

Chlorophyll Content of Leaves and Salt Ions (Na^+ and Cl^-) Content in the Seeds of *Zea mays* L. Treated with Different Concentrations of Salt (NaCl)

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Abstract

This study which aims at investigating the chlorophyll content of leaves and Salt ions (Na^+ and Cl^-) content in the seeds of *Zea mays* L. treated with different concentrations of salt (NaCl) as plants are often faced with salt stress. This study was carried out at the Botanical Garden of the Department of Plant Science and Biotechnology (PSB) in Rivers State University (RSU), Port Harcourt having latitude and longitude of (4.804°N and 6.980°E), annual rainfall of (200.45mm) and annual temperature range of (25°C-27°C), Soil used for the study was collected from the aforementioned study area. Maize seeds (SAMMAZ-51) were subjected to viability test and viable seeds were sown into polybags filled with 15kg of soil at the rate of 2-seeds per bag having 4 treatments (10mM-T1, 30mM-T2, 50mM-T3, Control-T4) replicated 8-times and arranged in a Completely Randomized Design (CRD). Treatments were applied at 2-days interval and plant was allowed to grow for a period of two months before taking samples to the laboratory for chlorophyll and salt ion contents using standard methods. Chlorophyll content for Month 1 and 2 revealed T4 to have highest content (1137ug/g and 1973ug/g) while T2 had least content (724ug/g) at month 1 and T1 had least content (1046 ug/g) at month 2. For the salt ions content, T3 recorded highest values (359mg/kg and 400mg/kg) for sodium and chloride contents respectively, with T4 recording least values (222mg/kg and 269mg/kg) respectively for chloride and sodium contents. This study reveals that salt stress has an impact in the chlorophyll content and salt ion compositions of the different parts of maize plant studied with an increase in NaCl contents.

Keywords: Maize, Salinity Stress, Photosynthetic Pigments, Chloride Accumulation, Sodium Accumulation

Introduction

Plants are often faced with salt stress which is as a result of excessive accumulation of sodium chloride (NaCl) contents in the soil. Salinity is a major abiotic stressor reducing global maize productivity with NaCl being the most prevalent form. Numerous studies have shown that plant growth, development and proximate compositions are affected by NaCl compositions at different concentrations (Memon *et al.*, 2010). These salt stress increases the intracellular osmotic pressure causing a toxic level of accumulation of sodium (Zhao *et al.*, 2021). Salt stress which is due to over-accumulation of NaCl is usually of great concern

and most injurious factor to plants. These saline soils are widespread on earth, sometimes they occur naturally or by man through intensive use of water resources, high evaporation rate and various human activities (Lambers, 2003; Arzani, 2008). Despite the essentiality of chloride as a micronutrient for higher plants and sodium as mineral nutrients for halophytes, salt accumulation may reduce local biodiversity, limit growth and development of plants as salt stress causes reduction in vegetative growth, photosynthetic rate (Neto *et al.*, 2004) and also water availability and nutrient uptake imbalance are affected by salt stress with seed germination inhibition due to ionic disturbance, osmotic and toxic effects (Turkmen *et al.*, 2002). The salt causes the wilting of the leaves even when moisture content is present and causes adverse effects in form of physiological dysfunction and decrease in yield of a crop (Zahra *et al.*, 2018). While chlorophyll decline is well-documented across vegetative stages, fewer studies track ion partitioning specifically into mature maize seeds under varying NaCl treatments. Most work examines germination rather than harvest stage content. This study addresses that gap by simultaneously quantifying leaf chlorophyll and seed ion content in the same plant.

Statement of the Research Problem

Soil salt stress is a problem in the world, which is one of the main limiting factors that hinder the production of maize. Salt stress significantly affects the growth and development of maize (Amira, 2011). Many researchers have conducted studies to assess salt concentrations on *Amaranthus hybridus* L. and *Celosia argentea* L. growth and reported decline in germination percentage with an increase in NaCl concentrations (Amukali *et al.*, 2014). Amira (2011) also reported that agronomic trait of plants decreased with increasing salt concentrations and salt stress also reduces chlorophyll, carotenoids and protein contents of maize.

