GROWTH AND PHYSIOLOGICAL RESPONSES OF BAMBARA GROUNDNUT TO SODIUM CHLORIDE SALINITY

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ABSTRACT

Bambara groundnut is one of the most neglected and under-utilized indigenous African legume with potential to alleviate food insecurity, malnutrition and poverty in tropical semi-arid regions of Africa. The crop is drought tolerant, resistant to pests and diseases, produces reasonable yields in low fertile soils and has high nutritional value. Salinity affects plant growth, development and productivity in agricultural soils world wide. It is caused by the accumulation of soluble salts especially sodium and chloride ions in the root zone. This crop may be grown in some semi-arid areas or under irrigation, both of which offer potentially saline conditions. There is limited research on the effect of salinity on growth and physiology of this plant, more so the landraces cultivated in Kenya. The study investigated the effects of NaCl salinity on growth and physiological responses of Bambara groundnut grown in Western Kenya, Kakamega 2 (Kk) and Mumias 2 (Mm).The experiments were laid out in the laboratory and greenhouse at Maseno University botanic garden as a completely randomized design consisting of 5 treatments and 3 replica. Seeds and plants were exposed to NaCl concentrations of electrical conductivity : 0 mm ho cm^{-1}, 6.96 mm ho cm^{-1}, 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} in the growth media. Germination percentage was determined, shoot and root length measured using a meter rule, seedling root and shoot fresh and dry weights measured using an electronic weighing balance, leaf growth, root to shoot biomass ratio, and percentage water content in shoots and roots were determined. Net photosynthesis was measured using an infra-red gas analyzer, chlorophyll fluorescence parameters measured using chlorophyll fluorescent monitoring system, leaf chlorophyll content measured using spectrophotometer and nitrogen content determined using the soil plant analysis device. Data was subjected to analysis of variance using Costat statistical computer package to determine whether the treatment effects were significant or non-significant at 5% level. Least significant difference was used to separate the means. NaCl salinity significantly ($P<0.01$) decreased and delayed germination. Plant growth parameters were significantly ($P<0.01$) reduced by salinity in both landraces however, Mm landrace was more salt tolerant. Salinity significantly ($P<0.01$) reduced chlorophyll and nitrogen content, and net photosynthesis. The Mm landrace had significantly ($P<0.05$) higher chlorophyll a, b and t compared to Kk landrace. Salinity significantly ($P<0.05$) decreased the Fv/Fm ratio and electron transport rate in the two landraces hence decreased plant growth and ultimately productivity, however there were no significant ($P>0.05$) differences in the Fv/Fm values for Mm as compared to the control indicating the ability of its PSII system to function under stressful conditions and thus may contribute to salt tolerance. The Mm landrace seeds seemed to be more salt tolerant at higher salinity (12.93 mm ho cm^{-1} and 19.89 6.96 mm ho cm^{-1}) as Kk landrace was at lower salinity (6.96 mm ho cm^{-1}). Both landrace seeds may be tried in saline soils with electrical conductivity as indicated.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General introduction

Kenya has a rich diversity of plant genetic resources yet she has serious food insecurity and malnutrition problems (Mwai, 2001). Agriculture production in Kenya has stagnated since 1980s resulting in malnutrition in over 89% of Kenya's population (Musyimi, 2005). Food insecurity has been identified as the prime cause of malnutrition in many Kenyan households (Mwamburi and Too, 2004). Her rapidly increasing population was estimated to be 28.7 million in 1999, has been increasing at a rate of 2.4 % per annum and it was estimated at 38.6 million in 2009 (KNBS, 2009). Growth in population, poor living standards and lack of employment have led to the need to increase and diversify production of food for family consumption or as a source of income which is a basic pre-requisite for improved household security (FAO, 1997). Land for agriculture is an increasingly shrinking resource, some land is being taken out of production all the time and diverted to other uses such as construction of roads, housing and industry hence the entire country depends on only about 20% suitable arable land (Musyimi, 2005).

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is one of the most neglected and underutilized crops in Kenya. It has been reported as one of the indigenous food crops found in Western Kenya that has potential of reducing food and nutritional insecurity (Musotsi, 2004). Due to their great genetic diversity, adaptability and resilience to drought, Bambara groundnut is a good candidate for research in its salt tolerance, which could help us identify cultivars suitable for cultivation in the saline agro-ecological zones and consequently to advice on the growing of the most tolerant and high yielding landraces. Ongoing research (Botany Department, Maseno University) on "Effects of water deficit on the growth and physiological responses" on the six commonly cultivated landraces in Kenya indicated that, the landraces under current study (Kakamega 2 and Mumias 2) gave reasonably higher yields on relatively poor soil, were resistant to pests, and indicated greater drought tolerance (Personal communication, Godfrey Netondo, 2011) compared to the rest. Research on these landraces (Kakamega 2 and Mumias 2) therefore, could help determine traits for salinity tolerance and identify cultivars suitable for cultivation in other agro-ecological zones

including the potentially saline areas. Furthermore, the superior characters identified may be used in breeding for tolerant and high yielding varieties. The landraces could be studied further as a source of genes for salt tolerance that could be exploited in breeding programs.

Bambara groundnut is an indigenous Africa crop that has been cultivated in Africa for centuries (Heller *et al.*, 1997). It's a highly nutritious plant which plays a crucial role in people's diet and is currently grown throughout Africa (Stephens, 2003). It is ranked the second most important underground pod legume in much of Africa after groundnut (*Arachis hypogea*) (Ntundu *et al.*, 2006). Important attributes of the crop reported in literature include tolerance to drought, high nutritional value, relative resistance to pests and diseases, produce reasonable yields in relatively low fertile soils, wide agro-ecological potential and great genetic diversity (Linnemann and Azam-Ali, 1993). Despite its usefulness, Bambara groundnut remains one of the most neglected crops in Kenya and by scientific community and it is commonly referred to as 'a poor mans crop' (Heller *et al.*, 1997).

Out of a total surface area of approximately 582,646 km^2, Kenya is 80% arid or semi-arid (Netondo, 1999). Some semi-arid areas are potentially saline. In the arid and semi-arid areas, salinity could be caused by (1) poor irrigation water which contains dissolved inorganic ions in soil solution, that include Na^+, Mg^{2+},Ca^{2+}, K^+, Cl^-, SO_4^{2-}, among others. (2) accumulation of salts in the top layer of the soil due to over-irrigation, (3) proximity to the sea, and (4) the capillarity rise of salts from underground water into the root zone due to excessive evaporation. Also, low rainfall, high evaporation rate and poor water management could cause salinity related problems in these areas (Netondo, 1999; Musyimi, 2005). The salt affected soils in Kenya are estimated to cover nearly 4.42 million hectares and generally these areas occur in poorly drained soils such as in Nyanza Province, in semi-arid savannah, and in irrigated and waterlogged areas (Netondo, 1999). Modern agriculture management practices often worsen the extent of salinity sometimes by remobilizing salts from deep soil layers. Kenya has suitable agro-ecological areas that can support Bambara production in the drier regions of the country and there is potential to increase acreage since the crop is drought tolerant. The crop also has a lot of potential to be grown under irrigation. In Kenya some of the potentially saline areas include parts of Coastal, North Eastern, Rift Valley and Nyanza

provinces. If these areas can be exploited by the production of salt tolerant food crops, food security would be improved in Kenya.

Salinization leads to an increase in water-soluble salts/ions such as sodium, potassium, magnesium, calcium, chloride, sulphate, carbonate and bicarbonate in the soil. A distinction can be made between primary and secondary salinization processes. Primary salinization involves salt accumulation through natural processes due to a high salt content of the parent material or in groundwater. Secondary salinization is caused by human activities such as inappropriate irrigation practices, especially with salt-rich irrigation water and/or insufficient drainage. In saline soil, salt induced water deficit is one of the major constraints for plant growth (Zadeh *et al.*, 2008). Salinity affects crop growth and development, and productivity of agricultural soils (Tester and Davenport, 2003; Munns and Tester, 2008). Despite the advanced management technology today, salinization of millions of hectares of land continues to reduce crop production severely world-wide (Alam *et al.*, 2004).

1.2 Statement of the problem

Salinity is one of the major problems to plant growth, development and productivity in agricultural soils world wide (Tester and Davenport, 2003; Alam *et al.*, 2004; Munns and Tester, 2008). Salinization of agricultural lands is widespread and occur in the semi-arid, low-lying, irrigated and poorly drained areas (Netondo, 1999; Musyimi, 2005). In Kenya the potentially saline areas include parts of Coastal, North Eastern, Rift Valley and Nyanza provinces (Netondo, 1999; Mwai, 2001; Musyimi, 2005). The effect of climate change threatens to increase salinity by causing areas that were initially not saline to be in this category, such include the semi-arid agro-ecological regions in tropical Africa (Netondo, 1999). If these areas can be exploited by the production of salt tolerant food crops, Kenya's food security would be improved. With the current shrinking of arable land, Bambara groundnuts may be grown in the potentially saline conditions or under irrigation. Already, some Bambara groundnut landraces are being grown in potentially saline areas such as Coast province. Those landraces currently being grown in non saline areas such as Kakamega and Mumias, could also be grown in such areas. Bambara groundnut has sustained human nutrition for generations yet it is one of the most neglected and under-utilized African food crops (Heller *et al.*, 1997). It has received scanty attention (Ntundu *et al.*, 2006) through research despite its potential to alleviate poverty, malnutrition and contribute to food security in Kenya. As Kenya's population continue to rise, there is pressure on land for diversified food production and increased yield. To reach this goal, research on indigenous, neglected and under-utilized food crops which have the potential to be grown in many agro-ecological zones including the salinized lands is important.

There is also limited scientific initiative documented on morphology, physiology and biochemical responses of this crop to forms of salinity locally and elsewhere in the world hence the need to assess the effects of salinity in commonly cultivated Bambara groundnut landraces in Kenya.

1.3 General objective

To investigate the physiological, morphological and biochemical responses associated with tolerance to salinity in two landraces of Bambara groundnuts grown in Western Kenya.

1.3.1 Specific objectives

a) To determine the effects of NaCl salinity on seed germination in Kakamega and Mumias Bambara groundnut landraces.

b) To determine the effects of NaCl salinity on the morphological parameters of Kakamega and Mumias Bambara groundnut landraces.

c) To investigate the effects of NaCl salinity on the physiological parameters of Kakamega and Mumias Bambara groundnut landraces.

d) To investigate the effects of NaCl salinity on biochemical parameters of Kakamega and Mumias Bambara groundnut landraces.

1.4 Hypothesis

Sodium chloride salinity has significant negative effect on morphological, physiological and biochemical parameters of Kakamega and Mumias Bambara groundnut landraces.

1.5 Justification

The increasing demand for food as a consequence of rapid rate of population growth necessitates that the country's agricultural potential be fully developed in order to address this challenge. Salinity is one of the major problems to plant growth, development and productivity in agricultural soils world wide. Salt-affected land comprises 19% of the 2.8 billion hectares of arable land on earth, and an increase in this menace is posing a serious threat to agriculture globally (Pessarakli and Szabolcs, 1999). Agricultural losses caused by salinity are difficult to assess but estimated to be substantial and expected to increase with time. In Kenya the potentially saline areas include parts of Coastal, North Eastern, Rift Valley and Nyanza provinces. If these areas can be exploited by the production of salt tolerant food crops, food security would be improved in Kenya. Research on salt tolerance of crop plants is one of the possible approaches to bring the saline or potentially saline areas under cultivation.

Bambara groundnut is one of the most neglected and under-utilized African indigenous legumes particularly in Kenya although it has been reported to be drought tolerant, resistant to pests and diseases, has high nutritional value and produce reasonable yields in low fertile soils (Linnemann and Azam-Ali, 1993). Research has not adequately addressed morphological, physiological and biochemical responses of Bambara groundnuts to salinity stress hence the need to assess the effects of salinity in commonly cultivated landraces in Kenya. Due to their great genetic diversity, adaptability and resilience to drought, Bambara groundnut is a good candidate for research in its salt tolerance, which could help us identify cultivars suitable for cultivation in the saline agro-ecological zones and consequently to advice on the growing of the most tolerant and high yielding landraces.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany and production of Bambara groundnut

According to (NRC, 2006) Bambara groundnut is classified as follows:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Tribe: Phaseoleae

Genus: *Vigna*

Species: *Vigna subterranea* (L.) Verdc.

The local names are njugumawe (Kiswahili), tsimbande (Luhya) and bande (luo). It is an annual herb that grows up to a height of 30 cm with creeping, multi-branched, leafy lateral stems just above the ground level. The landraces differ in many aspects from each other, with a wide variety of seed and pod colours, and growth habits vary from bunch type to semi bunch and spreading. It has small peduncles, which arise from the leaf axis formed (Linnemann and Azam- Ali, 1993). Recent research suggests that the plant is mainly self pollinated in most environments (Massawe *et al.*, 2003). Its pods develop underground and may be up to 3.7cm in diameter, depending on the landraces and number of seeds they contain. Mature pods are indehiscent, ranging from yellow to reddish to dark brown or even black in colour (Pasquet and Fotso, 1997). Lack of seeds, unsuitable varieties, pod losses during harvesting and superstitions related to traditional preferences and practices have contributed to the limited cultivation of this crop in Africa (Swanevelder, 1998). The crop is harvested when the leaves turn yellow (80 % of pods have matured) between 120-145 days after sowing depending on landrace and environmental factors. Seed vigour deteriorates after shelling hence shelling is done just prior to planting. Breeding of cultivars through hybridization is very difficult due to small flowers. Major factors associated with low production of the plant include drought, unimproved cultivars and low germination due to

poor seed storage. The development of high yielding, drought and salt tolerant varieties is one of the approaches to improving the plant productivity. It is ranked the second most important underground pod legume in much of Africa after groundnut (Ntundu *et al.*, 2006). Major producers of Bambara are West African countries such as Nigeria, Niger, Ghana and Cote d'Ivore, but it is also widely grown in Eastern Africa and Madagascar (Linnemann and Azam- Ali, 1993). It is also found in South and Central America and in Asia, particularly in India, Indonesia, Malaysia, Philippines and Sri-lanka (Linnemann and Azam-Ali, 1993).

2.2 Ecological requirements

Bambara groundnuts will grow on well drained soil, but light, sandy loams with a pH of 5.0 to 6.5 are most suitable. The nuts grow poorly in calcareous soils. They can be cultivated up to 1600m above sea level. An average day temperature of (20-28)° C is ideal for the crop. Well spread rain during the growing season (600-700) mm is ideal, though too much rain at harvest time may damage the crop (Swanevelder, 1998). It gives best yields on a deeply ploughed field with a fine levelled seedbed (Swanevelder, 1998; Masideni, 2006). Information on fertilizer requirements is limited, while rhizobial inoculation is practiced in some areas (Swanevelder, 1998). However, nitrogen and phosphorous application have no influence on the yield, infact nitrates only encourage vegetative growth at the expense of the yield (Swanevelder, 1998). Large and healthy seeds are recommended for sowing at 2 to 3 cm deep and spacing of 10-15 cm in single row 45 to 90 cm apart (Swanevelder, 1998).

2.3 Pests and diseases

The few reports on pests and diseases indicate that the crop has the tendency to resist pests and diseases however very little is known about the kinds of pests and diseases that attack it and the extent of damage to the plant, pods or seeds (Linnemann and Azam-Ali, 1993). Due to underutilization, pests and diseases may increase with intensified cultivation. The above warrants need for further research in this area.

2.4 Uses and nutritional value

Bambara groundnut is a complete food containing proteins, carbohydrates and fat in sufficient proportions to provide a nutritious food (table 1) and it has been reported to be the second most important legume in Africa after cowpea (Williams, 1995). It is rich in iron unlike most legumes in the range of 2.0-10.0 mg/100g, vegetable protein with a lot of lysine and methionine contents, fibre, potassium, sodium, calcium, carbohydrate, oil and energy (Rowland, 1993). Traditionally the seeds are milled into flour and used to make small flat cakes or biscuits. The flour can be mixed with cereals and used to make porridge, the seeds can be boiled and mixed with plantains or boiled together with maize and eaten or they can be roasted and eaten as a snack (Williams, 1995). In Kenya the beans are roasted, pulverized and used in preparing soup. Seeds can be eaten fresh or grilled while immature. The flour is used in bread making. Recent research has established its potential use in various food products such as vegetable milk, weaning food and processed products and it has even turned out that mashed Bambara seeds can be used as coagulants in solar water disinfection (Wambete and Mpotokwane, 2003). The seeds have been used to feed poultry while their foliage is suitable as livestock fodder. It fixes atmospheric nitrogen in symbiosis with *Bradyrhizobium* strains through nodulation process improving the soil hence useful in crop rotations and in intercropping with cereals such as millets, maize and sorghum (Masideni, 2006). Despite the importance of the crop as a food in traditional farming systems in Africa, no significant efforts have been made scientifically to improve this crop; no commercial production and no industrial use of the crop take place. Previous research concentrated only on agronomic aspects while other aspects such as physiological responses to salinity, temperature and many others have been neglected.

Table 1. Nutritional value of Bambara groundnuts

Constituent food	**Amount %**
Carbohydrates	(51-70)
Oil	(6-12)
Vegetable protein	(18-24)
Minerals	**(mg/100g)**
Iron	(4.9-48)
Potassium	(1144-1935)
Sodium	(2.9-12.0)
Calcium	(95.8-99)
Energy (Cal/100mg)	(365-414)
Fibre (%)	(5.0-12.0)

Source: Rowland (1993).

2.5 Definition, origin and general distribution of soil salinity

Salinity refers to the occurrence of high concentrations of dissolved inorganic ions in soil solution, that include Na^+, Mg^{2+},Ca^{2+}, K^+, Cl^-, SO_4^{2-} and HCO^{3-}. It can be defined as the accumulation of soluble salts in the rooting region of the soil (rhizosphere) to concentrations that are high enough to affect plant growth and development (Mwai, 2001). All saline soils have excess salts in the surface soil and in the root zone. The most common ions at high concentration in such soils are Na^+ and Cl^-. They usually have high pH above 7, low soluble Ca and occur in poorly drained areas where soils are shallow and where precipitation is limited. The main source of all salts in the soil is the primary minerals in the exposed layer of the earth's crust which are released gradually through chemical weathering involving hydrolysis, hydration, solution, oxidation, reduction and carbonation (Netondo, 1999). They also originate from volcanic eruptions, discharge from deep thermal sources and oceans. These salts are distributed from their areas of origin mainly by water either as surface run-off or as ground water streams usually accumulating in the valley basins, which have poor drainage. There are various ways in which soils become saline such as through saline seeps, tidal waves, salt sprays, irrigation using underground saline water or irrigation of semi-arid and arid areas using river water and localized redistribution and accumulation of salts in

areas of poor drainage. The occurrence of salinized soils primarily, although not exclusively in arid and semi-arid regions of the world is a result of faulty irrigation. The soils and substrata of these regions are rich in water-soluble salts, particularly sodium salts (Yeo and Flowers, 1986). Salinization due to injudicious irrigation is responsible for the loss of large tracts of agriculturally productive land for cultivation (Flowers *et al.*, 1976).

It is estimated that about 10% of the world's earth surface is sufficiently affected by salts, limiting its utilization for crop production. In Africa, about 43.6 million hectares of the land is affected by salinity and the situation is expected to worsen as more areas of the semi-arid and arid lands are increasingly being put under cultivation. Even some areas that receive adequate rainfall have the potential to become saline. The cost of salinity to agriculture is expected to increase as soils are further affected (Ghassemi *et al.*, 1995). When soils contain high amounts of soluble salts or high amounts of Na^+, they develop unsuitable characters becoming both unproductive and unmanageable. Therefore, an understanding of the physiology of salt tolerance in agricultural plants is essential for an effective approach to solving the problem of salinity (Ziska *et al.*, 1989).

2.5.1 The salinity problem in Kenya

Generally the salt affected soils in Kenya occur in semi-arid and arid regions, along the coast and poorly drained soils in Nyanza and are estimated to cover nearly 4.42 million hectares (Netondo, 1999). Modern agriculture management practices often worsen the extent of salinity by remobilizing salts from deep soil layers and leaching. Currently, more irrigated land is being abandoned due to salinity than there is new one coming under irrigation (Musyimi, 2005). Leaching away the salts alone cannot easily reclaim the poorly drained soils. The draining is also quite expensive. The presence of salts in the ground water and river water makes it unsuitable for use in irrigation (Netondo, 1999). It is the Kenyan government policy to encourage the growing of crop genotypes that can withstand low soil moisture content and salinity with reasonable productivity and acceptable food quality and other desirable agronomic characteristics (Netondo, 1999). Kenya has suitable agro-ecological areas that can support Bambara production in some of the drier regions of the

country and there is potential to increase acreage since the crop is drought tolerant. The crop has a lot of potential to be grown under irrigation.

2.6 Effects of salinity on plant growth and productivity

Salt stress as defined by Levitt (1980) is the presence of excess amounts of salt in the rooting medium to concentrations that are sufficiently high to lower the chemical potential of soil water appreciably by between 0.05 MPa and 0.1 MPa. Although other salts (Ca, Mg and K) play a role in the development of salt stress, most of the salt stress in nature is due to sodium salts, especially NaCl (Mwai, 2001).

Salinity stress affects crop growth, yield and productivity (Tester and Davenport, 2003; Munns and Tester, 2008). The general effect of salinity stress on plants is to produce dwarfed, stunted plants with dull coloured leaves which are often coated with wax deposits. The effects of high salt concentrations in rooting medium may include: a loss of turgor followed by a cessation of growth or if the stress is severe enough, an actual killing of the plant tissue in the form of necrotic spots, marginal burns, falling of leaves or death of the whole plant. Such effects have been observed to rise with increasing salinity (Levitt, 1980). The extent of salt stress injury and possible death varies from one species to another and even between varieties in the same species depending on such factors as the salt resistance of the species, age, genotype and salt concentration (Levitt, 1980).

The effect of salinity on plant growth is a complex syndrome that involves:
Osmotic effects, specific ion toxicity effects and nutritional effects (Neumann *et al.*, 1988). Sodium and chloride ions are the two key ions responsible for both osmotic and ion-specific damage that significantly reduces crop growth and yield (Munns and Tester, 2008). In addition to the above effects on plants, high salt concentrations in the soil also affect plant growth indirectly through the effects of the salts on the soil itself. Such effects are more pronounced in sodic soils, where structural deterioration (deflocculation) is likely to occur. Such deflocculation is followed by a decrease in moisture transmission and aeration of the soil, which could in turn cause root damage and accelerated death (Mwai, 2001). Strictly saline soils are categorized into three types: sodic (or alkali), saline and saline-sodic. Saline

soils occur in arid regions, estuaries, and coastal fringes. They are dominated by sodium cations with electrical conductivity (EC) of more than 4 dSm^{-1}, but the dominant anions are usually soluble chlorides and sulphates. Exchangeable sodium percentage (ESP < 15) and pH values of these soils are much lower than in sodic soils. Sodic or alkaline soils are widely distributed in arid and semi arid regions. They have high concentrations of free carbonate and bicarbonate and excess of sodium on the exchangeable site of clay particles. They are deficient in nitrogen, phosphorus and zinc. Such soils have high pH (greater than 8.5 and sometimes up to 10.7) with a high ESP (>15) and poor soil structure. Clay fraction and organic matter are dispersed, thus soils are sticky when wet and hard when dry. There is very poor hydraulic conductivity and high impedance to root growth. Saline-sodic soils or saline-alkali, have both high ESP and EC (Mwai, 2001).

