EFFECT OF SALINITY ON THE GROWTH OF *Telfaria occidentalis* Hook.f.

BY

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A PROJECT DISSERTATION SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC HONS) DEGREE IN PLANT BIOLOGY AND BIOTECHNOLOGY.

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CERTIFICATION

This is to certify that this work was carried out by **KOLAWOLE ANDREW LEKE** in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

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Prof. J. F. Bamidele **DATE**
(Project supervisor)

PROF. V. O. Vwioko **DATE**
(Head of Department)

DEDICATION

This work is dedicated to God Almighty, I also dedicate this work to my esteemed parents, Mr. and Mrs. Leke, and my honourable supervisor Prof. J. F. Bamidele.

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First and foremost, my profound gratitude goes to God Almighty, the giver of life, wisdom and strength for giving me the opportunity to complete this work and for His inspiration, guidance, protection and in making this work a reality.

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ABSTRACT

Fluted pumpkin (Telfairia occidentale Hook. F.) is a tropical plant belonging to the Cucurbitaceae family and holds significant economic value in Nigeria. Despite its high nutritional benefits and importance, there is a lack of information on how salinity impacts this plant. This study aims to evaluate the effects of salinity on the growth of T. occidentalis. The research was conducted over a four-week period. Seedlings of Telfairia occidentalis were placed in plastic bowls (30 cm in diameter) with drainage holes at the bottom to allow for free water drainage. Two weeks after planting, the seedlings exhibited uniform growth and were moved to the screen house of the Department of Plant Biology and Biotechnology. The plants were irrigated with saline water at varying concentrations: 0 (control), 70, 140, and 280 mM NaCl, with three replicates for each treatment. The results indicated that high salinity negatively affects T. occidentalis. The highest leaf length (19.67±1.53 cm) was observed in the 70 mM NaCl treatment, while the lowest leaf count (4.67±8.08) was recorded in week 4 for plants subjected to the highest salt concentration (280 mM NaCl). Additionally, the study found that salt stress adversely impacted the content of photosynthetic pigments, leading to a decrease in the chlorophyll content index and overall biomass yield. The findings suggest that T. occidentalis can thrive in low to moderate salinity levels (70-140 mM NaCl), but its growth is significantly hindered at high salinity levels.

CHAPTER ONE

INTRODUCTION

Saline soils are characterized by an electrical conductance (ECe) exceeding 4 dS/m, which indicates a high concentration of ions in the soil. At this threshold, approximately equivalent to 40 mM NaCl, many plants begin to experience significant yield reductions (Munns and Tester, 2008). Globally, over 800 million hectares are impacted by saline soils, representing more than 6% of the Earth's total land area (FAO, 2008). The majority of this salt-affected land results from natural processes, such as the weathering of rocks that releases various soluble salts, including chloride, sodium, calcium, magnesium, sulfates, and carbonates (Szabolcs, 1989). Additional sources of salt accumulation include the deposition of salts from seawater carried by wind and rain, as well as salts present in rainwater itself. It is estimated that rainwater can contain between 6 and 50 mg/kg of sodium chloride (NaCl), which can lead to significant salt deposits over time (Munns and Tester, 2008).

In addition to natural salinization processes, agricultural lands are also affected by secondary salinity, which arises from human activities such as land clearing and irrigation. This secondary salinity leads to elevated water tables and increased salt concentrations around plant roots. Approximately 32 million hectares of the 1,500 million hectares cultivated through dryland agriculture are impacted by secondary salinity, while 45 million hectares of the 230 million hectares of irrigated land are affected by salt (FAO, 2008). Although irrigated land constitutes only 15% of the total cultivated area, it is twice as productive as dryland agriculture. Therefore, yield losses due to increased soil salinization in irrigated regions have a disproportionately large impact. Unfortunately, the extent of farmland affected by salinization is on the rise, with irrigated land being particularly vulnerable (Munns and Tester, 2008).

The negative impact of soil salinity on agricultural yields is substantial. Addressing this issue will require a multifaceted approach, including changes in farming practices to prevent salinization, the implementation of remediation strategies to remove salt from soils, and initiatives aimed at enhancing the salt tolerance of crop plants through traditional breeding, genetic engineering, or the domestication of halophytes—plants that naturally thrive in saline environments. By improving crop salt tolerance, it is possible to develop plant varieties that can grow on marginal saline soils while longer-term land management practices are established. However, to enhance crop salt tolerance, it is essential to understand the two distinct stresses that plants face when grown in saline soils: osmotic stress and ionic stress.

Salt stress can adversely affect crop growth in several ways, including:

1. **Reduced Water Uptake**: Salt stress can dehydrate plant roots, leading to decreased water absorption (Szabolcs, 1989).

2. **Increased Osmotic Stress**: Salt stress can make plant cells hypertonic, resulting in various issues, such as diminished photosynthesis and growth (Munns, 2002).

3. **Ion Toxicity**: The accumulation of ions in plant tissues due to salt stress can be toxic (Flowers *et al.*, 1991).

