Screening of sorghum (*Sorghum bicolor* L. Moench) somaclonal variants and X-ray radiated seed for salinity tolerance for growth in the Asals

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DECLARATION

This thesis is my original work and has not been presented for a degree to any other university.

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

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DEDICATION

I give glory to God for granting me health, grace, wisdom and favor to accomplish this work. I dedicate this thesis with love to my family.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ASAL's	Arid and Semi-arid areas
CEC	Cation Exchange Capacity
CO_2	Carbon dioxide
Gs	Stomatal Conductance
kV	Kilovolts
mA	Milli Ampere
NaCl	Sodium chloride
PAR	Photosynthetic Active Radiation
r	Rads
ss	Somaclone
VPD	Vapour Pressure Deficit

ABSTRACT

Sorghum bicolor (L) Moench is an annual crop adapted to a wide range of environmental conditions. It has a number of morphological and physiological characteristics that contribute to its adaptation to arid and semi arid lands (ASALs), including an extensive root system, low osmotic potential, increased cell wall thickness, small cell size and waxy bloom on the leaves that reduces water loss and the ability to survive for years through generations from fresh tillers developing on the old bases. One of the factors that characterize the ASALs is land salinization. The salinization process occurs because of incomplete weathering and intensive soil evaporation hence soil salinity is one of the main problems for world agriculture. The aim of this study was to evaluate the salinity tolerance levels of three selected *Sorghum bicolor* (L) Moench cultivars. The three cultivars namely; Mtama 1, El-gadam, and Seredo represent the three major categories of cultivated sorghum. They were obtained from Kenya Agricultural Research Institute (K.A.R.I.) - Katumani, in the Eastern province of Kenya; their generated somaclones and their corresponding X-ray treated seeds were also screened for salinity tolerance during seedling growth period in Shive and Robbin's nutrient solution at 5, 10 and 15 dS/m salinity levels against a control (0.22 dS/m) based on morphological aspects. During the hydroponics screening experiment a check cultivar/ standard (Serena) documented as saline tolerant was also used as a control. The inter- and intra-cultivar effect of salinity on root length, root: shoot ratio, root and shoot morphology of seedlings was assessed after seven days growth period in hydroponics system. The ANOVA results for all the cultivars for the seven days growth in Shive and

Robbin's nutrient solution as well as treatment × cultivar interaction was significant ($p \leq 0.05$). Cultivar Seredo had higher seedling root length than cultivar Mtama 1 and El-gadam at 10 and 15dS/m. This was attributed to the effect of osmotic stress on salinity sensitive cultivars (Mtama 1 and El-gadam) which led to accelerated leaf senescence thereby inhibiting seedling leaf growth. Better root and shoot growth was however, obtained after X-ray seed treatment of the three cultivars. Further screening of the three sorghum cultivars, their somaclones and X-ray treated seed based on their physiological traits was carried out in the field. Results clearly indicated that basic seed cultivars Elgadam, Mtama 1 and Seredo, had significant differences in physiological traits in comparison with their somaclones and plants obtained via X-ray treatment of the basic seed. Plants obtained via X-ray treatment had significantly better physiological performance, during vegetative growth. Cultivar El-gadam, its somaclone and X-ray treated seeds showed the lowest growth potential when grown both in the hydroponics system and in the field showing the salinity sensitivity characteristics of the white sorghum category. In conclusion, cultivar Seredo representing the red sorghum exhibited optimal efficiency in physiological performance. X-ray seed treatment therefore, has potential in breeding for salinity because it is able to discriminate saline sensitive and Asals sensitive sorghum cultivars at an early stage. However, further research should be undertaken under field conditions to test the physiological and morphological performance of subsequent generations of the X-ray radiated seed.

CHAPTER ONE

1.0 INTRODUCTION

1.1Background information

The Sorghum genus as currently proscribed consists of twenty five species (USDA ARS, 2007). The genus is separated into five taxonomic subgenera or sections: Eu-Sorghum, Chaetosorghum, Heterosorghum, Para-Sorghum and Stiposorghum (Garber, 1950). Section Eu-Sorghum contains all domesticated/cultivated sorghum races and varieties such as *Sorghum bicolor* (L.) Moench as well as the wild and weed species *Sorghum halepense* (L.) Pers (Harlan and de Wet, 1971). *Sorghum bicolor* races of cultivated sorghum are recognizable on spikelet/panicle morphology (Smith and Frederiksen, 2000).

Salinity is characterized as one of the main factors of world soil degradation (Grieve and Maas, 1984; Grieve and Shannon, 2001). Crops grown in salt affected soils suffer decline in growth due to NaCl stress leading to reduced plant growth and crop productivity (Netondo *et al.*, 2004). The inhibition of growth in long term exposures (days) results from osmotic effects on water availability, reduction in net CO_2 assimilation, specific ion effects, or ion imbalance due to interference with uptake of essential nutrient ions. It has previously been demonstrated for sorghum that the primary effects of salt stress are to reduce leaf growth rate, leaf emergence rate and overall shoot development (Bernstein *et al.*, 1993). Adaptation and resistance responses to NaCl salinity have been expressed in *Sorghum bicolor* L. Moench by exposure to 150molm^{-3} NaCl respectively for 8 and 21 days after germination (Amzallag, 1994).

The quantitative nature of salinity stress tolerance and the problems associated with developing appropriate and replicable testing environments pose a challenge to distinguish salt tolerant cultivars from salt sensitive ones. Field selection for salinity tolerance is a laborious task therefore hydroponics is a reliable means of assessing salt tolerance of sorghum germplasm. Cell and tissue culture techniques have also been used to obtain salt tolerant plants employing *in vitro* culture approaches (Arzani, 2008).

Sorghum bicolor, a vigorous annual crop (FAO, 1991) and a diploid, is essentially unique among the major cereals in that it has a relatively small genome (735 Mbp), which although larger than rice (389 Mbp) is smaller than the other important cereals (wheat 16900 Mbp, maize 2600 Mbp) (Paterson *et al*., 2004).

The plant molecular and physiological traits provide the basis for its efficient germplasm screening although both breeding and screening for its salt tolerance encounters limitations such as different phenotypic responses of plants, different physiological mechanisms and complicated genotype × environment interactions (Reddy *et al*., 2006). The identification of varieties and lines with naturally high levels of pre-anthesis drought tolerance "where plants are stressed during panicle differentiation prior to flowering", can develop sorghum varieties with stable, high yields (Ellis *et al*., 1997). Some of its varieties, the so-called tannin, bitter, bird-proof, bird-resistant or red sorghum types contain condensed tannin. The condensed tannins, otherwise known as proanthocyanidins, are located in the testa (seed coat) and pericarp of the grain. Tannins confer considerable agronomic advantages to these tannin sorghums (Serna and Rooney, 1995). Bird-predation, a major problem, is reduced as the tannins are bitter. Further, the

tannins protect these sorghums from insect and fungal attack. However a drawback of the tannin sorghums is that the tannins can bind with both the grain protein (Emmambux and Taylor 2003) and with enzymes of the digestive tract (Price and Butler 1980). The tannins can also adversely affect the quality of malt made from high-tannin sorghum by reducing its enzymatic activity (Daiber, 1975). In addition, certain sorghum varieties posses "stay green" genes that enable them to continue to photosynthesize during environmental stress and have a high capacity for osmotic adjustment to maintain turgor pressure in cells (Nguyen *et al*., 1997).

1.2 Statement of the problem

Each year more and more land becomes non-productive owing to salt accumulation and at least 25% of currently cultivated land throughout the world suffers from excess salinity principally from sodium chloride (NaCl). In dryland areas, the accumulation of salt in the soil surface frequently occurs due to intensive evaporation demand of the dry atmosphere. Soil salinity is invariably associated with drylands as the most important modulating factors responsible for salt accumulation are least expressed under dry environment. In dry areas incomplete weathering/ lixiviation of constituent soil minerals ensures a steady source of salts. In addition the inadequate rainfall favours the deposition of salts on the soil surface. Further more, attempting to improve productivity in arid areas by use of saline irrigation water has resulted in a gradual increase in salinity. All these factors have contributed to increased salinity which causes osmotic drought,

strongly influencing the morphological and physiological traits of crops resulting into toxicity and subsequent reduction in plant growth.

Traditionally in dryland, crops are grown mainly for subsistence and are selected out of the locally available crops. These crops are not necessarily the most efficient with regard to productivity, moisture use, economic returns and labour utilization potential and lack of technological support on the crop such as mutation breeding in the dryland areas is the main reason in respect of modern farming. Thus the guiding principle is the need for choice of crops with a growth rhythm that fits in with the water availability for arid and semi arid areas. This was the basis for this study in order to evaluate hardy sorghum cultivars by X-ray seed treatment and assess selected *Sorghum bicolor* (L) Moench generated somaclones both in the field and hydroponically.

1.3 Justification

Sorghum bicolor (L) Moench has been ranked the fourth in importance among the cereal crops namely wheat, rice and maize (Smith and Frederiksen, 2000). Together with pearl millet (*Pennisetum americanum* (L) and finger millet (*Eleusine coracana* (L) Gaertn) it represents Africa's main contribution to the world food supply (de Vries and Toenniessen, 2001). According to the National Development Plans and National Food Policies (Republic of Kenya, 1989 and 1994) the top-ranking policy of the agricultural sector is to attain self-sufficiency in basic food. Therefore, increasing crop production through techniques that optimize the use of soil water is imperative. Therefore by attempting to improve productivity of arid and semiarid lands (ASALs) there is need to try alternative methods to find crop varieties that can withstand salinity stress. The breeding of crop plants for environmental stress tolerance has been difficult and slow and the quantitative nature of stress tolerance and the problems associated with developing appropriate and replicable testing environments make it difficult to distinguish stress-tolerant lines from sensitive lines (Robinson and Jones, 1986) hence the justification to use hydroponics experiments.

Sorghum fills a unique and highly significant place in salinity tolerance in arid zones (Ahloowalia and Meluzynski, 2004). The sorghum crop has been considered as having a few of its cultivars relatively more tolerant to salinity hence has the potential as a grain and fodder crop in saline soils. Sorghum yields more consistently than maize under conditions of moisture stress and is able to withstand low soil fertility and yet produce

grain of similar food value even though plant growth on such conditions is affected by higher osmotic tensions (osmotic stress) of soil solutions, poor physical conditions of soil created by sodium, toxicity of specific ions such as boron, nutrient imbalance and deficiency in soil solutions (Munns, 1993, 2002). The presence of drought and salt stress which limit crop production in arid regions has made plant breeders realize that in the light of biotechnology these stress problems can be minimized by altering the plant to suit the soil conditions other than relying on natural selection and in daaition generation of additional genetic markers through mutation by seed mutagenesis facilitates genetic and breeding studies (ICRISAT, 1981). X-ray treatment and somaclonal variation however, has a potential in breeding for salinity tolerance because it is able to discriminate saline sensitive and drought sensitive sorghum cultivars at an early stage.

Sorghum has not received full attention of the scientific community and by governments. Therefore, much of its production is on small scale as compared to maize and it is also limited in the commercial urban markets (Deu *et al*., 2006; Kayode *et al*., 2006).

1.4 Objectives

1.4.1 Overall objective

To screen *Sorghum bicolor* (L) Moench selected cultivars for improved salinity stress tolerance.

1.4.2 Specific objectives

1. Evaluate for salinity tolerance by screening among selected sorghum cultivars, their corresponding somaclonal variants and seedlings obtained via X-ray seed exposure hydroponically.
2. Screen for the effect of X-ray seed irradiation among the three selected sorghum cultivars.
3. To evaluate physiological parameters associated with drought and salinity tolerance under field conditions among selected sorghum cultivars, their corresponding somaclonal variants and seedlings obtained via X-ray seed radiation.

1.5 Hypotheses (Ho)

Ho (1): The three selected sorghum cultivars, their somaclones and X-ray exposed seeds have no tolerance to salinity.

Ho (2): X-ray irradiation of *Sorghum bicolor* seeds does not produce mutants.

Ho (3): The three selected sorghum cultivars, their somaclones and X-ray irradiated seeds have no tolerance to salinity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Environmental factors that impact on crop physiology in ASALs

2.1.1 Sunshine

Sunshine is one of the most advantageous aspects of the ASALs in respect of plant growth and the prime source of energy directly required by plants for photosynthesis (Jordan and Sullivan, 1982). In comparison to the wet areas the dryland areas have more sunny days, and mainly due to these reasons the average sunshine hour in dryland areas is high. Except in very hot periods when intensity of sunshine is too high for physiological activities, the plants are able to produce good yield throughout the year (Rosenzweig and Parry, 1994).

2.1.2 Evaporation and transpiration

Evaporation is a process of loosing water from a moist or wet surface to the atmosphere due to vapour pressure difference, which is of tapering nature from the ground level upwards in the sorghum crop (Ferguson *et al.*, 1998). Water is evaporated continuously from the surface of plants leaves (especially from tropics to subtropics) at about the same rate as from a free water surface having the same exposure and temperature. Evaporation from the crop is of two types: One is the common physical process of evaporation at the plant surface including the water that comes out through diffusion from the cuticle while the other is the physiological process by which the plants

evaporate the excess water drawn from the soil by their root system while absorbing the nutrients which is the main component of water loss from the plants in arid regions (Osmanzai, 1992). This latter process of water loss from the plant surface is called transpiration. About 99% of water absorbed by the roots is lost to the atmosphere through transpiration and is sufficiently significant in respect of localized atmosphere of a dryland area particularly to create microclimate hence humidity of a locality depends entirely on the continuous evaporation of moisture from the area. Humidity refers to the water content of the atmosphere. In the dryland areas, in general, the humidity is very low due to very low moisture. Liquid water is converted into water vapour by evaporation for which necessary energy is provided by solar radiation and the amount of minute droplets of water suspended on the air (water vapour) in atmosphere depends upon wind and temperature (Lang and Volz, 1993).

2.1.3 Temperature

Temperature of about 30^0C is always helpful for biological as well as biochemical activity of non-thermo sensitive plants although high temperature (40^0C) is a common phenomenon in ASALs which seriously reduces the vegetative growth of crops and adversely disturbs the pre-reproductive age (Bramlage and Weis, 1997).

The higher the temperature, the more is the amount of water vapour that can be held by the atmosphere. The troposphere, which is the lower most layer of the atmosphere, contains all the water vapour present in the atmosphere. In this troposphere the condensation of vapour to form clouds and to produce precipitation is likely to occur. In

hot humid areas it is up to 4 percent by volume, whereas in areas of inclement climate it is up to 0.01 percent (Marlow and Loescher, 2004).

2.1.4 Water stress

Inadequate rainfall favours the deposition of salts on the soil surface leading to water or moisture unavailability to crops hence scarcity of water is the main factor that limits the growth of plants in arid areas (Ghosh and Jana, 2004). In the ASALs scarcity is also associated with: - low rainfall or moderate to good rainfall with unimodal and erratic distribution, large scale surface runoff, undulating topography with low soil infiltration that provides little scope to retain rain water either on the surface or underground and highly permeable soils. Plants therefore, adapt to water stress by: - maintenance of higher root to shoot ratio, tolerance to water stress and higher temperature by physiological characteristics such as leaf rolling and membrane permeability useful for stress tolerance of the growing or mature plant (Whaley and Scott, 1997).

2.2 Sorghum domestication

The domestication of sorghum [*Sorghum bicolor* (L.) Moench] has its origins in Ethiopia and surrounding countries, commencing around 4000–3000 BC. Numerous varieties of sorghum were created through the practice of disruptive selection for more than one level of a particular character within a population (Sally et al., 2007). This resulted from balancing farmer selection for cultivated traits and natural selection for wild

characteristics, generating both improved sorghum types, wild types and intermediate types (Sally *et al*., 2007). These improved sorghum types were spread via the movement of people and trade routes into other regions of Africa, India (approx. 1500–1000 BC), the Middle East (approx. 900–700 BC) and eventually into the Far East by AD 400 (Doggett, 1988). Initial domestication of sorghum would have focused primarily on converting wild types with small, shattering (dehiscent) seed to improved types with larger, non-shattering seed with more compact panicles but disruptive selection resulted in sorghum types with vastly different characteristics in height, inflorescence type, and of course, end use that is, food, fodder, fibre, building materials, etc (Clayton and Renvoize, 1986).