2.6.1 Osmotic effects of salinity

Osmotic stress involves the water relations of the plant growing under saline conditions. The primary effect of salinity is that it renders less water available to plants although it is present in the root zone. Salinity reduces soil osmotic potential and hence makes soil water less available for extraction by the plant (Munns and Termaat, 1986; Yeo *et al.*, 1991). It follows that salinity exposes the plant to a salt-induced physiological drought stress (Ziska *et al.*, 1989). It has been argued (Munns, 2002) that osmotic stress is most active in the early stages of exposure to salinity, when the salts have not entered the plant to cause ionic toxicity. When a plant is subjected to a salt solution, it immediately suffers osmotic dehydration that is analogous to drought/water deficit induced by transpiration/evaporative water loss. The water in the plant tends to move out, the cells decrease in their volume and their water potentials fall leading to decreased cell turgor and growth. This first response is referred to as osmotic shock. The osmotic effects of salinity stress observed immediately after salt application are believed to continue for the duration of exposure, resulting in inhibited cell expansion and cell division, as well as stomatal closure (Flowers *et al.*, 2000; Munns, 2002; Munns and Tester, 2008). The osmotic dehydration is the immediate cause of such responses as the depression of growth, decreased yield and transpiration, which in turn is attributed to an increase in the hydraulic resistance of the roots and leaves upon loss of turgor (Mwai, 2001). The osmotic effect of the salt in the soil solution produces effects similar to those of water

stress caused by drought such as increased leaf RNase activity (Levitt,1980), stomatal closure hence decrease in transpiration.

High salt concentration in root medium affects the growth and economic yield of many important crops (Munns, 2002; Alam *et al.*, 2004; Hajer *et al.*, 2006). Salinity reduces growth and yield of non halophytes plants by decreasing the availability of water to the roots due to the osmotic effects of external salt and by toxic effects of excessive salt accumulation within the plant (Taffouo *et al.*, 2008). Thus excessive uptake of Na^+ and Cl^- may lead to ionic disturbance of whole plants. Although most salt-tolerant species control the accumulation of inorganic ions as the basic mechanism to adjust their internal tissue osmotic potential against external salinity, they differ widely in the extent to which they accumulate organic ions (Munns, 1993).

The osmotic effect may also occur when salts accumulate in the root cell walls. This is likely to cause cell dehydration since the water potential in the cell wall is lower than that in the cytoplasm resulting in a tendency for water to move from the cell cytoplasm to cell wall causing dehydration of the protoplasm (Munns, 1993).

2.6.2 Nutritional effects (mineral deficiencies) of salinity

The decrease in growth due to salinity has been partly explained by a suppression of nutrient absorption due to the uptake of Na^+ and Cl^- in competition with other nutrient ions on the plasma membrane. This is attributed to the fact that Na^+ competes with K^+ for binding sites essential for cellular function (Tester and Davenport, 2003). This role makes K^+ an important element as more than 50 enzymes are activated by K^+, and Na^+ cannot substitute in this role (Bhandal *et al.*, 1988). The implication of these two macronutrients in salinity is thought to be one of the factors responsible for reduction in the biomass and yield components. On the other hand, the reduction in growth is generally the consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behaviour, photosynthetic efficiency and carbon allocation and utilization (Greenway and Munns, 1980; Munns and Termaat, 1986).

Increased soil concentration of Na^+ and Cl^- ions is usually accompanied by a decrease in growth and in the uptake of K, Mg and Ca in many crop plants, such as spinach and beans (Flowers *et al.*, 1986; Kingsbury *et al.*, 1983). Both chlorides and sulfates are reported to cause a decrease in total inorganic phosphorous in some crop plants such as salinized tomato (Levitt, 1980). Salinity has been seen to inhibit ion absorption and translocation and the high pH of sodic soils depresses nutrient uptake by causing the precipitation of micronutrients such as Fe, Mn and Zn. The reduced nutrient uptake has partially been attributed to the osmotically induced restricted root growth, which in turn lowers the ability of roots to explore greater soil volume for the nutrients (Mwai, 2001

2.6.3 Specific ion toxicity effects of salinity

Salt may injure a plant by way of specific toxic effects, independent of osmotic and nutritional effects. Such ion-specific effects are effective at the levels of the organ, tissue, cell and sub-cellullar entities (Kingsbury *et al.*, 1983). There are two aspects of specific ion toxicity (Levitt, 1980; Kingsbury *et al.*, 1983): Inhibition of growth and development, and metabolic disturbances. The specific ion toxicity injury increases with time as more salts are absorbed (Levitt 1980).

The ion toxicity affects plants by:

a) Directly causing injury to the plasma membrane proton pump or H^+-ATpase. The pump generates the proton motive force that drives the trans-plasma membrane fluxes of solutes including ions, sugars and amino acids (Rausch *et al.*, 1996). The pump may play a key role in re-establishing and maintaining turgor under saline and osmotic stress, and restricting the concentration of toxic ions in the cytosol (Rausch *et al.*, 1996). The water channel proteins (aquaporins) in the plasma membrane facilitate trans-membrane water flux in plants. Generally, the trans-membrane ion and osmolyte flux is considerably slower than water flux as are metabolic changes resulting in internal osmotic potential. There is reported control of aquaporin gene expression by salinity or water stress (Yamada *et al.*, 1995).

b) The ions directly penetrating the cells and causing injury to the internal contents of the protoplasts, and as such cause inhibition of enzyme activity (Flowers *et al.*, 1977), cell death through DNA destruction and degradation of chloroplast and mitochondrial

membranes (Flowers *et al.*, 1985) decreasing growth. In case the ions build up in the cytoplasm, then they cause death to the cell due to cell poisoning or induce dehydration. The main site of Na^+ toxicity for most plants is the leaf blade rather than the root tips as Na^+ accumulates in the leaf blades due to continuous translocation and deposition due to transpiration. Thus, it is very important that Na^+ doest not reach the leaf blades in excess as the Na^+ relocation from leaves to roots is likely to be only a small portion of what was delivered to the leaf. Ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis, necrosis) in mature leaves of crop plants (Munns, 2002; Tester and Davenport, 2003; Munns *et al.*, 2006), due to high Na^+ concentration disrupting protein synthesis and interfering with enzyme activity (Bhandal *et al.*, 1988). Leaf senescence is therefore accelerated starting from older leaves.

2.7 Physiological effects of salinity on photosynthesis

Generally salinity reduces photosynthetic rates by decreasing CO_2 assimilation rates, photosynthetic products, leaf expansion and chlorophyll content as well as lowering CO_2 compensation points, increasing stomatal and mesophyll resistance and changing activities of various enzymes (Ziska *et al.*, 1989). Processes in photosynthetic metabolism which may be impaired by water stress include ribulose bisphosphate carboxylase /oxygenase (Rubisco) enzyme activity, ribulose bisphosphate (RuBP) regeneration, ATP supply, electron transport rate and efficiency of light capture in the photosystems (Lawlor and Cornic, 2002). It is widely known that salinity is one of the main environmental stresses that decrease growth, transpiration and photosynthesis (Munns and Termaat, 1986). Photosynthesis is, however, reported to be more sensitive to salinity compared to growth and transpiration.

Chlorophyll content of leaves is a useful indicator of both potential photosynthetic productivity and general plant vigour (Alonso *et al.*, 2002). Salinity causes metabolic disturbances associated with photosynthesis such as depressing chlorophyll content (Lutts *et al.*, 1996) and hence reduced photosynthetic capacity of the plant, thus affecting the amount of assimilate translocated and distributed from leaves to growing tissues which further limit growth (Munns and Termaat, 1986). Chlorophyll is widely used as a basis for determination of photosynthesis because the reaction components essential for photosynthesis (such as the

reaction centres of PS1 and PS11, electron carriers and enzymes related to ATP synthesis and CO_2 fixation) are present in chloroplast at fixed molar ratios to chlorophyll (Kura-Hotta *et al.*, 1987).

It has been suggested that photosynthesis in salt stressed plants may be inhibited due to photo inhibition. This is where the photosynthetic apparatus is damaged in the presence of excess light. Plants that grow in the tropics such as sorghum are likely to experience photo inhibition in addition to salt stress (Netondo, 1999).

The effects of salinity on photosynthesis depend on the plant species' adaptation to salinity, the length of time that the plant has been exposed to salinity, the concentration and composition of the salt, and the interaction of salinity effects with other prevailing environmental factors such as humidity and soil nutrient status, making photosynthetic responses to salinity to be varied for various plant groups (Mwai, 2001). The effect of salinity on photosynthetic apparatus, however, is a complex process.

Chlorophyll pigments absorb radiant energy which is used for photosynthesis and part of this energy is reradiated as longer wavelengths through a process called chlorophyll fluorescence. Chlorophyll fluorescence can be used to determine the effects of environmental stress in plants. Chlorophyll fluorescence parameters are direct indicators of the photosynthetic activity or efficiency and provide basic information on the functioning of the photosynthetic apparatus especially photosystem II. The ratio Fv/Fm refers to photosynthetic efficiency. This is when the leaf is predarkened and the photosystems and electrons carriers are oxidized. The flow of electrons through PS II is indicative under many conditions of the overall rate of photosynthesis (Pereira *et al.*, 2004). It has been used in determining plant tolerance to environmental stresses such as cold, drought, temperature, light and salinity in sorghum (Netondo, 1999). Cornic and Massacci (1996) reported no significant difference in the data collected from dehydrated and non dehydrated plants for both bean and maize leaves. Results on the effect of water deficit on the Fv/Fm ratio reflecting potential quantum yield of PS II is used as a parameter of the physiological state of the photosynthetic apparatus in intact plant leaves (Pospisil *et al.*, 1998) and damage to PSII will often be the first manifestation of stress in a leaf (Maxwell and Johnson, 2000), hence fluorescence can give insights into the ability of a plant to tolerate environmental stresses. Under optimal

physiological conditions, this parameter was found to have the value of 0.832 (Demmig and Bjorkman, 1987). Environmental stresses affect PS II efficiency and lower the Fv/Fm values (Pospisil *et al.*, 1998).

Gas exchange parameters are a common practice in investigations of plants exposed to salinity. Salinity reduces transpiration rate due to direct influence of the stomata whose aperture determines how much water is lost. Reduced transpiration has also the tendency to lower the rate of salt loading into leaves (Everard *et al.*, 1994). This is due to the fact that salts reach the leaves through the transpiration stream. This tends to maintain the salts at subtoxic levels for a long time (Everard *et al.*, 1994). In the process water is conserved in order to maintain a high water status in the plant. This complements the reduced leaf area which minimizes the amount of water lost per unit leaf area. Therefore, stomatal closure in response to salinity in one way, is a limitation to photosynthetic capacity. However, it also in another way, offers a protective mechanism which aids the survival of plants that are exposed to salinity stress by minimizing salt loading in leaves and conserving water (Everard *et al.*, 1994). A plant with better compromise between opening the stomata to allow water to evaporate (hence more salts enter) and the closure to minimize the entry of salts will do better.

The intercellular CO_2 concentration declines with increasing salinity showing that the salt induced reduction in CO_2 assimilation is partially as a result of stomatal closure. In most cases the effect of salinity on photosynthesis is attributed to stomatal limitations (stomatal closure) which curtails the entry of CO_2 hence restricted carboxylation.

Salinity causes increased water use efficiency (WUE). It reduces transpiration rate due to direct influence on the stomata whose aperture closes, meaning that there is reduced water transpired for a unit quantity of CO_2 fixed. This is important since it allows less salt to go to the leaves through the transpiration stream. This parameter can be useful for breeding since an increase of WUE due to salinity could be advantageous in offering salt tolerance to a plant (Netondo, 1999).

2.8 Biochemical effects of salinity on plant nitrogen content

Nitrogen is a macronutrient required by plants as a component of amino acids that are precursors of protein synthesis, component of nucleic acids and also heterocyclic and azo compounds such as pyrol molecule found in chlorophyll. It is stored in various forms, in different organs and at different times. Herbaceous plants have better capacity to store nitrates in their larger vacuoles and their faster rates of photosynthesis enhance higher capacity for nitrate reduction. There is a strong relationship between leaf chlorophyll content and leaf nitrogen concentration that may assist predict the crop nitrogen status, the onset of nitrogen stress in a plant and also provide good comparison of nitrogen status between plants of a given cultivar (Richharia *et al.*, 1997).

Salinity can interfere with nitrogen nutrition in a direct and indirect way, usually simultaneously at several points of the assimilation pathway of the inorganic nitrogen compounds. Nitrogen uptake and assimilation is inhibited by high concentration of Na^+, K^+ and Mg^{2+} to different extents. Salinity has a tendency to differentially stimulate the synthesis of nitrate reductase (NR) in shoots and roots (Ullrich, 1983). The first metabolic step of nitrate assimilation that usually takes place within the cells is nitrate reduction catalyzed by nitrate reductase (NR), a highly regulated enzyme (Solomonson and Barber, 1990) that often plays the role of a bottle neck for the whole process. The bulk activity of NR is located within the cytosol of root or leaf cells under normal conditions. The salt effects on nitrate reduction depend very much on salt concentration and species investigated. Moderate salinities up to 20 or 40mM NaCl may cause changes in NR activities. They increase it in the roots and remain little affected or unchanged in leaves. Salinity diverts large proportion of NR from shoots to roots as observed in tomato plants treated with 100 mM NaCl although it is generally regarded as a leaf reducer and not root reducer (Cramer and Lips, 1995). In rice, 1M NaCl caused up to 85 or 90% loss of NR activity (Richharia *et al.*, 1997). NR is regulated by gene expression that is certainly involved in adaptation to salinity.

2.9 Plant responses to salinity

The difference in a plants' response to a given level of salinity is dependent upon the concentrations and composition of the ions in solution, stage of development as well as the genotype exposed to salinity. Plants hence differ with regard to their tolerance to salinity with the seedling stage being most sensitive.

Plants are categorized as either halophytes (salt tolerant), adapted to grow optimally and reproduce in saline environment or glycophytes (salt sensitive) growing well in non-saline environment and cannot grow, or are severely inhibited in saline environments. Even though most crop plants are glycophytes and can tolerate only moderate amounts of salts, they exhibit a wide range of salt tolerance among them ranging from a maximum in beet roots, to a minimum in carrots (Levit, 1980; Kingsbury *et al.*, 1983).Table1 shows plant salt tolerance groupings.

Table 2: Soil and water salinity criteria based on plant salt tolerance groupings

Plant salt tolerance grouping	Water or soil salinity rating	Average root zone salinity (dS/m or mm ho/cm) EC
sensitive crops	very low	< 0.95
moderately sensitive crops	low	0.95–1.9
moderately tolerant crops	medium	1.9–4.5
tolerant crops	high	4.5–7.7
very tolerant crops	very high	7.7–12.2
generally too saline	extreme	> 12.2

Source: Maas (1990).

Research of recent decades has established that most halophytes and glycophytes tolerate salinity by rather similar mechanisms (Hasegawa *et al.*, 2000). It follows then that many of the molecular entities that mediate ion homeostasis and salt stress signaling are similar in all plants (Hasegawa *et al.*, 2000). In general, the mechanisms of salinity tolerance in plants can be categorized into three: (1) tolerance to osmotic stress, (2) Na^+ exclusion and (3) tissue tolerance (Munns and Tester, 2008).

Since osmotic stress causes immediate reduction in cell expansion in roots and young leaves, tolerance involves the ability of the plant to tolerate the drought aspect of salinity stress and to maintain leaf expansion and stomatal conductance. While the mechanisms involved in this process are not fully understood, it can be demonstrated that the response of the plant to osmotic stress is independent of nutrient levels in the growth medium (Hu *et al.*, 2007). Plants which have high osmotic tolerance will maintain growth rates, particularly over the first few days after exposure to Na^+, whereas those with low leaf senescence and either low or high shoot Na^+ concentrations will be Na^+ excluders or tissue tolerators, respectively. Rapid growth has been observed to have a diluting effect by stimulating rapid water uptake which results in the absorption of water in sufficient quantities to prevent an increase in salt concentration due to passive leakage of salt into the tissues as found in barley, wheat and tomato (Levit, 1980).

Osmotic adjustments may contribute to the maintaining of the water potential gradient between soil water and the transpiring leaves and of cell turgor. It significantly improves soil water uptake under dry conditions and allows maintenance of open stomata with larger apertures and a higher stomatal conductance (Otieno *et al.*, 2005) hence sustains photosynthesis by maintaining leaf water content at reduced water potential. Osmotic adjustment to loss of turgor occurs by either dehydration avoidance or dehydration tolerance (Levitt, 1980) for cells growing in a saline medium. Dehydration tolerance permits the cell to survive turgor loss, but the cell can only remain in the non-growing state until the stress is relieved. Dehydration avoidance allows cell rehydration, regaining turgor and recommencing cell growth (Ziska *et al.*, 1989).

Salt exclusion regulatory systems may occur at either the root surface preventing or restricting salt absorption or within the roots preventing the translocation of the salt to the

shoot, leaves, tender or young parts hence the plant escapes osmotic and ion toxicity of the salt (Mwai, 2001). Plants transport the toxic ions to the older leaves and leaf sheaths which are sacrificed for early senescence and/or death at the cost of saving young growing meristematic tissues. The ability of rice cultivars to compartmentalize ions in older leaves and structural tissues could crucially affect plant survival (Fukuda *et al.*, 2004). Maintaining younger leaves at low salt concentrations probably contributes to the ability of certain varieties to survive saline conditions if they maintain their rates of leaf initiation at least equal to rates of leaf death.

Ionic tolerance may involve tolerance of ionic imbalance and high ion concentrations by protoplasmic organelles (Kingsbury *et al.*, 1983). In this case the membranes and enzymes must possess special properties that permit normal cell functioning even in the presence of high ionic concentrations and ionic imbalance (Levitt, 1980). In saline soils, Ashraf and Harria (2004) suggested that the maintenance of a high tissue K^+/Na^+ ratio by plants may serve as a reliable criterion of salt-tolerance.

Salt tolerance induced nutrient deficiency may be due to a replacement of the deficient nutrient element by the excessively absorbed one. The observations of increased K^+ absorption by some salt resistant plants may also be evidence of salt tolerance due to avoidance of salt induced nutrient deficiency (Levitt, 1980).

Ionic stress on the plant is minimized by the amount of Na^+ that accumulates in the cytosol of cells, particularly in the transpiring leaves. It has been suggested that salinity tolerance in wheat (Munns and James, 2003) and other cereals (Garthwaite *et al.*, 2005) is particularly associated with the ability to exclude Na^+ from the shoot. Research into improving salinity tolerance of wheat cultivars has identified mechanisms for Na^+ exclusion such as the *Knal* locus on chromosome 4D of bread wheat (Dubcovsky *et al.*, 1996) and the *Nax1* and *Nax2* loci in durum wheat (James *et al.*, 2006). Na^+ enters roots passively, via voltage independent non selective cation channels and via other Na^+ transporters (Laurie *et al.*, 2002). Most of the Na^+ that enters root cells in the outer part of the root is likely to be pumped back out again via plasma membrane Na^+/H^+ antiporters (Tester and Davenport, 2003). Although identities of the genes that encode the Na^+ efflux proteins are yet to be found out, recent research has

confirmed the involvement of a plasma membrane protein SOS (salt overly sensitive) 1 in Na^+/H^+ antiporter activity (Apse *et al.*, 1999; Zhang and Blumwald, 2001).

Ion homeostasis in cell is taken care of by the ions pumps like antiporters and carrier proteins on membranes (plasma membrane or tonoplast membrane). SOS regulatory pathway is activated when salt stress is perceived to alter protein activity and gene transcription resulting in a Na^+/H^+ antiporter activity in the plasma membrane (Chinnuswamy and Zhu, 2003) of *Arabidopsis thaliana* (Qui *et al.*, 2002; Quitero *et al.*, 2002; Guo *et al.*, 2004). Since active Na^+ efflux is required in all cells through out the plant, it is likely that other genes coding for Na^+/H^+ antiporters also exist (Munns and Tester, 2008). The Na^+ that enters into the xylem through the transpirational stream may also be retrieved. In *Arabidopsis* root, a gene is involved in the retrieval of Na^+ from xylem before it reaches the shoot (Davenport *et al.*, 2007). Involvement of genes in maintaining higher K^+ / Na^+ ratio is becoming more evident in rice and wheat (Blumwald, 1987; Ren *et al.*, 2005).

In tree plants like olive trees, the salt tolerance was related to their ability to decrease leaf osmotic potential and Na^+/Cl^- ion exclusion mechanisms in the roots (Tattini *et al.*, 1994). Most of the olive cultivars show an exclusion capacity of Na^+ such that accumulation of potentially toxic ions in the aerial parts is prevented.

Once the potentially toxic ions start accumulating in the leaf tissues, due to enhanced absorption and translocation, it has to be tolerated. Na^+ which enters leaf cells, is pumped into vacuoles before it reaches to toxic level for enzymatic activities. This pumping activity is controlled by vacuolar Na^+/H^+ antiporters (Blumwald *et al.*, 2000). Addition of salt induce the Na^+/H^+ antiporter activity but it increases more in salt tolerant than salt sensitive species (Staal *et al.*, 1991). This mechanism has been emphasized by certain experiments where over- expressing of vacuolar transporter has increased the salinity tolerance of *Arabidopsis* (Apse *et al.*, 1999), Tomato (Zhang and Blumwald, 2001), *Brassica napus* (Zhang *et al.*, 2001) and rice (Fukuda *et al.*, 2004). This increase uptake of Na^+ to shoot vacuoles facilitating enhanced storage of Na^+ and ultimately conferring greater tolerance by reducing Na^+ in cytosol. A salt-inducible Na^+/H^+ antiporter gene is involved in the compartmentalization of Na^+ in the vacuoles (Apse *et al.*, 1999). Over expression of this tonoplast Na^+/H^+ antiporter gene has improved salt tolerance in *Arabidopsis* (Apse *et al.*, 1999), tomato and *Brassica napus* (Zhang *et al.*, 2001), and wheat (Xue *et al.*, 2004). The

cytotoxic ions in saline environments, typically Na^+ and Cl^-, are compartmentalized in the vacuole and used as osmotic solutes (Blumwald *et al.*, 2000; Niu *et al.*, 1995). Apart from extruding Na^+ from leaves (Tester and Davenport, 2003; Møller and Tester, 2007; Munns and Tester, 2008), efficient compartmentalizing of Na^+ in the vacuole or in particular cell types where the damage to metabolism is kept to a minimum (Munns and Tester, 2008) occurs. Both processes involve up- and down- regulation of the expression of specific ion channels and transporters, allowing the control of Na^+ transport throughout the plant (Davenport *et al.*, 2007).

Salinity stress has also been shown to induce a switch from C_3 to CAM (Crassulacean Acid Metabolism) in Aizoaceae such as *Mesembryanthemum crystallinum* (Cheffings *et al.*, 1997) due to apparent stimulation of the C_4 enzyme, phosphenol pyruvate carboxylase (PEPcase) by salt ions (Levit, 1980). C_3 plants open their stomata during the day. CAM plants are able to conserve water by opening their stomata at night and closing them during the day when evaporative demand is higher.