Justification of the Study

The lack of information and research on the effect of salt stress on the photosynthetic pigments and sodium and chloride accumulation of maize grown in Port Harcourt, Rivers State has prompted this research which will provide data on the effect of salt stress on the chlorophyll content and sodium and chloride accumulation of maize grown in Port Harcourt, Rivers State.

Aim and Objectives of the Study

This study aims at assessing the chlorophyll content of leaves and the salt ions (Na^+ and Cl^-) content in the seeds of *Zea mays* L. treated with different concentrations of salt (NaCl). Specifically, the research sought:

1. To assess the chlorophyll content on the leaves of treated maize.
2. To determine the Cation and Anion composition of treated maize

Research Hypotheses

This study was designed to assess the following hypothesis regarding chlorophyll content of leaves and the salt ions (Na^+ and Cl^-) content in the seeds of *Zea mays* L. treated with different concentrations of salt (NaCl):

Null hypothesis:

- i. There is no significant difference in the chlorophyll content in leaves of maize treated with different NaCl concentrations.
- ii. There is no significant difference in the salt ions (Na^+ and Cl^-) in maize seeds among plants treated with different concentrations NaCl.

Alternate hypothesis:

- i. The chlorophyll content of maize leaves decreases with increasing NaCl concentrations.
- ii. The salt ions (Na^+ and Cl^-) contents in the seeds of maize increases significantly with increased NaCl concentrations.

Literature Review

Maize is an annual, staple food crop which is a native of south and Central America (Olaniyi and Adewale, 2012), but cultivated extensively by farmers in other countries around the world. It is a crop that is cultivated in a well-drained soil in both tropical and temperate regions (Kumar *et al.*, 2013; Dowsell, 2019). The plant is known to be one of the major cereal food crops cultivated widely as it is an important source for animal feed, industrial resources and as food for human consumption (Quan *et al.*, 2020). Maize is slowly becoming an industrial important crop (Olaniyan, 2015) as it is been used in recent times in the production of ethanol (Antar *et al.*, 2021). Kumar and Jhariya (2013) reported that Nigeria is the largest producer of maize amongst other African countries, making her one of the major exporters of maize to continuously aid in combating food shortage and in feeding the fast-growing population across the country. Maize being one of the most important grain crops cultivated around the world with other crops like cowpea, millet, soybean, rice and sorghum (Alabi, 2006) with its production rising as indicated by food and agricultural organization (FAO) data as the plant has the ability to thrive under different ecological zones and environmental conditions (Adiaha, 2018). Various factors affect crop yields which can be classified into abiotic and biotic constraints. The abiotic constraint includes soil properties (soil components, pH, physiochemical and biological properties) and climatic stresses (cold, drought, flood, heat and salinity), on the other hand; biotic constraints include beneficial organisms (pollinators, decomposers and natural enemies), pests (arthropods, pathogens, weeds, vertebrate pests) and anthropogenic evolution.

Salinity which refers to the amount of salt in the soil. Salinity stress affects crop production in over 30% of irrigated crops and 7% of dry land agriculture worldwide (Schroeder *et al.*, 2013). It is one of the major problems affecting crop production all over the world since

around 20% of cultivated land and 33% of irrigated land are salt affected in the world (Machado and Serralheiro, 2017).

Salt stress causes osmotic stress and ionic toxicity in crop plants. Maize which is one of the most important crops in the world is moderately sensitive to salinity (Farooq *et al.*, 2015), just like other plants; salinity negatively affects a maize plant's relative growth rate, osmotic status, transpiration, ion transport, photosynthetic activity and senescence (Negrao *et al.*, 2017; Zhong *et al.*, 2020). Huo *et al.*, (2020) stated that an excessive accumulation of salt in maize plant cell can produce reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radicals and superoxide anions, all of which inhibits photosynthetic activity.

In maize plant cells, salt-induced osmotic effects alter the general metabolic processes and activity levels of enzymes, leading to an excessive accumulation of ROS, which results to a rise in oxidative stress (Ali *et al.*, 2021). When plants are exposed to salt stress during its development, its root system being the first organ to sense the signal of stress is incited to adapt its morphological structure to counteract the unfavourable environmental condition it is exposed to (Kong *et al.*, 2016).