4. **Reduced Photosynthesis**: Salt stress can damage the photosynthetic machinery of plants, leading to lower photosynthesis rates (Munns *et al.*, 2006).

5. **Altered Gene Expression**: Salt stress can trigger changes in gene expression, affecting the plant's physiological processes (Yeo, 1991).

Salt Tolerance in Plants:

Plants exhibit a wide range of salt tolerance, from highly tolerant halophytes to sensitive glycophytes. Salt tolerance is a complex trait influenced by several factors, including:

1. **Genotype**: The plant's genetic makeup is the most significant determinant of salt tolerance (Frensch and Hsiao, 1994).

2. **Environmental Conditions**: Factors such as temperature and water availability also play a role in influencing salt tolerance (Hu, 2007).

3. **Developmental Stage**: The stage of plant development can affect its ability to tolerate salt (Hu *et al*., 2005).

Strategies for Enhancing Salt Tolerance in Crops

Several approaches can be employed to enhance salt tolerance in crops, including:

1. **Breeding Salt-Tolerant Varieties**: Developing crop varieties that are specifically bred for salt tolerance is one of the most effective strategies.

2. **Utilizing Salt-Tolerant Rootstocks**: Implementing salt-tolerant rootstocks can significantly improve the salt tolerance of various crops.

3. **Soil Salinity Management**: Effective management of soil salinity levels can contribute to increased salt tolerance in crops.

4. **Application of Biostimulants**: Biostimulants represent a promising new method for enhancing salt tolerance in crops.

Charles Telfair (1778-1835), an Irish botanist residing in Mauritius, sent a species from the African cucumber family (Cucurbitaceae) to Sir William Jackson Hooker (1785-1865) for identification. In recognition of Dr. Telfair's contributions, Sir Hooker named the plant Telfairia occidentalis in his honor (Verma and Baksh, 2013). However, the first documented mention of Telfairia was by Oliver in 1871, which noted its presence in Upper Guinea regions of Sierra Leone, Fernando Po, and Abeokuta (Nigeria) (Akoroda, 1990). Telfairia occidentalis is widely cultivated for its delicious and nutritious leaves, which have a higher nutritional value compared to other tropical vegetables. With a protein content of 21%, its leaves surpass those of many commonly consumed leafy greens. Additionally, the leaves are rich in essential vitamins and minerals, including calcium, phosphorus, and iron. The seeds are also edible, and the oil extracted from them is used in cooking (Akoroda, 1990). There are also claims regarding the plant's medicinal properties.

LITERATURE REVIEW

BOTANICAL CLASSIFICATION OF Telfairia occidentalis

According to the Integrated Taxonomy Information System, the taxonomic hierarchy of Telfairia occidentalis is as follows:

- **Kingdom:** Plantae (plants)

- **Subkingdom:** Viridaeplantae (green plants)

- **Infrakingdom:** Streptophyta (land plants)

- **Division:** Tracheophyta (vascular plants)

- **Subdivision:** Spermatophytina (seed plants)

- **Infradivision:** Angiospermae (flowering plants)

- **Class:** Magnoliopsida

- **Superorder:** Rosanae

- **Order:** Cucurbitales

- **Family:** Cucurbitaceae (gourds and squashes)

- **Genus:** Telfairia Hook.

- **Species:** Telfairia occidentalis Hook. f. (oysternut)

POPULAR AND COMMON NAMES

Telfairia occidentalis is known by various names across different languages and regions, including: Fluted pumpkin, oyster nut, oil nut, fluted gourd, and Telfairia nut (English); Costillada (Spanish); Krobonko (Ghana); Oroko, pondokoko, and Gonugbe (Sierra Leone); Ugwu (Igbo-Nigeria); Aworoko and Eweroko (Yoruba-Nigeria); and Ikong (Efik/Ibibio-Nigeria) (Gbile, 1984).

BOTANICAL DESCRIPTION

Telfairia occidentalis is a large perennial climbing plant that utilizes bifid tendrils, which are typically coiled. The stem features five ridges and is often covered with multicellular hairs, particularly in its younger stages. The leaves are compound, usually consisting of 3-5 leaflets, with both the blades and petioles also exhibiting multicellular hairs. The fruits are notable for their 10 prominent longitudinal ridges and are among the largest known, measuring 16-50 cm in length and 9 cm in diameter. The seeds, encased in a bright-yellow fibrous endocarp, are large, non-endospermic, and typically dark red (Akoroda, 1990).