Most plants such as sorghum, wheat, rice, bacteria and many other organisms accumulate certain organic solutes during or for osmotic adjustment. Such organic osmolytes mediate osmotic adjustment in the cytosol or organelles. They include simple sugars (mainly fructose and glucose), sugar alcohols such as glycerol and complex sugars (trehalose and fructans), quaternary amino acid derivatives (proline, glycine, betaine, alanine) and sulfonium compounds like choline osulfate in response to osmotic stress, hence they are called osmoprotectants. They are also termed as compatible solutes because even in high concentration they do not interfere with enzymatic activities (Johnson *et al.*, 1968). They are localized in the cytoplasm while the inorganic ions such as Na^+ and Cl^- are preferentially sequestered into vacuole, thus leading to the turgor maintenance for the cell under osmotic stress (Flowers *et al.*, 1977; Bohnert *et al.*, 1995). Vacuolar compartmentalization prevents metabolic poisoning of the cytosol and organelles (Hasegawa *et al.*, 2000). The net uptake of these ions across the plasma membrane is restricted to minimize cytoplasmic toxicity during vacuolar compartmentalization. Though osmoprotectants enable plants to tolerate high salinity, a significant amount of Na^+ needs to be compartmentalized for better tolerance. Therefore, it is desirable that overproduction of osmoprotectants is governed by the pleiotropic control of vacuolar Na^+/H^+ antiporter activity. Trehlose, a non-reducing sugar,

possess a unique feature of reversible water storage capacity to protect biological molecules from desiccation damages. There has been growing interest of utilization of trehlose metabolism to ameliorate the effects of abiotic stresses. Garg *et al.*, 2002) have demonstrated that expression of trehlose biosynthesis conferred tolerance to multiple abiotic stress. The increase in trehlose levels in transgenic rice lines of Pusa basmati-1 using either tissue specific or stress dependent promoter, resulted into higher capacity for photosynthesis and concomitant decrease in photo-oxidative damage during salt drought and low temperature stress. Plants produce many kinds of stress responsive proteins such as dehydrins induced by various kind of stresses like heat, cold, salt or drought. These are reported to play an important protective role during desiccation /salt stress in rice plants (Moons *et al.*, 1995). An adaptive biochemical function of osmoprotectants is the scavenging of reactive oxygen species (ROS) that are by products of hyperosmotic and ionic stresses and cause membrane dysfunction and cell death (Bohnert and Jensen, 1996).

Salt stress in plants induce higher concentration of ROS intermediates such as superoxide, H_2O_2 and hydroxy-radicals due to the impaired election transport processes in chloroplast, mitochondria and photorespiration pathway. Under normal growth conditions, the production of ROS in cells is as low as $240\mu MS^{-1}$ superoxide and the steady state level of H_2O_2 in chloroplast is $0.5\mu M$ (Mittler, 2002; Polle 2001). However, under salinity, the level of ROS reaches as high as $720\mu MS^{-1}$ (3 fold) and H_2O_2 level as high as $15\mu M$ (30 fold). It is reported that H_2O_2 concentration of $10\mu M$ reduces the net photosynthesis rate by 50% (Bohnert and Jensen, 1996). Superoxide and H_2O_2 toxicity have been attributed to the reactions that result into the production of hydroxyl radicals and other destructive species like lipid peroxidases. Indeed hydroxyl radicals are very reactive and can damage vital macromolecules by protein denaturation, mutation and peroxidation of lipids. Plants have developed different systems for scavenging of ROS by using the detoxifying enzymes like catalases, and antioxidants like ascorbate and reduced glutathione.

Transcription factor acts in response to stress by binding to specific sequence of the promoter regions of target genes which needs to be activated collectively or sequentially in response to stress such as drought, salinity or temperature. These promoter regions include dehydration-responsive elements (DRE's) and abscic acid (ABA) responsive elements (ABRE's) which are involved in the plant responses to the osmotic effect. The transcription factor DREB1A

specifically interacts with DRE and induces the expression of stress tolerance genes. Over-expression of source of the genes coding for these protein can induce the constitutive expression of many genes resulting into increased tolerance but it associated with reduced growth even under unstressed condition. DREB1A activated at least 12 genes in *Arabidopsis* (James *et al.*, 2006) but caused dwarfism of the plants. Hence, the stress inducible promoters are preferred to have normal plants showing enhanced stress tolerance (James *et al.*, 2006).

Bambara groundnut is widely considered a drought resistant crop (Collinson *et al.*, 1997). Begemann (1988) proposes two traits that help the crop to adapt to dry environment, a short growing season and a deep root system. It is suggested that drought tolerance of Bambara groundnut is a result of osmotic adjustment, reduction of leaf area index and low water loss through the stomata (Collinson *et al.*, 1997). Nyamudeza (1989) reported a high root to total dry matter ratio in Bambara compared to other crops, while Shamudzarira (1996) found a high water use efficiency, both characteristics confer drought tolerance. Research has shown that the plant is capable of producing good yields under water deficit where other legumes such as groundnuts failed completely (Collinson *et al.*, 1997). The vegetative growth of the crops under salinity may be severely restricted resulting into reduced total dry matter and smaller leaf areas as reported in many crops including legumes like *Arachis hypogaea* (Collino *et al.*, 2001). The reduced leaf area of plants may limit light interception hence lowered net photosynthesis (Singh, 1991).

Developing salt tolerant crops is not an easy task because salt tolerance is a polygenic trait. Therefore, integration of knowledge on morphological, physiological, biochemical and genetic aspects of salt tolerance is essential to make any progress in this regard (Ashraf and Foolad, 2007). Breeding for salt tolerance can be successful if sufficient variations exist between the varieties. Research to overcome salt related problems is based on two approaches: Changing the growing environment (making it normal) suitable for the normal growth of plants. It involves major engineering and soil amelioration processes which need enormous resources, that are often out of the reach of small and marginal farmers.

The second approach involves selection of the crop and/or modification of plant genotype so that it could be grown in saline areas. In this case, breeding crop varieties with in-built salt tolerance is done. It is the most promising, less resource consuming /economical and socially

acceptable approach. Selection of crops that are tolerant to salinity should focus on physiological processes, which directly influence dry matter production such as photosynthesis. The crop plants must be capable of satisfactory biomass production in a saline environment (yield stability). There is usually a keen interest on the photosynthetic efficiency, photosynthate translocation and photosynthetic pigment composition. Research should as much as possible expose the groups of plants being screened to uniform salt stress. Therefore, most screening experiments are performed under solution culture or sand culture in controlled conditions (Netondo, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiments were carried out in the laboratory and in the green house at Maseno University Botanic garden in Nyanza province, Kenya, starting from October, 2009 to January, 2010. Maseno is approximately 1500m above the sea level and receives an annual mean precipitation of 1750 mm with bimodal pattern of distribution. The mean temperature is 28.7 ^{0}C with a relative humidity of 40% (Mwai, 2001). The soils are classified as acrisol, deep reddish brown friable clay with pH ranging from 4.5 to 5.5, soil organic carbon and phosphorus contents are 1.8 % and 4.5mg/ kg, respectively (Netondo, 1999). Maseno area has no salinity problem, the soils are well drained, deep and have high extractable Ca and K. The minimum and maximum temperatures inside the green house were 26 ± 6^o C and 35 ± 6^{0}C, respectively and a relative humidity of 38 ± 5%.

3.2 Laboratory experiment

3.2.1 Germination test

Large, similar sized seeds of two local Bambara groundnut landraces obtained from researchers at Maseno University, Botany Department harvested in the long rain season (February- May) of 2009 were sterilized for five minutes in 10% sodium hypochlorite and then rinsed five (5) times with distilled water. Kakamega 2 (Kk) and Mumias 2 (Mm) seeds were used (names based on the locality where they were initially collected from). Ongoing research shows that they are drought tolerant, they give reasonable yields on relatively poor soil and are resistant to pests. To determine whether seed germination could be affected by various concentrations of NaCl, the seeds were germinated in sterile 9 cm diameter plastic petri dishes lined with whatman No.1 filter papers. The petri dishes were arranged in a completely randomized design (CRD) consisting of 5 treatments and 3 replica. The petri dishes were then moistened with 10ml of the respective treatment solutions. Treatments consisted of 5 levels of NaCl concentrations of 0 mol/litre, 0.07 mol/l, 0.13mol/l, 0.20 mol/l and 0.26 mol/l which exerted soil EC of : 0 mm ho cm^{-1} (control), 6.96 mm ho cm^{-1}, 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} in the rooting media respectively (Personal communication, Harun Ogindo, 2011). The above concentrations in mm ho cm^{-1}

were derived as follows: 1 mole. of NaCl is 58.5g and 1 g/l is equivalent to 1.7 m mho/cm. Therefore, 0 mole NaCl gives 0 g, 0.07mol gives 4.095g, 0.13mole gives 7.605g, 0.20mole gives 11.7g while 0.26 mole gives 15.21g that correspond to EC: 0 mm ho cm^{-1}, 6.96 mm ho cm^{-1}, 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} respectively.

Each petri dish containing 30 seeds was then covered to minimize microbial contamination and water loss. The filter paper linings were constantly moistened until the seeds germinated. Observations were made by counting the number of seeds germinated daily for 14 days prior to the green house experiments.

3.3 Green house experiments

These involved two sets of experiments set up in the green house as described below in the month of November, 2009. The two experiments had similar design and set up except that experiment one involved destructive measurements (morphological and chlorophyll content parameters) while experiment two involved non-destructive measurements (physiological and nitrogen content parameters).

3.3.1 Plant material and culture technique

During the first experiment, the soil collected from the botanic garden was filled in 20 litre PVC pots (20 Kg soil per pot) after being solarized (sun sterilized) for 3 days mainly to prevent fungal growth. The pots had perforated bottoms to facilitate drainage. Large, similar sized seeds of the two local Bambara groundnut landraces harvested in the long rain season of 2009 were treated as in **3.2.1** above before planting. Ten seeds were planted in each of the 30 pots at 2 to 3 cm depth and at the recommended spacing of 10-15 cm. Each pot was irrigated daily with sufficient tap water to ensure successful germination and establishment of the crop in readiness for treatment. Germination commenced on the sixth day after sowing. Thinning was done to leave 6 uniformly spaced plants per pot.

3.3.2 Treatments and experimental design

Treatments were applied as from 20 days after sowing (DAS). Five treatments involving various NaCl salt concentrations were applied as follows: A control experiment (T1) in which the pots were irrigated with sufficient tap water (salt content assumed negligible) only up to the end of the experiment. The other salinity treatments comprised irrigating the pots with NaCl concentrations of EC : 6.96 mm ho cm^{-1}, 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1}

and 25.86 mm ho cm^{-1}. The salt solutions (T2, T3, T4 and T5) were made by dissolving commercial table salt in tap water. The salt is reported to contain various salt ions: Na= 36%, K= 1.1%, Ca= 1.2%, Cl= 55% among other ions (Onkware, 1986). All the pots except (T1) were first irrigated with 6.96 mm ho cm^{-1} salt solution on the first day of treatment application. To reduce osmotic shock, saline treatment was imposed incrementally, increasing the concentration by 6.96 mm ho cm^{-1} every second day until the final concentration of 25.86 mm ho cm^{-1} was reached. Irrigating the pots after every three days with a litre of their respective solutions was assumed to eliminate any matrix effects that may have arisen due to soil drying.

Milraz WP 76 fungicide (3.3-5) g/litre of water was sprayed after every 2 weeks from 0 day after treatment to control pests and diseases. The experiment was laid out as a completely randomized design (CRD) consisting of two landraces, 5 treatments and 3 replicates

3.4 Measurement of Parameters

Both experiments were sampled on the whole plant basis. On the day of measurement a single plant per pot was selected randomly and used.

3.4.1 Plant height: This was measured using meter rule from stem base/soil surface to shoot apex/tallest leaf tip after every 7 days from 0 days after treatment (DAT) to end of the experiment.

3.4.2 Leaf number: This was determined by counting fully expanded leaflets after every 7 days from 0 DAT to the end of the experiment.

3.4.3 Leaf area

Plant leaf area (A_{plant}) was measured after every 7 days from 0 DAT to end of experiment, using Cornelissen *et al.*, (2005) formula. The length and width of the middle-leaflet were measured and leaf area was calculated using formula,

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

Where: (A_{plant}) -Plant leaf area , L - Length of the middle-leaflet (cm), W- Width of the middle- leaflet (cm), $\pi = 3.1416$ and N_1 - total number of leaves.

3.4.4 Root Length

One plant per pot was carefully scooped with all its roots intact using a trowel and hand washed over a fine sieve with tap water after every 7 days from 0 DAT to the end of experiment. Root length was measured using a meter rule from the base of the stem to the furthest root tip.

3.4.5 Plant root and shoot biomass

One plant per pot was carefully scooped with all its roots intact using a trowel and hand washed over a fine sieve with tap water collecting all roots one at a time after every 7 days from 0 DAT to end of experiment. Each plant was then separated into shoot and root. Their

fresh weights were determined after which they were oven dried at 72^{0}C for 48 hours and their dry weight determined using an electronic weighing balance (Denver instrument, Model XL – 31000).

3.4.6 Root: Shoot biomass percentage.

The procedure in **3. 4. 5** was repeated and root to shoot biomass ratio was then computed as percentage.

$$Root: Shoot\ biomass\ percentage = \frac{\text{Root dry weight}}{Shoot\ dry\ weight} \times 100$$

3.4.7 Percentage water content (% WC)

The plants were treated as in **3. 4. 5** and (% WC) per plant was calculated as:

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

3.4.8 Gas exchange parameters

Measurement of gas exchange parameters involved a plant per replicate chosen at random. Three readings were taken from two leaflets per plant. Net photosynthesis was determined by use of a portable infrared gas analyser (IRGA) (Model, CIRAS 1-pp system, USA). These measurements were determined from an area of 2.5 cm^2 of two leaflets per plant on the first fully opened and exposed leaf of the main axis after every 2 weeks from 0 DAT to end of the experiment in brightly lit greenhouse just before irrigation.

Measurements were done in the morning (10.00 – 12.00) hours to avoid high afternoon temperatures after warming of the equipment for 10 minutes to achieve steady- state conditions of gas exchange.

3.4.9 Chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured using a portable fluorescent monitoring system (Hansatech model FMS 2 Hansatech instruments, Germany). The measurements were done on the first fully opened and exposed leaf of the main axis after every 2 weeks from 0 DAT to end of the experiment. Measurements were carried out on one a plant chosen randomly from all replicates. The leaves to be used for the measurements were dark adapted for 15

minutes using the dark adaptation clips and then illuminated for 6 seconds to induce fluorescence. The initial fluorescence (F_O) and the maximum fluorescence (F_M) were measured and the variable fluorescence (Fv = Fm-Fo) and the Fv/ Fm ratio were calculated. The potential minimum efficiency of PSII (Fv/Fm) of dark adapted leaves was calculated as Fv /Fm = (Fm-Fo)/ Fm.

Plate 1: Demonstration of chlorophyll fluorescence measurements using a portable fluorescent monitoring system on five week old Bambara groundnuts.

3.4.10 Chlorophyll content determination

Chlorophyll content was determined using the methods of Arnon (1949) as described by Netondo (1999). The fourth youngest fully expanded leaflet was randomly sampled from all the replicates at every 2 weeks interval from 0 DAT to the end of experiment. In the laboratory 0.5g of the fresh leaf tissue was cut into small pieces and placed into a specimen bottle. Ten milliliters of 80% acetone was added and the set up kept in the dark for 7 days for chlorophyll to be extracted by the acetone. One millilitre of the filtered extract was diluted

with 20 ml of 80% acetone and the absorbance of chlorophyll solution measured using a spectrophotometer (Model, Novaspec 11) at 645nm and 663nm to determine the content of chlorophyll a and b respectively. The respective chlorophyll concentration in milligrams (mg) of chlorophyll per gram (g) of leaf tissue were calculated using the formula of Arnon (1949) as follows,

$$Chl\ a\ (mg/g) = 12.7\ (D_{663}) - 2.69\ (D_{645}) \times (V/1000) \times W$$

$$Chl\ b\ (mg/g) = 22.9\ (D_{645}) - 4.68\ (D_{663}) \times (V/1000) \times W$$

$$total\ Chl\ (mg/g) = 20.2\ (D_{645}) + 8.02\ (D_{663}) \times V/(1000 \times W)$$

Where,

D = absorbance measured at wavelengths 645nm and 663nm.

V =Volume in ml of the acetone extract used

W = Fresh weight (g) of leaf tissue from which the extract was made.

3.4.11 Nitrogen content determination

Leaf nitrogen content percentage was directly estimated using the soil plant analysis device (model SPAD-502, Minolta Campany, Germany). Measurements involved 3 plants per replicate chosen randomly. Measurements were done on healthy uppermost fully expanded leaflets and midway between base and tip and midway between midrib and margin after every 2 weeks interval from 0 DAT to end of the experiment.

3.5 Statistical Data Analysis

Analysis of variance (ANOVA) was carried out on the data using Costat statistical computer package (Costat Version 6.204 (Cohort Software), CA,USA) to determine whether there were any significant effects of the NaCl treatments on plant morphological, physiological and biochemical parameters measured. Means were separated using the least significant difference (LSD) test at the 5% level. Correlation analysis was done to determine the relationship between variables and whether treatment effects were significant or non-significant at 5 % level.

CHAPTER FOUR

4.0 RESULTS:

4.1 Percentage seed germination

The results of percentage seed germination are shown in Figs.1a and 1b. In the control experiments percentage seed germination increased rapidly and reached over 70 % by 8 DAT for both Kk and Mm landraces. The salt treatments significantly ($p<0.01$) reduced germination as compared to the control in both landraces. Both Bambara groundnut landraces showed similar response to NaCl salinity during this study. However 6.96 mm ho cm^{-1} salinity affected Mm landrace more than Kk between 6DAT and 14DAT. Seed germination delayed with increasing level of applied NaCl salinity, the lowest (6.96 mm ho cm^{-1}) salinity by one day, 12.93 mm ho cm^{-1} by 9DAT, 19.89 mm ho cm^{-1} by 13 DAT and it was completely inhibited in the highest (25.86 mm ho cm^{-1}) NaCl treatment since all the seeds failed to germinate. Differences in seed germination between Kk and Mm landraces became clearer with time of salt application as the Kk landrace showed greater response at 6.96 mm ho cm^{-1}. However, Mm landrace showed greater response at 12.93 mm ho cm^{-1} (higher salinity).

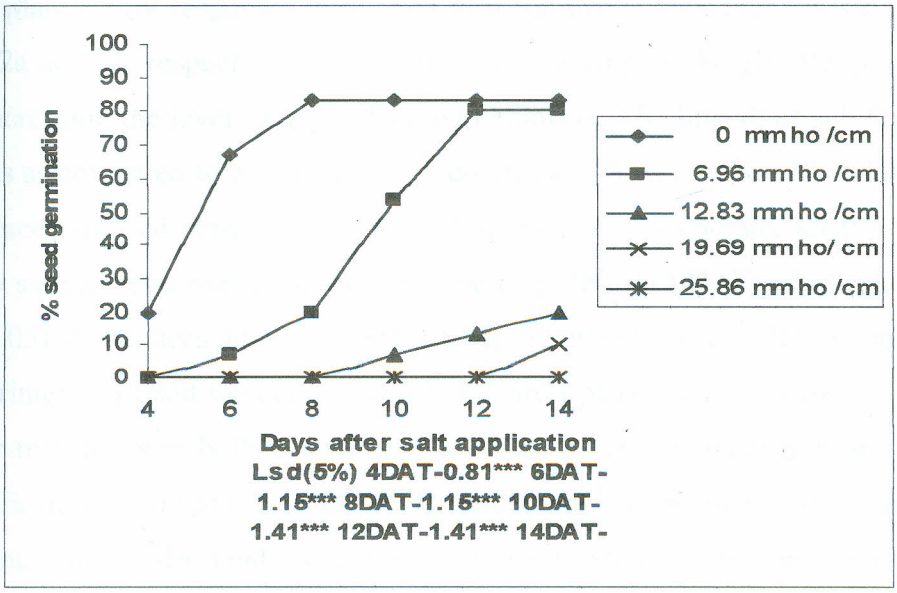

Fig.1a: The effect of sodium chloride salinity on % seed germination in Kakamega Bambara groundnut landrace.

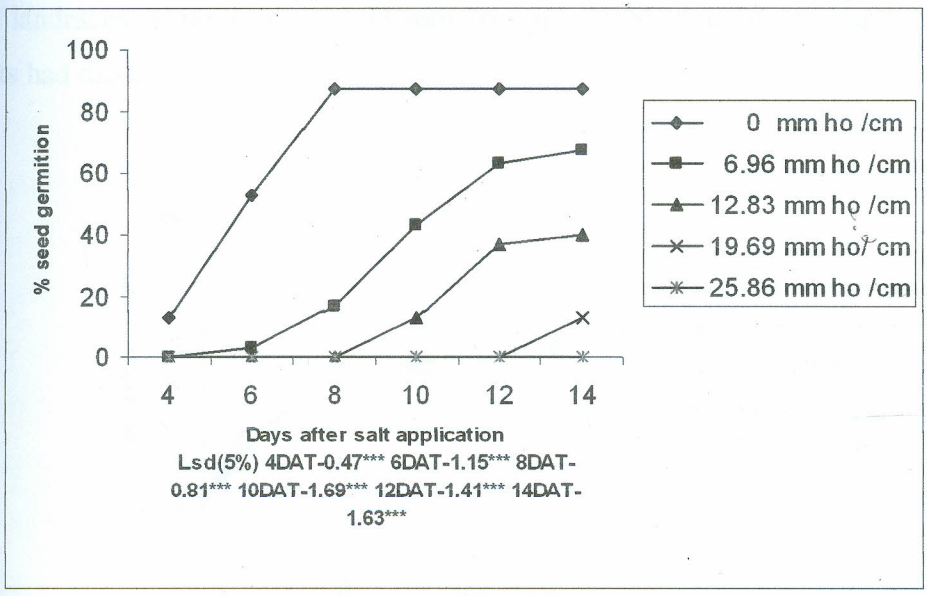

Fig.1b: The effect of sodium chloride salinity on % seed germination in Mumias Bambara groundnut landrace.

4.2 Morphological parameters

4.2.1 Plant height

The plant height responses in Kk and Mm Bambara groundnut landraces are represented in Fig. 2a and 2b, respectively. Generally, increase in plant height was progressively inhibited by increasing the level of applied sodium chloride. The impact of salinity was greater in Kk plants as compared to Mm plants. The control and 6.96 mm ho cm^{-1} treatment plants in both landraces showed similar response during this study. Generally all the Mm plants seem to show similar responses at any given time (Fig 2b). At 0DAT, plants showed no significant ($p>0.05$) differences in height for all the treatments. From 7DAT up to the end of the experimental period the control (0 mm ho cm^{-1}) plants were consistently taller than those of other treatments in both landraces. The plants given treatment 6.96 mm ho cm^{-1} were also significantly ($p<0.05$) taller than 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants. In the Mm landrace the most stressed 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants were similar in height unlike in the Kk landrace where the most stressed plants had the least height significantly ($p<0.01$) different from the rest. From 35DAT to 42 DAT, the control and 6.96 mm ho cm^{-1} plants showed non significant ($P>0.05$) differences in height of

both landraces while all the 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants had died.

