EFFECT OF SALT STRESS ON MAIZE (*ZEA MAYS L.*) VARIETIES AT EARLY SEEDLING STAGE

BY

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APPROVAL FORM

This is to certify that the dissertation entitled **"The effect of salt stress on maize (*Zea mays l.*) varieties at early seedling stage"**, submitted in partial fulfilment of the requirements for Bachelor of Science Honors Degree in Applied Biosciences and Biotechnology at Midlands State University, is a record of the original research carried out by Tafadzwa Lionel Vheremu R154897W under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

The assistance and the help received during the course of this research have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the dissertation requirements.

Name of supervisor(s) …………………………………..

Signature …………………..……………..

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ABSTRACT

Maize (*Zea mays L.*) is grown in different parts of the world, where it is a staple crop and many people rely on it as a primary source of nutrition. Salt stress is an external abiotic constraint that reduces maize ability to convert energy to biomass when it is in high concentrations. We conducted an experiment to investigate the effect of sodium chloride concentration on morphology of seedlings of maize varieties Agri Seeds R201, SC 513 and SC 403. The practical importance of the study was to develop high yielding maize varieties, which are adaptable to the salt stressed environments thereby increase in maize productivity in Zimbabwe. Maize varieties seeds were germinated in plastic pots inside the greenhouse for seven days. Different concentrations of sodium chloride, 25, 50, 75, 100% and 0% (distilled water) as a control were prepared in the laboratory. Maize seedlings were washed with distilled water before different parameters, root and shoot length, seedling height, fresh and dry weight were measured. All the data was collected and noted down for each maize seedling variety. Maize seedling roots were immersed into the saline solution with their leaves hanging out of the different plastic bottles through a circular opening on the lid for six days. The parameters, root and shoot length, seedling height, fresh and dry weight were measured after the seedlings were removed from the saline treatments. The final data was recorded by calculating the differences between the data recorded before treatment and after salt treatment. Results showed that an increase in salt concentration resulted in a decrease in root and shoot length, seedling height, fresh and dry weight of all maize varieties seedlings. Sodium chloride concentrations 25, 50, 75 and 100% had a significant mean difference when compared to the control treatment (0%) for root and shoot length, seedling height, fresh and dry weight of all maize varieties seedlings (Dunnett's test, Multiple Comparisons). Maize seedling varieties were salt tolerant and much growth and development was favoured by sodium chloride concentration at 25%, apart from the control treatment (0%). The retardatory effects of sodium chloride on the growth of maize seedlings, require a plant breeding approach to minimize the effects of salinity, which results in the development of maize varieties that can grow and produce economic yields under saline conditions.

ACKNOWLEDGEMENTS

I would like to thank the Lord Almighty God for guiding me throughout my studies. I am forever grateful for my loving parents, for being my biggest support system as they endured with me emotionally and financially throughout my studies. A special thank you to my academic supervisor Dr F. Songwe. This work would not have been successful without his wisdom, encouragement and tireless efforts. I also extend my gratitude to Mr. C. Mabhugu for his assistance in the laboratory. Thank you to my friends Kasava, Kwari, Takudzwa, Michael and Kudakwashe for your help throughout this project.

DEDICATION

This is for my parents and my late grandfather (May his soul rest in eternal peace).

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND INFORMATION

Maize (*Zea mays L.*) occupies a key position as one of the most important cereals for both human and animal consumption (Idris, 2015). It is grown under various conditions in different parts of the world and plays a major role as a staple food (Cairns *et al.*, 2013) . In sub-Saharan Africa it is grown on over 25 million hectares mostly by smallholder farmers producing about 38 tonnes of maize grain (Setimela *et al.*, 2017). Salt stress is an external abiotic constraint that limits maize plant rate of photosynthesis and reduces its ability to convert energy to biomass when it is in high concentrations. It also reduces leaf growth rate of maize due to a reduction in the number of elongating cells (Nawaz and Majeed, 2018). Sodium chloride (NaCl) is the main salt found in saline soils, however, sodium sulphate, magnesium sulphate and potassium chloride are also responsible and can dominate different saline environments (Bot, 2017).

Early maize seedling growths are more sensitive to salinity than later developmental stages (Mehraj, 2015). The root is the first organ exposed to salt stress, however shoots are more sensitive to salt stress than roots. Salt in the root zone decreases the water potential of the soil solution (Ali, 2011). Effects of salinity are mostly in arid and semi-arid regions where there is limited rainfall, high evapo-transpiration and high temperature associated with poor water and soil management practices are the major contributing factors (Nawaz and Majeed, 2018). There is an increase in the pH-level of soil as a result of high salinity in which most crop plants do not grow well under these levels.

Studies have been carried out to observe the effects of salt stress on maize as it is a salt-sensitive crop. It was observed that seed germination is a critical stage in seedling establishment which determines the success of crop production on salt-affected soils (Aliu, 2015). The germination percentage, water absorption of the seeds, water uptake percentage and the growth parameters were observed to decrease with increasing sodium chloride concentrations (Katembe, 2006). Shoot growth in maize is strongly inhibited in the first phase of different concentrations of salt stress. Stunted maize growth with dark green leaves without any toxicity symptoms during the first phase of salt stress were observed. This was as a result of impaired extension growth as osmotic adjustment and turgor maintenance were not limiting.

Generally the growth of maize plant is reduced by salinity but it may vary from variety to variety in their tolerance. This investigation is to be undertaken to study the response of maize seedlings of varieties SC 403, SC 513 and Agri Seeds R201 to different concentrations of sodium chloride.

1.2 PROBLEM STATEMENT

Salt stress is a major obstacle for ensuring global food security (Shtereva *et al.*, 2015). Soil salinity is the amount of soluble salts in soil solution. It is also a major abiotic stress and its development in maize cultivating soils is a crucial problem. Maize yields in sub-Saharan Africa remain low ranging from 2 tonnes per hectare to less than 1.5 tonnes per hectare under smallholder farmer conditions compared to other regions due to salt stressed soils (Setimela *et al.*, 2017). In Zimbabwe more than 70% of maize is grown in areas with a 40-60% frequency of a failed season due to low rainfalls and high temperatures (Villiers, 2012). Soil salinity is a major factor limiting agricultural productivity, which has increased cost of productivity and crop failures in irrigation schemes located in south-east Zimbabwe such as Chipinge district (Chidoko *et al.*, 2014). The use of poor quality irrigation water and the continued use of this water as well as reduced water infiltration leads to soil salinization problems (Krista, 2003). Electrical conductivity, Sodium Adsorption Ratio (SAR) and Exchangeable Sodium Percentage (ESP) are indices of salinity in soils which are used for assessing quality of the water used for irrigation and the status of the soils that receive the irrigation water. The thresholds for SAR and ESP in Zimbabwe are 13% and 9% respectively (Chidoko *et al.*, 2014). Saline soils are non-productive for maize growth which in turn to low economic returns for the farmers. Soil salinity affects maize development aspects which include germination, vegetative growth and reproductive development. It also results in inhibition and malfunctioning of vital physiological functions such leaf blade thickness, photosynthesis, stem and root length (Hassan and Shaddam, 2018).