PHARMACOLOGICAL AND BIOLOGICAL ACTIVITIES

Antioxidant Properties

Telfairia occidentalis has been recognized for its antioxidant properties. The aqueous extract demonstrated higher levels of total phenols, reducing power, and free radical scavenging ability (12.2%, 1.9 OD700, and 92%, respectively) compared to the ethanolic extract, which showed total phenols, reducing power, and free radical scavenging ability of 5.5%, 1.5 OD700, and 25%, respectively (Oboh *et al.*, 2010). The leaf's free soluble polyphenols, which were more abundant than the bound polyphenols, exhibited greater antioxidant activity, as indicated by their superior reducing power and free radical scavenging ability (Nwanna and Oboh, 2007). Additionally, the leaves contained significantly higher amounts of vitamin C, total flavonoids, and phenolics than the stem bark of Psidium guajava, and they inhibited more free radicals than the latter (Aminu *et al*., 2012). The n-hexane fraction of the plant had the highest flavonoid content and free radical scavenging activity, comparable to the commercial antioxidant BHT. The leaf's ability to reduce iron (III) to iron (II) has also been documented. The antioxidant properties of Telfairia occidentalis are largely attributed to its high polyphenol content, particularly flavonoids.

Oxidative stress, often initiated by free radicals such as superoxide anions, hydrogen peroxide, hydroxyl radicals, and nitric oxide, is responsible for many human diseases. These free radicals can damage macromolecules like DNA, proteins, and lipids, leading to conditions such as diabetes, hypertension, atherosclerosis, cancer, myocardial infarction, arthritis, anemia, asthma, inflammation, and neurodegenerative diseases (Potterat, 1997). Endogenous antioxidants, including superoxide dismutase, catalase, glutathione reductase, ascorbic acid, and tocopherol, help protect the body from the harmful effects of free radicals. However, certain pathological conditions can impair the protective functions of these endogenous antioxidants, necessitating the use of exogenous antioxidants to safeguard against the damaging effects of free radicals.

Anticancer activity

Cancer refers to a broad category of diseases that can impact any part of the body, with metastasis being a notable characteristic. The World Health Organization (WHO) identifies cancer as a leading global cause of death, responsible for approximately 7.6 million fatalities (around 13% of all deaths) in 2008. Projections indicate that cancer-related deaths worldwide could exceed 13.1 million by 2030. Research suggests that Telfairia occidentalis has potential anticancer properties. The crude extract from its seeds demonstrated anticancer activity by significantly inhibiting oxidative burst activity in whole blood, as well as in isolated polymorphonuclear cells (PMNs) and mononuclear cells (MNCs). The effectiveness of the extracts was ranked as follows: hexane fraction > dichloromethane fraction > ethyl acetate fraction > butanol fraction > aqueous fraction > crude extract (Okokon *et al.*, 2012). Additionally, the seeds of T. occidentalis were found to lower serum prostatic acid phosphatase levels, enhance the testosterone to estradiol ratio, and diminish both the size and secretory function of the enlarged prostate, indicating their potential in managing experimental benign prostatic hyperplasia in rats (Ejike and Ezeanyika, 2011). The extract reduced the mean relative prostate weight, protein content (mg/tissue) in the rats' prostates, and serum prostatic acid phosphatase, likely by increasing serum testosterone levels while simultaneously decreasing serum estradiol levels. The leaves of Telfairia occidentalis contain phenolic compounds, flavonoids, phytosterols, tannins, saponins, chlorophyll, and glycosides, all of which have been reported to exhibit chemosuppressive effects.

Anti-inflammatory and Analgesic Activity

The leaves of T. occidentalis significantly inhibited carrageenan-induced edema in the sub-planar hind paw of vegetable extracts (Oluwole *et al.*, 2005). The seed extract demonstrated notable anti-inflammatory effects against egg albumin and xylene-induced edema and showed a dose-dependent reduction in pain during acetic acid-induced writhing, formalin-induced hind paw licking, and thermal pain models. The extract's ability to inhibit both neurogenic and non-neurogenic pain, as well as narcotic pain, may partially elucidate its mechanisms of action (Okokon *et al.*, 2012).

Effects of Salt Stress on Plant Growth

Osmotic Stress

Osmotic stress occurs when the salt concentration around a plant's roots reaches approximately 4 dS/m, leading to a rapid decline in shoot growth (Munns and Tester, 2008). This stress diminishes the expansion rate of growing leaves, the emergence of new leaves, and the development of lateral buds. Consequently, osmotic stress causes stomatal closure, elevated leaf temperatures, and a direct inhibition of photosynthesis shortly after salt exposure. In dicotyledonous crops like soybean, this stress results in smaller leaves and fewer branches, while in monocotyledonous cereals such as wheat, barley, and rice, the primary impact is a reduction in leaf elongation and tiller numbers, ultimately decreasing total leaf area (Munns *et al*., 2006).

Salinity stress can significantly impact root structure, affecting both primary and lateral root growth (Geng, 2013). The recovery of growth in these roots is at least partly linked to the stress hormone abscisic acid (ABA) (Mian, 2011). Additionally, salinity stress has been shown to negatively influence the mechanical properties of cell walls. Following exposure to saline conditions, the cell wall in the root's elongation zone becomes softer as sodium ions replace the load-bearing boron and calcium ions that typically crosslink the cell wall components. The stiffness of the cell wall is restored through the action of the plasma membrane-localized receptor kinase FERONIA, which is thought to work in conjunction with transient increases in cytosolic Ca2+ (Feng, 2018).

