of salinity tolerance mechanism to manipulate in

crops plants will depend on the underlying salt tolerance mechanisms within individual crop species.

**2.8.2.1 Ion exclusion**

The high affinity potassium transporter (HKT) gene family (Munns and Tester, 2008; Xue *et al.*, 2010) and the salt overly sensitive (SOS) pathway (Yang *et al.*, 2009; Kudla *et al.*, 2010) have an important role in regulating Na+ transport within a plant. Manipulation of the expression of these genes increases the $Na^+$ levels in the shoot. In this regard, the HKT1 group of HKTs has the greatest potential for improving the salinity tolerance of crops, (Rus *et al.,* 2001; Ahmadi *et al.,* 2011). However, an HKT2 gene has been reported to increase salinity tolerance but not through $Na^+$ exclusion (Mian *et al.,* 2011).

More over these genes require cell type specific expression to be effective. If stress inducible and cell type specific expression of these genes can be realized in an effective way in crops, the potential for improving crop salinity tolerance through ion exclusion is possible (Møller *et al.,* 2009).

**2.8.2.2 Shoot tissue tolerance**

The vacuolar $Na^{+/}H^+$ antiporters (NHX) gene family and vacuolar pyrophosphatases (AVP1) are proteins involved in the synthesis of compatible solutes and enzymes responsible for the detoxification of reactive oxygen species in the plants. Salinity tolerant plants have been developed by the overexpression of NHX and vacuolar pyrophosphatase genes (Barragan *et al.,* 2012).

Success has also been reported in the development of shoot tissue tolerance of crops to salinity through increasing compatible solutes and enzymes involved in

ROS metabolism (Hmida-Sayari *et al.,* 2005). Reports indicate that when constitutive promoters are used pleiotropic effects and gene silencing are observed. However such effects can be avoided by use of stress-inducible promoters to drive the target genes (Su and Wu, 2004).

**2.8.2.3 Osmotic tolerance**

Modulation of signal transduction during salt stress from the roots to the shoots and control of cell cycle are the main processes involved in plant osmotic tolerance.

Introgression of TmHKT1; 5-A from *Triticum monococcum* into the durum wheat, Tamaroi, resulted in improvement in grain yield in high saline environment, by increasing its ion exclusion mechanism (Munns *et al.,* 2012). These observations were similar to that observed in the Tamaroi cultivar without the introgressed gene, under low and moderate saline conditions of field grown durum wheat. In conclusion these researchers therefore suggesting that osmotic stress was having a greater effect on the end yield of these plants growing in low to moderate salinity, than ionic stress (Munns *et al.,* 2012).

**2.8.2.4 Signaling/regulatory pathways**

This involves the over expression of genes involved in the detection, signaling and regulatory pathways in global salinity tolerance. Various researchers have reported that altering one pathway could have beneficial effects such as ion exclusion, tissue tolerance and osmotic tolerance. Incidentally, some the mechanisms involved in controlling shoot $Na^+$ accumulation is ROS signaling

through the regulation of vasculature $Na^+$ concentrations (Mittler *et al.,* 2011; Jiang *et al.,* 2012).

Overexpression of genes encoding proteins in $Ca^{2+}$ signaling pathways improved the growth of crop plants, such as rice, apple, barley, tobacco and tomato during salt stress (Kudla *et al.,* 2010; Batistic and Kudla, 2012). Similarly, salinity tolerance in plants may be achieved through the overexpression of transcription factors regulating the induction and/or repression of a range of salinity tolerance genes (Kasuga *et al.,* 1999). However, yield penalties are observed in low stress environments as a result of silencing and pleiotropic effect when transcription factors are constitutively expressed. This can be avoided by use of stress inducible promoters to regulate their expression levels (Liu *et al.,* 1998; AbuQamar *et al.,* 2009).

**2.9 Thidiazuron supplementation in *in vitro* plant regeneration**

Thidiazuron (TDZ), N-phenyl-N'-l, 2, 3-thidiazol-5-ylurea, is a light yellow-crystalline compound synthetic growth regulator sparingly soluble in water but highly soluble in ethanol, and other organic solvents. When explants are exposed for short periods to low concentration, up to 10pM, of TDZ they are able to show regeneration activity. TDZ has auxin and cytokinin like effects, a characteristic that distinguish TDZ from other naturally occurring or synthetic plant growth regulators (Hutchinson and Saxena, 1996).

Structurally, TDZ has two functional groups; a phenyl and thidiazol groups with complimentary roles in activity (Mok *et al.,* 1982). Interestingly, the replacement of either of these groups with other ring structures results in the reduction in

activity (Mok *et al.,* 1982). Again, the efficacy of TDZ owes to its storage as short-length polymers that are subsequently released during the culture period.

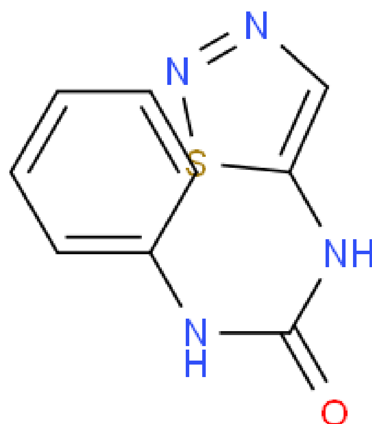


Figure 2.8: Structure of TDZ

### 2.9.1 Tissue culture

Cells in a plant body are totipotent via *de novo* formation of organs or somatic embryos. Plant growth hormones such as auxins and cytokinins regulate the acquisition of competency, dedifferentiation, and redifferentiation. However, TDZ is effective either alone or in combination with other growth-regulating substances in the induction media (Murthy *et al.*, 1998).

The first stage in the morphogenic process is the induction of cell proliferation and callus growth by the use of auxins such as NAA; and growth regulators with auxin-like activity such as 2, 4-D in culture media. Primarily, auxins are part of signaling molecules involved in the regulation of cell division, cell elongation and cell differentiation in higher plants (Bennett *et al.,* 1994).

TDZ is a strong callus induction agent with higher rate of cell proliferation, up to 30-fold, than that obtained with other growth regulators. At lower concentrations TDZ promote the formation of compact-green nodular callus (Murthy and

Saxena, 1998). A wide range of crop species, have been regenerated via TDZ-induced shoot production. For this purpose, TDZ has been shown to promote differentiation of cultured tissues at much lower concentrations, with far greater efficiency of shoot regeneration than that of other cytokinins (Malik and Saxena, 1992).

The best success story in the application of TDZ was in the regeneration of woody plant species *Cydonia oblonga* (Baker and Bhatia, 1993). Organogenesis of these species required very high concentrations of adenine-type of cytokinins as opposed to TDZ induced organogenesis at much lower concentrations with formation of adventitious buds directly on the cultured shoot tips (Huetteman and Preece, 1993; Lu, 1993).

**2.9.2 Somatic embryogenesis**

Among the requirements for the formation of embryogenic tissues from somatic cells is the alteration of either the concentration of exogenously applied auxins or the ratio of auxins to cytokinins. Alternatively, for somatic embryogenesis, TDZ alone can be applied as a substitute for both the auxin and cytokinin requirement in many species (Gill *et al.,* 1993).

Higher rate of *in vitro* somatic embryogenesis have been achieved by addition of TDZ in the culture media in a number of species including tobacco (Gill and Saxena, 1993), peanut (Murthy *et al.,* 1995), geranium (Visser *et al.,* 1992), chickpea (Murthy *et al.,* 1996) and neem (Murthy and Saxena, 1998). In addition, inclusion of TDZ in the culture media may lead to simultaneous production of shoots and somatic embryos (Bates *et al.,* 1992).

### 2.9.3 *In vivo* regeneration

Other applications of TDZ include the formation of regenerative outgrowths on root tissues and adventitious shoots at the crown region of greenhouse-grown geraniums (Sanago *et al.,* 1996). Similarly, pot-grown *Spathiphyllum* plants sprayed with TDZ produced a large number of shoots (Zhao et al., 2012). While spraying ginseng tap roots with TDZ lead to formation of adventitious buds on their shoulders (Proctor *et al.,* 1996).

# CHAPTER THREE

# *IN VITRO* REGENERATION OF SWEETPOTATO (*Ipomoea Batatas*) THROUGH DIRECT SHOOT ORGANOGENESIS

## 3.1 INTRODUCTION

*In vitro* organogenesis refers to organ formation through dedifferentiation of differentiated cells into callus and reorganization of cell division to form specific organ primordia and meristems (Sugiyama, 1999). In direct shoot organogenesis, the shoots are formed directly without a callus intermediate stage, a process is known as adventitious bud formation. The process is quick, faster and attractive for plant regeneration and improvement for desirable traits such as salt tolerance. However, this regeneration system has high number of chimeras and somaclonal variation (Gamborg and Phillips, 1995). Organogenic callus induction from explants requires a high auxin/cytokinin ratio in the media whereas shoot formation is triggered by a high cytokinin/auxin ratio or high cytokinin levels with no auxin (Sefasi *et al*., 2013). Such shoots originate from unipolar and callus-derived organ primordia that form roots upon transfer to medium without hormones or containing natural rooting auxins such as isobutyric acid (IBA) and α naphthalene acetic acid (NAA) (Gamborg and Phillips, 1995).

Different explants have been used for organogenic callus cultures with subsequent shoot organogenesis such as tobacco (*Nicotiana tabacum* L.) protoplasts, *Arabidopsis thaliana* root and leaf explants (Anami *et al.,* 2013) and strawberry shoot organogenesis using leaf disks and petioles (Debnath *et al.,* 2006)

Sweetpotato regeneration through adventitious bud formation at a high frequency has been restricted to a few exotic genotypes such as Jewel, Huachano and

Jonathan, since a wide range of African cultivars are recalcitrant or respond at low frequencies (Al-Mazrooei *et al.,* 1997; Luo *et al.,* 2006; Anwar *et al.*, 2010). Besides, most of the reported regeneration protocols that have worked with exotic genotypes in some laboratories are difficult to reproduce in others, particularly when recalcitrant African sweetpotato genotypes are used, thereby further compounding the problem (Luo *et al.,* 2006). Indeed it has been reported by different researchers' that different media formulation and explants have been shown to influence *in vitro* regeneration (Song *et al.,* 2004; Anwar *et al.,* 2010). Thus, there is need to develop an efficient reproducible protocol of plant regeneration for recalcitrant African sweetpotato genotypes that can be used for improvement through marker assisted selection (MAS) and or genetic transformation such as to enhance salt tolerance among others. This chapter outlines *in vitro* regeneration of selected Kenyan sweetpotato genotypes via direct shoot organogenesis.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Chemical and reagents

All chemicals and reagents used were supplied by Sigma-Aldrich Chemie GMBH, schenstrasse, Taufkirchen.

### 3.2.2 Plant material and culture conditions

Six selected Kenyan sweetpotato cultivars were obtained from KALRO biotechnology center courtesy of Dr. Eustous Nyamongo. These are *SPK004, KSP36, KEMB 36, SPK 013, Enaironi, Mugande* and *Kalamb Nyerere* whereas the model variety *Jewel* was obtained from CIP Nairobi.

Vigorously growing vines from screen house plants were used to provide cuttings for establishment of *in vitro* stock cultures. The cuttings were surface sterilized according to the protocol described by Song *et al*. (2004) with slight modifications. Briefly, the explants were immersed in commercial Sodium hypochlorite solution containing 3.85% NaOCl and two drops of Tween 20 ® for 10 minutes before transfer to 70% ethanol for further disinfection and removal of the sodium hypochlorite in the laminar flow chamber for a period of 5 minutes. Thereafter, the cuttings were rinsed three times with sterile double distilled water.

The sterile cuttings were incubated into sweetpotato propagation (SP) medium in culture bottles. The medium contained MS (Murashige and Skoog, 1962) salts (4.3 g/l), sucrose (30 g/l), myo-inositol (0.1 g/l ), Indole-3 acetic acid (IAA) (1.0 µM), 5 ml/l sweetpotato vitamin stock comprising 40 g l Ascorbic acid, 20 g/l, L-arginine, 4 g/l putrescine HCl, 5.8 µM gibberellic acid (GA3) and 0.4 g/l calcium pantothenate (Untiveros *et al.,* 2008). The medium pH was adjusted to 5.8 before

adding 3 g/L gelrite, followed by autoclaving at 121 °C for 15 minutes under 15 kPa.

### 3.2.3 Adventitious bud induction on TDZ medium

Bud induction medium was composed of MS basal salts (4.3g/l), sucrose (30 g/l), myo-inositol (0.1 g/l) and sweetpotato vitamin stock (1 ml/l). Two concentrations of Thidiazuron (TDZ), 0.15mg/l or 0.25mg/l, were added to the medium after autoclaving. Stem internodes segments (0.6 - 1.0 cm) were cut from 4-week-old *in vitro* cultures growing in SP medium in culture bottles. The explants were horizontally placed and partially pressed into the medium and cultured on 25 ml of induction medium in plastic petri-dishes. The petri-dishes containing the cultures were placed in dark for 7 days to induce adventitious bud before transfer to light for shoot regeneration for 4 weeks as reported by Sefasi *et al*. (2013). The cultures were then transferred onto fresh regeneration media after the induction period.

### 3.2.4 Effect of light on adventitious bud induction on TDZ medium

To assess the effect of light on bud induction of explants on medium containing TDZ (0.15mg/l or 0.25 mg/l), half of the explants were placed on the medium with TDZ for 7 days in the dark at $28^{0}$C, while the other half were kept on the same medium for 7 days in 16 hours of light. Afterwards, the petri-dishes containing the cultures were transferred to light for shoot regeneration for 4 weeks as earlier described in section 3.2.3.

### 3.2.5 Adventitious bud regeneration on auxin containing medium

Due to low frequency of conversion of adventitious buds into shoots, the effect of α Naphthalene acetic acid (NAA) on conversion of adventitious buds into shoots was investigated. In this experiment adventitious buds were induced in both light and dark as described in section 3.2.3 in two TDZ concentrations. Thereafter, each group of explants was then further divided into three equal groups; one group was transferred into medium that did not contain any NAA while the other groups were transferred media containing 0.1 mg/l NAA and 0.25 mg/l NAA respectively. They were then incubated in 16 hours of light at $26^0$C for shoot regeneration as reported by Sefasi *et al*. (2013).

### 3.2.6 Experimental design and statistical analysis

All experiments were laid out in a completely randomized design. Three petri-dishes, containing 10 explants each, were used in each experiment that was replicated three times. Data on total number of explants with adventitious shoot and number of buds per explants were recorded after 4 weeks in culture. The number of explants with adventitious shoots with developed leaves and roots and number of adventitious shoots per explants were collected after 12 weeks. The frequency of explants regenerating adventitious buds and shoots was calculated by expressing the number of explants regenerating buds or shoots as a percent of the total number of explants investigated. Statistical analyses were done using two way analysis of variance (ANOVA) and means were compared using the least significant difference (LSD) test at $P \leq 0.05$ confidence level using SAS computer software (version 9.1.3).

## 3.3 RESULTS

### 3.3.1 Adventitious bud induction frequencies

When explants obtained from *in vitro* grown plants were incubated in induction media containing two different concentration of TDZ, they swelled and their colour changed to yellowish within 3 days. The explants incubated in the dark tended to be pale yellow while those in light were light green in colour (Figure 3.1a.). At the end of induction period, adventitious buds were observed on the explant surfaces. The developing adventitious buds were surrounded by callus tissue in all the regenerated genotypes (Figure 3.1b).

The mean number of adventitious bud-like structures induced in light conditions using 0.15mg/l TDZ level were significantly highest in *Kalamb nyerere* (4.33) and significantly lowest in *Mugande* (1.33) ($p \leq 0.05$;Table 3.1). There was no significant difference in the mean number of adventitious buds induced in *Jewel* and *KSP 36* ($p > 0.05$) but were significantly lower than from *Enaironi* and *SPK 004*.

Induction frequencies from 0.25mg/l TDZ hormone level in the light, showed a 25% higher mean numbers of adventitious buds induced compared to 0.15mg/l TDZ. At this level, *Kalamb nyerere* (5.33) again had the highest mean number and the lowest mean number was from *Mugande* (2.33) that were significantly different ($p \leq 0.05$) from all the other genotypes. However, no significant differences were observed among *Kalamb nyerere, Kemb 36* and *KSP 36* as well as *Enaironi, SPK004* and *Jewel* ($p > 0.05$; table 3.1).

When the explants were incubated in the dark, they produced higher mean numbers of buds than those recovered from light conditions at the two levels of

TDZ hormone tested. There were no significant difference in the mean number of buds induced in *Jewel, SPK004* and *Kemb 36* ($p>0.05$; table 3.1).

On the other hand, the mean number of buds induced at the 0.25mg/l TDZ hormone level in the dark showed the highest mean number of buds from *Kalamb nyerere* (8.00) and the lowest from *Mugande* (4.37). Results from all the genotypes showed that there was significant difference in the mean number of adventitious buds induced except in *Jewel, SPK004* and *Kemb 36* (Table 3.1).

Generally it was observed that the levels of adventitious bud induction was significantly ($P\leq0.05$) affected by treatment and photoperiod.

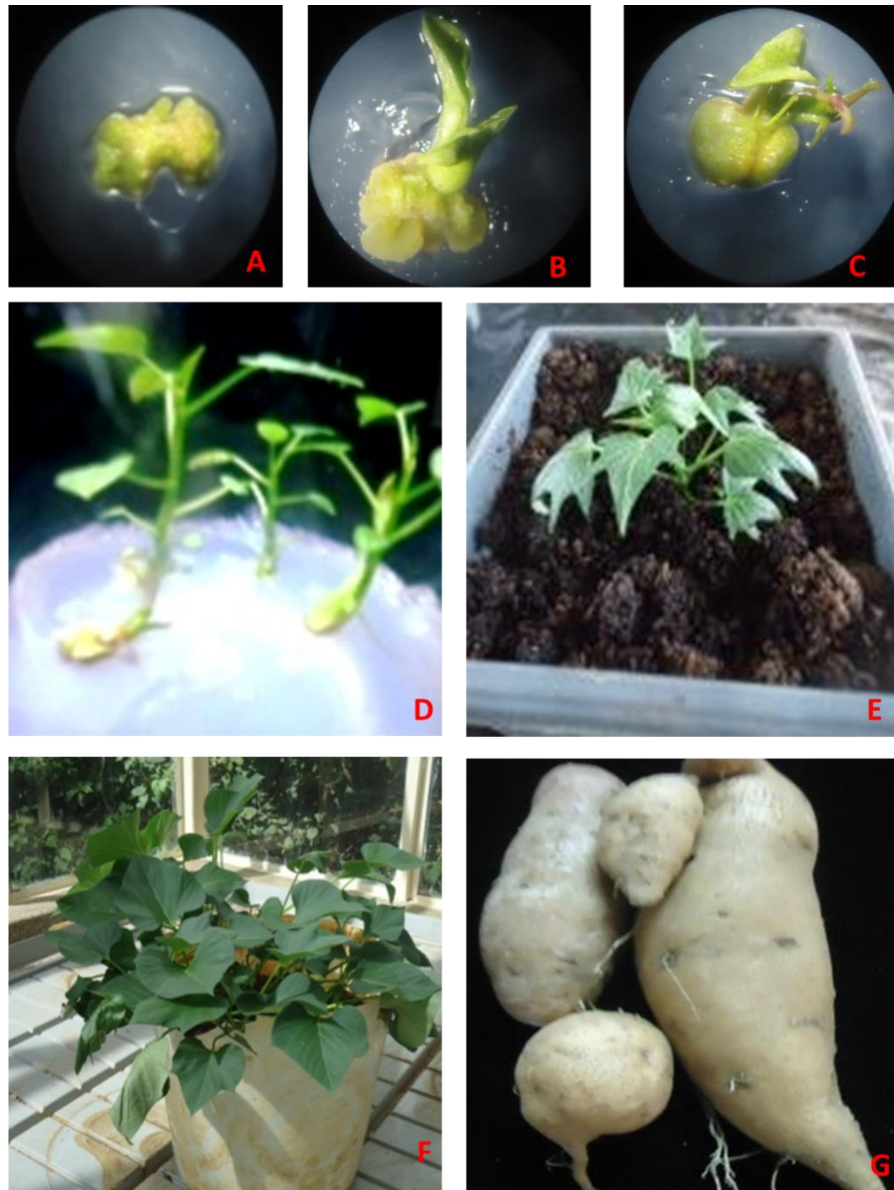


**Figure 3.1: Regeneration of sweetpotato genotypes through organogenesis**
A. Adventitious bud forming after induction in TDZ hormone in the dark
B. Germinating bud forming plantlet surrounded by callus tissue
C. Plantlets in regeneration media
D. Plants in shooting media
E. *Kalamb Nyere* plants in peat moss during hardening
F. *Jewel* sweetpotato plants in the glass house
G. Harvest from mature jewel plant after four months

**Table 3.1: Adventitious bud induction in seven sweetpotato genotypes using thidiazuron hormone (TDZ)**

| | **TDZ level (Dark)** | | **TDZ level (Light)** | |
|---|---|---|---|---|
| **Genotype/hormone** | **0.15mg** | **0.25mg** | **0.15mg** | **0.25mg** |
| ***Enaironi*** | $3.00 \pm 0.41^{c}$ | $5.00 \pm 0.41^{cd}$ | $2.00 \pm 0.50^{bc}$ | $3.33 \pm 0.44^{ab}$ |
| ***Jewel*** | $5.00 \pm 0.41^{ab}$ | $7.00 \pm 0.41^{ab}$ | $3.67 \pm 0.33^{ab}$ | $4.00 \pm 0.41^{ab}$ |
| ***K. Nyerere*** | $6.00 \pm 0.41^{a}$ | $8.00 \pm 0.41^{a}$ | $4.33 \pm 0.44^{a}$ | $5.33 \pm 0.53^{a}$ |
| ***KSP 36*** | $4.00 \pm 0.41^{bc}$ | $5.67 \pm 0.33^{bcd}$ | $3.00 \pm 0.41^{abc}$ | $4.67 \pm 0.50^{a}$ |
| ***Kemb 36*** | $5.33 \pm 0.33^{ab}$ | $7.00 \pm 0.41^{ab}$ | $3.33 \pm 0.41^{ab}$ | $5.00 \pm 0.58^{a}$ |
| ***Mugande*** | $3.67 \pm 0.53^{bc}$ | $4.33 \pm 0.44^{d}$ | $1.33 \pm 0.33^{c}$ | $2.33 \pm 0.33^{b}$ |
| ***SPK 004*** | $5.00 \pm 0.41^{ab}$ | $6.33 \pm 0.33^{ab}$ | $2.00 \pm 0.41^{bc}$ | $3.33 \pm 0.41^{ab}$ |
| **LSD** | **1.81** | **1.70** | **1.77** | **2.00** |

Values are means and standard errors of 3 replicates with 10 explants per petri plate replicated 3 times within genotypes for each treatment. Means followed by the same letter in each column are not significantly different from each other (Tukey's test; $p \leq 0.05$)

The results revealed three groupings such that *Kalamb nyerere* and *Kemb 36* were in the first; *Jewel* and *KSP 36* in the second while *Enaironi* and *SPK 004* in the last. These groups had significantly different mean number of adventitious buds between the groups but no difference within them. On the other hand mean adventitious bud induction averages from the dark condition showed slightly higher average values. However, the results showed that the average mean number of adventitious buds were significantly different from each other ($p \leq 0.05$; Figure 3.2).

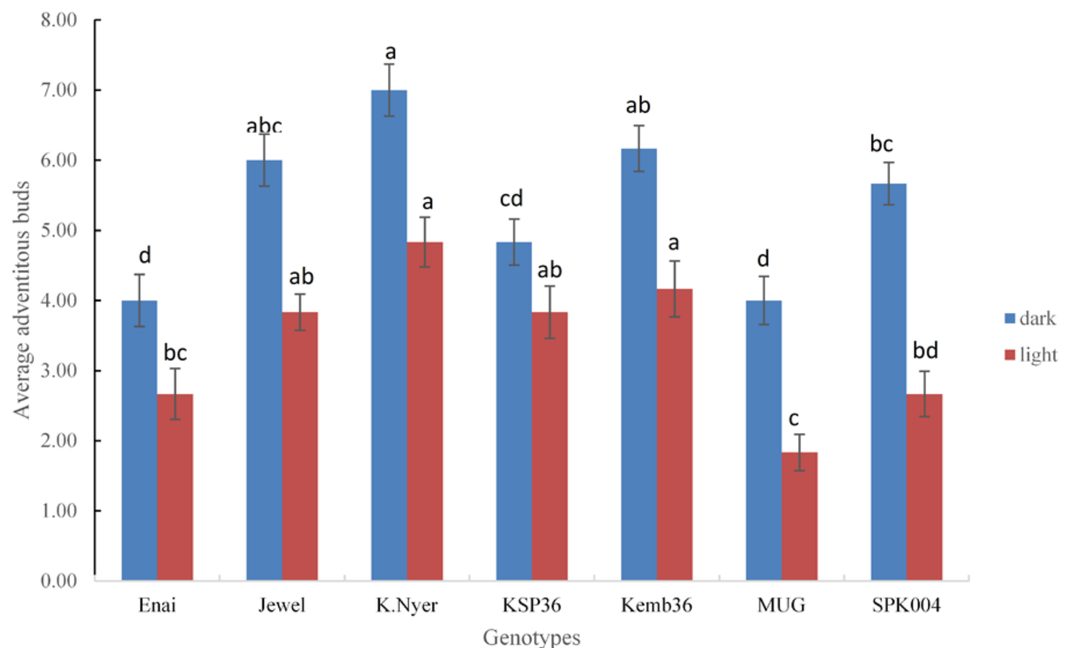


**Figure 3.2: Average number of adventitious bud in light and darkness**. Values are means and standard errors. Means followed by the same letter in each column are not significantly different from each other (Tukey's test; $p \leq 0.05$)

### 3.3.2 Regeneration frequencies in NAA media

When the explants were transferred to regeneration media, the adventitious buds were converted to plantlets. The efficiency of conversion depended largely on the amount of NAA in the regeneration media and the genotype.

Adventitious buds induced at 0.15 mg/l TDZ level when transferred to hormone free regeneration media (Table 3.2). The results shows that only one genotype (*Kalamb nyerere*), in light induction, and one (*SPK 004*), in dark induction were able to regenerate significantly ($p \leq .05$) higher plantlets compared to other genotypes. The rest of the genotypes showed adventitious bud abortion irrespective of the induction source and photoperiod when no hormone was added (Table 3.2).

On the other hand, regeneration media supplemented with 0.1mg/l NAA had the highest adventitious bud conversions to plantlets whether the buds were induced in light or darkness. At 0.1mg/l NAA level, two genotypes, *KSP 36* and *Mugande* showed total adventitious bud abortion with no regenerants formed. However, the remaining genotypes had significant ($p \leq 0.05$) regeneration frequencies for both adventitious bud induction conditions. On the other hand adventitious bud conversion to plantlets showed significantly ($p \leq 0.05$) variable regeneration percentages for all genotypes when buds were recovered in the dark (Table 3.2).

Interestingly, the results from 0.25mg/l NAA level showed only one genotype (*Kalamb nyerere*) with significantly different percentage plant regeneration while the other varieties suffered adventitious bud abortions in light. Adventitious bud induced in the dark had three genotypes (*Enaironi, Jewel* and *Kalamb nyerere*) regenerated significantly ($p \leq 0.05$) number of adventitious buds while the rest suffered bud abortions (Table 3.2; Appendix 1B).