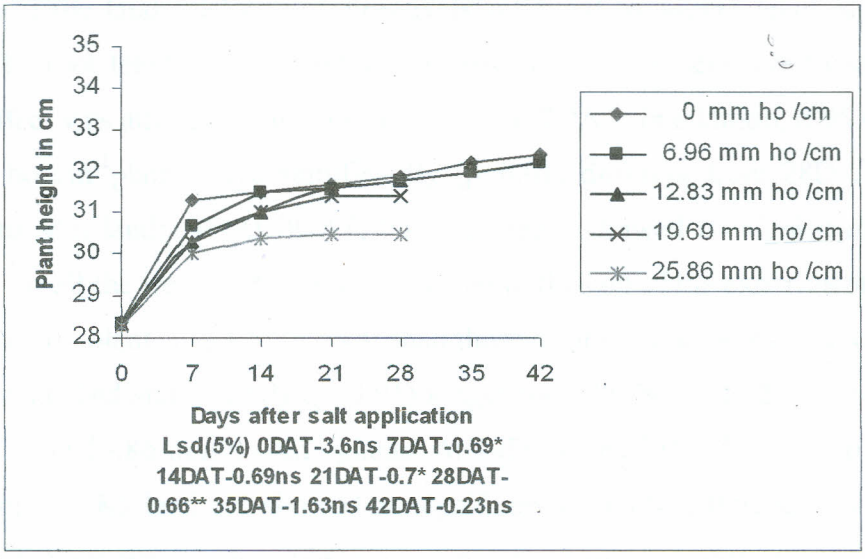

Fig 2a: The effect of NaCl salinity on plant height in Kakamega Bambara groundnut landrace.

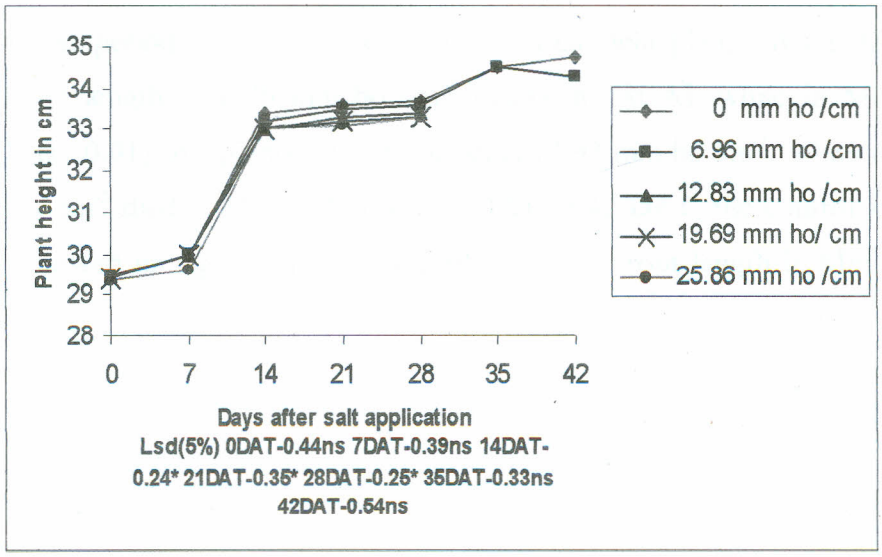

Fig 2b: The effect of NaCl salinity on plant height in Mumias Bambara groundnut landrace.

4.2.2 Root Length

Generally there were significant ($p<0.01$) differences in the root length between the two Bambara groundnut landraces during the experimental period (Fig. 3a and 3b). Increase in root length of the landraces was progressively inhibited by higher level of applied sodium chloride. The root length in Kk landrace seemed to be more sensitive to salinity than Mm since the effect was already significant ($p<0.001$) at 7DAT. In addition, 6.96 mm ho cm^{-1} and 12.93 mm ho cm^{-1}plants were significantly ($p<0.01$) different from 28DAT to 35DAT as compared to Mm landrace. At 0DAT, both landraces showed no significant differences in root length for all the treatments. From 7DAT up to the end of the experiment the control and 6.96 mm ho cm^{-1} plants had longer roots than those of other treatments. The 6.96 mm ho cm^{-1} treatment plants had significantly ($p<0.01$) longer roots to those of 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants. At 14DAT and 21DAT the control and 6.96 mm ho cm^{-1} plants in Kk landrace showed no significant ($p>0.05$) differences in length unlike in Mm. Root damage was evident physically from the hardened root specimens especially in treatments 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1}. Deflocculation (structural deterioration) of the soil may have occurred, hence poor drainage of irrigated water in pots was observed, especially in treatments 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} at the end of experimental period. The 12.93 mm ho cm^{-1} treatment plants in the Kk landrace had equivalent root length to 6.96 mm ho cm^{-1} plants at 28DAT while in Mm they differed significantly ($p<0.01$). All plants given treatments 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} died by 28 DAT. From 35DAT to 42 DAT, the control and 6.96 mm ho cm^{-1} plants showed no significant $p>0.05$) differences in root length in Mm landrace unlike Kk landrace.

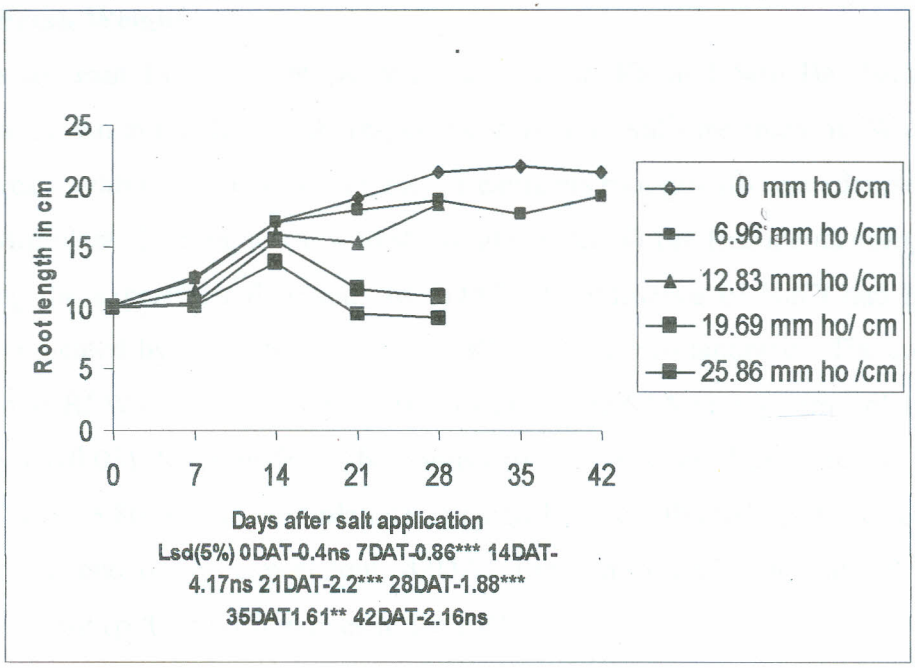

Fig 3a: The effect of NaCl salinity on root length in Kakamega Bambara groundnut landrace.

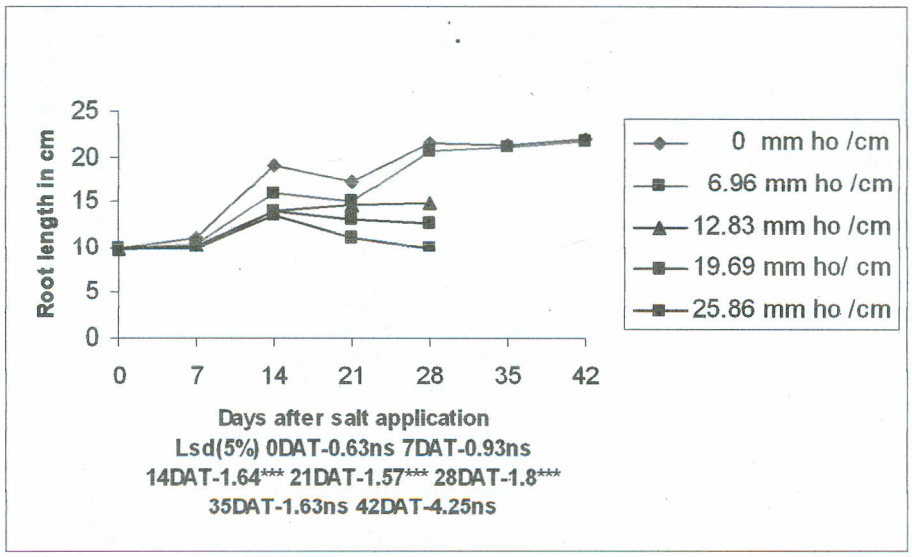

Fig 3b: The effect of NaCl salinity on root length in Mumias Bambara groundnut landrace.

4.2.3 Root Fresh Weight

The results for root fresh weight (RFW) responses in Kk and Mm Bambara groundnut landraces are shown in Fig 4a and 4b, respectively. At low NaCl treatment RFW continued to steadily increase with time but at higher NaCl treatments root growth was adversely affected. Generally Mm plants had higher root fresh weight under all the treatments compared to Kk plants during the experimental period. At 7DAT, the influence of NaCl had begun to be effective as indicated by the differences in the RFW of the two landraces. The control plants had the highest RFW compared to other treatments while 6.96 mm ho cm^{-1} plants were not significantly ($p<0.05$) different from the control plants however, 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants were significantly affected ($p<0.01$) and they died by 28 DAT. At end of the experiment, 42DAT the control and 6.96 mm ho cm^{-1} plants showed significant ($p<0.05$) differences in their RFW.

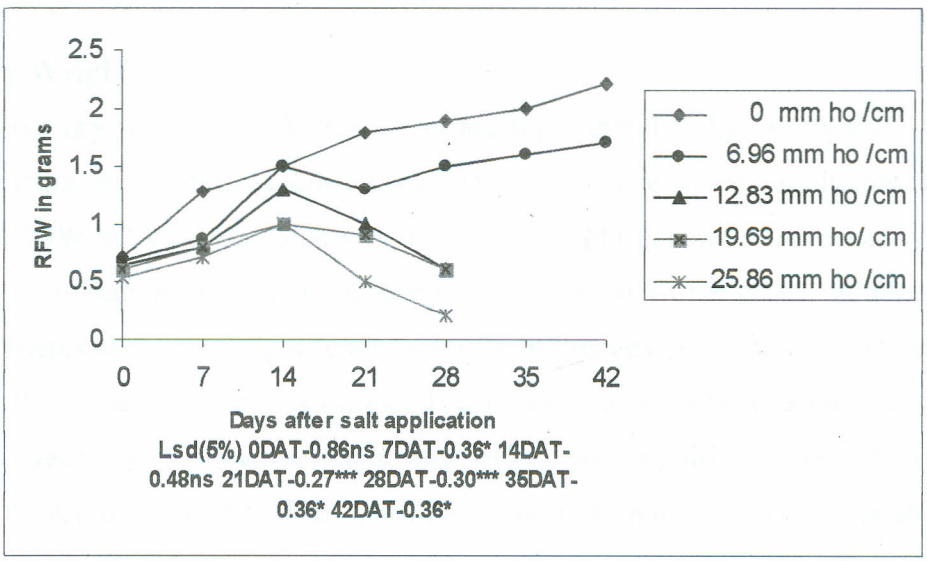

Fig 4a: The effect of NaCl salinity on root fresh wt in Kakamega Bambara groundnut landrace.

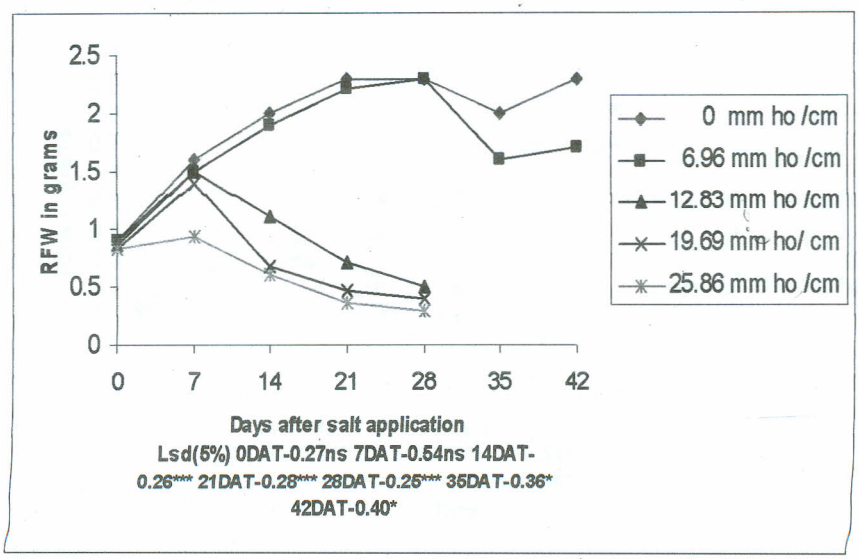

Fig 4b: The effect of NaCl salinity on root fresh wt in Mumias Bambara groundnut landrace.

4.2.4 Root Dry Weight

The data for root dry weight (RDW) responses are represented in figures 5a and 5b for Kk and Mm Bambara groundnut landraces, respectively. There were significant ($p<0.05$) differences in RDW between the landraces. Root dry weight generally increased with time, but the influence of salinity was more apparent with time. In all the cases, salinity reduced RDW. All the plants showed no significant ($p>0.05$) differences in RDW in both Mm and Kk landraces at 7DAT and 14DAT. Continued exposure to salinity caused the observed differences between the treatments. Treatment effect was significant ($p<0.001$) in both landraces at 21DAT and 28DAT. The control and the 6.96 mm ho cm^{-1} plants showed no significant ($p>0.05$) RDW differences in Mm landrace from 35DAT to 42DAT.

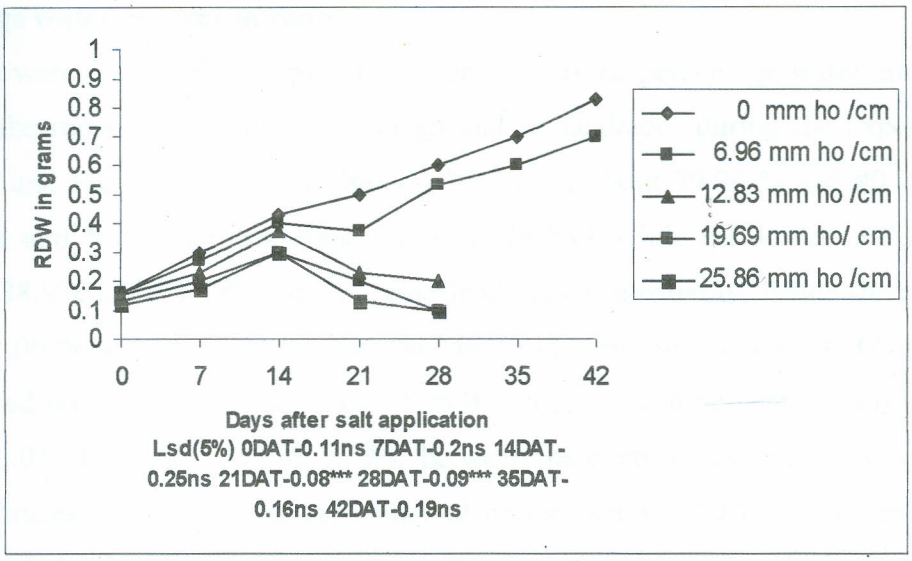

Fig 5a: The effect of NaCl salinity on root dry wt in Kakamega Bambara groundnut landrace.

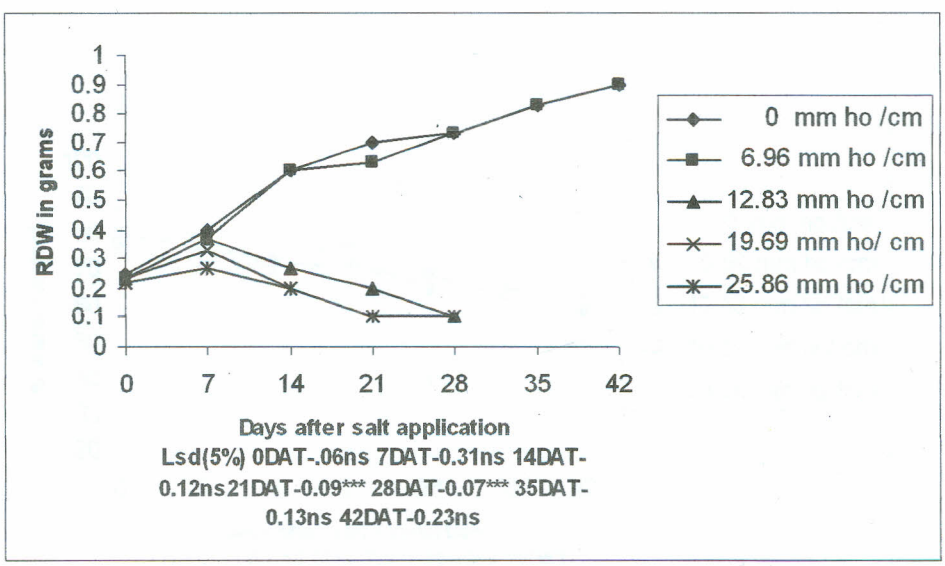

Fig 5b: The effect of NaCl salinity on root dry wt in Mumias Bambara groundnut landrace.

4.2.5 Percentage water content in roots

Generally there were no significant ($p>0.05$) differences in the percentage water content (% WC) between the roots of the two Bambara groundnut landraces during the experimental period (Fig. 5c and Fig. 5d). The mean highest % WC of about 79.96 % and 80.23% was recorded in Kk and Mm landraces respectively at 28DAT while the lowest values were approximately 38.9% and 93.89% for the same landraces respectively, hence there was % reduction of 41.06 % and 16.34 % for Kk and Mm landraces respectively. At 0DAT, both landraces showed no significant differences in %WC for all the treatments. There were no significant ($p>0.05$) differences in root % WC between the controls and the other treatments in the two landraces throughout the experimental period. From 28DAT to the end of the experimental period, 42DAT, the control and 6.96 mm ho cm^{-1} plants in both Kk and Mm landraces showed no significant ($p>0.05$) differences, however all 12.93 mm ho cm^{-1},19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants died by 28 DAT.

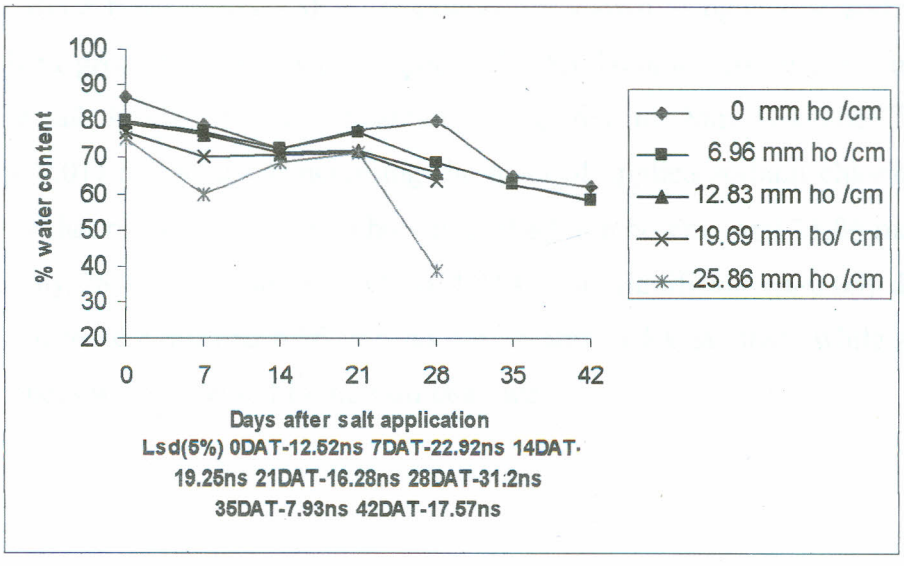

Fig 5c: The effect of NaCl salinity on % water content in roots of Kakamega Bambara groundnut landrace.

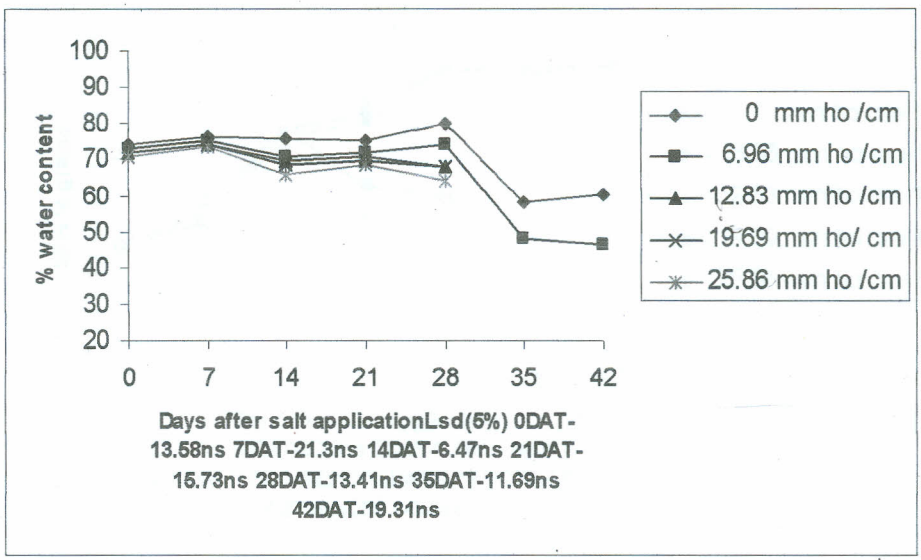

Fig 5d : The effect of NaCl salinity on % water content in roots of Mumias Bambara groundnut landrace.

4.2.6 Shoot Fresh Weight

The results for shoot fresh weight (SFW) responses are shown in figures 6a and 6b for Kk and Mm Bambara groundnut landraces, respectively. Kk landrace consistently maintained higher SFW at all levels of NaCl treatment compared to Mm landrace. SFW was significantly ($p<0.01$) inhibited by increasing the level of applied sodium chloride in both landraces. Plants that received 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} treatments died by 28 DAT. From 35DAT to 42DAT, no significant ($p>0.05$) differences occurred between the control and 6.96 mm ho cm^{-1} plants in Kk landrace while significant ($p<0.01$) differences were observed in the Mm landrace.