The mechanisms behind the reduction in leaf growth and shoot development remain unclear; however, a decrease in shoot area likely helps the plant conserve water, thereby maintaining soil moisture and preventing an increase in soil salinity. Osmotic stress not only affects vegetative growth but also impacts the reproductive development of crop plants, with osmotically stressed plants often exhibiting early flowering and/or a reduced number of flowers (Munns and Tester, 2008). Furthermore, osmotic stress leads to a decline in CO2 assimilation, which in turn reduces carbohydrate production, adversely affecting crop yield. Some plants can partially compensate for decreased CO2 uptake by developing smaller, thicker leaves with more densely packed chloroplasts, although this adaptation requires significant energy expenditure (Munns and Tester, 2008).

Ionic Stress

Ionic stress develops more gradually than osmotic stress and occurs when Na+ or Cl- accumulates to high levels in older leaves, leading to premature leaf senescence (Roy, 2013). In certain plant species, such as citrus, soybean, and grapevines, the accumulation of Cl- ions in the shoot is particularly toxic, as Na+ tends to be retained in the roots and stem (Lauchli, 1984). Recent studies have identified proteins that facilitate the long-distance transport of Cl-, enhancing Cl- exclusion and salinity tolerance. However, in most crop plants, including cereals, Na+ reaches toxic levels before Cl-, causing the majority of damage. Na+ and Cl- are transported to the shoot via the transpiration stream, which carries water from the roots through the xylem.

Potassium (K+) is essential for the activation of over 50 key enzymes. Elevated levels of cellular Na+ can disrupt various enzymatic processes by increasing the Na+: K+ ratio and reducing K+ availability. Additionally, protein synthesis relies on sufficient K+ concentrations for tRNA binding to ribosomes, so high Na+ levels can hinder this process. Ionic stress also has an osmotic component; high extracellular ion concentrations can lead to water loss from cells, severely affecting cellular function. Elevated Na+ and Cl- levels in leaves create osmotic challenges, as they must maintain cellular water potential below that of the soil to facilitate water uptake for growth. In saline conditions, plants must accumulate solutes to sustain water uptake, with Na+ and Cl- being the most readily available and energy-efficient options, despite their toxicity at high concentrations.

While Na+ and Cl- can be stored in vacuoles or the apoplastic space, maintaining low cytosolic levels of these ions is challenging for plant cells (Gorham, 1990). To lower water potential within cells, plants must synthesize compatible solutes that can be accumulated in high concentrations without disrupting metabolism (Hu *et al*., 2000). However, the synthesis of these solutes is energetically costly and places significant demands on a plant's energy resources. Overall, salt-stressed plants exhibit slower growth and increased mortality compared to non-stressed plants. The ionic effects become more pronounced only when salt levels are high or if a plant is particularly sensitive to salinity.

Variation in Plant Salinity Tolerance

Plants exhibit a wide range of responses to saline soils. While many species experience decreased growth and yield, some, like the salt-tolerant saltbush (Atriplex amnicola), thrive optimally at moderate salt levels (Azlam, 1986). Based on their sensitivity to salinity, plants can be categorized into two main groups: glycophytes, which are relatively susceptible to salt damage, and halophytes, which can withstand and may even benefit from high salt concentrations in the soil. Notably, halophytic saltbush can survive in salt concentrations comparable to or exceeding that of seawater. It is estimated that only 2% of plant species are true halophytes, while the majority, including most crops, fall into the glycophyte category (Glenn *et al.*, 1999).

Among crops, hexaploid bread wheat (Triticum aestivum), which contains the genomes of three wheat species (AABBDD), demonstrates moderate salt tolerance, capable of excluding 97–99% of Na+ from entering its shoots. In contrast, tetraploid durum wheat (T. turgidum ssp durum), with two genomes (AABB), is more sensitive to salt due to the absence of salinity tolerance genes found in the D genome of bread wheat, allowing it to exclude only 94–95% of Na+ from its roots. In durum wheat, there is a clear negative correlation between Na+ accumulation in the shoots and plant yield: higher Na+ levels result in lower yields. The variation in salinity tolerance among dicotyledonous crop species is even more pronounced than in cereals. On a salt sensitivity scale, sugar beet is considered salt tolerant, while cotton and tomatoes show intermediate tolerance, and chickpeas, beans, and soybeans are classified as salt-sensitive (Flowers, 2010). Quinoa, regarded as a promising new crop, demonstrates high salinity tolerance.