**Table 3.2: Regeneration frequencies of adventitious buds induced in 0.15mg/l TDZ from seven sweetpotato genotypes in media supplemented with NAA**

| **Genotype/** | **Light** | **Dark** | **Light** | **Dark** | **Light** | **Dark** |
|---|---|---|---|---|---|---|
| **NAA (mg/l)** | 0.00 | 0.00 | 0.10 | 0.10 | 0.25 | 0.25 |
| ***Enaironi*** | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ | $20.20\pm 3.84^{bc}$ | $47.63\pm 1.89^{b}$ | $0.00\pm 0.00^{b}$ | $20.27\pm 3.43^{a}$ |
| ***Jewel*** | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ | $20.30\pm 2.76^{bc}$ | $34.40\pm 2.77^{bc}$ | $0.00\pm 0.00^{b}$ | $25.07\pm 3.10^{a}$ |
| ***K. Nyerere*** | $20.30\pm 4.23^{a}$ | $0.00\pm 0.00^{b}$ | $83.20\pm 3.84^{a}$ | $99.93\pm 4.62^{a}$ | $50.03\pm 4.33^{a}$ | $25.07\pm 3.23^{a}$ |
| ***KSP 36*** | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{d}$ | $20.23\pm 4.50^{cd}$ | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ |
| ***Kemb 36*** | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ | $17.20\pm 2.86^{c}$ | $47.93\pm 3.48^{b}$ | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ |
| ***Mugande*** | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{d}$ | $17.17\pm 2.89^{d}$ | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ |
| ***SPK004*** | $0.00\pm 0.00^{b}$ | $20.13\pm 4.63^{a}$ | $32.10\pm 1.65^{b}$ | $48.13\pm 0.63^{b}$ | $0.00\pm 0.00^{b}$ | $0.00\pm 0.00^{b}$ |
| **LSD** | **7.73** | **8.45** | **12.70** | **16.68** | **7.90** | **10.28** |

Values are means and standard errors of 3 replicates with 10 explants per petri plate replicated 3 times within genotypes for each treatment. Means followed by the same letter in each column are not significantly different from each other (Tukey's test; $p \leq 0.05$)

Explants from 0.25 mg/l TDZ hormone induction level had higher adventitious bud conversion to plantlets on average. Adventitious buds were aborted in all genotypes except in two (*Jewel* and *Kalam nyerere*) which were induced in light and three (*Jewel, Kalamb nyerere* and *SPK 004*) that were induced in the dark when the explants were transferred to NAA free regeneration media (Table 3.3).

Regeneration media supplemented with 0.1 mg/l NAA registered significantly higher adventitious bud regeneration frequencies for all the genotypes used whether in light or in dark. However values of regeneration percentages shows that adventitious bud conversion was higher for those buds recovered from the dark than from light in all genotypes (Table 3.3).

Higher levels of NAA (0.25 mg/l) had an inhibitory effect on the adventitious bud conversion to plantlets for majority of the genotypes tested. However two genotypes (*KSP 36* and *SPK004*) induced in light and two genotypes (*Mugande* and *SPK 004*) induced in darkness had adventitious bud abortions. At 0.25 mg/l NAA level the regeneration frequencies were significantly lower as compared to those recovered from 0.1 mg/l NAA level (Table 3.3). Overall, the pooled regeneration frequencies at the genotype level showed that incubation in the dark is the best condition for most of the sweetpotato genotypes than light condition with the exception of *Kalamb nyerere* and *SPK 004* (Figure 3.3; Appendix 1C).

**Table 3.3: Regeneration frequencies of adventitious buds induced in 0.25mg/l TDZ from seven sweetpotato genotypes**

| Genotype / | Light | Dark | Light | Dark | Light | Dark |
|---|---|---|---|---|---|---|
| NAA (mg/l) | 0.00 | 0.00 | 0.10 | 0.10 | 0.25 | 0.25 |
| *Enaironi* | 0.00± 0.00[b] | 0.00± 0.00[b] | 19.87± 1.95[d] | 49.63± 2.70b[c] | 25.23± 1.36[b] | 20.10± 2.15[b] |
| *Jewel* | 20.20± 1.89[a] | 25.13± 3.91[a] | 60.23± 2.69[a] | 83.20± 2.86[a] | 40.57± 3.42[a] | 40.50± 3.12[a] |
| *K.Nyerere* | 25.30± 2.76[a] | 20.30± 2.88[a] | 60.23± 2.26[a] | 80.10± 3.29[a] | 17.60± 1.83[b] | 20.13± 3.48[b] |
| *KSP36* | 0.00± 0.00[b] | 0.00± 0.00[b] | 16.80± 2.14[d] | 40.20± 3.75[c] | 0.00± 0.00[c] | 25.13± 3.14[b] |
| *Kemb36* | 0.00± 0.00[b] | 0.00± 0.00[b] | 35.60± 1.35[c] | 60.10± 3.31[b] | 20.30± 1.42[b] | 40.13± 2.18[a] |
| *Mugande* | 0.00± 0.00[b] | 0.00± 0.00[b] | 50.17± 2.17[b] | 50.27± 1.83[bc] | 25.80± 1.56[b] | 0.00± 0.00[c] |
| *SPK004* | 0.00± 0.00[b] | 17.07± 1.48[a] | 36.30± 1.35[c] | 50.30± 2.88b[c] | 0.00± 0.00[c] | 0.00± 0.00[c] |
| **LSD** | **6.11** | **9.27** | **9.83** | **14.48** | **8.44** | **11.70** |

Values are means and standard errors of 3 replicates with 10 explants per petri plate replicated 3 times within genotypes for each treatment. Means followed by the same letter in each column are not significantly different from each other (Tukey's test; $p \leq 0.05$)

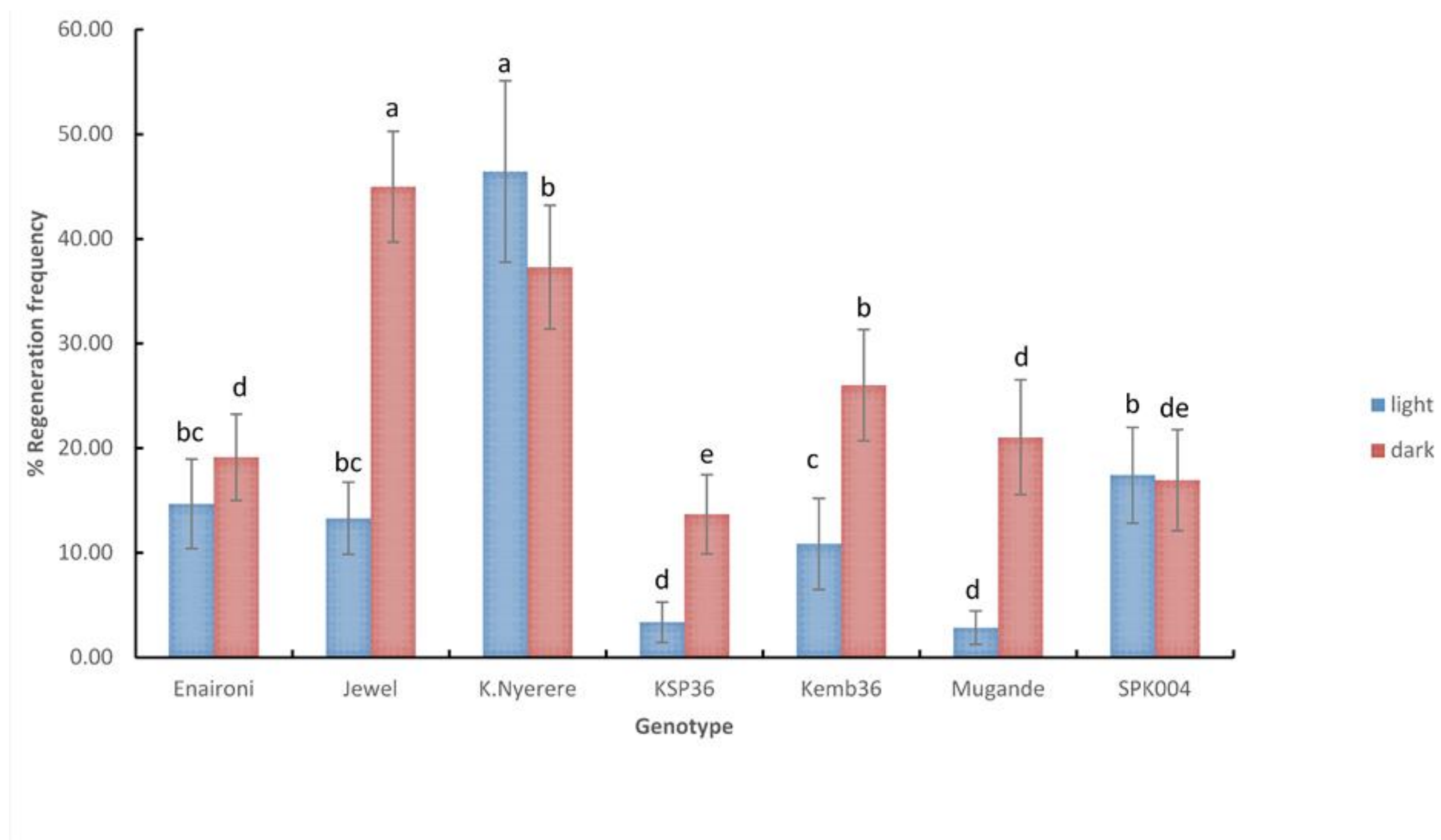


**Figure 3.3: Summary of mean regeneration frequency for 7 sweetpotato genotypes in light and darkness irrespective of NAA and TDZ levels.**

Data represents Mean ± standard error. Means followed by same letter are statistically not different at p=0.05 according to Tukey's mean separation.

The results on regeneration frequency on the basis of regeneration media, irrespective of the genotype in the two photoperiods shows that media supplemented with 0.1 mg/l NAA had significantly highest regeneration frequencies as compared with media without or with higher levels of NAA (Figure 3.4 A and B). Equally, the overall regeneration frequencies irrespective of induction hormonal level, genotype and photoperiod showed significantly higher regeneration frequency for media supplemented with 0.1 mg/l NAA (Figure 3.5; Appendix 1D)

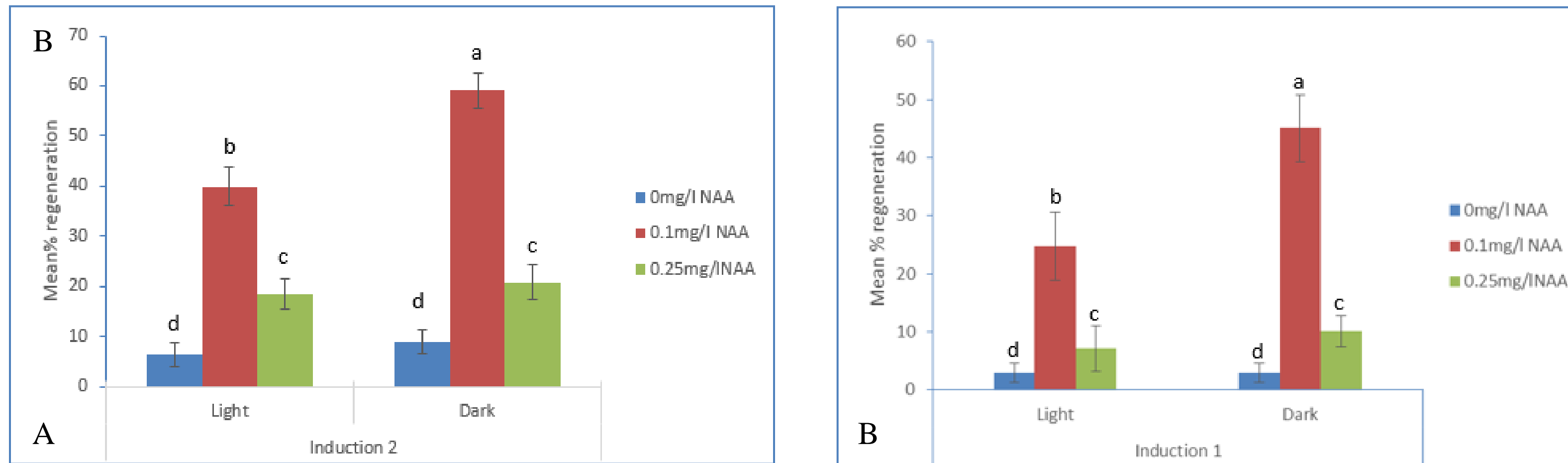


**Figure 3.4: Mean regeneration frequencies at three NAA levels.**

A: Adventitious buds induced in 0.15mg/l TDZ hormone.

B: Adventitious buds induced in 0.25mg/l TDZ hormone.

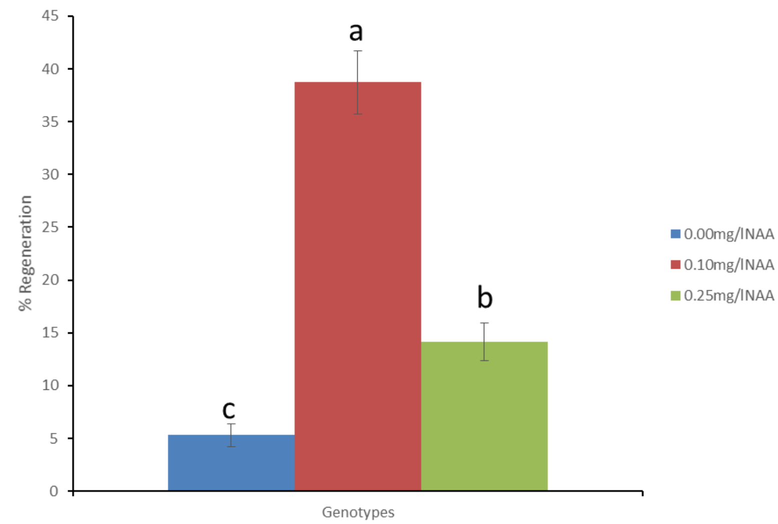


**Figure 3.5: Overall pooled mean regeneration frequencies at three NAA levels irrespective of induction TDZ hormone level and photoperiod**

### 3.3.3 Survival of regenerants

In the present study, an average of 67 % of the regenerants from all genotypes was able to survive and be transferred to the green house. At the genotype level the highest number of regenerants recovered were from *Kalamb nyerere* (72%) while the lowest survival rates was from *Enaironi* and *KSP 36* (60%)(Table 3.4). Most of the plants developed normally with no visible somaclonal variation or vitrification and reached maturity after four months.

**Table 3.4: % survival of regenerants recovered**

| Variety | No of regenerants | Surviving plants | % survival |
|---|---|---|---|
| ***Enaironi*** | **10** | **6** | **60** |
| ***Jewel*** | **17** | **12** | **71** |
| ***Kalamb Nyerere*** | **25** | **18** | **72** |
| ***KSP36*** | **5** | **3** | **60** |
| ***Kemb36*** | **11** | **7** | **64** |
| ***Mugande*** | **7** | **5** | **71** |
| ***SPK004*** | **10** | **7** | **70** |
| **Total** | **85** | **58** | **67** |

### 3.4 DISCUSSION

Successful regeneration of seven Kenyan selected sweetpotato genotypes through *in vitro* shoot organogenesis using stem explants has been established in this study. The variable bud induction frequency observed when using stem explants was genotype dependent. The cells of the explants in the presence of TDZ, a cytikinin, alone were able to acquire competence, dedifferentiate and redifferentiate to form adventitious buds that later were able to form entire plants in regeneration media. This is in agreement with Sefasi *et al.* (2013) working with Ugandan sweetpotato genotypes showed that cytokinin level and the genotype affected the induced adventitious buds using TDZ.

Other reports indicate that TDZ is effective either alone or in combination with other growth-regulating substances in the induction of regenerative processes in tissue cell cultures (Sefasi *et al.,* 2013). Other types of explants have been used for bud induction in sweetpotato with varying degree of success such as root, leaves, nodal segments, auxiliary buds and even leaf stalks (Hamant *et al.,* 2013; Sefasi *et al.,* 2013). Similar effects were previously investigated by several authors who used low levels of cytokinin to induce adventitious buds such as in *Phaseolus angularis* and *Stevia rebaudiana* respectively (Varisai *et al.,* 2006; Hamant *et al.,* 2013).

In the present study we report that photoperiod affected the number of buds induced. Results indicate that when the explants were incubated in the dark the number of adventitious bud induced were higher. Similar findings have been reported by Varisai *et al.* (2006) who observed 50% frequency increase of regenerating buds when explants were pre-cultured in the dark.

The efficacy of TDZ in producing high rates of adventitious bud followed by regeneration and axillary shoot proliferation in a number of plants is supported by several reports from different researchers. These include bertoni (Hamant *et al.,* 2013), sweetpotato (Sefasi *et al.,* 2012), legumes (Varisai *et al.,* 2006) and peanuts (Murthy *et al.,* 1995). This could be related to the fact that TDZ is a substituted phenyl urea derivative that exhibits strong cytokinin-like activity (Thomas and Katterman, 1986).

Structurally, there are two functional groups in a TDZ molecule; phenyl (adenine type) and thidiazol groups that have complimentary roles in TDZ-induced responses (Mok *et al.,* 1982). Reports indicate that adenine - and phenylurea - type cytokinins have a common binding receptor in the plant cell, a cytokinin - binding protein (CBP) with two different binding sites at the molecular level. One site binds adenine-type cytokinins naturally, while the other is able to bind phenylurea - type cytokinins (Nielsen *et al.,* 1995).

Binding of an adenine-type cytokinin to CBP induces the expression of the rapid cytokinin genes, IBC6 and IBC7, thereby promoting cell division as well as stimulating tissue and shoot formation (Brandstatter and Kieber, 1998).

Reports indicate that exogenous application of TDZ the phenylurea CBP site becomes occupied that enhances the effect of exogenous and or endogenous adenine - type cytokinin already bound to CBP. Alternatively TDZ promote growth due to its own biological cytokinin activity and also induces the synthesis or accumulation of endogenous cytokinins (Bretagane *et al.,* 1994). TDZ also

enhances the availability and accumulation of endogenous cytokinins through non-competitively inhibition of cytokinin oxidase activity (Eapen *et al.,* 1998).

The adventitious bud induction and regeneration frequency in the present study was observed to be genotype dependent as was reported by Sefasi *et al.,* (2013). This could be due to the fact that different cells within the same explant can have varying concentrations of innate plant growth regulators and additional variation in receptor affinity or cellular sensitivity to plant growth regulator as such it is reasonable to expect that in vitro responses will vary within genotypes (Kim *et al.,* 1997).

In this study low NAA concentrations stimulated shoot formation with variable regeneration frequencies that was genotypic dependent. However, high NAA concentration levels decreased sharply the regeneration frequency due to inhibition of root formation. Similar observation was reported by Sefasi *et al.* (2013) when regenerating Ugandan sweetpotato genotypes. Response of stem explant to varying NAA concentrations in the media could be a reflection of probable differences of endogenous hormonal levels in the explant or tissue sensitivity to plant growth regulator (Lisowska and Wysonkinska 2000; Gong *et al.*,2001). However, higher concentrations of NAA have been found to suppress rooting in shoots of some species such C*hrysanthemum morifolium* (Belarmino and Gabon, 1999).

TDZ was observed to induce developmental abnormalities including vitrification in some species in higher concentrations and long incubation period (Huetteman and Preece, 1993; Li *et al.,* 1994). It was suggested that very short periods (7±2 d)

of induction were sufficient to induce morphogenetic potential (Li *et al.,* 1994; Murthy *et al.,* 1995). Throughout this study, shoots that were normal in appearance (without vitrification) were obtained due to the relatively short period of exposure of the explant to TDZ.

# CHAPTER FOUR

# GROWTH ANALYSIS AND *IN VITRO* EFFECTS OF SALT AND OSMOTIC STRESS ON GROWTH OF SELECTED KENYAN SWEETPOTATO GENOTYPES

## 4.1 INTRODUCTION

Plant growth is affected by light, water availability, temperature and nutrient availability (Poorter *et al.,* 2012). The primary factors affecting carbon acquisition and biomass allocation are light intensity and nutrients availability. Additionally, environmental constraints affect the optimal biomass allocation within the plant body. Carbon from photosynthesis can be used either for maintenance or growth within the roots or shoots. In the case of roots, carbon from the stem can be respired to provide energy for metabolic processes such as nutrient uptake and assimilation, or used in growth to explore the soil for further nutrients (Wright *et al.,* 2004).

*In vitro* experiments are used to study the effects of abiotic stress on plants growth. They are used as representatives for the complex field environmental conditions in which plants experience the stress. Primarily, such experimental setups involve the addition of compounds to the growth medium (Claeys *et al.,* 2014). For drought experiments, an osmotica such as mannitol, sorbitol, or polyethylene glycol is added to the media to lowers the water potential (Verslues *et al.,* 2006). In studying of salt stress NaCl is added to the medium to impose a combination of osmotic stress and $Na^+$ toxicity to the growing plants (Munns and Tester, 2008). Oxidative stress is simulated by the addition of hydrogen peroxide ($H_2O_2$) or methyl viologen to the media to induce formation of toxic reactive

oxygen species ($O^-_2$) in plant tissues. The practical advantages of *in vitro* experiments include, control of stress level and onset, low experimental variability, and the ability to grow many plants using limited space as well as provide additional information about stress sensitivity in plants (Lawlor, 2013).

In this chapter growth analysis of selected Kenyan sweetpotato genotypes to normal environmental conditions with no profounding constraints and the results of *in vitro* leaf disc assays subjected to different levels of osmotic and salt stress (sudden shock treatment) are reported.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Plant material

Fourteen locally adapted sweetpotato varieties selected randomly were obtained from KALRO Biotechnology Center, courtesy of Dr, Nyamongo E. namely *KSP 36, KSP 20, KSP28, KEMB 36, KEMB 10, Kemb 23 Kalamb Nyerere, Mweu Mutheki, Enaironi, Mugande, Zambezi, SPK 004, SPK 013* and *SPK 203*. The exotic variety *Jewel* was obtained from CIP Nairobi, which was used as model cultivar in all experiments. They were then planted in the green house and served as a source of planting material for the different experiments. Plants were maintained in the greenhouse under natural sunlight; average day/night temperatures were 30±2/26±2°C.

### 4.2.2 Plant growth analysis

The performance of each sweetpotato genotype under natural environmental conditions was determined by evaluating the growth of 4$^{th}$ leaf for a period of two weeks. Plant growth was monitored daily by measuring the length of 4$^{th}$ leaf of all sweetpotato vines in centimeters immediately the 4th leaf emerged according to a

method described by Olive (2013). The length measurement of leaf 4 in all sweetpotato plants was done until no further growth was recorded. Measurements were taken early in the morning every day. Leaf elongation rate was determined by getting the difference between growths of leaf four on daily intervals.

**4.2.3 *In vitro* (sudden shock treatment) leaf disc assays**

In order to analyze physiological responses of sweetpotato genotypes to sudden shock treatment of osmotic and salt stress, varying concentrations of mannitol and sodium chloride (NaCl) were used as described by Olive (2013) with slight modifications. Leaf discs of about 0.3 cm in diameter were excised from the leaf obtained from the first four internodes of three vines per genotype using a paper punch. An average of ten leaf discs, equivalent to 0.1g, were floated in 15 ml solution of NaCl of different concentrations (100, 150, 200 mM) and mannitol (100, 400, 800 mM) separately in 90 ml petri plates and were placed under 16/8 hour photoperiod at $28^0$C for 48 hours. In both experiments, distilled water was used as a control. Both the concentrations of NaCl and mannitol were optimized such that 100, 150, 200 mM NaCl and 100, 400, 800 mM mannitol were found to induce observable senescence in all the sweetpotato varieties after 48 hours. The effects of these treatments on the leaf discs were observed by checking chlorophyll and carotenoid content in response to the treatments. A completely randomized design was used and the experiment replicated three times

### 4.2.4 Leaf pigment extraction and measurements

Leaf pigments were extracted from approximately 0.1 g of leaf samples for analysis according to a method described by Wellburn (1994) with slight modifications using Dimethyl sulphoxide (DMSO). This method has the following advantages over other methods; firstly, the method is faster, largely because grinding and centrifuging is not required. Secondly, the Chlorophyll extracts are more stable, up to 5 days, in DMSO compared to those in acetone.

Three replicates of each sweetpotato varieties were placed in falcon tubes containing 7ml of Dimethyl sulphoxide (DMSO) and incubated in a water bath maintained at $60^0$C for 20 minutes. Immediately after, the volume was toped up to 10 ml and thereafter 2 ml of the mixture was pipetted into glass cuvettes where the absorbance was recorded at 665 nm, 649 nm and 480 nm using a 722 N spectrophotometer (Everich Medicare import and export Co. LTD, Nanjing China). The spectrophotometer was calibrated to zero absorbance using a blank of pure DMSO. Measurements and calculations were done as follows;

$C_a = 11.75A_{665} - 2.350A_{649}$

$C_b = 18.61A_{649} - 3.960A_{665}$

$C_{x+c} = 1000A_{480} - 2.27C_a - 81.4C_b/227$

Where, $C_a$ is Chlorophyll a (mg/ml), $C_b$ is Chlorophyll b (mg/ml), $C_{x+c}$ are total carotenoids (mg/ml) and A is the absorbance at 649, 665 and 480 wavelengths.

### 4.2.5 Statistical data analysis

Growth parameters (leaf four length and growth rate) of sweetpotato plants and leaf pigment content (chlorophyll a, chlorophyll b, total chlorophyll a+b, total

carotenoids, chlorophyll a/b; total chlorophyll/total carotenoids and total carotenoids/ total chlorophyll ratios of the selected genotypes) from *in vitro* experiments were analyzed using ANOVA at 95 % confidence interval. Mean separation was carried out using Tukey's pairwise comparison test at 5 % probability level. Statistical analyses were done using SAS statistical computer software (version 9.1.3).

## 4.3 RESULTS

### 4.3.1 Plant growth analysis

#### 4.3.1.1 Length of leaf four growth analysis

Generally, there was rapid growth at the start of the observation that progressively decreased and leveling off at day thirteen. At the end of the thirteenth day, the highest growth was observed in *Kalamb Nyerere, Kemb10, Mweu mutheki* and *KSP28*. At the end of the same period, the lowest growth was observed in *SPK 013, KSP 20, Kemb 23* and *Jewel* in that order (Figure 4.1)

The remaining genotypes also had medium growth within the same period, irrespective of the genotypes growth habit whether upright growing or crawling type (Figure 4.1).

Analysis of variance of the fifteen tested genotypes showed that, on average at the genotype level, growth of leaf four was significantly different in all the fifteen genotypes tested ($p \leq 0.05$*;* Table 4.2).

Analysis of variance of the average growth rate of the fifteen genotypes showed that the growth rate of *Kalamb nyerere* and *Spk 203* were not significantly different but significantly higher than the rest of the genotypes. *Kemb 23* and *jewel* recorded intermediate average growth rate that was significant different

from all the remaining genotypes (Table 4.2) while *kemb23, kemb 10, Ksp36,* and *Spk013* recorded low average growth rates that was significantly different from the rest of the genotypes. In addition, no significant difference in average growth rate between *Mugande, Ksp20* and *Zambezi* but were significantly different from all the remaining genotypes (Table 4.2).