In addition to these morphological adaptations, maize plants also develop biochemical responses to salt stress as many studies confirmed the inhibitory effects of salinity on biochemical processes of which photosynthetic activity is the most important. The salinity effect on the photosynthesis can be gauged using the effects on the photosynthetic pigment as specific studies of (Misra *et al.*, 2006; Tafouo, 2010) who indicated that salinity reduced the content of photosynthetic pigments in treated plants.

Effects of Salt Stress

Salt stress affects growth and development of maize, however, plants response to salinity varies with the degree of stress and crop growth stage. The short-term exposure of maize plants to salt stress influences plant growth owing to osmotic stress in the first phase of salt stress (Sumer *et al.*, 2004).

In salt-affected soils, excess amount of sodium and chloride ions in the rhizosphere leads to nutritional imbalances in maize due to strong interference of these ions with the essential mineral elements such as potassium, calcium, nitrogen, phosphorus, magnesium, iron, manganese, copper, and zinc (Hasegawa *et al.*, 2000; Karimi *et al.*, 2005; Turan *et al.*, 2010). Salt stress reduces the uptake and absorption of nitrogen, potassium, calcium, magnesium, and iron (Turan *et al.*, 2010; Kaya *et al.*, 2010; Shahzad *et al.*, 2012; Qu *et al.*, 2012; Yasmeen *et al.*, 2013). Sodium which is the principal toxic ion affecting maize (Sümer *et al.*, 2004) interferes with potassium uptake and transport leading to disturbance in stomatal balance and causing water loss and necrosis.

Materials and Methods

Experimental Site and Soil Collection: The study was conducted at the Department of Plant Science and Biotechnology, Botanical Garden, Rivers State University, Port Harcourt,

River State and Loam soil for the study was also obtained from the above study area. 15kg of soil was bagged into a polybag.

Collection and Preparation of Salt Concentrations: Salt (Sodium Chloride-NaCl, 1500grams) was purchased from JoeChem Ventures, UNIPORT Road, Port Harcourt, Rivers State and brought Rivers State University, to the Department of Plant Science and Biotechnology laboratory for preparation of different salt concentrations to be used as treatments (10mM, 30mM and 50mM) using the methods as described by Aicha *et al.*, (2022).

Source and Sowing of Seed: The seeds of maize (SAMMAZ 51) were obtained from Rivers State Ministry of Agriculture, Rivers State. The seeds of maize (SAMMAZ 51) were sown into polythene bags (55 x 49 x 49 cm) containing 15kg of sandy-loam soil at the rate of two seeds per bag.

Experimental Design and Layout: The maize seeds planted in the polythene bags with four treatments (Control, 10mM, 30mM and 50mM) which was replicated eight times and arranged in a Completely Randomized Design in the open field giving a sample size of 32 experimental units. The treatments were applied every 2 days for the period of two months before harvest was done and weeding and earthing was done when necessary.

Determination of Total Chlorophyll: The method of described by Lichtenthaler and Buschmann (2001) was adopted. The chlorophyll was extracted with aqueous acetone, transferred into ether and the optical density measured at 660nm and 643nm.

Sodium and Chloride Test

Salinity Measurement by Silver Nitrate Titration: All glasswares were washed and rinsed with enough distilled water. 8.243gm of dried NaCl should be dissolved in 500mls-distilled water. 50ml of this solution was diluted to 1000mls-distilled water to give 0.05mg CT.

Standard Silver Nitrate: 2.396gm silver nitrate in 1 litre distilled water. Each ml is equivalent to 0.50mg CT. Standardize Silver Nitrate against the Sodium Chloride. Potassium chromate: 8gm of Potassium chromate in 100ml distilled water.

Calculation:

Salinity mg/l = $1.805 \times \text{Chloride value} + 0.03$

Sodium Ion

589nm wavelength was selected. Air and gas pressure were adjusted slit width and other settings as recommended for the equipment employed were adjusted. The instrument was calibrated with standard sodium ion concentration to obtain a standard sodium ion graph. The sample was aspirated into the instrument and its concentrations obtained by extrapolation from the standard graph in mg/l.