Mechanisms of Salt Tolerance

Osmotic Tolerance

Osmotic tolerance is the capacity of plants to sustain growth and water absorption despite increased Na+ levels around their roots. Osmotic stress can quickly hinder the growth of both shoots and roots, leading to the closure of stomatal pores. Plants that demonstrate higher osmotic stress tolerance tend to recover growth more swiftly, experiencing only a minor decrease in transpiration and photosynthetic rates (Awlia, 2016). This trait is advantageous in irrigated agricultural settings where water is abundant, but it may pose challenges in dryland farming if soil moisture is depleted before the growing season concludes. While it was previously believed that significant variation in osmotic tolerance existed among crop species, measuring this variation has been difficult until recently. Estimating growth rates typically requires daily monitoring of leaf growth or stomatal conductance, both of which can be time-consuming or necessitate destructive sampling for accuracy. However, advancements in nondestructive measurement techniques have greatly enhanced our understanding of salt stress physiology. By capturing digital images of plants over time, researchers can now assess relative growth rates immediately following salinity exposure, thereby gauging osmotic tolerance. New imaging technologies, such as infrared, fluorescence, and hyperspectral imaging, are being integrated into high-throughput phenotyping systems. These advanced methods can evaluate plant growth and the impact of salinity on this growth, as well as measure stomatal conductance, photosynthetic efficiency, and carotenoid levels. The combination of these innovative technologies and analytical tools will enable scientists to explore additional salinity tolerance mechanisms that have previously been difficult to characterize. Future genetic research is expected to uncover new genetic factors and processes related to osmotic tolerance found in the wild relatives of our elite crop varieties.

Ionic Tolerance

Na+ can accumulate in plant shoots to toxic levels at concentrations lower than those required for Cl toxicity. As a result, much research has concentrated on identifying variations in shoot Na+ accumulation and its transport within the plant. Ion concentrations in specific tissues can be easily assessed at particular developmental stages, using either image analysis (Rajendran *et al.*, 2009) or chlorophyll content meters to evaluate leaf senescence.

Ionic Tolerance: Tissue Tolerance

Tissue tolerance refers to a plant's ability to maintain tissue function while accumulating high levels of internal Na+ or Cl ions. Achieving this tolerance necessitates the compartmentalization of toxic ions in areas where they cannot cause harm. At the cellular level, this typically involves preventing the accumulation of Na+ and Cl in the cytoplasm, where critical metabolic processes occur. One approach to tissue tolerance is the compartmentalization of ions within the vacuole, a large organelle in plant cells that serves as a storage site. By utilizing this mechanism, a plant can store high concentrations of Na+ and Cl in its shoots while mitigating the toxic effects. There is already substantial evidence indicating variation among different crop varieties regarding the rates of Na+ and Cl accumulation in shoots, as well as the concentrations of these ions that various varieties can tolerate (Negrao, 2007).

Generation of Salt Tolerant Crops

Salt-tolerant crop plants can be developed once we have a comprehensive understanding of the mechanisms that confer salinity tolerance and the differences among plant species in utilizing these mechanisms. After identifying these mechanisms, it is essential to evaluate the advantages of incorporating them into crops. For instance, introducing salinity tolerance mechanisms from a slow-growing, highly tolerant halophyte into a cereal may not be practical if it results in a slow growth phenotype, delaying the cereal's maturity for years. Furthermore, a salt-tolerant crop must perform comparably to a sensitive variety when grown without salt. A high-yielding, salt-sensitive crop that experiences a 50% yield reduction under salt stress would still be more valuable to a farmer than a salt-tolerant variety that, despite showing minimal yield reduction, produces only 40% of the grain yield of the salt-sensitive variety.

It is also important to consider that different salinity tolerance mechanisms may need to be developed for crops depending on their growing environment. For example, crops cultivated in dryland agriculture may particularly benefit from tissue tolerance mechanisms, as the accumulation of high ion concentrations in plant cell vacuoles can help the plant extract more water from the soil. In contrast, an osmotic tolerance strategy combined with Na+ exclusion may be more advantageous for irrigated crops, where water availability is not a concern, but the Na+ content in the irrigation water may be elevated. Research has shown that the most salinity-tolerant Australian barley varieties possess multiple tolerance mechanisms (Tilbrook, 2017).