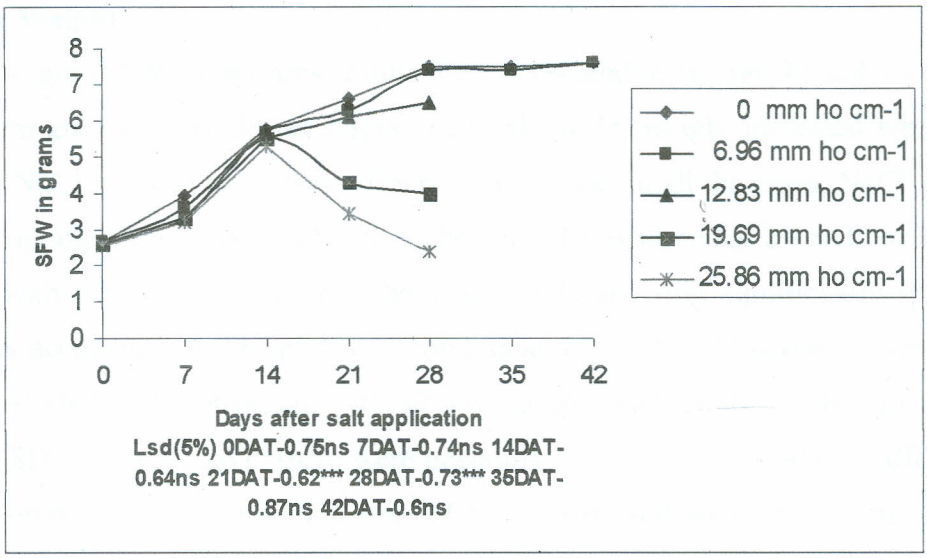

Fig 6a: The effect of NaCl salinity on shoot fresh wt in Kakamega Bambara groundnut landrace.

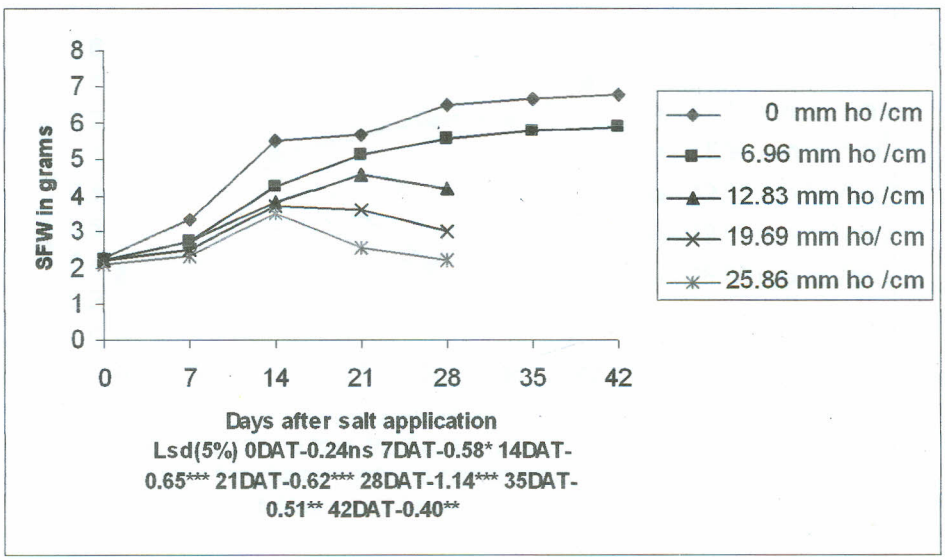

Fig 6b: The effect of NaCl salinity on shoot fresh wt in Mumias Bambara groundnut landrace.

4.2.7 Shoot Dry Weight

The shoot dry weight (SDW) responses results are represented in figures 7a and 7b for Kk and Mm Bambara groundnut landraces respectively. Shoot dry weight increased with time, but the effect of NaCl treatment was more observed with time. In all the cases, NaCl salinity reduced SDW. Significant ($p<0.001$) differences begun to be exhibited at 21DAT for Kk and Mm landraces with control and 6.96 mm ho cm^{-1} plants showing significantly ($p<0.01$) higher dry matter accumulation. The plants growing under higher NaCl treatments were more affected such that all 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants were killed by 28DAT. By 42DAT, there were generally no significant ($p>0.05$) differences in SDW of the control and the 6.96 mm ho cm^{-1} plants of both landraces. The effects of NaCl treatment on shoot dry matter of Kk and Mm landraces, were significant ($p<0.01$).

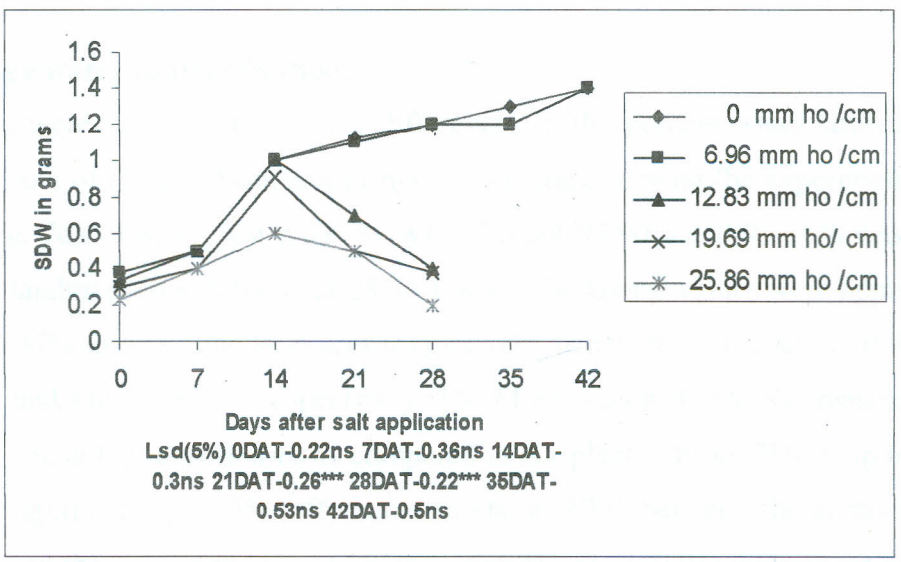

Fig 7a: The effect of NaCl salinity on shoot dry wt in Kakamega Bambara groundnut landrace.

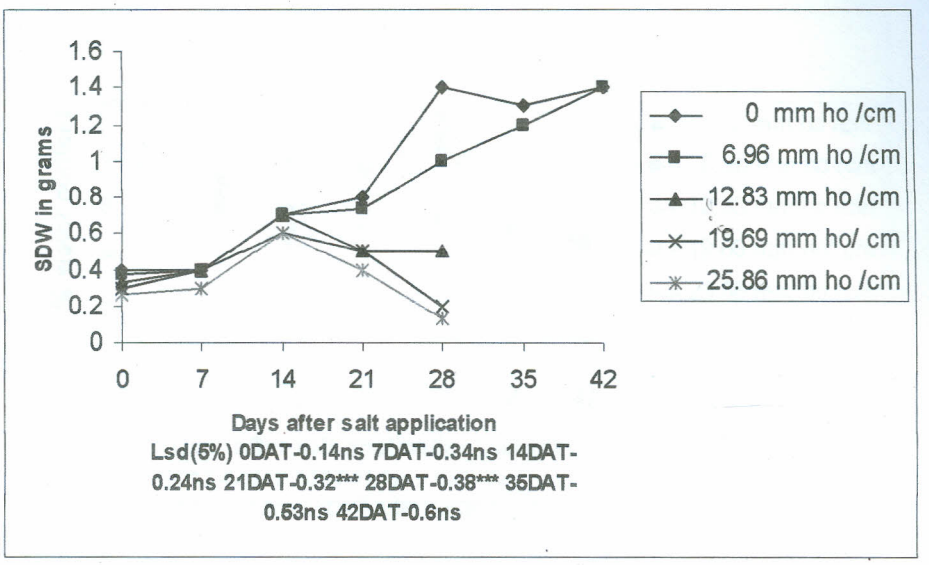

Fig 7b: The effect of NaCl salinity on shoot dry wt in Mumias Bambara groundnut landrace.

4.2.8 Percentage water content in shoots

Generally there were significant ($p<0.05$) differences in the percent water content (%WC) between the shoots of the two Bambara groundnut landraces during the experimental period (Fig. 7c and Fig. 7d). The highest mean % WC of about 93.90% and 95.16% was recorded in Kk and Mm landraces, respectively at 28DAT while the lowest values were approximately 83.79% and 78.47% for the same landraces respectively, hence the % reduction of 9.11% and 16.69% for Kk and Mm landraces respectively. The Mm plants had values consistently closer to the control even at higher salinities as compared to Kk plants. From 7DAT up to 28DAT, there were no significant ($p>0.05$) differences in shoot RWC between the controls and the other treatments in the two landraces. At 28DAT, significant ($p<0.01$) differences occurred in both landraces. From 28DAT to the end of the experimental period, 42DAT, the 6.96 mm ho cm^{-1} salinity treatment plants were not significantly ($p>0.05$) different from the control plants in both landraces, however 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants died by 28DAT.

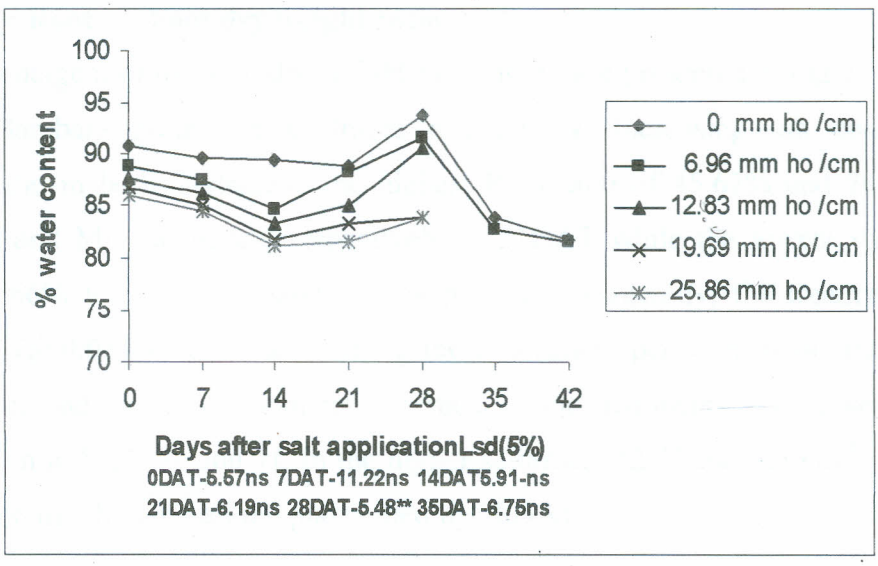

Fig 7c: The effect of NaCl salinity on % water content in shoots of Kakamega Bambara groundnut landrace.

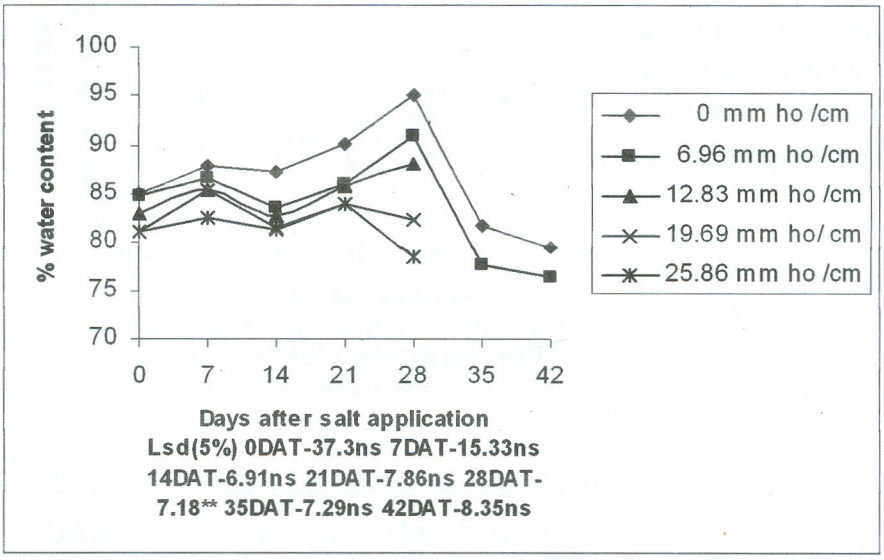

Fig 7d : The effect of NaCl salinity on % water content in shoots of Mumias Bambara groundnut landrace.

4.2.9 Percentage Root to shoot dry weight ratio

Results for percentage root to shoot dry weight ratio (R: S) are presented in Fig.7e and 7f for Kk and Mm Bambara groundnut landraces respectively. Increasing salt concentration decreased the ratio in both landraces. The highest R: S ratio of 45.67% and 74.67% was recorded in Kk and Mm landraces, respectively at 21DAT while the lowest values were 26.33% and 20.66% for the same landraces respectively. Generally, the treatment effects were significant ($p<0.05$) for the plants during the experiment period in both landraces. Kk Bambara landrace had low root: shoot ratio in most of the treatments as compared to Mm landrace as shown in Figs. 7e and 7f. In the higher salinities, 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} all the plants died by 28DAT.

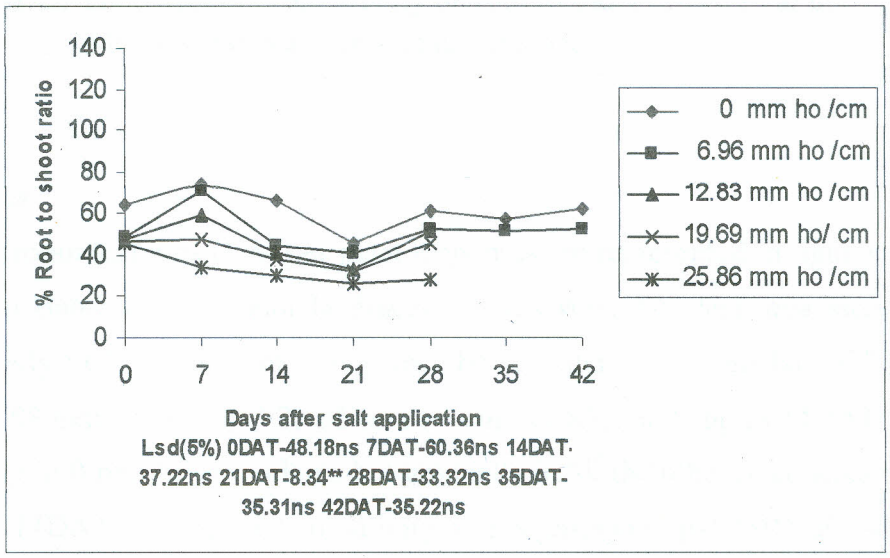

Fig 7e: The effect of NaCl salinity on root to shoot dry wt ratio in Kakamega Bambara groundnut landrace.

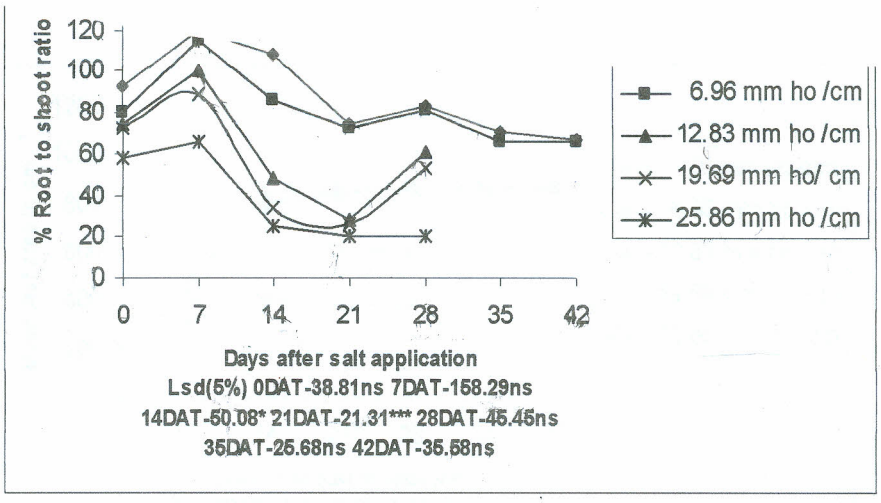

Fig 7f: The effect of NaCl salinity on root to shoot dry wt ratio in Mumias Bambara groundnut landrace.

4.2.10 Leaf Area

The results for mean leaf area (LA) per plant responses are represented in figures 8a and 8b for Kk and Mm Bambara groundnut landraces, respectively. The leaf area increased with time, more rapidly in 0 mm ho cm^{-1}, 6.96 mm ho $cm^{-1}$and 12.93 mm ho cm^{-1} plants but gradually in 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} of Kk plants up to 14DAT. Leaf area increased rapidly in 0 mm ho cm^{-1} plants but gradually in all the other treatments in the Mm landrace up to 14DAT. The impact of salinity was significant ($p<0.001$) at 14DAT. The highest LA of 850 cm^2 and 972cm^2 was recorded in Kk and Mm landraces, respectively at 14DAT while the lowest values were 400 cm^2 and 442 cm^2 for the same landraces, respectively. From14DAT increasing salt concentration progressively decreased leaf area in both landraces. The plants in the control experiment in Mm landrace recorded the highest leaf area compared to all other treatments in the two landraces. All 6.96 mm ho cm^{-1} plants in the Mm landrace showed no significant ($p>0.05$) difference in leaf area to control and 6.96 mm ho cm^{-1} plants in the Kk landrace. There was more salinity effect on 12.93 mm ho cm^{-1} plants in Mm landrace as compared to Kk landrace. Plants in 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} treatments had the least leaf area in both landraces that were not different. By

28DAT all plants given treatment 12.93 mm ho cm^{-1},19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} died.

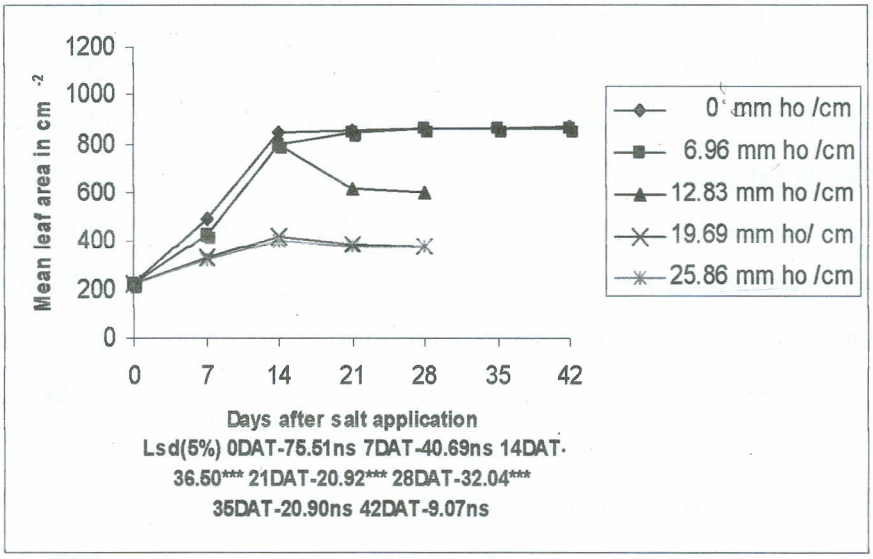

Fig 8a: The effect of NaCl salinity on leaf area in Kakamega Bambara groundnut landrace.

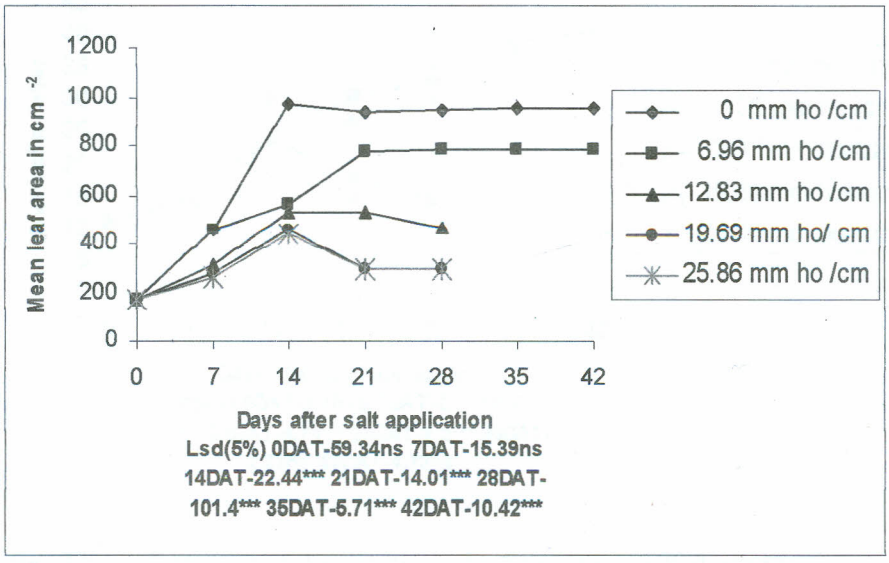

Fig 8b: The effect of NaCl salinity on leaf area in Kakamega Bambara groundnut landrace.

4.2.11 Leaf number

The data for mean plant leaf number responses are presented in figures 9a and 9b for Kk and Mm Bambara groundnut landraces, respectively. Leaf number was significantly ($p<0.01$) reduced by increasing the level of sodium chloride in both landraces. The leaf number gradually increased with time, in all treatments in both landraces up to 14DAT. Premature senescence of older leaves, and extensive leaf destruction in form of marginal burns, necrotic patches and leaf death were observed especially in treatments 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} after 28DAT hence significantly ($p<0.01$) slow leaf production. Throughout the experiment period, the control plants had the highest number of leaves while the most stressed plants had the lowest. The 12.93 mm ho cm^{-1} treatment plants within the Kk landrace maintained significantly ($p<0.01$) higher leaf number compared to 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants up to 28DAT. All the plants in treatments 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} died by 28DAT.

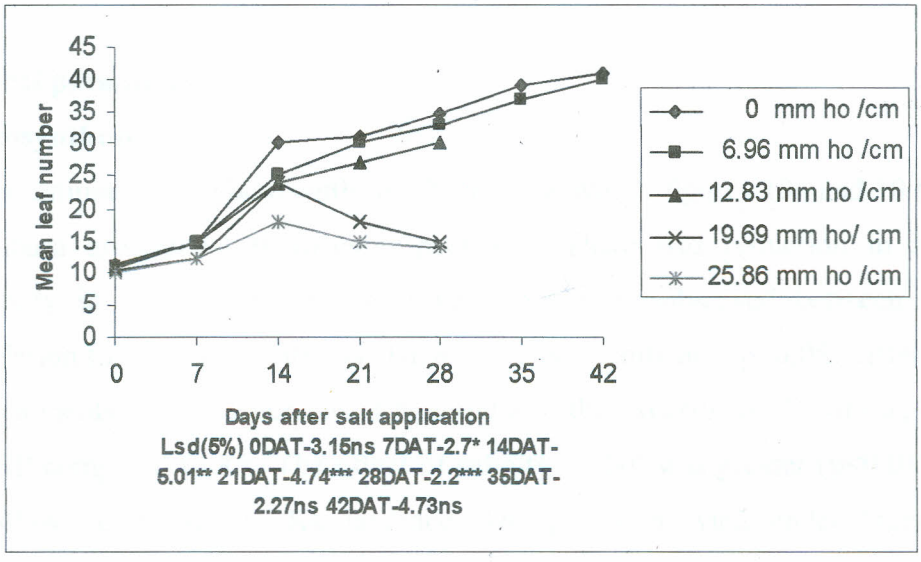

Fig 9a: The effect of NaCl salinity on leaf number in Kakamega Bambara groundnut landrace.