Overall, it was observed that growth and growth rate were significantly ($P \leq 0.05$) affected by time and genotype used. The interaction between the time and the genotype was highly significant at $P \leq 0.05$ (Appendix 2 B)

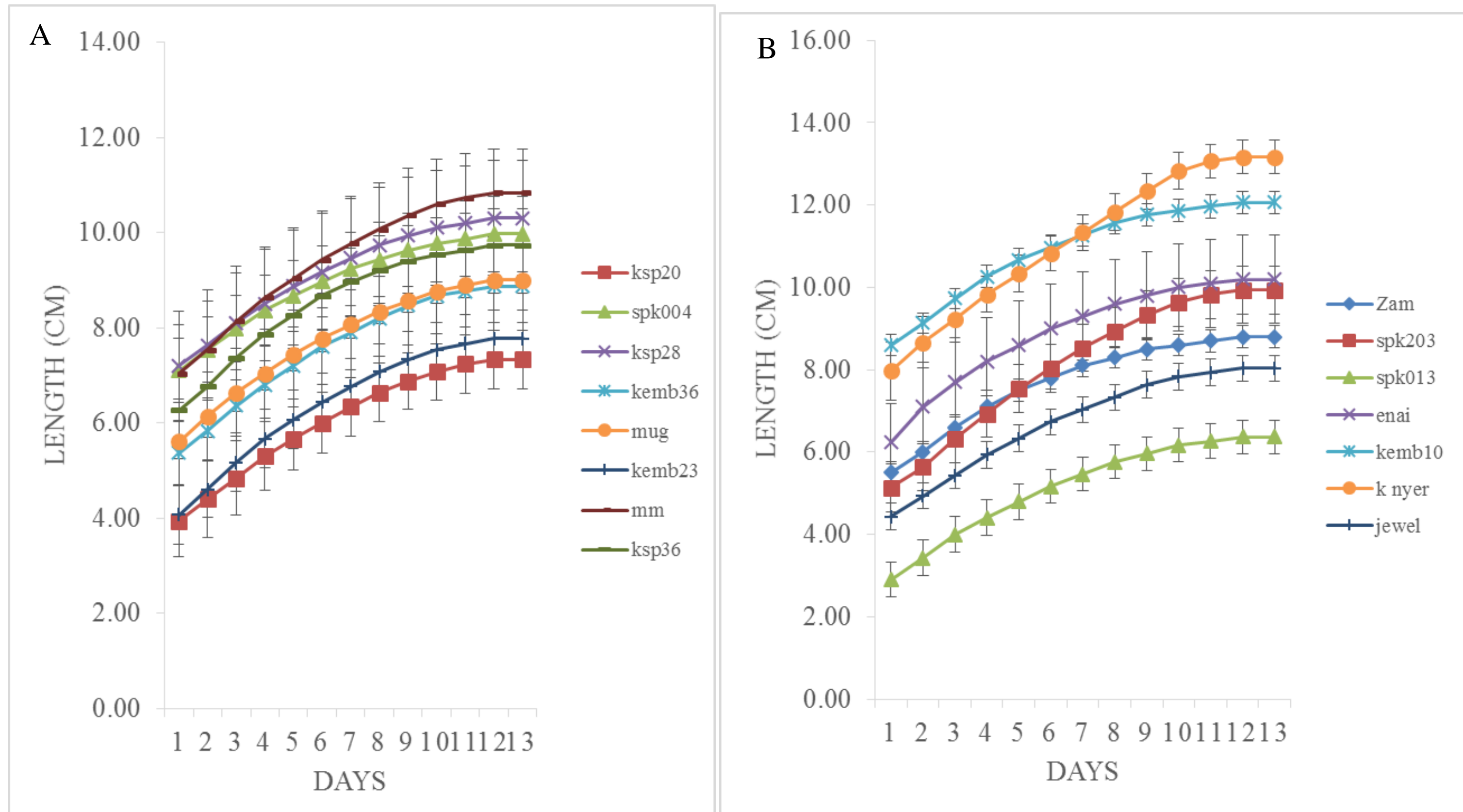


**Figure 4.1: A and B Growth of leaf four length of sweetpotato genotypes**
Values are means and standard error for three measurements of genotypes for a period of thirteen days.

**Table 4.1: Length of leaf four growth summary of fifteen sweetpotato genotypes**

| | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Genoype** | 1 | 3 | 5 | 7 | 9 | 11 | 13 | M |
| ***ksp20*** | 3.93±0.75[a] | 4.83±0.78[a] | 5.67±0.67[a] | 6.33±0.62[a] | 6.87±0.59[a] | 7.23±0.62[a] | 7.33±0.62[a] | 6.07±0.24[gh] |
| ***spk004*** | 7.10±0.67[a] | 7.97±0.70[a] | 8.67±0.74[a] | 9.23±0.78[a] | 9.63±0.78[a] | 9.87±0.80[a] | 9.97±0.80[a] | 8.96±0.23[bc] |
| ***ksp28*** | 7.20±1.15[a] | 8.10±1.20[a] | 8.87±1.23[a] | 9.47±1.23[a] | 9.93±1.22[a] | 10.20±1.21[a] | 10.30±1.21[a] | 9.19±0.32[b] |
| ***kemb36*** | 5.37±0.12[h] | 6.37±0.12[fg] | 7.20±0.12[de] | 7.90±0.12[bc] | 8.47±0.15[ab] | 8.77±0.15[a] | 8.87±0.15[a] | 7.610.19[ef] |
| ***mugande*** | 5.60±0.90[a] | 6.63±0.93[a] | 7.43±0.93[a] | 8.07±0.96[a] | 8.57±0.92[a] | 8.90±0.90[a] | 9.00±0.90[a] | 7.79±0.28[de] |
| ***kemb23*** | 4.07±0.62[c] | 5.17±0.62[abc] | 6.07±0.62[abc] | 6.77±0.58[abc] | 7.33±0.58[ab] | 7.67±0.61[ab] | 7.77±0.61[a] | 6.45±0.24[g] |
| ***MM*** | 7.03±1.02[a] | 8.13±1.02[a] | 9.03±1.02[a] | 9.77±0.98[a] | 10.37±0.98[a] | 10.73±0.92[a] | 10.83±0.92[a] | 9.46±0.30[b] |
| ***ksp36*** | 6.27±0.71[a] | 7.37±0.71[a] | 8.27±0.71[a] | 8.97±0.71[a] | 9.40±0.74[a] | 9.63±0.77[a] | 9.73±0.77[a] | 8.57±0.25[bcd] |
| ***Zam*** | 5.50±0.26[e] | 6.60±0.26[cde] | 7.50±0.26[abc] | 8.10±0.26[ab] | 8.50±.26[a] | 8.70±0.26[a] | 8.80±0.26[a] | 7.72±0.18[de] |
| ***spk203*** | 5.13±0.58[e] | 6.33±0.58[cde] | 7.53±0.58[abcde] | 8.53±0.58[abcd] | 9.33±0.58[ab] | 9.83±0.58[ab] | 9.93±0.58[a] | 8.13±0.29[cde] |
| ***spk013*** | 2.90±0.42[d] | 4.00±0.44[bcd] | 4.80±0.44[abcd] | 5.47±0.41[abc] | 5.97±0.41[ab] | 6.27±0.41[a] | 6.37±0.41[a] | 5.16±0.21[h] |
| ***enaironi*** | 6.23±0.95[a] | 7.70±1.07[a] | 8.60±1.07[a] | 9.30±1.07[a] | 9.80±1.07[a] | 10.10±1.07[a] | 10.20±1.07[a] | 8.93±0.32[bc] |
| ***kemb10*** | 8.60±0.26[f] | 9.73±0.24[def] | 10.67±0.27[bcd] | 11.27±0.27[abc] | 11.77±0.27[ab] | 11.97±0.27[ab] | 12.07±0.27[a] | 10.92±0.19[a] |
| ***k.nyer*** | 7.97±0.71[f] | 9.23±0.55[def] | 10.33±0.44[bcdef] | 11.33±0.44[abcd] | 12.33±0.44[ab] | 13.07±0.41[a] | 13.17±0.41[a] | 11.12±0.30[a] |
| ***jewel*** | 4.43±0.32[f] | 5.43±0.32[def] | 6.33±0.32[bcde] | 7.03±0.32[abcd] | 7.63±0.32[ab] | 7.93±0.32[ab] | 8.03±0.32a | 6.74±0.21[gf] |

Values are means and standard error of three daily measurements of the each genotypes. Means with the same letters in each column are not significantly different from each other (Tukey's test; $p \leq 0.05$). M -overall mean growth rate over the growth period for the genotype

**Table 4.2: Length of leaf four growth rates summary of fifteen selected Kenyan sweetpotato genotypes**

| | | | | **Days** | | | | |
|---|---|---|---|---|---|---|---|---|
| **Days** | 1 | 3 | 5 | 7 | 9 | 11 | 12 | Average |
| ***Ksp20*** | 0.47±0.07$^{a}$ | 0.47±0.07$^{a}$ | 0.33±0.03$^{abc}$ | 0.30±0.00$^{abcd}$ | 0.20±0.00$^{cd}$ | 0.10±0.00$^{de}$ | 0.00±0.00$^{e}$ | 0.28±0.03$^{de}$ |
| ***Spk004*** | 0.43±0.03$^{a}$ | 0.40±0.06$^{a}$ | 0.30±0.00$^{ab}$ | 0.20±0.00$^{bc}$ | 0.13±0.03$^{c}$ | 0.11±0.01$^{cd}$ | 0.00±0.00$^{d}$ | 0.24±0.02$^{f}$ |
| ***Ksp28*** | 0.43±0.03$^{a}$ | 0.40±0.00$^{ab}$ | 0.31±0.01$^{bcd}$ | 0.27±0.03$^{cde}$ | 0.17±0.03$^{ef}$ | 0.11±0.01$^{fg}$ | 0.00±0.00$^{g}$ | 0.26±0.02$^{ef}$ |
| ***Kemb36*** | 0.47±0.03$^{ab}$ | 0.43±0.03$^{ab}$ | 0.40±0.00$^{bc}$ | 0.30±0.00$^{cde}$ | 0.20±0.00$^{ef}$ | 0.11±0.01$^{f}$ | 0.00±0.00$^{g}$ | 0.30±0.03$^{cde}$ |
| ***Mugande*** | 0.53±0.03$^{a}$ | 0.40±0.00$^{ab}$ | 0.33±0.03$^{bc}$ | 0.27±0.03$^{bcd}$ | 0.20±0.06$^{cde}$ | 0.10±0.00$^{ef}$ | 0.00±0.00$^{f}$ | 0.29±0.03$^{de}$ |
| ***Kemb23*** | 0.53±0.03$^{a}$ | 0.51±0.01$^{ab}$ | 0.37±0.03$^{cd}$ | 0.31±0.01$^{cde}$ | 0.20±0.00$^{ef}$ | 0.11±0.01$^{fg}$ | 0.00±0.00$^{g}$ | 0.31±0.03$^{bcd}$ |
| ***M. Mutheki*** | 0.50±0.00$^{b}$ | 0.50±0.00$^{b}$ | 0.41±0.01$^{c}$ | 0.31±0.01$^{de}$ | 0.23±0.03$^{e}$ | 0.11±0.01$^{f}$ | 0.00±0.00$^{g}$ | 0.32±0.03$^{bc}$ |
| ***Ksp36*** | 0.51±0.01$^{b}$ | 0.51±0.01$^{b}$ | 0.40±0.00$^{c}$ | 0.23±0.03$^{de}$ | 0.13±0.03$^{fg}$ | 0.11±0.01$^{g}$ | 0.00±0.00$^{h}$ | 0.29±0.03$^{cde}$ |
| ***Zambezi*** | 0.51±0.01$^{b}$ | 0.51±0.01$^{b}$ | 0.31±0.01$^{d}$ | 0.20±0.00$^{e}$ | 0.10±0.00$^{f}$ | 0.10±0.00$^{f}$ | 0.00±0.00$^{g}$ | 0.28±0.03$^{de}$ |
| ***Spk203*** | 0.51±0.01$^{c}$ | 0.60±0.00$^{b}$ | 0.50±0.00$^{c}$ | 0.40±0.00$^{d}$ | 0.30±0.00$^{e}$ | 0.11±0.01$^{g}$ | 0.00±0.00$^{h}$ | 0.41±0.03$^{a}$ |
| ***Spk013*** | 0.53±0.03$^{a}$ | 0.40±0.00$^{b}$ | 0.37±0.03$^{bc}$ | 0.30±0.00$^{c}$ | 0.20±0.00$^{d}$ | 0.11±0.01$^{e}$ | 0.00±0.00$^{f}$ | 0.29±0.03$^{cde}$ |
| ***Enaironi*** | 0.87±0.12$^{a}$ | 0.50±0.00$^{bc}$ | 0.40±0.00$^{cd}$ | 0.30±0.00$^{de}$ | 0.20±0.00$^{ef}$ | 0.11±0.01$^{fg}$ | 0.00±0.00$^{g}$ | 0.34±0.04$^{b}$ |
| ***Kemb10*** | 0.53±0.03$^{a}$ | 0.53±0.03$^{a}$ | 0.30±0.00$^{c}$ | 0.31±0.01$^{c}$ | 0.10±0.00$^{e}$ | 0.10±0.00$^{e}$ | 0.00±0.00$^{f}$ | 0.29±0.03$^{cde}$ |
| ***K. Nyerere*** | 0.70±0.10$^{a}$ | 0.60±0.12$^{a}$ | 0.51±0.01$^{a}$ | 0.50±0.00$^{a}$ | 0.50±0.00$^{a}$ | 0.11±0.01$^{b}$ | 0.00±0.00$^{b}$ | 0.44±0.04$^{a}$ |
| ***Jewel*** | 0.50±0.00$^{a}$ | 0.51±0.01$^{a}$ | 0.40±0.00$^{b}$ | 0.31±0.01$^{c}$ | 0.20±0.00$^{d}$ | 0.11±0.01$^{e}$ | 0.00±0.00$^{f}$ | 0.31±0.03$^{bcd}$ |

The figures represent means and standard error of three daily growth rate of each genotypes. Means with the same letters in each column are not significantly different from each other (Tukey's test; $p\leq0.05$).

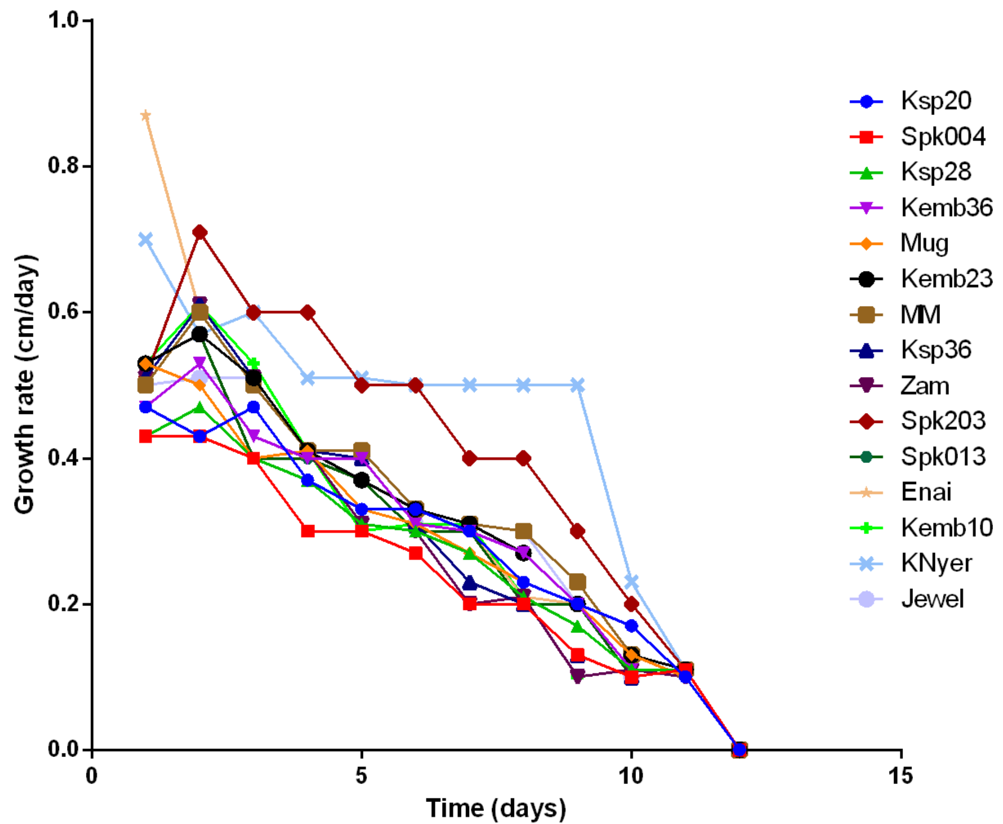


**Figure 4.2: Growth rate summary showing high growth rate at the start of measurement decreasing to zero at the end of observation**

### 4.3.2 In vitro analysis of photosynthetic pigments

#### 4.3.2.1 Effect of *in vitro* osmotic stress on chlorophyll a and b

In the control leaf disc assay experiment, the genotypes with the highest amount of chlorophyll a were *Kemb 10* (15.97), then *Mugande* (15.83) and *Kalamb Nyerere* (15.79) while those with the lowest amounts were *Kemb 23* (13.52), *Ksp 20* (13.99) and *Ksp 36* (14.61) in that order (Figure 4.3A and B). Analysis of variance for the controls showed significant difference among the individual genotypes tested ($P \leq 0.05$).

Results show that when leaf discs were subjected to moderate and high *in vitro* osmotic stress assays the amount of chlorophyll a decreased from a high of 16.20 (*Spk004*) in low osmotic stress to 14.53 (*Ksp 36*) and 14.36 (*Zambezi*) in moderate and high osmotic stress respectively(Figure 4.3 A and B).

In terms of chlorophyll a deterioration, it ranged from +1.44% to -69.07% in all the levels of *in vitro* osmotic stress such that the least amount of chlorophyll a was in high *in vitro* osmotic stress 4.94 (*Kemb 10*), then in moderate osmotic stress 5.53 (*Kemb 36*) and finally 10.17 (*Kemb 36*) in low osmotic stress (Figure 4.3 A and B). *Ksp 36* was the most tolerant genotype in *vitro* osmotic stress leaf disc assays whereas the most sensitive genotype was *Kemb 36* for all the three levels. However all the genotypes showed the highest deterioration of chlorophyll a under high *in vitro* osmotic stress leaf disc assays (Figure 4.3 A).

Generally the amount of chlorophyll a was significantly affected at all osmotic stress levels (low, moderate and high), $P \leq 0.05$. Equally, the interaction between genotype and all levels of *in vitro* osmotic treatment was highly significant ($P \leq 0.05$ (Appendix 3A and B).

The amount of chlorophyll b in sweetpotato leaf discs under *in vitro* osmotic stress varied depending on the level of *in vitro* osmotic stress applied. In the control experiment the genotypes with the highest amount of chlorophyll b were *Kemb 10* (18.32), *Zambezi* (18.18) and *Spk 004* (18.12). The genotypes with the lowest amount of chlorophyll b on the other hand were *Enaironi* (10.43), *Kemb 36* (10.47) and *Kemb 23* (11.01) (Figure 4.4 A and B). Analysis of variance showed no significance difference among the control genotypes tested ($P \leq 0.05$).

Under *in vitro* osmotic stress the tolerant genotypes with the highest amount of chlorophyll b shows a downward trend in the three levels of stress comparatively. The highest amount of chlorophyll b was 14.92 (*Zambezi*) in low osmotic stress, then 12.03 (*Ksp 36*) in moderate stress and finally 10.43 (*Ksp 36*) in high osmotic stress.

Comparative analysis of the sensitive genotypes, with the lowest amount of chlorophyll b, showed a decreasing trend in the three salt stress levels. The lowest amount of chlorophyll b was 1.79 (*Kalamb Nyerere*) in high osmotic stress, 3.40 (*Kemb 10*) in moderate stress and lastly 5.48 (*Kemb 36*) in low osmotic stress (Figure 4.4A and B).

High *in vitro* osmotic stress exerted highest deteriorating effect on the amount of chlorophyll b in all the genotypes tested resulting in very low amounts of chlorophyll b. The amounts were *Kalamb Nyerere* (1.79), *Enaironi* (2.91) and *Spk 013* (3.21) respectively (Figure 4.4 A and B). On the basis of the amount of chlorophyll b, the most tolerant genotypes under *in vitro* osmotic stress leaf disc assays were *Zambezi* and *Ksp 36*.

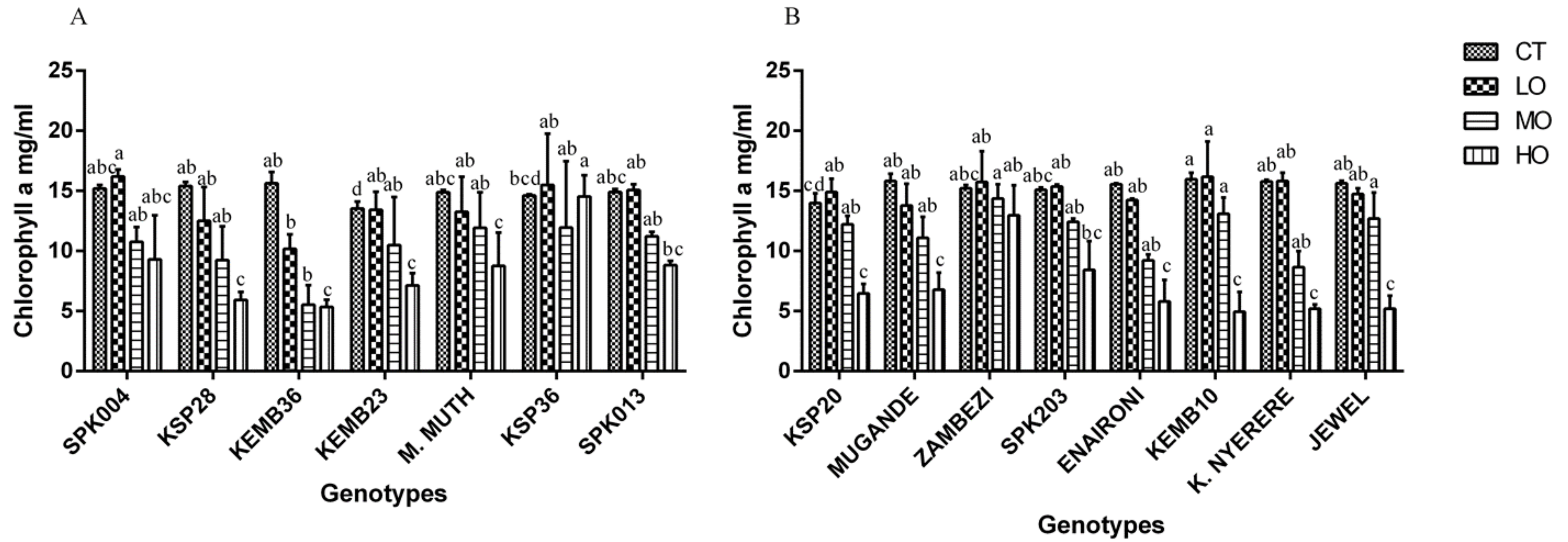


**Figure 4.3: A and B Effect of osmotic stress on the amount of chlorophyll a compared to control**
Bars are means of three measurements and standard errors of treatment and control for each genotype. Means with same letters in the same column have no significant difference (Tukey's test; $p \leq 0.05$). CT-Control; LO- low osmotic stress; MO- Moderate stress; HO – High Osmotic stress.

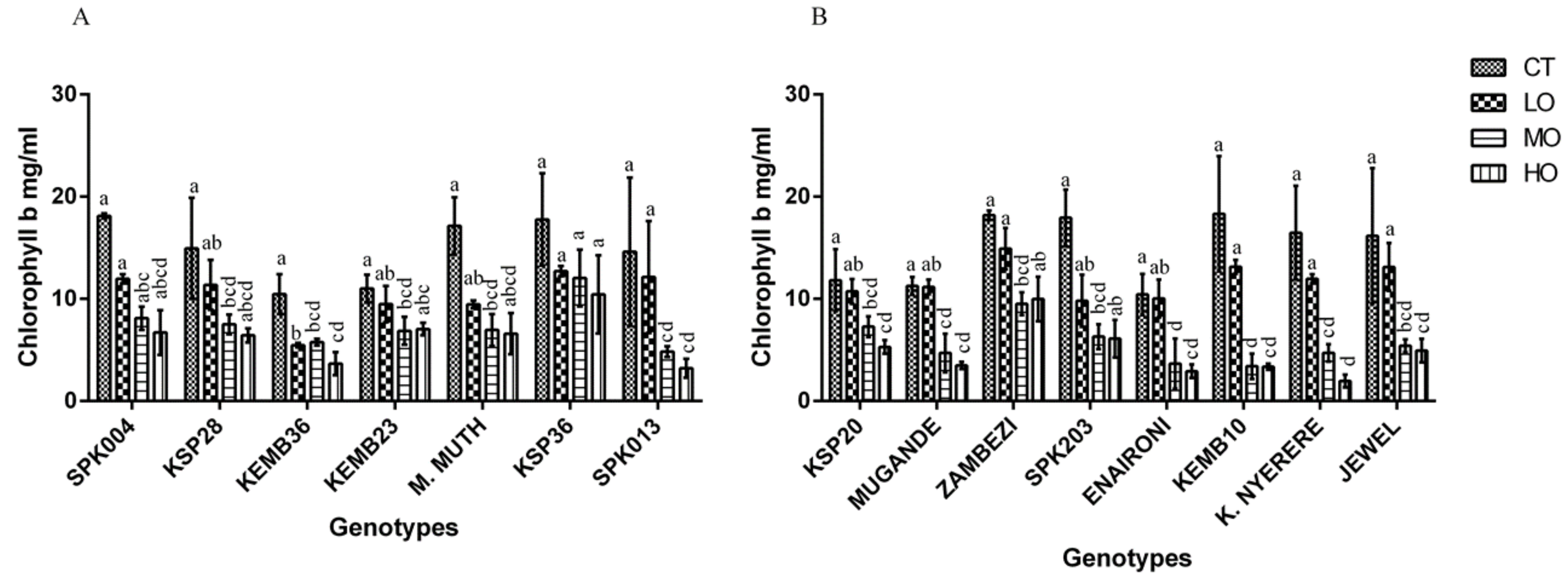


**Figure 4.4 A and B Effect of osmotic stress on the amount of chlorophyll b compared to control**

Bars are means of three measurements and standard errors of treatment and control for each genotype. Means with same letters in the same column have no significant difference (Tukey's test; $p \leq 0.05$). CT-Control; LO- Low osmotic stress; MO- Moderate osmotic stress; HO – High osmotic stress.

**4.3.2.2 Effect of *in vitro* salt stress on chlorophyll a and b**

The sensitivity of chlorophyll a to *in vitro* salt stress leaf disc assays varied depending on the concentration of salt used compared to the control experiment in all genotypes tested.

Results from *in vitro* salt stress assays indicate that the highest amount of chlorophyll a was 17.48 (*Zambezi*) in low salt stress, 16.02 (*Zambezi*) in moderate stress and 14.90 (*Ksp 36*) in high salt stress. In contrast, comparative analysis of the least amount of chlorophyll a, shows that the most sensitive genotype was *Spk 013* (2.67) in high salt stress, then *Kemb 36* (8.17) and (9.33) in both moderate and low salt stress (Figure 4.5 A and B).

The highest deterioration of chlorophyll a in any given genotype and treatment was observed under high *in vitro* salt stress leaf disc assays. Two (2) genotypes; *Zambezi* and *Ksp 36* performed exceptionally well in moderate and high *in vitro* salt stress. The best overall tolerant sweetpotato genotype under *in vitro* salinity stress was *Ksp 36* (Figure 4.5A and B).

Analysis of variance indicated that *in vitro* salt stress affected the chlorophyll a levels significantly ($P \leq 0.05$) under in vitro salt treatment. The interaction between salt treatment and genotype was significant at $P \leq 0.05$ probability level using Turkeys' means separation (Appendix 3A).

*In vitro* salt stress assays on sweetpotato reveals that the amount of chlorophyll b depended largely on the level of stress and the genotype used. Data from *in vitro* salt stress shows a mixed trend in the highest amount of chlorophyll b in the three salt stress levels. The highest amount was 13.42 (*Zambezi)* in low stress, 15.13

(*Ksp 36*) for moderate stress level and 14.19 (*Ksp 36*) for high salt stress level (Figure 4.6 A and B).

Moderate *in vitro* salt stress leaf disc assays revealed the genotypes with the highest levels of chlorophyll b were *Ksp 36* (15.13), *Spk 203* (13.47) and *Zambezi* (12.76) correspondingly. The genotypes with the lowest amount of chlorophyll b in the same salt stress level were *Spk 013* (2.46), *Kalamb Nyerere* (3.08) and *Enaironi* (3.08) respectively (Figure 4.6 A and B).

It was also observed that the sensitivity of the genotypes showed a downward trend in the three levels of salt stress. The lowest amounts was 1.67 (*Kalamb Nyerere*) in high salt stress, 2.46 (*Spk 013*) in moderate salt stress and finally 3.97 (*Spk 013*) in low salt stress. Additionally, from comparative analysis based on the effect of salt stress on chlorophyll b, the most salt tolerant genotypes were *Ksp 36* and *Zambezi* (Figure 4.6 A and B).

Analysis of variance revealed that the amount of chlorophyll b is affected significantly ($P \leq 0.05$) by salt stress. Again the interaction between salt treatment and genotype was highly significant at $P \leq 0.05$ probability level based on Turkey's' mean separation (Appendix 3A).