1.3 JUSTIFICATION

The responses and resistance mechanism of different maize varieties found locally that are adapted to the salinity conditions needs to be investigated. This helps to devise strategies for improved maize performance in saline environments (Farooq, 2015). It also ensures that salty agricultural environments are not out of the agricultural plan. This will result in the development of improved and high yielding maize varieties that are adaptable to the salt stressed environments thereby increase in maize productivity in Zimbabwe. Soil salinity from irrigation occurs over time wherever irrigation occurs, since all water contains some dissolved salts (Johnson, 2012). The excessive use of water for irrigation in dry climates where there are clay texture soils causes salt accumulation because they cannot be washed out by rainfall**.** Therefore, any irrigation system has the potential to deliver an increased amount of salt to the soil.

1.4 OBJECTIVES

1.4.1 MAIN OBJECTIVE

- To determine the optimum salt concentration favouring growth of maize seedlings of SC 403, SC513 and Agri Seeds R201 varieties at seedling stage

1.4.2 SPECIFIC OBJECTIVES

- To determine extent of tolerance of maize varieties SC 403, SC513 and Agri Seeds R201 seedlings to different levels of salinity
- To assess the morphology of maize varieties exposed to different salt concentrations

CHAPTER 2

2.1 LITERATURE REVIEW

2.2 SCIENTIFIC CLASSIFICATION OF MAIZE

Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Cyperales
Family	Poaceae
Genus	Zea
Species	*Zea mays L.*

2.2.1 MAIZE (*ZEA MAYS L.*) DISTRIBUTION

Maize is grown under various conditions in different parts of the world and most maize production is in Africa (Idris, 2015). It is grown over a wider range of altitudes and latitudes than any other food crop, under temperatures ranging from cool to very hot, on wet to semi-arid lands and in many different types of soils (Delgado, 2019). It is also an important food grain which farmers grow in all climates from tropical to warm prevailed in various parts of the world (Bose, 2018). Maize is grown in all natural regions of Zimbabwe, including dry marginal areas of natural regions four and five that receive between 450 to 650 millimeters rainfalls annually (Mutungwe *et al.,* 2017).

2.2.2 MAIZE NUTRITION

Globally, maize is a staple crop and many people rely on it as a primary source of nutrition (Shah, Prasad and Kumar, 2016). Maize kernel is an edible and nutritive part of the plant as it contains starch, which provides approximately 70 % of the kernel weight, proteins, oils from the germ, vitamins C, E and K as well as potassium. According to Chaudhary *et al* (2014), phytochemicals such as carotenoids are bioactive chemical compounds naturally present in plants that provide human health benefits and reduce the risk of major chronic diseases are found in maize. Maize is highly nutritious, highly appetizing and it prevents constipation as it is easily broken down in the body.

2.3 SOIL SALINITY

Salinity in agricultural terms is the excess of soluble salts which are above the level of plants require, resulting in the reduction of growth of the crop plants (Ahmad, 2011). Several different soluble salts such as sodium sulphate, magnesium chloride, and calcium sulphate are responsible for salinity, with the most common salt causing soil salinity sodium chloride (common table salt). When sodium chloride is ionized by water it produces sodium (Na^+) and chloride (Cl^-) ions. There are four levels of soil salinity based on saline irrigation water which are low, medium, high and very high salinity.

Table 1: Soil salinity in quantities

SALINITY CLASS	ELECTRICAL CONDUCTIVITY (micro mhos/cm)
Low	<250
Medium	250-750
High	750-2250
Very high	2250-5000

2.3.1 SOIL SALINITY DISTRIBUTION

Salinity is worldwide problem that spreads in all the continents except Antarctica (Hussain and Rawahy, 2006)**.** According to the United Nations Educational, Scientific and Cultural Organization (UNESCO) soil map of the world, the total area of saline soils is 397 million hectares and that of sodic soils is 434 million hectares. Salinization of agricultural lands is widespread as it occurs in arid, semiarid, low-lying, irrigated and poorly drained areas (Ambede, 2012). Soil affected by salt also widely exists in sub-humid and humid that is high rainfall region. Rietz (2014) observed that induced salinity was marked in the lower slope than the upper slope position of gently sloping fields in the Zimbabwean low-veld under furrow irrigation. Salt affected soils contain a higher amount of sodium ions that are absorbed onto soil particles. Salinity is often seen in sandy soils containing lower amounts of clay and organic matter.

2.4 CAUSES OF SOIL SALINITY

Salinity occurs through natural or human induced processes that result in the accumulation of dissolved salts in the soil to an extent that inhibits plant growth.

2.4.1 SECONDARY SALINITY

Human activities during different cultural practices, irrigation operations in agriculture, releasing polluted effluent and sewages into the soils and waterways by the industry and residential areas induce soil salinity (Hussain and Rawahy, 2006). Underground water also becomes more concentrated as a result of the induction of soil salinity. The total amount of dissolved salts in the irrigation water and their composition influence soil salinity. When the soil dries, the concentration of salts in the soil solution is increased. Salts accumulate in specific profiles according to the irrigation regimen and the type of irrigation used. When irrigating using sprinklers, water and salts move deeper according to the soil's infiltration capacity and the water quantity until they stop at a certain depth. When using drip irrigation there is also a lateral movement of water and salts. Following irrigation in the absence of proper drainage, the water table rises which causes the salty groundwater to reach the upper soil layers thereby supplying salts to the root zone.

Salts in the soil occur as ions which are released from weathering minerals in the soil, may also be applied as chemical fertilizers for crops and migrate upward in the soil from shallow groundwater (Shrivastava and Kumar, 2018). The type and amount of fertilizers applied to soil, affect its salinity. Some fertilizers contain high levels of potentially harmful salts which when overused and misused leads to salinity buildup. Through physical and chemical weathering such as hydrolysis, hydration and dissolution, there is a gradual release of salts from the bedrock after becoming soluble. The released salts dissolve into the surface water and groundwater. When precipitation is not enough to leach ions from the soil profile, salts accumulate in the soil resulting soil salinity. Chemicals from industrial emissions may accumulate in the soil and if the concentration is high enough it can result in salt accumulation in the upper layer of soil. Deforestation is recognized as a major cause of salinization of soils as a result of the effects of salt migration in both the upper and lower layers (Ahmad, 2011).

2.4.2 CLIMATIC CONDITIONS

Climatic factors and water management accelerate soil salinization in arid and semi-arid lands as evapo-transpiration plays a very important role in the formation of saline soils (Ahmad, 2011). Low rainfall accompanied with high temperatures cause accumulation of salts on the surface of the soil as the salts cannot be leached underground completely (Hussain and Rawahy, 2006). There is gradual accumulation of salts as the little rainfall is evaporated thereby leaving the salts on the surface of the soil or nearer underneath. In dry seasons in arid and semi-arid climates the process is active. Rainwater contains salts which come from pollutants dissolved in atmospheric moisture and airborne salts from ocean spray, which are then deposited on land and water when it rains.

2.4.3 NEARNESS TO SEA

Soil salinity is a threat to places near the sea as soil forms from minerals which are high in soluble salt content, saline water floods and sprays (Hussain and Rawahy, 2006). Salinity occurs in coastal areas since the soil in those areas is exposed to seawater, subjected to tides and the main cause is intrusion of saline water into rivers. Coastal crops are frequently affected by exposure to sea water brought in by cyclones around the Indian Ocean (Ahmad, 2011). The ocean salts are carried inland by wind and deposited by rainfall, which is mainly sodium chloride.