SALINITY INDUCES DECLINE OF BIOMASS AND YIELD LOSSES

Crop plants can achieve high yields and produce quality grains, fibers, and significant amounts of sugar, oil, or protein when their growth conditions are optimally managed. This includes proper nutrient and water management, as well as ensuring that light and temperature align with their ideal growth requirements. However, yield-related traits are negatively impacted by abiotic stressors such as salinity and drought during plant development. For instance, if salt stress occurs early in a plant's growth, it can lead to a substantial decline in yield and compromise the quality and quantity of the plant's products. It's crucial to recognize that salinity can result in crop yield losses, even when the effects are not immediately apparent. The salt tolerance and sensitivity of a specific crop depend on its capacity to absorb water and nutrients from saline soils while preventing excessive accumulation of salt ions in its tissues (Ahmad *et al*. 2017; Khan *et al*. 2017; Kaleem *et al*. 2018). Most vegetable crops are particularly sensitive to salt. According to the model proposed by Maas and Hoffman (1977), relative crop yield remains unaffected until a certain salinity threshold (ECt) is surpassed, as described by the equation: $Y = 100 – S\ (ECe–ECt)$. In this equation, Y represents the relative crop yield (with 100 being the maximum), ECe is the salinity of the saturated soil extract, and ECt (dS m^-1) is the threshold value of electrical conductivity (EC) that is anticipated to cause a significant initial reduction in maximum expected yield. The slope S indicates the percentage of yield reduction expected for each unit increase in salinity above the ECt. Salt tolerance in crops is assessed based on the salinity threshold (ECt) and the percentage reduction in relative yield per unit increase in soil salinity above this threshold (S). High concentrations of Na+ and Cl- can be toxic to plants, particularly when they accumulate in the cytosol. Despite the importance of this issue, there is limited understanding of the cytosolic processes that are disrupted by excessive salt ion concentrations. For example, the toxic effects of chloride in the cytosol are still not fully understood (Geilfus, 2018). When plants are subjected to high levels of salt ions, they can become poisoned and ultimately perish. The toxicity of ions can lead to various consequences, including ionic imbalances due to competition for uptake between Na+ and essential nutrients like K+, Ca2+, and Mg2+. This can accelerate the senescence of transpiring leaves, not only due to toxic ion concentrations in photosynthetically active tissues but also because of the reduced availability of beneficial nutrients. The energy dynamics of a crop under salinity stress are illustrated in Fig. 1. At any given moment, a plant has a limited amount of energy and resources that can be harnessed through photosynthesis or utilized metabolically (Munns and Gilliham 2015). Under non-stressed conditions, plants allocate most of their energy to maintenance and both vegetative and reproductive growth.

SALINITY TOLERANCE OF VEGETABLE CROPS AND FLESHY FRUITS

Most vegetable crops have a low salinity threshold of 2.5 dS m^{-1} (Snapp *et al*., 1991). Consequently, the area of soils that restrict vegetable crop production is larger than the area classified as 'salinized.' Saline soils are typically defined as those with an electrical conductivity (EC) value of the saturation extract (ECe) in the root zone exceeding 4 dS m^{-1} (approximately 40 mM NaCl) at 25 °C, along with an exchangeable Na^+ level of 15% (Shrivastava and Kumar, 2014). The relationship between soil salinity and the performance of sensitive and tolerant crops can be illustrated in a simplified diagram, as shown in Fig. 1B. Salt sensitivity varies across different growth stages; for example, salinity primarily impacted cauliflower growth during its initial growth phase (Giuffrida *et al*., 2016). The EC of irrigation water also plays a role in salt tolerance (Grattan, 2002). The lowest threshold level of irrigation water that does not hinder crop growth is 0.7 dS m^{-1}. For major crops like wheat and maize, a 10% yield reduction occurs at soil extract salinity (ECe) levels of 2.5 or 7.2 dS m^{-1}, while a 50% yield decrease is observed at ECe levels of 5.5 or 13 dS m^{-1} (for further details, see the review by Panta *et al*., 2014). The following section will examine the physiological ion adjustments in various grain crops and their mechanisms for coping with salt stress.

PERTURBATION OF CELLULAR ION HOMEOSTASIS

In non-saline conditions, nutrient ion fluxes in crops such as maize, wheat, barley, and broad bean are regulated according to developmental and cellular needs, thereby maintaining ion homeostasis (Niu *et al.*, 1995). A reduction in K^+ uptake induced by Na^+ can hinder shoot growth (Zorb *et al.*, 2014). High concentrations of Cl^- can also disrupt nutrient uptake by interfering with anion absorption (Geilfus, 2018). An antagonistic relationship between Cl^- and nitrate (NO_3^-) uptake has been observed at elevated external Cl^- concentrations (Cerezo *et al.*, 1999; Abdelgadir *et al.*, 2005), which can negatively affect growth and yield in wheat (Hu and Schmidhalter, 1998), although this effect was not confirmed in maize (Hutsch *et al.*, 2016). Competition for uptake between Cl^- and phosphate (PO_4^{3-}) has also been documented. Research on tomato (Papadopoulos and Rendig, 1983) and roses (Massa *et al.*, 2009) supports the notion of specific competitive inhibition of PO_4^{3-} uptake by Cl^-, while such an effect was not observed in melon (Navarro *et al.*, 2001). There is even less information regarding competition between Cl^- and sulfate (SO_4^{2-}), which may occur in peas (Mor & Manchanda, 1992). It seems that Cl^- may inhibit growth under NaCl salinity by causing deficiencies in phosphorus and sulfur due to reduced uptake of PO_4^{3-} and SO_4^{2-}; however, drawing general conclusions from the available data is challenging. For the validity of such studies, it is crucial to differentiate between the effects of Cl^- and those of the counter-cation.