2.3 Sorghum production

Sorghum is the fourth most important cereal crop behind wheat, rice and maize, (Smith and Frederiksen, 2000). The world annual sorghum production is over sixty million tonnes. Of the total world area devoted to sorghum, over 80 percent is in developing countries. Africa produces about twenty million per annum, about one-third of the world crop whereby sorghum is grown in a large belt that spreads from the Atlantic coast to Ethiopia and Somalia, bordering the Sahara in the north and the equatorial forest in the south. This area extends through the drier parts of eastern and southern Africa, where rainfall is too low for the successful cultivation of maize (Taylor, 2002). Table I shows the major sorghum producing countries of Africa, with the northern African countries of Nigeria, Sudan, Ethiopia and Burkina Faso accounting for nearly 70% of Africa's production.

Table 1: Countries in Africa with an annual production of at least one hundred thousand tonnes of sorghum as at 2001.

Country	**Production (tonnes x 10^3)**
Nigeria	7 081 (33.8)[a]
Sudan	4 470 (21.4)
Ethiopia	1 538 (7.3)
Burkina Faso	1 372 (6.6)
Egypt	862 (4.1)
Tanzania	736 (3.5)
Niger	656 (3.1)
Mali	517 (2.5)
Chad	497 (2.4)
Cameroon	450 (2.1)
Uganda	423 (2.0)
Mozambique	314 (1.5)
Ghana	280 (1.3)
South Africa	211 (1.0)
Rwanda	175 (0.8)
Benin	165 (0.8)
Togo	141 (0.7)
Senegal	140 (0.7)
Kenya	133 (0.6)
Zimbabwe	103 (0.5)
Somalia	100 (0.5)

[a] Percentage of Africa's sorghum production

Source: Taylor, 2002.

Consumption of sorghum follows the global pattern of output, since most of it is consumed in the countries where it is grown. Sorghum is used for two distinct purposes: human food and animal feed (Masi, 1988).

In Kenya, agriculture provides three quarter of the national food requirements and raw materials for the agro-industrial sector. The current attempt to improve sorghum productivity in the ASALs takes place behind a background of inadequate soil water, and crop failures (Boursier and Lauchli, 1990). Salinity stress is a major constraint to

sorghum productivity in the Asals (Rosenow and Clark, 1995). Sorghum is grown predominantly in low-rainfall, high temperatures, arid and semi-arid tropics although trials conducted in Kenya during the short rainy season showed that an improved sorghum variety KAT-369 yielded 4.1 tonnes/ha in comparison to the 3.2 tonnes/ha for maize (ICRISAT, 1994). It has been the only viable food grain for many of the world's most food insecure people (Taylor, 2002). This pronounced sorghum environmental tolerance is associated with its very deep penetrating and extensive roots (National Research Council, 1996), moisture conservation which reduces transpiration when stressed, by leaf rolling and stomatal closure. Higher than normal levels of epicuticular wax also appear to be of importance in this respect (Jordan and Sullivan, 1982).

More than 800 million hectares of land throughout the world are salt affected (including both saline and sodic soils). Some of the most serious examples of salinity occur in the arid and semi arid lands (Arzani *et al*., 2008) because of the use of irrigation in attempting to improve productivity has led to a gradual increase in salinity (Republic of Kenya, 1982).

2.4 Adaptation of sorghum to salinity

Sorghum has a number of morphological and physiological characteristics that contribute to its adaptation to dry conditions, including an extensive root system, predominantly self-pollinating, low osmotic potential, increased cell wall thickness, decreased cell size, waxy bloom on the leaves that reduces water loss and the ability to stop growth in periods of drought and resume it again when conditions become favorable. These modifications arose from random mutations and recombinations that

occur in plants growing in dry habitats but have become preserved by natural selection in dry habitats (Culter *et al*., 1977). It is, however, primarily a crop of hot, semi-arid tropical environments with 400 – 600 mm rainfall that are too dry for maize. It is also widely grown in temperate regions and at altitudes of up to 2300 m in the tropics (Taylor, 2002).

High salinity leading to water stress has important effects on the physiology and biochemistry of plants; accumulation of substances like proline and betaine help in lowering osmotic potential of cells without denaturing enzymes essential for metabolic processes (Salisbury and Ross, 1986) while Abscisic Acid (ABA) is a universal stress hormone whose production is triggered by stress factors including nutrients deficiency, toxicity, salinity, chilling and water-logging (Hanson and Hitz, 1982). Accumulation of ABA decreases shoot growth conserving water and promotes root growth to increase water supply. ABA plays a role in drought tolerance in that it also affects membrane permeability and causes stomatal closure (Rains, 1989). Its level increases due to disturbance in normal nitrogen metabolism caused by water stress but has resulted in some cultivars of the gramineae family to become adapted to ASALs (Naylor, 1972).

2.5 Mutation breeding

Mutation breeding is the improvement of crops through the induction of mutations at specific loci controlling economically important traits and/or eliminating undesirable genes from elite breeding lines. Mutations are important as the major source of genetic variation which fuels evolutionary change**.** Changes may occur in gametes (germline mutations) or somatic mutations. The selected mutants can be developed directly as cultivars or they can be used as source of population variations in a breeding program. In addition, mutants are valuable as genetic markers and as testers for linkage studies (Till *et al*., 2003).

Mutants have long been a valuable resource in plant breeding, both natural and artificially induced and provide an alternate source of genetic diversity (Henikoff and Comai, 2003; Henikoff *et al*., 2004). The method employed (irradiation or chemical) to induce a mutated population can affect its usefulness and application for genomics research. As a result of the random nature of mutation induction, by physical and chemical means, each individual in a population is likely to contain a unique range of gene mutations. This provides a powerful resource for genome analysis employing recent molecular technologies (Sally *et al*., 2007).

Ionizing radiations and chemical mutagens have been the principal agents employed to increase mutation frequency in plants. The radiations include X-rays, neutrons, gamma rays, Ultraviolet and laser beams. The radiation dose is determined by the intensity of the radiations and the length of exposure. If ionization occurs in or near a chromosome, its force can split chemical bonds, causing various structural changes within the DNA,

such as a change in a single nucleotide base of a gene (point mutation), replacement of one nucleotide base by another, or deletion of one or more bases in the DNA sequence (Sleper and Poehlman, 2004).

X- and gamma-rays are energetic enough that they produce reactive ions (charged atoms or molecules) when they react with biological molecules; thus they are referred to as ionizing radiation. Ionizing radiation produces a range of damage to cells and organisms primarily due to the production of free radicals of water (the hydroxyl or OH radical). Free radicals possess unpaired electrons and are chemically very reactive and will interact with DNA, proteins, lipids in cell membranes, etc. Thus X-rays can cause DNA and protein damage which may result in organ failure, block cell division, or cause cell death.

The objective of seed treatment is to produce seedling mutants that are valuable for plant breeders, especially if they can be found linked to a particular gene of interest that can also be selected at the seedling stage, where they can practice selection at an early stage (Neuffer 1993). Seedling mutants have been helpful in genetics and breeding studies for induction of early flowering in spring rape (Thurling and Depittayanan 1992) and male sterility in wheat (Maan and Williams 1984).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of experimental materials

Four cultivars of sorghum were obtained from K.A.R.I. Katumani, a research station in the Eastern province of Kenya. These were: Mtama 1, El-gadam, Seredo and Serena. The latter (Serena) has been documented as drought and salinity tolerant cultivar and therefore it was used in hydroponics screening experiments as the check cultivar/ standard cultivar. Mtama 1, El-gadam and Seredo represent the three major categories of cultivated sorghum based on tannin level because entire sorghum cultivars fall in three categories as follows:

Category I represented by Mtama 1: Creamish in colour with less testa and no tannins

Category II represented by El-gadam: Chalky white with testa and low tannins

Category III represented by Seredo: Brown/red in colour with testa and high tannins

Somaclonal seed - this refers to seed obtained via tissue culture of the three sorghum cultivars (Mtama 1, El-gadam and Seredo). During the tissue culture propagation the callus formed was exposed to 100mM NaCl to change the ploidy level and harden the resulting plants for saline growth conditions (Makobe, 2002).

3.1.1 X-ray seed treatment

The JKUAT Hospital provided the necessary equipment for the X-ray seed treatment. Seeds of the four *Sorghum bicolor* (L) Moench cultivars (sourced from K.A.R.I. Katumani) were exposed to X-rays. The X-ray radiation levels of exposure were

achieved based on the current (mA), voltage (kV) and period of exposure (seconds). The X-ray treatment was done at three strength levels: 15,000r, 17,500r and 20,000r followed by screening for salinity tolerance in hydroponics system and in the field.

3.2 Hydroponics screening for salinity tolerance

3.2.1 Pregermination of seeds

Prior to seed germination, preparation involved washing seeds in running tap-water for thirty minutes before rinsing three to four times with distilled water. Seeds of the three selected cultivars (basic seed, somaclonal seed and X-ray treated seed) in addition to the check variety (Serena) of sorghum were pregerminated in petri dishes (90mm diameter) fitted with moist cotton wool in a germination chamber at 27^0C for a period of 3 days. Each pregermination experiment was replicated three times and each Petri dish contained 35 seeds of a particular cultivar. (Experiment 1 had the four selected cultivars each represented by four petridishes of 35 seeds replicated three times which added upto 1,680 seeds. Experiment 2 had the three somaclones of cultivars Mtama 1, El-gadam and Seredo each represented by four petridishes of 35 seeds replicated three times which added upto 1,260 seeds. Experiment 3 had the X-ray treated seed of cultivars Mtama 1, El-gadam and Seredo each represented by four petridishes of 35 seeds replicated three times which added upto 1,260 seeds). A complete randomized design was used in the pregermination chamber. After 3 days the seedlings chosen for uniformity of both the plumule and radicle length of 0.5 cm were transferred to 4 litre containers (30 seedlings

per container) containing 3litres of Shive and Robbin's nutrient solution culture which contained macronutrients and micronutrients.

3.2.2 Preparation of stock solution

Stock solution was prepared by weighing the following salts, i.e macronutrients namely: KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4.7H_2O$, NH_4SO_2 and micronutrients which were sourced from $FeSO_4$, $MnCl_2.4H_2O$, H_3BO_3, $(NH_4)_6$ $MO_7O_{24}.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ on an electronic weighing balance. All salts were dissolved in distilled water. Major salts (macronutrients) were dissolved to make a concentration of 1M in a volume of 250ml and the micronutrients obtained by dissolving salts containing the minor elements to make a concentration of 1ppm in a volume of 100ml since they are required in relatively small amounts.

3.2.3 Preparation of working solution and Sodium chloride (NaCl) treatment

A working solution was obtained by pippeting precise amounts of stock solution (micro and macronutrients respectively) and transferring into 4L containers. This was immediately followed by topping up the mixture of macro and micro nutrients to three litres per container using distilled water.

Four treatments were imposed, corresponding to a Shive and Robbin's nutrient solution Electrical conductivity (EC) levels of 5, 10 and 15dS/m obtained by dissolving 7g, 14.5g and 23.6g of NaCl in 3 litres of the basal nutrient solution against a Shive and Robbin's basal nutrient solution (control; 0.22dS/m).

3.2.4 Hydroponics system

A hydroponics system in the laboratory was set for the growth of seedlings with provision for aeration whereby the nutrient solution was kept circulating to optimize gaseous exchange for plant growth. Treatment application was done immediately after transfer of the seedlings from the pregermination chamber into the nutrient solution. Supplemental lighting (4750lux) was provided for 12 hours a day by use of fluorescent tubes through out the growing season and temperature maintained at 28^0C and 25^0C for day and night respectively. The seedlings were allowed to grow in this condition for seven days but the nutrient solution was replaced after every 48 hours as well as pH adjustment to pH5.5. Following 7 days exposure to non lethal NaCl levels (5, 10 and15dS/m), selected cultivars of *Sorghum bicolor* (L.) Moench plants were assessed of their capacity to survive in and tolerate NaCl concentration as an adaptation to salinity. The 7 days exposure to non lethal NaCl levels was replicated three times for each cultivated variety.

3.2.5 Data collection and Analysis

The results of non-destructive daily shoot length measurements were obtained during the rapid phase of leaf elongation for plants growing in hydroponics solution for five consecutive days, starting on the second day after transferring the seedlings into the nutrient solution. After 7 days, shoot length and root length were measured using a graduated 30cm ruler while fresh weight and dry weight of all the seedlings from each treatment were measured using an electronic precision weighing balance. Dry weight was obtained after drying the seedlings in the oven at 65^0C for 24 hours. The data

obtained was entered and stored in Excel spreadsheet. The effect of salt treatment was tested by ANOVA and means separated by SNK Test then a SAS statistical package used for further analysis. In addition photographs of salinity sensitive and tolerant varieties were taken and shown in plates.

3.3 Field experiment

Two field experiments were conducted at JKUAT demonstration farm during September to December, 2007 and March to June 2008 respectively. The three selected sorghum cultivars (Mtama 1, El-gadam and Seredo) together with their somaclonal variants and X-ray treated seeds were screened for salinity tolerance based on C_4 physiological aspects of growth. The parameters used were: Photosynthetic Active Radiation (PAR), Stomatal Conductance, Transpiration rate, Carbon dioxide Assimilation Rate and Photosynthesis rate since the osmotic growth inhibition of sorghum by salinity closely relates to the regulatory physiological changes.

3.3.1 Field experimental layout and design

In the field, a Randomized Complete Block Design was adopted to avoid bias on treatment randomization. This was after obtaining soil samples from the site and conducting laboratory analysis to determine the pH in a 1:1 (soil: water) suspension according to McKeague, 1978 and McLean 1982, EC (Electrical Conductivity) based on the methodology of EC measurement given in the USDA Handbook (Richards, 1954), CO_3^-, HCO^-_3 using the titrimetric method (FAO, 1974), Cl^- , K (by absorption spectrophotometry) and CEC (Cation Exchange Capacity) of the soil according to Richards, 1954. Soil imported from other ASALs such as Yatta and Amboseli, Kenya, were analysed for the same to determine their sutability for growth of sorghum. The Randomization was performed before planting to avoid biased placement of treatments.

At planting, seeds were drilled in rows with spacing of 30×60 between plants and rows respectively. Three seeds were directly seeded per hole and thinning followed soon after establishment. During thinning a single seedling was maintained per hole. Each of the three cultivars was represented by 4×4m plots with guard rows.

3.3.2 Characterization of mechanisms for salinity tolerance

During the growth period of the three selected cultivars in the field, measurement of some selected C_4 physiological parameters which characterize drought and salinity tolerance was carried out. The parameters were: Photosynthetic Active Radiation (PAR), Stomatal Conductance, transpiration rate, CO_2 assimilation rate and photosynthesis rate.

3.3.3 Data collection and Analysis

Measurements of the C_4 physiological parameters were made at 09:00, 12:00 and 15:00 hrs fortnightly. The measurements commenced fourteen days (two weeks) after seed germination. Subsequent measurements were done at four weeks, six weeks, eight weeks and ten weeks respectively on the second leaf from the leaf sheath. These parameters were measured and recorded by the infrared gas analyzer (IRGA), model: CIRAS-1. The data obtained was downloaded and stored in Excel spreadsheet and the effect of stress at the peak of vegetative growth (eight weeks when significant differences became evident), for all the treatments tested by ANOVA. Means were separated by SNK Test then a SAS statistical package used for further analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Hydroponics screening for Salinity Tolerance in *Sorghum bicolor*

4.1.1 Hydroponics screening of basic seed

The aim of the hydroponics experiment was to establish intercultivar differences in response to known salinity levels since it was not easy to separate the effects of salinity and nullify other variables that affect plant growth in the soil. The root length, root dry weight, shoot dry weight, root and shoot morphology were the parameters used to establish intercultivar variations because monocotyledonous plants have predominant leaf growth from the intercalary meristem at the leaf base hence a unidirectional growth.