2.4.4 FALLOWING

Leaving the soil uncultivated for longer periods invites salinity build up because upwards movement of groundwater due to capillary action can bring salts from groundwater to surface soil (Laosheng, 2010).

2.5 EFFECTS OF SALT STRESS

Salt stress has several effects on maize growth and development and on soils.

2.5.1 IMPACT ON MAIZE

Salinization severely affects the agricultural productivity (Johnson, 2012). The impacts of salinity on maize include low agricultural productivity and low economic returns. Salinity has a negative impact as it disrupts or hinders the plant’s morpho-physiological processes due to osmotic disturbance and ionic stress (Ahmad, 2011). Increasing the concentration of sodium chloride stress on maize seedlings significantly increases the uptake of sodium and chloride ions by the roots and shoots (Sugirtharan, 2008). Shoot growth of maize seedlings is reduced by salinity as it suppresses leaf initiation and expansion as well as internodes growth (Ali, 2011). Salinity reduces the ability of plants to take up water and this causes reductions in seedling growth rate by inducing drought in the plant (Johnson, 2012). Root growth and nutrient extraction are restricted by salinity from subsoil resulting in poorer plant growth and lower crop yields (Haidarizadeh and Zarei, 2018). Soil salinity results in osmotic stress, ion toxicity and nutrient deficiency on plants thereby limiting the rate of water uptake from the soil (Shrivastava and Kumar, 2018).

At high concentrations, salt suppresses plant growth such as germination ability of maize seeds, seedling establishment, growth and vigour as the plant has to use more energy to absorb water. Under extreme salinity conditions, maize may be unable to absorb water and will wilt, even when the surrounding soil is saturated resulting in the death of the maize plant thereby ultimately reducing crop yield (Khodarahmpour, 2012). Sodium chloride increases salt concentrations in the root and shoot of maize plants. Shoots are generally more sensitive to cation disturbances than roots and there are differences among plant species in their ability to prevent or tolerate the excess salt concentrations (Turan, 2010). Different cultivars of maize seedling growth responses vary among them when exposed to saline soils (Okçu, 2005). An effective indicator during seedling growth period is plant height (Yu, Liao and Oladipo, 2014). Different salinity levels of irrigation water and different soil salt content have great effects on maize height. Depending on the salinity level, saline soils and water causes plant growth inhibition, dwarfism and lower yields**.**

2.5.2 IMPACT ON SOILS

High concentrations of salinity can adversely influence the physical, chemical, and biological properties of soils (Mancer and Bouhoun, 2019). Salinity is one of the most widespread soil degradation processes on earth as it causes desertification (Ladeiro, 2012). Recent studies of desertification in the Arab World indicated that about 2% of the Arab lands are deteriorating as a result of salinity increasing (Mohmmad and Soufan, 2018). In saline soils, sodium replaces calcium and magnesium, which are adsorbed to the surface of clay particles in the soil. Thus, aggregation of soil particles is reduced and the soil will tend to disperse. Soils are subjected to increase in erosion as salinity is often associated with weakening soil surface cover. Soil physical properties are affected by soil water salinity as fine particles of soil bind together to form aggregates which prevents soil aeration, root penetration, and root growth (Krista, 2003)**.** Soil permeability is reduced by soil dispersion, which causes clay particles to plug soil pores. This hardens the soil and blocks water infiltration thus making it difficult for plants to establish and grow. Saline soils are wholly or partly unproductive especially where there is a general upward movement of soluble salts**.**

CHAPTER 3

3.1 MATERIALS AND METHODS

3.2 STUDY AREA

This study was carried out at the Midlands State University (MSU) main campus laboratory in Gweru, Zimbabwe. The MSU campus is situated ten kilometers to the south east of the Midlands Provincial capital of Gweru.

3.3 SAMPLE COLLECTION

Treated maize seeds of varieties namely SC 403 (Tsoko), SC 513 (Mbizi) and Agri seeds R201 were acquired from the MSU Agriculture department. A total of 15 seeds for each variety were randomly selected and were placed in labeled beakers.

3.4 MAIZE SEEDS GERMINATION

Pine bark shavings were filled into six plastic pots that were perforated with small holes at the bottom. The plastic pots were placed in the greenhouse and were watered before maize seeds were placed in them. A total of ten maize seeds of varieties namely SC 403, SC 513 and Agri seeds R201 were selected for germination. Two plastic pots were assigned for each maize variety and were labeled accordingly. Seeds were placed and covered with the pine bark shavings in the plastic pots. Distilled water was used to wet the pine bark and seeds until they germinated on the seventh day. The seeds were considered to have germinated when hypocotyls and epicotyl emerged on the surface of the pine bark shavings in the plastic pots.

Figure 1: Germinated maize seedlings in a plastic pot

3.5 MEDIA PREPARATION

Different concentrations of sodium chloride prepared inside the laboratory were 25, 50, 75 and 100%. The control used was 0%, which was distilled water without sodium chloride. The numbers of plastic bottles for each sodium chloride concentration was 12. Sodium chloride in each plastic bottle was diluted with 150 milliliters of distilled water and a spatula was used to stir the solutions. After stirring the solutions, the plastic bottles were closed tightly with their lids.

3.6 TRANSFER OF SEEDLINGS TO THE LABORATORY

On the seventh day, all the maize seeds in the plastic pots had germinated to produce seedlings. Pine bark shavings were carefully removed from the plastic pots to get hold of the different 10 maize seedling varieties with their roots intact. The maize seedlings were put in three clearly labeled beakers according to their varieties. Each maize seedling was thoroughly washed especially the roots with distilled water to remove stuck pine bark shavings and other debri. Paper towels were used to completely dry the washed maize seedlings and were transferred to the laboratory.

3.7 BEFORE SALT STRESS TREATMENT

Four seedlings were selected randomly for each maize variety seedlings. Different parameters, root and shoot length, seedling height, fresh and dry weight, were measured before the maize seedlings were subjected to different sodium chloride treatments. A ruler was used to measure root and shoot lengths as well as the seedling height. For each maize seedling, the roots were measured to find the total lengths and the mean length was recorded. The fresh weight of the maize seedlings was also measured on a balance. All the data was collected and noted down for each maize seedling variety. Plastic bottle lids were cut using a surgical blade to form small circular openings of about two cm in diameter. The plastic bottles were labeled according to maize variety and respective saline concentrations. Each maize variety had four seedlings for each concentration. Maize seedling roots were made to immerse into the saline solution with their leaves hanging out of the plastic bottle through the circular opening on the lid. The plastic bottles with the maize seedlings were then placed on laboratory desks and were left for six days.

Figure 2: Plastic bottles with different saline concentrations and maize seedling varieties

3.8 ASSESSMENT OF PARAMETERS AFTER STRESS TREATEMENT

On the sixth day, the following parameters were assessed after the seedlings were removed from the treatments and were dried using paper towels. Root and shoot lengths were measured using a ruler. Root length for each maize seedling was recorded after the mean of the roots on the seedling were measured. Seedling height was also measured using a ruler. Seedling fresh and dry weight were measured on a balance and the recordings were noted down. For dry weight, seedlings were placed in a drying oven at 60 ^{O}C for five hours and were weighed immediately. The differences between the data which were recorded initially before treatment and after treatment of root and shoot length, seedling height and fresh weight were recorded to be the final data.