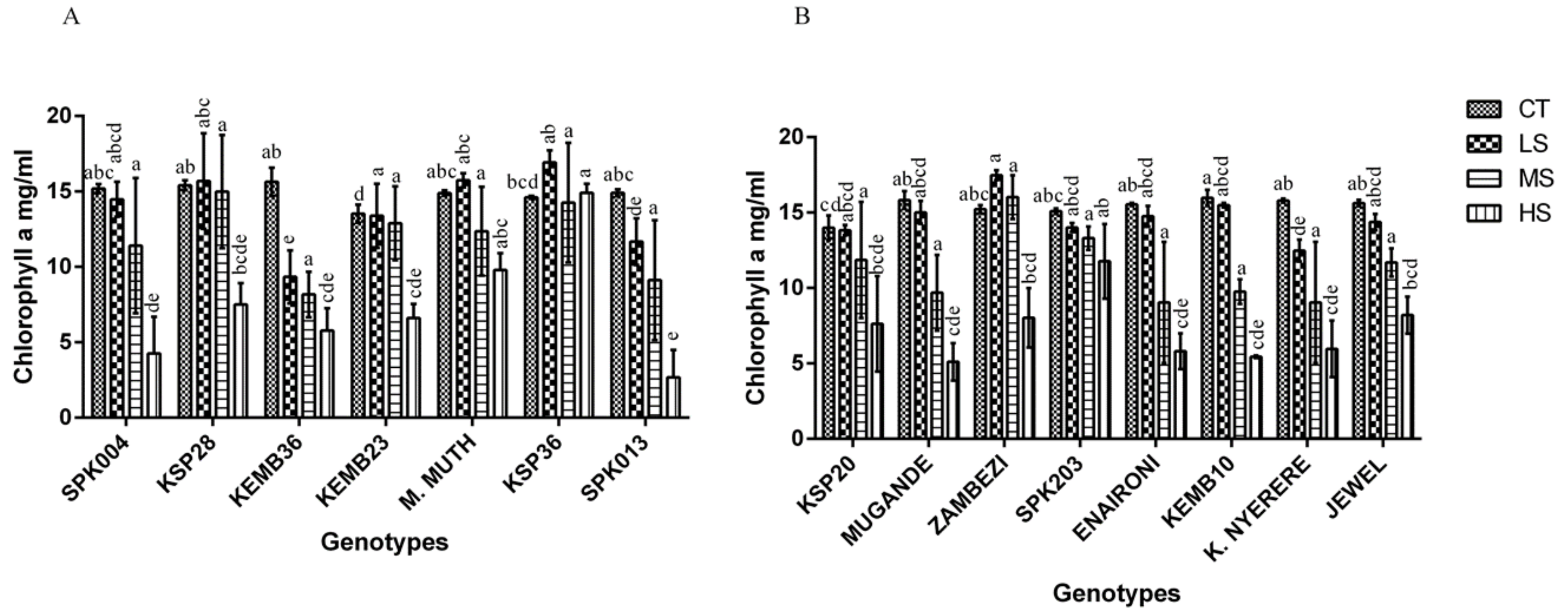


**Figure 4.5: A and B Effect of *in vitro* salt stress on the amount of chlorophyll a as compared to control**

Bars represent means of three replicate and standard error of treatment and control genotypes. Means with same letters in the same column have no significant difference (Tukey's test; $p \leq 0.05$). CT-Control; LS- Low salt stress; MS- Moderate salt stress; HS – High salt stress.

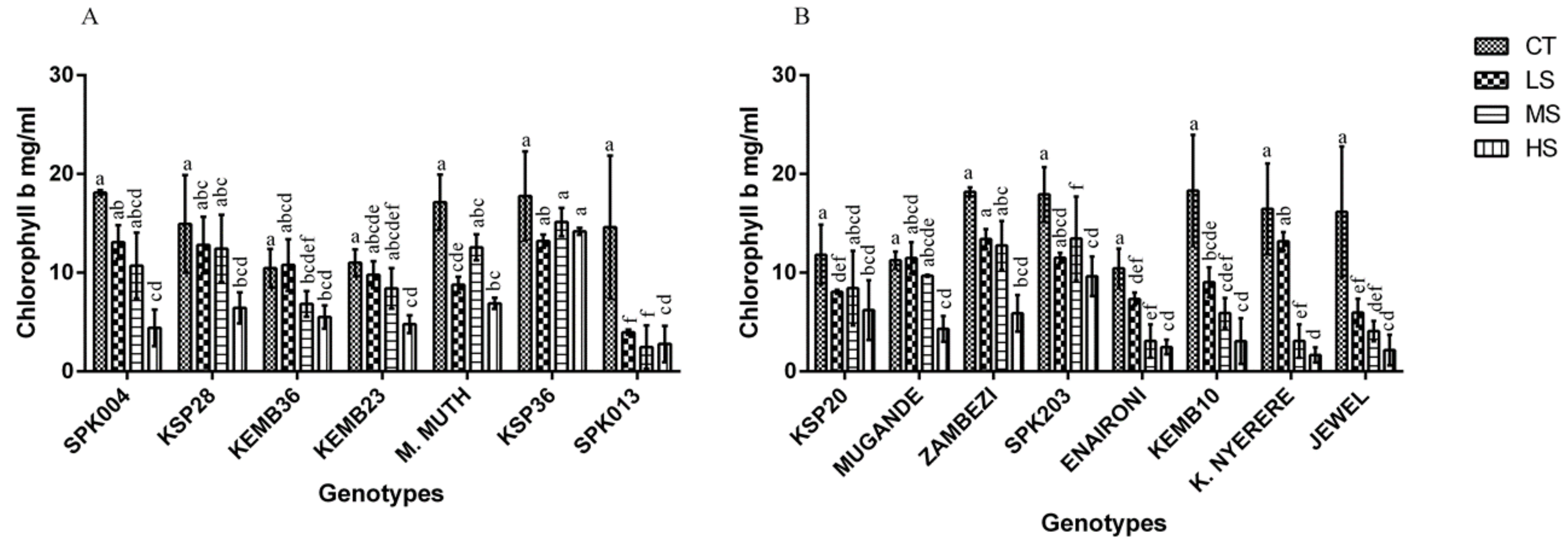


**Figure 4.6: A and B Effect of *in vitro* salt stress on the amount of chlorophyll b compared to control**

Bars are means of three measurements and standard errors of treatment and control for each genotype. Means with same letters in the same column have no significant difference (Tukey's test; $p \leq 0.05$). CT-Control; LS- Low salt stress; MS- Moderate salt stress; HS – High salt stress

**4.3.2.3 Effect of *in vitro* osmotic and salinity stress on total chlorophyll**

*In vitro* leaf disc osmotic and salt stress assays affect the total chlorophyll in similar ways to the effect on chlorophyll a and b as previously reported. Results indicate that the total chlorophyll variation in the control experiment occurs due to time factor and the genetic makeup. Therefore, the highest amount of total chlorophyll was 34.30 (*Kemb 10*), 33.41 (*Zambezi*) and 33.31 (*Spk 004*) in that order. The genotypes with the lowest chlorophyll amount were *Kemb 23* (24.53), *Ksp 20* (25.81) and 25.97 (*Enaironi*) respectively (Table 4.3).

It was observed that *in vitro* leaf disc osmotic stress assays had a mixed trend in the highest amount of total chlorophyll in the three levels of stress comparatively. Interestingly, a downward trend was observed in the highest amount of total chlorophyll in the three levels of *in vitro* salt stress.

Results show that the highest amount of total chlorophyll was 30.65 (*Zambezi*) in low osmotic stress, moderate osmotic stress had 23.98 (*Ksp 36*) and finally it was 24.91 (*Ksp 36*) in high osmotic stress. On the other hand the highest amount of total chlorophyll was 30.90 (Zambezi) in low salt stress, 29.39 (*Ksp 36*) for moderate salt stress and lastly 29.09 (*Ksp 36*) in high salt stress (Table 4.3).

Data showed a downward trend in the lowest amount of total chlorophyll for both the *in vitro* salt and osmotic stress levels. The lowest amount of total chlorophyll were 15.65 (*Kemb 36*), 11.30 (*Kemb 36*) and 7.17 (*Kalamb Nyerere*) for low, moderate and high *in vitro* osmotic stress levels respectively. Likewise, the lowest amount of total chlorophyll were 15.65 (*Spk 203*), 11.58 (*Spk 013*) and 5.45 (*Spk*

*013*) for low, moderate and high *in vitro* salt stress levels correspondingly (Table 4.3).

Analysis of variance showed that total chlorophyll was affected by both osmotic and salt stress significantly (P≤0.05) in all the genotypes tested. Equally, the interaction between genotype and treatment was highly significant at P≤0.05 probability level based on Turkeys' mean separation (Appendix 3A and B).

**4.3.2.4 Effect of osmotic and salt stress on chlorophyll a/b ratio**

Results from *in vitro* leaf disc assays of the control show that chlorophyll a/b ratio was affected by the combined levels of the chlorophyll a and b. Generally the ratios shows that the genotypes with highest amount of total chlorophyll had lower chlorophyll a/b ratios and vice versa. As an illustration, data shows that the highest total chlorophyll amount was 34.30 (*Kemb 10*) and 33.41 (*Zambezi*) with a chlorophyll a/b ratio of 0.95 and 0.84 respectively. Again, the lowest amounts of total chlorophyll were 24.53 (*Kemb 23*) and 25.97 (*Enaironi*) with a chlorophyll a/b ratio of 1.25 and 1.53 respectively (Table 4.4).

The same similarity was observed in all the levels of *in vitro* osmotic and salt leaf disc assays. Results from the three levels of *in vitro* osmotic assays shows that the highest amount of total chlorophyll was 30.65 (*Zambezi*) and 29.31 (*Kemb 10*) with 1.06 and 1.23 chlorophyll a/b ratios in low osmotic level; 23.98 (*Ksp 36*) and 23.88 (*Zambezi)* which had 0.96 and 0.83 ratios in moderate osmotic stress and finally 24.96 (*Ksp 36*) and 22.97 (*Zambezi*) with 0.96 and 1.51 chlorophyll a/b ratios in high osmotic stress (Table 4.4)

**Table 4.3: Effect of *in vitro* salt and osmotic stress on total chlorophyll compared to control**

| Genotype | Control | Osmotic stress | | | Salt stress | | |
|---|---|---|---|---|---|---|---|
| | | Low | moderate | High | Low | Moderate | High |
| ***KSP 20*** | 25.81±1.29$^{a}$ | 25.64±0.63$^{a}$ | 19.49±0.72$^{ab}$ | 11.77±0.27$^{bcd}$ | 21.89±0.29$^{cde}$ | 20.30±1.43$^{abcd}$ | 13.84±0.54$^{cd}$ |
| ***SPK 004*** | 33.31±0.04$^{a}$ | 28.16±0.54$^{a}$ | 18.85±1.35$^{abc}$ | 16.01±1.16$^{b}$ | 27.58±1.38$^{abc}$ | 22.13±4.52$^{abcd}$ | 8.67±0.42$^{de}$ |
| ***KSP28*** | 30.36±2.66$^{a}$ | 23.88±2.46$^{ab}$ | 16.75±2.07$^{bcde}$ | 12.35±0.76$^{bcd}$ | 28.50±2.19$^{ab}$ | 27.44±3.19$^{a}$ | 13.94±1.73$^{cd}$ |
| ***KEMB 36*** | 26.10±1.45$^{a}$ | 15.65±0.83$^{b}$ | 11.30±0.83$^{e}$ | 9.00±0.99$^{cd}$ | 20.12±2.51$^{de}$ | 15.00±1.62$^{cd}$ | 11.29±1.37$^{cde}$ |
| ***Mugande*** | 27.09±0.54$^{a}$ | 24.96±1.28$^{a}$ | 15.79±0.69$^{bcde}$ | 10.27±0.78$^{bcd}$ | 26.49±1.25$^{abcd}$ | 19.36±1.41$^{abcd}$ | 9.42±0.47d$^{e}$ |
| ***KEMB 23*** | 24.53±0.44$^{a}$ | 22.92±0.86$^{ab}$ | 17.36±0.34$^{bcd}$ | 14.19±0.94$^{bc}$ | 23.18±1.78$^{bcd}$ | 21.30±2.19$^{abcd}$ | 11.39±0.72$^{cde}$ |
| ***M.Mutheki*** | 32.02±1.55$^{a}$ | 22.71±1.68$^{ab}$ | 18.88±0.81$^{abc}$ | 15.36±1.52$^{b}$ | 24.52±0.74$^{abcd}$ | 24.93±2.43$^{abc}$ | 16.69±0.64$^{bc}$ |
| ***KSP 36*** | 32.38±2.55$^{a}$ | 28.19±2.19$^{a}$ | 23.98±1.43$^{a}$ | 24.96±3.19$^{a}$ | 30.12±0.83$^{a}$ | 29.39±3.11$^{a}$ | 29.09±0.41$^{a}$ |
| ***Zambezi*** | 33.41±0.30$^{a}$ | 30.65±2.15$^{a}$ | 23.88±1.32$^{a}$ | 22.97±1.48$^{a}$ | 30.90±0.76$^{a}$ | 28.78±2.26$^{a}$ | 13.91±2.20$^{cd}$ |
| ***SPK 203*** | 33.02±1.51$^{a}$ | 25.18±1.56$^{a}$ | 18.73±0.69$^{abcd}$ | 14.53±0.35$^{bc}$ | 25.52±0.41$^{abcd}$ | 26.77±2.02$^{ab}$ | 21.41±2.54$^{b}$ |
| ***SPK013*** | 29.53±4.25$^{a}$ | 27.22±3.42$^{a}$ | 16.05±0.50$^{bcde}$ | 12.03±0.34$^{bcd}$ | 15.65±0.91$^{e}$ | 11.58±1.07$^{d}$ | 5.45±0.29d$^{e}$ |
| ***Enaironi*** | 25.97±1.17$^{a}$ | 24.28±1.11$^{ab}$ | 12.88±1.37$^{de}$ | 8.72±1.01$^{cd}$ | 22.11±0.59$^{bcd}$ | 12.12±1.70$^{d}$ | 8.27±0.27d$^{e}$ |
| ***KEMB 10*** | 34.30±2.95$^{a}$ | 29.31±1.55$^{a}$ | 16.49±1.48$^{bcde}$ | 8.30±1.12$^{cd}$ | 24.52±0.94$^{abcd}$ | 15.68±0.41$^{bcd}$ | 8.5±1.05$^{de}$ |
| ***K. Nyerer*** | 32.25±2.66$^{a}$ | 27.79±0.66$^{a}$ | 13.38±0.33$^{cde}$ | 7.17±0.34$^{d}$ | 25.66±0.55$^{abcd}$ | 12.12±1.70$^{d}$ | 7.63±1.17$^{de}$ |
| ***Jewel*** | 31.78±3.72$^{a}$ | 27.87±1.59$^{a}$ | 18.09±1.39$^{abcd}$ | 10.14±0.16$^{bcd}$ | 20.32±0.85$^{de}$ | 15.77±0.93$^{bcd}$ | 10.37±0.38$^{de}$ |
| **LSD** | 11.39 | 8.80 | 5.90 | 6.28 | 6.43 | 11.63 | 6.13 |

Values are means and standard errors of 3 replicates for each treatment and control. Means with same letters in each column indicate non-significant difference from one another at P≤0.05 probability level using Turkeys' mean separation

Equally, the lowest amount of total chlorophyll was 15.65 (*Kemb 36*) and 22.72 (*Mweu mutheki*) with 1.85 and 1.32 chlorophyll a/b ratios in low osmotic stress; the values were 12.88 (*Enaironi*) and 13.38 (*Kalamb nyerere*) which had 3.82 and 1.92 chlorophyll a/b ratio in moderate osmotic stress lastly the values were 7.17 (*Kalamb nyerere*) and 8.72 (*Enaironi*) with 2.90 and 3.82 chlorophyll a/b ratio in high osmotic stress (Table 4.4).

The same was observed for the three levels of salt stress. Among the highest ratios for chlorophyll a/b were 2.51 (*Jewel*) and 2.01 (*Spk 203*) with 20.32 and 15.65 total chlorophyll in low salt stress; the values were 5.49 (*Spk 013*) and 3.07 (*Kalamb Nyerere*) which had 11.58 and 12.12 total chlorophyll in moderate salt stress level and lastly the values were 2.58 (*Enaironi*) and 2.07 (*Spk 013*) with 8.27 and 5.45 amount of total chlorophyll in high salt stress level (Table 4.4).

The lowest amount of chlorophyll a/b ratios were 1.22 (*ksp 28*) and 1.51 (*Zambezi*) with 28.50 and 30.90 total chlorophyll in low salt stress level; they were 0.93 (*Ksp 36*) and 1.25 (*Ksp 28*) with 29.39 and 27.44 total chlorophyll in moderate salt stress level and 1.05 (*Ksp 36*) and 1.42 (*Mweu Mutheki*) which had 29.09 and 16.69 total chlorophyll in high salt stress level (Table 4.4).

**Table 4.4: Effect of *in vitro* salt and osmotic stress on the chlorophyll a/b ratio compared to control**

| Genotype | Control | Osmotic stress | | | Salt stress | | |
|---|---|---|---|---|---|---|---|
| | | low | moderate | High | Low | Moderate | High |
| ***KSP 20*** | 1.25±0.21[a] | 1.41±0.14[a] | 1.70±0.14[ab] | 1.25±0.17[ab] | 1.72±0.02[cde] | 1.45±0.09[b] | 1.28±0.10[a] |
| ***SPK 004*** | 0.84±0.02[a] | 1.36±0.02[a] | 1.33±0.05[ab] | 1.36±0.10[ab] | 1.11±0.08[def] | 1.04±0.06[b] | 0.91±0.14[a] |
| ***KSA 28*** | 1.11±0.21[a] | 1.13±0.19[a] | 1.22±0.17[ab] | 0.92±0.02[b] | 1.27±0.21[cdef] | 1.25±0.21[b] | 1.18±0.05[a] |
| ***KEMB 36*** | 1.52±0.13[a] | 1.85±0.09[a] | 0.97±0.19[b] | 1.53±0.22[ab] | 0.87±0.03[f] | 1.20±0.01[b] | 1.05±0.12[a] |
| ***Muganda*** | 1.41±0.08[a] | 1.23±0.09[a] | 2.55±0.44[ab] | 1.97±0.30[ab] | 1.32±0.08[cdef] | 1.00±0.15[b] | 1.19±0.04[a] |
| ***KEMB 23*** | 1.25±0.13[a] | 1.46±0.24[a] | 1.56±0.37[ab] | 1.01±0.03[b] | 1.37±0.12[cdef] | 1.57±0.24[b] | 1.42±0.19[a] |
| ***M.Mutheki*** | 0.89±0.10[a] | 1.41±0.19[a] | 1.77±0.32[ab] | 1.48±0.51[ab] | 1.80±0.06[bcd] | 0.97±0.09[b] | 1.43±0.13[a] |
| ***KSP 36*** | 0.87±0.15[a] | 1.23±0.22[a] | 0.96±0.13[b] | 1.47±0.18[ab] | 1.28±0.02[cdef] | 0.93±0.11[b] | 1.05±0.03[a] |
| ***Zambezi*** | 0.84±0.02[a] | 1.06±0.12[a] | 1.51±0.03[ab] | 1.35±0.24[ab] | 1.31±0.04[cdef] | 1.27±0.09[b] | 1.38±0.05[a] |
| ***SPK 203*** | 0.86±0.08[a] | 1.63±0.22[a] | 2.02±0.21[ab] | 1.54±0.47[ab] | 1.22±0.03[def] | 1.09±0.28[b] | 1.22±0.05[a] |
| ***SPK013*** | 1.30±0.49[a] | 1.49±0.47[a] | 2.33±0.10[ab] | 2.91±0.50[a] | 2.95±0.25[a] | 5.49±2.37[a] | 2.07±1.59[a] |
| ***Enaironi*** | 1.53±0.17[a] | 1.45±0.17[a] | 3.82±1.84[ab] | 1.97±0.10[ab] | 2.02±0.10[bc] | 3.07±0.24[ab] | 2.59±0.70[a] |
| ***KEMB 10*** | 0.95±0.21[a] | 1.24±0.15[a] | 4.09±0.59a | 1.45±0.20[ab] | 1.75±0.16[bcd] | 1.75±0.36[b] | 2.55±0.96[a] |
| ***K. Nyerere*** | 1.01±0.17[a] | 1.32±0.01[a] | 1.92±0.39[ab] | 2.90±0.70[a] | 0.95±0.06[ef] | 3.07±0.24[ab] | 4.15±1.17[a] |
| ***Jewel*** | 1.07±0.21[a] | 1.14±0.10[a] | 2.36±0.24[ab] | 1.13±0.30[b] | 2.51±0.39[ab] | 2.99±0.47[ab] | 6.55±3.72[a] |
| **LSD** | 1.01 | 1.00 | 2.87 | 1.72 | 0.78 | 3.38 | 5.91 |

Values are means and standard errors of 3 replicates for control and treatment. Means followed by the same letter in each column are not significantly different from each other (Tukey's test; $p \leq 0.05$)

#### 4.3.2.4 Effect of *in vitro* osmotic and salinity stress on carotenoids

When leaf discs were subjected to *in vitro* osmotic stress assays, the amount of total carotenoids affected was dependent on the genotype and the level of stress applied.

In the control experiment the genotypes with the highest amount of carotenoids were *Mugande* (4.08), *Enaironi* (3.52) and *Ksp 28* (3.42). The lowest amount of carotenoids was recorded from *Ksp 36* (2.35), *Kemb 23* (2.42) and *Mweu Mutheki* (2.49). (Figure 4.7 A and B)

Data from *in vitro* leaf disc osmotic stress assays showed mixed results in terms of performance in the different osmotic levels. The results show mixed trend in the highest amounts of carotenoids from the three levels of osmotic stress. From the low osmotic stress, the amount was 3.05 (*Spk 203*), then 3.94 (*Spk 203*) in moderate osmotic stress and 3.94 (*Mweu Mutheki*) in high osmotic stress (Figure 4.7A and B).

Comparative analysis of the lowest amount of carotenoids from the three levels of osmotic stress revealed a downward trend. The amount was 0.60 (*Ksp 28*) from high osmotic stress, 0.77 (*Kemb 36*) from moderate osmotic stress and finally 1.54 (*Ksp 28*) from low osmotic stress (Figure 4.7 A and B).

Analysis of variance showed that carotenoids were affected significantly by osmotic stress and that the interaction between treatment and genotype were also highly significant at $P \leq 0.05$ probability level based on Turkeys' mean separation (Appendix 3A)

Data on the performance of sweetpotato genotypes when subjected to *in vitro* salt stress assays revealed a downward trend on the highest amount of carotenoids in the three levels of salt stress. Results shows that the amount was 3.08 (*Ksp 20*) in low salt stress, 2.06 (*Jewel*) from moderate salt stress and lastly 1.96 (*Kemb 10*) from high salt stress (Figure 4.8 A and B).

Results also show that the most sensitive genotypes hence the lowest amount of carotenoids had a downward trend for sensitivity but upward trend for amount in the three levels of salt stress starting from the highest to the lowest. The least amount of carotenoid was 0.15 (*Mugande*) from high salt stress, 0.85 (*Spk 004*) from moderate salt stress and 1.11 (*Kalamb Nyerere*) from low salt stress (Figure 4.8 A and B).

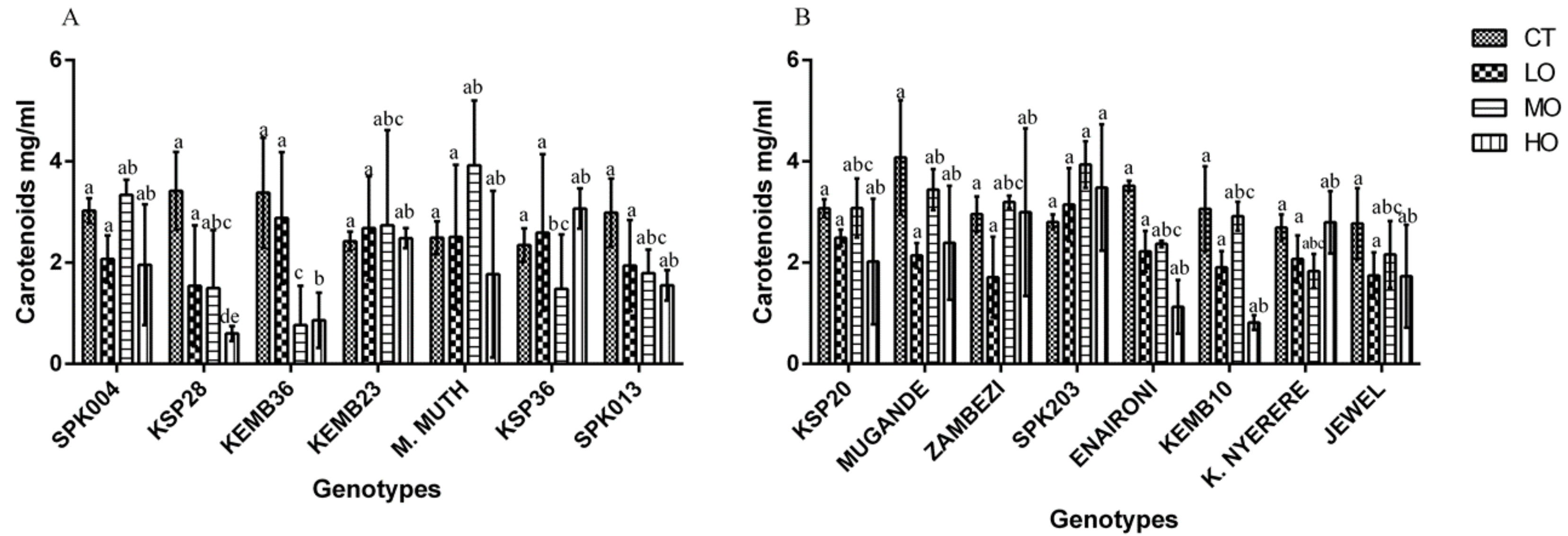


**Figure 4.7: A and B Effect of *in vitro* osmotic stress on carotenoids amount compared to control**

Bars are means of three measurements and standard errors of treatment and control for each genotype. Means with same letters in the same column have no significant difference (Tukey's test; $p \leq 0.05$). CT-Control; LO- Low osmotic stress; MO- Moderate osmotic stress; HO – High osmotic stress.

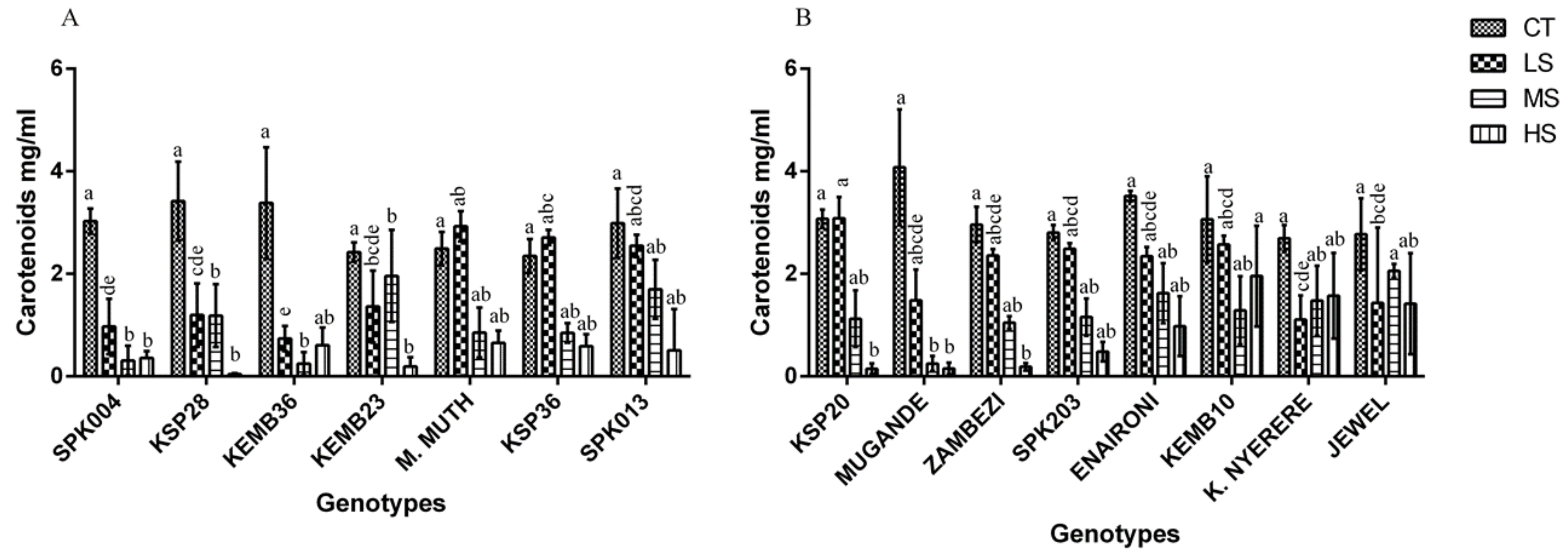


**Figure 4.8: A and B Carotenoids under *in vitro* salinity stress compared to control**

Bars are means of three measurements and standard errors of treatment and control for each genotype. Means with same letters in the same column have no significant difference (Tukey's test; $p \leq 0.05$). CT-Control; LS- Low salt stress; MS- Moderate salt stress; HS – High salt stress.