Fig. 1 shows the results of root length of the four basic seed cultivars grown in hydroponics at 0.22, 5, 10 and 15dS/m {$LSD_{0.05} = 0.1825$}

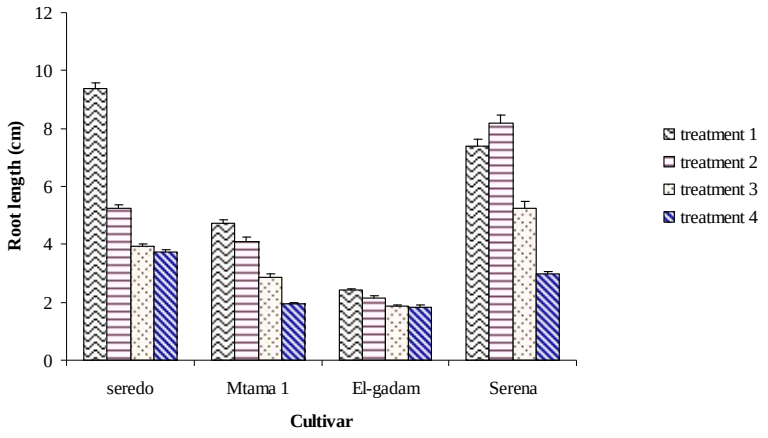

Figure 1: Effect of salinity level on root length of basic seed cultivars (Where treatment 1, 2, 3 and 4 correspond to Electrical conductivity 0.22, 5, 10 and 15dS/m)

The root growth of the basic seed cultivars varied with treatment. This followed the same pattern, i.e., decreasing with increase in EC as follows: Treatment 1(control = 0.22 dS/m) > Treatment 2(5 dS/m)> Treatment 3(10dS/m) > Treatment 4(15 dS/m) in the selected cultivars. The least root length was observed in El-gadam, indicating its sensitivity to increase in EC. Mtama 1 had relatively longer roots than El-gadam at all levels of EC but this difference was insignificant. Root length differences observed in cultivars Seredo and the check/standard cultivar (Serena) were insignificant in the four EC levels i.e they responded to treatment in the same way with the root length decreasing with increasing EC. However the comparison of root length between Mtama 1 versus El-gadam and Seredo versus Serena were significantly different ($P \leq 0.05$). Basing on this comparison, Seredo and Serena were salinity tolerant while Mtama 1 and El-gadam were sensitive to salinity. This was further justified by obtaining the root: shoot ratio (Fig. 2) {$LSD_{0.05} = 0.14$}

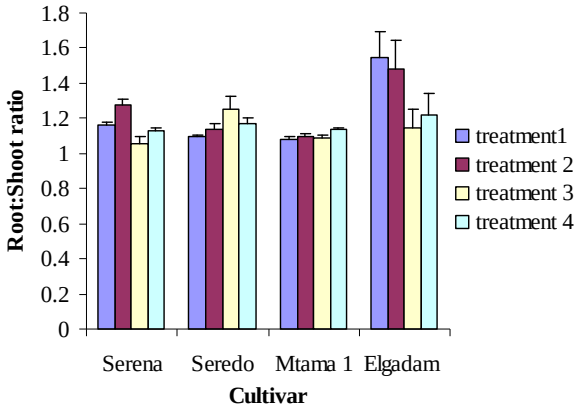

Figure 2: Root: shoot ratio of basic seed (Where treatment 1, 2, 3 and 4 correspond to Electrical conductivity 0.22, 5, 10 and 15dS/m)

Root dry weight was divided by shoot dry weight to determine the effect of increasing the EC on root: shoot ratio. Root ratio was negatively affected by increase in salinity levels of the growth medium (nutrient solution) and intercultivar differences were observed (Fig. 2). However difference in salinity tolerance was observed in relation to cultivar Serena (check/ standard cultivar). Shoot dry weight was less affected in Seredo, Serena and Mtama1 in the different levels of salinity but high root:shoot ratio observed in El-gadam showed that the shoot dry weight was negatively affected by an increase in salinity level. The impact of increasing EC on root: shoot ratio of the four cultivars had a significant effect ($p \leq 0.05$). Root dry weight was also differentially inhibited at low salinity levels (0.22dS/m and 5dS/m) and this was attributed to inter-cultivar differences. From the results, root: shoot ratio of cultivars Seredo and Serena indicate that there was balanced root-shoot growth hence were grouped as salinity tolerant cultivars while El-gadam was salinity sensitive basing on both the root length and root: shoot ratio

4.1.2 Hydroponics screening of X-ray treated seed

Figures 3{$LSD_{0.05} = 1.05$} and 4{$LSD_{0.05} = 0.04$} represent the effect of 15dS/m EC on three different levels of X-ray seed treatment for the three selected cultivars against a control (the basic seed cultivars).

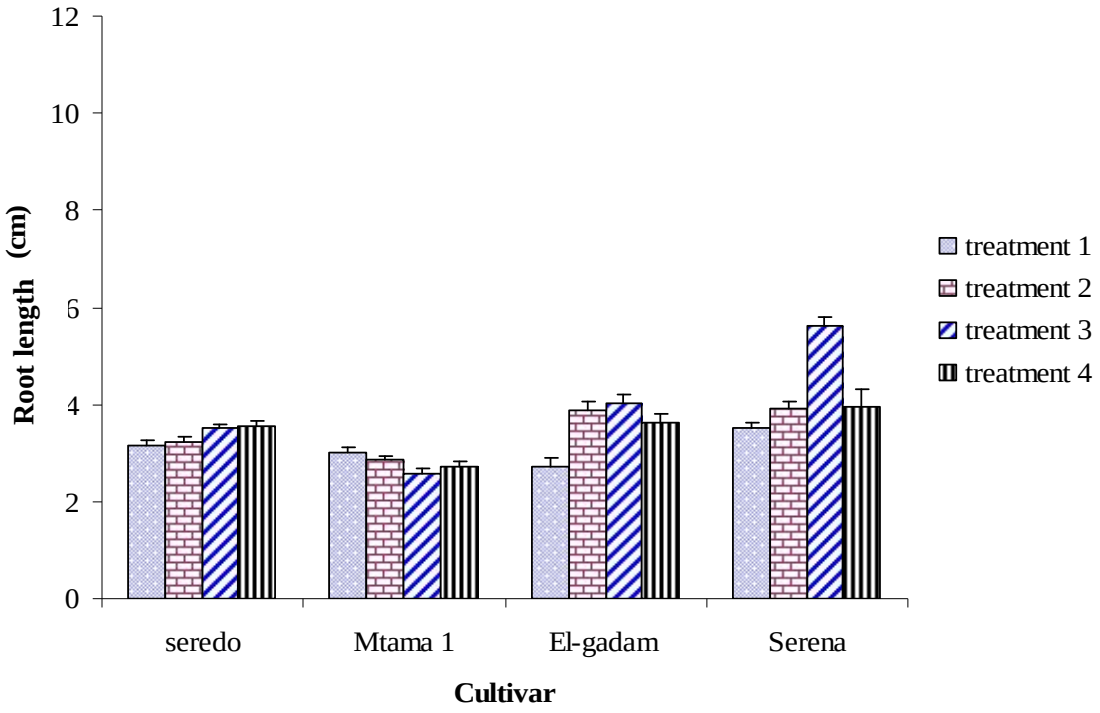

Figure 3: Effect of 15dS/m salinity level on root length of X-ray treated seeds (Where treatment 1, 2, 3 and 4 correspond to control (basic seed), X-ray seed treatment 1 (15000r), X-ray seed treatment 2 (17 500r) and X-ray seed treatment 3 (20 000r)

Root length was different after by X-ray seed treatment in cultivars Seredo, Serena and El-gadam. The lowest level of X-ray exposure (15,000r) had relatively longer roots for the basic seeds (control) at 15dS/m ($p \leq 0.05$). Treatment 2 (17 500r), resulted into plants with the longest roots in three cultivars except for cultivar Mtama 1(2.49cm). This middle level of X-ray seed exposure also resulted into longer roots in comparison to the original (basic) seeds. The highest level of X-ray seed treatment (20 000r) resulted into plants with shorter roots as compared to treatment 2 (middle level of X-ray seed exposure =17,500r) hence a significant root growth inhibition. Therefore these results

indicate that 17, 500r X-ray seed irradiation had better root growth. However, there were significant differences (P< 0.05) in intercultivar root length. The root length was rated as Serena > El-gadam > Seredo >Mtama 1. As far as root length is concerned, X-ray seed treatment promoted salinity tolerance in El-gadam by improving shoot dry weight. Fig. 4 shows the effect of X-ray seed treatment on inter cultivar root: shoot ratio at 15dS/m.

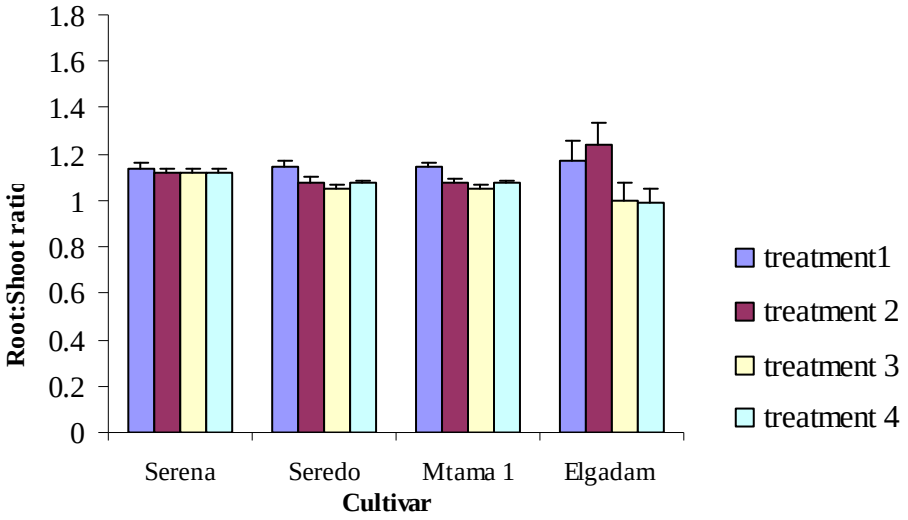

Figure 4: Root:shoot ratio of X-ray treated seed (Where treatment 1, 2, 3 and 4 correspond to control, X-ray seed treatment 1 (15000r), X-ray seed treatment 2 (17 500r), X-ray seed treatment 3 (20 000r) screened at an Electrical conductivity of 15dS/m)

Shoot growth sensitivity was reduced upon X-ray seed treatment. This created a balance between the root dry weight and shoot dry weight. The two way Anova, cultivar × treatment interaction showed significant differences ($p \leq 0.05$). This was a clear indication of built-in salinity tolerance of the selected sorghum cultivars. The relatively low root: shoot ratios in all the cultivars at 17,500r (as compared to the control, figure 2) shows a balance in root and shoot dry weight hence tolerance of high salinity level

(15dS/m) without negatively affecting shoot growth except in El-gadam control and 15,000r X-ray seed exposure. Nevertheless there was growth inhibition at 20.000r in all the cultivars.

4.1.3 Hydroponics screening of somaclonal variants (somaclones)

The results of screening somaclones of the three selected cultivars at Electrical conductivities of 0.22, 5, 10 and 15dS/m are as summarized in Fig. 5{LSD $_{0.041}$ = 0.7}.

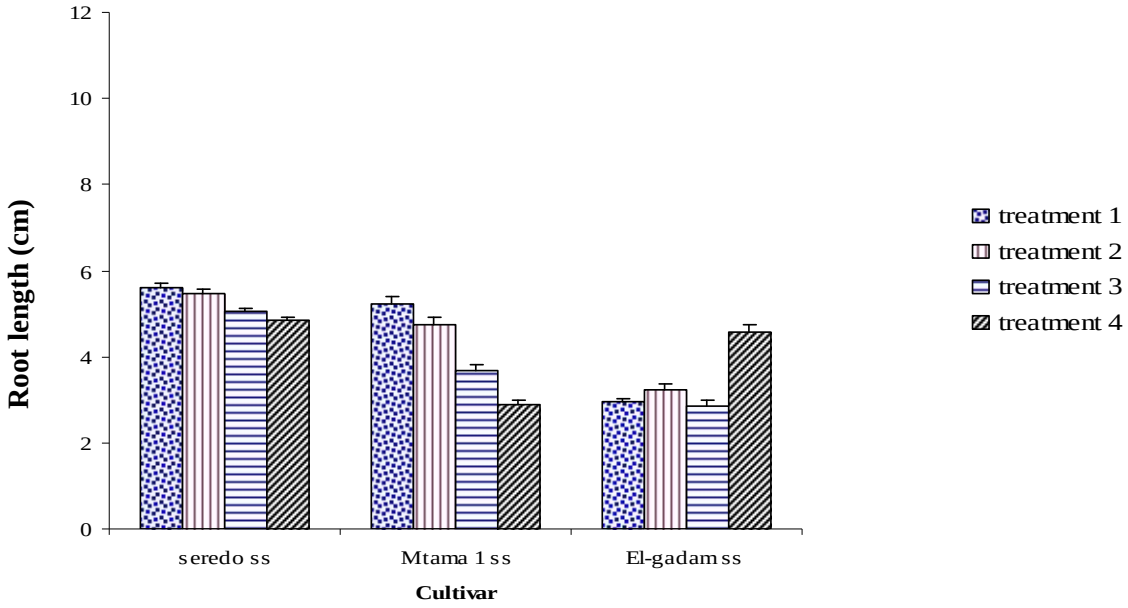

Figure 5: Effect of salinity levels on root length of somaclonal variants (Where treatment 1, 2, 3 and 4 correspond to Electrical conductivity 0.22, 5, 10 and 15dS/m)

There were significant differences in cultivar×treatment interaction ($p \leq 0.041$) among the three somaclones. Seredo ss and Mtama 1 ss followed a similar trend in which low salinity level corresponded to long roots. The root length systematically decreased with increasing salinity. On the other hand El-gadam did not follow the same trend but high

level of salinity triggered lengthening of the roots as a means of adaptation to acquire salinity tolerance. The root: shoot ratio results are as shown in Fig.6 {LSD $_{0.05}$ =0.18}.

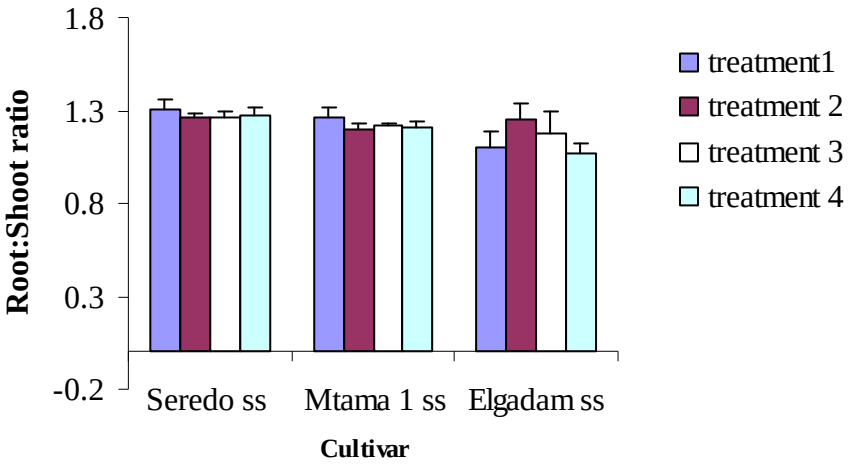

Figure 6: Root: shoot ratio of somaclonal variants (Where treatment 1, 2, 3 and 4 correspond to Electrical conductivity 0.22, 5, 10 and 15dS/m)

Two of the three somaclones tested for salinity tolerance i.e, Seredo ss and Mtama1ss had insignificant differences in their root and shoot dry weights meaning that they responded to the treatments in the same way. For both of them (Seredo and Mtama 1) at high and low EC, the root growth in saline media transformed to better shoot growth as compared to the control. Strongest suppression of root growth for El-gadam somaclone was observed at low EC (0.22dS/m) inhibiting root dry weight resulting into low root:shoot ratio while growth in 5dS/m and 10dS/m promoted shoot dry weight as well as root dry weight. At high EC level (15dS/m) root dry weight was promoted in an attempt to counteract high salinity level meaning that El-gadam somaclone had an adaptation to tolerate high salinity.

4.2 Root and shoot morphology

The genotypes differed in root branching as shown by variations in the number of root hairs which appeared to be different among the cultivars in rooting patterns in various root sections. Root and shoot morphology of salinity tolerant cultivar Seredo (Plate 1a - 4b) and root and shoot morphology of salinity sensitive cultivar El-gadam (Plate 5a -8b) at 0.22, 5, 10 and 15dS/m were associated with mass per unit area within which roots are distributed in the nutrient solution. The cultivars with greater branching were associated with greater root dry matter, longer roots and smaller shoot: root ratios. The opposite was true for the lesser branched genotypes. Root elongation of salt stressed sorghum cultivars (El-gadam and Mtama 1) was found to improve after X-ray seed exposure and somaclone screening for salinity. The given plates summarize the root and shoot morphology of extreme salinity tolerance and extreme salinity sensitivity which excludes cultivar Mtama 1 because its root and shoot characteristics were intermediate as compared to the two extremes.