3.9 DATA ANALYSIS

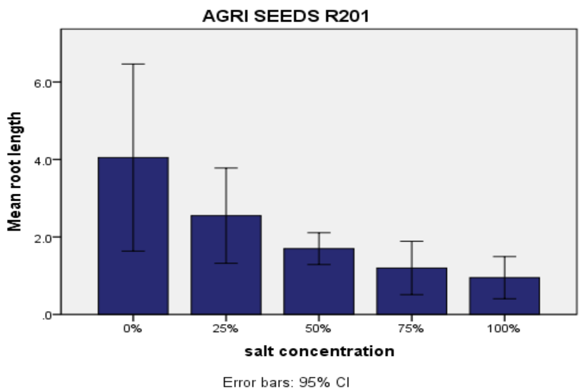
One-Way Analysis of variance (ANOVA) was conducted to analyse data in SPSS. The predictor variables were the different concentrations of sodium chloride and the response variables were root and shoot length, seedling height, dry and fresh weight of the maize varieties Agri Seeds R201, SC 513 and SC 403. Tukey tests and Multiple Comparisons were done to locate differences among varieties where ANOVA showed significant differences. Dunnet's test was also conducted to compare each of the sodium chloride concentrations with the control treatment.

CHAPTER 4

4.1 RESULTS

4.2 EFFECT OF SALT CONCENTRATION ON MAIZE ROOT LENGTH

Means in root lengths ranged from Agri Seeds R201 (0.950±01708; 4.050±0.7588), SC 513 (0.9500±3.8500; 3.8500±0.43493) to SC 403 (1.050±0.1708 ; 2.800±0.4690). Root lengths differed significantly among maize varieties (ANOVA, $p < 0.05$). Maize variety SC 403 had the least salt concentration mean difference compared to other maize varieties which had significant difference (Tukey, Multiple Comparisons). Seedlings of maize varieties were salt tolerant and had general long root lengths at 25% salt concentration compared to 50, 75 and 100%. Salt concentrations 25, 50, 75 and 100% had a significant mean difference when compared to the control treatment (0%) for root lengths of all maize varieties (Dunnett's test, Multiple Comparisons).



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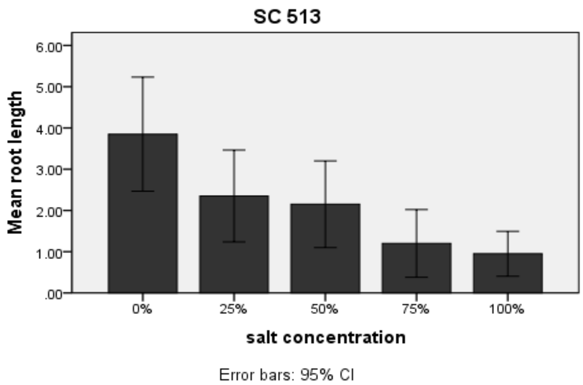

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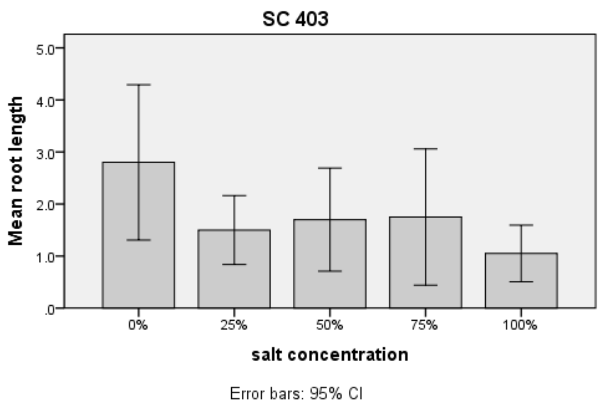

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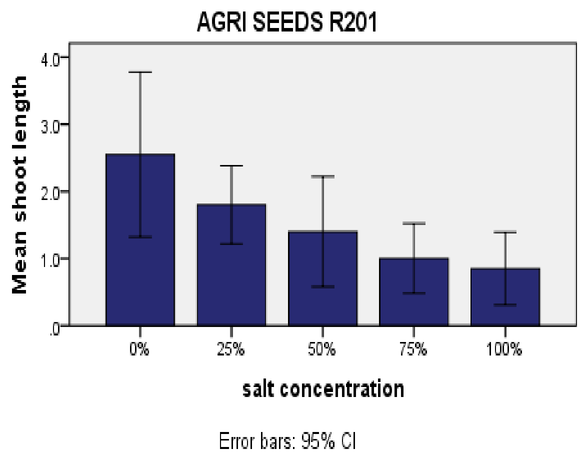
Figure 3: Effect of salt concentration on root length of maize varieties a) Agri Seeds R201, b) SC 513 and c) SC 403, where an increase in salt concentration resulted in a decrease in the length of roots in all maize varieties

4.3 EFFECT OF SALT CONCENTRATION ON MAIZE SHOOT LENGTH

Means in shoot length ranged from Agri Seeds R201 (0.850±0.1708 ; 2.550±0.3862), SC 513 (0.9000±0.12910 ; 2.8000±0.66833) to SC 403 (1.600±0.3916 ; 3.050±0.7411). There were statistically significant differences in shoot lengths among three maize varieties (ANOVA, $p < 0.05$). Maize varieties Agri Seeds R201 and SC 513 had significant salt concentrations mean differences except for SC 403 (Tukey, Multiple Comparisons). Highest shoot lengths of maize varieties seedlings were observed at 0 and 25% of the salt concentration compared to other salt concentrations. Salt concentrations 25, 50, 75 and 100% had a significant mean difference when compared to the control treatment (0%) for shoot lengths of maize varieties Agri Seeds R201 and SC 513 (Dunnett's test, Multiple Comparisons).



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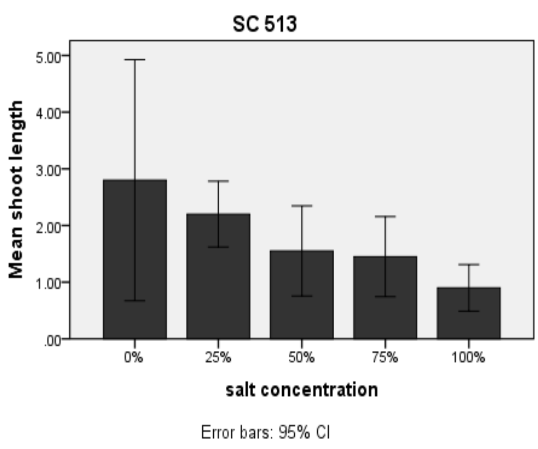