PHYSIOLOGY OF YIELD LOSSES

Salinity stress is primarily influenced by the presence of NaCl, although other ions also play a role in determining stress levels. The toxic effects of different ions and their combinations can vary (Kaiser *et al.*, 1988; Hawkins and Lewis, 1993). At low salinity, the transport of salt ions into the vacuole helps manage the influx of ions across the plasma membrane into the cell

(Blumwald *et al*., 2000). However, at higher influx rates, cellular ion balance is disrupted, leading to the accumulation of anions such as Cl, SO4, or PO4, and cations like Na+, and sometimes Mg2+ or Ca2+, in the cytosol, matrix, and stroma instead of the vacuole. The activity of pyruvate kinase (Vmax) can increase by 400% in the presence of K+ (Oria-Hernandez *et al*., 2005), while substituting K+ with Na+ can inhibit this activity by up to 92% (Kayne, 1971). The peptidyl transferase activity of eukaryotic ribosomes relies on high K+ concentrations, achieving a turnover rate of 20 s^-1 (Ioannou & Coutsogeorgopoulos, 1997). A decrease in K+ concentration coupled with an increase in Na+ concentration can lead to metabolic imbalances, affecting redox and energy metabolism.

In the leaves of salt-sensitive plants, photosynthesis is inhibited. Photosynthetic electron transport becomes over-reduced in light conditions, resulting in the release of reactive oxygen species (ROS) (Miller *et al*., 2010). Mitochondrial respiration is also disrupted, further increasing ROS production during respiratory electron transport (Jacoby *et al*., 2010). Additionally, if stomata close and intracellular CO2 levels drop, photorespiration rates rise. Common biochemical markers of abiotic stress, including salinity, are ROS, lipid peroxidation products, carbonylated proteins, and increased ion leakage (Dietz, 2010). For example, in a NaCl-tolerant cotton cultivar, Pora, the activities of superoxide dismutase and glutathione reductase increased, while they remained unchanged in the sensitive cultivar, Guazuncho (Meloni *et al*., 2003). The relationship between tolerance and the increased activity of antioxidant enzymes and repair mechanisms is complex, as it is influenced by salinity levels, exposure duration, and circadian rhythms. Under lower stress conditions, tolerant species can maintain homeostasis through effective compartmentalization and nutrient uptake without needing to upregulate defense mechanisms, whereas sensitive species may experience homeostatic disturbances and activate general defense responses. Generally, tolerant species tend to fine-tune their antioxidant systems. For instance, in sugar beet, salt exposure leads to the upregulation of antioxidant enzymes such as superoxide dismutase, alternative oxidase, and plastid terminal oxidases, while transcripts for NADPH oxidase decrease, resulting in lower ROS production in salt-exposed tissues compared to control plants (Hossain *et al*., 2017).

SIGNALING AND THE ROLE OF PLANT HORMONES

Addressing salinity without considering the roles of signaling pathways and hormones would be incomplete. In this discussion, we highlight several recent advancements. The discovery of the salt overly sensitive (SOS) signaling pathway during a seedling-based screening for Na+-sensitive mutants of Arabidopsis thaliana in 1997 (Liu and Zhu, 1997; Zhu *et al*., 1998) marked the beginning of an in-depth investigation into the cellular mechanisms underlying salt acclimation. While halophytes consistently express tolerance genes, most other species, including crops, activate these mechanisms only in response to specific stressors. This activation initiates a salinity acclimation response that includes changes in physiological processes, metabolic adjustments, enhanced defense and repair systems, and alterations in developmental programs to adapt morphological and anatomical characteristics. Such extensive reorganization indicates the involvement of multiple regulatory pathways. Numerous studies, particularly those involving Arabidopsis mutants and transcript profiling, have underscored the essential roles of plant hormones in the acclimation process, growth, and organ development under saline conditions.

****Progress in Crop Improvement Over the Last Decade:** For crop production, achieving salt tolerance is crucial for increasing yields. While experiments using seedlings, hydroponics, or greenhouse settings can clarify mechanisms, they often do not provide conclusive evidence for crop performance in real-world scenarios. Cross-scale studies are rare, and in Europe, the deployment of genetically modified crops in the field faces significant challenges. Despite the complex nature of salt tolerance, literature frequently suggests that transferring a single or a few genes can greatly enhance plant resilience to saline environments (Parmar *et al*., 2017). A review from the past decade evaluated genetically improved crops based on just 19 reports that included quantitative growth data, concluding that while these studies may illuminate aspects of tolerance, they do not support claims of improved tolerance at the whole-plant level (Flowers, 2004).For example, the introduction of a gene for Na+ exclusion (Nax2) from the wheat relative Triticum monococcum into durum wheat (Triticum durum) effectively reduced leaf Na+ concentration by promoting its removal from the xylem (James *et al*., 2006). Field trials on saline soils involving the overexpression of TmHKT1;5-A, a gene within the Nax2 locus, showed decreased leaf Na+ levels and increased grain yield in durum wheat (Munns *et al*., 2012). Unfortunately, recent instances of successful field-verified increases in salt tolerance for genetically modified crops are limited. A major challenge in developing salt-tolerant crops is that many screening experiments evaluate candidate plants' performance under salt stress for only a short duration, often during the vegetative phase (e.g., assessing plants during germination or the early weeks of growth). It is crucial to implement screening methods that assess performance throughout both the vegetative and reproductive stages, as demonstrated by the salt-tolerant rice variety BRRI Dhan 10 (Islam *et al*., 2008). Ismail and Horie (2017) provide a summary of advancements in breeding strategies aimed at enhancing salt tolerance in rice, wheat, and barley, directing readers to a recent review for further insights.