Plate 1a: Cultivar Seredo, shoot at 0.22dS/m

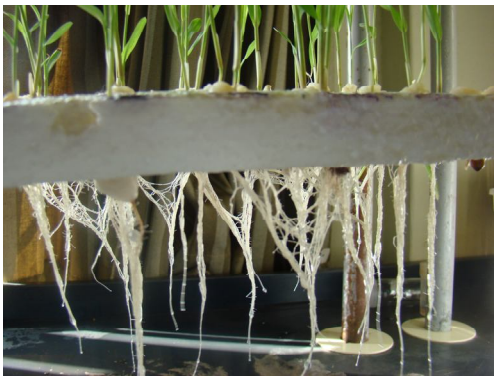

Plate 1b: Cultivar Seredo, root a0.22dS/m

Plate 2a: Cultivar Seredo, shoot at 5dS/m

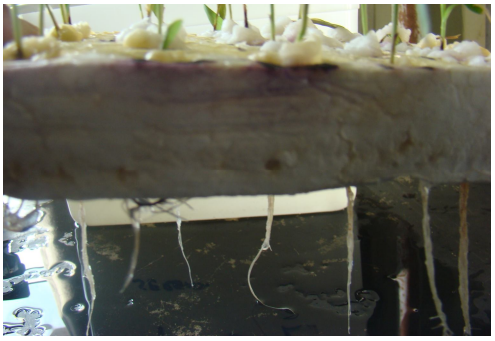

Plate 2b: Cultivar Seredo, root at 5dS/m

Plate 3a: Cultivar Seredo, shoot at 10dS/m

Plate 3b: Cultivar Seredo, root at 10dS/m

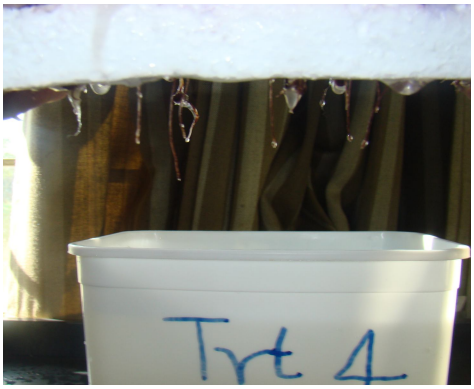

Plate 4a: Cultivar Seredo, shoot at 15dS/m

Plate 4b: Cultivar Seredo, root at 15dS/m

Root and shoot morphology of a salinity tolerant cultivar (Seredo) at 0.22, 5, 10 15dS/m

Plate 5a: El-gadam, shoot at 0.22dS/m

Plate 5b: El-gadam, root at 0.22dS/m

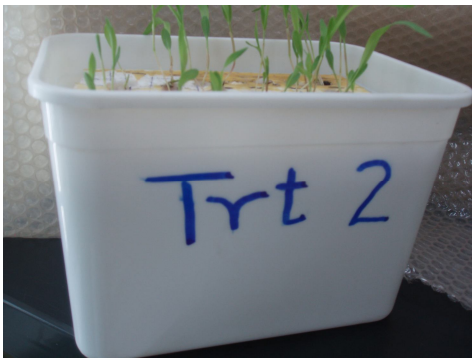

Plate 6a: El-gadam, shoot at 5dS/m

Plate 6b: El-gadam, root at 5dS/m

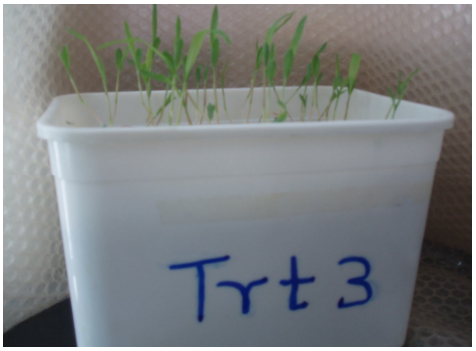

Plate 7a: El-gadam, shoot at 10dS/m

Plate 7b: El-gadam, root at 10dS/m

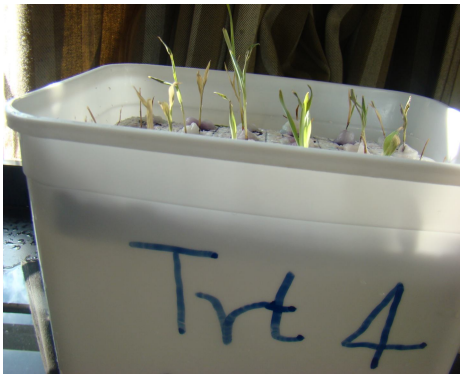

Plate 8a: El-gadam, shoot at 15dS/m

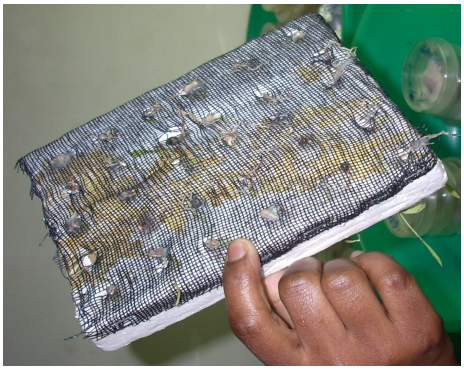

Plate 8b: El-gadam, root at 15dS/m

Root and shoot morphology of a salinity sensitive cultivar (El-gadam) at 0.22, 5, 10 15dS/m

4.3 Field results

4.3.1 Results for season one on C_4 physiological parameters

4.3.1.1 Photosynthetic Active Radiation (PAR)

The average PAR recorded during season one followed the trend as shown in Fig. 7, being lowest at 9:00hrs (143 µmol/m^2/s) and reaching the peak at 12:00hrs (387 µmol/m^2/s). The PAR was the major factor influencing the C_4 physiological parameters.

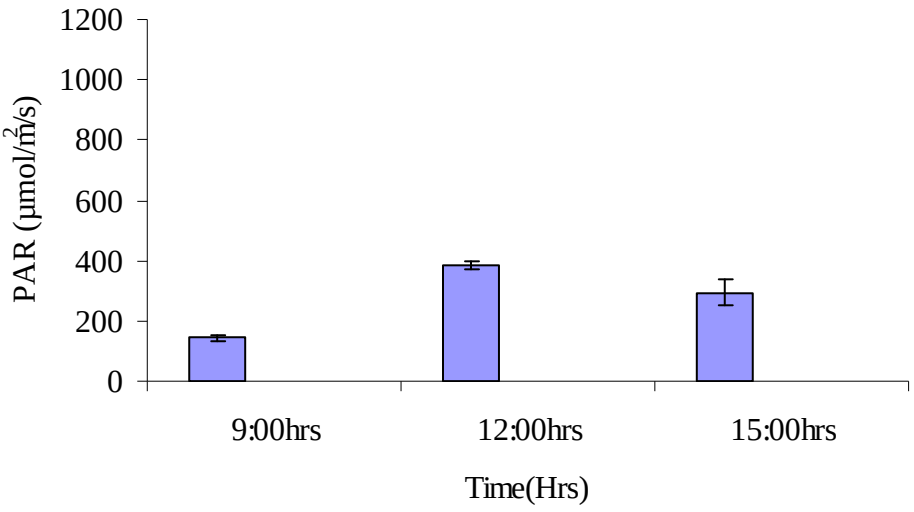

Figure 7: Photosynthetic Active Radiation (µmol/m^2/s) recorded during season one

4.3.1.2 Stomatal conductance (Gs)

Figure 8 (a) {LSD = 3.1}, (b) {LSD = 3.17} and (c) {LSD = 4.5} represent the Stomatal conductance (Gs) of the basic seed, their somaclones and X-ray treated seed cultivars grown during season one.

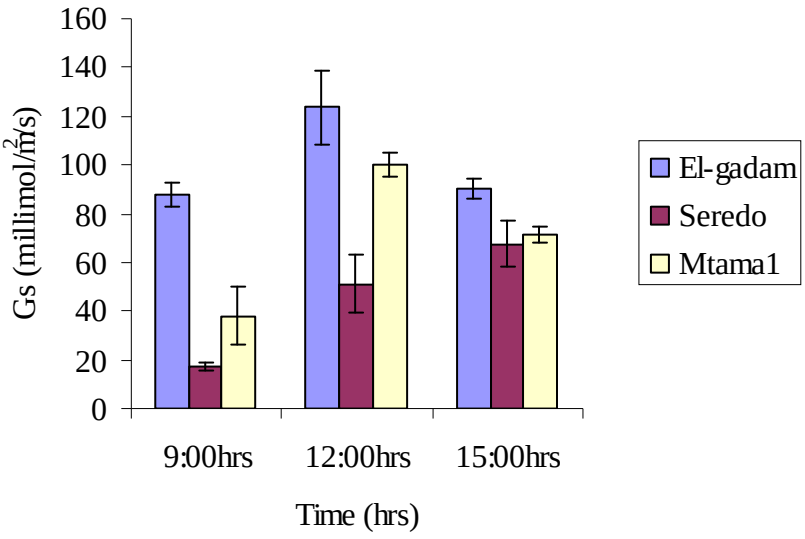

Figure 8(a): Stomatal conductance of basic seed cultivars during season one

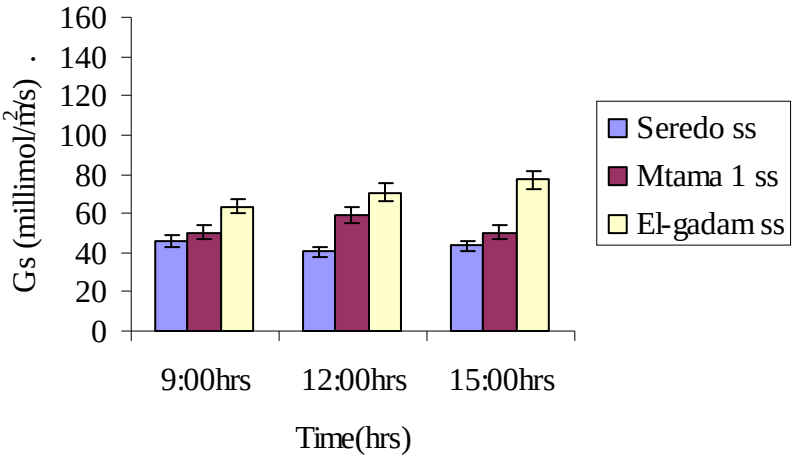

Figure 8(b): Stomatal conductance of somaclones during season one

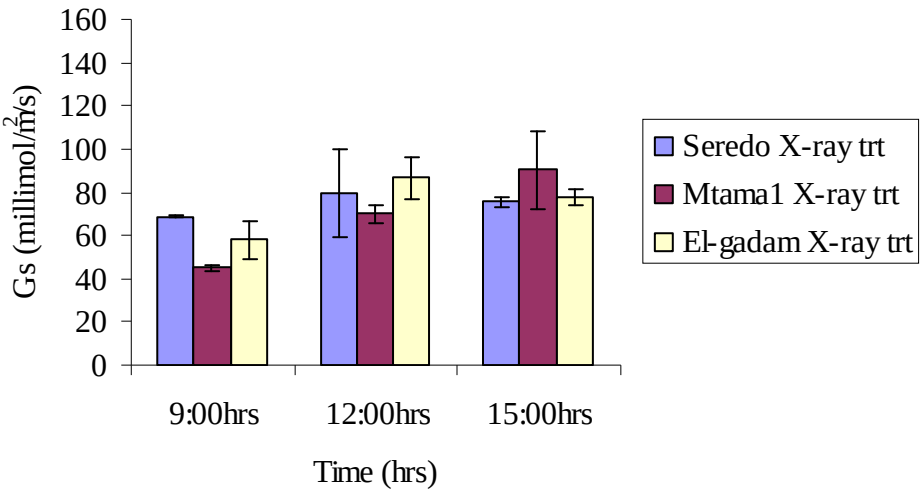

Figure 8(c): Stomatal conductance of X-ray treated seed cultivars during season one

Figure 8 (a), (b) and (c): Gs (Stomatal conductance) in (millimol/m^2/s) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season one season.

Inter-cultivar differences in stomatal conductance were observed in basic seed (Fig. 8 (a). These differences narrowed down in somaclones (Fig. 8 (b) and further after X-ray seed treatment resulting into low stomatal conductance as a result of X-ray seed exposure. Partial stomatal closure was recorded earlier in the morning (9:00hrs), corresponding to reduced water loss thruogh transpiration in all the selected sorghum cultivars. At 12:00hrs the stomatal conductance attained a peak followed by reducing stomatal conductance in the afternoon which was as recorded at 15:00hrs. Following an

increase in transpiration due to exposure to high PAR, 12:00hrs, the X-ray treatment resulted into reduced stomatal conductance which was associated with salinity tolerance (recycling of CO_2 between mesophyll and bundle sheath cells).

4.3.1.3 Transpiration rate

Fig. 9 a {LSD = 0.075}, b {LSD = 0.105}, and c {LSD = 0.3}, show the rate of transpiration in season one. The rate of transpiration increased with increase in PAR and stomatal conductance reaching a peak at12:00hrs and decreased in the afternoon (15:00hrs) following a marked decrease in PAR and stomatal conductance in all the cultivars. However, rate of transpiration among treatments was rated as Basic seed cultivars > somaclones > X-ray treated seed cultivars. There was a significant difference ($p \leq 0.05$) in treatment×time interaction and intercultivar differences were observed; Basic seed cultivars ($p= 0.017$), X-ray treated seed cultivars ($p= 0.047$) while differences among the somaclones were insignificant. The overall rate of water loss among the cultivars was rated as Elgadam> Mtama 1> Seredo, which also reflected the same ranking in stomatal conductance. Cultivar El-gadam performed poorly an indication that it was not well adapted to arid conditions but after X-ray seed treatment its physiological behaviour showed relative adaptation. On the other hand Seredo exhibited relatively a lower rate of transpiration and was rated the best performer in these field conditions while Mtama 1was averagely adapted. Low rates of transpiration reflected that most of the water was entering the growing cells by a high resistance pathway while with

increasing transpiration most of the water was by-passing the protoplasts following a low resistance pathway.