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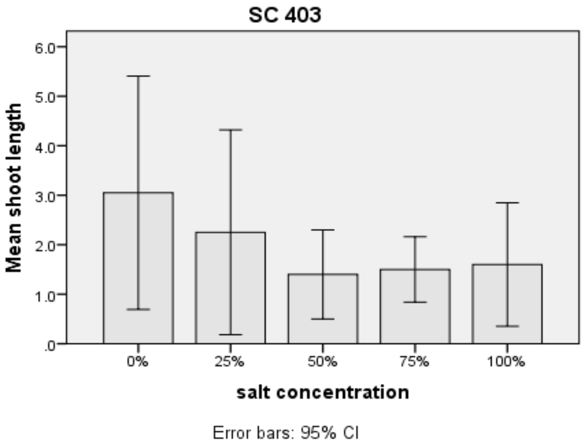

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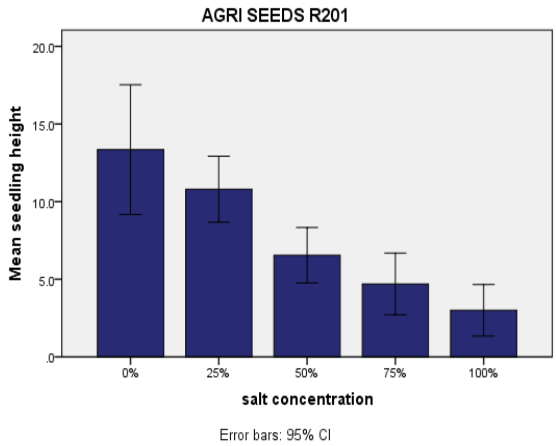
Figure 4: Effect of salt concentration on shoot length of maize varieties a) Agri Seeds R201, b) SC 513 and c) SC 403, an increase in salt concentration resulted in decreased shoot lengths in all maize varieties

4.4 EFFECT OF SALT CONCENTRATION ON MAIZE SEEDLING HEIGHT

Mean seedling height of maize varieties in different salt concentrations ranged from, Agri Seeds R201 (3,000±0,5228 ; 13.350±1.3124), SC 513 (4.000±0,82057 ; 15.4500±1.03401) to SC 403 (5.150±1.1786 ; 12.300±0.8963). There were statistically significant differences in seedling heights between Agri Seeds R201 and SC513 (ANOVA, $p = 0.000$) and SC 403 (ANOVA, $p = 0.002$). Seedling height for all maize varieties had significant mean differences across all salt concentrations (Tukey, Multiple comparisons). Salt concentration of 0 and 25% favoured high seedling height in all maize varieties with less seedling height recorded at 100% salt concentration. Salt concentrations 25, 50, 75 and 100% had a significant mean difference when compared to the control treatment for seedling heights of all maize varieties (Dunnett's test, Multiple Comparisons).



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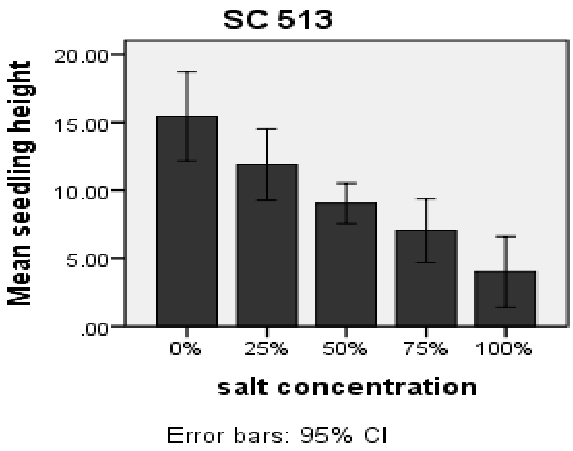

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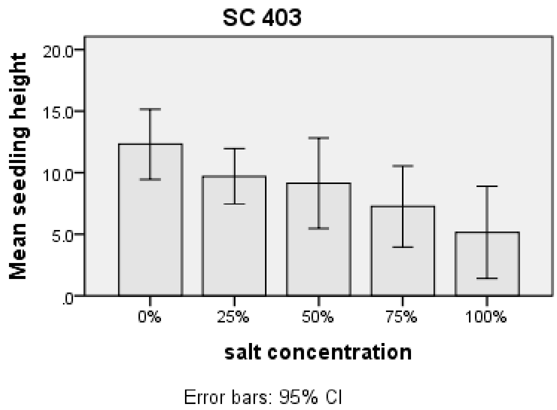

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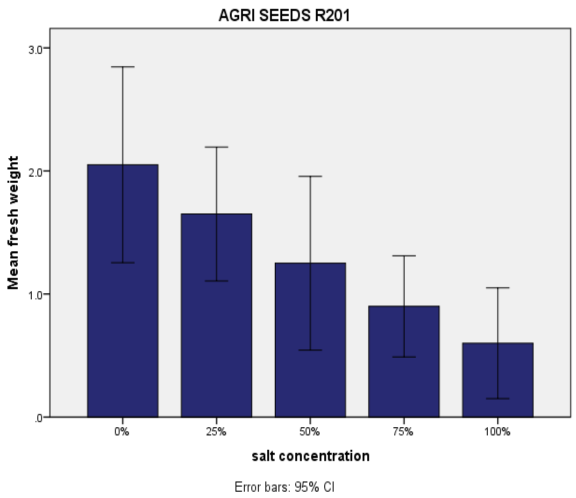
Figure 5: Effect of salt concentration on seedling height of maize varieties a) Agri Seeds R201, b) SC 513 and c) SC 403, an increase in salt concentration resulted in a general decrease in seedling heights in all maize varieties

4.5 EFFECT OF SALT CONCENTRATION ON MAIZE FRESH WEIGHT

Mean fresh weight of maize varieties in different salt concentrations ranged from, Agri Seeds R201 (0.600±0.1414 ; 2.050±0.2500), SC 513 (0.8500±0.17078 ; 2.5000±0.26458) to SC 403 (0.600±0.1826 ; 2.650±0.3304). There were statistically significant differences in fresh weight among three maize varieties (ANOVA, $p < 0.05$). Fresh weight for all maize varieties had significant mean differences across all salt concentrations (Tukey, Multiple comparisons). Seedlings of maize varieties were salt tolerant and had high fresh weight recorded at 0 and 25% of the salt concentration and the lowest at 100%. Salt concentrations 25, 50, 75 and 100% had a significant mean difference when compared to the control treatment for fresh weight of all maize varieties (Dunnett's test, Multiple Comparisons).