#### 4.3.2.5 Effect of *in vitro* osmotic and salt stress on carotenoids/ total chlorophyll ratio

The ratio of chlorophyll a/b and carotenoids/total chlorophyll shows similar trends in *in vitro* control, osmotic and salt stress levels. Observation also shows that the genotypes with the highest chlorophyll a/b ratio also had highest carotenoid / total chlorophyll ratio in the different stress levels and vice versa.

Carotenoids/total chlorophyll ratio results shows that the highest values were 0.18 (*Kemb 36*), 0.23 (*Mugande*) and 0.39 (*Kalamb Nyerere*) on the other hand the lowest values were 0.06 (*Zambezi*), 0.06 (*Ksp 36*) and 0.05 (*Ksp 28*) for low, moderate and high levels osmotic stresses respectively (Table 4.5).

When the leaf discs were subjected to *in vitro* salt stress the carotenoids/ total chlorophyll ratio, the highest values were 0.16 (*Spk 203*), 0.16 (*Spk 203*) and 0.14 (*Jewel*) in contrast the lowest values were 0.04 (*Kemb 36*), 0.03 (*Mweu Murtheki*) and 0.04 for low moderate and high salt stress levels in that order (Table 4.5).

**Table 4.5: Effect of *in vitro* salt and osmotic stress on carotenoid/total chlorophyll compared to control**

| Genotype | Control | Osmotic stress | | | Salt stress | | |
|---|---|---|---|---|---|---|---|
| | | Low | moderate | High | Low | Moderate | High |
| *KSP 20* | 0.12±0.01[a] | 0.10±0.01[ab] | 0.16±0.02[abc] | 0.17±0.06[b] | 0.14±0.01[ab] | 0.05±0.00[cd] | 0.01±0.01[b] |
| *SPK 004* | 0.09±0.00[a] | 0.07±0.01[ab] | 0.18±0.02[abc] | 0.11±0.03[b] | 0.04±0.01[d] | 0.01±0.00[d] | 0.04±0.00[b] |
| *KSP 28* | 0.12±0.02[a] | 0.06±0.03[b] | 0.08±0.03[bc] | 0.05±0.01[b] | 0.04±0.01[cd] | 0.04±0.01[cd] | 0.00±0.00[b] |
| *KEMB 36* | 0.13±0.02[a] | 0.18±0.04[a] | 0.06±0.03[c] | 0.10±0.03[b] | 0.04±0.01[d] | 0.02±0.01[d] | 0.05±0.01[ab] |
| *Muganda* | 0.15±0.02[a] | 0.09±0.00[ab] | 0.23±0.04[a] | 0.23±0.05[ab] | 0.06±0.01[cd] | 0.01±0.00[d] | 0.02±0.01[b] |
| *KEMB 23* | 0.10±0.01[a] | 0.12±0.03[ab] | 0.15±0.05[abc] | 0.18±0.02[b] | 0.06±0.02[cd] | 0.09±0.02[abc] | 0.02±0.01[b] |
| *M.Mutheki* | 0.08±0.00[a] | 0.11±0.03[ab] | 0.21±0.04[ab] | 0.11±0.06[b] | 0.12±0.01[abc] | 0.03±0.01[cd] | 0.04±0.01[b] |
| *KSP 36* | 0.07±0.01[a] | 0.09±0.03[ab] | 0.06±0.01[c] | 0.13±0.02[b] | 0.09±0.01[abcd] | 0.03±0.00[cd] | 0.02±0.00[b] |
| *Zambezi* | 0.09±0.01[a] | 0.06±0.01[ab] | 0.14±0.01[ab] | 0.13±0.05[b] | 0.08±0.00[bcd] | 0.04±0.00[cd] | 0.01±0.00[b] |
| *SPK 203* | 0.09±0.01[a] | 0.13±0.02[b] | 0.21±0.02[ab] | 0.24±0.05[ab] | 0.10±0.00[abcd] | 0.04±0.01[cd] | 0.02±0.00[b] |
| *SPK013* | 0.11±0.03[a] | 0.08±0.03[ab] | 0.11±0.02[abc] | 0.13±0.02[b] | 0.16±0.00[a] | 0.16±0.02[a] | 0.09±0.08[ab] |
| *Enaironi* | 0.14±0.01[a] | 0.09±0.01[ab] | 0.19±0.02[abc] | 0.13±0.01[b] | 0.11±0.01[abcd] | 0.14±0.01[ab] | 0.12±0.04[ab] |
| *KEMB 10* | 0.09±0.02[a] | 0.06±0.00[b] | 0.18±0.02[abc] | 0.10±0.01[b] | 0.11±0.01[abcd] | 0.08±0.03[bc] | 0.26±0.10[a] |
| *K. Nyerere* | 0.09±0.01[a] | 0.07±0.01[ab] | 0.14±0.01[abc] | 0.39±0.05[a] | 0.04±0.01[cd] | 0.12±0.01[ab] | 0.20±0.05[ab] |
| *Jewel* | 0.09±0.02[a] | 0.06±0.01[b] | 0.12±0.01[abc] | 0.17±0.06[b] | 0.07±0.05[bcd] | 0.13±0.00[ab] | 0.14±0.06[ab] |
| **LSD** | 0.09 | 0.11 | 0.14 | 0.20 | 0.08 | 0.07 | 0.21 |

Values are means and standard errors of 3 replicates for each treatment and control. Values with same letters in each column are not significantly different from one another at P≤0.05 probability level using Turkey's' mean separation

**4.4 DISCUSSION**

The growth rate potential varies widely in all the plant species. Indeed, the relative primary and secondary growth rate vary from one genotype to another when plants are grown under optimal conditions. In the present study the sweetpotato genotypes with the highest growth rate (*Kalamb nyerere, Spk 203, Enaironi* and *Mweu mutheki*) and those with slowest growth rate (*SPK 004, KSP 28, Zambezi* and *KSP 20*) were identified. The results is consistent with that observed by Olive (2013) and Badu-Apraku *et al*. (2011), who observed lack of uniformity of growth rate in maize genotypes.

The identification of such genotypes is important since high growth rate is an adaptation for plants to survive adverse conditions such as salt stress. Such plants are capable of utilizing resources by having high metabolic activity and rapid growth before the setting in of adverse effects of stress (Sherrard and Maherali, 2006). However, the slow growth rate could be a mechanism by the plant to delay growth and later resume its optimal genetic potential when conditions are favorable (Chaves *et al.*, 2002).

Osmotic and salt tolerance variability either inter or intra specific, is present in almost all plant species (Chaitanya *et al.*, 2003; Wentworth *et al.,* 2006). Chlorophyll a, chlorophyll b, chlorophyll a/b and carotenoid contents in sweetpotato, showed increase and decrease depending on exposure to the level of osmotic and NaCl stress (Doganlar *et al.,* 2010).

The present study revealed different response of sweetpotato genotypes to various levels of mannitol and NaCl concentrations. Higher concentration of NaCl at 200mM/l significantly reduced chlorophyll a, chlorophyll b, total chlorophyll, and

chlorophyll a/b in susceptible genotypes like *Kemb 36*, *Spk 013*and *Kalamb Nyerere* but not with tolerant genotypes such as *Ksp 36, Ksp 28* and *Zambezi.*

These results are in agreement with many reports which show the reduction of photosynthetic pigments amounts in the salt-stressed leaves of different plants such as bean (Parida and Das, 2005), sunflower (Santos, 2004), castor bean (Pinheiro *et al.,* 2008), winter wheat (Zheng *et al.,* 2008), rice (Chaum *et al.,* 2007) and cotton (Meloni *et al.,* 2003).

Besides, increase in pigment content was observed in salinity stressed plant such as Rice (Doganlar *et al.,* 2010) and Purslane (Rahdari *et al.,* 2012), that this increment may be due to increase in the number of chloroplast in the stressed plant leaves (Chaum and Kirdmanee, 2010).

Under salinity stress, the photosynthetic apparatus, especially chloroplast may be damaged by toxic ion, especially sodium ion ($Na^+$). Since, the $Na^+$ can generate the lipid peroxidation in the chloroplast to damage the ultra-structure and chloroplast functions as well as produce reactive oxygen species (ROSs) (Vaidyanathan *et al.,* 2003) to disturb the function of photosynthetic pigments (Demiral and Türkan, 2005).

Photosynthetic pigments present in the photo systems are damaged by the stress factors resulting in reduced light-absorbing efficiency of both photo systems (PSI and PSII) and hence a reduced photosynthetic capacity (Geissler *et al.*, 2009; Zhang *et al.,* 2011). Salt induced weakening of protein-pigment-lipid complex as well as increased chlorophyllase enzyme activity are the causes of pigment content reduction due to salt stress (Turan *et al.,* 2009).

# CHAPTER FIVE

# *IN VIVO* EFFECT OF SALT STRESS ON GROWTH, PIGMENT CONTENT AND HARVEST ON SELECTED KENYAN SWEETPOTATO GENOTYPES

## 5.1 INTRODUCTION

The world's population continues to increase at a tremendous rate such that more food needs to be grown to feed the people. Feeding such a population can be achieved by an increase in cultivated land and an increase in crop productivity per area through the planting of high yielding and drought tolerant crops in drought prone areas. Again, crop production under irrigation can be carried out in arid and semi-arid areas but such practice has the effect of increased salinity to the soils in the long run.

Salinity affects yield and developmental parameters in specific ways to plants due to osmotic and ionic effects of salt solution in the soil (Grieve and Shannon, 1999; Munns, 2002). The reduction in growth and development is partly due to excessive salt absorption, which causes plants to suffer ionic stress, leading to premature leaf aging due to a reduction in the available photosynthetic area to maintain growth (Munns, 2002).

Studies have shown that salt stress tolerant species have increased chlorophyll content than sensitive ones. Thus an accumulation of photosynthetic pigments has been proposed as one of the potential biochemical indicators of salt tolerance in different crops species, for example, in wheat (Raza *et al.,* 2014, Arfan *et al.,* 2007), pea (Noreen *et al.,* 2010), melon (*Cucumis melo*) (Romero *et al.,* 1997),

sunflower (Akram and Ashraf, 2011) and proso millet (*Panicum miliaceum*); (Sabir *et al.,* 2009).

In view of the above, in this chapter *in vivo* salt stress treatment were used to assess the effect on leaf pigments, growth and physiological parameters as well as yield on fifteen selected Kenyan sweetpotato varieties to serve as a criterion for selection.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Plant material

Fourteen locally adapted sweetpotato genotypes selected randomly were obtained from KALRO Biotechnology Center, courtesy of Dr, Nyamongo E. namely *KSP 36, KSP 20, KSP28, KEMB 36, KEMB 10, Kemb 23 Kalamb Nyerere, Mweu Mutheki, Enaironi, Mugande, Zambezi, SPK 004, SPK 013* and*spk203*. The exotic variety *Jewel* was obtained from CIP Nairobi, which was used as model cultivar in all experiments. They were then planted in the green house and served as a source of planting material for the different experiments. Plants were maintained in the greenhouse under natural sunlight; average day/night temperatures were 30±2/26±2°C. (Figure 5.1)

### 3.2.2 *In vivo* salt stress assays

Soil mixed with manure at a ratio of 2:1 was placed in twenty liter plastic pots. Stem cuttings of fifteen genotypes measuring approximately six centimeters were sown individually in each pot containing 10 kg of the soil mixture and arranged in a completely randomized design in triplicate, according to a method described by Amirjani (2013) with slight modifications. The plants were watered daily with 400 ml of tap water and maintained in the natural environment for four weeks, which is the critical vine development and storage root initiation stage. Thereafter, the pots were divided into two groups. In the first group, pots were irrigated with water at 1-day interval, which served as a control while the second group; salt stress experiment, the plants were irrigated at 1-day interval as the control but with 400ml of 10g/l of NaCl solution for 8 weeks.

After the treatment period, the experimental block was allowed to recover for 4 weeks by being watered daily with 400 ml of tap water. The pots were arranged in complete randomized blocks design where the control and the test plants were represented in each block. Three samples were collected from each treatment at 4 week interval for a period of 12 weeks. Immediately after sample collection, growth parameters (vine length and leaf relative water content), leaf chlorophyll and carotenoid content were measured. At the end of the 12 week period, the tubers were harvested and the weight of well-defined tubers was measured in triplicate. Data recorded were the means of the parameters tested in the experimental setup.

**5.2.3 Relative water content (RWC)**

In order to monitor the state of dehydration of the plants, the relative water content (RWC) of the leaves was determined from test treatments and the control sweetpotato genotypes. This parameter was used to determine leaf water status in plants and serves a reflection of the tolerance ability of a plant during salt stress. RWC is measured in terms of fresh weight and dry weight (Beadle *et al.,* 1987). Three leaves from the treatment were randomly selected and the method described by Turner was followed (Turner, 1981).

About 0.35 g leaf sample was cut and weighed using a Mettler Toledo PB 1502-S/fact analytical scale (Goindustry Dovebid European instruments, Switzerland), to determine initial weight (Wi). The leaf samples were then floated in freshly de-ionized water for 14-18 h and weighed thereafter to determine fully turgid weight (Wf). Samples were oven-dried at 80°C for three (3) days and weighed and the dry weight (Wd) was recorded. The relative water content (RWC) expressed in percentage was determined using the following formula below. The experiment was replicated three times.

$RWC = (Wi - Wd)\ (Wf - Wd)^{-1} \times 100.$

### 5.2.3 Leaf pigment extraction and measurements

Leaf pigments were extracted from approximately 0.1 g of leaf samples for analysis according to a method described by Wellburn (1994) with slight modifications using Dimethyl sulphoxide (DMSO). This method has the following advantages over other methods; firstly, the method is faster, largely because grinding and centrifuging is not required. Secondly, the Chlorophyll extracts are more stable, up to 5 days, in DMSO compared to those in acetone.

Three replicates of each sweetpotato varieties were placed in falcon tubes containing 7ml of Dimethyl sulphoxide (DMSO) and incubated in a water bath maintained at $60^0$C for 20 minutes. Immediately after, the volume was toped up to 10 ml and thereafter 2 ml of the mixture was pipetted into glass cuvettes and absorbance was recorded at 665 nm, 649 nm and 480 nm using a 722 N spectrophotometer (Everich medicare import and export Co. LTD, Nanjing China). The spectrophotometer was calibrated to zero absorbance using a blank of pure DMSO. Measurements and calculations were done as follows;

$$C_a = 11.75A_{665} - 2.350A_{649}$$

$$C_b = 18.61A_{649} - 3.960A_{665}$$

$$C_{x+c} = 1000A_{480} - 2.27C_a - 81.4C_b/227$$

Where, $C_a$ is Chlorophyll a (mg/ml), $C_b$ is Chlorophyll b (mg/ml), $C_{x+c}$ are total carotenoids (mg/ml) and A is the absorbance at 649, 665 and 480 wavelengths.

### 3.2.7 Yield analysis

The storage roots of the whole potted plant were harvested at 150 days after planting (DAP). The storage roots were thereafter selected based on their shapes, sizes, weights and defects. Root tubers with a diameter of 1cm and above were measured. In contrast, the roots whose diameter was less than 1cm or showing defects (cracks, irregular shape) and pest infestation were discarded.

### 3.2.8 Statistical data analysis

Growth parameters (leaf relative water content and vine length), leaf pigment (chlorophyll a, chlorophyll b, total chlorophyll a+b, total carotenoids, chlorophyll a: b and total chlorophyll/total carotenoids ratios) of the selected genotypes and yield of control and salt treated plants were analyzed using ANOVA at 95 % confidence interval. Mean separation was carried out using Tukey's pairwise comparison test at 5 % probability level and simple linear regression analysis of the results were carried out. Pearson's coefficient of correlation between leaf pigment, vine length, and yield in relation to treatment was determined. Statistical analyses were done using SAS statistical computer software (version 9.1.3; SAS). Yield reduction, compared to control, was calculated by subtracting the weight value of the salt stressed cultivar from the control, and expressing the yield decline as a percentage of the control.

## 5.3 RESULTS

### 5.3.1 *In vivo* sweetpotato growth analysis

#### 5.3.1.1 *In vivo* effect of salt stress on vine length

Salinity was observed to affect vine length of the selected Kenyan sweetpotato genotypes that depended largely on the duration of exposure to the stress and the genotype (Figure 5.1).

In the first month, the vine length ranged from 26.67 (*Spk 203*) to 95.00 (*Kemb 36*) in the control experiment while that of salt stressed sweetpotato plants ranged from 16.50 (*Mugande*) to 76.83 (*Kemb 23*). In general the salt stressed plants vine length growth ranged from -48.65% (*Ksp 28*) to +92.00 (*Spk 013*). All the genotypes had their vine length decreasing significantly (P≤0.05) except that of *Spk 203* (26.83), *Spk 013* (72.33) and *Kemb 23* (76.83) compared to the control (Figure 5.2 A and B).

In the second month, it was observed that the vine length ranged from 37.67 (*Mugande*) to 145.00 (*Kemb 36*) in the control sweetpotato genotypes while data revealed that for the salt stressed plants, the vine length ranged from20.17 (*Mugande*) to 79.33(*Kemb 36*). In general, the percentage increase in growth ranged from -51.39 (*Spk 004*) to +43.49 (*Spk 013*) in the salt stressed sweetpotato genotypes. Conversely, only two sweetpotato genotypes *Spk 013* (+92.00) and *Kemb 23* (+5.20) had a higher vine length in salt stressed plants than that of the control while all the remaining genotypes were observed to have significantly shorter vines (P≤0.05; Figure 5.2 A and B)

In the third month, it was observed that vine length of all sweetpotato genotypes tested ranged from 41.00 (*Mugande*) to 186.00 (*Kemb 36*) in the control while it

ranged from 20.33 (*Mugande*) to 74.33 (*Kemb 36*) in salt stressed sweetpotato plants.

In terms of percentages, it was observed that salinity stress depressed the sweetpotato vine length significantly such that it ranged from -62.35 (*Spk 004*) to +36.77 (*Spk 013*). Again all the genotypes had significantly ($P \leq 0.05$) lower vine length compared to the control except *Spk013* (+36.77) (Figure 5.3 A and B).

**Figure 5.1: Sweetpotato genotypes experimental setup in the greenhouse**
A: control experiment in month one
B: control experiment month two
C: salt experiment in month one
D: salt treatment in month two.

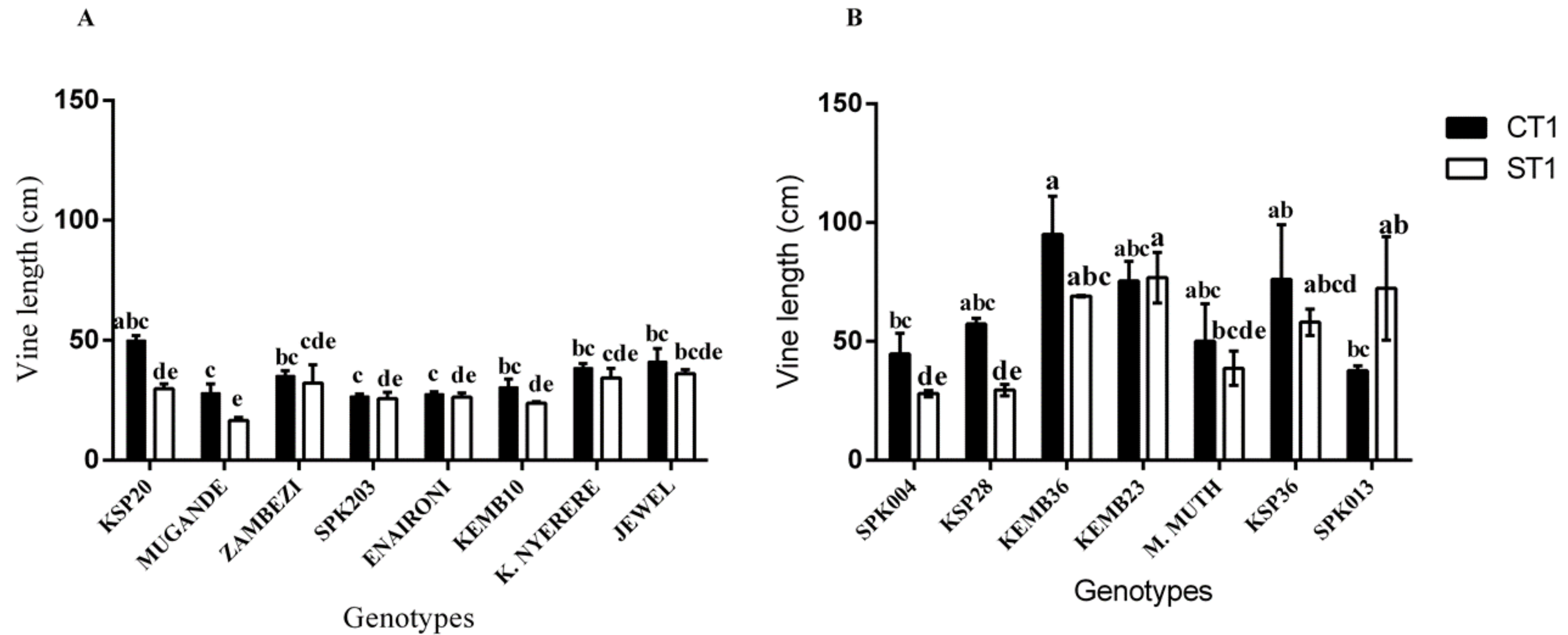


**Figure 5.2: A and B Vine length of sweetpotato genotypes after one month**

Values are means and standard error vine length. Bars of a particular treatment with the same letter are not significantly different from one another (P≤0.05). CT control; ST- salt treatment

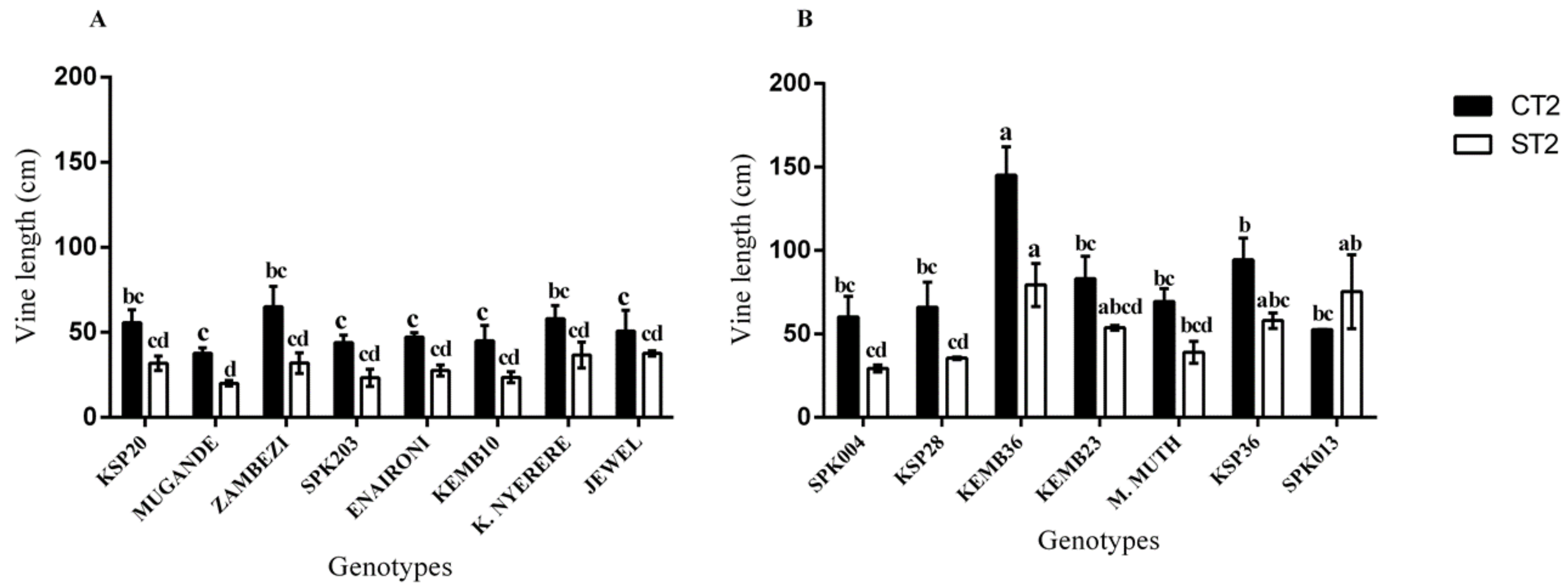


**Figure 5.3: A and B. Vine length of sweetpotato genotypes after two month**

Values are means and standard errors vine length. Bars of a particular treatment with the same letter are not significantly different from one another (P≤0.05). CT control; ST – salt treatment

Comparison of percentage growth in all the selected sweetpotato genotypes within the three months revealed that most of the genotypes registered an increase in growth of vine length between the first and second month in saline environment with a few exceptions but generally the growth ranged from -30.15% (*Kemb 23*) to +22.24 % (*Mugande*). The genotypes with decreased vine lengths were *Kemb 23* and *Kemb 20* (-30.15), *Zambezi* (-10.29), *Spk 203* (-8.32), and *Kemb 10* (-0.84). Interestingly, a few sweetpotato genotypes *Kemb 23* (+19.86), *Ksp 20* (+15.21), *Spk 203* (+8.57), *Kemb 10*, (+5.62), *Ksp 36* (+4.02), *Kalamb Nyerere* (+3.63) and *Mugande* (+0.79) were able to significantly (P≤0.05) grow and have an increase in vine length during the recovery period between the second and third month (Figure 5.4, 5.5 and 5.6).

Analysis of variance showed that vine length was affected by salt stress significantly (P≤0.05) in all the genotypes tested during the second month. Additionally, the interaction between genotype and treatment in the second month was highly significant at P≤0.05 probability level based on Turkey's' mean separation (Appendix 4A B and C).