Cultural Practices

Watering of the plant was done every two-days by applying one litre of the prepared different salt concentrations solution per replicated bag respectively; weeding and earthing of the plants were carried out when necessary.

Statistical Analysis

One way analysis of the variance (ANOVA) and Tukey Post Hoc test were used for the establishment of significant difference ($p < 0.05$).

Results

Chlorophyll Content analysis

The average chlorophyll content for maize plant for two months is shown in Figure 1. For Month One; highest chlorophyll composition was recorded from T₄ (1137.91 ± 1.24^a) with lowest values from T₂ (724.59 ± 9.24^b). For Month Two; lowest values were recorded from T₁ (1046 ± 0.00^d) when compared to other treatments as highest value was recorded from T₄ (1973 ± 0.00^a). There was significant difference in the chlorophyll compositions for both months one and two respectively.

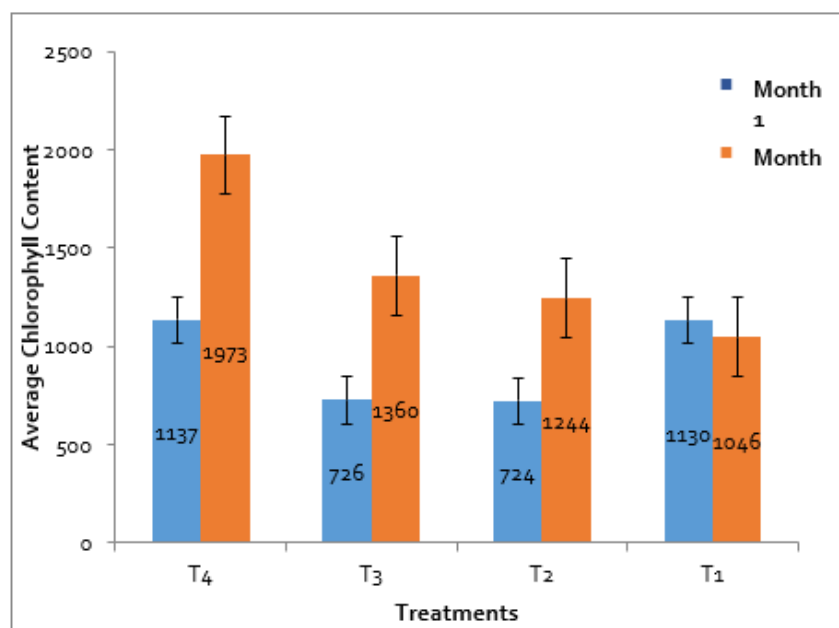
Salt Ion Analysis

Anion analysis (Chloride ion Cl⁻ content)

The result for anion composition is shown in Figure 2. T₃ had the highest composition (400 ± 0.011^a) when compared to other treatments, while T₄ had the least composition (222 ± 0.00^a).

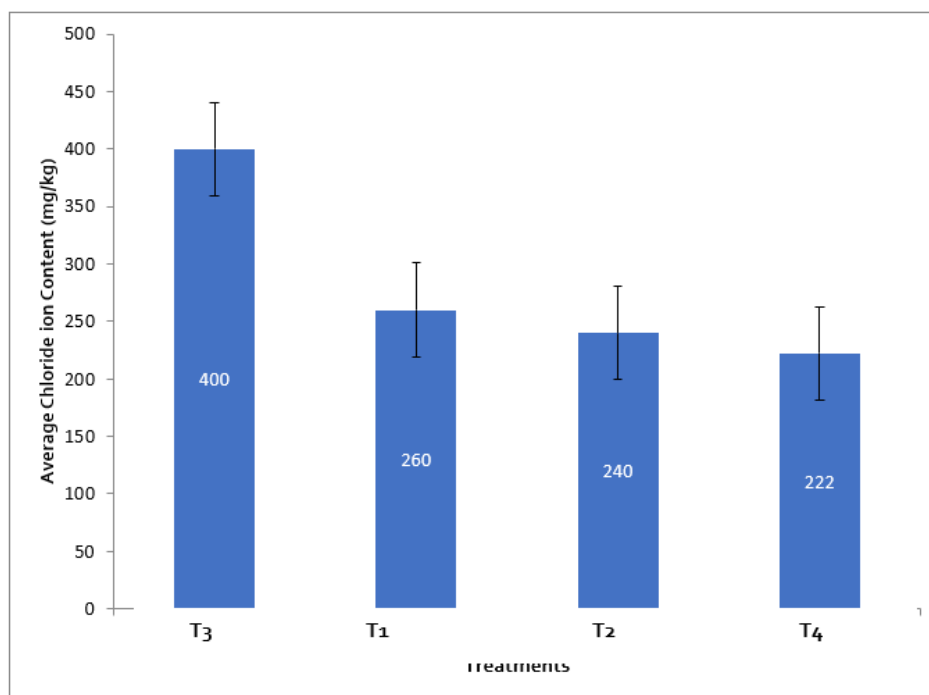
Cation analysis (Sodium ion Na⁺ content)