Aim and Objectives

Aim of the Experiment

The aim of the experiment is to evaluate the effect of salinity on the growth of *Telfaria occidentalis*

Objectives of the Experiment

The objectives of the experiment are to:

1. Study the growth of *Telfaria occidentalis*
2. Evaluate the effect of salinity on the chlorophyll content of *Telfaria occidentalis,*

CHAPTER FOUR

DISCUSSION

The current study investigated the impact of salinity on the growth of Telfaria occidentalis, focusing on various parameters such as chlorophyll content index, leaf count, leaf length, and the fresh and dry weights of the plant's stem, leaves, and roots. The findings indicate that salinity significantly influences the physiological and biochemical processes of plants, which are critical for their growth. Chlorophyll content was measured using a specialized chlorophyll probe meter, as shown in Figure 3.1. The results revealed that plants exposed to high concentrations of saline mixtures exhibited the lowest chlorophyll content index (CCI). Several factors may contribute to these effects, as previous research has shown that salinity is a major abiotic stressor that disrupts plant cells. Under saline conditions, the rate of photosynthesis decreases, likely due to irregular stomatal closure, which reduces intercellular CO2 concentration, along with other non-stomatal factors.

There is substantial evidence that salt impacts photosynthetic enzymes, chlorophyll, and carotenoids (Munns, 1993). This observation aligns with the findings of Jing *et al.* (2015), who reported a significant decrease in total chlorophyll concentration in Suaeda aralocaspica when exposed to higher salinity, attributing this decline to damage to chloroplast structures. Similarly, Amira and Abdul (2010) noted reductions in chlorophyll 'a', 'b', and total chlorophyll in V. faba subjected to saline stress. Tort and Turkyilmaz (2004) also documented a decrease in chlorophyll 'a', 'b', and total chlorophyll content in barley (Hordeum vulgare L.) exposed to varying concentrations of sodium chloride. This decline may be linked to the suppression of chlorophyll biosynthesis precursors (Santos, 2004) and reduced magnesium uptake (Giri and Mukerji, 2004).

High salinity levels significantly affected leaf length, leaf number, chlorophyll content index, and plant weights. These observations are consistent with a study by Anoliefo and Vwioko (1995), which examined the effects of high chromium concentrations on plant growth. Most elements, particularly sodium, carry ionic charges. When sodium chloride (NaCl) dissolves in water, it breaks down into ions, altering the electrical conductivity (EC) of the water. As ion concentration increases, so does the EC (measured in dS/cm^2 or dS/m) (Richard, 2004). Plant activities can be completely inhibited at EC levels comparable to seawater, approximately 54 dS/m or 54,000 µS/cm. Even at salt stress concentrations lower than seawater, plants face challenges in acquiring soil water and nutrients due to negative osmotic effects, direct cellular damage, or failure of soil particles to aggregate. These issues can lead to reduced yields, lower farmer incomes, and compromised nutrition. Salt-sensitive plants are adversely affected by salt concentrations at all developmental stages, with germination being the most vulnerable process (Shahi *et al.*, 2010).

The decline in growth parameters due to salt concentration is illustrated in Tables 3.1 to 3.3. The stunted growth observed in plants treated with high salinity is consistent with findings by Foy *et al*. (2008). Azmar and Haider (2007) reported that high concentrations of toxic media resulted in reduced leaf lengths, plant weights, and chlorophyll content. Jones *et al*. (1973) noted that plants submerged in soil contaminated with heavy metals suffer from oxygen deprivation, leading to slow growth and the inhibitory effects of toxic metals on root development. Slow growth may also result from nutrient imbalances in soils with high chromium concentrations, which can hinder physiological processes essential for plant growth and crude protein accumulation. The decline in nutrient uptake, particularly in chromium-laden soils, has been previously reported by Eun *et al*. (2002). Sharma and Pant (1994) also found reduced uptake of Fe, Mn, and Zn in maize due to chromium application. This observed reduction corresponded to significant decreases in the fresh and dry weights of leaves, stems, and roots.

To mitigate the severe effects of salinity on plants and soils, effective regulatory measures should be implemented to reduce salt deposits in water and on land. Nitrogen-containing fertilizers have been recognized as highly effective in alleviating stress effects and enhancing plant growth and productivity (Malhi *et al*., 2001).

Conclusion

The findings of this research indicate that soil salinity is one of the most significant abiotic stresses impacting plant growth and production globally, as demonstrated in this study. The results revealed that salt stress adversely affected the contents of photosynthetic pigments, leading to a reduced chlorophyll content index and diminished biomass yield.

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