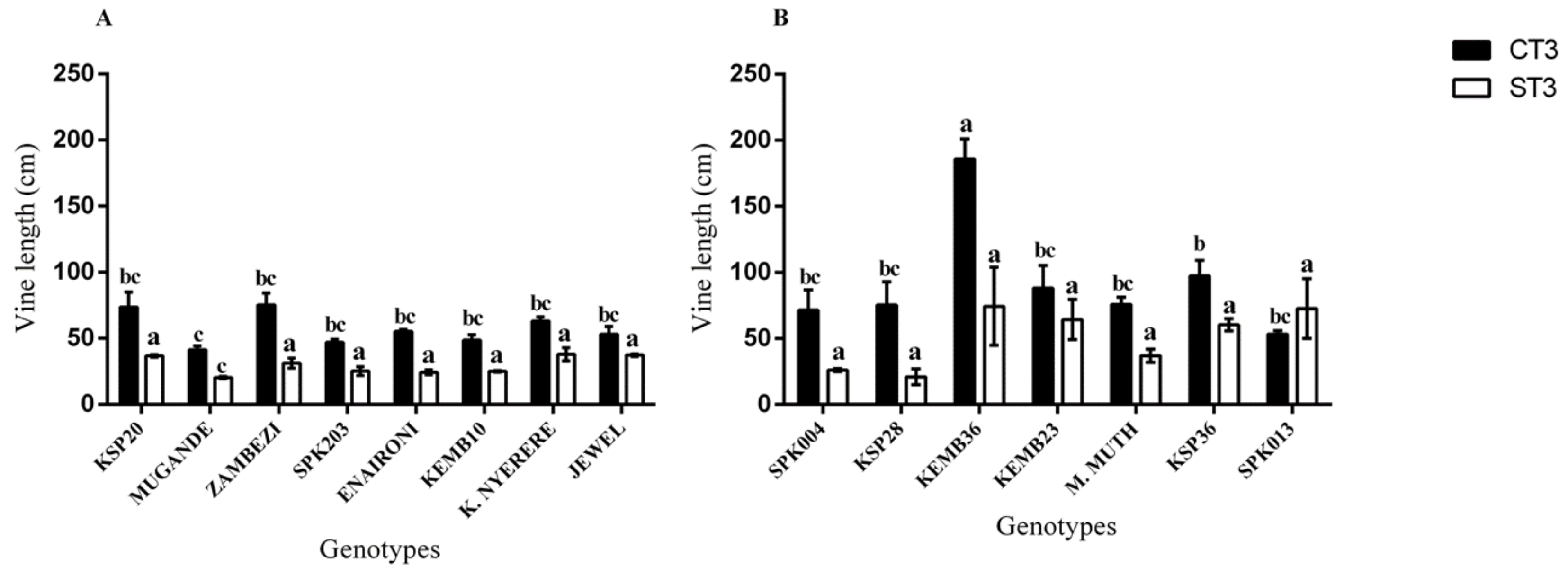


**Figure 5.4: A and B. Vine length of sweetpotato genotypes after three month of salt stress**
Values are means and standard error of chlorophyll a amounts. Bars of a particular treatment with the same letter are not significantly different from one another (P≤0.05)

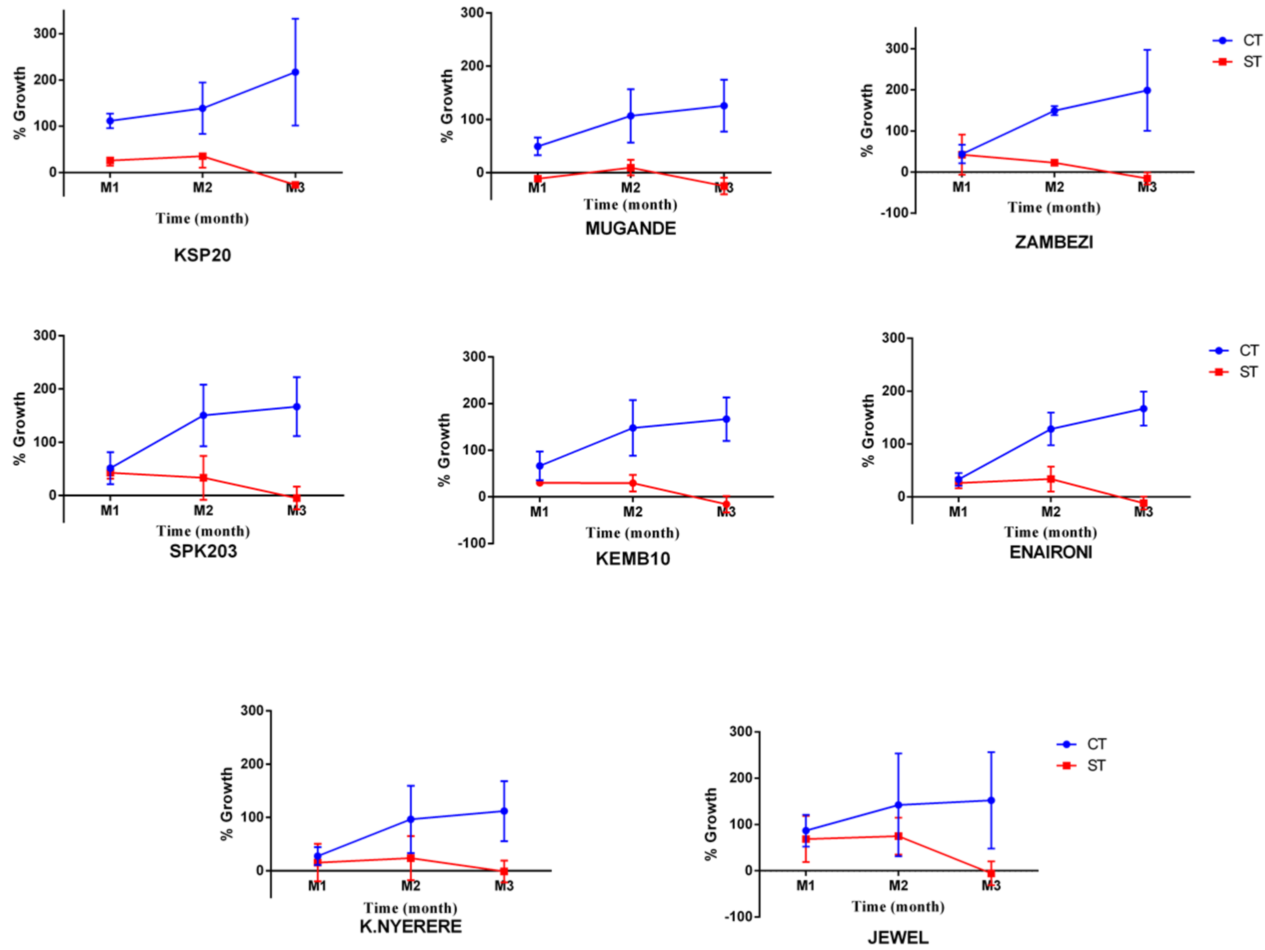


**Figure 5.5: Sweetpotato genotypes showing percentage growth of vines for three month period for salt stressed vines compared to control**

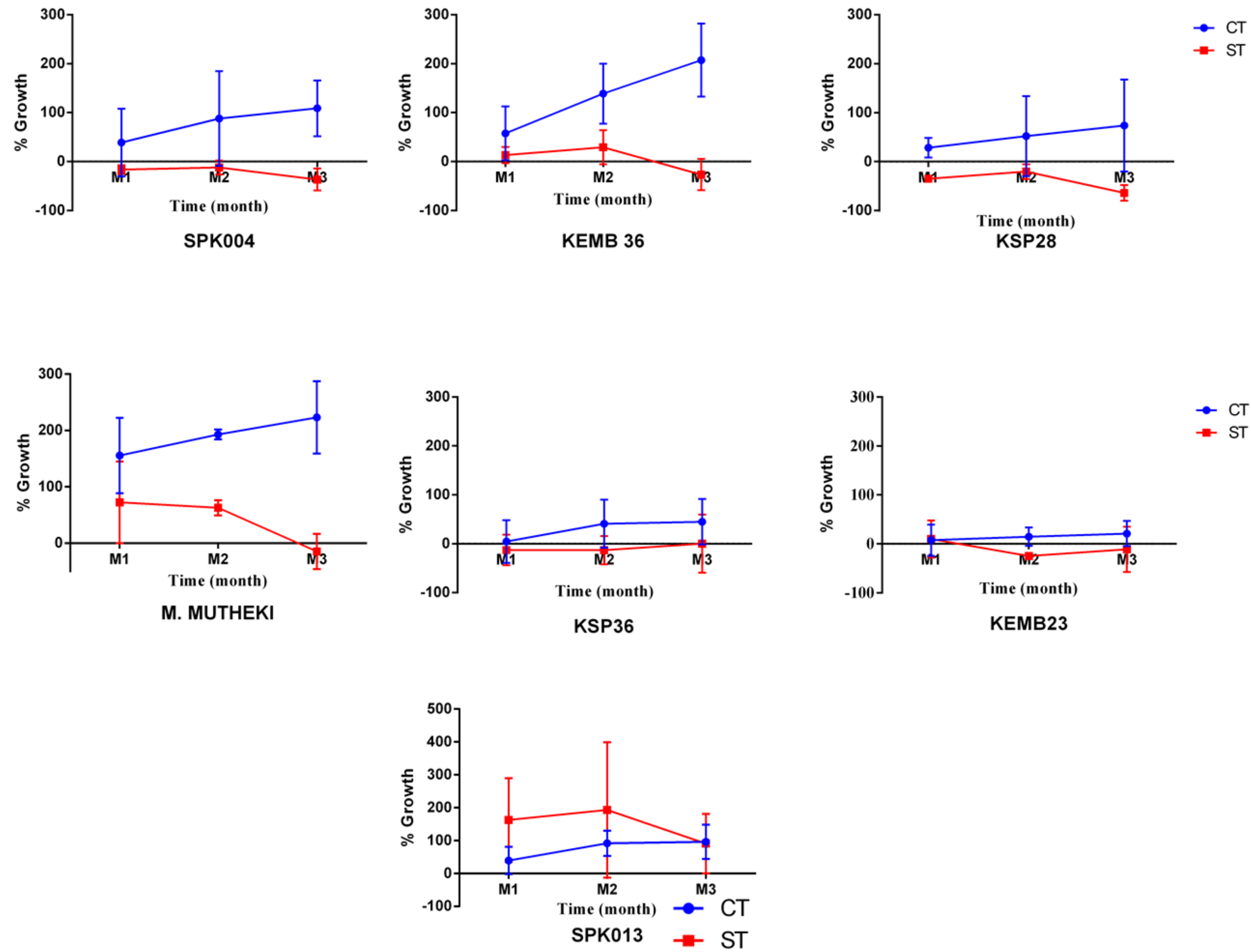


**Figure 5.6: Sweetpotato genotypes showing percentage growth of vines for three month period for salt stressed vines compared to control**

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**5.3.1.2 Regression analysis of salt stress effects on vine length**

The results indicate that the effect of salt stress on vine length was highly varied depending on the genotype. Regression analysis indicates that some genotypes had positive while others had negative regression coefficient. The genotypes with negative regression coefficient were *Mweu Mutheki* ($R^2$ = 0.99), *Kemb 10* ($R^2$= 0.87), *Ksp 20* ($R^2$= 0.66), *Spk 013* ($R^2$= 0.32), *Kemb 23* ($R^2$ = 0.16) and *Spk 004* ($R^2$ = 0.11) respectively. (Figure 5.7 and 5.8).

Among the remaining genotypes *Ksp 36* ($R^2$ = 0.00) had a slight change in vine length while the rest had positive regression coefficients. Very high negative regression coefficient in a given genotype is indicative of sensitivity to salt stress. Positive regression coefficient shows genotypes that are tolerant to salt stress and are able to adapt to the stress to maintain growth.

However, too high negative or positive regression coefficient fall above the goodness of fit and therefore are rejected. For this reason the genotypes that are tolerant are *Ksp 36*, *Kalamb nyerere, Ksp 28* and *Kemb 36* (Figure 5.7 and 5.8).

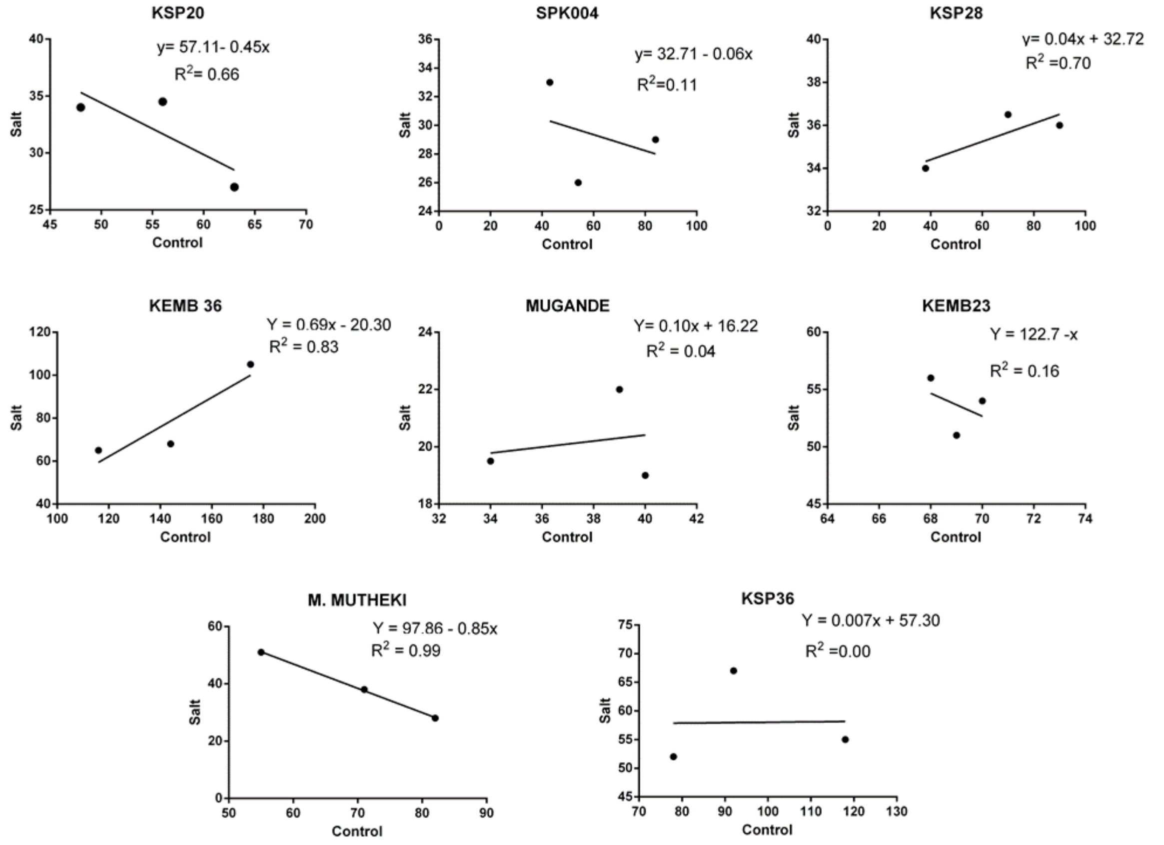


**Figure 5.7: Regression analysis of effect of salt stress on vine length on sweetpotato genotypes**

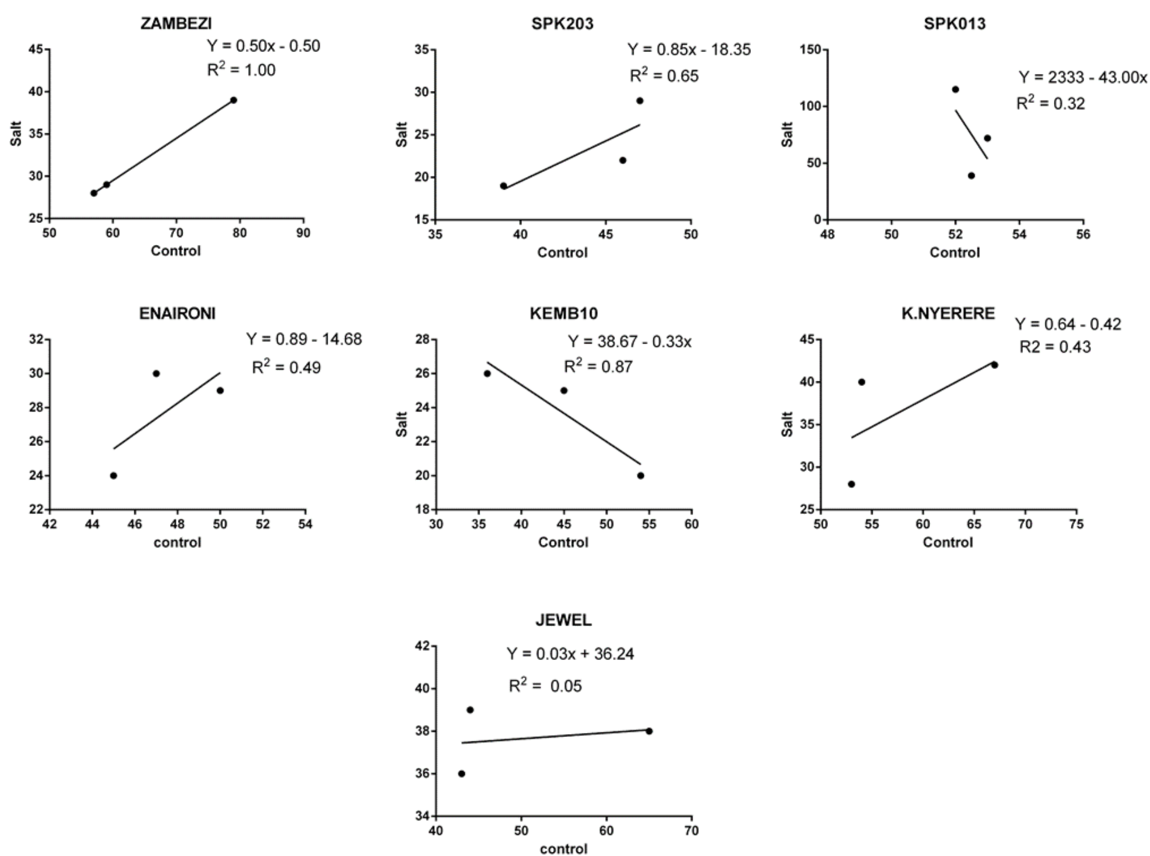


**Figure 5.8: Effect of salt stress on vine length of sweetpotato genotypes**

**5.3.1.3 Effect of salt stress on relative water content (RWC)**

It was observed that salt stress affected the relative water content (RWC) of selected Kenyan sweetpotato genotypes. Data revealed that the RWC ranged from the lowest 46.85 (*Kemb 10*) to the highest 68.07 (*Ksp 20*) for salt stressed plants while that of control ranged from 72.53 (*Kemb 36*) to 85.00 for *Spk 203* (Table 5.1).

Comparison of RWC between the control and salt stressed sweetpotato leaves showed that it depended on the genotypes ability to tolerate the stress induced. Data indicate that the reduction of RWC ranged from 20.13 (*Ksp 20*) to 39.91 (*Mugande*). The tolerant genotypes were *Ksp 20* (20.13), *Jewel* (26.13) and *Kalamb Nyerere* (27.79) respectively with lowest RWC reduction values. On the other hand the sensitive genotypes were *Mugande* (39.31), *Kemb 10* (38.40) and *Spk 004* (34.62) in that order (Table 5.1).

Analysis of variance showed that relative water content was affected by salt stress significantly ($P \leq 0.05$) in all the genotypes tested. Additionally, the interaction between genotype and treatment was highly significant at $P \leq 0.05$ probability level based on Turkey's' mean separation (Appendix 4B).

**Table 5.1: Relative water content of fifteen selected Kenyan sweetpotato genotypes after one month of salt treatment**

| Genotype | Control | Month 1 | % Reduction |
|---|---|---|---|
| *KSP 20* | 85.23±3.90a | 68.07±3.73a | 20.13 |
| *SPK 004* | 78.43±19.19a | 51.28±8.21ab | 34.62 |
| *KSP 28* | 81.22±6.86a | 52.69±2.91ab | 31.13 |
| *KEMB 36* | 72.53±2.29a | 51.44±6.47ab | 29.08 |
| *MUGANDE* | 83.63±6.53a | 50.76±3.02ab | 39.31 |
| *KEMB 23* | 83.36±2.09a | 59.76±2.19ab | 28.32 |
| *M. MUTHEKI* | 82.78±10.11a | 56.88±2.02ab | 31.29 |
| *KSP 36* | 81.82±2.62a | 55.20±4.73ab | 32.53 |
| *ZAMBEZI* | 84.93±1.74a | 59.62±2.28ab | 29.80 |
| *SPK 203* | 85.30±3.15a | 61.31±3.51ab | 28.12 |
| *SPK 013* | 83.53±3.38a | 56.63±0.99ab | 32.20 |
| *ENAIRONI* | 79.82±15.22a | 55.05±3.89ab | 31.93 |
| *KEMB 10* | 76.05±7.16a | 46.85±0.93b | 38.40 |
| *K. NYERERE* | 80.23±12.53a | 57.95±2.28ab | 27.79 |
| *JEWEL* | 79.07±3.72a | 58.41±2.05ab | 26.13 |
| **LSD** | 44.02 | 19.77 | |

Values are means and standard error of relative water content in selected Kenyan sweetpotato genotypes. Means with the same letters within a column are not significantly different from one another at P≤0.05 probability level based on Turkey's' mean separation

### 5.3.2 *In vivo* sweetptato pigment content analysis

#### 5.3.2.1 *In vivo* effect of salt stress on chlorophyll a and b

The amount of chlorophyll a was observed to deteriorate with increasing time the sweetpotato plants are subjected to salt stress in the environment. In the first month, it was observed that the amount of chlorophyll a ranged from 15.72 (*Kemb 10*) to 19.40 (*Ksp 36*) in the control while it was 15.76 (*Spk 203*) to 18.00 (*Spk 013*) in salt stressed sweetpotato plants (Figure 5.9: A and B)

Data indicate that chlorophyll a reduced in all the selected sweetpotato genotypes when subjected to salt stress. However only four genotypes *Spk 004* (17.83), *Kemb 36* (17.66), *Enaironi* (17.33) and *Kemb 10* (16.79) were observed to have higher chlorophyll a content than the control (Figure 5.9 A and B)

Analysis of variance of indicated that salt stress affected chlorophyll a in the second month significantly ($P \leq 0.05$). The interaction between genotype and treatment in the same period was highly significant using Turkey's' means separation (Appendix A and C)

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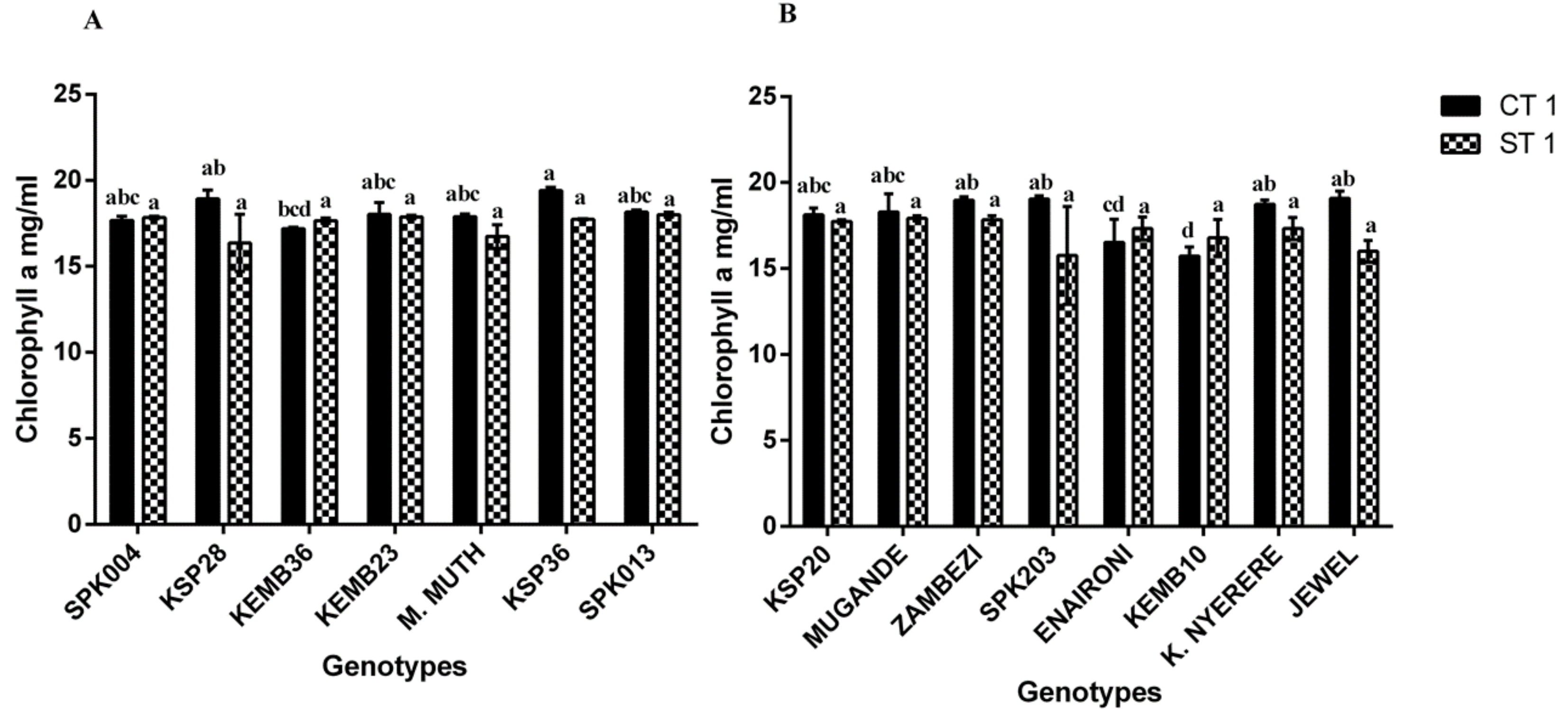


**Figure 5.9: A and B. Amount of chlorophyll a in leaves of sweetpotato genotypes after one month of salt stress**

Values are means and standard error of chlorophyll a amounts. Bars of a particular treatment with the same letter are not significantly different from one another (P≤0.05)

During the second month, the amount of chlorophyll a reduced dramatically in all the genotypes tested compared to the control. Data indicated that the reduction in the amount of chlorophyll a in relation to the control ranged from -85.59% (*Spk 203*) to -25.75% (*Kemb 36*; Figure 5.10 A and B).

Generally, it was observed that the levels of chlorophyll a was significantly ($P \leq 0.05$) affected by salt stress in the second month compared to the control. A highly significant interaction between the treatment and the genotypes at $P \leq 0.05$ probability level was also observed in the second month (Appendix 4 A and C)

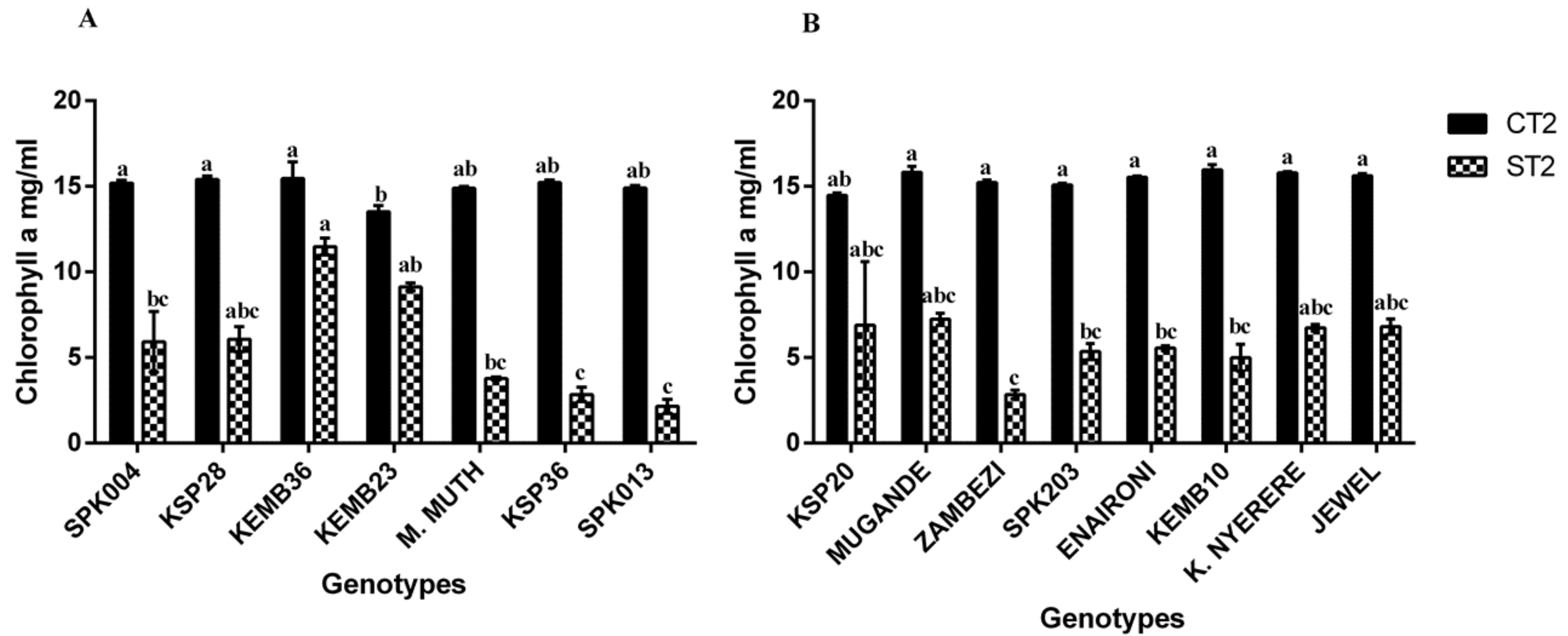


**Figure 5.10: A and B. Amount of Chlorophyll a of fifteen sweetpotato genotypes in the second month of salt treatment**
Bars represent means and standard error of chlorophyll a amount. Bars with the same letter in the same treatment are not significantly different from one another at P≤0.05 probability level.