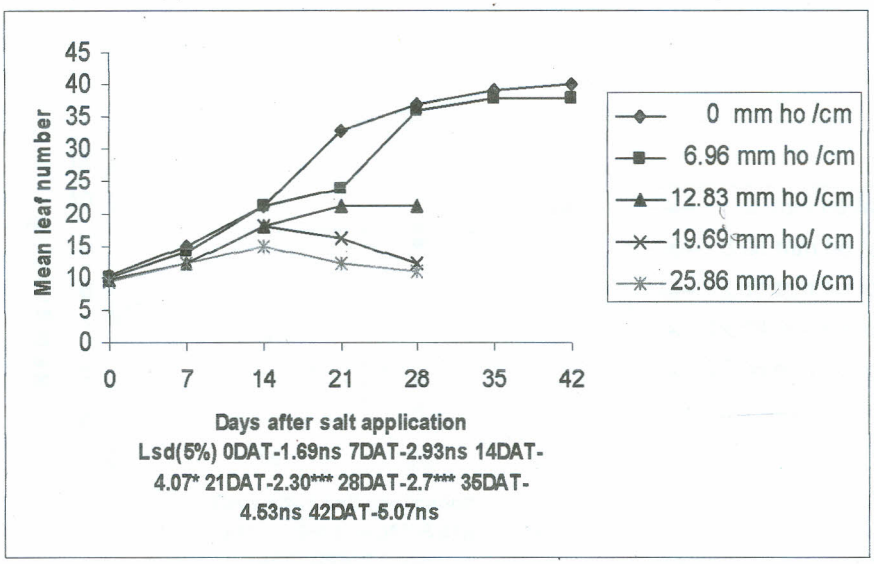

Fig 9b: The effect of NaCl salinity on leaf number in Mumias Bambara groundnut landrace.

4.3 Physiological parameters

4.3.1 Net photosynthesis

The data for the estimated net photosynthesis (NP) are shown in figures 10a and 10 b for Kk and Mm Bambara groundnut landraces respectively. There was reduction in NP with increasing salinity in both landraces. Wide variations were observed between the two landraces in relation to net photosynthesis. There were no significant ($p>0.05$) differences in NP between treatments in both landraces at beginning of the experiment. However, Mm had higher initial NP compared to Kk. The effect of salinity on NP was greater ($p<0.01$) in Mm landrace at 14DAT compared to Kk landrace. The plants growing under higher NaCl treatments were more affected with time as the most stressed indicated the least NP. By 28DAT, there were significant ($p<0.001$) differences in NP of the two landraces. All the 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} treatment plants died at 28 DAT. The control and 6.96 mm ho cm^{-1} treatment plants did not differ in NP at 42 DAT for Kk, but for Mm the difference was significant ($p<0.05$).

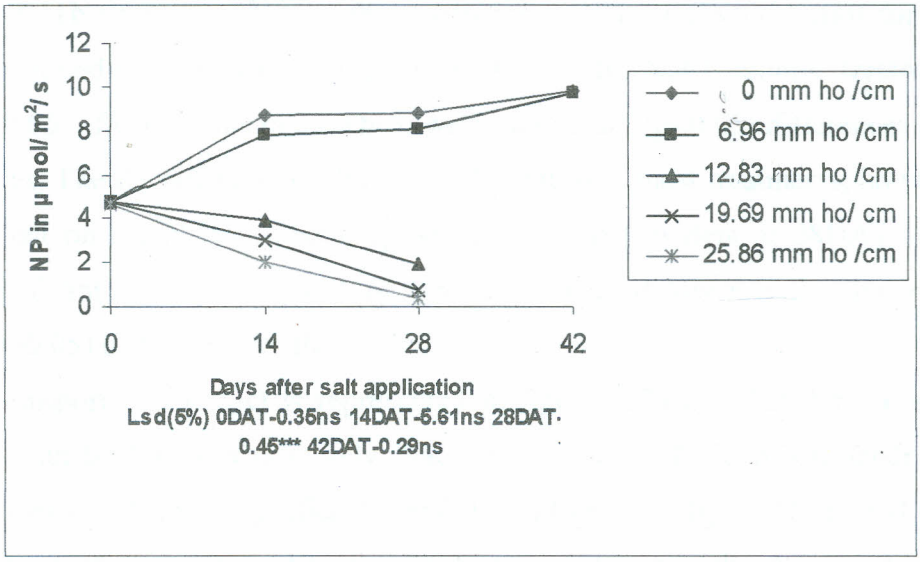

Fig 10a: The effect of NaCl salinity on net photosynthesis in Bambara in Kakamega Bambara groundnut landrace.

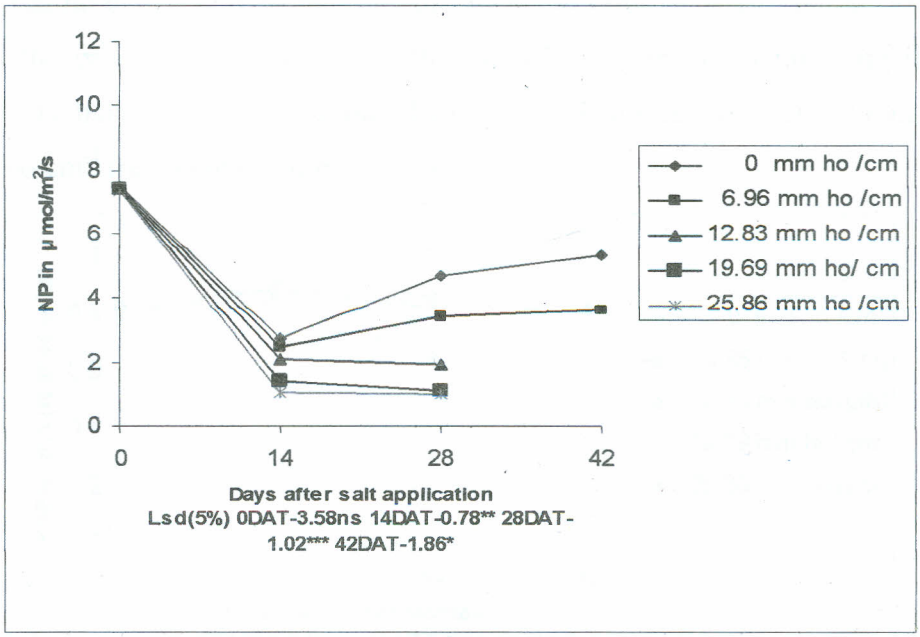

Fig 10b: The effect of NaCl salinity on net photosynthesis in Mumias Bambara groundnut landrace.

4.3.2 Chlorophyll fluorescence parameters

Figures 11a and 11b show the photosynthetic efficiency of PSII (Fv/Fm ratio) for Kk and Mm Bambara groundnut landraces, respectively. Generally, NaCl salinity treatment had significant ($p<0.05$) effect on this ratio. There were significant ($p<0.05$) differences between the two landraces. The Kk landrace had higher Fv/Fm ratios at most treatments, however, the impact of the salt on the photosynthetic apparatus was most evident at 28DAT when the effect was significant ($p<0.001$). The Mm landrace indicated lower ratios that were not significantly ($p>0.05$) different from the control.

The electron transport rate (ETR) is represented in figures 12a and 12b for Kk and Mm Bambara groundnut landraces, respectively. NaCl salinity varied ETR in both landraces. Kk and Mm landraces indicated significant ($p<0.01$) differences by 14DAT and 28DAT respectively. The control maintained higher ETR compared to all other treatments for both landraces. The 6.96 mm ho cm^{-1} treatment plants also had significantly ($p<0.01$) higher ETR compared to 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants. The ETR increased in 0 mm ho cm^{-1} and 6.96 mm ho cm^{-1} plants in both landraces as the system was stimulated and the two landraces did not differ significantly ($p<0.05$) implying that their response to salinity may be similar. All the plants in 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} treatments died by 28 DAT.

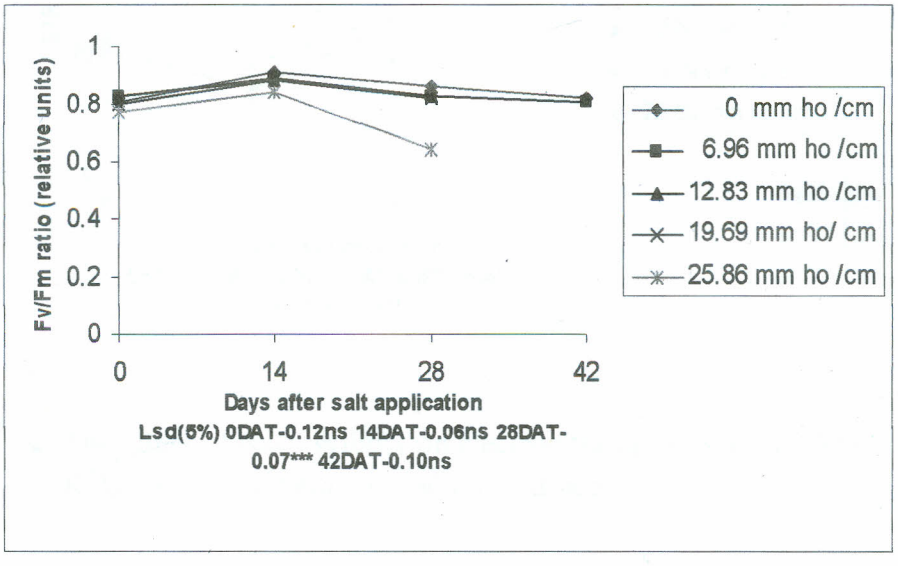

Fig 11a: The effect of NaCl salinity on Fv /Fm ratio in Kakamega Bambara groundnut landrace.

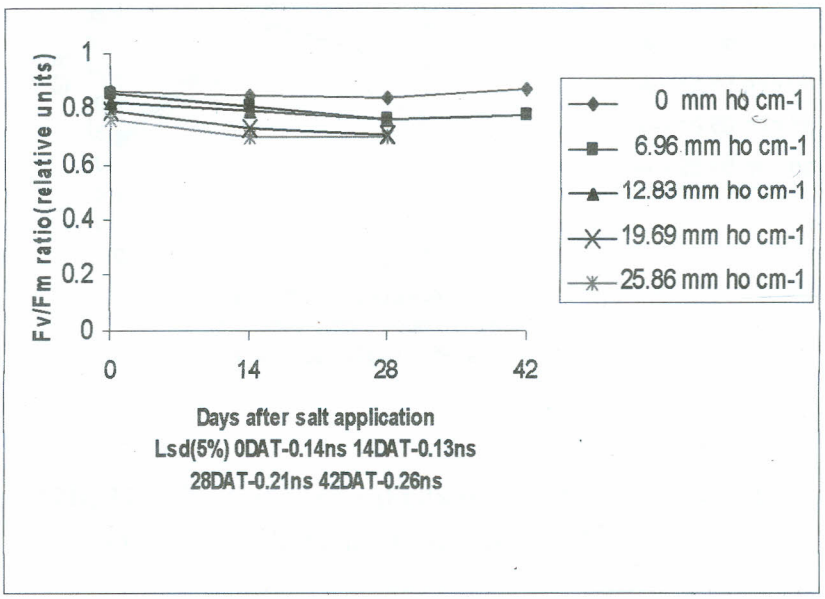

Fig 11b: The effect NaCl salinity on Fv /Fm ratio in Mumias Bambara groundnut landrace.

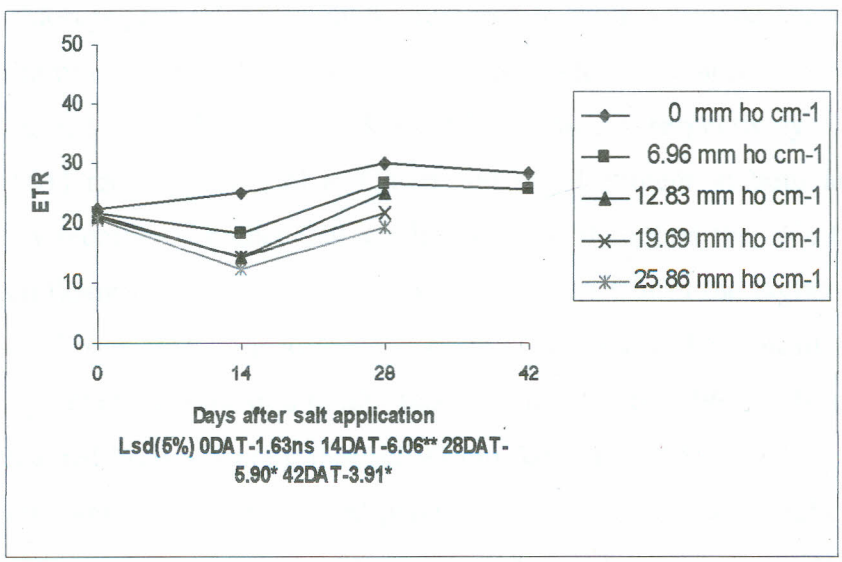

Fig 12a: The effect of NaCl salinity on electron transport Rate (ETR) in Kakamega Bambara groundnut landrace.

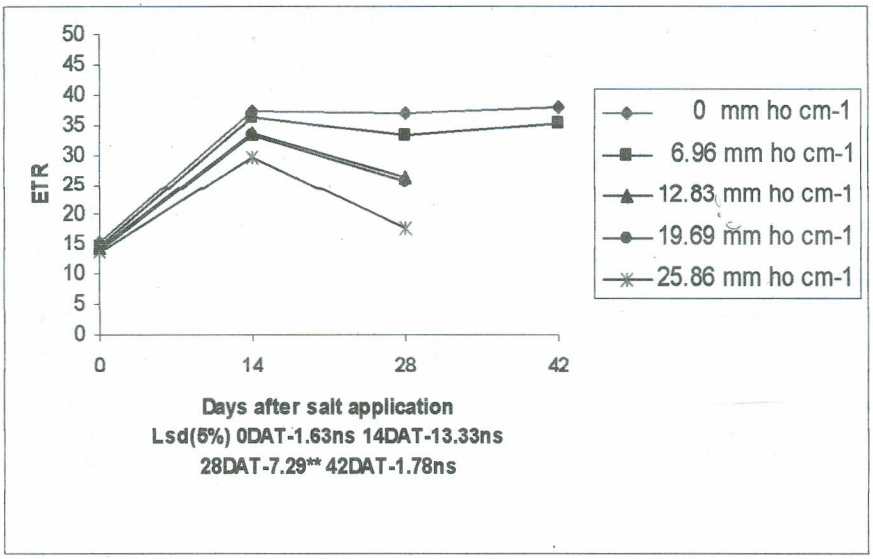

Fig 12b: The effect of NaCl salinity on electron transport Rate (ETR) in Mumias Bambara groundnut landrace.

4.4 Biochemical parameters

4.4.1 Chlorophyll content

The results for chlorophyll a (Chl a) content are presented in Fig13a and Fig 13b. Those for chlorophyll b (Chl b) content in Fig 14a and Fig 14 b, while total chlorophyll (t Chl) content is represented Fig in 15a and Fig 15b in Kk and Mm landraces, respectively. Salinity caused significant ($p<0.01$) reduction in Chl a, Chl b and t Chl content in both landraces. Mm landrace generally recorded a higher Chl a, Chl b and t Chl content than Kk landrace. Mm landrace had significantly ($p<0.05$) more Chl a, Chl b and t Chl in the higher NaCl salinity than Kk landrace. From 7DAT up to the end of the experiment the control plants had the highest chlorophyll content compared to all other treatments. The 6.96 mm ho cm^{-1} treatment plants also maintained higher content than 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants while the most stressed plants recorded the lowest. Significant ($P<0.001$) differences in Chl a content of Kk and Mm and Chl b of Kk occurred at 14DAT while the same occurred in Chl b and t Chl of Mm and t Chl of Kk from 28DAT. All the plants in treatments 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} died by 28 DAT. At the end of the experiment, the Kk control plants were generally significantly ($P<0.05$)

different in Chl a, Chl b and t Chl content from the 6.96 mm ho cm^{-1} plants while in Mm the differences were not significant (P>0.05).

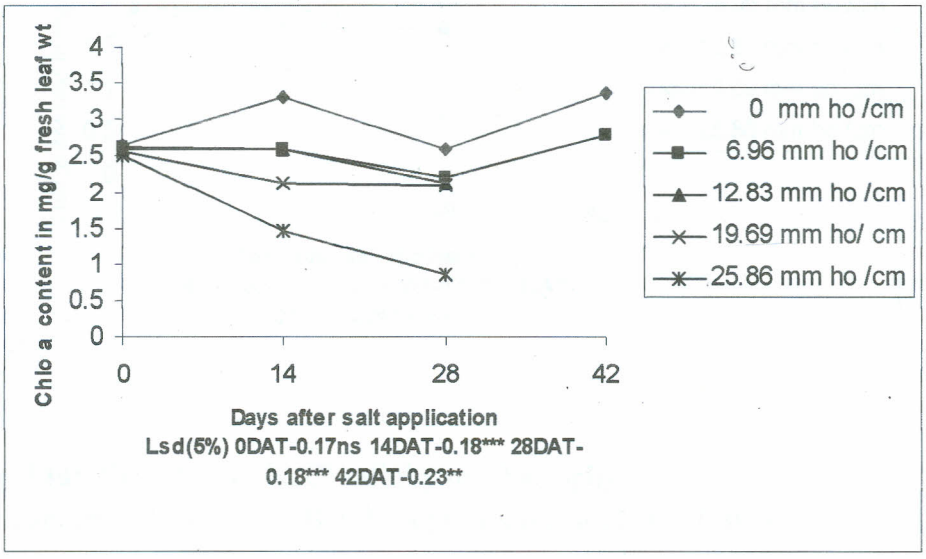

Fig 13a: The effect of NaCl salinity on chlorophyll a content in Kakamega Bambara groundnut landrace leaves.

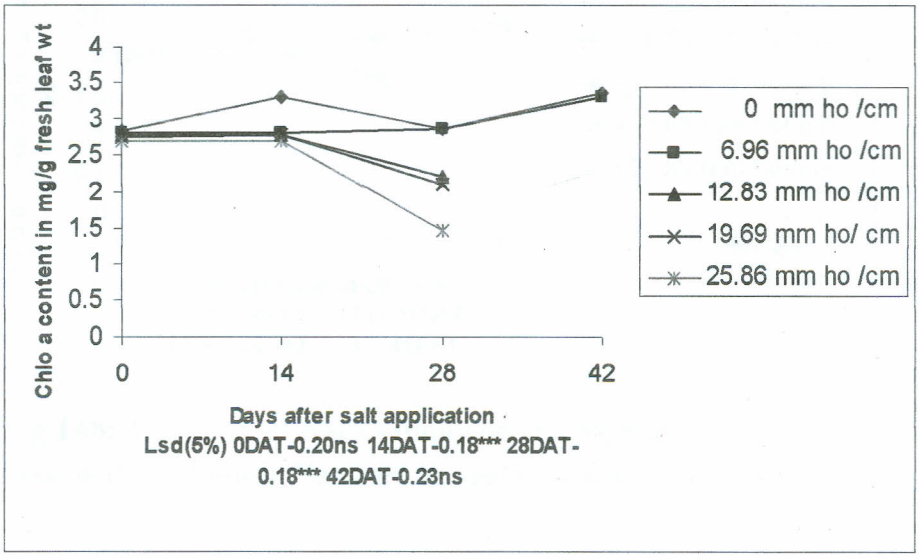

Fig 13b: The effect of NaCl salinity on chlorophyll a content in Mumias Bambara groundnut landrace leaves.

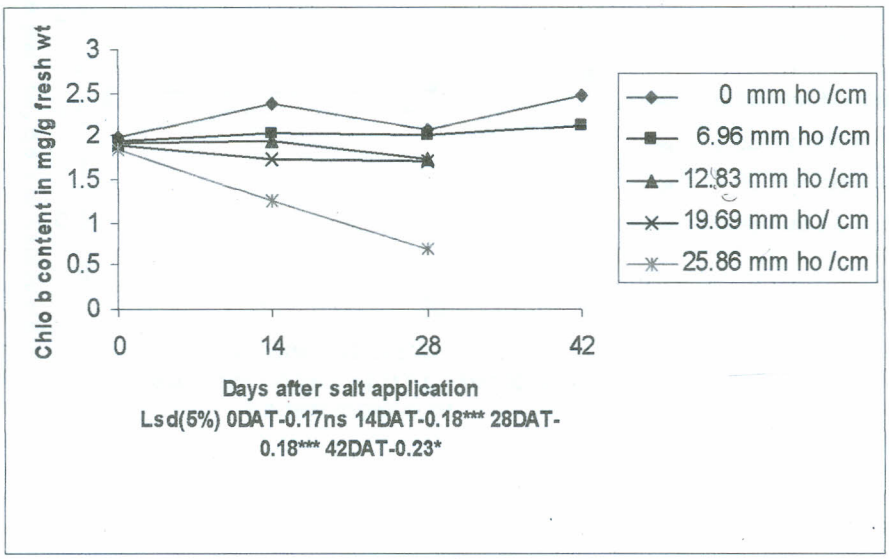

Fig 14a: The effect of NaCl salinity on chlorophyll b content in Kakamega Bambara groundnut landrace leaves.

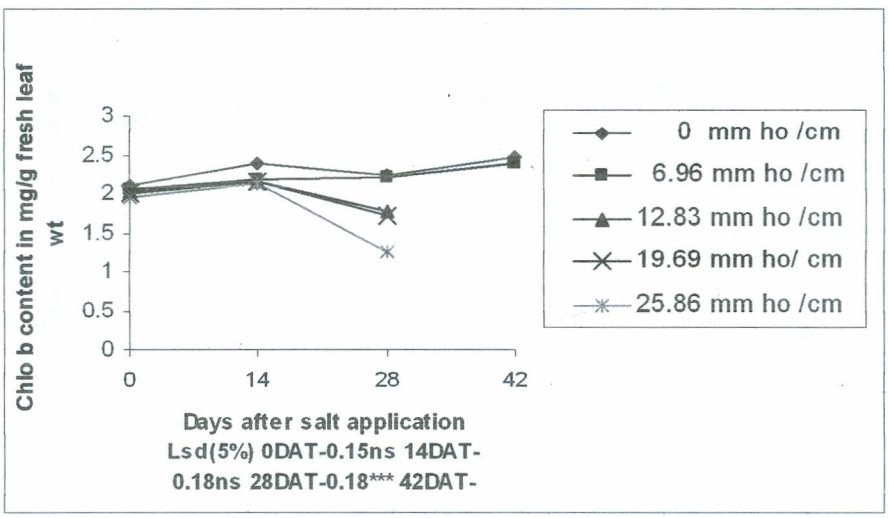

Fig 14b: The effect of NaCl salinity on chlorophyll b content in Mumias Bambara groundnut landrace leaves.