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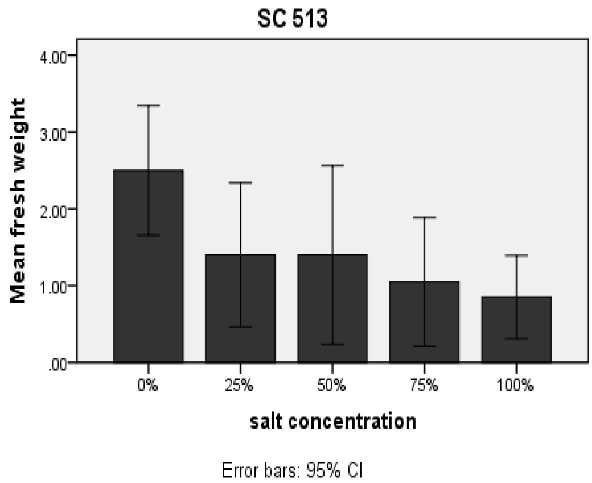

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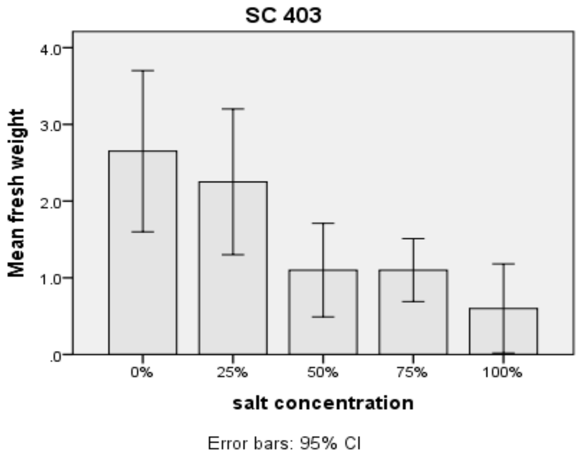

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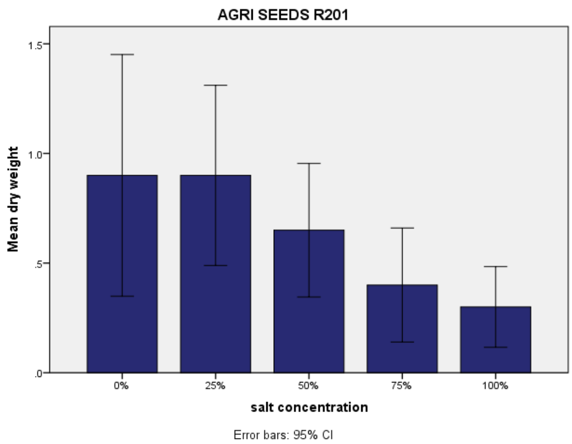
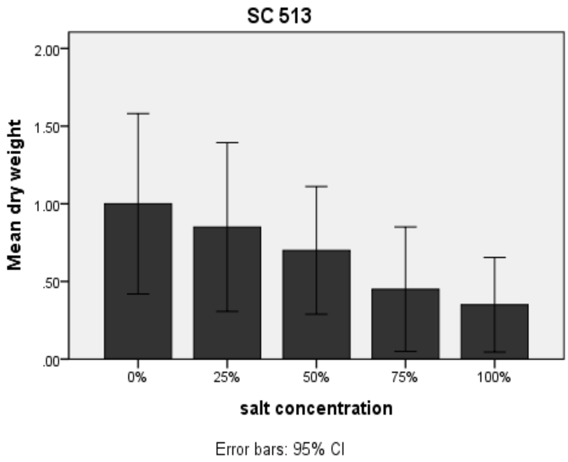
Figure 6: Effect of salt concentration on fresh weight of maize varieties a) Agri Seeds R201, b) SC 513 and c) SC 403, an increase in salt concentration resulted in a decrease in fresh weights of maize varieties

4.6 EFFECT OF SALT CONCENTRATION ON MAIZE DRY WEIGHT

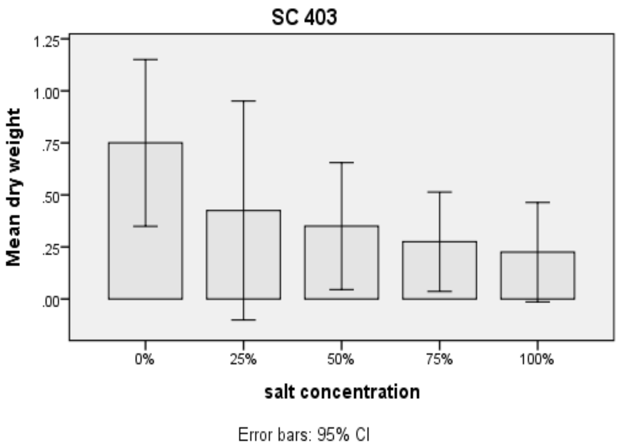
Mean dry weight of maize varieties in different salt concentrations ranged from Agri Seeds R201 (0.300±0.0577 ; 0.900±0.1732), SC 513 (0.3500±0.09574 ; 1.0000±0.18257) to SC 403 (0.225±0.0750 ; 0.750±0.1258). There were statistically significant differences in fresh weight among three maize varieties (ANOVA, $p < 0.05$). Dry weight for all maize varieties had significant mean differences (Tukey, Multiple comparisons). Maize varieties dry weight was high with 0 and 25% salt concentration compared to other high salt concentrations. Salt concentrations 25, 50, 75 and 100% had a significant mean difference when compared to the control treatment (0%) for dry weight of all maize varieties (Dunnett's test, Multiple Comparisons).



a)



b)



c)

Figure 7: Effect of salt concentration on dry weight of maize varieties a) Agri Seeds R201, b) SC 513 and c) SC 403, an increase in salt concentration resulted in a decrease in dry weights of maize varieties

CHAPTER 5

5.0 DISCUSSION

This study was aimed at investigating the response of maize seedlings of varieties SC 403, SC 513 and Agri Seeds R201 to different concentrations of sodium chloride. The optimum salt concentration favouring growth of maize seedlings varieties was determined by measuring different parameters, which were root and shoot length, seedling height, fresh and dry weight.

Results showed that there were significant mean differences of salt concentrations on root lengths of maize varieties Agri Seeds R201, SC 513 and SC 403. Sodium chloride concentration of 25% significantly enhanced root length compared to 100%, hence for all maize varieties root length decreased with an increase in sodium chloride concentrations. The increase of salt concentration caused a significant decrease in the growth of shoot lengths of the maize varieties Agri Seeds R201, SC 513 and SC 403 (Figure 4). For all maize varieties, shoot length was more pronounced with the contol treatment (0%) and 25% sodium chloride concentration and less at 50, 75 and 100% of sodium chloride concentration. Results also showed that maize variety SC 513 had the lowest reduction in seedling height when exposed to different concentrations of salt stress compared to Agri Seeds R201 and SC 403. There were significant mean differences when 0%, the control treatment, was compared to the sodium chloride concentrations. Increase in salinity from 0% to 25% had no effect on all maize varieties fresh weights, while further increase from 50% onwards significantly reduced fresh weight. Dry weights of maize varieties Agri Seeds R201, SC 513 and SC 403, decreased with increased levels of salt stress. The obtained results mean that seedlings of maize varieties were variably affected in growth and development at 25% salt concentration. This is the optimal level of salt concentration compared to higher salt concentrations 50, 75 and 100%. Seedlings of maize varieties Agri Seeds R201, SC 513 and SC 403 were less tolerant to high salt concentrations, hence there was a general reduction on growth and development of the maize seedlings.