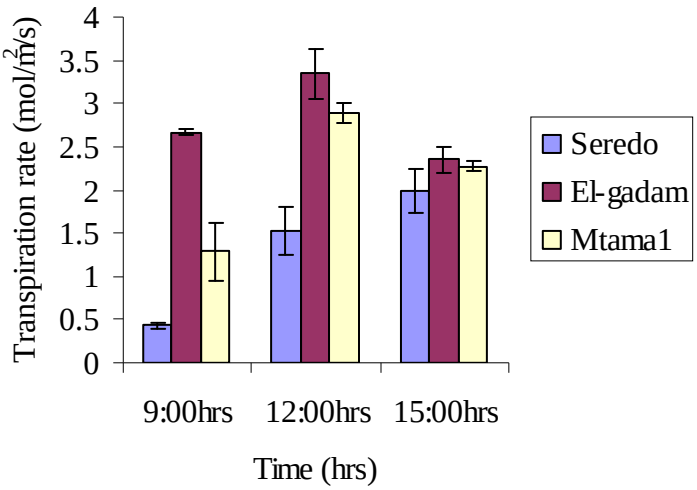

Figure 9(a): Transpiration rate of basic seed cultivars during season one

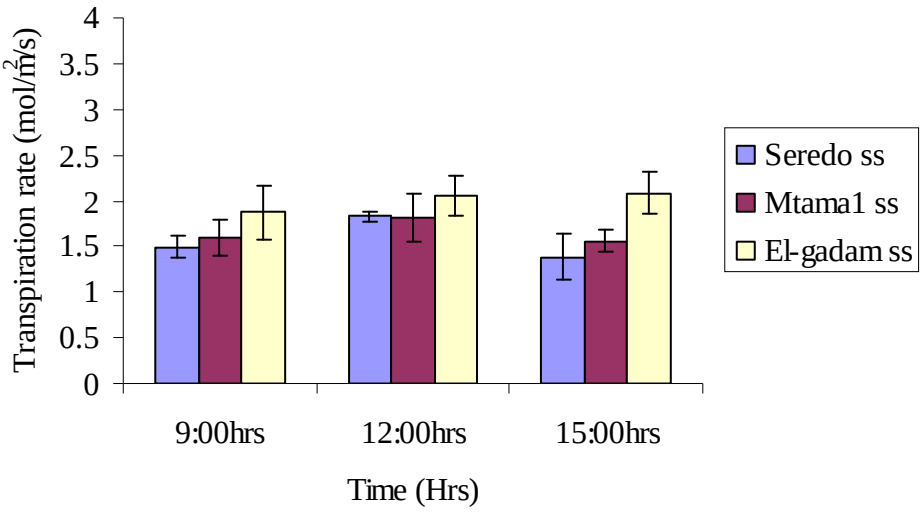

Figure 9(b): Transpiration rate of somaclones during season one

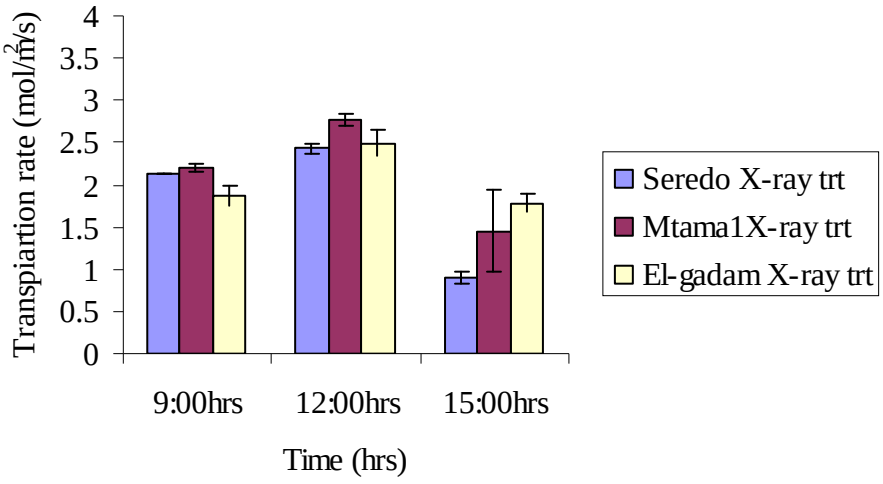

Figure 9(c): Transpiration rate of X-ray treated seed cultivars during season one

Figure 9(a), (b) and (c): Transpiration rate (mol/m^2/s) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season one.

Rates of transpiration varied significantly with treatment and time. The control (basic seed) had highest rates of transpiration at 12:00hrs and 15:00 hrs (Fig. 9 (a) compared to the somaclones (ss) (Fig.9 (b) and X-ray treatment (Fig. 9 (c). At 9:00hrs the rate of transpiration is lowest in all the cultivars although inter-cultivar differences arise coinciding with the stomatal conductance at the same time. This rate increased with increasing Photosynthetic Active Radiation attaining maximum water loss at 12:00hrs.

4.3.1.4 Carbon dioxide assimilation rate

Carbon dioxide assimilation rates of the three cultivars, their somaclones and X-ray treated seeds were as shown in Fig.10 a {LSD = 3.75}, b {LSD = 0.567} and c {LSD = 2.05}.

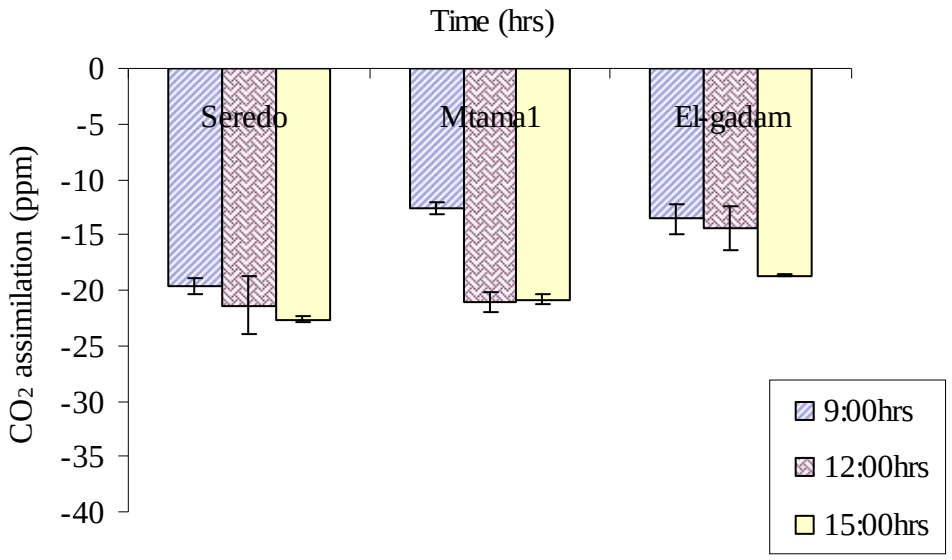

Figure 10(a): Carbon dioxide assimilation rate of basic seed cultivars during season one

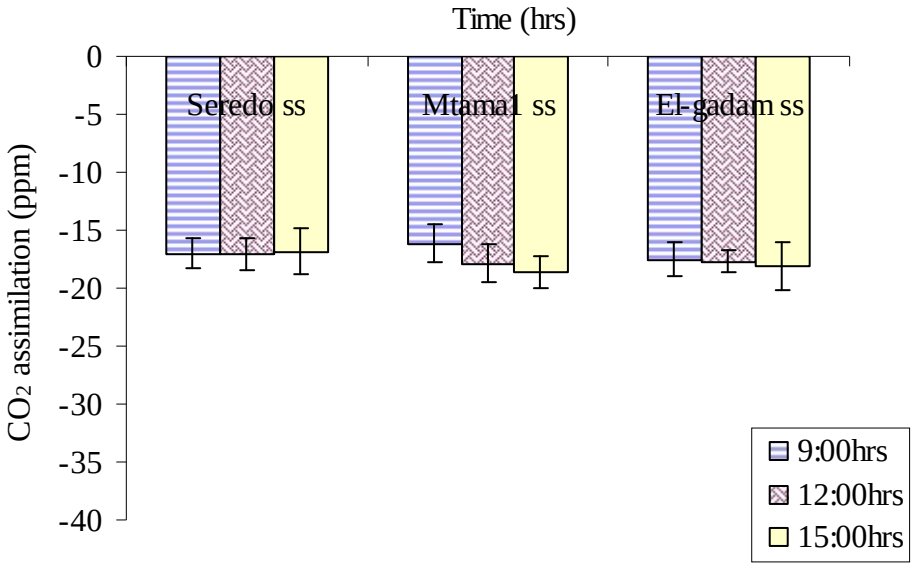

Figure 10(b): Carbon dioxide assimilation rate of somaclones during season one

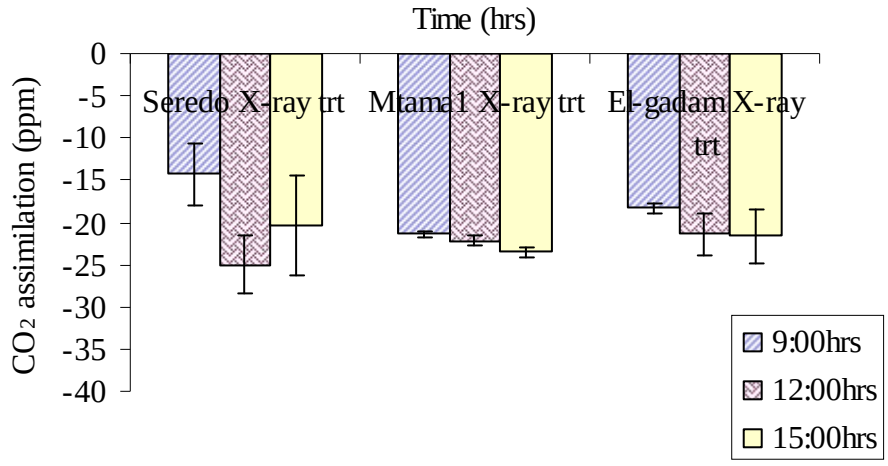

Figure 10(c): Carbon dioxide assimilation rate of X-ray treated seed cultivars during season one

Figure 10 (a), (b) and (c): Carbon dioxide assimilation rate (ppm) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season one.

There was a significant difference in the carbon dioxide assimilation rate ($p<0.05$) considering the time×cultivar interaction. The rate of assimilation increased with time of the day as a response to the elevated PAR (Fig.7) attaining a peak at 12:00hrs. There being no photoinhibition, the assimilation slightly decreased between 12:00hrs and 15:00hrs in the three cultivars. Highest assimilation rates were recorded in X-ray treated seeds (Fig. 10 (c) followed by somaclonal variants of the three cultivars (Fig.10 (b) while the basic seed (control) had lower rates of assimilation (Fig. 10 (a). There was a marked increase in the rate of assimilation especially in Mtama 1 ss, El-gadam ss (Fig. 10 (b) and Mtama 1 X-ray seed treatment, El-gadam X-ray treatment (Fig. 10 (c) respectively as compared to the control (Fig. 10 (a).

4.3.2.5 Photosynthesis rate

Figure 11(a) {LSD = 0.8}, (b) {LSD = 0.016} and (c) {LSD = 0.95}: Photosynthesis rate (µmol/m^2/s) of (a) control (parent seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season one.

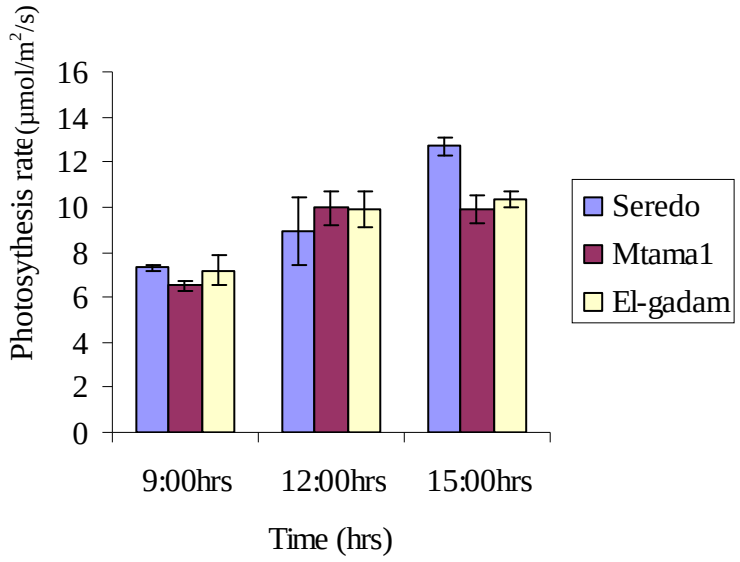

Figure 11(a): Photosynthesis rate of basic seed cultivars during season one

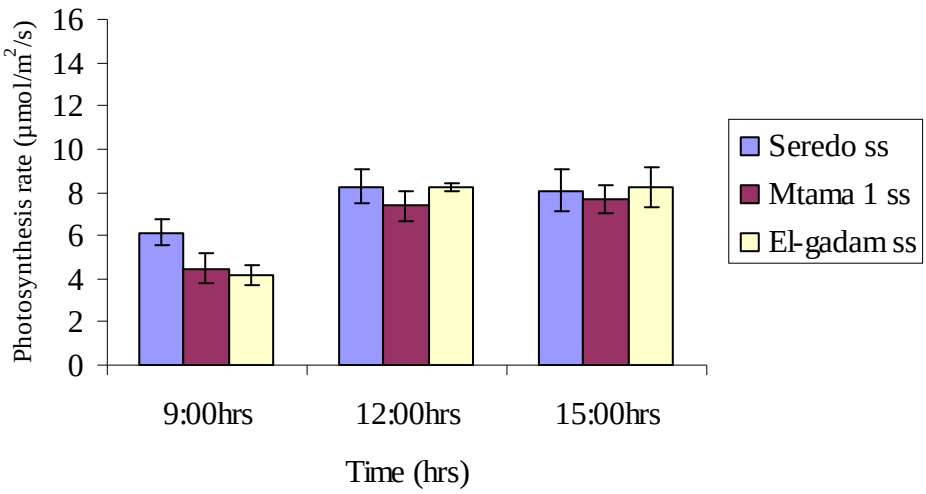

Figure 11(b): Photosynthesis rate of somaclones during season one

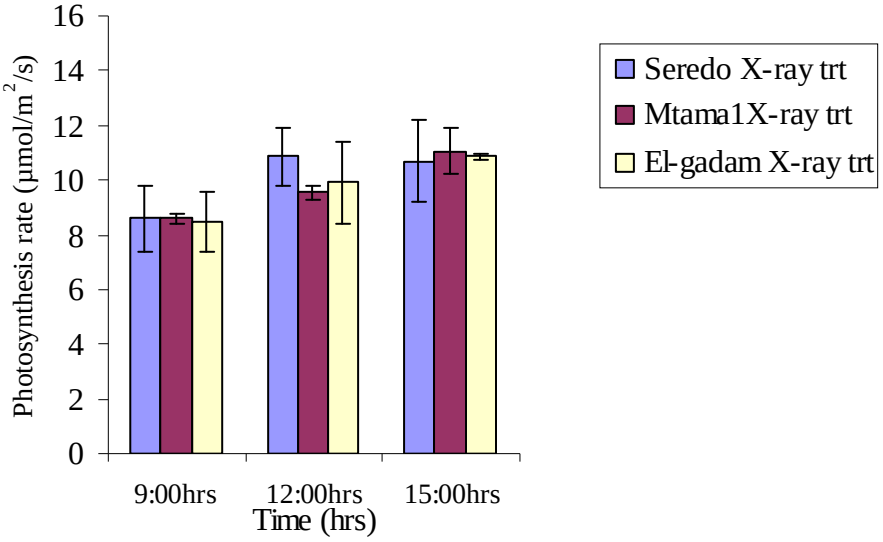

Figure 11(c): Photosynthesis rate of X-ray treated seed cultivars during season one

The somaclonal variants, (Fig.11 (b) depicted low photosynthesis rate at 9:00hrs, 12:00hrs and 15:00hrs in comparison to the control. At 9:00hrs the rate of

photosynthesis was lowest in all the cultivars but increased reaching a peak 12:00hrs. This rate increased with time after X-ray seed treatment up to and including 15:00hrs (Fig. 11(c) at which the highest rates were observed.

4.3.2 Results for season two on C_4 physiological parameters

Similar to season one experiment, the basic seed of the three selected cultivars was the control for the field screening experiments inseason two. The field experiment comprised of three treatments, that is, the selected three basic seed cultivars (control), their generated somaclones and X-ray treated seeds of the three cultivars replicated three times in homogeneous field.

4.3.2.1 Photosynthetic Active Radiation (PAR)

The results presented in Fig. 12 show the changes in PAR levels during season two. During this season the rains failed and the PAR escalated attaining a peak of 976.5 µmol/m^2/s at 12:00hrs.

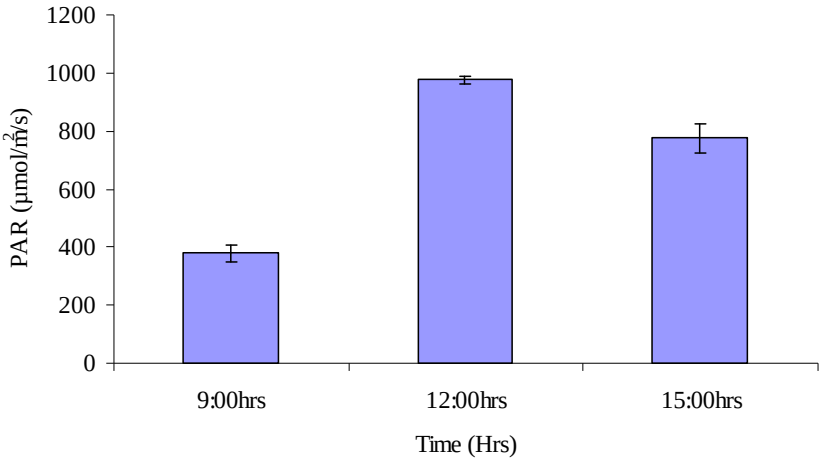

Figure 12: Photosynthetic Active Radiation (µmol/m^2/s) recorded during season two

The values of PAR obtained during the vegetative growth period of the selected sorghum cultivars in the second season (Fig. 12) were significantly different at 9:00hrs (386 µmol/m^2/s), 12:00hrs (986.5 µmol/m^2/s) and 15:00hrs (774.5 µmol/m^2/s). This was characterized by low PAR at 9:00hrs which increased attaining a peak at12:00hrs followed by a steady decrease up to and including15:00hrs.