Chlorophyll b was affected by salt stress in all the selected Kenyan sweetpotato genotypes tested. In the first month, the results show that the highest amount of chlorophyll b was 15.32 (*Spk 004*) and the lowest amount was 10.90 (*Kemb 10*) in the control experiment. In the treatment experiment, the highest amount of chlorophyll b was 14.26 (*Spk 004*) and the lowest amount was 10.61 (*Kemb 10*) sweetpotato genotype (Figure 5.11 A and B).

All selected Kenyan sweetpotato genotypes were observed to have reduced amount of chlorophyll b when subjected to salt stress except two genotypes; *Ksp 28* (12.96) and *Ksp 20* (14.15), which had higher amounts compared to the control (Figure 5.11, A and B).

Analysis of variance indicates that chlorophyll b was insignificantly affected by salt stress in the first month, but there was significant difference among the genotypes tested however the interaction between genotype and treatment was not significant ($P \leq 0.05$; Appendix 4A, B and C).

In the second month, results show that there was a remarkable decrease in the amount of chlorophyll b when sweetpotato genotypes were subjected to salt stress (Figure 5.12 A and B). It was observed that all the sweetpotato genotypes had reduced chlorophyll b content compared to the control after the experiment. The highest amount of chlorophyll b was 18.32 (*Kemb 10*) while the lowest amount was 8.48 (*Ksp 20*) in the control experiment. On the other hand the highest amount was 4.45 (*Kemb 36*) and the lowest amount as 1.57 (*Mugande*) in the salt stress experimental setup (Figure 5.12 A and B).

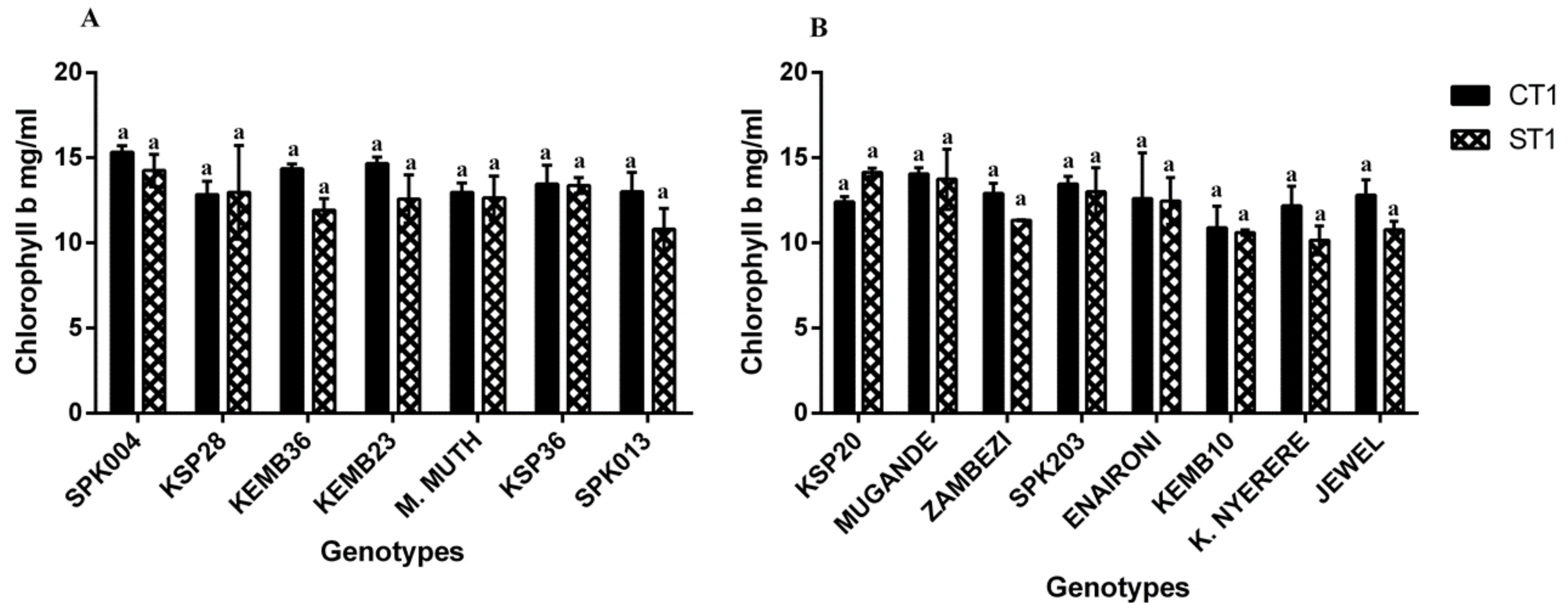


**Figure 5.11: A and B. Amount of chlorophyll b of fifteen selected sweetpotato genotypes under salt stress in the first month.** Bars are means and standard error of chlorophyll b amount. Bars with the same letters in the same treatment are not significantly different from each at P≥0.05 probability level

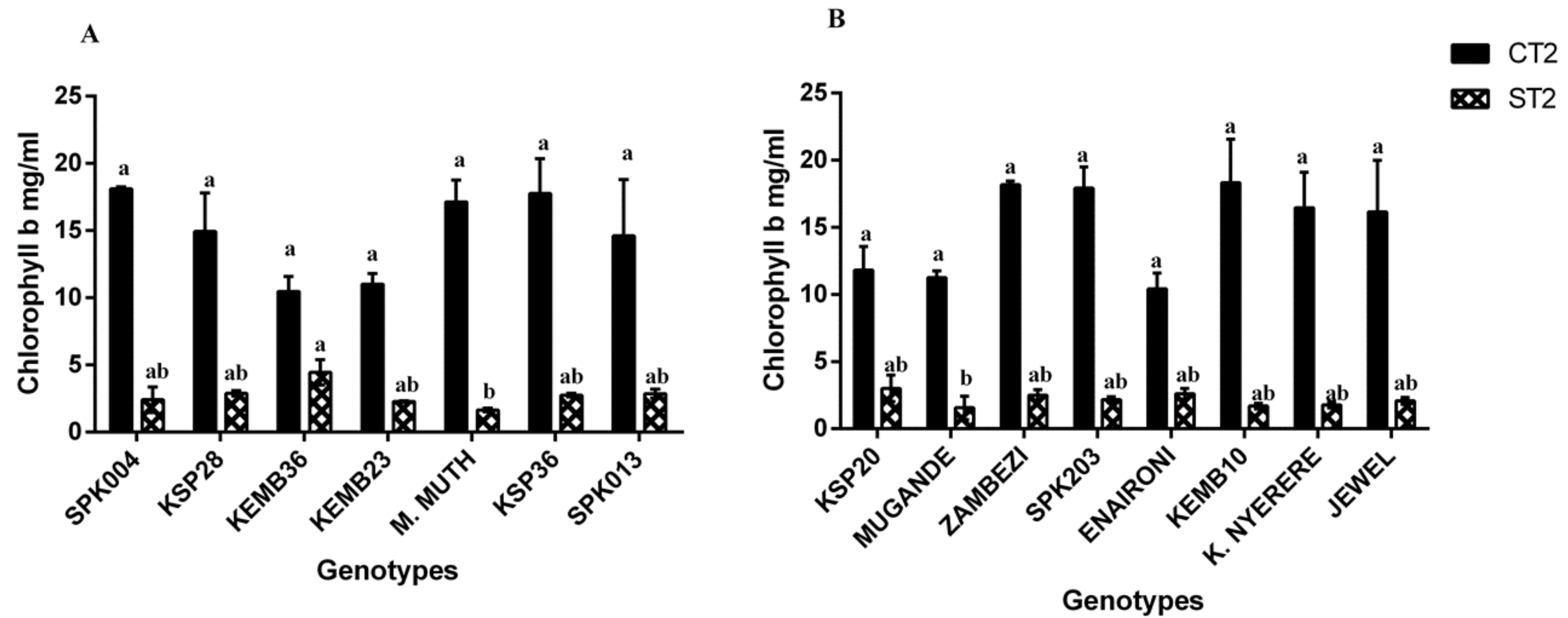


**Figure 5. 12: A and B. Chlorophyll b of fifteen selected sweetpotato genotypes under salt stress in the second month**

Bars are means and standard error of chlorophyll b. Bars with the same letters in the same treatment are not significantly different from each at P≤0.05 probability level.

**5.3.2.2 *In vivo* effect of salt stress on carotenoids**

Carotenoids are important components in plant defense system and like all other plant pigments, they are affected by salt stress. In the first month, the results from selected Kenyan sweetpotato genotypes subjected to salt stress shows that the carotenoids ranged from 2.22 (*Kemb 36*) to 3.57 (*Mweu Mutheki*) in control experimental setup whereas in the salt treatment it ranged from 2.43 (*Spk 004*) to 3.59 in the *Spk 013* genotype (Figure 5.13 A and B).

Within the same period, it was observed that almost all the genotypes had the amount of carotenoids deteriorated in comparison to the control in treatment setup. However four genotypes; *Spk 203* (3.59), *Zambezi* (3.50), *Kemb 36* (3.20) and *Kemb 23* (2.97), were observed to have increased carotenoids than the control (Figure 5.13 A and B).

Analysis of variance for carotenoids shows that they were affected by salt stress significantly in the first month, but there was high significant difference among the genotypes tested as well as the interaction between genotype and treatment ($P \geq 0.05$; Appendix 4 A and C).

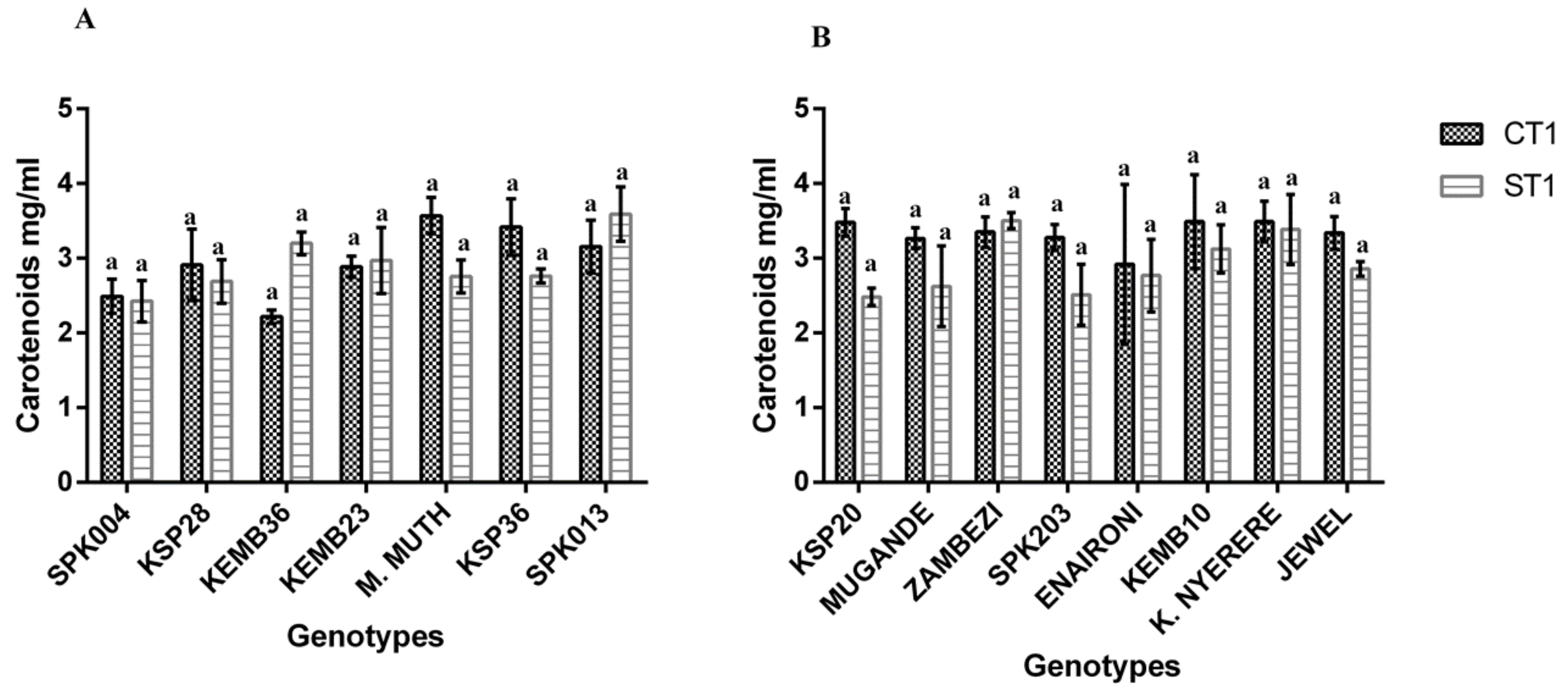


**Figure 5.13: A and B. Carotenoids of fifteen selected sweetpotato genotypes under salt stress in the first month**
Bars are means and standard error of carotenoid amount. Bars with the same letters in the same treatment are not significantly different from each at P≤0.05 probability level.

In the second month, there was a dramatic reduction of carotenoids in all the sweetpotato genotypes compared to the control. Data revealed that the carotenoids amounts in the selected sweetpotato genotypes ranged from 0.90 (*Kemb 23*) to 4.08 (*Mugande*) in the controls whereas in the salt stress treatment the range was 0.31 (*Zambezi*) to 2.60 for *Kemb 36* genotype (Figure 5.14 A and B).

Results also show that almost all the genotypes had reduced carotenoid amount when the sweetpotato plants were grown in salt environment for extended period of time (2 months). In contrast only one genotype, *Kemb 36* (2.60), had significantly ($P \leq 0.05$) increased carotenoid amounts compared to the control (Figure 5.14 A and B).

Additionally, when analysis of variance was done for carotenoids in the second month, it showed that salt stress had significant ($P \leq 0.05$) effects in all genotypes tested. Furthermore the interaction between genotype and treatment was highly significant at $P \leq 0.05$ probability level (Appendix 4 A, B and C).

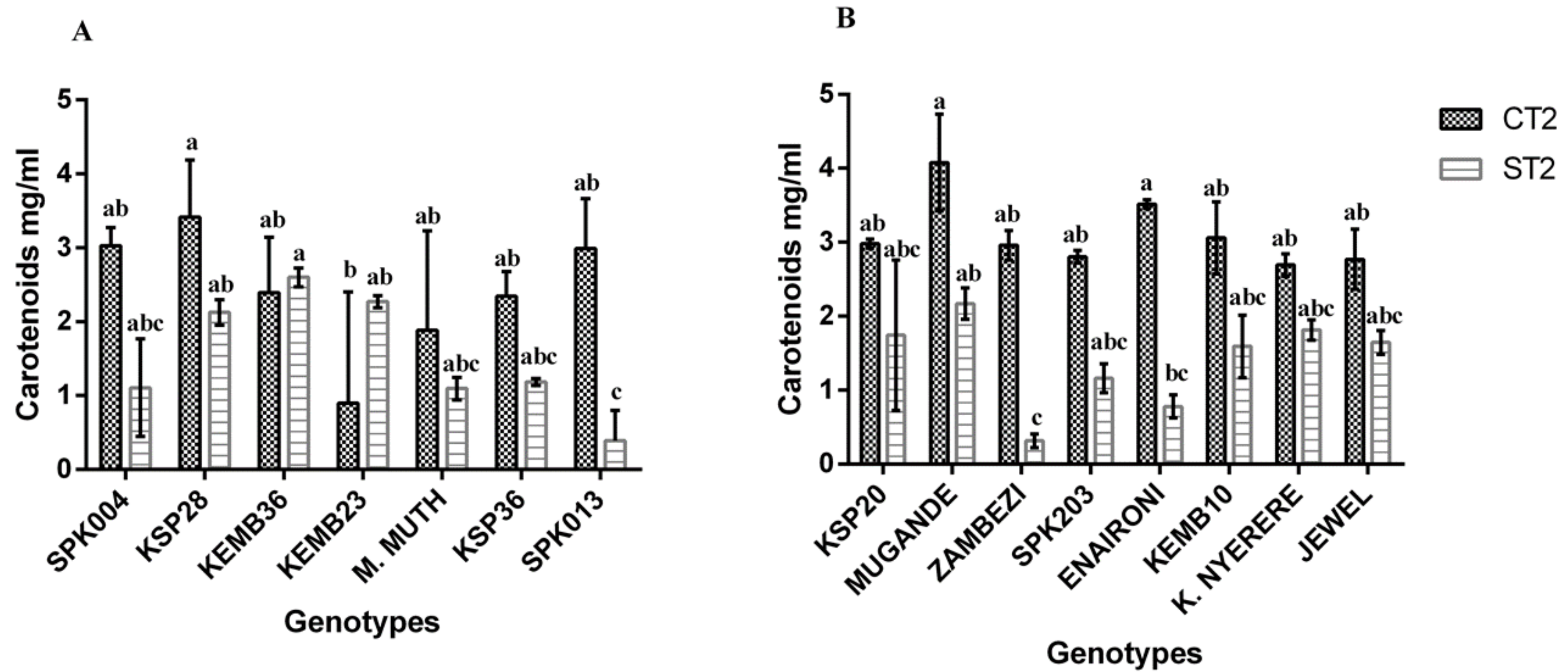


**Figure 5.14: A and B Carotenoids of fifteen selected sweetpotato genotypes under salt stress in the second month**
Bars represent means and standard error carotenoids. Bars with the same letters in the same treatment are not significantly different from each at P≤0.05 probability level.

#### 5.3.2.3 Effect of *in vivo* salt stress on total chlorophyll (TC), chlorophyll a/b and total carotenoids/total chlorophyll ratios

It was observed that the total chlorophyll, chlorophyll a/b and total carotenoids/ total chlorophyll were affected by salt stress just like other photosynthetic pigment. Primarily the effect depends largely on the genotype and the duration of exposure to the stress.

In the first month, data indicate that the total chlorophyll amount ranged from 26.62 (*Kemb 10*) to 32.99 (*Spk 004*) in the control while the range was 26.78 (*Jewel*) to 32.10 (*Spk 004*) for the salt stress treatment. It was observed that almost all sweetpotato genotypes registered reduction in total chlorophyll under salt stress compared to the control. However three selected sweetpotato genotypes; *Ksp 20* (31.90 vs. 30.53) and *Enaironi* (29.79 vs. 29.14) were observed to have higher total chlorophyll compared to the control (Table 5.2).

In the second month, the total chlorophyll in all the tested sweetpotato genotypes ranged from 22.95 (*Ksp 36*) to 34.30 (*Kemb 10*) and 4.25 (*Ksp 36*) to 15.93 (*Kemb 36*) for control and salt stress experimental setup respectively. All selected Kenyan sweetpotato genotypes had drastic reduction of total chlorophyll in this period (Table 5.2).

Analysis of variance revealed that total chlorophyll was highly affected by salt stress in all the selected sweetpotato genotypes that was not significant $P \geq 0.05$. On the other hand the interaction between genotype and salt treatment was highly significant at $P \leq 0.05$ probability level in the second month (Appendix 4 A and C)

In the first month, the chlorophyll a/b ratio in the selected sweetpotato genotype ranged from 1.16 (*Spk 004*) to 1.56 (*Kalamb Nyerere*) and 1.25 (*Ksp 20*) for control and salt stress treatment respectively. The results also showed that seven genotypes; *Kalamb Nyerere* (1.74vs 1.56), *Zambezi* (1.58 vs. 1.48), *Kemb 10* (1.58 vs. 1.48), Mugande (1.36 vs. 1.30), *Kemb 23* (1.46 vs. 1.20), *Kemb 36* (1.49 vs. 1.20) and finally *Spk 004* (1.26 vs. 1.16) had higher chlorophyll a/b ratio than the control (Table 5.2).

In the second month, the chlorophyll a/b ratio ranged from 0.84 (*Zambezi*) to 2.31 (*Kemb 36*) and from 0.80 (*Spk 013*) to 10.94 (*Mugande*) for control and salt treatment respectively (Table 5.2). Analysis of variance reveled that salt stress affected chlorophyll a/b ratio significantly however the interaction between genotype and treatment was not significant at $P \leq 0.05$ probability level using Turkeys' mean separation (Appendix 6).

In the first month, carotenoid /total chlorophyll ratio ranged from 0.07 (*Kemb 36*) to 0.13 (*Kemb 10*) and 0.08(*Spk 004*) to 0.13 (*Spk 013*) in the control and salt stress treatment (Table 5.2). Most of the genotypes showed reduced ratios that was not significantly ($P \geq 0.05$) in the selected sweetpotato genotypes in comparison to the control. However five genotypes; *Kemb 23* (0.10 vs. 0.09), *Zambezi* (0.12 vs. 0.11), *Kemb 36* (0.11 vs. 0.07), *Spk 013* (0.13 vs. 0.10), and *Kalamb Nyerere* (0.12 vs. 0.11) had higher carotenoids/ total chlorophyll ratios that not significant ($P \geq 0.05$) in the salt stress treatment compared to the control (Table 5.2).

In the second month, the carotenoids/ total chlorophyll ratio was significantly ($P \leq 0.05$) reduced by salt stress in all the genotypes compared to the control (Table 5.2). Generally analysis of variance revealed that salt stress affected the carotenoids/ total chlorophyll ratio significantly. Genotype and treatment was highly significant ($P \leq 0.05$) as well (Appendix 4A).

**Table 5.2: Total chlorophyll, chlorophyll a/b and total carotenoids/ total chlorophyll for control, salt stress and time**

| Genotype | Total chorophyll | | | Chlorophyll a/b | | | Carotenoids/total chlorophyll | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Month 1 | Month 2 | Control | Month 1 | Month 2 | Control | Month 1 | Month 2 |
| *Enaironi* | 29.14±2.47[ab] | 29.79±1.35[a] | 8.17±0.30[bc] | 1.49±0.41[a] | 1.43±0.17[a] | 2.24±0.40[a] | 0.11±0.05[a] | 0.09±0.02[a] | 0.10±0.02[cde] |
| *Jewel* | 31.90±0.82[a] | 26.78±0.87[a] | 9.57±0.46[bc] | 1.51±0.11[a] | 1.49±0.04[a] | 2.47±0.18[a] | 0.11±0.01[a] | 0.11±0.00[a] | 0.17±0.02[abcde] |
| *K.nyerere* | 32.25±2.66[a] | 27.47±0.46[a] | 9.20±0.12[bc] | 1.56±0.13[a] | 1.74±0.19[a] | 2.78±0.22[a] | 0.11±0.01[a] | 0.12±0.02[a] | 0.20±0.01[abcde] |
| *Kemb 10* | 34.30±2.95[a] | 27.40±0.51[a] | 6.69±0.99[bc] | 1.48±0.14[a] | 1.58±0.08[a] | 2.91±0.17[a] | 0.13±0.03[a] | 0.11±0.01[a] | 0.23±0.03[abc] |
| *Kemb 23* | 32.68±0.99[a] | 30.45±1.31[a] | 11.41±0.16[ab] | 1.23±0.03[a] | 1.46±0.19[a] | 4.01±0.09[a] | 0.09±0.01[a] | 0.10±0.02[a] | 0.20±0.01[abcd] |
| *Kemb 36* | 31.56±0.37[ab] | 29.59±0.53[a] | 15.93±0.86[a] | 1.20±0.02[a] | 1.49±0.10[a] | 2.88±0.74[a] | 0.07±0.00[a] | 0.11±0.01[a] | 0.16±0.00[abcde] |
| *Ksp 20* | 30.53±0.12[ab] | 31.89±0.23[a] | 9.91±4.29[abc] | 1.46±0.06[a] | 1.25±0.02[a] | 2.79±1.35[a] | 0.11±0.01[a] | 0.08±0.00[a] | 0.15±0.03[abcde] |
| *Ksp 28* | 31.76±0.28[a] | 29.34±4.41[a] | 8.98±0.50[bc] | 1.49±0.14[a] | 1.35±0.20[a] | 2.12±0.18[a] | 0.10±0.01[a] | 0.09±0.03[a] | 0.24±0.01[ab] |
| *Ksp 36* | 32.86±0.97[a] | 31.11±0.43[a] | 4.25±0.14[c] | 1.46±0.13[a] | 1.33±0.05[a] | 5.04±0.96[a] | 0.10±0.01[a] | 0.09±0.00[a] | 0.28±0.02[a] |
| *M.m* | 30.87±0.68[ab] | 29.39±1.86[a] | 5.42±0.08[bc] | 1.38±0.05[a] | 1.34±0.10[a] | 2.33±0.19[a] | 0.12±0.01[a] | 0.10±0.01[a] | 0.20±0.02[abcd] |
| *Mugande* | 32.35±0.28[a] | 31.66±1.70[a] | 8.80±0.74[bc] | 1.30±0.08[a] | 1.36±0.21[a] | 10.94±6.99[a] | 0.10±0.00[a] | 0.09±0.02[a] | 0.25±0.04[ab] |
| *Spk 004* | 32.99±0.47[a] | 32.10±1.00[a] | 8.34±0.19[bc] | 1.15±0.03[a] | 1.26±0.09[a] | 4.48±2.82[a] | 0.08±0.01[a] | 0.08±0.01[a] | 0.13±0.04[bcde] |
| *Spk 013* | 31.16±1.03[ab] | 28.81±1.29[a] | 5.02±0.16[c] | 1.42±0.15[a] | 1.71±0.21[a] | 0.79±0.19[a] | 0.10±0.02[a] | 0.13±0.02[a] | 0.08±0.05[de] |
| *Spk 203* | 33.02±1.51[a] | 28.77±0.61[a] | 7.53±0.41[bc] | 1.42±0.05[a] | 1.26±0.23[a] | 2.52±0.39[a] | 0.10±0.01[a] | 0.09±0.01[a] | 0.15±0.02[abcde] |
| *Zambezi* | 33.41±0.30[a] | 29.17±0.11[a] | 5.36±0.16[bc] | 1.48±0.06[a] | 1.58±0.02[a] | 1.24±0.34[a] | 0.11±0.01[a] | 0.12±0.00[a] | 0.06±0.02[e] |
| **LSD** | 5.21 | 7.90 | 6.24 | 0.74 | 0.77 | 10.49 | 0.09 | 0.08 | 0.14 |

Values are means and standard error for all tested parameters. Means with same letters in the column are not significantly different from one another at $P \leq 0.05$ probability level.

### 5.3.3 Effect of *in vivo* salt stress on selected Kenyan sweetpotato yield

The results indicate that the yield reduction varies with the genotype and duration of the exposure to salt stress (Table 5.3, Figure 5.15). The mean sweetpotato yield ranged from 43.50 (*Enaironi*) to 163.00 (*Ksp 36*) and 16.77 (*Enaironi*) to 105.73 (*Ksp 36*) for the control and salt stress setups respectively (Table5.3). Some of the sweetpotato genotypes were observed to have elongated roots that could not be could not fit in the selection criteria (Figure 5.15c).