Previous studies done by Nizam (2011) also showed that root length decreased with increase in sodium chloride concentrations. The significant differences observed among maize varieties Agri Seeds R201, SC 513 and SC 403and salinity levels with respect to root length is similar to the study of Hoque (2015). According to Jamil (2006), reduction in maize root lengths may be due to toxic effects of the sodium chloride used as well as unbalanced nutrient uptake by the seedlings. All the maize varieties had decreased shoot growth with increased sodium chloride concentration (Pessarakli and Kopec, 2009). According to Bose (2018), the reduction in shoot length growth was due to excessive accumulation of salts in the cell wall elasticity. The increase of salinity concentration led to a decrease in maize seedling heights, which is comparable to the results obtained by Mohmmad (2018). Osmotic differences is the reason why maize seedlings growth reduced, as lower salinity levels positively influenced solutes to readily cross the cell membranes into the cytoplasm of the cells but at highly salinity levels active metabolic pumps prevented accumulation of ions (Mensah, 2015). Reduction in maize varieties seedling fresh weights can be attributed to water deficits as established by Mensah (2015). The presence of salt in solution reduces the ability of the maize seedlings to take up water leading to reduction in maize fresh weight and growth (Ahmad, 2011). Bose (2018) reported that dry weight of maize varieties declined with increasing salinity. Reduction in dry weight of maize varieties reflects salt impact on tissues and attainment of maximum salt concentration tolerated by the maize seedling (Jamil, 2006).

The study had its limitations, but they did not affect the final results. Maize varieties seedling roots seemed to fall off in the process of removing the seedlings from the plastic pots after seed germination and also during measuring them in the laboratory. This did not affect the final results as the maize seedlings were handled carefully during the experiment. Another limitation of the study was that of the incubation period of the maize seedlings in sodium chloride concentration. Maize seedlings subjected in high sodium chloride concentration seemed to be stunted or die to the extent of not being able to measure the physiological parameters or they would produce a much negative result. This did not affect the final result as the incubation period of maize seedlings in sodium chloride was reduced from ten to six days.

In conclusion, the study showed that increase in salt stress decreased maize varieties' root and shoot lengths growth, seedling height, fresh and dry weights due to excessive accumulation of salts in the cells as well as decrease in osmotic potential. Considering the retardatory effects of sodium chloride on the growth of maize seedlings, plant breeding is a complementary and a more permanent approach for minimizing the effects of salinity, with the development of maize varieties that can grow and produce economic yield under saline conditions. The investigated maize varieties Agri Seeds R201, SC 513 and SC 403, can be further used to develop new lines for salt tolerance. The development of bioinformatics tools and high-throughput platforms will boost the acquisition of physiological data, with consequent benefits to research on salinity tolerance in maize varieties.

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APPENDICES

APPENDIX 1: MEANS AMONG MAIZE VARIETIES IN DIFFERENT SALT CONCENTRATIONS

1a. AGRI SEEDS R201

i. Root length

Salt concentration (%)	Mean ± SE
0	4.050±0.7588
25	2.550±0.3862
50	1.700±0.1291
75	1.200±0.2160
100	0.950±0.1780

ii. Shoot length

Salt concentration (%)	Mean ± SE
0	2.550±0.3862
25	1.800±0.1826
50	1.400±0.2582
75	1.000±0.1633
100	0.850±0.1708

iii. Seedling height

Salt concentration (%)	Mean ± SE
0	13.350±1.3124
25	10.800±0.6683
50	6.550±0.5620
75	4.700±0.6245
100	3.000±0.5228

iv. Fresh weight

Salt concentration (%)	Mean ± SE
0	2.050±0.2500
25	1.1650±0.1708
50	1.250±0.2217
75	0.900±0.1291
100	0.600±0.1414

v. Dry weight

Salt concentration (%)	Mean ± SE
0	0.900±0.1732
25	0.900±0.1291
50	0.650±0.0957
75	0.400±0.0816
100	0.300±0.0577

1b. SC 513

i. Root length

Salt concentration (%)	Mean ± SE
0	3.8500±0.43493
25	2.3500±0.3500
50	2.1500±0.33040
75	1.200±0.25820
100	0.9500±0.17078

ii. Shoot length

Salt concentration	Mean ± SE
0	2.8000±0.66833
25	2.2000±0.18257
50	1.5500±0.2500
75	1.4500±0.22174
100	0.9000±0.2910

iii. **Seedling height**

Salt concentration (%)	Mean ± SE
0	15.4500±1.03401
25	11.9000±0.82260
50	9.0500±0.46458
75	7.0500±0.74106
100	4.0000±0.82057

iv. **Fresh weight**

Salt concentration (%)	Mean ± SE
0	2.5000±0.26458
25	1.4000±0.29439
50	1.4000±0.36515
75	1.0500±0.26300
100	0.8500±0.17078

v. **Dry weight**

Salt concentration (%)	Mean ± SE
0	1.0000±0.18257
25	0.8500±0.17078
50	0.7000±0.12910
75	0.4500±0.12583
100	0.3500±0.09574

1c. SC 403

i. **Root length**

Salt concentration (%)	Mean ± SE
0	2.800±0.4690
25	1.500±0.2082
50	1.700±0.3109
75	1.750±0.4113
100	1.050±0.1708

ii. Shoot length

Salt concentration (%)	Mean ± SE
0	3.050±0.7411
25	2.250±0.6500
50	1.400±0.2828
75	1.500±0.2082
100	1.600±0.3916

iii. Seedling height

Salt concentration (%)	Mean ± SE
0	12.300±0.8963
25	9.700±0.7047
50	9.150±1.1587
75	7.250±1.0340
100	5.150±1.1786

iv. Fresh weight

Salt concentration (%)	Mean ± SE
0	2.650±0.3304
25	2.250±0.2986
50	1.100±0.1915
75	1.100±0.1291
100	0.600±0.1826

v. Dry weight

Salt concentration (%)	Mean ± SE
0	0.750±0.1258
25	0.425±0.1652
50	0.350±0.0957
75	0.275±0.0750
100	0.225±0.0750

APPENDIX 2: ANOVA SPSS OUTPUTS

2a. AGRI SEEDS R201

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
root length	Between Groups	25.188	4	6.297	9.628	.000
	Within Groups	9.810	15	.654		
	Total	34.998	19			
shoot length	Between Groups	7.492	4	1.873	7.676	.001
	Within Groups	3.660	15	.244		
	Total	11.152	19			
seedling height	Between Groups	295.772	4	73.943	29.358	.000
	Within Groups	37.780	15	2.519		
	Total	333.552	19			
fresh weight	Between Groups	5.348	4	1.337	9.415	.001
	Within Groups	2.130	15	.142		
	Total	7.478	19			
dry weight	Between Groups	1.232	4	.308	5.848	.005
	Within Groups	.790	15	.053		
	Total	2.022	19			

2b. SC 513

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
root length	Between Groups	21.040	4	5.260	12.726	.000
	Within Groups	6.200	15	.413		
	Total	27.240	19			
shoot length	Between Groups	8.612	4	2.153	4.424	.015
	Within Groups	7.300	15	.487		
	Total	15.912	19			
seedling height	Between Groups	310.468	4	77.617	30.470	.000
	Within Groups	38.210	15	2.547		
	Total	348.678	19			
fresh weight	Between Groups	6.508	4	1.627	5.237	.008
	Within Groups	4.660	15	.311		
	Total	11.168	19			
dry weight	Between Groups	1.172	4	.293	3.516	.032
	Within Groups	1.250	15	.083		
	Total	2.422	19			