The cation composition is illustrated in Figure 3. Highest composition was recorded from T₃ (359 ± 0.001^a) and lowest composition was recorded from T₄ (269 ± 0.00^a) when compared to the other treatments used.



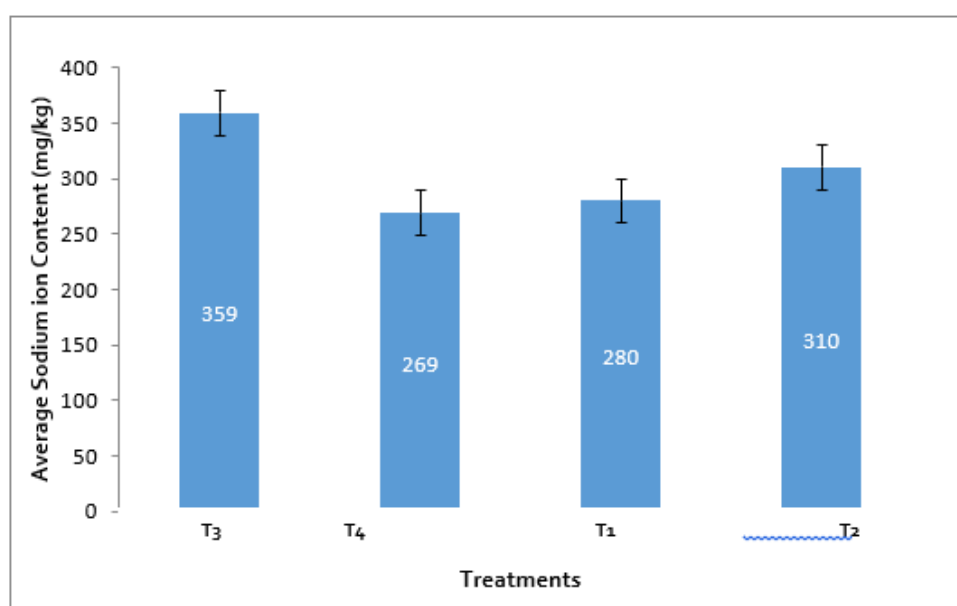
Keys: T1-10mM NaCl, T2-30mM NaCl, T3-50mM NaCl, T4-Control

Figure 1: Effect of salt concentrations on the chlorophyll content of *Z. May*



Keys: T1-10mM NaCl, T2-30mM NaCl, T3-50mM NaCl, T4-Control

Figure 2: Effect of salt concentrations on the chloride ion content of *Z. Mays*



Keys: T₁-10mM NaCl, T₂-30mM NaCl, T₃-50mM NaCl, T₄-Control

Figure 3: Effect of salt concentrations on sodium ion content of *Z. mays*

Discussion

Salt Stress Impact on Chlorophyll Content

The results for chlorophyll content amongst the different treatments used, revealed that there were significant differences at ($P \leq 0.05$). Photosynthesis is one of the most important biochemical pathways by which plants convert solar energy into chemical energy to enable them grow. The reduction in photosynthetic rate in plants under salt stress is mainly due to reduction in chlorophyll content and water potentials. The findings from this research are in line with the findings of (Cha-um and Kirdmanee, 2009) who observed a reduction in total chlorophyll contents in maize with increased salt stress. The reduction in chlorophyll contents of the plants can be attributed to the inhibitive effect of salt stress in the leaf expansion which causes a decline in photosynthetic pigment (Muhammad *et al.*, 2015). Photosynthesis is also inhibited when high concentrations of Na^+ and Cl^- are accumulated in the chloroplasts and chlorophyll being important content of photosynthesis directly correlates to the plant's healthiness (Gururani *et al.*, 2015).