In terms of percentage reduction of the yield, it ranged from -70.68 (*Kemb 10*) in the most sensitive genotype to -31.13 (*Spk 004*) in the tolerant genotype (Table 5.3). The overall tolerant genotypes in terms of yield reduction were *Spk 004* (-31.13), *Mweu Mutheki* (-31.43), Ksp 36 (-35.29) and *Kalamb Nyerere* (-36.85) in that order (Table 5.3)

Analysis of variance shows that salt stress significantly reduced the yield of selected Kenyan sweetpotato genotype ($P \leq 0.05$). The interaction between genotype and treatment was highly significant as well at $P \leq 0.05$ probability level (Appendix 4 B)

**Figure 5.15: Sweetpotato yield**
A: tubers from salt treatment from *ksp 20*
B: tubers from control experiment
C: elongated tubers from salt treatment

**Table 5.3: Effect of salt stress on yield of selected Kenyan sweetpotato genotypes compared to control.**

| Genotype | Control | Salt | % Reduction |
|---|---|---|---|
| ***Ksp 20*** | 89.23±6.27$^{cd}$ | 39.13±6.79$^{cdefgh}$ | 56.15 |
| ***Spk 004*** | 87.90±2.94$^{cd}$ | 60.53±0.90$^{bcd}$ | 31.13 |
| ***Ksp 28*** | 63.93±0.83$^{ef}$ | 38.87±3.39$^{cdefgh}$ | 39.21 |
| ***Kemb 36*** | 63.37±3.45$^{ef}$ | 24.30±0.53$^{gh}$ | 61.65 |
| ***Mugande*** | 106.70±1.50$^{bc}$ | 50.77±1.00$^{bcdef}$ | 52.42 |
| ***Kemb 23*** | 91.27±2.19$^{cd}$ | 46.13±4.26$^{cdefg}$ | 49.45 |
| ***M. Mutheki*** | 105.33±0.97$^{bc}$ | 72.23±3.61$^{b}$ | 31.42 |
| ***Ksp 36*** | 163.40±6.86$^{a}$ | 105.73±6.46$^{a}$ | 35.29 |
| ***Zamb*** | 116.57±5.38$^{b}$ | 61.43±9.69$^{bc}$ | 47.30 |
| ***Spk 203*** | 87.30±6.49$^{cd}$ | 46.57±5.02$^{cdefg}$ | 46.66 |
| ***Spk 013*** | 105.53±0.41$^{bc}$ | 52.87±0.96$^{bcde}$ | 49.90 |
| ***Enaironi*** | 43.50±3.67$^{f}$ | 16.77±2.19$^{h}$ | 61.46 |
| ***Kemb 10*** | 61.97±3.91$^{ef}$ | 18.17±3.43$^{h}$ | 70.68 |
| ***K Nyerere*** | 58.43±1.51$^{ef}$ | 36.90±3.05$^{efgh}$ | 36.85 |
| ***Jewel*** | 77.77±7.43$^{de}$ | 29.37±1.33$^{fgh}$ | 62.24 |
| **LSD** | 22.24 | 22.53 | |

Values are means and standard error of mean harvest. Different letters in each column show significant difference at P≤0.05by Turkey's' mean separation.

### 5.3.4 Physiological and biochemical relationships of different parameters in selected Kenyan sweetpotato genotype.

The relationship between the parameters used in testing sweetpotato genotypes under salt stress varied widely in terms of significance and magnitude. There was high correlation coefficient for total carotenoids/ total chlorophyll ratio with chlorophyll a, b, total chlorophyll, total carotenoids and chlorophyll a/b with total carotenoids that was highly significant ($P < 0.05$). However the association for total carotenoid/ total chlorophyll with chlorophyll b and total chlorophyll was negative (Table 5.4). The correlation coefficients for vine length and relative water content with chlorophyll a, b and total chlorophyll were negative and not significant ($P < 0.05$).

The results also showed that the correlation coefficient for harvest with chlorophyll a was negative and moderately significant ($P < 0.05$). However the association was negative and insignificant for harvest with chlorophyll a/b and total carotenoids/ total chlorophyll ratios. Interestingly a positive association was found between chlorophyll b, total carotenoids, total chlorophyll, vine length and RWC with harvest that was not significant under salt stress condition (Table 5.4).

**Table 5.4: Relationship between physiological and biochemical parameters for fifteen selected sweetpotato genotypes**

| Parameter | Chl a | Chl b | Cx+c | a+b | a/b | Cx+c/TC | VL | RWC | Harvest |
|---|---|---|---|---|---|---|---|---|---|
| **Chl a** | 1.00 | | | | | | | | |
| **Chl b** | -0.10$^{ns}$ | 1.00 | | | | | | | |
| **Cx+c** | 0.69*** | -0.30* | 1.00 | | | | | | |
| **a+b** | 0.05$^{ns}$ | 0.99*** | -0.19$^{ns}$ | 1.00 | | | | | |
| **a/b** | 0.36** | -0.81*** | 0.29* | -0.75*** | 1.00 | | | | |
| **Cx+c/TC** | 0.57*** | -0.63*** | 0.92*** | -0.54*** | 0.58*** | 1.00 | | | |
| **VL** | -0.12$^{ns}$ | -0.05$^{ns}$ | -0.29* | -0.07$^{ns}$ | 0.27$^{ns}$ | -0.19$^{ns}$ | 1.00 | | |
| **RWC** | -0.01$^{ns}$ | -0.04$^{ns}$ | 0.10$^{ns}$ | -0.04$^{ns}$ | 0.00$^{ns}$ | 0.10$^{ns}$ | -0.14$^{ns}$ | 1.00 | |
| **Harvest** | -0.35** | 0.15$^{ns}$ | 0.14$^{ns}$ | 0.10$^{ns}$ | -0.22$^{ns}$ | -0.17$^{ns}$ | 0.13$^{ns}$ | 0.12$^{ns}$ | 1.00 |

*** Highly significant, ** Intermediate significant,* significant; ns - Not significant at P≤0.05 probability level.
Chl a - Chlorophyll a; Chl b - Chlorophyll b; Cx+c - Total carotenoids; a+b – Chlorophyll a +b ; TC – Total chlorophyll; RWC – relative water content

## 5.4 DISCUSSION

Cell growth is one of the physiological processes that is most sensitive to salt stress due to the reduction in turgor pressure. Cell division and expansion produced by meristematic tissue within the plant is reduced or inhibited by salt stress. Our study shows sweetpotato genotypes having varied growth of vines under salt stress. Variation in growth responses of sweetpotato genotypes might have been due to their different genetic makeup involved in the absorption of water and changes in biochemical mechanisms under saline conditions (Ziaf *et al.,* 2009).

In the present study, the growth of sweetpotato genotypes was estimated by measuring vine length and relative water content (RWC), which decreased significantly under salt stress as compared to control. This was because plants need more energy for their survival to cope with salinity stress, consequently depressing the growth. Additionally, such decline in vine length response to salt stress might be due to either decrease in cell elongation resulting from the inhibitory effect of water shortage on growth promoting hormones, which, in turn, led to a decrease in cell turgor, cell volume and eventually cell growth. Sometimes this might be due to blockage of xylem and phloem vessels thus hindering any translocation within the plant (Lavisolo and Schuber, 1998).

Reduction in growth and relative water content of plants caused by salinity has been observed by several researchers, in many plants, such as sorghum (Lacerda *et al*., 2001), rice (Morsy *et al.*, 2007), pea (Hernandez *et al.,* 2000), sunflower (Santos, 2004) and Rumex (Chen *et al.,* 2004).

This may be a reflection of the decline in water consumption causing water stress, affecting the photosynthetic capacity of plants (Stępień and Kłbus, 2006). Consequently, this leads to the reduction in specific metabolic processes such as carbon assimilation or nitrogen absorption or a combination of both (Zhang *et al.*, 2009).

Additionally, the decrease in the vine length due to treatment with sodium chloride could be due to the negative effect of the salt on the rate of photosynthesis, the changes in enzyme activity, and also the decrease in the level of carbohydrates and growth hormones, all of which can lead to inhibition of the growth (Mazher *et al.,* 2011).

In the present study, three of the selected Kenyan sweetpotato genotypes *Spk 203*, *Spk 013* and *Kemb 23,* were observed to have increased vine length upon exposure to salinity for a short period of time (four weeks). The elongation of the stem when treated with low concentrations of salts may induce osmotic adjustment and dry matter partitioning activity of plants for sustaining salt stress situation, which may improve growth. Besides, plants cope with stress situations by storing starch in stems and ensuring optimal yield for survival (Kulkarni and Phalke, 2009).

These findings are in agreement with studies done in other plants such as *Oryza sativa* L. (Misra *et al.,* 1997), *Vigna unguiculata* L (Memon *et al*., 2010) and *Brassica campestris* L (Dantus *et al*., 2005) which showed that the use of low concentrations of sodium chloride led to increases in plants lengths.

Chlorophyll a and b as well as Carotenoids are the main photosynthetic pigments that play an important role in photosynthesis. In the present study, the effect of salt stress on selected Kenyan sweetpotato genotypes in a particular salt concentration and exposure times was determined.

Pigment responses of all selected Kenyan sweetpotato genotypes were found to differ from each other in the different exposure times. In my results, some sweetpotato genotypes were observed to have increased chlorophyll a (*Spk 004, Kemb 36, Enaironi* and *Kemb 10),* increased chlrophyll b (*Ksp 28* and *Ksp 20)* and total chlorophyll (*Ksp 20, Enaironi* and *Kemb 10)*. My observation on increased total Chlorophyll content under salt stress is in agreement with that made in other plants such *Cucumis sp*., wheat, broad bean and rice plants (Kusvuran *et al.,* 2009). Such an increase in chlorophyll content is a plant adaptation to cope with salt stress through increased pigment synthesis by increasing the number of chloroplasts in the stressed leaves, to mitigate the adverse effect (Misra *et al.,* 1997).

In the present study, it was observed that the reduction of chlorophyll content on exposure to salt stress increases with increase in time. This observation is in agreement with that reported on barley *Hordeum vulgare* L (Tort and Turkyilmaz, 2004) that the exposure of barley to salt stress led to the decrease in chlorophyll a, chlorophyll b and total chlorophyll content.

The degree of the chlorophyll content reduction is largely dependent on the salt tolerance of a given sweetpotato genotype. Reports indicate that salt tolerant genotype, chlorophyll content increases but decreases in salt-sensitive genotype

under saline regimes (Khan *et al.,* 2009, Akram and Ashraf, 2011). Reduction in the content of photosynthetic pigments in leaves of selected sweetpotato genotypes upon salt treatment may be due to the reduced protein synthesis at the LHC complexes that protect the photosynthetic apparatus (Kauser *et al.*, 2012).

The degradation of photosynthetic pigments results from oxidative damage of chloroplast lipids, pigments and proteins in thylakoid membranes. This leads to the degradation of PS II thus reducing trapping and electron transport. The degradation of PS II will also lead to reduced production of ATP and NADPH, and ultimately reduced $CO_2$ fixation (Abdalla and El-koshiban, 2007). Reduced chlorophyll ratio (a/b) shows that the PSII complexes were increased allowing increased absorption of photons, leading to the modification of the active reaction centers (Lehtimäki *et al.,* 2010).

In the present study, the carotenoid content was observed to increase with salinity treatment in one genotype (*Kemb 36)* while in all the remaining genotypes the amount of total carotenoids reduced. The results obtained, regarding the reduction of carotene content is in agreement with that reported for barley (*Hordeum vulgare* L) (Tort and Turkyilmaz, 2004) and dogwood, (*Cornus sericea* L) (Mustard and Renault, 2006). On the other hand, the increase in carotene content under salt stress findings, agrees with that reported for rice *Oryza sativa* L (Misra *et al.*, 1997).

Carotenoids form part of complex antioxidant defense system developed by plant cells to protect themselves against salt stress and the adverse effects of reactive oxygen species (Hossain *et al.*, 2007; Turkan and Demiral, 2009). Specifically,

carotenoids quench triplet chlorophyll; scavenge singlet oxygen which damage membranes and proteins; dissipate excess energy via xanthophyll-mediated non-photochemical quenching (NPQ) (Cazzonelli and Pogson, 2010).

Yield reduction is one of the observable results when sweetpotato genotypes are grown under salt stress environment. In this study, it was observed that salt stress reduced sweetpotato yield in all the tested genotypes. The results are consistent with that obtained under field condition for sweetpotato (Amin *et. al.,* 2011). Amin et al. (2011) observed a decrease in yield of up to 50% of sweetpotato at field salinity level of more than 8 dS-m. This findings are also in agreement with those reported for soybean (Kokubun *et al.,* 2001), pea (Duzdemir *et al.,* 2009) and mungbean (Mannan *et al*., 2013).

Such observation may be explained in part that salinity leads to metabolic changes and salt specific effects. Again accumulation of the toxic level of $Na^+$ in leaves results in necrosis and premature leaf senescence (Munns, 2002). Leaf senescence or defoliation leads to short supply of photosynthates that ultimately reduces yield under salinity and water stress (Grodzinski *et al.,* 1998).

The physiological, biochemical and morphological changes as well as yield reduction has played an important role as indices in salt stress tolerant screening of rice breeding programs. Surprisingly, low reduction in chlorophyll content serves as selection criteria for salt tolerant genotypes in soybean (Essa and Dawood 2001), proso millet (*Panicum miliaceum*) (Sabir *et al.,* 2009). In general changes of pigment contents under salt stress are used as parameter for selection of tolerant and sensitive cultivars in crop plants (Eryilmaz, 2006).

# CHAPTER SIX

# SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 SUMMARY

Adventitious bud and shoot regeneration of sweetpotato (*Ipomoea batatas)* was achieved from five Kenyan recalcitrant genotypes, within 12 weeks. However, the number of plants regenerated using the reported protocol was highly dependent on the type of explants and genotypes used Nevertheless, the results achieved in this study are very important for the breeding of *I. batatas*, particularly Kenyan genotypes, which have been reported to be difficult to regenerate through both somatic embryogenesis and organogenesis; and are still considered recalcitrant to *in vitro* regeneration (Luo *et al.,* 2006).

Indeed, the results presented in this study, confirms that most *I. batatas* genotypes that were thought to be recalcitrant can be regenerated following optimisation of media composition. The regeneration protocol reported in this study can be used to improve salt resistance of sweetpotato genotypes. Current knowledge and the findings from this study show that Kenyan sweetpotato genotypes can be regenerated through adventitious bud regeneration provided the conditions are optimized. In our study, the results indicate that the best genotypes to regenerate through organogenesis are *Kalamb Nyerere, Kemb 36* and *Spk 004*.

The production and productivity of several crops such as sweetpotato continues to be adversely affected by abiotic stresses resulting in enormous economic losses worldwide. Never the less traditional breeding technologies and proper crop husbandry strategies continue to play an important role in crop improvement. The conventional breeding programs are being used to introgress favorable genes of

interest from inter crossing genera and species into the crops to induce salt stress tolerance but with little success (Purohit *et al.*, 1998). New methods such as genetic engineering for introgression of genes that are known to be involved in stress response and putative tolerance have been adopted. However, the major limiting factors of this technique are the silencing of transgene, consequently reduction of gene expression and low transformation frequency (Meza *et al.*, 2001).

For the success of these techniques the selection in terms of tolerance and sensitive genotype is the starting point. The physiological and biochemical techniques described in this study serves as criterion for the selection of salt tolerance in the selected sweetpotato genotypes in particular vine length, RWC, and pigment content. Indeed, the accumulation of Chlorophyll content has been proposed as one of the potential biochemical indicators of salt tolerance in different crops, for example wheat (Arfan *et al.*, 2007), pea (Noreen *et al.,* 2010), melon (*Cucumis melo*) (Romero *et al.,* 1997), sunflower (Akram and Ashraf 2011) and proso millet (*Panicum miliaceum*) (Sabir *et al.*, 2009).

In order to improve salinity tolerance in crops, genotype variation should be observed to select the potential genotypes for salt affected areas (Sabir *et al.,* 2009). The study of salt stress influence on plant water status in association with photosynthetic parameters is important for understanding the physiological mechanisms of salt tolerance and identification of tolerant genotypes of some species including sweetpotato (Omar *et al.,* 2007). Taken together from the above criterion the following genotypes were identified as salt tolerant *Spk 004, Mweu*

*Mutheki, Ksp 36, Kemb 36* and *Kalamb Nyerere*. These genotypes can be in cooperated in breeding programs so as to introgress tolerance to sensitive sweetpotato genotypes.

**6.2 CONCLUSIONS**

The present study report success in the following;

1. Adventitious bud regeneration of Kenyan sweetpotato genotypes that had not been reported before. The regenerability of any given genotype is one of the requirements for crop improvement through biotechnology approaches such as genetic engineering.

2. This study was able to identify Kenyan sweetpotato genotypes that are salt tolerant by use of physiological and biochemical criterion. It is worth noting that not one criterion is sufficient to identify a tolerant genotypes.

3. I have established that the use of multi dimension approach for the identification of salt tolerance in sweetpotato as the efficient and reliable criteria than the use of one dimension approach. From this study the following are recommended.

**6.3 RECOMMENDATION**

1. The following sweetpotato genotypes; *Kalamb nyerere, Kemb 36* and *Spk 004* should be used for direct shoot organogenesis. For adventitious bud induction, the optimal TDZ hormone level of 0.25mg/l in the dark condition should be used. Then bud conversion to plantlets should be done in media containing 0.10 mg/l NAA.

2. Sweetpotato genotypes with the highest mean growth rates K*alamb nyerere, Spk203, Enaironi* and *Mweu mutheki* should be used for growth analysis experiments.

3. Salt tolerant sweetpotato genotypes *Spk 004, Mweu Mutheki, Ksp 36, Kemb 36* and *Kalamb Nyerere* can be in cooperated in conventional breeding or MAS programs so as to introgress tolerance to sensitive sweetpotato genotypes.

4. The following genotypes *Spk 004, Mweu Mutheki, Ksp 36, Kemb 36, Kalamb Nyerere* and *Ksp 28* are recommended for use in salt environment or ASAL areas with low yield reduction.

## 6.4 SUGGESTIONS FOR FURTHER RESEARCH

1. The identified genotypes should be analysed further using molecular tools to identify the genetic factors that make them salt tolerant or otherwise. This would confirm the basis of their tolerance hence making further research into development of genotypes suitable for use in ASAL areas of Kenya more efficient.

2. Indeed, the identified genotypes should be incooperated into MAS programs for the development of new salt tolerant genotypes suitable for saline environments in Kenya.

3. Further research should also be undertaken to evaluate more Kenyan sweetptotato genotypes for salt tolerance. There is need to expand the pool of the tolerant sweetpotato genotypes suitable to the different physiographic regions in Kenya.

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# Appendix

## Appendix 1: ANOVA mean squares (MS) summary tables

A. ANOVA of Photoperiod effects on adventitous bud induction

| Parameters | DF | Light | Dark |
|---|---|---|---|
| **Genotype (G)** | 6 | 19.64* | 23.45* |
| **Treatment (T)** | 1 | 44.64* | 82.57* |
| **G x T** | 6 | 0.98 | 1.07 |
| **Mean** | | 3.40 | 5.38 |

* Significant at p≤0.05 Turkey's means separation level.

B. ANOVA of regeneration frequencies at different levels of NAA hormone level (TDZ I)

| | **NAA level mg/l** | **0.00** | | **0.10** | | **0.25** | |
|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **Light** | **Dark** | **Light** | **Dark** | **Light** | **Dark** |
| **Model** | 6 | 1057.66* | 1042.33* | 14379.15* | 13630.33* | 6437.15* | 2878.17* |
| **Error** | 14 | 107.54 | 128.51 | 288.32 | 442.72 | 112.41 | 190.48 |
| **Corrected Total** | 20 | 1167.2 | 1170.84 | 14667.47 | 14073.05 | 6549.55 | 3068.65 |
| **Mean** | | 2.90 | 2.87 | 24.71 | 45.06 | 7.15 | 10.06 |

* Significant at p≤0.05 Turkey's means separation level.

C. ANOVA of regeneration frequencies at different levels of NAA hormone level (TDZ II)

| | **NAA level mg/l** | **0.00** | | **0.10** | | **0.25** | |
|---|---|---|---|---|---|---|---|
| **Source** | DF | Light | Dark | Light | Dark | Light | Dark |
| **Model** | 6 | 2257.14* | 2331.03* | 5696.06* | 4875.31 | 3822.35* | 4940.51* |
| **Error** | 14 | 67.18 | 154.67 | 174.17 | 377.37 | 128.35 | 246.56 |
| **Corrected Total** | 20 | 2324.32 | 2485.7 | 5870.23 | 5252.69 | 3950.7 | 5187.07 |
| **Mean** | | 6.50 | 8.92 | 39.89 | 59.11 | 18.5 | 20.86 |

* Significant at p≤0.05 Turkey's means separation level.

D. Overall Regeneration frequencies from two bud induction levels

| Source | DF | RF1 | RF 2 |
|---|---|---|---|
| **Model** | 41 | 68329.23* | 66824.02* |
| **Error** | 84 | 1269.97 | 1148.31 |
| **Corrected Total** | 125 | 69599.2 | 67972.33 |
| **Mean** | | 15.46 | 25.63 |

* Significant at p≤0.05 Turkey's means separation level.

E. ANOVA of I5 sweetpotato genotypes for organogenesis regeneration

| Variety | DF | SSquares | Mean Square | F Value | Pr > F | Sig |
|---|---|---|---|---|---|---|
| **KSP20** | 11 | 0.71 | 0.06 | 13.68 | <.0001 | **** |
| **Spk004** | 11 | 0.65 | 0.06 | 30.08 | <.0001 | **** |
| **Ksp28** | 11 | 0.70 | 0.06 | 43.91 | <.0001 | **** |
| **Kemb36** | 11 | 0.88 | 0.08 | 66.33 | <.0001 | **** |
| **Mugan** | 11 | 0.87 | 0.08 | 34.72 | <.0001 | **** |
| **Kemb23** | 11 | 1.05 | 0.10 | 55.26 | <.0001 | **** |
| **MM** | 11 | 1.06 | 0.10 | 101.37 | <.0001 | **** |
| **Ksp 36** | 11 | 1.22 | 0.11 | 164.01 | <.0001 | **** |
| **Zam** | 11 | 1.25 | 0.11 | 693.10 | <.0001 | **** |
| **Spk203** | 11 | 1.53 | 0.14 | 1135.46 | <.0001 | **** |
| **Spk013** | 11 | 1.00 | 0.09 | 103.78 | <.0001 | **** |
| **Enai** | 11 | 1.93 | 0.18 | 47.14 | <.0001 | **** |
| **Kemb10** | 11 | 1.28 | 0.12 | 169.47 | <.0001 | **** |
| **K Nyer** | 11 | 1.44 | 0.13 | 18.07 | <.0001 | **** |
| **Jewel** | 11 | 0.98 | 0.09 | 626.61 | <.0001 | **** |

*** Highly significant at P ≤0.05 probability level

**Appendix 2: ANOVA mean squares (MS) summary tables**

A. ANOVA of length of leaf 4 at end of growth period.

| Parameters | DF | Leaf length |
|---|---|---|
| **Days (D)** | 12 | 69.84* |
| **Genotype (G)** | 14 | 106.92* |
| **G x D** | 168 | 0.16 |
| **Mean** | | 8.19 |

* Significant at p≤0.05 Turkey's means separation level.

B. ANOVA of growth rate analysis

| Parameters | DF | GR |
|---|---|---|
| **Days (D)** | 11 | 1.40* |
| **Genotype (G)** | 14 | 0.09* |
| **G x D** | 154 | 0.01* |
| **Mean** | | 0.31 |

* Significant at p≤0.05 Turkey's means separation level.

**Appendix 3: ANOVA mean squares (MS) summary tables**

A.ANOVA of overall *in vitro* effect of salt and physiological stress on leaf pigments

| Parameters | DF | Chl a | Chlb | Cart | Tot Chl | Chl a/b | car/ Tot chl |
|---|---|---|---|---|---|---|---|
| **Genotype (G)** | 14 | 48.28* | 102.59* | 1.79* | 272.54* | 6.07* | 0.01* |
| **Treatment (T)** | 6 | 430.36* | 556.62* | 29.26* | 1958.11* | 5.15* | 0.06* |
| **G x T** | 84 | 9.75* | 14.40* | 1.41* | 32.87* | 1.78* | 0.01* |
| **Mean** | | 11.65 | 8.89 | 1.93 | 20.54 | 1.65 | 0.10 |

* Significant at p≤0.05 Turkey's means separation level.

**B.** ANOVA of *in vitro* salt and osmotic stress treatment effects

| Treatment | Chl a | Chl b | Car | Tot chl | Chl a/b | Car/tot chl |
|---|---|---|---|---|---|---|
| **High salt** | 28.12* | 31.54* | 1.00* | 110.63 | 6.97* | 0.02* |
| **moderate salt** | 10.99 | 15.62* | 2.74* | 2.57* | 0.01* | 17.13* |
| **low salt** | 12.69* | 25.76* | 1.87* | 50.07* | 1.00* | 0.00* |
| **high osmotic** | 29.63* | 18.79* | 2.38 | 79.14* | 1.08* | 0.02* |
| **moderate osmotic** | 10.99 | 15.62* | 2.74* | 2.57* | 0.01* | 17.45* |
| **low osmotic** | 7.96* | 14.54* | 0.63 | 39.61* | 0.13 | 0.00 |

* Significant at p≤0.05 Turkey's means separation level.

**Appendix 4: ANOVA mean squares (MS) summary tables**

A. ANOVA of overall effect of *in vivo* salt stress on photosynthetic pigment and vine length

Month 1

| Parameter | DF | Chl a | Chl b | Car | Tot Chl | Chl a/b | Car/ tot Chl | Vine length |
|---|---|---|---|---|---|---|---|---|
| **Genotype (G)** | 14 | 2.06 | 6.84* | 0.47 | 11.57 | 0.08 | 0.00 | 1896.62 |
| **Treatment (T)** | 1 | 16.50* | 17.3 | 1.75 | 67.58* | 0.04 | 0.00 | 1033.61 |
| **G x T** | 14 | 2.75* | 2.03 | 0.44 | 4.7 | 0.04 | 0.00 | 343.75 |
| **Mean** | | 17.69 | 12.76 | 3.03 | 30.44 | 1.42 | 0.10 | 44.16 |

* Significant at p≤0.05 Turkey’s means separation level.

Month 2

| Parameters | DF | Chl a | Chl b | Car | Tot Chl | Chl a/b | Car/ tot Chl | Vine length |
|---|---|---|---|---|---|---|---|---|
| **Genotype (G)** | 14 | 9.09* | 14.6 | 1.26* | 13.9 | 9.2 | 0.01* | 2608.36* |
| **Treatment (T)** | 1 | 1932.16* | 3472.17* | 39.26* | 10584.60* | 89.43* | 0.12* | 12840.28* |
| **G x T** | 14 | 9.48* | 20.16 | 1.75* | 46.49* | 8.38* | 0.01* | 495.02* |
| **Mean** | | 10.53 | 8.66 | 2.13 | 19.19 | 2.19 | 0.13 | 52.16 |

* Significant at p≤0.05 Turkey’s means separation level.

B. ANOVA of the effect of *in vivo* salt stress on RWC and yield

| Parameters | DF | RWC | Yield |
|---|---|---|---|
| **Genotype (G)** | 14 | 97.7 | 3981.28* |
| **Treatment (T)** | 1 | 14140.66* | 38742.33* |
| **G x T** | 14 | 22.48 | 223.28* |
| **Mean** | | 68.66 | 67.4 |

* Significant at p≤0.05 Turkey’s means separation level.

C. ANOVA of *in vivo* salt stress summary of effect

| Period | Chl a | Chl b | Car | Tot chl | Chl a/b | Car/tot chl | V. length |
|---|---|---|---|---|---|---|---|
| **Month 1** | 1.69 | 5.31 | 0.45 | 8.08 | 0.07 | 0.00 | 1027.38 |
| **Month 2** | 17.30* | 1.60* | 1.39* | 22.85* | 17.08 | 0.01* | 1002.42* |

* Significant at p≤0.05 Turkey’s means separation level.