2c. SC 403

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
root length	Between Groups	6.628	4	1.657	3.710	.027
	Within Groups	6.700	15	.447		
	Total	13.328	19			
shoot length	Between Groups	7.708	4	1.927	1.930	.158
	Within Groups	14.980	15	.999		
	Total	22.688	19			
seedling height	Between Groups	115.468	4	28.867	7.074	.002
	Within Groups	61.210	15	4.081		
	Total	176.678	19			
fresh weight	Between Groups	12.028	4	3.007	13.189	.000
	Within Groups	3.420	15	.228		
	Total	15.448	19			
dry weight	Between Groups	.687	4	.172	3.379	.037
	Within Groups	.763	15	.051		
	Total	1.450	19			

APPENDIX 3: TUKEY, MULTIPLE COMPARISONS

3a. AGRI SEEDS R201

Multiple Comparisons

Tukey HSD

Dependent variable	**(I)Salt concentration (%)**	**(J)Salt concentration (%)**	**Mean difference (I-J)**	**Sig.**
Root length	0	50	2.3500*	0.007
		75	2.8500*	0.001
		100	3.1000*	0.001
Shoot length	0	50	1.1500*	0.034
		75	1.5500*	0.004
		100	1.7000*	0.002
Seedling height	0	50	6.8000*	0.000
		75	8.6500*	0.000
		100	10.3500*	0.000
	25	50	4.2500*	0.013
		75	7.8000*	0.001
		100		0.000
	50	100	3.5500*	0.043
Fresh weight	0	75	1.1500*	0.005
		100	1.4500*	0.001
	25	100	1.0500*	0.010
Dry weight	0	100	0.6000*	0.016
	25	100	0.0600*	0.016

The mean difference is significant at the 0.05 level

3b. SC 513 Tukey HSD

Dependent variable	**(I)Salt concentration (%)**	**(J)Salt concentration (%)**	**Mean difference (I-J)**	**Sig.**
Root length	0	25	1.50000*	0.034
		50	1.70000*	0.014
		75	2.65000*	0.000
		100	2.90000*	0.000
Shoot length	0	100	1.90000*	0.012
Seedling height	0	25	3.55000*	0.045
		50	6.40000*	0.000
		75	8.40000*	0.000
		100	11.45000*	0.000
	25	75	4.85000*	0.005
		100	7.90000*	0.000
	50	100	5.05000*	0.003
Fresh weight	0	75	1.45000*	0.016
		100	1.65000*	0.006
Dry weight	0	100	0.65000*	0.042

The mean difference is significant at the 0.05 level

3c. SC 403

Dependent variable	**(I)Salt concentration (%)**	**(J)Salt concentration (%)**	**Mean difference (I-J)**	**Sig.**
Root length	0	100	1.7500*	0.015
Seedling height	0	75	5.0500*	0.021
		100	7.1500*	0.001
	25	100	4.5500*	0.042
Fresh weight	0	50	1.5500*	0.003
		75	1.1500*	0.003
		100	2.0500*	0.000
	25	50	1.1500*	0.027
		75	1.1500*	0.027
		100	1.6500*	0.002
Dry weight	0	100	0.5250*	0.034

The mean difference is significant at the 0.05 level

APPENDIX 4: DUNNETT'S TEST, MULTIPLE COMPARISONS

4a. AGRI SEEDS R201

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) saltconcentrations	(J) saltconcentrations	Mean Difference (I-J)	Std. Error	Sig.	9
						L
root length	25%	0%	-1.5000	.5718	.061	-
	50%	0%	-2.3500*	.5718	.003	-
	75%	0%	-2.8500*	.5718	.001	-
	100%	0%	-3.1000*	.5718	.000	-
shoot length	25%	0%	-.7500	.3493	.145	-
	50%	0%	-1.1500*	.3493	.017	-
	75%	0%	-1.5500*	.3493	.002	-
	100%	0%	-1.7000*	.3493	.001	-
seedling height	25%	0%	-2.5500	1.1222	.116	-
	50%	0%	-6.8000*	1.1222	.000	-
	75%	0%	-8.6500*	1.1222	.000	-
	100%	0%	-10.3500*	1.1222	.000	-
fresh weight	25%	0%	-.4000	.2665	.400	-
	50%	0%	-.8000*	.2665	.029	-
	75%	0%	-1.1500*	.2665	.002	-
	100%	0%	-1.4500*	.2665	.000	-
dry weight	25%	0%	.0000	.1623	1.000	-
	50%	0%	-.2500	.1623	.378	-
	75%	0%	-.5000*	.1623	.025	-
	100%	0%	-.6000*	.1623	.007	-

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

4b. SC 513

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) salt concentration	(J) salt concentration	Mean Difference (I-J)	Std. Error	Sig.	95%
						Low
root length	25%	0%	-1.50000*	.45461	.016	-2.7
	50%	0%	-1.70000*	.45461	.007	-2.9
	75%	0%	-2.65000*	.45461	.000	-3.8
	100%	0%	-2.90000*	.45461	.000	-4.1
shoot length	25%	0%	-.60000	.49329	.573	-1.9
	50%	0%	-1.25000	.49329	.072	-2.5
	75%	0%	-1.35000*	.49329	.049	-2.6
	100%	0%	-1.90000*	.49329	.005	-3.2
seedling height	25%	0%	-3.55000*	1.12857	.022	-6.6
	50%	0%	-6.40000*	1.12857	.000	-9.4
	75%	0%	-8.40000*	1.12857	.000	-11
	100%	0%	-11.45000*	1.12857	.000	-14
fresh weight	25%	0%	-1.10000*	.39412	.044	-2.1
	50%	0%	-1.10000*	.39412	.044	-2.1
	75%	0%	-1.45000*	.39412	.008	-2.5
	100%	0%	-1.65000*	.39412	.003	-2.7
dry weight	25%	0%	-.15000	.20412	.873	-.70
	50%	0%	-.30000	.20412	.417	-.85
	75%	0%	-.55000	.20412	.053	-1.1
	100%	0%	-.65000*	.20412	.021	-1.2

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

4c. SC 403

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) salt concentration	(J) salt concentration	Mean Difference (I-J)	Std. Error	Sig.	95
						Lo
root length	25%	0%	-1.3000*	.4726	.048	-2.5
	50%	0%	-1.1000	.4726	.105	-2.3
	75%	0%	-1.0500	.4726	.127	-2.3
	100%	0%	-1.7500*	.4726	.007	-3.0
shoot length	25%	0%	-.8000	.7066	.629	-2.7
	50%	0%	-1.6500	.7066	.104	-3.5
	75%	0%	-1.5500	.7066	.133	-3.4
	100%	0%	-1.4500	.7066	.170	-3.3
seedling height	25%	0%	-2.6000	1.4284	.249	-6.4
	50%	0%	-3.1500	1.4284	.131	-7.0
	75%	0%	-5.0500*	1.4284	.010	-8.9
	100%	0%	-7.1500*	1.4284	.001	-11
fresh weight	25%	0%	-.4000	.3376	.594	-1.3
	50%	0%	-1.5500*	.3376	.001	-2.4
	75%	0%	-1.5500*	.3376	.001	-2.4
	100%	0%	-2.0500*	.3376	.000	-2.9
dry weight	25%	0%	-.3250	.1594	.174	-.76
	50%	0%	-.4000	.1594	.075	-.83
	75%	0%	-.4750*	.1594	.031	-.91
	100%	0%	-.5250*	.1594	.017	-.96

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.