4.3.2.2 Stomatal Conductance (Gs)

Figure 13 a {LSD = 5.56}, b {LSD = 3.5} and c {LSD = 4.5}: Stomatal conductance (millimol/m^2/s) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season two.

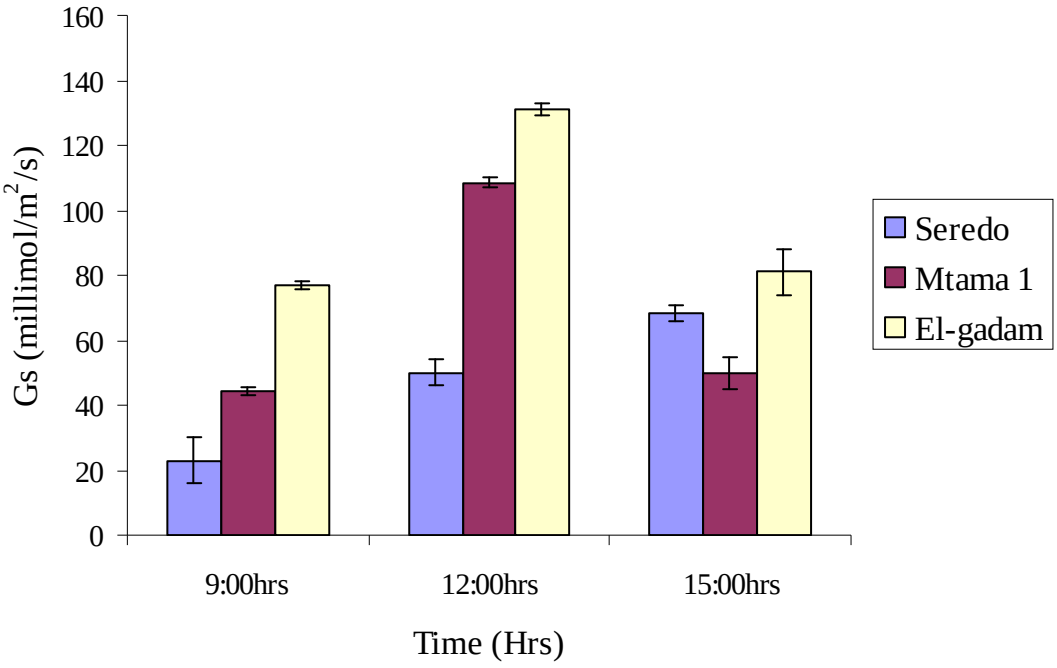

Figure 13(a): Stomatal conductance of basic seed cultivars during season two

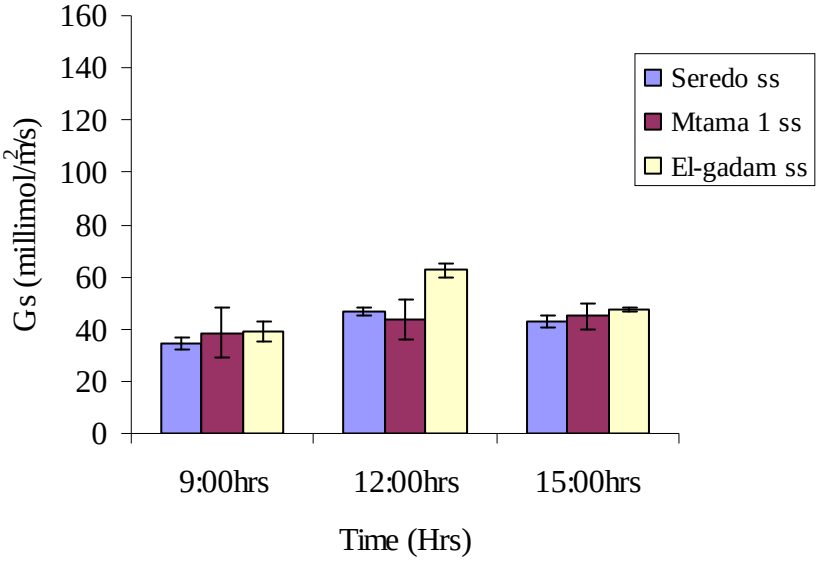

Figure 13(b): Stomatal conductance of somaclones during season two

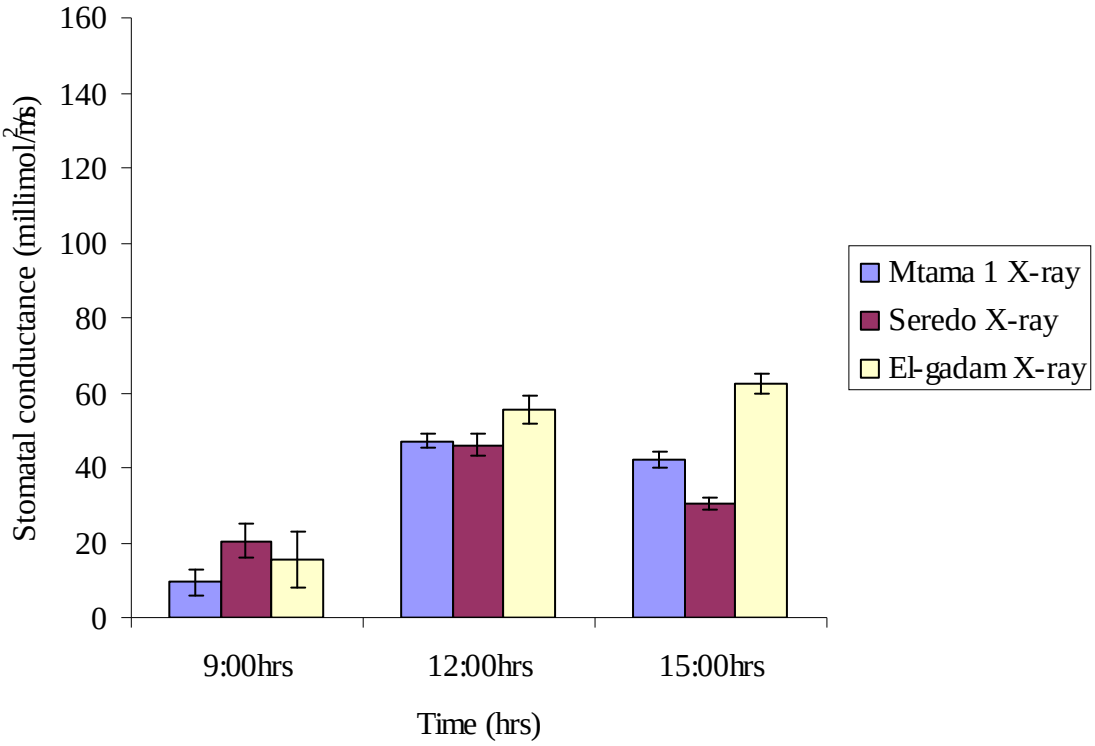

Figure 13(c): Stomatal conductance of X-ray treated seed cultivars during season two

4.3.2.3 Transpiration rate

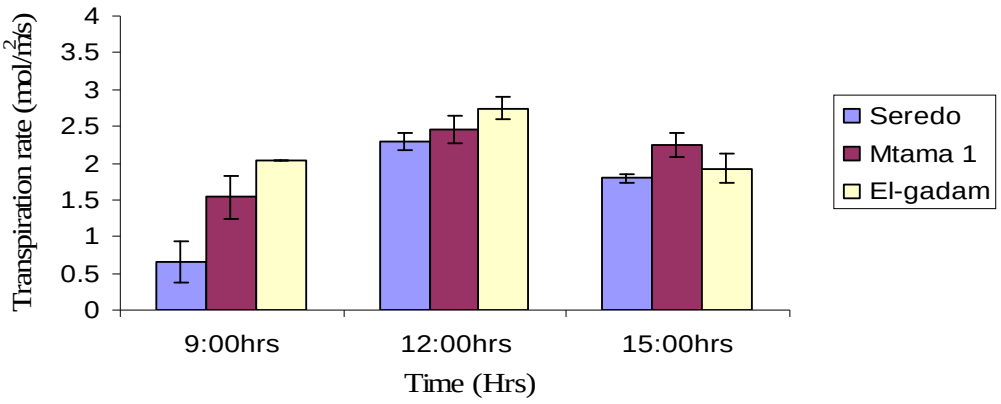

Figure 14(a): Transpiration rate of basic seed cultivars during season two

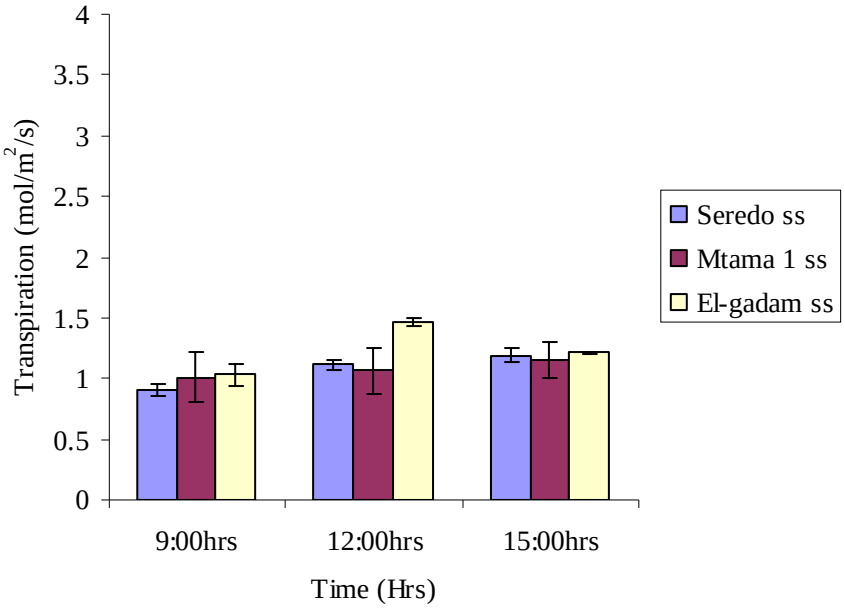

Figure 14(b): Transpiration rate of somaclones during season two

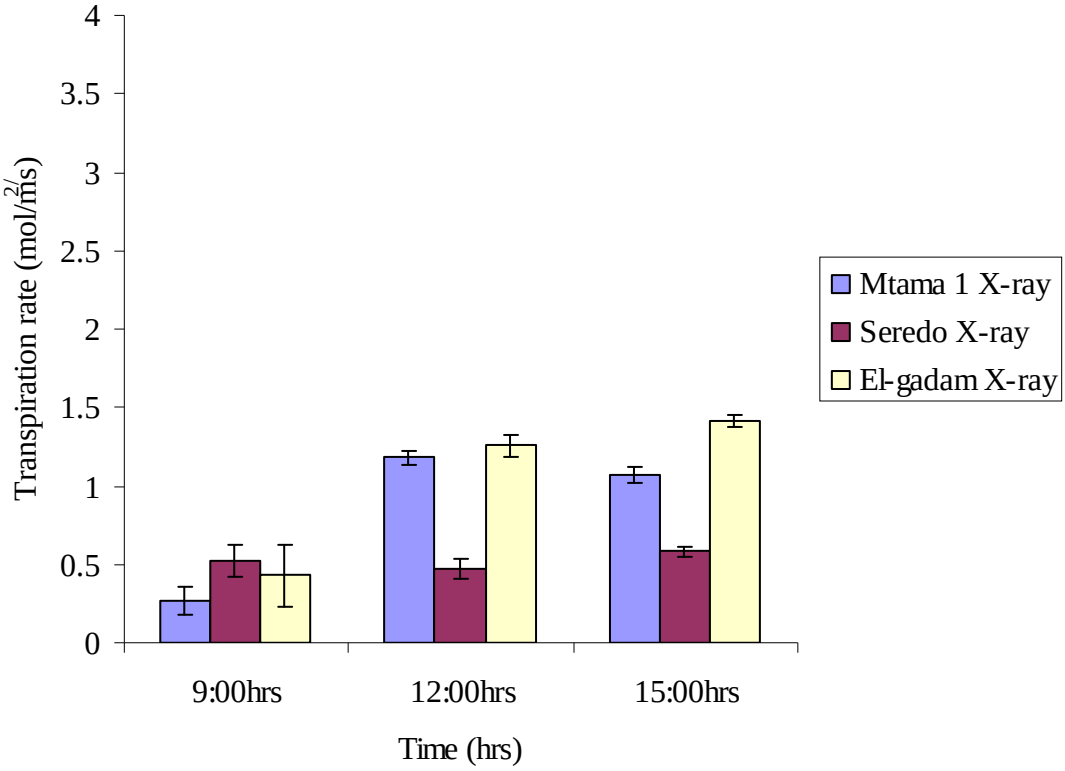

Figure 14(c): Transpiration rate of X-ray treated seed cultivars during season two

Figure 14(a) {LSD = 0.775}, (b) {LSD = 0.21} and (c) {LSD = 0.105}: Transpiration rate (mol/m^2/s) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season two.

Time×cultivar interaction was significant during this second season. In the control treatment, figure 14(a), El-gadam had highest rates of transpiration recorded at 9:00hrs and 12:00hrs. Least transpiration rate was found to be in cultivar Seredo while Mtama 1 had relatively reduced water loss (El-gadam > Mtama 1 > Seredo). Generally, the rate of transpiration was high for the three cultivars, in the control than in somaclones and X-

ray treated seed. The somaclones had reduced rate of transpiration at 9:00hrs, 12:00hrs and 15:00hrs as compared to the control while the X-ray treated seed had lowest rates at 9:00hrs which increased surpassing those of the somaclones except for Seredo X-ray trt.

4.3.2.4 Carbon dioxide assimilation rate

Fig. 15 a, b and c show the Carbon dioxide assimilation rates of a control, somaclones and X-ray treated seed grown in homogeneous field conditions.