Chlorophyll content reduction under salt stress has been reported in various studies. El Sayed (2011) and Qu *et al.*, (2012) also reported decline in chlorophyll and carotenoids content as a result of maize under salt stress, this is also in agreement with the findings of Chutipaijiti *et al.*, (2011) who reported similar results as *O. sativa* exposed to different NaCl showed a decrease in chlorophyll and carotenoid contents when compared to *O. sativa* not exposed to NaCl treatments. Total chlorophyll content was also reported to reduce due to

salt as leaf expansion, leaf abscission is affected by prolonged salt stress (Munns and Tester, 2008), this agrees with the findings of this present research as chlorophyll content also reduced with increased salt stress.

The reduction in chlorophyll content of maize plant under salt stress was also revealed by (Omoto *et al.*, 2012) who reported that a reduced photosynthetic activity in maize under salinity stress is attributed to the decrease in chlorophyll content with increased concentrations of salt, likewise, according to Gong *et al.*, (2011) different salt concentrations impacted photosynthesis differently, as a result of reduced chlorophyll content which is also similar to the results of (Amirjani, 2011) who reported that the chlorophyll contents in the leaves of *Oryza sativa* reduced when treated with NaCl treatments.

Salt Stress Impact on Salt Ions

There is lack of information on the effects of salinity on the salt ion composition in the seeds of maize, this study reveals that the harvested seeds of maize accumulated sodium and chloride ion and this could be attributed to an increase in the osmotic pressure in the plants. Other studies have revealed the accumulation of these ions in the leaves, (Munns and Tester, 2008) and that under salinity older leaves of plants accumulates more Na^+ than younger leaves. The results also agree with the findings of Hnilickova *et al.*, (2019) who reported an increase Na^+ accumulation in kiwifruit as similar increase was also revealed in rice genotypes under various salt concentrations. Though Cl^- ion is an important essential nutrient as it plays photosynthetic, stomata regulation and cytoplasmic regulation roles (Franco-Navarro *et al.*, 2019), yet a higher content can be toxic for metabolic activities (Tavakkoli *et al.*, 2011). The accumulation of Cl^- ion is at par with the study of Richter *et al.*, (2019) where they observed the concentrations of Cl^- ion to be higher in the shoots of *Vicia faba* after 7 days of salt treatments, similar results was reported by Pollastri *et al.*, (2018) where *Arundo donax* plant exhibited an excessive Cl^- ion accumulation under salt stress and saline soil.

In a study by Babu *et al.*, (2012) they reported that NaCl concentrations showed significant elevation in the levels of Na^+ concentrations in mature leaves and fruits of tomatoes. Similar studies were carried out and same outcomes were found by Beck *et al.*, (2004); Akram *et al.*, (2007). Flowers *et al.*, (1991) in their study reported that Na^+ ion variedly increased at different salt concentrations and that Na^+ transport from root to shoot is unidirectional and the resultant buildup of sodium ions in the leaves and fruits cause osmotic damages.

Conclusion and Recommendations

This present study has revealed that salt stress has an impact in the chlorophyll content and salt ion compositions of the different parts of maize plant studied with an increase in NaCl contents. This study conforms with previous researches who reported the inhibiting effect of salinity to plant performance. The impact on the chlorophyll content could be as a result

of inhibitory effect of salt stress in leaf expansion leading to a decline in photosynthetic pigment formations. Also, the salt ion contents in the seeds are attributed to excessive accumulation of sodium and chloride ion from the soil through the roots and increased osmotic pressure of the plant grown in saline soils.

Based on the findings of this study, the following recommendations are proposed:

It is recommended that soil used for cultivation should be analyzed as maize have proven not to thrive in soils with high sodium and chloride contents. Also, further study on the inhibitory effect and activity of salinity to leaf expansion should be carried out.

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