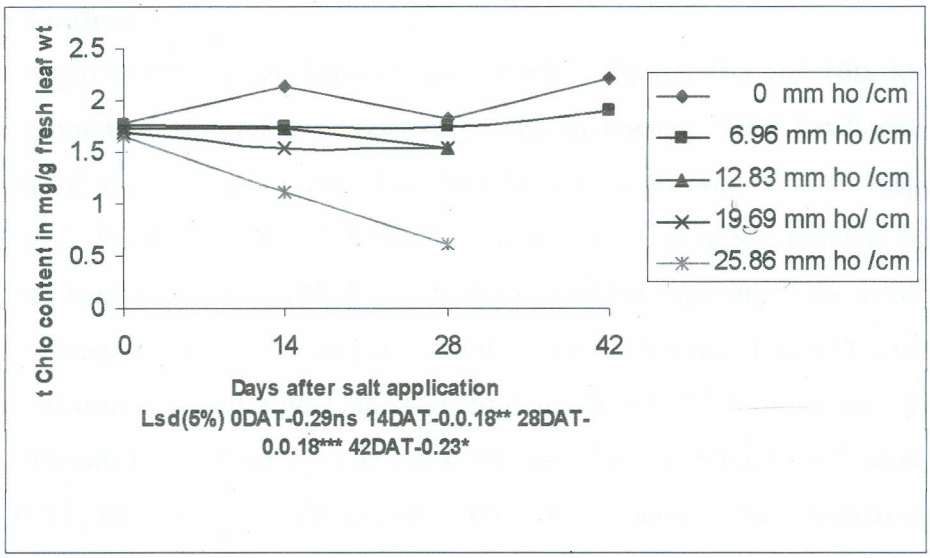

Fig 15a: The effect of NaCl salinity on t chlorophyll content in Kakamega Bambara groundnut landrace leaves.

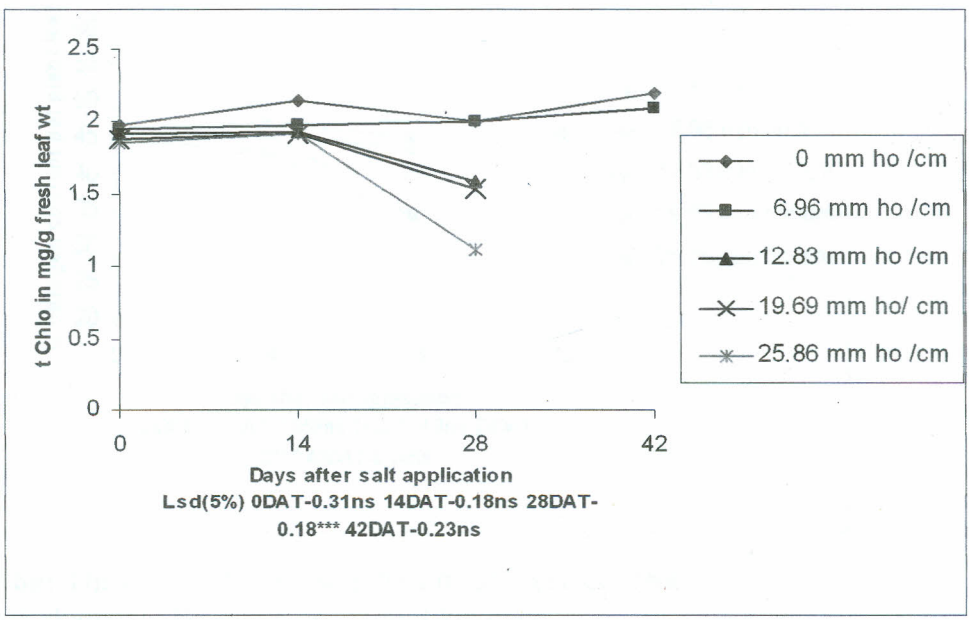

Fig 15b: The effect of NaCl salinity on t chlorophyll content in Mumias Bambara groundnut landrace leaves.

4.4.2 Nitrogen Content

The percent nitrogen content in the leaves is presented in figures 16a and 16b, for Kk and Mm Bambara groundnut landraces, respectively. The increasing NaCl treatment reduced nitrogen content of the two landraces. The two landraces showed similar responses to salinity. At 0DAT, there were no significant differences in nitrogen content among all treatments in both landraces. From 14DAT up to the end of the experiment the control plants had the highest nitrogen content as compared to all other treatments. The 6.96 mm ho cm^{-1} treatment plants also maintained higher nitrogen content than 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} plants. The most stressed plants recorded the lowest nitrogen content. At 28DAT, there were significant ($p<0.001$) differences in the nitrogen content of the Kk and Mm landraces leaves. All the plants given treatments 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} died by 28 DAT.

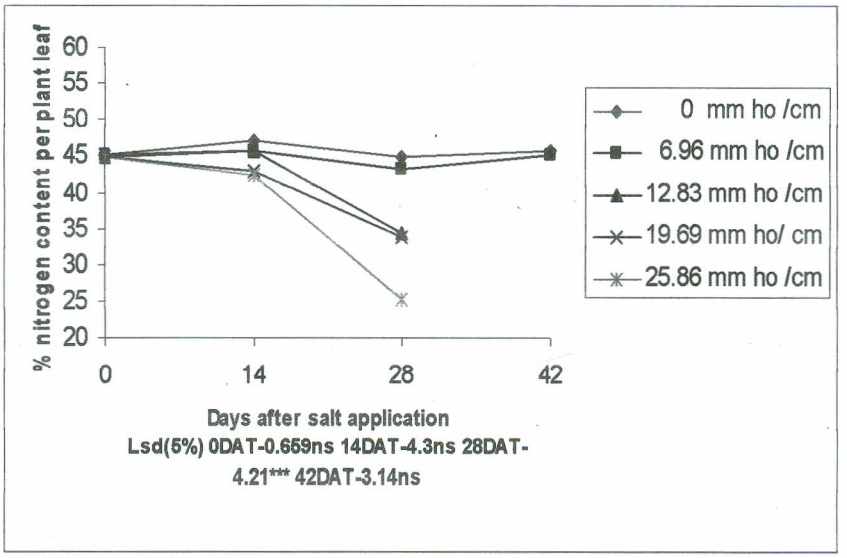

Fig 16a: The effect of NaCl salinity on nitrogen content in Kakamega Bambara groundnut landrace.

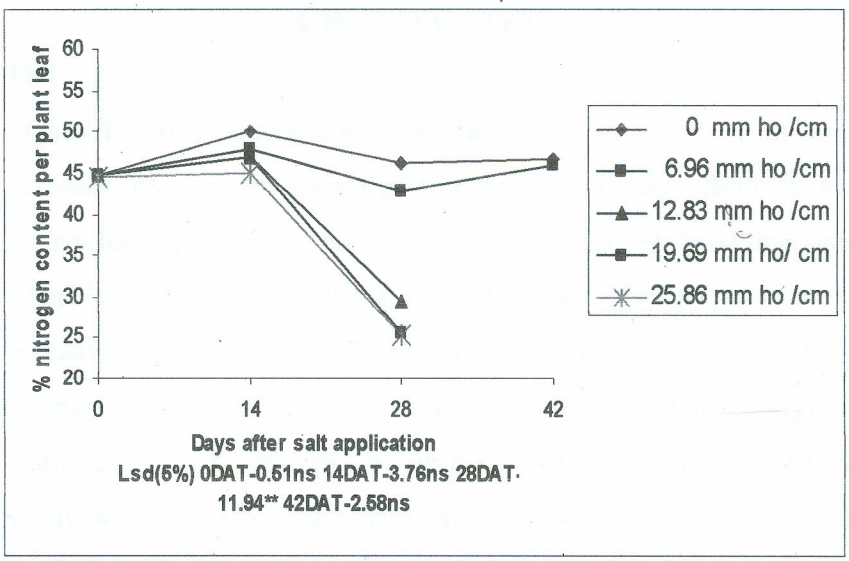

Fig 16b: The effect of NaCl salinity on nitrogen content in Mumas Bambara groundnut landrace.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The effect of NaCl salinity on seed germination

Environmental stresses interfere with seed germination, hence the attention on the effects of salinity on seed germination in plants. The results of this study showed that seed germination was affected by NaCl salinity and the effect varied depending on salinity level and landrace. Exposure to increasing salinity delayed germination and also caused decrease in final germination percentage. Similar delay in germination was also found in sorghum (Francois *et al.*, 1984; Netondo, 1999). The process of germination begins by the dry seed imbibing water, which is followed by increased hormonal and enzyme activity that lead to a higher metabolic rate, cell division and expansion. Any process that may interfere with germination at any phase may delay or inhibit seed germination. Younis *et al.*, (1987) suggest that the reduction in germination percentage may be attributed to a decrease in the rate of water uptake (osmotic effect) and ion toxicity of the accumulated sodium chloride, which lowers the enzyme activity. The seeds exposed to NaCl salinity are likely to suffer from ion toxicity since the sodium and chloride ions interact with cell protoplasm and interfere with various cell processes such as reduction of enzyme synthesis and activity, increased membrane permeability and dispersal of the protoplasm contents (Netondo, 1999). When the cell membranes leak, then solutes may easily flow out of the cell resulting into energy loss in form of metabolites. Dry seeds have very low water content (vary for different seeds between -6 and -20 MPa) and when they are exposed to moisture, they start to imbibe water. The rate of imbibing depends on the water potential of the germination medium compared to that of the seed. Salt treatments significantly ($p<0.001$) reduced percentage germination in both Bambara groundnut landraces, in fact it completely inhibited it in the most stressed (25.86mm ho cm^{-1}) plants. Salinity reduced osmotic potential of the germination medium and hence made soil water less available for extraction by the seeds. The seeds were exposed to a salt-induced physiological drought stress (Ziska *et al.*, 1989). The results indicate that both Bambara groundnut landraces can tolerate up to 19.89 mm ho cm^{-1} salinity during seed germination. Mm landrace showed greater response at higher NaCl salinity (12.93 mm ho cm^{-1} and 19.89 mm ho cm^{-1}) as Kk landrace showed similar response at lower NaCl salinity (6.96 mm ho cm^{-1}). Mm landrace therefore showed more osmotolerance compared to Kk.

5.2 The effect of NaCl salinity on Bambara plant growth

The results of this experiment showed that the Bambara plant growth was significantly ($p<0.01$) affected by sodium chloride salinity and the effect varied depending on salinity level and landrace. Not all growth parameters were similarly affected by NaCl salinity. It was revealed that NaCl salinity had significant ($p<0.01$) effect on the height of Bambara groundnut plants. There was a reduction in plant height with increase in salinity in both landraces and the magnitude of the reduction varied between the landraces. The detrimental effects of salts on plants are the consequence of both a water deficit that results from the relatively high solute concentrations in the soil as well as a stress specific to Cl^- and Na^+, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development and disturb photosynthesis, proteins synthesis and nucleic acid metabolism (Sairam *et al.*, 2004). Decreased plant height in response to salinity has been reported in many species (Netondo,1999; Mwai, 2001; Alam *et al.*, 2004; Zadeh and Naeini, 2007; Taffouo *et al.*, 2008, 2009, 2010). According to Alam *et al.*, (2004), the observed decrease in plant height in salinized plants were possibly due to: salinity reduced photosynthesis, which in turn limited the supply of carbohydrate needed for growth, salinity reduced shoot and root growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium and a disturbance in mineral supply, either an excess or deficiency, induced by changes in concentrations of specific ions in the growth medium that might have directly affected growth.

The salt may also have affected the internode length and also the leaf since the plant height measurements included the longest leaf. The reduced increase in plant height may have been due to growth involving both cell growth and development processes that consist of cell division, cell enlargement and differentiation, all of which are very sensitive to osmotic stress because of their dependence upon turgor (Jones and Lazenby, 1988). Ion toxicity may have decreased cell division and expansion hence reduced vertical growth since it has been reported that accumulation of Na^+ and Cl^- ions in the stem tissues of sorghum (Netondo, 1999) and finger millet (Onkware,1986) lead to ion toxicity at high concentration.

Osmotic effects may also have played a role in reducing stem growth as salinity leads to low water potential in the plant. In salt-sensitive plants, shoot, and to lesser extent, root growth is

permanently reduced within hours of salt stress and this does not appear to depend on Na^+ concentrations in the growing tissues, but rather is a response to the osmolarity of the external solution (Munns *et al.*, 2000; Munns, 2002). Ghassemi *et al.*, (1995) observed that cotton growth was retarded severely by salinity, through a decrease in the osmotic potential and reduced availability of nutrients. The occurrence of stunting reduced main stem height and biological yield, probably due to a drop in osmotic potential of soil solution and non-availability of water. The inhibition of cell expansion is usually followed closely by a reduction in cell wall synthesis (Salisbury and Ross, 1992). The low turgor may have led to retarded cell expansion hence growth. Reduced rates of photosynthesis generated less photosynthetic products under these stressful conditions. This, coupled with increased respiration under salinity, may have increased breakdown of metabolites directly reducing growth. The increase in height was slow at the beginning of the experiment but the rate progressively increased with time in 6.96 mm ho cm^{-1} treatment. This may have been due to the fact that the germinating seedlings had fewer cells capable of growth but the growth progressively increased as more cells were formed (Salisbury and Ross, 1992). In the current study, Mm Bambara groundnut landrace had higher plant height in all the treatments that were not significantly ($p<0.05$) different as compared to Kk landrace. This may have been due to higher chances of osmotic tolerance involving maintenance of turgor and presumably normal metabolic activity at a lower water potential than would have been possible. It has been suggested that drought tolerance of Bambara groundnut is a result of osmotic adjustment, reduction of leaf area index and low water loss through the stomata (Collinson *et al.*, 1997).

Leaf area and number measured during the study period were significantly ($p<0.01$) reduced by salinity. This response to salinity is common in plants. The vegetative growth of the crops under salinity stress may be severely restricted resulting into smaller leaf areas as reported in many crops including legumes like *Arachis hypogea* (Collino *et al.*, 2001). Reduced leaf growth due to salinity has been observed in plant species such as beans (Neumann *et al.*, 1988) and maize (Cramer *et al.*,1994). In the present experiment, the reduction in leaf area and number at higher NaCl treatments may be attributed to the retarded leaf development processes, leaf senescence and death, reduced leaf growth and delayed leaf emergence whose

overall effect is malformed leaves that offers less leaf area leading to reduced leaf size of the plants. The reduced leaf area is an adaptation to reduced ion uptake by roots (Neumann *et al.*,1988). Plant development is affected since the reduced leaf area contributes to less photosynthesis hence less dry matter accumulation. The primary effect of salinity in many species is to reduce leaf growth rate, leaf emergence rate and overall shoot development (Netondo, 1999). The reduction in leaf growth of plants exposed to salinity has been attributed to reduced turgor (Neumann *et al.*, 1988) or reduction in extensibility of expanding cell walls (Neumann, 1993). This inhibition of leaf growth in the short term may be due to water stress while on long term scale, leaf growth is affected by ion toxicity when the ions move through the transpiration stream and accumulate in the leaves (Yeo *et al.*, 1991); which eventually leads to increased leaf mortality and senescence. Lack of vasculature to the meristems reduce transport of Na^+ and Cl^- ions to these cells and the fully expanded leaves that are ion sinks may abscise. Plants hence minimize exposure of these cells to the ions in the tissues.

Salinity reduces transpiration due to direct influence on the stomata whose aperture determines how much water is lost. Reduced transpiration also has the tendency to lower the rate of salt loading into leaves (Everard *et al.*, 1994). This is due to the fact that salts reach the leaves through the transpiration stream. This tends to maintain the salts at subtoxic levels for a long time (Everard *et al.*, 1994). Consequently, water is conserved in order to maintain a high water status in the plant as the reduced leaf area minimizes the amount of water lost per unit leaf area. Therefore, stomatal closure in response to salinity in one way, is a limitation to photosynthetic capacity. However, it also in another way offers a protective mechanism which aids the survival of plants that are exposed to salinity stress by minimizing salt loading in leaves and conserving water (Everard *et al.*, 1994).

Research of recent decades has established that most halophytes and glycophytes tolerate salinity by rather similar strategies. Generally, the mechanisms of salinity tolerance in plants can be categorized into three: (1) tolerance to osmotic stress, (2) Na^+ exclusion and (3) tissue tolerance (Munns and Tester, 2008). The degree to which each of these factors affects growth depends on the plant genotype and environmental conditions (Zadeh *et al.*, 2008).

Osmotic stress causes immediate reduction in cell expansion in roots and young leaves, tolerance to osmotic stress results in less reduction in leaf growth and stomatal conductance (Munns and Tester, 2008). The main site of Na^+ toxicity for most plants is the leaf blade rather than the root tips as Na^+ accumulates in the leaf blades due to continuous translocation and deposition due to transpiration. Thus, it is very important that Na^+ does not reach the leaf blades in excess as the Na^+ relocation from leaves to roots is likely to be only a small portion of what was delivered to the leaf (Munns and Tester, 2008). Plants therefore transport the toxic ions to the older leaves and leaf sheaths which are sacrificed for early senescence and/or death at the cost of saving young growing meristematic tissues. The ability of rice cultivars to compartmentalize ions in older leaves and structural tissues crucially affect plant survival (Fukuda *et al.*, 2004).

Ionic stress on the plant is minimized by the amount of Na^+ that accumulates in the cytosol of cells, particularly in the transpiring leaves. It has been suggested that salinity tolerance in wheat (Munns and James, 2003) and other cereals (Garthwaite *et al.*, 2005) is particularly associated with the ability to exclude Na^+ from the shoot. Research into improving salinity tolerance of wheat cultivars has identified mechanisms for Na^+ exclusion such as the *Knal* locus on chromosome 4D of bread wheat (Dubcovsky *et al.*, 1996) and the *Nax1* and *Nax2* loci in durum wheat (James *et al.*, 2006; Davenport *et al.*, 2007). A part from extruding Na^+ from leaves (Tester and Davenport, 2003; Møller and Tester, 2007; Munns and Tester, 2008), efficient compartmentalizing of Na^+ in the vacuole or in particular cell types where the damage to metabolism is kept to a minimum (Munns and Tester, 2008) occurs. Both processes involve regulation of the expression of specific ion channels and transporters, allowing the control of Na^+ transport throughout the plant (Davenport *et al.*, 2007).

Na^+ enters roots passively, via voltage independent non selective cation channels and via other Na^+ transporters (Laurie *et al.*, 2002). Most of the Na^+ that enters root cells in the outer part of the root is likely to be pumped back out again via plasma membrane Na^+/H^+ antiporters (Tester and Davenport, 2003). Plants unable to regulate salt ion concentration in this way and whose tissues are not innately salt tolerant *per se*, encounter severe physiological dysfunction (Netondo,1999; Mwai, 2001).

The extensive leaf destruction in form of marginal burns, necrotic patches and leaf death that were observed in lower leaves especially in treatments 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} and the premature senescence of leaves in the same treatments could be attributed to specific ion toxicity of either Na^+ ions, Cl^- ions or both in tissues. The high mortality and responses shown by these plants at 28 day after salt application indicate ion toxicity damage. Ionic stress results in premature senescence of older leaves and in toxicity symptoms that is chlorosis and necrosis in mature leaves (Munns, 2002; Tester and Davenport, 2003; Munns *et al.*, 2006), due to high Na^+ concentration disrupting protein synthesis and interfering with enzyme activity (Bhandal *et al.*, 1988). Specific ion toxicity effects of the salts, either cause injury to cell membranes, salt accumulation in cell wall resulting in dehydration or disturbance to other plant metabolic processes such as protein synthesis or hydrolysis (Levitt, 1980) or after penetration through the membrane into protoplast, the effect of ions such as Na^+, Ca^{2+}, Mg^{2+} and Cl^- is important as a result of ions causing injury to the internal contents of the protoplasts, such as inhibition of enzyme activity (Flowers *et al.*, 1977), cell death through DNA destruction and degradation of chloroplast and mitochondrial membranes (Flowers *et al.*, 1985).

Leaf growth in both Kk and Mm Bambara groundnut landraces was generally higher during the first 14 days from the start of the experiment. This could be suggesting osmotic tolerance in these plants. Plants with high osmotic tolerance maintain high growth rates, particularly over the first few days after exposure to Na^+, whereas those with low leaf senescence and either low or high shoot Na^+ concentrations are Na^+ excluders or tissue tolerators, respectively (Munns and Tester, 2008).

Shoot and root dry and fresh weights decreased with increasing level of salt stress. Comparable results were obtained in sorghum (Netondo, 1999) and spider plant (Mwai, 2001). Root, stem and leaf dry weights decreased at high salt treatment (200 mM NaCl) Taffouo *et al.*, (2008, 2010) in the white seed coat Bambara groundnuts and in the tropical salt tolerant species curcubit species (*Lagenaria siceraria*). Root fresh and dry weights significantly ($p<0.01$) declined in the highly stressed plants prior to death in plants exposed to 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1} treatments.

The reduction of the plant dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl^- and Na^+ (Turan *et al.*, 2007; Taffouo *et al.*, 2010). According to Alam *et al.*, (2004) many nutrients have an essential role in the process of cell division and cell extension and those would cease soon after the supply were halted, especially in tissues with little nutrient storage. The reduced Bambara plant growth in the present study under salt stress could be due to disturbed and imbalanced nutrition.

The reduction in shoot dry weight could also be associated with reduced rate of leaf production hence low number of leaves leading to reduced photosynthesis and accumulation of dry matter. Root damage and death due to salinity may have affected water and mineral salt absorption from the soil hence decreased water and minerals in the transpiration stream reaching the leaves resulting in decreased net photosynthesis which in turn may have affected shoot growth.

Reduction in dry weight of plant tissues reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation (Richardson and McCree, 1985; Netondo *et al.*, 2004). It also reflects salt impact on tissues (Greenway and Munns, 1980), reduction in photosynthetic rates per unit of leaf area (McCree, 1986; Netondo *et al.*, 2004), and attainment of maximum salt concentration tolerated by the fully expanded leaves (Munns and Termaat, 1986). These findings agree with those from sorghum (Boursier and Läuchli, 1990; Maas *et al.*, 1986; Weimberg *et al.*, 1984). At high salt concentration 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1}, the plants could not regulate ion concentration since there may have been severe physiological dysfunctions leading to decreased growth rates and eventually cell death leading to death of whole plant. Shoot and root damage caused by ion toxicity, osmotic effects or both may have contributed to the observed sharp drop in fresh and dry weights preceding the death of highly stressed plants. Inhibition of long distance transport of nutrient ions by salinity has been proposed to explain reduced nutrient content in the shoot due to displacement of K^+ and Ca^{2+} by Na^+ on the membranes (Cramer *et al.*, 1985) hence reduced shoot growth.

The vegetative growth of the crops under salinity stress may be severely restricted resulting into reduced total dry matter as reported in many crops including legumes like *Arachis hypogaea* (Collino *et al.*, 2001). Total dry matter in Bambara was reduced by water deficit (Collinson *et al.*, 1996; 1997). Reduced biomass due to water deficit is partly a consequence of restricted leaf area of the plants, which in turn reduces light interception (Singh, 1991) and partly a direct effect of low net photosynthesis due to stomatal closure.

The two landrace root fresh and dry weights were generally similar when subjected to NaCl salinity. Shoot dry weight accumulation were significantly ($p<0.01$) affected by NaCl treatment in both landraces however the control and 6.96 mm ho cm^{-1} treatment plants in both Bambara groundnut landraces were not significantly ($p<0.05$) different. Shoot fresh weights were not significantly ($p<0.05$) different in the two landraces, however Kk landrace indicated greater salt tolerance during fresh weight accumulation.