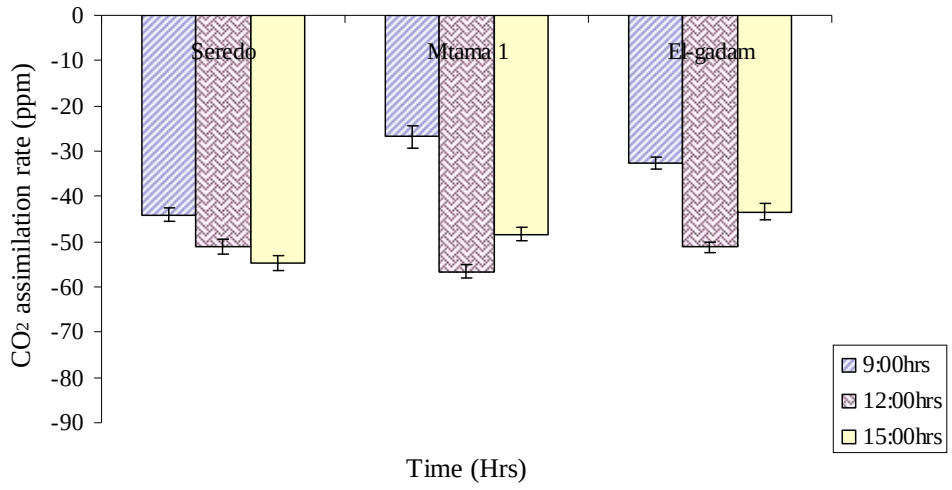

Figure 15(a): Carbon dioxide assimilation rate of basic seed cultivars during season two

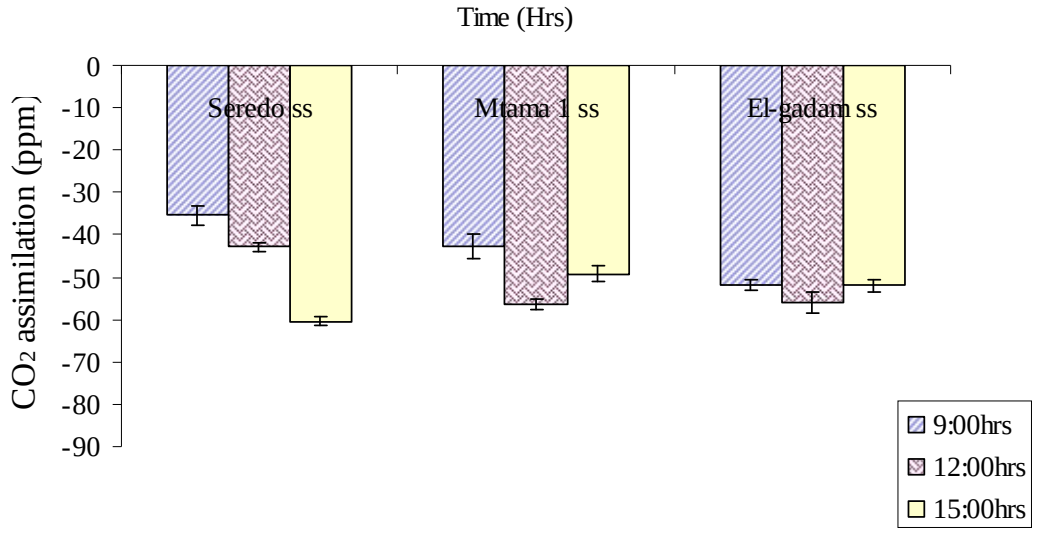

Figure 15(b): Carbon dioxide assimilation rate of somaclones during season two

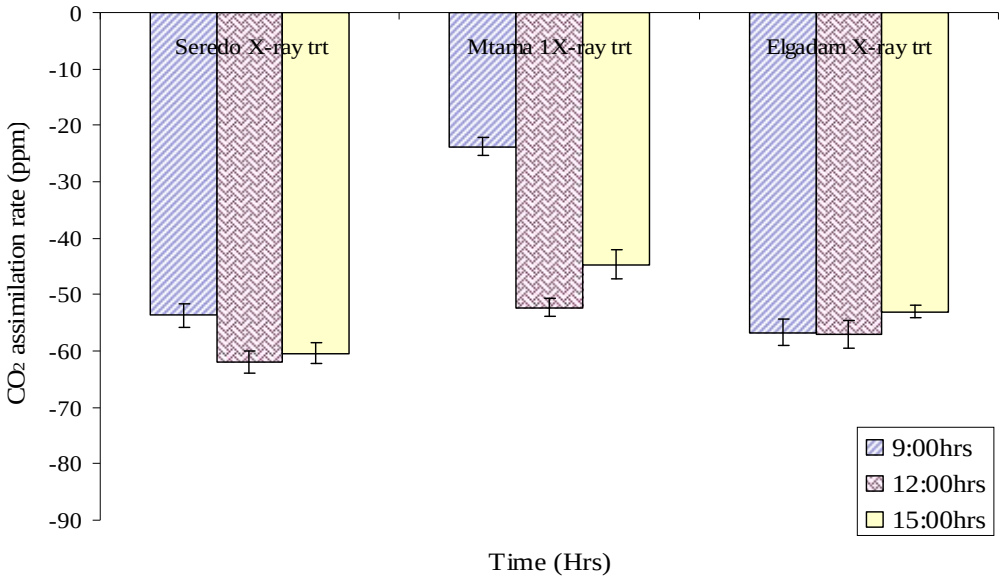

Figure 15(c): Carbon dioxide assimilation rate of X-ray treated seed cultivars during season two

Figure 15 (a) {LSD = 5.85}, (b) {LSD = 1.5}and (c) {LSD = 6.35}: Carbon dioxide assimilation rate (ppm) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) X-ray seed treatment of the three cultivars.

Figure 15 a, b and c represent the Carbon dioxide assimilation rates sorghum plants grown in homogeneous field conditions. In the control experiment Mtama 1 exhibited low Carbon dioxide assimilation rate at 9:00hrs among the three cultivars. The peak assimilation rate was attained at 12:00hrs for the three cultivars. This rate decreased slightly by 15:00hrs. The somaclonal variants on the other hand, had low assimilation rates at 9:00hrs with Seredo ss being the least (Seredo ss < Mtama 1 ss < El-gadam ss). The rate followed the same trend in the control by attaining the peak at 12:00hrs and slightly decreasing slightly by 15:00hrs. In the X-ray treatment the rates were accelerated especially in the salinity sensitive cultivars (Mtama1 and El-gadam) and continued to rise following the same trend in control and somaclones.

4.3.2.5 Photosynthesis rate

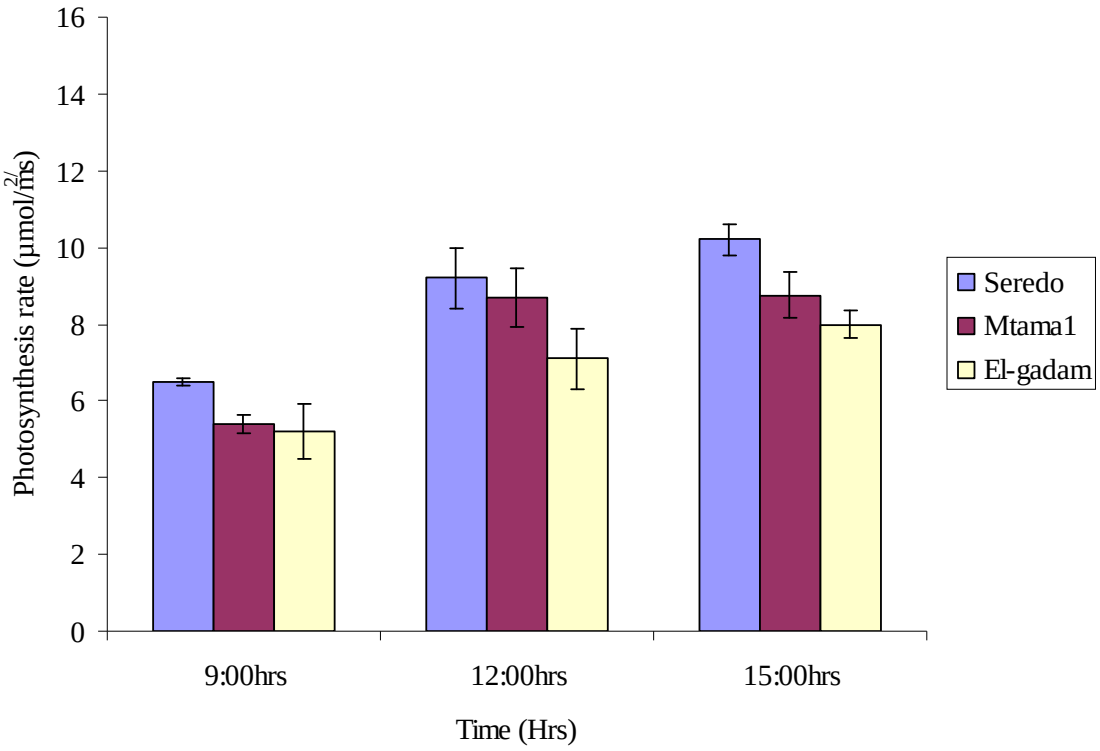

Figure 16(a): Photosynthesis rate of basic seed cultivars during season two

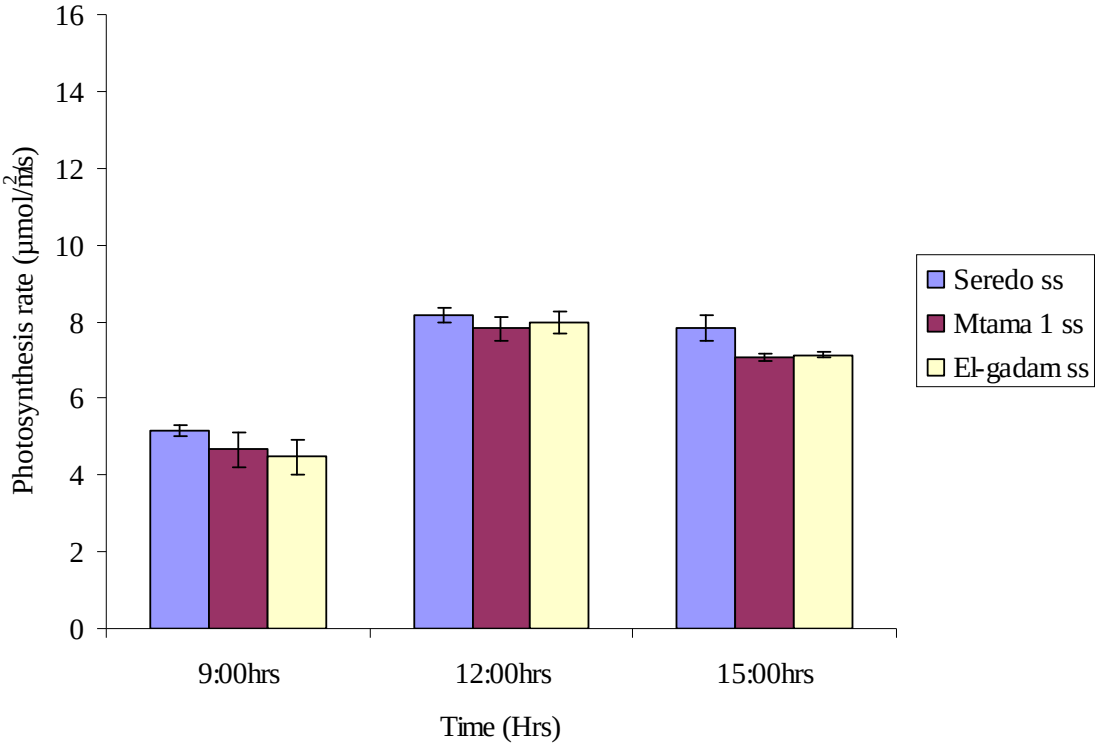

Figure 16(b): Photosynthesis rate of somaclones during season two

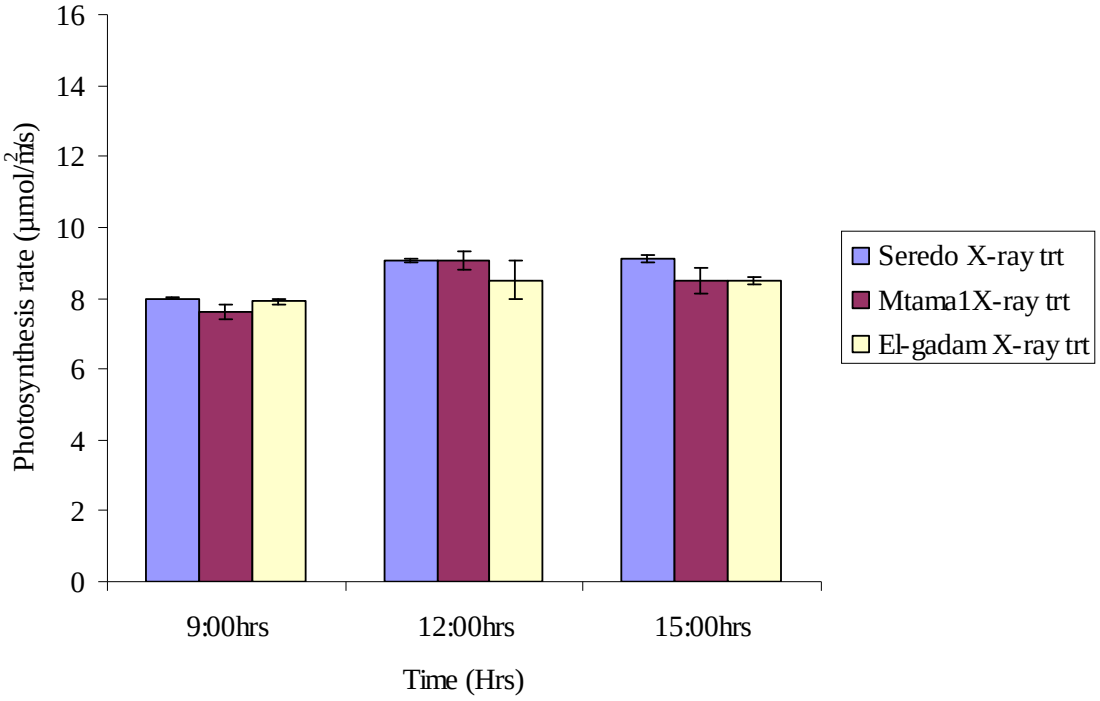

Figure 16(c): Photosynthesis rate of X-ray treated seed cultivars during season two

Figure 16 (a) {LSD = 1.30}, (b) {LSD = 0.338} and (c) {LSD = 0.56}: Photosynthesis rate (µmol/m^2/s) of (a) control (basic seed of Mtama 1, Elgadam and Seredo); (b) somaclones of the three cultivars and (c) plants resulting from X-ray seed treatment of the three cultivars during season two.

The photosynthesis rate of control treatment (Fig. 16 (a) was found to be low at 9:00hrs and increased in the three cultivars at differing rates optimizing at 12:00hrs. There was a slight decrease in the rate of photosynthesis in cultivar Mtama 1 whereas the El-gadam and Seredo had a steady increase in the afternoon recorded at 15:00hrs. The three somaclones had a relatively low photosynthetic rate at 9:00hrs and a slight decrease in photosynthetic rate between 12:00hrs and15:00hrs whereas the X-ray treated cultivars

were able to increase their photosynthetic carbon gain by incomplete stomatal closure, Fig. 16 (c) early in the morning which was advantageous in the arid environment because of a higher potential for early morning carbon gain when temperatures and Vapour Pressure Deficit are lower. Stomata responses to light influenced the total daily carbon gain. In addition they didn't show any evidence of injury from photoinhibition in any of the three cultivars and the photosynthesis rate was relatively high at 9:00hrs steadily increased until 12:00hrs and then leveled by 15:00hrs. The X-ray treatment therefore resulted into highest rates of photosynthesis in comparison to the control and the somaclones.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Hydroponics screening for Salinity Tolerance in *Sorghum bicolor*

This study indicated that basic seed cultivars of sorghum significantly differed in root length when subjected to the four EC levels of the nutrient solution for seven days. The roots of basic seed cultivars Serena and Seredo penetrated deeper into the nutrient solution compared to those of cultivars Mtama 1 and El-gadam (Fig. 1). This was in agreement with Cayuela *et al*., 1996, that the characteristics for salinity tolerance are associated with the ability of the cultivars to cope with temporal and spatial variability in water status within their root environment since the induction of plant adaptation is possible only during a short time and early stage of plant development (between 5 and 10 days in sorghum plants). On the other hand, cultivar El-gadam and Mtama 1 were unable to cope with adverse effects of the high NaCl content (10 and 15dS/m) in the nutrient solution due to their shorter roots. These differences in salinity tolerance in early response to salinity were basically due to the osmotic effects of the salt surrounding the roots (Munns, 1993). Based on the results obtained in this experiment it was clear that basic seed cultivar Serena (the check variety) exhibited tolerance to high EC levels by developing extensive roots among the four basic seed cultivars screened for salinity tolerance while El-gadam was most sensitive to salinity as Seredo and Mtama 1 were intermediate. Hydroponics screening for salinity tolerance was associated with low available water as well as osmotic effects related with salinity.

The impact of X-ray seed treatment on the basic seed cultivars was increased salinity tolerance at high EC level (15dS/m) reflected by the relatively longer roots which penetrated more deeply into the nutrient solution (Fig. 3). This response was probably under the control of hormonal signals from roots (Munns, 2002). Apparently, the tolerance to high saline concentrations after X-ray seed treatment seems to be related to the cultivar ability to develop extensive and highly branched roots. For instance the root length of salinity sensitive basic cultivars Mtama 1 and El-gadam at 15dS/m (2cm and 1.8 cm) was amplified to 2.98, 2.5, 2.7cm and 4, 4.2 and 3.3cm after X-ray seed treatment at 15,000r, 17,500r and 20,000r respectively. Since ionizing radiation, produces a range of damage to cells depending on the level of exposure an optimal X-ray radiation was required to improve root length of the salinity sensitive cultivars. On the other hand the generated somaclones of the selected three cultivars had decreased root growth ((Fig. 5) as compared to the basic seed cultivars and the X-ray radiated seeds, a general consequence of osmotic stresses, including water deficit and high salinity levels (Incharoensakdi *et al*., 1986).