The landraces were not significantly ($p<0.05$) different during dry matter accumulation. The observed reduction in root length and root to shoot ratios as salinity increased in both landraces may have been caused by a reduction in plant growth and increased root death. The reduction in plant growth depends on cells would be the first to be affected directly by ionic toxicity of the salt and hence the roots die. In most cases, roots are less affected by increasing salinity as compared with the shoot (Yang *et al.*, 1990) leading to a higher root: shoot ratio in various plant parts. The roots are directly in contact with saline soil hence the root epidermal the plants exposed to higher salt concentrations. In this study, the treatment effects were generally significant ($p<0.05$) on this ratio in both Bambara groundnut landraces. The increase in the root to shoot ratio observed on the 7DAS and in 12.93 mm ho cm^{-1}, 19.89 mm ho cm^{-1} treatments and on the 21DAS suggest higher chances of osmotolerance involving maintenance of turgor at a lower water potential than would have been possible. The higher root: shoot ratio for Mumias landrace in most of the treatments as compared to Kakamega may be a response of the landrace for survival under salt stress conditions since increased root surface area allows more water and nutrients to be absorbed from the soil. A reduction in shoot growth coupled with increased root growth would result in an improved plant water status under the saline conditions. Nyamudeza (1989) reported a high root to total dry matter ratio in Bambara groundnuts under water stress. Although there is a noted general reduction

in plant growth rates under osmotic stress, shoot growth is more inhibited than root growth (Richardson and Mcree, 1985).

In the present study, the root length of both landraces was significantly ($p<0.01$) affected by the increasing NaCl salinity reducing their length. The root in Kk groundnut landrace was more sensitive to salinity than Mm since the effect of salinity was already significant ($p<0.001$) at 7DAT. Root elongation rate is reduced by salinity due to reduced rates of cell production and growth, reduced final length of epidermal cells and shorter apical meristem (Zidan *et al.*, 1990). Salt induced death of root cells has been reported in barley by Katsuhara (1977) and this has been attributed to osmotically induced turgor loss and Na^+ ion toxicity in root meristem, causing reduced instant cell extension rates. Physiologically, reduction of root epidermal cell elongation and production may be attributed to accumulation of Na^+ to toxic levels in some of the meristematic cells. The reduced cell length as a result of salinity may result from reduced cell extension rates and or in the duration of extension period. Neumann *et al.*, 1994) also reported inhibition of root growth in salt stressed maize as a result of reduced extensibility of root tip tissues due to hardening of the expanding cell walls.

Decreased root growth due to increased salinity stress may have also been through suppression of nutrient absorption due to the uptake of NaCl in competition with other nutrient ions. Reduced nutrient uptake is partially attributed to the osmoticallly induced restricted root growth since osmotic restriction of root growth reduces the ability of roots to explore greater soil volume for the nutrients (Mwai, 2001).

Root damage may also have been due to destruction of soil structure as a result of high Na^+ concentration and subsequent high exchangeable sodium percentage (ESP) in the soil ion exchange complex (Wild, 1988). Poor drainage of irrigated water in pots and crumpled soils were observed at the end of experiment particularly in the most stressed treatments. The high Na^+ ions concentration in soil would have caused structural deterioration of the soil leading to poor aeration and deflocculation that accelerated root death. The reduction in root growth reduce plant growth since the available surface area for absorption of water and mineral salts is reduced (Neumann *et a.*, 1994).

5.3 Effect of NaCl salinity on plant water status

Percentage water content (%WC) measured was used as an indicator to estimate plant water status. The results show that %WC declined in a similar way for the two landraces with increase in NaCl treatment. The reduction in %WC may have led to inadequate water in the cells. The gentle decrease observed in Mm landrace suggests that the landrace may have had a better chance of osmotic adjustment at this stage of growth. The plant (shoot and root) water potential may have reduced with increasing salinity as soil water was less available for absorption by plant roots in both landraces. The results indicate that the plants exposed to NaCl salinity experienced water deficit. Similar results were reported by Greenway and Munns (1980) and Neumann (1997) who suggested that water stress occurs throughout the time plants are exposed to salinity, leading to turgor loss and subsequent depressed growth, transpiration and yield.

The results show that the %WC of the roots of the two Bambara groundnut landraces was not significantly ($p<0.05$) affected by NaCl salinity throughout the experimental period, however Mm showed an increase in %WC after 0DAT. The shoots showed a similar response.

Water content values of white seed coat Bambara groundnut landrace roots, stems and leaves grown under saline conditions were not significantly ($p<0.05$) different from the control plants (Taffouo *et al.*, 2008). Similar results were found in the same organs of *Phaseolus adenanthus* and *Avicennia germinans*, two natural halophytes (Taffouo *et al.*, 2008) and *Lagenaria siceraria*, a salt-tolerant plant (Taffouo *et al.*, 2008). This is attributed to the significant storage of monovalent cations in leaves, facilitated by the increase in Na^+ concentrations from roots to leaves. At 28DAT no significant ($p>0.05$) differences were observed in %WC in the roots of all plants in all treatments of the two Bambara groundnut landraces in this study. The %WC in the shoots was generally much higher than in the roots. This may suggest that the root was accumulating more dry matter, an a response for survival under saline conditions since increased root surface area allows more water to be absorbed from the soil resulting in an improved plant water status under the saline conditions. Rapid growth has been observed in barley, wheat and tomato (Levitt, 1980). This may also be explained by salt dilution effect depending on rapid growth of the plant or development of succulence in the shoots. The succulence system depends on high plastic extensibility of the

cell walls, mainly of parenchyma cells that enlarge due to an increase in water content as a result of changes in cell wall properties thus preventing excessive salt concentration in the cell sap and maintaining turgor (Levitt, 1980).

Most plant species have capacity to recover from the adverse effects of environmental stresses such as drought and salinity after they have been exposed to the stress for a long time (Levitt, 1980). Such acclimation has been observed in wheat varieties (Kingsbury *et al.*, 1983), sorghum varieties (Netondo, 1999) and spider plant (Mwai, 2001). Such acclimation response may explain the increase in %WC seen during the study period after salt treatment in the Mm Bambara groundnut landrace. The plant may adapt to osmotic stress by regulating water loss through leaves and the roots or osmotic dehydration of the root cells may have been regulated by lowering their osmotic potential below that of the surrounding soil solution hence subsequent observed increase in %WC as the plant was able to absorb water from the salty soil. This is only possible if the root cells have lower water potential than the soil. This dehydration avoidance and recovery of %WC by lowering the plant water potential is possible only as a result of an increase in the solute content of the plant cells sufficiently to lower the plant's osmotic potential to compensate for the external osmotic stress. This adaptive response of plants exposed to water and salt stress is called osmotic adjustment (Turner and Jones, 1980; Munns, 1988). In the current study, the increase in %WC observed may be interpreted as evidence that Mm Bambara groundnut landrace is capable of osmotic adjustment under sodium chloride salinity. The capacity for osmotic adjustment can also be explained by the degree of salt resistance since different plant species are reported to accumulate various types of organic solutes for this purpose (McCree, 1986; Shalhevet and Hsiao, 1986). The high salt resistance of the halophytes is thought to involve their ability to absorb large amounts of inorganic ions for osmotic adjustment. This may be coupled with either tolerance of the plant tissues and metabolic apparatus to these high ion concentrations (Levitt, 1980). Once the potentially toxic ions start accumulating in the leaf tissues, due to enhanced absorption and translocation, it has to be tolerated. The cytotoxic ions in saline environments, typically Na^+ and Cl^-, are preferentially compartmentalized into the vacuole and used as osmotic solutes (Blumwald *et al.*, 2000; Niu *et al.*, 1995) thus leading to the turgor maintenance for the cell under osmotic stress (Flowers *et al.*, 1977; Bohnert *et al.*,

1995). Vacuolar compartmentalization prevents metabolic poisoning of the cytosol and organelles (Hasegawa *et al.*, 2000). The net uptake of these ions across the plasma membrane is restricted to minimize cytoplasmic toxicity during vacuolar compartmentalization.

In glycophytes, the ability to compartmentalize the salt ions is limited (Greenway and Munns, 1980). Salt resistance in most glycophytes depends on their ability to exclude the salt ions at the root surface, coupled with osmotic adjustment through the accumulation of organic solutes such as glycine and proline in sorghum (Nagy *et al.*, 1994) and amylase in finger millet (Onkware 1986). These compatible solutes, even in high concentration they do not interfere with enzymatic activities (Johnson *et al.*, 1968) in the cytoplasm. Some glycophytes such as sorghum possibly accumulate both organic solutes and inorganic ions (Netondo, 1999). Osmotic adjustments contribute to the maintenance of the water potential gradient between soil water and the transpiring leaves and of cell turgor. It significantly improves soil water uptake under dry conditions and allows maintenance of open stomata and a higher stomatal conductance (Otieno *et al.*, 2005) hence sustains photosynthesis by maintaining leaf water content at reduced water potential. Water stress significantly increased ($p<0.05$) proline concentration in Bambara groundnut plants water stressed at the vegetative, flowering and pod filling stages compared to the non-stressed plants (Vurayai *et al.*, 2011). Although proline's role in plant osmotolerance remains controversial it is however, thought to contribute to osmotic adjustment, detoxification of reactive oxygen species and protection of membrane integrity during water stress (Hare and Cress, 1997). Although both the organic solutes and inorganic ions were not measured in the current study, the responses shown by the salinized plants supports the argument that these plants suffered both water stress and ion toxicity damage.

5.4 Effects of NaCl salinity on photosynthesis

Sodium chloride salinity had significant ($p<0.01$) effect on net photosynthesis of Bambara groundnut plants. There was reduction in net photosynthesis with increase in salinity. The underlying cause may have been osmotic stress, ion toxicity or both. The effects of salinity on photosynthesis depend on the plant species' adaptation to salinity, the length of time that the plant has been exposed to salinity, the concentration and composition of the salt, and the interaction of salinity effects with other prevailing environmental factors such as humidity

and soil nutrient status, making photosynthetic responses to salinity to be varied for various plant groups (Mwai, 2001). Generally, salinity reduces photosynthetic rates by decreasing CO_2 assimilation rates. This may be attributed to stomatal or non-stomatal causes. In most cases the effect of salinity on photosynthesis is attributed to stomatal closure, limiting the amount of CO_2 that reach the site of carboxylation directly leading to a reduction in CO_2 assimilation. Salinity also reduces photosynthetic products, leaf expansion, mesophyll resistance and changes activities of various enzymes (Ziska *et al.*, 1989). This may suggest that the chlorophyll content may have been affected by this stress, or enzymatic processes responsible for CO_2 fixation, or both of these factors. Salinity causes metabolic disturbances associated with photosynthesis such as depressing chlorophyll content (Lutts *et al.*, 1996) and hence reduced photosynthetic capacity of the plant affecting the amount of assimilate translocated and distributed from leaves to growing tissues which further limit growth (Munns and Termaat, 1986). Spinach plants exposed to salt stress have decreased osmotic potentials, yet photosynthetic capacity is not decreased, suggesting that osmotic adjustment in the chloroplasts, somehow prevents the inhibition which is observed when chloroplasts are exposed to decreased osmotic potentials in *vitro* (Robinson, 1985). Their photosynthetic capacity for instance, suggests that osmotic adjustment somehow prevents inhibition indicating possession of certain degree of salt resistance.

Wide variations in net photosynthesis were observed between the two Bambara groundnut landraces. The reduction in leaf growth limited light interception and CO_2 absorption hence lowered net photosynthesis resulting in the overall retarded plant growth as the resources required for growth and development processes become limited in supply (Mwai, 2001). Mm Bambara groundnut landrace net photosynthesis was more affected by salinity compared to Kk landrace. The observed drop in net photosynthesis at 14DAT may have been due to the effect of environmental factors on the landraces such as limiting the amount of CO_2 that reach the site of carboxylation hence decreased CO_2 assimilation rates. Kk landrace improved responses such as leaf production and leaf area may have improved light interception and CO_2 absorption hence net photosynthesis.

Chlorophyll fluorescence is a useful parameter for assessing the response of plants exposed to stress. Chlorophyll fluorescence parameters are direct, non destructive indicators of the photosynthetic activity and provide basic information on the functioning of the photosynthetic apparatus especially photosystem II (Netondo, 1999). Environmental stresses affect PS II efficiency and lower the Fv/Fm values (Pospisil *et al.*, 1998). Cornic and Massacci (1996) reported no significant difference in the data collected from dehydrated and non dehydrated plants for both bean and maize leaves. Results on the effect of water deficit on the Fv/Fm ratio which reflects potential quantum yield of PS II was used as a parameter of the physiological state of the photosynthetic apparatus in intact plant leaves (Pospisil *et al.*, 1998) and damage to PSII will often be the first manifestation of stress in a leaf (Maxwell and Johnson, 2000), hence fluorescence can give insights into the ability of a plant to tolerate environmental stresses. Lutts *et al.*, (1996) exposed rice varieties to salinity and obtained significant differences between the control and higher NaCl after 9 to 12 days and between varieties after 18 days of treatment initiation.

The Fv/ Fm ratio declined with increase in NaCl treatment. This could be attributed to the effects of the salts on the reaction centres of PS II system directly or through accelerated senescence. There were no significant ($p<0.05$) differences between the two landraces until at 28DAT when the Fv/Fm ratios differed significantly ($p<0.001$) for Kk landrace. One lower Fv/Fm ratio outside the normal Fv/Fm range that highly differed from the control was indicated at 28DAT for Kk landrace. The Mm landrace showed no significant ($p>0.05$) differences in the ratio for all the treatments throughout the experiment. Although the Mm landrace had lower Fv/Fm ratios outside the normal range they were not significantly different from the control treatment. Generally the leaf photochemical efficiency of the PS11 was not severely affected by salinity in both landraces except at the highest salinities, 19.89 mm ho cm^{-1} and 25.86 mm ho cm^{-1}. This indicates that both landraces may withstand higher levels of salinity in the early stages of life, meaning that the PSII is not damaged in the early stages of salinity exposure. The photosynthetic apparatus of Kk landrace may have maintained higher Fv/Fm ratios under high salinity however severe damage occurred at 28DAT. Mm landrace Fv/Fm ratio indicate slightly lower photochemical efficiency of PS11 that was not severely affected by high salinity levels implying that its photosynthetic apparatus is more salt tolerant.

The effects of NaCl on electron transport rate in both landraces appear mild on 6.96 mm ho cm^{-1} plants since the rate is not significantly ($p<0.05$) different from that of 0 mm ho cm^{-1} treatment. The higher ETR at low NaCl levels may indicate stimulated electron transport in the thylakoid membranes. Electron transport rate declined with increase in NaCl salinity. The ions could have affected the thylakoid membrane by disrupting the lipid bilayer or lipid protein complex impairing electron transport activity. Salt stress in plants also induce higher concentration of ROS (reactive oxygen species) intermediates due to the impaired electron transport processes in chloroplast, mitochondria and photorespiration pathways (Mittler, 2002; Polle, 2001). It is reported that H_2O_2 concentration of 10μM reduces the net photosynthesis rate by 50% (Mittler, 2002). The flow of electrons through PS II is indicative under many conditions of the overall rate of photosynthesis (Pereira *et al.*, 2004). It has been used in determining plant tolerance to environmental perturbations such as cold, drought, temperature, light and salinity in sorghum (Netondo, 1999). Cornic and Massacci (1996) reported no significant difference between dehydrated and non dehydrated bean and maize plant leaves.

5.5 Effects of NaCl salinity on leaf chlorophyll and nitrogen content

NaCl salinity caused a reduction in leaf chlorophyll content in both Bambara groundnut landraces. The decrease in chlorophyll content under salt stress is a commonly reported phenomenon in various studies, because of the adverse effects of the salts on membrane stability (Ashraf and Bhatti, 2000).

Similar results have been reported for finger millet (Onkware, 1986), sorghum (Netondo,1999) and spider plant (Mwai, 2001). The total chlorophyll concentration of Kk and Mm landrace leaves was significantly ($p<0.01$) reduced under salt stress except in 0 mm ho cm^{-1} and -6.96 mm ho cm^{-1} treatments. Similar results were reported for total leaf chlorophyll concentration of curcubit species (Taffouo *et al.*, 2008), Bambara groundnut landraces (white seed coat) (Taffouo *et al.*, 2010) and lentil plants (Turan *et al.*, 2007). The effect of NaCl salinity was attributed to salt-induced weakening of protein-pigment-lipid complex (Strogonov *et al.*, 1970) and increasing chlorophyllase activity (Stivsev *et al.*, 1973).

Significant ($p<0.05$) differences were observed between the two Bambara groundnut landraces, with Mm landrace having significantly ($p<0.05$) more chlorophyll a, b and total chlorophyll compared to that of Kk landrace at higher NaCl treatment. Unlike the results of sorghum (Netondo, 1999) that chlorophyll a was reduced to a greater extent, all the three (chlorophyll a, chlorophyll b and total chlorophyll) parameters were found to be equally sensitive to increasing NaCl salinity since all were reduced to comparable levels. Similar findings have been reported for spider plant (Mwai, 2001).

Chlorophyll content of leaves is a useful indicator of both potential photosynthetic productivity and general plant vigour (Alonso *et al.*, C, 2002). According to Levitt (1980), chlorophyll content in plants often decreases with increased mesophyll resistance commonly observed in dry areas. Chlorophyll is widely used as a basis for determination of photosynthesis because the reaction components essential for photosynthesis (such as the reaction centres of PS1 and PS11, electron carriers and enzymes related to ATP synthesis and CO_2 fixation) are present in chloroplast at fixed molar ratios to chlorophyll (Kura Hotta *et al.*, 1987). The reduction of chlorophyll in salt stressed plants may be due to a reduction in the lamellar content of the light harvesting chlorophyll a/b protein (Randall *et al.*, 1977). Salinity may have caused metabolic disturbances associated with photosynthesis such that the absorbed ions ($Na^+$and Cl^-) interfered with the synthesis of the pigments by inhibiting the enzymes from functioning. The ions could have also interfered with absorption of magnesium component of chlorophyll, depressed chlorophyll synthesis and content (Lutts *et al.*, 1996) resulting in reduced photosynthetic capacity of the plant affecting the amount of assimilate translocated and distributed from leaves to growing tissues which further limit growth (Munns and Termaat, 1986).

Osmotic stress reduces chlorophyll a synthesis and may cause breakdown of already formed chlorophyll. The maintenance of higher chlorophyll a content in Mm landrace indicates that the fluorescence system of the photosynthetic apparatus may have been less severely affected. The higher values of chlorophyll b in the Mm landrace may imply that its PS11 antenna system structure was not severely modified by osmotic and/or ion toxicity reducing photosynthesis. Another possible explanation may be that chlorophyll synthesis could have

induced by salinity especially chlorophyll b (Luvaha, 2005). The maintenance of higher chlorophyll a and b in treatments 12.93 mm ho cm^{-1} and 19.89 mm ho cm^{-1} not significantly ($p<0.05$) different from the controls, suggests that the chlorophyll pigments in Mm leaves were more tolerant to NaCl salinity. The maintenance of high total chlorophyll in Mm under NaCl salinity suggests that the chlorophyll pigments in Mm leaves may tolerate salinity. In the present experiment, Kk and Mm Bambara groundnut landraces may tolerate salinity. However, Mm may be more tolerant since more chlorophyll a and b are directly correlated with light capture.

Plant leaf nitrogen content was significantly ($p<0.05$) reduced by the increasing NaCl salinity in both Bambara groundnut landraces. The amount of chlorophyll is closely related to leaf nitrogen content. Water stress significantly increased ($p<0.05$) proline concentration in Bambara groundnut plants water stressed at the vegetative, flowering and pod filling stages compared to the non-stressed plants (Vurayai *et al.*, 2011). Water stress results in proteolysis hence higher nitrogen content. The observed salinity induced decline in leaf nitrogen content may have been due to interference with chlorophyll synthesis, photosynthetic pigment composition, various enzyme activities such as nitrate reductase and the capacity for plant to store nitrates (Solomonson and Barber, 1990). The reduced chlorophyll concentration contributes to a reduction in photosynthesis and ultimately plant growth. Mm Bambara groundnut landrace had higher leaf nitrogen content although not significantly ($p<0.05$) different from that of Kk landrace. The NaCl salinity effects appear mild and indicate that the damage to the nitrogen regulation mechanism was less severe for Mm compared to Kk landrace.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results obtained from this study have indicated some important morphological, physiological and biochemical responses of Bambara groundnuts to NaCl salinity. Some conclusions can be made from the findings of this research.

NaCl salinity significantly reduced seed germination in both Bambara groundnut landraces. Both Bambara groundnut landraces were tolerant to up to19.89 mm ho cm^{-1} NaCl salinity during seed germination. Mm landrace seems to be more salt tolerant at higher NaCl salinity (12.93 mm ho cm^{-1}) however, at lower NaCl salinity (6.96 mm ho cm^{-1}) the Kk landrace seem more tolerant.

NaCl salinity significantly reduced plant height, root length, root and shoot fresh and dry weight, leaf growth (leaf number, emergence and area), root to shoot ratio and percentage water content. It reduced net photosynthesis, electron transport rate, chlorophyll concentration (chlorophyll a, chlorophyll b and total chlorophyll) and nitrogen content in both Bambara groundnut landraces.

The overall results provided evidence that increasing NaCl salinity inflicted damage to the seeds and plants of the two landraces to different extents. The results indicate that Bambara groundnut vegetative stage is sensitive to NaCl salinity. Even though, the study did not go up to yield as a result of some of the plants dying, it indicated greater response in most parameters up to 6.96 mm ho cm^{-1} salinity in both landraces. Mm Bambara groundnut landrace indicated more salt tolerance at morphological, physiological and biochemical parameters of the plant level. There is significant promise of various parameters therefore to be used for screening for salt tolerance and are potentially useful for plant breeders to improve on plant response to NaCl salinity.

6.2 Recommendations

Both Mm and Kk Bambara groundnut landraces were tolerant to NaCl salinity up to 19.89 mm ho cm^{-1} during seed germination. Mm landrace seems to be more salt tolerant at higher NaCl salinity (12.93 mm ho cm^{-1} and 19.89 mm ho cm^{-1}) however, Kk seem more tolerant at lower NaCl salinity (6.96 mm ho cm^{-1}) during seed germination. The leaf photochemical efficiency of the PS11 was also not severely affected by salinity in Mm landrace. Both landrace seeds may be grown in more saline environments up to (19.89 mm ho cm^{-1}) however, the responses strongly suggest that Mumias landrace may be more salt tolerant and thus recommended for cultivation in the agro ecological areas of Kenya prone to water deficit and potentially saline. This landrace could be studied further as a source of genes for salt tolerance that could be exploited in breeding programs.

Osmotolerance study could be conducted for more locally grown landraces in addition to the above landraces to assess the effects of osmolytes such as proline during osmotic adjustment.

6.3 Suggestions for further research

The research has opened up potential areas for further investigations. It is clear from the results that NaCl salinity is a complex process involving varied and diverse traits for many species. Further research is therefore recommended in the following areas:

1. Study on osmotolerance due to osmotic adjustment to involve more locally grown Bambara groundnut landraces collected from varied ecological zones for further comparisons.
2. Determine mineral nutrient (Na^+ and Cl^- ions) content of roots, stems and leaves of Bambara groundnut landraces in response to salinity to establish ion toxicity stress.
3. Determine gas exchange and chlorophyll fluorescence of Bambara groundnut plants at different stages of development in response to salinity.

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