Some effects of roots on shoots, such as their role in absorption and anchorage, seem obvious but some effects such as damage to root systems appeared serious enough to inhibit shoot growth thus considerable emphasis was placed on the root: shoot ratio (based on the root dry weight and shoot dry weight of the seedlings) as an indicator of a balanced growth between the roots and shoots (Figures 2, 4 and 6), since the roots conserve water and promote increased water supply to the shoot (Rains, 1989). In this

respect, successful growth of tolerant seedlings of cultivars Serena and Seredo therefore depended on maintenance of a balance in growth between roots and shoots regardless of the saline treatment. Basic seed cultivars Serena and Seredo which have large root: shoot ratios, are relatively tolerant to salinity because the root: shoot ratio is regarded as the result of a source-sink in which lack of carbohydrates limit root growth and lack of water and minerals limits shoot growth. The largest root: shoot ratio of the salinity tolerant cultivars at 0.22 and 5dS/m and the smallest reduction of this ratio after increased salinity level of 10 and 15dS/m observed suggest that this plant characteristic could be an important tolerance indicator of salt stress in sorghum. Salt induced changes, however, in the seedlings of salinity sensitive basic seed cultivars Mtama 1 and El-gadam contributed to growth suppression with the low root dry weight and subsequent low shoot dry weight hence an overall low root: shoot ratio.

On exposure to X-ray radiation salinity tolerant basic seed cultivars developed long, profusely branched root systems. At the same time the salinity sensitive cultivars also had relatively longer roots which translated to higher root dry weight and subsequently more shoot dry weight (Fig. 4). The longer root system absorbed water from a large volume of the nutrient solution boosting the translocation physiological process hence deep rooting and high root dry weight are important factors in salinity tolerance of cultivated sorghum. The mechanism involved in salinity tolerance appears to be that of avoidance by fast growth in the nutrient solution similar to results observed in other plant species (Rains, 1989).

Al-Naber *et al*., 1998 while working on wheat somaclones found out that the decreased number of leaves with increasing salinity had an effect on the root: shoot ratio and this supports the findings of Fig.6 that, the three somaclones suffered decline in both root and shoot dry weight following increased salinity in both the tolerant cultivar Seredo and sensitive cultivars hence, reduced shoot dry weights. Significant reduction of the dry matter yield and length of the root system was observed especially in the sensitive cultivars (Mtama 1 amd El-gadam respectively) even though root systems have been considered as the basic system to counteract salinity stress (Smith and Frederiksen, 2000). This can be attributed to their short roots at varying levels of EC in the nutrient solution conforming to the findings of Fawzi, 2004 that growth at high levels of salinity (10 and 15dS/m) is very limited.

5.1.1 Root and shoot morphology

According to Rendig and Taylor, 1989, roots have been reported to be less sensitive to salinity than leaves however, root morphology was negatively affected by the highest level of salinity (15dS/m) in this study. The adverse effect of salinity on roots was not as obvious at the 0.22, 5 and 10 dS/m EC level as it was at the highest salinity level (15dS/m) at seven days after starting the salt treatment when the lack of continued seedling growth of salinity sensitive cultivars Mtama 1 and El-gadam in hydroponics solution at highest level of salinity (15dS/m) was used as a bench mark for the seedling tolerance level at which fresh and dry weights were obtained and having observed a significant interaction between cultivars and salinity levels. Less root branching was also evident amidst the roots suberized at their tips. These roots with less growth capacity

remained short and thin contributing to the low biomass production and reducing their capacity to absorb water. Seedlings shoot and root growth reduction and damage caused to them due to salinity was used as a measure to separate sorghum cultivars that are susceptible or tolerant to soil salinity. Highest salinity level ($15dSm^{-1}$) substantially reduced both root length and root hair development for cultivars Mtama 1 and El-gadam while cultivar Seredo had improved root length and root surface area per plant enabling greater exploration for water and nutrients. The osmotic stress of the salt outside the roots reduced the rate of formation of new leaves (Munns, 2002) in cultivars Mtama 1 and El-gadam and subsequent shoot dry weight reduction. This is a reflection of the low tolerance levels to saline growth conditions. In addition, shallow root systems and lack of root hairs in the two basic seed cultivars was accompanied by leaf chlorosis, wilting and subsequent yellowing of leaves (plate 5a – 8b) at high levels of salinity. Netondo *et al*., 2004, claimed that, this was due to an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid synthesized in the roots then translocated to the shoots where it was converted into ethylene which was observed in the lower leaves first and the high level of ethylene concentration stimulated cellulase activity which in turn caused cell breakdown; although water stress due to increased salinity play a role, the major factor in injury to the shoots is disturbance of nitrogen metabolism, including premature translocation out of older leaves.

Cultivars Serena and Seredo which showed adaptation to saline growth conditions had the characteristics of being deep rooted with a greater partitioning of dry matter to roots (plate 1a-4b), hence regarded as more saline-tolerant than Mtama 1 and El-gadam. These

tolerant cultivars also had greater root branching patterns, root dry matter and higher root: shoot ratios. The roots hairs were found primarily in the upper portion of the root profile. This was in agreement with Masoud *et al*. 2008 that plants have adaptive strategies to cope with high salt, water deficits and osmotic stress by different mechanisms in root morphology with altered patterns of development. In general, as the root system grows and becomes larger (greater total length or mass), branching increases and the root architecture becomes more complex in order to acquire resources. Either an excess or a deficiency in water/ moisture limits root growth and functioning but water is not directly injurious to roots as shown by the vigorous growth of the salinity tolerant cultivars in well-aerated nutrient solution. Root development (presence of root hairs and root length) as a function of NaCl concentrations therefore provides a useful guideline for salt tolerance (Khan *et al*., 2006). The manner in which the root system develops in salinity tolerant cultivars Serena and Seredo (the red sorghum cultivars) can also be attributed to internal factors such as its internal hereditary potentialities and on environmental factors but there are also important interactions between shoots and roots which affect the size of root systems because differences in type of seedling root systems significantly affected establishment and survival of seedlings in saline nutrient growth media.

5.2 Field experiment

5.2.1 C_4 physiological parameters

The Photosynthetic Active Radiation (PAR) for both season one and season two (Fig. 7 and 12 respectively) reflect hot and dry habitats where plants such as sorghum exhibiting the C_4 carbon pathway often occur. The PAR increased significantly from 9:00hrs attaining a peak at 12:00hrs and subsequently reduced by 15:00hrs ($p<0.05$) during the two seasons with season one being of a 'cloudy and dull weather'. It was observed that there were physiological differences among the three selected basic seed cultivars, their somaclones and plants arising from X-ray seed treatment with respect to PAR at which stomatal conductance, transpiration rate, CO_2 assimilation rate and photosynthesis rates occurred. The resulting traits were associated with changes in light intensity coinciding with the peak PAR at 12:00hrs. Light intensity/irradiance however, is one of the principal environmental factors affecting physiological behaviour in C_4 plants while other factors are vapour pressure deficit of the air, water supply, internal carbon dioxide concentration caused by photosynthesis and temperature besides plant factors that include leaf area, leaf structure and exposure and the effectiveness of the root system as an absorbing surface which differ in salinity tolerance as observed under hydroponics (Wieser and Havranek, 1993).

In this field experiment light intensity significantly affected stomatal conductance and as reported by Bucci *et al*., 2004; Dodd *et al*., 2005; Howard and Donovan, 2007, for many species, gradual increases in stomatal opening during daytime hours have been found

under natural field conditions as well as in controlled environments causing relatively high rates of transpiration when the guard cells that surround the stomatal pores bulge and separate while the middle lamella disintegrates leaving an opening between them. Stomatal opening or closing however, involves a complex series of processes and depends on the turgor of the guard cells such that when they are turgid, the pore between them is large but when they lose turgor the pore decreases in size optimizing the requirements for carbon dioxide uptake and control of water loss. Therefore cycling or oscillation between the open and closed conditions occurs chiefly in water stressed plants and has a periodicity ranging from minutes to hours but can also be caused by shocks such as sudden change in temperature, humidity or light intensity (Dodd *et al*., 2005). Generally, the stomatal conductance increased with increasing PAR among the mother plants and somaclones (Fig. 8 a, b and Fig. 13 a, b) and this trend was maintained during both seasons. The changes in irradiation affected stomatal aperture hence the opening of stomata in hot, sunny weather coinciding with the peak PAR at 12:00hrs while a reduction in stomatal conductance between the peak and 15:00 hours was due to reducing irradiation.

The X-ray treated seeds, however, were characterized by relatively low stomatal conductance at 9:00hrs during times of low PAR (Fig. 8c and 13c), and higher photosynthesis rate (Fig. 11c and 16c) in season one and season two respectively. Lower stomatal conductance at 9:00hrs and 15:00hrs was associated with decreased plant water status in response to a water stress and reduced transpiration rate as the stomata tended to close (Ludwig *et al*., 2006) hence the difference in stomatal conductance exhibited

after X-ray seed treatment of the basic seed cultivars is an indication that when seeds of the selected three cultivars were subjected to X-ray exposure they acquired salinity tolerance; had the ability to close stomata more than is commonly observed in the basic seed cultivars (control) at early hours of the day, midday and late hours of the day, alongside increasing PAR thus measurements of maximal stomatal closure (minimum stomatal conductance) corresponding to low transpiration rate since according to Bucci *et al*., 2004, 2005; Daley and Phillips, 2006, the magnitude of water loss occurring during the day depends on stomatal conductance. Such plants had better stomatal control of transpiration than basic seed cultivars and their somaclones thus they can conserve soil water until late in the growing season. The maintenance of a continuous water stream through the plant during the day however, results in enhanced movement of mobile mineral nutrients within the xylem of a plant by transpiration-driven mass flow from the soil solution in addition to supply of nutrients to roots, distribution of nutrients within plants and depends on the xylem flow rate and duration of transpiration (Mairgareth *et al*., 2009).

Optimal efficiency (optimization of cost to benefit, costs being loss of water and benefits, carbon dioxide assimilation and photosynthesis) in the selected three cultivars was observed in X-ray seed treatment, when stomatal aperture during the day varied in a manner that resulted in minimum transpiration for maximum photosynthesis Fig. 8(c), 9(c) (season one) and Fig. 13(c), 14(c) (season two). This stomatal control system produced an optimum degree of opening for the entrance and subsequent assimilation of carbon dioxide (Fig. 10a, 10b, 10c) as shown in both season one and season two (Fig.

15a, 15b, 15c) which in turn resulted in efficient control of water loss although water loss is an inevitable consequence of stomatal opening for photosynthetic carbon gain centrally to some species whereby the stomata tend to open in low concentrations of carbon dioxide and close in high concentrations (Barber, 1995).

The positive effects of X-ray seed treatment on physiological aspects observed suggest that an adaptation mechanism (tolerance to salinity) had been developed by the plants mainly in the leaves during the growth period with the measured photosynthetic rates significantly differing from those of somaclonal variants and basic seed cultivars as the latter had elevated transpiration rates and stomatal conductance but without an equivalent increase in photosynthesis rate or carbon dioxide assimilation rates. The possible explanation for this occurrence as observed by Scholz *et al*., 2007, is that, the adapted protoplasm did not become rigid and brittle as soon as that of non-treated seeds, and was better capable to hold water against dehydrating force maintaining green leaf photosynthetic capability under moisture stress. Therefore, higher rates of photosynthesis were recorded and lower rates of transpiration characterized these plants.

Even though the stomata occupy a central position in the pathways for both water lost by transpiration and exchange of carbon dioxide for photosynthesis (Meyer *et al*., 1973), highest stomatal conductance observed in the field was desirable for photosynthesis, but undesirable for conserving water. It is commonly assumed that they therefore provide the main control of both transpiration and photosynthesis (Hamlyn, 1997) justifying this study of variation in magnitude and the responses of stomatal conductance among cultivars as important considerations in determining how large an impact daytime

stomatal opening or closure have on transpiration, photosynthesis and CO_2 assimilation rates.

From the results obtained it is clear that a change in one of the factors affecting leaf photosynthetic capability under moisture stress, transpiration does not necessarily produce a proportional change in rate of transpiration because the rate is controlled by more than one factor (Richards, 2006). This poses a challenge to modern genetics for improving salinity tolerance by genetic manipulation such as the X-ray seed treatment which significantly resulted into plants more adapted to the arid conditions during the two growing periods in comparison to the basic seed cultivars and their somaclonal variants simply for the reason that stomata constitute the principal transpiration control system, and 80-90% of the water usually escapes through them (Howard and Donovan, 2007). This response may be attributed to plant capacity to accumulate high concentrations of leaf apoplastic solutes (Mairgareth *et al*., 2009), which in turn affects stomatal regulation.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

- Based on the results obtained in these experiments it was clear that among the three selected sorghum cultivars screened for salinity tolerance, Seredo (which represents the red sorghum in this study) was the most tolerant cultivar to salinity stress while cultivar El-gadam was most sensitive based on root length, root: shoot ratio and root shoot morphology. Mtama 1 was intermediate and significantly different from Seredo and El-gadam in root length, root: shoot ratio and root shoot morphology. Their root length and root: shoot ratio differences observed at lower salinity levels (0.22 and 5dS/m) were however, attributed to inter-cultivar differences.
- Hydroponics screening of Seredo, El-gadam and Mtama 1 somaclones confirmed that seedling root elongation and root: shoot ratio of Mtama 1 and El-gadam were adversely affected at 10 and 15dS/m in comparison to the significantly tolerant Seredo somaclone.
- X-ray seed treatment of the three selected seed cultivars improved tolerance to salinity. Seredo X-ray treated seed was the most tolerant at 15dS/m based on root length, root: shoot ratio and root shoot morphology. Mtama1 X-ray treated seed was intermediate while El-gadam was most sensitive to salinity.
- Results obtained during the field experiments clearly indicated that Seredo, El-gadam and Mtama 1 had significant differences in physiological traits in comparison with their somaclones and plants obtained via X-ray treatment of the basic seed. Seredo X-ray

treated seed had low transpiration rate, high carbon dioxide assimilation rate and a subsequent high photosynthesis rate. Thus regarded as the most suitable for growth in the Asals. Mtama 1 and El-gadam X-ray treated seed on the other hand exhibited high transpiration rates, high stomatal conductance but without equitable photosynthesis rate and carbon dioxide assimilation rate. Seredo, El-gadam and Mtama 1 commercial seed and their somaclones had relatively low photosynthesis rate, high stomatal conductance, and high transpiration rates.

6.2 Recommendation

- Field screening should be undertaken to verify the inheritance of physiological and morphological traits of X-ray treated seed and its subsequent generations.

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APPENDIX

Soil analysis results of four ASAL sites

Laboratory soil analysis was carried out before growing the seeds of the three cultivars in field plots at JKUAT farm. Three soil samples obtained from ASALS where wild sorghum grows were analysed to establish the nutrient content and pH in comparison to the soil of the experimental site. The results of the parameters measured are as shown in

Site	*pH in H_2O*	*EC (dS/m)*	*CO_3^{2-}*	*HCO_3^-*	*Cl^-*	*K (ppm)*	**CEC Meq/100**
Experiment site (JKUAT)	6.70	0.10	Trace	Trace	6.25	164	15
JKUAT virgin land	5.00	0.07	Trace	Trace	6.25	64	17
Yatta soil	9.10	3.00	Trace	Trace	7.50	90	12
Amboseli soil	10.70	4.70	100	Trace	74.0	224	11

Table 2.

Table 2: Soil analysis results of four sites in Kenya

Extreme salinity and pH was found in Amboseli soil followed by the Yatta soil where cultivated sorghum failed to grow. Sorghum grew well in 0.10 dS/m (EC) and pH= 6.7. This was the basis for choosing the experimental site as the JKUAT farm where the three selected sorghum cultivars, their somaclones and their X-ray treated seeds were grown during season one and season two. *Sorghum bicolor* thrives well at pH 5.5 – pH 8 (FAO,

1974) therefore, at high pH values (pH 9.10 – Yatta soil and pH 10.70 – Amboseli soil), availability of phosphorus and most micronutrients tend to decrease.

The results on soil analysis showed varying levels of EC for the four soil samples, that is 0.10dS/m – JKUAT Experiment site; 0.07dS/m – JKUAT Virgin soil; 3.00dS/m – Yatta soil and 4.70dS/m Amboseli soil. On the basis of saturation extract, values of 0 - 2dS/m are safe for all crops; very sensitive crops are affected between 2 – 4dS/m; while only the tolerant crops grow well above that level (Richards, 1954). This justified the important laboratory measurement (EC) since it reflects the extent to which the soil is suitable for growth of sorghum. In other words, the EC measures the concentration of soluble inorganic salts in the soil.